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Morphological variation in the distal phalanges of the springbok

Marine seismic surveys for hydrocarbon exploration

Insecticide resistance in *Anopheles arabiensis* in KwaZulu-Natal

Industry–university research collaboration partnerships

Determining safe retirement withdrawal rates

Energy poverty, burns and the need for a safe and inclusive energy transition

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Book Reviews

Invited Commentaries

Commentaries

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Research Articles

Research Letters

Cover caption
A springbok (*Antidorcas marsupialis*). In an article on page 98, Rossouw compares the distal phalanges of adult springbok and shows distinctive morphological differences between the subspecies *Antidorcas marsupialis marsupialis* and *Antidorcas marsupialis hofmeyri*.
Photo: Leon Pauleikhoff

Our Journal in 2021: The year that was

The mission of our Journal is to *promote the visibility and impact of South African and African research* by publishing high-quality *original research from Africa or on African-relevant issues* that will be of interest to readers in *any discipline and for the benefit of scholars, educators, the general public and policymakers*.

Each year we gather data on the previous year to see how well we are doing in achieving that mission, as well as how well we are performing as a journal globally.

Publishing original research with African relevance

In 2021, a total of 490 original research and review articles were submitted to the Journal (down from 550 in 2020) – 43% of these submissions were from South Africa, and 24% were from elsewhere on the continent. We published 80 peer-reviewed articles across the six issues published in 2021. About 75% of the published authors were from South Africa, with only 10% from the rest of Africa. Most of the published articles (70 of the 80) were multi-authored. Our overall rejection rate in 2021 was 91%. The rejection rate is typically high because of the criterion of African relevance, as evidenced by 50% of desk rejections being submissions from outside of Africa, with about 25% each from South Africa and the rest of Africa.

Our average turnaround time from submission to final decision (which includes revision rounds) was 133 days in 2021. As is the case with many other journals, we struggle to find reviewers to review for us – on average per submission in 2021, we approached six reviewers to secure at least two peer reviews; on occasion we approached many more than six. In total, 870 reviewers were approached in 2021, of whom 228 completed a peer review and 8 completed two peer reviews. Of those reviewers who completed reviews, 60% were based in South Africa. A list of reviewers who reviewed for our Journal in 2021 can be found here. We are grateful to every reviewer who completed a review, as well as to those who, if unable to review, responded and recommended others. One of our biggest challenges in improving turnaround times is the high number of reviewers invited who do not respond, either to accept or to decline a review invitation.

In addition to publishing the Journal, we participated in initiatives such as Peer Review Week and held a very well-attended virtual workshop on writing for a scholarly journal. We host a monthly open forum on Zoom for any early career authors to raise questions about the publication process; this is for all authors and not only those hoping to publish in our Journal. We are planning more support and training activities in peer reviewing, both as a capacity-building service and to broaden and further diversify the pool of reviewers we call upon.

Promoting the visibility and impact of African research

There were 113 000 visits to our website in 2021 (based on Google Analytics), with 57% of visits coming from readers in South Africa. Although the Journal is read in almost every country, 15% of our readers in 2021 were based in the USA, and under 10% were from the rest of Africa. There were 320 000 article downloads from our website in 2021. The 2021 article with the most views in 2021 (12 500 views) was co-authored by researchers in South Africa and Madagascar.

The Web of Science Journal Impact Factor from Clarivate released in 2021 is 2.197. Based on this impact factor, the Journal ranks 39/73 multidisciplinary science journals and 3/33 South African journals in the Science Citation Index Expanded of the Web of Science.

For the benefit of scholars, educators, the general public and policymakers

Before each issue is published, we compile and send a media release, which includes the table of contents and abstracts of each article, to local mainstream media. Media reports on articles published in the Journal can be viewed on our website here. In 2021, there were 129 such media reports on articles published in the Journal, with a global online reach of almost 11 million.

We also have an active and growing social media presence, and we encourage our readership to follow us on Twitter, Facebook and LinkedIn and to engage with us through these platforms.

The articles published in 2021 contributed to 14 of the 17 Sustainable Development Goals (SDGs), with SDG13 (Climate Action) and SDG15 (Life on Land) being predominant.

Writing a journal article for a broad audience is more difficult than writing an article for specialist readers. In 2021, we introduced an annual Outstanding Article Award for an outstanding peer-reviewed article published in the Journal that advances the mission of our Journal, makes an important contribution to knowledge in its field, and is eloquent and accessible to the broad readership of our Journal. The winning article was announced this month.

How did we do?

The *South African Journal of Science* was established in 1905 and is still going strong, thanks to the support of our authors and readers, and, in recent years, our becoming part of the Academy of Science of South Africa (ASSAf). We are committed to continuing to produce a multidisciplinary African journal of high quality. We would like greater participation in the Journal from African countries outside of South Africa, as continental debates are important. We are committed to supporting constructive debate across the spectrum of science and research disciplines, and hope to continue to provide a platform for discussion. Diversity and access are important issues for us, and we are working in a range of ways to widen participation in the Journal. Readers will hear more about this in forthcoming issues.

As always, we are dependent on feedback from our authors and readers about the things we are doing well but also, crucially, about where we can do better. We are fortunate that our Journal is funded from the public purse in South Africa – the Journal belongs not only to the science community but to the community more broadly, and we are aware of the responsibility that comes with this. We are happy with the growth and interest evidenced in the statistics gathered about our Journal (for space reasons we have shared just a few), but we can always do better. For this, we need your help. It is also important to note that every achievement of the Journal is attributable in large part to our authors, reviewers, associate editors, and readers.

Thank you.

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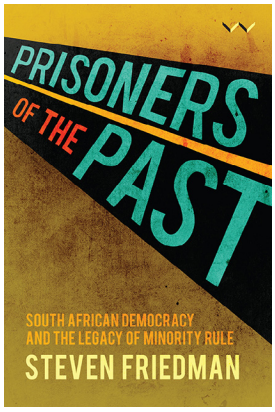
Prisoners of the past: South African democracy and the legacy of minority rule



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Negotiating with the past by negotiating in the present: A review of *Prisoners of the Past*

When I was asked to review Steven Friedman's new book, *Prisoners of the Past: South African Democracy and the Legacy of Minority Rule*, I felt bemused. It was surely unusual – even outrageous – for a clinical psychologist to assess the work of a political scientist. But I understood the nature of the request on reading the author's opening quote, taken from William Faulkner: 'The past is never dead. It's not even past.' That we are never done with history goes without saying in the psychoanalytic canon.

Drawing on the corpus of Douglass North, Friedman diagnoses the present South African malaise as a case of *path dependence*, which involves 'retaining the same ways of doing things, the same set of economic and social values, and the same connections even when societies experience great change in their formal rules' (p. 34). In a non-trivial sense, his study is a psychological one: he wants to understand the intractability of our country's social and economic problems by focusing on behavioural patterns, cognitive structures and social relationships.

Towards that end, *Prisoners of the Past* unpacks a series of common myths, one of which is the view that state corruption is a post-apartheid novelty. Friedman describes just how venal the colonial and apartheid orders were, quoting for good measure the words of one scholar – that '[t]here is a tradition of corruption in South Africa and it's a white tradition' (p. 59). But he also makes the crucial point that persistent (i.e. path-dependent) racial hierarchies in the market have created a ceiling effect for an aspirant black middle class – with the *state* becoming, for some, 'their primary economic vehicle' (p. 12). It all started at the dawn of democracy when the old economic and new political elites doomed us to path dependence, assuming that 'a democratic South Africa should extend to everyone what the white minority enjoyed' (p. 44). They failed to appreciate that 'the minority would have to give up some of what it had enjoyed if everyone was to enjoy full economic citizenship. And so, the need to negotiate new economic rules which would open opportunity was ignored' (p. 14).

Another myth that Friedman tackles is the fear among white South Africans that the country is always on the brink of all-out war. He reminds us, rather, that the underclasses are unable to organise themselves into a political force *because* of their economic exclusion. Politics, therefore, becomes about insiders, and insider politics is 'more often than not racial politics' (p. 104). The paradox is that insider demands are often articulated in the language of the left, with frequent reference being made to social and economic inequality. Friedman cites as examples the #FeesMustFall movement and the pushback against e-tolling in Gauteng; in both cases, the benefits accrued not to poor people but to the affluent classes instead.

Friedman insists, however, that insider politics 'is not a sham' (p. 102) because 'something more subtle and important is at play' (p. 110). What he is driving at, is the anger of a frustrated black bourgeoisie: 'middle-class black people enthusiastically support demands which appear to assist outsiders because they want change, not necessarily because the issue is important to them. The assumption [is that] nothing will change unless there is a crisis' (p. 96). In theoretical terms, that is, 'path dependence can be changed if either the elites' perception of their interests changes (because pressure forces a change or because existing patterns do not offer the benefits they once did) or if the elite which favours the existing arrangements is replaced by one which does not' (p. 43).

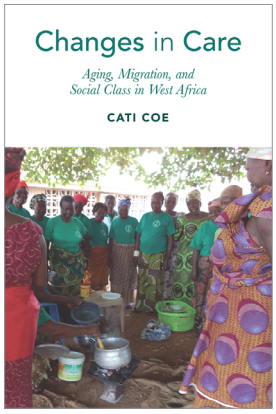
Next up is a myth about the South African Constitution. Some regard this document as part of the problem – particularly on the question of land – and so decry it as a continuation of colonisation by other means. But, for Friedman, the constitution is *necessarily* open-ended because 'the more [it] decides what social policy should be, the more does power shift from politicians and the voters to whom they account to judges and lawyers who are not bound to reflect the wishes of citizens' (p. 133). South Africans should not expect the Constitution to stipulate everything right down to the last detail, nor should we wait on some miracle summit of power brokers that will generate a new social compact to replace the one fraying before our eyes. Our fractures run too deep for a national vision to emerge suddenly.

Instead, the responsibility of citizens is to pressure the state into driving a process where stakeholders with competing interests – the white elite, the black elite, the unions and the unemployed – are compelled to engage with one another in a dialectical, back-and-forth manner that is all about hard bargaining and compromise. With the post-1994 deal no longer working, 'any attempt to begin movement away from path dependence must begin with a strategy designed to ensure that bargaining begins because the key interests recognise the need for it' (p. 162).

There is a realism at the heart of Friedman's argument that is to be admired and not mistaken for reformism. He notes that '[a] total break with the past order cannot provide a better future even if this rupture were possible, which it may well not be since no one has yet succeeded anywhere in building a new order which contained no traces of that which it replaced' (p. 153). And that is about as psychological as it gets, that traces of the past will forever be with us. Indeed, it is not always the radical act that produces the radical outcome: 'incremental changes to a path-dependent society can produce fundamental change' (p. 154).

But what specifically generates change? Friedman offers the classical psychoanalytic answer according to which knowledge that is consciously held, is key: 'if changes are born of a *recognition* of path dependence, and are *consciously* designed to weaken it, there is no reason why the result should entrench the past and the power relationships which sustain it' (ibid., added emphases). Yet psychoanalytic theory – and psychology more broadly – has moved on since then. It is arguably not insight *per se* that precipitates change, but *an act of will*. The trouble is that our nation's emotional life is racked with shame, guiltiness and, as Friedman himself acknowledges, bitter resentment. None of these affects is likely to produce acts of will that are consistent with what we know to be true about the world. We may well be prisoners of the past after all.

BOOK TITLE:
Changes in care: Aging, migration, and social class in West Africa



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Changing long-term care realities and futures for older persons in (West) Africa

The provision of and need for long-term care (LTC) for older persons in sub-Saharan Africa are already extensive[1] and predicted to rise further given demographic[2] and epidemiological trends[3]. There is, however, a scarcity of publications on this topic.[1] We thus find *Changes in Care* to be an influential volume of sound scholarship because it contributes towards filling this gap by comprehensively examining the LTC practices that are emerging in southern Ghana to address the growing demand for LTC. It explains, through the lens of ageing, how social change is occurring within and beyond kin groups. Indeed, it is an exciting and excellent contribution to the small corpus of work on care for older persons in sub-Saharan Africa. What makes this monograph particularly noteworthy is that these themes are approached as processes that are complex, quiet, organic and sporadic, rather than simplistically linear as is still often proposed by exponents of ageing and modernisation theory.

The introduction presents a background to ageing in Ghana. It is fascinating how the author uses her own experiences to explain relevant theories, such as the concept of inscriptions. Inscriptions are some people's responses to problems (caused by conflicting norms), which lie within the continuum between shared norms and individual actions. In terms of norms related to ageing and care in Ghana, the orthodox position of families caring for older persons (kin care) may be challenged by alternative solutions (heterodoxies) when it no longer provides a desirable result and forces people to explore other options. Similarly, some care inscriptions (alterodoxies) emerge, in quieter, less visible ways, as extensions of kin care. The processes by which social change occurs regarding age and care inscriptions and the actors that drive these processes are well covered.

Chapter 1 discusses the orthodoxy of kin care as illustrated in policy architecture and popular discourse in Ghana, and which LTC practices it ignores. The author explains how history helps with understanding the patterns of kin work, ranging from domestic slavery in the 1860s to the move by daughters back to their hometowns in the 1990s to care for their elderly parents. This chapter reveals the dynamism of kin care arrangements in Ghana.

Part I (Chapters 2–4) lays out how social changes related to ageing and care take place in the rural towns in Ghana's eastern region. Chapters 2 and 3 focus on the fears and realities of familial neglect that have forced older persons to consider alternative practices. Chapter 2 largely explores heterodox residential facilities that rural older persons in Ghana are attracted to, despite this still being a relatively unfamiliar Western concept. Alterodox solutions, examined in Chapter 3, include: adult children paying for and managing their parents' LTC provided by domestic workers and foster children; older women moving into the homes of their migrant daughters; and kin care being supplemented by neighbours. Understandably, these approaches seem likely to become more widely accepted than residential facilities, as they are more closely aligned with Ghanaian social norms. Chapter 4 outlines the role of the church in providing beyond-kin care options for mobile and relatively healthy older persons.

Chapters 5–7, which form Part II, cover emerging commercial LTC initiatives in the urban area of Accra-Tema that are available for the small number of middle-class and elite older persons who can afford them. These consist mainly of private agencies providing home care to older persons and a few small, struggling residential care facilities. Chapter 5 insightfully illustrates how commercial LTC services associated with Western societies are 'assembled' and modified, by the uncoordinated actions of various actors, as heterodoxies or alterodoxies. Focusing on the experiences of owners, consumers, and employees of LTC services, this chapter describes how transnational migration has assisted in creating and sustaining the LTC market. The education and training of home carers, elaborated in Chapter 6, includes the complex relations between the private and public healthcare sectors in the context of neoliberalism and the ways in which home carers sought and were denied cultural capital. Chapter 7 examines the new inscription of the home carer, highlighting emergent forms of social inequality between and within households. It discusses how home carers position themselves as nurses, despite being viewed as domestic workers by most of their employers. This chapter candidly describes the difficulties experienced by young, mainly female, home carers in Ghana when they look for employment with status, respect, and a salary that can support themselves and their families.

Finally, the conclusion presents recommendations to address issues examined in the previous chapters in order to improve the LTC sector in Ghana for all actors. It discusses the possibility that older Ghanaians could become a political force through their critique of the neglect they face by other social groups.

Changes in Care is a well-conceptualised and well-structured book, whose tone neither patronises Africa nor apologises for problematising issues and concepts. It is elegantly written in a manner that is both scholarly and accessible to wider audiences, including academics, practitioners, and policymakers. Professor Coe draws the reader into the intimacy of her personal research journey, critically but sensitively exploring the complex concept of social change without falling into tired binaries. She understands LTC, ageing, and social change in Africa, as well as the diversity of social change in the context of the broader globalised world. Hers is a voice and approach that are much needed in African scholarship.

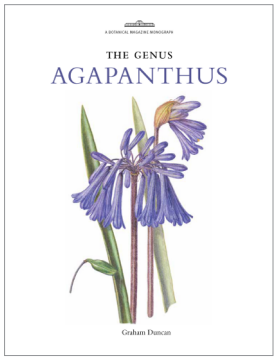
In addition, even though this book focuses predominately on middle-class and elite older persons (a limitation that the author acknowledges), it supplements existing literature and contributes towards a scientific understanding of LTC systems in Africa.[4] The practices presented in it can be applied to drive the expansion of innovative, culturally appropriate, affordable, and accessible LTC services and, ultimately, to influence policy and practice.

Changes in Care is a relevant and significant piece of work from Ghana for Africa and beyond. It offers value to everyone – from those unfamiliar with LTC and social change to subject experts who seek better understanding of LTC, social change, and ageing in the African context.

References

1. Aboderin I, Epping-Jordan J. Towards long-term care systems in sub-Saharan Africa: WHO series on long-term care. Geneva: World Health Organization; 2017. Available from: https://www.who.int/publications/i/item/9789241513388
2. United Nations Department of Economic and Social Affairs Population Division. World population prospects 2019: Highlights. New York: United Nations; 2019. Available from: https://www.un.org/development/desa/publications/world-population-prospects-2019-highlights.html
3. Bloom DE, Canning D, Lubet A. Global population aging: Facts, challenges, solutions and perspectives. Daedalus. 2015;144(2):80–92. https://doi.org/10.1162/DAED_a_00332
4. De-Graft Aikins A, Apt NA. Aging in Ghana: Setting priorities for research, intervention and policy. Ghana Stud. 2016;19(1):35–45. https://doi.org/10.1353/ghs.2016.0002

BOOK TITLE:
The genus *Agapanthus*



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A new revision of *Agapanthus*

Agapanthus is a genus of plants in the family Amaryllidaceae, where it is the only genus in the subfamily Agapanthoideae. The genus includes eight species, with four subspecies recognised under *Agapanthus inapertus*. These plants are endemic to southern Africa; all eight species are found in South Africa, and three species also occur in Lesotho (two species), eSwatini (two species) and southern Mozambique (one species). One might be forgiven for wondering how it could be possible to fill over 240 pages on a description of just eight species, but this book offers much more than just a revision of a small genus of plants. The taxonomic account is presented at the end of the book, and occupies about a third of the pages. *Agapanthus* are also globally popular garden plants. The different species show an extraordinary propensity for hybridising in cultivation, giving rise to at least 625 recognised cultivars, of which 155 (90 deciduous and 65 evergreen) are covered in a further third of the book. The selection is intended to portray 'some of the better recommended cultivars, and for which a reasonably good chance exists of their being obtained in the nursery trade from at least one outlet'. Each cultivar is also rated in terms of its hardiness with respect to cold temperatures.

The book is a testament to the difficulties encountered in unravelling the identity of species. *Agapanthus* species have proved to be difficult in this regard because of the paucity of unique characters that could be used to separate them, as well as the extraordinarily high level of morphological plasticity that exists in some species depending on the conditions found at the sites where they grow. The genus has also been ping-ponged back and forth between its own family Agapanthaceae and a subfamily in the Amaryllidaceae many times since 1836. Frances Leighton's treatment of the genus in 1965, in which 10 species were recognised[1], has stood as the only comprehensive account of the genus for over half a century.

The taxonomic treatment provides a comprehensive description of each species' characteristics and distinguishing features, phenology, distribution, habitat and conservation status. The reader is also introduced to a new species (*A. pondoensis*), which was first collected in 1953, but considered to be a subspecies of *A. praecox* – 65 years were to pass before the author was able to collect further material and confirm its status as a separate species. Judging by the photographs of its habitat (sheer cliffs alongside spectacular waterfalls), it is no wonder that it remained unstudied for so long. The taxonomic text is complemented by many colour photographs of each species in its natural habitat, as well as distribution maps and superb full-page colour plates by the artist Elbe Joubert.

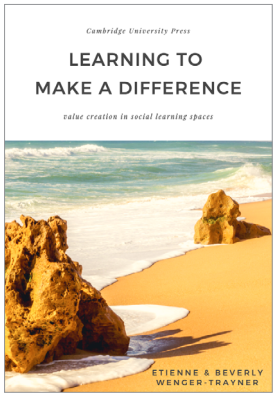
The remaining third of the book is made up by chapters addressing the history of discovery and classification, cultivation and propagation, ecology and conservation, and biology. Together these chapters cover just about everything that is known about where these plants come from, how they live, reproduce and die, and how they are faring in the modern world. Many of the species are not yet under threat in their native habitats, as they are fairly widely distributed and grow in relatively inaccessible sites. The exception is *A. walshii*, which is classified as Endangered due to its restricted range, expanding human settlements, invasive alien acacias and changing fire regimes.

This book is a welcome addition to the growing South African botanical literature. Graham Duncan is to be congratulated on an impressive volume, which is in line with his earlier treatments of the genus *Lachenalia* (published by Kew Publishing in 2012), and of the family Amaryllidaceae (published by Umdaus Press in 2017). I would recommend this book unreservedly to naturalists and gardeners, as well as to collectors of Africana.

Reference

1. Leighton FM. The genus *Agapanthus* L'Héritier. J S Afr Bot. 1965;supplementary volume 4.

BOOK TITLE:
Learning to make a difference: Value creation in social learning spaces



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Elevating everyday learning to a level of awareness where it can make a difference

Thirty years ago, social learning theorist Etienne Wenger (now Wenger-Trayner) and social anthropologist Jean Lave coined the term 'community of practice' (CoP), based on their observations of learning among traditional tailor apprentices in West Africa. Since then, the concept has developed into an influential branch of social learning theory, supported by a large body of literature with wide-ranging application including in business, education, health care, knowledge management and sustainable development. Through ongoing development of the concept, Etienne remained at the forefront of CoP thinking and his name is inextricably linked to the concept.

In *Learning to Make a Difference*, Etienne and Beverly Wenger-Trayner (who became work and life partners in 2008), present an 'extension and refinement of' CoP theory. They argue that, over time, the term CoP has been used with increasing freedom, to the point where the concept was stretched well beyond its intended meaning. There was a need to both tighten the definition of a CoP and to expand their theory to encompass social learning that takes place beyond the relatively narrow confines of a CoP.

In short, a CoP refers to an ongoing learning partnership, which over time results in a shared practice related to a specific competence (e.g. tailoring, wine tasting or curation of specimens). Members identify with the community and in turn are recognised based on their competence in the specific practice. For mutual engagement and learning outside of what takes place in a CoP, the authors introduce the concept of a social learning space (SLS). SLSs are described as simpler, more pervasive structures than CoPs. They do not require a shared competence or the longevity of a CoP. Rather, meaning and identity in a SLS are based on making a difference to a bigger cause, while the learning takes place in the context of a broader and more diverse social landscape. Even so, while social learning can involve all kinds of interactions (e.g. a productive conversation, reflecting with colleagues on lessons learned during a field trip, visiting another location or a collaborative research project), these do not necessarily give rise to SLSs.

Part 1 (five chapters) of the book is devoted to theorising the conditions for learning in SLSs, as well as the nature of value being created through such learning. The text is rich and the authors show great care in their selection of words, explanation of new concepts, and situating their theory within the existing strands of learning scholarship. In essence, SLSs are created through participation (mere presence is not enough) of individuals who *care to make a difference* (even implicitly), are willing to *uncover and share their own uncertainties* about how to make a difference, and are committed to *paying attention* to see new things. The authors explain that 'a social learning space is a delicate thing' (p. 23), sensitive to various enablers and disablers. Enablers include a full range of voices (diversity of perspectives), mutual engagement of uncertainty (acknowledging that no one has full understanding or knows the final destination) and experiencing agency (the power to make a difference). The authors stress that engagement with uncertainty does not refer to 'a lazy uncertainty, a leisurely satisfaction with not knowing. We are talking about a restless uncertainty – on the move, driven demanding, productive – uncertainty as an edge: pulling learning, insisting that learning help make a difference' (p. 26). Furthermore, paying attention is hard work and might be as much about unlearning old beliefs as about learning new things. On the disabling side, a SLS can be undermined by different types of 'intruders' who may be insensitive to/or lacking self-awareness of power dynamics, and sometimes unwittingly take control of the SLS. Or they might confound mutual engagement of uncertainties by filling the space with their apparent certainty (they know it all and have the right answers).

Next to SLSs, value creation represents a foundational concept of the expanded theory. Participants find value in SLSs to the extent that their participation is seen as leading to a difference that matters to them/their particular endeavour (e.g. business, sport or research). At the heart of realising such a difference is the human experience of agency and meaningfulness (the will to make a difference), which in turn finds expression in four learning modes that are inherent in all SLSs: generating value (producing something of value towards making a difference), translating value (taking something of value to the space of doing something with it), framing social learning (setting expectations and aspirations for value creation), and evaluating social learning (inspecting the difference social learning is making or not). These four modes of learning are theorised in depth and serve as the backbone for understanding how SLSs function.

In Part 2 (13 chapters) of the book, the authors present a framework for operationalising the process of value creation in SLSs. The framework addresses each of the social learning modes mentioned above. Central to the framework are eight value-creation cycles (generating value mode), each of which creates a specific kind of value. For each cycle, examples are provided of positive and negative value, as well as ways and actions for producing the particular kind of value. The sometimes intricate details are summarised in useful tables. Furthermore, value created in one cycle can be translated into value at the next, and sometimes the flow of value between cycles can feed back through learning loops to introduce an enriched understanding to an earlier cycle along the flow. These *flows* and *loops* are key to the second learning mode of translating value. The third learning mode (framing) is explained through chapters on framing participation, framing value creation and conducting a framing event. Here framing is viewed as an emotionally deeper and more open-ended process than, for example, designing. Four chapters are dedicated to evaluation (fourth mode of social learning), covering the development of monitoring indicators, collecting value-creation stories (as a form of narrative data), consolidating and analysing data, and making value visible.

In summary, this book is about presenting a learning theory for the 21st century that can be used to enable individuals, groups, communities and organisations caring to make a difference. Its rationale is that 'we need to

learn to live together on a small planet, where we don't know what's going to happen next and where survival of our species appears to be at stake' (p. 3). An outstanding feature of the theory is its focus on why people participate in the first place: 'to make a difference to something they care about' (p. 69). The authors admit that social learning, and what they call SLSs, have always existed. However, what this book offers is a language to talk about, reflect on, and be more intentional about, enabling learning that happens in interactions between people.

Etienne and Beverly Wenger-Trayner refer to themselves as practitioner-theorists. This book also reflects that theory-practice duality, or perhaps complementarity. In a similar vein, the authors intended this book to be useful to researchers, educators and practitioners (those who convene and facilitate, or wish to evaluate, social learning in various sectors). Part 1 (conceptual foundation) may appeal more to academic-oriented readers and Part 2 (operational framework) to practitioners. However, each part is enriched by the other, and both are needed to make the theory complete. I found the theory in Part 1 an accessible and a stimulating read, and Part 2 an incredibly rich source of practical ideas and examples of when and how to cultivate or evaluate SLSs.

Who should read this book? CoP enthusiasts should note that the book is not about CoPs, except for a couple of pages to clarify how and when this concept should be used. The focus here is on establishing new concepts as the foundation for a learning theory that goes well beyond the boundaries of CoPs. For those with a scholarly interest in social learning this is probably a must-read. Facilitators of social learning processes and evaluators of projects or programmes in which learning is important are likely to find the book ground-breaking and stimulating. The book might also work more generally as a casual read and as a source of ideas for those who are serious about learning and mindful engagement with the world around them.

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Energy impoverishment and burns: The case for an expedited, safe and inclusive energy transition in South Africa

Significance:

Energy poverty is the constrained access to modern forms of energy. In South Africa, energy impoverished communities are dependent on a mixture of solid fuels (e.g. wood or coal) and hydrocarbons such as paraffin. These, especially paraffin use, are associated with significant negative health outcomes, particularly burns due to accidental fires and spillages, but also paraffin ingestion, and toxic fume inhalation. The energy-poor furthermore suffer disproportionate long-term social, economic and psychological impacts that entrench their impoverished conditions. There is both international and national recognition of these adverse effects of energy poverty and that the universal access to safe and sustainable energy is crucial for the attainment of health and other global social, economic and well-being goals. South Africa is called on to expedite access to modern energy usage, through the enactment of a substantive policy on the provision of safe, clean and affordable energy for energy-impoverished communities and households.

South Africa's energy access challenge

Energy poverty is a recognised obstacle to health and socio-economic well-being.[1] In South Africa, energy poverty is widespread, with about half of all households considered energy-poor in 2012.[2] In 2018, about 600 000 households, or 2 million people, were in extreme energy deprivation, relying on paraffin for domestic energy.[3] These families face disproportionate risks to health, with greater exposure to air pollution, poisoning and burn injury. Energy deprivation is concentrated in the country's informal urban settlements. These settlements remain largely unelectrified, despite South Africa's overall electrification rate of nearly 90% of dwellings. In the context of rapid population migration into the cities and urbanisation, the demand for safe and affordable energy in urban peripheries remains an unresolved political, social and economic issue that results in deleterious health outcomes. The health outcomes are exacerbated by crowded and inferior home structures, under-resourced and congested community spatial arrangements, and limited healthcare and support.[4]

Paraffin combustion is a leading cause of these consequent adverse health outcomes. Households that use paraffin manifest substantially higher risks for injury, directly through fires or indirectly through scalding, ingestion and toxic fume inhalation.[5] However, the health consequences are not necessarily exclusively due to the nature of the fuel. The use of unsound, unsafe and inefficient technologies, the fuel packaging and distribution system are contributors, along with an impoverished socio-environmental milieu.[1] Paraffin stove failures resulting in an explosion are often implicated in the ~5000 shack fires that South Africa's emergency services respond to every year.[6] The consequent flame-burn casualties are associated with the most severe burn injury outcomes, and likely comprise a significant proportion of the 100 000 burn injuries and 2000 deaths reported every year.[1,7]

In South Africa's informal settlements, such fire incidents are common and seemingly perennial, with informal settlement fires mainly caused by paraffin fuels (53%) and candles (30%).[5] Informal dwellings, typically made of flammable materials and often closely spaced, present a manifest vulnerability to runaway conflagrations. Accidental fires, often triggered by a faulty paraffin appliance, may be exacerbated by the flammable materials, congested internal spatial arrangements, and storage of paraffin or other combustibles.[8] If not immediately brought under control, an accidental fire in an individual structure may set off a widespread and devastating community conflagration.

These dangers have been widely recognised, through South Africa's electrification campaign, the 2006 promulgation of the paraffin stove safety standard, and campaigns for improved appliances by the Paraffin Safety Association of South Africa. Regrettably, these actions have not resulted in the desired outcomes. The quality of the available appliances has neither improved nor been effectively monitored. Underperforming appliances are still sold and widely used, even those approved by the South African Bureau of Standards (SABS).[9]

There are other dangers specific to the domestic use of paraffin. It is commonly decanted in neighbourhood spaza shops for sale in smaller, more affordable quantities. However, this perceived user benefit is also a significant ingestion hazard, whereby paraffin purchased and stored in beverage containers is easily mistaken for water or soft drinks. Paraffin ingestion and poisoning may affect up to 3.6% of paraffin-using households[10] and is a leading cause of poisoning to children, with 40% of cases developing chemical pneumonia[11]. There are concerns that paraffin emissions may over time impair lung function and increase the susceptibility to infectious illness, including tuberculosis, already rife in impoverished communities in South Africa, asthma and cancer.[12] There may also be central nervous system effects due to acute and chronic exposure to paraffin fumes, including irritability, restlessness, ataxia, convulsions, and coma.[13]

The survivors of exposure to burns, poisoning and inhalation may suffer immediate and long-term physical, psychological and socio-economic consequences. Burn injuries that require hospitalisation involve intensive, invasive, painful and often long-lasting treatment and care. Those who survive burns may face permanent scarring, in some cases with limited physical functionality or dexterity; those with visible scars may report social stigmatisation from strangers, peers, and even family members.[7] The physical recovery may be slow, and some

health consequences may endure due to the immune and inflammatory responses. Survivors report increased risks for cancer, cardiovascular disease, nervous system disorders, diabetes, musculoskeletal disorders, gastrointestinal disease, and infections over time.[14] Psychological outcomes are also reported in the immediate aftermath and may persist, with a significant incidence of post-traumatic stress disorder, depression, and anxiety.[7] The economic impacts are significant – burn care is expensive. The cost of hospitalisation and treatment for survivors who have sustained burns over 20% of their body ranges between ZAR103 000 and ZAR154 000.[1] At least ZAR490 million is spent caring for patients who have suffered paraffin burns.[15] Up to ZAR180 million is estimated to be lost annually in razed home structures.[6]

International mobilisation and demonstrations of alternative, safe and clean energy

The adverse health and social impacts of energy poverty have been recognised in the United Nations 2030 Agenda for Sustainable Development, which has declared universal access to safe, affordable, sustainable and modern energy a global priority.[16] The UN's Sustainable Development Goal 7 for universal access to safe and sustainable energy is considered a crucial point on which other global social, economic and well-being goals may depend, including poverty eradication, gender equality and the formation of sustainable cities and communities.[16] South African institutions, such as the Human Rights Commission, have noted that access to safe energy is constitutionally protected and should be recognised as a key aspect of the country's social justice agenda.[17]

This international and national recognition aligns with recent evidence that energy poverty can be addressed by substituting hazardous fuels with cleaner and safer alternatives.[1] In particular, electricity and liquid petroleum gas (LPG) have been globally recognised as clean, safe and health-promotive domestic energy technologies.[18] At the same time, in the case of South Africa, others may include ethanol, solar and biogas. Internationally, electricity is a preferred energy carrier due to its versatility, ease of use and 'cleanness' at the point of use. South Africa has made significant efforts with electrification, with 90% of households now connected to the grid compared to 35% three decades ago.

However, adequate electricity access is hampered by cost and grid limitations which often cause even on-grid low-income households to revert to paraffin and other 'dirty' fuels.[19] In some instances, LPG may be a more effective replacement for paraffin due to better technological attributes, a low emission profile, ease of use and greater suitability for remote off-grid settings.[20] The environmental footprint of LPG is negligible compared to biomass and other hydrocarbon fuels due to its efficient and complete combustion.[18] LPG and electricity provide specific respective advantages. The former is thus also recommended as a safe fuel alternative for resource-poor settings.[21] There have been encouraging experiences in implementing large LPG initiatives in countries with similar socio-economic and energy profiles to South Africa. These countries include Indonesia, where 50 million households were converted from paraffin to LPG between 2007 and 2011, thus reducing extreme energy poverty and saving on fuel expenses and paraffin subsidies.[22] Despite misperceptions about affordability and safety[17] and concerns that it is a non-renewable fuel, South Africa's and others' uses of LPG have provided encouraging results on user satisfaction[23] and well-being promotion. Despite these good experiences, there has been only limited implementation of LPG or electricity in South Africa's energy-poor locations.

A call to action

In February 2021, a multi-sectoral grouping of South African and international academics, civic coalitions, government and corporate partners joined a No Paraffin! Campaign Webinar Series[1], hosted by the Academy of Science of South Africa (ASSAf) and the University of South Africa. In these webinars, speakers explored South Africa's inequalities in energy risk, best and emerging safe energy practices, and the institutional and policy pathways for an inclusive domestic energy transition that foregrounds the country's impoverished communities.[1] An ASSAf Statement, 'The No Paraffin! Campaign: A Call to Action'[24] was authored by the authors of this commentary, drawing on the presentations and discussions arising from the Webinar Series. The Call makes an empirical case and an appeal for an accelerated transition to safe, affordable and modern energy, with this energy transition to be actioned, in part, through a No Paraffin! Campaign.[24] The Campaign is to be directed at:

- reducing health risks to the energy-poor, through the phasing out of paraffin as a domestic fuel;
- strengthening protections for paraffin users during phase-out; and
- scaling up implementation of a modern energy alternative, either electricity or LPG, and possibly other locally proven, feasible energy alternatives for energy-poor settings.

The Campaign thus recognises the challenges experienced with the domestic use of paraffin, especially in informal, minimally protected settings. Therefore, it calls on the South African government for more stringent measures to curtail and eventually eliminate the domestic use of paraffin while simultaneously advancing efforts to implement either electrification or, where not suitable, safe and cost-effective alternative energy such as LPG, or a locally proven renewable source. While the Campaign emphasises government leadership and championship, its success is also contingent on public mobilisation and support, local empirically produced information, and a regularised communication strategy, all constituents of previous successful health campaigns.[1] The impact of past initiatives has been maximised where government has championed an issue through partnerships with civil society and community actors, researchers and industry. Broad-based partnerships facilitate the mobilisation of public support for the adoption of behaviours required for the implementation of interventions.[1] The Campaign has proposed a staggered scaling-up of the required energy interventions through specific near- and longer-term measures.[24]

Near-term interim measures: Hazard control and removal

The South African government, licenced appliance manufacturers and distributors, and civic partners are called to champion key near-term policy and implementation recommendations. These recommendations align with established public health approaches that seek to strengthen hazard management through removal while paraffin is being phased out but still in use. The following steps are recommended:

- The manufacturers of paraffin stoves should strengthen efforts to ensure that domestic products comply with design and construction standards. The National Regulator for Compulsory Standards is to increase its enforcement of these standards and curtail, e.g. through harsher penalties, the local manufacture and distribution of risky sub-standard stoves. The current compulsory paraffin stove standard is dated.[25] SABS is requested to institute an urgent review to address its shortcomings.[9]
- SABS should formulate a standard for a bitterant to be added to illuminating paraffin to reduce accidental ingestion and poisoning cases.
- Safety educational campaigns should disseminate information on safe stove use behaviours and emergency responses to prevent or respond to poisoning or fire accidents. This could be further enabled through compulsory stove and paraffin packaging information on safe use.[4] The campaigns should be facilitated as a joint effort between government, industry, academic and relevant civil society groups.

Short- to medium-term measures: Engagement, policy, implementation and monitoring

The South African government is called on to prioritise, develop and enact a substantive policy on safe energy provision for energy-impoverished

communities and households (alongside other interventions to support suitable housing and regular household incomes in impoverished households and communities). Key elements of this policy should include:

- A White Paper to specify the milestones, timelines and necessary mechanisms for the gradual phase-out of paraffin and the introduction and upscaling of the chosen safer energy alternatives.
- SABS to develop design and construction standards for all emerging domestic energy appliances.
- An engagement strategy to facilitate public interest in an energy transition programme and the use of safe energy alternatives, specifically for affected communities and households. This strategy could include a national awareness campaign to highlight attributes of the replacement energy, the characteristics of replacement appliances, and the required safe stove use behaviour.
- A distribution infrastructure to support energy user access and safe use of the replacement energy. This would include distribution networks, financial support and incentive systems for a sustainable energy technology acquisition, and monitoring of energy distributors and stove producers.
- A monitoring and evaluation programme to determine the readiness of local users to adopt alternative energy, identify implementation issues, assess household energy technology performance and usage patterns, and report on local safety, health and cost outcomes.

Government has a model for the implementation of such campaigns. The District Development Model, or DDM, coordinates the government's response to the interconnected challenges of poverty and is proposed as the governmental focal point for implementing the Campaign in local communities.[26] The DDM was launched in 2019 as a model to coordinate the government's response to the challenges of poverty, unemployment and inequality and accelerate and integrate service delivery. Safe and healthy energy provision is key to its mandate. The DDM could initially provide a small-scale platform as a 'real-life' site for targeted implementation, testing, evaluation and demonstration of replacement energy to establish context-specific safe energy usage practices and concerns. This initial implementation would provide a platform for local empirical support to enable coordinated, phased, and broader scale roll-outs to transition energy-poor communities away from high-risk energy carriers and technologies.[24]

This Campaign therefore aligns with the United Nations 2030 Sustainable Development Goal on universal access to safe and sustainable energy. It responds to persisting and widespread energy impoverishment in South Africa, the complex health and safety challenges specifically to domestic paraffin use, and recent demonstrations of feasible alternatives. The Campaign calls for championship from government, civil society, research and industry; local empirical research into the use, impact and cost-effectiveness of alternative energy; a scaled-up implementation plan for the safe energy carrier; and the adoption of clear milestones to record progression towards more equitable, safe and healthy energy use in South Africa.[24]

Competing interests

We have no competing interests to declare.

References

1. Academy of Science of South Africa (ASSAf). 'No Paraffin! Campaign': National roundtable discussion webinar series. Pretoria: ASSAf; 2021. http://dx.doi.org/10.17159/assaf.2021/0078
2. South African Department of Energy (DoE). A survey of energy related behaviour and perceptions in South Africa: The residential sector. Pretoria: DoE; 2013. Available from: http://www.energy.gov.za/files/media/Pub/DoE-2013-Survey-of-EnergyRelated-Behaviour-and-Perception-in-SA.pdf
3. StatsSA. General household survey 2018: Statistical release P0318 [document on the Internet]. c2019 [cited 2021 May 12]. Available from: http://www.statssa.gov.za/publications/P0318/P03182018.pdf
4. Kimemia D, Van Niekerk A, Seedat M. Paraffin dangers, health and socio-economic consequences: Urgent need for policy action. S Afr Med J. 2021;111(1):17–19. https://doi.org/10.7196/SAMJ.2020.v111i1.15095
5. Kimemia D, Vermaak C, Pachauri S, Rhodes B. Burns, scalds and poisonings from household energy use in South Africa: Are the energy poor at greater risk? Energy Sustain Dev. 2014;18:1–8. https://doi.org/10.1016/j.esd.2013.11.011
6. Fire Protection Association of South Africa. Informal settlement fires 2018 [webpage on the Internet]. c2018 [cited 2021 May 11]. Available from: http://www.fpasa.co.za/140-informal-settlement-fires-2018
7. Van Niekerk A. Burn-related injuries. In: McQueen D, editor. Oxford Research Encyclopedia of Global Public Health. New York: Oxford University Press. Forthcoming 2022.
8. Van Niekerk A, Reimers A, Laflamme L. Area characteristics and determinants of childhood burn injury in Cape Town. Public Health. 2006;120(2):115–124. https://doi.org/10.1016/j.puhe.2005.08.015
9. Kimemia D, Van Niekerk A, Govender R, Seedat M. Burns and fires in South Africa's informal settlements: Have approved kerosene stoves improved safety? Burns. 2018;44(4):969–979. https://doi.org/10.1016/j.burns.2017.11.006
10. Matzopoulos R, Jordaan E, Carolissen G. Safety issues relating to paraffin usage in Eshane, Kwazulu-Natal. J Energy South Afr. 2006;17(3):4–9. https://doi.org/10.17159/2413-3051/2006/v17i3a3242
11. UCT Pathology Learning Centre. Paraffin [webpage on the Internet]. No date [cited 2021 Feb 24]. Available from: http://www.pathologylearningcentre.uct.ac.za/harmful-substances-paraffin
12. Lam NL, Smith KR, Gauthier A, Bateset MN. Kerosene: A review of household uses and their hazards in low- and middle-income countries. J Toxicol Environ Health B Crit Rev. 2012;15(6):396–432. https://doi.org/10.1080/10937404.2012.710134
13. Chilcott RP. Compendium of chemical hazards: Kerosene (fuel oil). Didcot: UK Health Protection Agency; 2006. Available from: https://www.who.int/ipcs/emergencies/kerosene.pdf
14. Barrett LW, Fear VS, Waithman JC, Wood FM, Fear MW. Understanding acute burn injury as a chronic disease. Burns & Trauma. 2019;7:23. https://doi.org/10.1186/s41038-019-0163-2
15. World Health Organization. Burns: Economic impact [webpage on the Internet]. c2018 [cited 2020 May 06]. Available from: https://www.who.int/news-room/fact-sheets/detail/burns
16. United Nations. Accelerating SDG 7 achievement: SDG 7 policy briefs in support of the high-level political forum [document on the Internet]. c2019 [cited 2020 Jun 20]. Available from: https://sustainabledevelopment.un.org/content/documents/22877UN_FINAL_ONLINE_20190523.pdf
17. Thipanye T. The right to energy and electricity. NewzRoom Africa. 13 May 2021.
18. World Health Organization. Increasing the use of liquefied petroleum gas in cooking in developing countries [document on the Internet]. c2017 [cited 2022 Jan 12]. Available from: https://documents1.worldbank.org/curated/en/707321494347176314/pdf/114846-REVISED-LW74-LJ-fin-logo-OKR.pdf
19. Tait L, Merven B, Senatla M. Investigating the current and future roles of paraffin in South Africa. Cape Town: Energy Research Centre, University of Cape Town; 2013. Available from: https://media.africaportal.org/documents/13Tait-etal_Paraffin_in_SA.pdf
20. Kimemia D, Van Niekerk A. Cookstove options for safety and health: Comparative analysis of technological and usability attributes. Energy Policy. 2017;105:451–457. https://doi.org/10.1016/j.enpol.2017.03.022
21. World Health Organization (WHO). Burning opportunity: Clean household energy for health, sustainable development, and wellbeing of women and children. Geneva: WHO; 2016. Available from: https://apps.who.int/iris/bitstream/handle/10665/204717/9789241565233_eng.pdf?sequence=1

22. Thoday K, Benjamin P, Gan M, Puzzolo E. The Mega Conversion Program from kerosene to LPG in Indonesia: Lessons learned and recommendations for future clean cooking energy expansion. Energy Sustain Dev. 2018;46:71–81. https://doi.org/10.1016/j.esd.2018.05.011

23. Kimemia D, Annegarn H. Domestic LPG interventions in South Africa: Challenges and lessons. Energy Policy. 2016;93:150–156. https://doi.org/10.1016/j.enpol.2016.03.005

24. Van Niekerk A, Kimemia D, Seedat M, Annegarn H. The No Paraffin! Campaign: A call to action 2021 [document on the Internet]. c2021 [cited 2021 Sep 03]. Available from: https://www.assaf.org.za/files/2021/No%20Paraffin/No%20Paraffin%20Campaign%20Statement%20for%20ASSAF%20Branding%2013%20July%202021_Final%20Version.pdf

25. SANS1906:2012 Ed3.1: Non-pressure kerosene stoves and heaters. Pretoria: Standards South Africa; 2012.

26. South African Cooperative Governance and Traditional Affairs. District Development Model [webpage on the Internet]. c2020 [cited 2021 Apr 26]. Available from: https://www.cogta.gov.za/ddm/DDM

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Marine seismic surveys for hydrocarbon exploration: What's at stake?

Significance:

We argue that the immediate, intermediate, and long-term implications of seismic surveys for hydrocarbon exploration merit noting. If seismic surveys detect feasible hydrocarbon deposits, they effectively serve as a precursor to hydrocarbon extraction and consumption. The additional greenhouse gas emissions that will originate from new oil and gas fields in South Africa will push the world closer to the tipping point of breaching the limit of 1.5 °C targeted at the 2021 COP26 UN climate summit, and should thus be avoided at all costs. South Africa's pursuit of energy self-sufficiency through local fossil fuel extraction should not come at the cost of its unique biodiversity nor planetary health.

With a coastline extending approximately 3900 km, South Africa exercises jurisdiction over a vast Exclusive Economic Zone (EEZ) that exceeds 1.5 million km^2.[1] South Africa is located at an ecologically important crossroad for inter-ocean exchange of heat, salt and biota[2] involving the warm, fast-flowing Agulhas current of the Indian Ocean and the cold, nutrient-rich Benguela upwellings of the Atlantic Ocean, and within the range of influence of the world's most biologically productive ocean[3], the Southern Ocean[4-6]. South Africa's marine territory is also characterised by spectacular topography, including dramatic canyons, slopes, plateaus, and seamounts.[7] Unsurprisingly, South Africa's complex oceanographic influences, coastal topography, and geology boasts 179 marine ecosystem types, with 150 around South Africa and 29 in the country's sub-Antarctic territory.[8] South Africa may also be richly endowed with hydrocarbon deposits.[9,10] Seismic surveys are a routine and key upstream component of the hydrocarbon sector and crucial to understanding where recoverable oil and gas resources likely exist. Hydrocarbon extraction is largely dependent on seismic data acquisition and processing technology, with exploration companies relying on seismic survey results to decide whether or where to extract hydrocarbon deposits. While seismic surveys pose an immediate threat to South Africa's exceptionally rich marine life, the downstream implications of such surveys – the extraction and use of non-renewable energy sources – are more profound. Actively seeking new hydrocarbon deposits to exploit, thus contributing to already dangerously high levels of greenhouse gases (GHG) in the earth's atmosphere, poses an existential threat to most life on earth. Seen in this context, rationalising the boring of wells to exploit hydrocarbons in the name of energy sovereignty and security, is short-sighted, nationalistic, environmentally irresponsible, and morally indefensible. We, as a country, need to rethink this strategy.

What do we know about South Africa's marine life?

Approximately 13 000 species have been documented in South Africa's marine realms[11], including almost a quarter of global cephalopods (octopus, squid and cuttlefish)[12]. With over 3800 species occurring nowhere else on earth[13], South Africa ranks third in the world for marine species endemism[12]. However, even these astonishing statistics may represent an incomplete picture as current knowledge of marine life in South African waters is limited and outdated.[11] For instance, coastal zone samples in South African waters were largely collected before 1980, primarily (83%) from depths shallower than 100 m.[11] More than 65% of South Africa's abyssal zone – which extends to 5700 m deep[11] – lies deeper than 2000 m. The abyssal plain in South African waters – where wells would ultimately have to be drilled to anchor rigs for hydrocarbon extraction – is completely unexplored and has not been surveyed for marine life.[11] Undoubtedly, many more species are yet to be discovered in South African waters.[14] With the recent declaration in South Africa of an additional 20 offshore Marine Protected Areas (MPAs), 5.4% of the marine environment within the South African mainland EEZ is now protected, of which 3% is zoned as 'restricted' or 'no-take'.[15,16] While admirable, this protection falls short of Goal 14.5 of the 2015 United Nations Sustainable Development Goals, which calls for the protection of a minimum of 10% of ocean ecosystems and habitats by 2020.[17]

Seismic surveys and potential harms

Short-term harms in the ocean

Marine seismic surveys are a key hydrocarbon exploration activity, and typically involve the use of airgun arrays that are towed behind vessels and produce high-intensity, low-frequency impulsive sounds at regular intervals. The sounds generated by seismic airguns can 'blanket' areas of up to 300 000 km^2 with noise[18], have been recorded at locations up to 4000 km from the source[19], and extend particularly well in deeper waters[20]. Such robust findings refute claims by oil companies, such as Shell, that 'a buffer zone of 5 km' from a MPA constitutes an adequate risk mitigation measure.[21] Seismic surveys are cause for concern for marine species reliant on sound for key life functions. Shell has argued that 'there is no evidence that any of [the 35 surveys conducted in South Africa have] caused any harm'[21]. Such arguments – encapsulated by the Latin phrase *Argumentum ad Ignorantiam* – capture the fallacy that a proposition is true simply on the basis that it has not been proven false or that it is false simply because it has not been proven true. Such misdirection is to be expected of a sector that is fighting to survive as the world rapidly transitions to greener energy sources. Contrary to what the hydrocarbon sector claims, there is ample evidence that seismic surveys detrimentally impact on a diverse range of marine species, including mammals, fish, invertebrates, plankton, and reptiles.[18,19,22-27] In the African context, for example, seismic surveys have been found to negatively affect humpback whale singing activity off northern Angola.[28] The predominant

frequency range of seismic airgun emissions is within the detectable hearing range of most fish and elasmobranchs and can also elicit a neurological response in cephalopods and decapods.[25] The relative paucity of data in the South African context does not equate to no harm nor a low likelihood of harm.[29] Instead, it highlights a need for local research on the issue, including in relation to microbiota and meiofauna, which typically dominate benthic faunal biomass at abyssal depths and are essential for deep-sea ecosystem functions.[30] Further, the mere fact that seismic surveys have previously been conducted in South African waters does not establish irrevocable precedent, nor legitimise such activities in perpetuity.

We cannot assess potential thresholds of harm[31] and mitigate associated risks if we have a poor understanding of *what* we could be harming and *how* different species suffer harm. While some international studies have found that seismic surveys have only a limited impact on demersal fish of commercial or recreational interest[32], such results are not necessarily generalisable elsewhere given the array of heterogeneity across marine environments, both in terms of biota and physical characteristics. Nor can such results be extrapolated to other marine species. In assessing risk, we should consider the impact of seismic surveys on all marine life, including those in bathyal and abyssal environments. All species, regardless of commercial value, play a vital role in the ecosystem value chain. Because we do not currently know what the impact of seismic surveys could be in the South African context, the Precautionary Principle – which is a key tenet of South African environmental legislation and has been described as enabling decision-makers 'to adopt precautionary measures when scientific evidence about an environmental or human health hazard is uncertain and the stakes are high'[33] – is warranted, in line with established international norms[34,35].

Long-term harms in the ocean

It would be short-sighted to only consider the immediate impact of seismic surveys. Downstream marine implications also bear noting. If seismic surveys detect feasible hydrocarbon deposits, they effectively serve as a precursor to hydrocarbon extraction and consumption. Climate change driven by fossil fuel extraction and consumption alter key factors that drive marine ecosystems, including winds, water temperatures, sea ice cover and ocean circulation.[36] Rising atmospheric CO_2 and the resulting increased oceanic CO_2 uptake is fuelling ocean acidification.[37,38] Such changes in ocean temperature and chemistry may alter the physiological functioning, behaviour, biological interactions, and productivity of organisms, which, in turn, could lead to shifts in marine life size structure, spatial range, seasonal abundance, community structure and ecosystem function.[39] For instance, disruptions related to climate change will transfer nutrients from surface waters down into the deep ocean, leaving less at the surface to support plankton growth.[40] Such an outcome will have a knock-on effect on the entire ocean food chain. Studies show that sustained high levels of GHG emissions could suppress marine biological productivity for a millennium.[36] Left unchecked, such changes will ultimately destroy the fisheries and marine tourism industries of all countries, including South Africa, resulting in devastating job losses, food insecurity, and other adverse socio-economic consequences.[41,42]

The Intergovernmental Panel on Climate Change (IPCC) has concluded with a degree of high confidence that climate change has caused substantial damages, and increasingly, irreversible losses, in terrestrial, freshwater and coastal and open ocean marine ecosystems.[42] In its report published in February 2022, the IPCC noted that the extent and magnitude of climate change impacts are larger than estimated in previous assessments. The IPCC has concluded with a high degree of confidence that '…hundreds of local losses of species have been driven by increases in the magnitude of heat extremes, as well as mass mortality events on land and in the ocean'[41]. Despite incontrovertible evidence underscoring the harmful impact of fossil fuels on the oceans and the wider planet, given that the hydrocarbon sector has everything to lose if the world seeks alternative energy sources, unsurprisingly, the sector has, and will, continue to rationalise its relevance and downplay its detrimental impact on the planet.

What is at stake for the hydrocarbon sector?

Seismic surveys represent an upstream component of the hydrocarbon sector. The global seismic survey market registered a revenue of almost USD8 billion in the year 2020 and is forecast to pass USD11.5 billion by the end of 2030, growing at a compound annual growth rate (CAGR) of 3.96% during the forecast period.[43] The Middle East and Africa market is expected to attain the fastest growth globally, with a CAGR of 5.41% between 2020 and 2030.[44] With multiple exploration licences already approved in South Africa and other licence applications pending[45], South Africa represents a key emerging market for seismic surveyors. It would be fair to say that the cessation of offshore seismic surveys in South Africa represents an existential threat to the upstream seismic survey market locally and would undoubtedly undermine Africa's projected market growth. Not surprisingly, given such vested interests, those who represent the seismic survey market have vociferously defended their sector against those urging caution.[46]

The downstream constituents of the hydrocarbon sector, though, have considerably more to lose if we cease seismic surveys in South African waters. South Africa's EEZ potentially holds nine billion barrels of oil, equivalent to 40 years of South African oil consumption, and natural gas deposits equivalent to 375 years of current South African gas consumption.[9] The South African government has signalled its enthusiasm to exploit these resources by openly promoting the drilling of 30 hydrocarbon exploration wells off South Africa's coast within a decade.[47] Besides the immediate risks to marine life that seismic surveys may hold, the exploitation of South Africa's offshore hydrocarbon deposits also carry dire downstream implications.

Medium-term risks: Extraction

Beyond the serious occupational health and safety risks implicit in offshore hydrocarbon extraction[48], extraction activities also pose dire risks for the environment. In creating an enabling environment to give the hydrocarbon sector 'the comfort to invest in this capital-intensive sector', the South African government has committed to, amongst other measures, conducting 'emergency response drills…to initiate the creation of a world-class oil spill response capacity in South Africa' and operationalising the International Oil Pollution and Compensation Fund.[9] While such measures may be touted as forward-thinking risk-mitigation measures, they also clearly illustrate that the government is under no illusion that offshore oil extraction, especially in South Africa's notoriously rough waters, carries immeasurable risks for the country's marine environment. The ocean circulation current of the Gulf of Mexico, which hosts numerous oil and gas rigs, moves up to 2 m/s, strong enough to severely damage the steel infrastructure of oil and gas rigs.[49] The Agulhas current along the eastern seaboard of South Africa has also been measured to flow at 2 m/s[50], a close second only to the Gulf Stream, which is considered the world fastest ocean current at approximately 2.5 m/s[51]. But the Agulhas current is remarkable for its strength[52], transporting up to 122.9 Sv. (122.9 million m^3/s), with an average of 69.7 Sv.[53], significantly more than the Gulf Stream, which transports approximately 30 Sv.[54] In 2014, French energy company, Total, had to abandon its deep-sea exploration off South Africa's east coast because rough seas damaged its rig[55,56], underscoring the risks implicit in drilling off South Africa's coast. 'Rogue waves' – waves that are abnormally large and unpredictable – occur with relative frequency off South Africa's east coast.[57-61] Rogue waves merit concern as they have been implicated in damaging oil and gas platforms in the open sea, including the North Sea's Draupner oil platform.[62] The massive release of crude oil from the breach of the Deepwater Horizon rig in the Gulf of Mexico[63] offers a sobering example of the devastating impact the release of a contaminant can have on ecosystems. These case studies collectively highlight the risks of sinking wells in oceans with abnormally high waves and strong currents, and how an oil spill from an offshore industrial accident in such conditions could devastate South Africa's exceptional coastline and sensitive marine ecosystems over a wide area, in a short time. Attempted damage control after the fact is no comfort when we can stop the possibility of an accident even occurring by not sinking wells in our EEZ. Prevention is better than cure. Seismic

surveillance and extraction risks aside, furtive emissions (unintentional leakage and discharge) resulting from the extraction of hydrocarbons[64,65] and the associated GHG from hydrocarbon consumption itself poses the greatest risk to our planet.

Long-term risks: Breaching the tipping point for planetary health

GHGs have a detrimental impact on human health[66,67] and on the planet[68]. In 2021, the International Energy Agency, of which South Africa is an associate member, noted[96]:

> *The energy sector is the source of around three-quarters of greenhouse gas emissions today and holds the key to averting the worst effects of climate change, perhaps the greatest challenge humankind has faced. Reducing global carbon dioxide (CO_2) emissions to net zero by 2050 is consistent with efforts to limit the long-term increase in average global temperatures to 1.5 °C.* ***This calls for nothing less than a complete transformation of how we produce, transport and consume energy****. [our emphasis]*

This warning echoes earlier evidence that showed that only a sharp and rapid decline in fossil fuel use will keep the world's temperature increase below 1.5 °C.[69,70] If we have any hope of keeping within a 1.5 °C 'carbon budget', by 2050 nearly 60% of oil and fossil methane gas and 90% of coal must remain unextracted.[71] Emissions do not respect borders. Humanity cannot achieve this collective goal if South Africa does not do its fair share. In February 2020, South Africa adopted a Low Emissions Development Strategy.[72] In September 2021, South Africa announced its intention to limit GHG emissions to 398–510 metric tons of CO_2 equivalent ($MtCO_2e$) by 2025, and to 350–420 $MtCO_2e$ by 2030.[73] While such commitments on paper are welcomed, they are incompatible with the government's open and vociferous support for hydrocarbon exploitation, evidenced by the publication of a draft Upstream Petroleum Resources Development Bill at the end of 2019[74], government's siding with the oil sector in court against concerned communities and environmentalists[75-77], and the Department of Mineral Resources and Energy (DMRE) pursuance of a 'gas master plan'[78]. The present MRE Minister's unflinching support of the oil and gas sector[79] and his attacks on those who oppose seismic surveys or simply ask inconvenient questions related to his portfolio, are also well documented[80]. Such factors raise the question of whether today's politicians are prepared to gamble the interests of future generations and the planet's sustainability for local, short-term gain.

Stemming the unfolding climate change emergency will require immediate action. On paper, South Africa has made changes towards the sustainable management of its marine habitats, such as becoming a signatory to the Convention on the Conservation of Migratory Species of Wild Animals (CMS)[81] and declaring additional MPAs in 2019[82]. These commitments require an accompanying meaningful change in policies and standard operating procedures. For example, Resolution 12.14 of the CMS (adopted in 2017)[83] mandates full environmental impact assessments before starting any noise-emitting operations that may negatively impact marine life. Given the promulgation of the additional 20 MPAs[15], the proven impact of seismic surveys[18,19,22-27], and our international obligations under the CMS, the Scientific Advisory Group on Emergencies (SAGE) recommends that all exploration applications approved before 28 June 2018, should be subject to environmental authorisation. Such review must be objective and robust. To not do so on the basis of 'transitional arrangements'[84] – a legal loophole to avoid 'red tape' – constitutes a moral wrong and suggests that our government's commitment to a sustainable future is mere lip service. But beyond robust environmental authorisation for upstream hydrocarbon activities, we need to consider the big picture. Hydrocarbon seismic surveys are a harbinger of GHGs. As South Africa is already one of the world's largest contributors of GHGs[85], the country needs to do its fair share to help reduce GHGs. To do so, the South African government needs to urgently rethink its long-term energy security strategy.

The cost of transitioning to a net-zero emission economy by 2050

In October 2019, South Africa published its revised Integrated Resource Plan (IRP), which outlines the country's future energy strategy.[86] The IRP notes that South Africa's energy sector contributes approximately 80% of the country's total greenhouse gas emissions, of which 50% are from electricity generation and liquid fuel production alone.[86] Disappointingly, despite South Africa's tremendous renewable energy potential, the 2019 IRP outlines new investments in hydrocarbon-based energy sources – including an additional 1500 MW of coal-to-power capacity – and sets an artificial limit of 33% of South Africa's energy needs to be met by renewables by 2030.[86] This counterintuitive strategy suggests that political and vested interests are impeding South Africa's renewable energy potential. Aside from the hydrocarbon sector supporting approximately 248 000 jobs in South Africa (directly, indirectly or through induced impact) – which equates to 1.5% of South African employment[87] – fossil fuels are also an important source of government revenue. In 2019–2020, South African taxes on fossil fuel consumption, production, and incomes amounted to approximately ZAR100.5 billion (2% of South Africa's GDP and 7.4% of general government revenue).[88] However, such revenue is discounted by significant government bailouts for state enterprises that are heavily dependent on fossil fuels, such as the South African Airways.[88] Revenue from fossil fuels is also dwarfed by the estimated ZAR172 billion in energy subsidies gifted by the government in the 2020/2021 financial year alone.[88] If one factors in government expenditure through bailouts to the state-owned energy company, Eskom (which further distorts the price of electricity generated from coal), government revenue from fossil fuels is rendered insignificant.[88] In fact, once the social costs of fossil fuels (climate change and air pollution related deaths and lost working days from fossil fuel combustion) are factored in – which are estimated to be at least five times higher than fossil fuel revenues – the net annual cost to society of fossil fuels is approximately ZAR550 billion.[88] The planned retirement of coal-fired power stations, technological changes (such as drops in the cost of green energy sources and rapid progress in energy storage), new energy market regulation, available finance, and geopolitics, will significantly impact on South Africa's ability and appetite to continue pursuing a hydrocarbons-centred economy. Seen in this light, South Africa should be moving away from fossil fuels as fast as possible. This necessity will prove costly.

South Africa's 'transition risk' – the cumulative impact to South African asset prices and revenues of transitioning to a low-carbon economy, in line with the country's international commitments, has been estimated at approximately USD125 billion (ZAR1.8 trillion in 2019 value terms) by 2035.[89] For South Africa to achieve its target of transitioning to a net-zero emission economy by 2050, the country will require an investment of ZAR887–1173 billion.[90] To optimally reduce emissions, South Africa may require aggregate new capital expenditure of up to ZAR2.9 trillion by 2050.[90] South Africa cannot achieve these goals without international support and has requested a minimum of USD8 billion support per year by 2030, with a view to equally distributing funding between adaptation and mitigation.[91]

Moral responsibility and international solidarity

As developed countries have historically been responsible for the majority of GHG emissions[92,93], they have committed to providing financial resources to assist developing countries meet their mitigation and adaptation obligations[94]. In November 2021, the governments of South Africa, France, Germany, the United Kingdom and the United States of America, along with the European Union, announced a long-term 'Just Energy Transition Partnership' to support South Africa's decarbonisation efforts.[95] The partnership will mobilise an initial commitment of USD8.5 billion for the first phase of financing through various mechanisms including grants, concessional loans and investments and risk sharing instruments.[95] The Partnership aims to prevent up to 1–1.5 gigatonnes of emissions over the next 20 years and support South Africa's move away from coal and its accelerated transition to a low emission, climate resilient economy. While this initiative is a welcome

first step, such assistance will have to be sustained for countries such as South Africa to transition to a net zero economy by 2050. Such support will be more forthcoming if South Africa's next IRP commits to ambitious green energy targets – at least 50% of South Africa's energy needs should come from renewable sources by 2030 – and government aggressively pursues its realisation.

Conclusion

Safeguarding and strengthening our natural systems is crucial to securing a liveable future. Marine seismic surveys for the hydrocarbon industry are effectively a harbinger of additional GHGs. If today's politicians fail to act as responsible stewards of our environment and resources for the sake of future generations and the long-term sustainability of our planet, concerned communities, civil society actors, environmentalists, and scientists have a moral obligation to speak out. South Africa is highly vulnerable to the impacts of climate change, so should do everything in its power to avert a rise in GHG emissions. While gas has been touted as a 'transition' energy source, investing in new gas infrastructure will unnecessarily lock South Africa into fossil fuel energy consumption for decades. Further, because such investments will take decades to recuperate, vested interests will ensure that these facilities are used for their full lifetimes or beyond, which will only delay the switch to renewable energy sources. Such an opportunity cost is economically, environmentally, and morally unacceptable. South Africa's cabinet should signify its unequivocal commitment to the sustainability of the planet by aggressively decarbonising its power sector, not unnecessarily prolonging its lifespan for as long as possible, or worse, ramping it up. It is time for the country to address its ambition gap and transition to a net-zero emission economy as soon as possible. Moreover, cabinet should review all offshore exploration licences already awarded, in light of South Africa's declaration of additional MPAs. The additional GHG emissions that will originate from new oil and gas fields in South Africa (inland and offshore), will push the world closer to the tipping point of breaching the limit of 1.5 °C targeted at the 2021 COP26 UN climate summit, and should thus be avoided at all costs. Instead, South Africa should harness its impressive scientific and technical capacity to develop and harness sustainable, renewable energy sources, in line with the country's vast potential. South Africa's pursuit of energy self-sufficiency through local fossil fuel extraction should not come at the cost of its unique biodiversity, nor planetary health. Energy security should be based on science, not narrow, short-sighted political and vested interests. History will judge the current South African government harshly if it fails to act decisively now for the sake of its people and the greater good of the planet.

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Competing interests

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References

1. South African Department of Forestry, Fisheries, and the Environment (DFFE). Operation Phakisa: Oceans Economy [document on the Internet]. No date [2022 Feb 23]. Available from: https://www.dffe.gov.za/sites/default/files/docs/publications/operationphakisa_businessreportadvert.pdf
2. Phoma S, Vikram S, Jansson JK, Ansorge IJ, Cowan DA, Van de Peer Y, Makhalanyane TP. Agulhas Current properties shape microbial community diversity and potential functionality. Sci Rep. 2018;8, Art. #10542. https://doi.org/10.1038/s41598-018-28939-0
3. Turner J, Bindschadler A, Convey P, Di Prisco G, Fahrbach E, Gutt J, et al., editors. Antarctic climate change and the environment. Chemistry International – Newsmagazine for IUPAC. 2010;32(2):25–26. https://doi.org/10.1515/ci.2010.32.2.25
4. Ansorge I, Lutjeharms JRE. The hydrography and dynamics of the ocean environment of the Prince Edward Islands (Southern Ocean). J Mar Syst. 2002;37:107–127. https://doi.org/10.1016/S0924-7963(02)00198-7
5. South African Department of Environmental Affairs (DEA). South Africa's oceans and coasts: Annual science report. Pretoria: DEA; 2018. Available from: https://www.datocms-assets.com/7245/1574920221-2018-oceans-coasts-annual-report.pdf
6. Rouault M, Monyela B, Kounge RAI, Stella A, Njouodo N, Dieppois B, et al. Ocean impact on southern African climate variability and water resources. Pretoria: Water Research Commission; 2019.
7. Sink K, Adams R, Mann J, Whitehead O, Franken M, Maze K. South Africa's New Marine Protected Areas. Pretoria: South African National Biodiversity Institute; 2019. Available from: http://opus.sanbi.org/jspui/handle/20.500.12143/6650
8. South African National Biodiversity Institute (SANBI). Tenfold increase in marine protected areas supports SA's sustainable oceans economy [webpage on the Internet]. c2019 [cited 2022 Feb 23]. Available from: https://www.sanbi.org/uncategorized/tenfold-increase-in-marine-protected-areas-supports-south-africas-sustainable-oceans-economy/
9. South African Department of Planning, Monitoring and Evaluation. Operation Phakisa: Offshore oil and gas exploration [webpage on the Internet]. No date [cited 2022 Feb 23]. Available from: https://www.operationphakisa.gov.za/operations/oel/oilgas/pages/default.aspx
10. United States Energy Information Administration. Technically recoverable shale oil and shale gas resources: South Africa [document on the Internet]. c2015 [cited 2022 Feb 23]. Available from: https://www.eia.gov/analysis/studies/worldshalegas/pdf/South_Africa_2013.pdf
11. Griffiths CL, Robinson TB, Lange L, Mead A. Marine biodiversity in South Africa: An evaluation of current states of knowledge. PLoS ONE. 2010;5(8), e12008. https://doi.org/10.1371/journal.pone.0012008
12. South African National Biodiversity Institute (SANBI). National biodiversity assessment: The status of South Africa's ecosystems and biodiversity. Synthesis report. Pretoria: SANBI; 2018. http://hdl.handle.net/20.500.12143/6362
13. Griffiths CL, Robinson TB, Lange L, Mead A. Marine biodiversity in South Africa: An evaluation of current states of knowledge. PLoS ONE. 2010;5(8), e12008. https://doi.org/10.1371/journal.pone.0012008
14. Atkinson LJ, Sink KJ, editors. Field guide to the offshore marine invertebrates of South Africa. Pretoria: Malachite Marketing and Media; 2018. Available from: http://www.saeon.ac.za/Field%20Guide%20to%20SA%20Offshore%20Marine%20Invertebrates_web%20full%20version.pdf
15. South African Department of Forestry, Fisheries, and the Environment (DEFF). South Africa's marine protection increased by the new representative network of Operation Phakisa: Oceans Economy Marine Protected Areas [media release]. 2019 May 28. Available from: https://www.dffe.gov.za/mediarelease/20marineprotectedareas_declared
16. Mann-Lang JB, Branch GM, Mann BQ, Sink KJ, Kirkman SP, Adams R. Social and economic effects of Marine Protected Areas in South Africa, with recommendations for future assessments. Afr J Mar Sci. 2021;43(3):367-387. https://doi.org/10.2989/1814232X.2021.1961166
17. United Nations General Assembly. Transforming our world: The 2030 Agenda for Sustainable Development. Document A/RES/70/1. New York: United Nations; 2015. Available from: https://www.refworld.org/docid/57b6e3e44.html
18. Weilgart L. A review of the impacts of seismic airgun surveys on marine life. Paper presented at: The CBD Expert Workshop on Underwater Noise and its Impacts on Marine and Coastal Biodiversity; 2014 February 25–27; London, UK. Available from: http://www.cbd.int/doc/?meeting=MCBEM-2014-01

19. Nieukirk SL, Mellinger DK, Moore SE, Klinck K, Dziak RP, Goslin J. Sounds from airguns and fin whales recorded in the mid-Atlantic Ocean, 1999–2009. J Acoust Soc Am. 2012;131:1102–1112. https://doi.org/10.1121/1.3672648

20. Folegot T, Clorennec D, Sutton G, Jessopp M. In: Popper A, Hawkin A, editors. The effects of noise on aquatic life II. Advances in Experimental Medicine and Biology Volume 875. New York: Springer; 2016. p. 313–320. https://doi.org/10.1007/978-1-4939-2981-8_37

21. Case No: 3865/2021 in the High Court of South Africa Eastern Cape Division, Grahamstown. Third to Fifth Respondents' Heads of Argument; 2021. Available from: https://cer.org.za/wp-content/uploads/2021/12/shell.heads-of-argument.1-December-2021.pdf

22. Gordon J, Gillespie D, Potter J, Frantzis A, Simmonds MP, Swift R, et al. A review of the effects of seismic surveys on marine mammals. Mar Technol Soc J. 2003;37:16–34. https://doi.org/10.4031/002533203787536998

23. Paxton AB, Taylor JC, Nowacek DP, Daled J, Colee E, Vossa CM, et al. Seismic survey noise disrupted fish use of a temperate reef. Mar Policy. 2017;78:68-73. https://doi.org/10.1016/j.marpol.2016.12.017

24. De Soto NA, Delorme N, Atkins J, Howard S, Williams J, Johnson M. Anthropogenic noise causes body malformations and delays development in marine larvae. Sci Rep. 2013;3, Art. #2831. https://doi.org/10.1038/srep02831

25. Carroll AG, Przeslawskia R, Duncan A, Gunning M, Bruce B. A critical review of the potential impacts of marine seismic surveys on fish & invertebrates. Mar Pollut Bull. 2017;114:9–24. https://doi.org/10.1016/j.marpolbul.2016.11.038

26. Kavanagh AS, Nykänen M, Hunt W, Richardson N, Jessopp MJ. Seismic surveys reduce cetacean sightings across a large marine ecosystem. Sci Rep. 2019;9, Art. #19164. https://doi.org/10.1038/s41598-019-55500-4

27. Castellote M, Clark CW, Lammers MO. Acoustic, and behavioural changes by fin whales (*Balaenoptera physalus*) in response to shipping and airgun noise. Biol Conserv. 2012;147:115–122. https://doi.org/10.1016/j.biocon.2011.12.021

28. Cerchio S, Strindberg S, Collins T, Bennett C, Rosenbaum H. Seismic surveys negatively affect humpback whale singing activity off northern Angola. PLoS ONE. 2014;9, e86464. https://doi.org/10.1371/journal.pone.0086464

29. Le Roux A, Singh JA, Ansorge I, Bornman T, Elwen S, Gammage L, et al. (Scientific Advisory Group on Emergencies (SAGE) Sub-committee on Marine Ecology and Risk Mitigation.) Advisory on the use of deep-sea seismic surveys to explore for oil and gas deposits in South African waters [document on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://www.assaf.org.za/files/2022/SAGE/SAGE%20Advisory%20on%20Shell%20Seismic%20Survey.pdf

30. Ingels J, Vanreusel A, Pape E, Pasotti F, Macheriotou L, Martínez Arbizu P, et al. Ecological variables for deep-ocean monitoring must include microbiota and meiofauna for effective conservation. Nat Ecol Evol. 2021;5:27–29. https://doi.org/10.1038/s41559-020-01335-6

31. Boebel O, Breitzke M, Burkhardt E, Bornemann H. Strategic assessment of the risk posed to marine mammals by the use of airguns in the Antarctic: Concepts, methods, results, controversies. Paper presented at: 24 Internationale Polartagung, 2010 September 6–10; Obergurgl, Austria. Available from: https://epic.awi.de/id/eprint/22668/

32. Meekan MG, Speed CW, McCauley RD, Fisher R, Birt MJ, Currey-Randall LM, et al. A large-scale experiment finds no evidence that a seismic survey impacts a demersal fish fauna. Proc Natl Acad Sci USA. 2021;118, e210086911. https://doi.org/10.1073/pnas.2100869118

33. Bourguignon D. The precautionary principle: Definitions, applications, and governance [In-Depth Analysis]. European Parliament Think Tank; 2015. Available from: https://www.europarl.europa.eu/thinktank/en/document/EPRS_IDA(2015)573876

34. Principle 15 of the Rio Declaration of the UN Conference on Environment and Development [webpage on the Internet]. c2006 [cited 2022 Feb 23]. Available from: https://www.cbd.int/doc/ref/rio-declaration.shtml

35. COP2 Decision II/10 on conservation and sustainable use of marine and coastal biological diversity, adopted by the Conference of the Parties to the CBD in November 1995 [webpage on the Internet]. c1995 [cited 2022 Feb 23]. Available from: https://www.cbd.int/decision/cop/?id=7083

36. Moore KJ, Fu W, Primeau F, Britten GL, Lindsay K, Long M, et al. Sustained climate warming drives declining marine biological productivity. Science. 2018;6380:1139–1143. https://doi.org/10.1126/science.aao6379

37. Feely RA, Doney SC, Cooley SR. Ocean acidification: present conditions and future changes in a high-CO_2 world. Oceanography. 2009;22:36–47. https://doi.org/10.5670/oceanog.2009.95

38. Doney SC, Fabry VJ, Feely RA, Kleypas JA. Ocean acidification: The other CO_2 problem. Annu Rev Mar Sci. 2009;1:169–192. https://doi.org/10.1146/annurev.marine.010908.163834

39. Doney SC, Ruckelshaus M, Duffy JE, Barry JP, Chan F, English CA, et al. Climate change impacts on marine ecosystems. Ann Rev Mar Sci. 2012;4:11–37. https://doi.org/10.1146/annurev-marine-041911-111611

40. Moore KM. Climate change could alter ocean food chains, leading to far fewer fish in the sea [webpage on the Internet]. c2018 [cited 2022 Feb 23]. Available from: https://phys.org/news/2018-04-climate-ocean-food-chains-fish.html

41. Intergovernmental Panel on Climate Change (IPCC). Climate change 2022: Impacts, adaptation, and vulnerability. Summary report for policymakers [document on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://report.ipcc.ch/ar6wg2/pdf/IPCC_AR6_WGII_SummaryForPolicymakers.pdf

42. Intergovernmental Panel on Climate Change. Climate change 2022: Impacts, adaptation, and vulnerability. Chapter 3: Oceans and coastal ecosystems and their services [document on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://report.ipcc.ch/ar6wg2/pdf/IPCC_AR6_WGII_FinalDraft_Chapter03.pdf

43. Nester Research. Seismic survey market: Global demand analysis & opportunity outlook 2020-2030 [webpage on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://www.researchnester.com/reports/seismic-survey-market/3590

44. Nester Research. Global seismic survey market to generate a revenue of USD 11658.2 million by 2030; rising need amongst oil & gas organizations to explore proven reserves for crude oil and natural gas to drive market growth [webpage on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://www.globenewswire.com/news-release/2022/02/03/2378226/0/en/Global-Seismic-Survey-Market-to-Generate-a-Revenue-of-USD-11658-2-Million-by-2030-Rising-Need-Amongst-Oil-Gas-Organizations-to-Explore-Proven-Reserves-for-Crude-Oil-and-Natural-Gas.html

45. Petroleum Agency of South Africa. Exploration and production: Current activities [webpage on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://www.petroleumagencysa.com/index.php/regulations/e-p-activities

46. Singh JA, Le Roux A, Naidoo S. Scientific Advisory Group on Emergencies (SAGE) Sub-committee on Marine Ecology and Risk Mitigation Response to EnerGeo Alliance (EA) and African Energy Chamber (AEC) – Advisory on the use of deep-sea seismic surveys to explore for oil and gas deposits in South African waters [document on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://www.assaf.org.za/files/2022/SAGE/Response%20to%20EnerGeo%20and%20AEC%20correpondence%20-%201%20Feb%202022%20-%20final2.pdf

47. The Presidency, Republic of South Africa. Operation Phakisa – Oceans Economy [document on the Internet]. c2014 [cited 2022 Feb 23]. Available from: https://www.operationphakisa.gov.za/cc/Documents/Open%20Day%20Operation%20Phakisa%20President%20Speech.pdf

48. Gardner R. Overview and characteristics of some occupational exposures and health risks on offshore oil and gas installations. Ann Occup Hyg. 2003;47:201–210.

49. Joint Ocean Commission Initiative. Gulf of Mexico. Ocean observation systems monitor the Loop Current and inform industry [webpage on the Internet]. c2017 [cited 2022 Feb 23]. Available from: https://oceanactionagenda.org/story/loop-current-gulf/

50. Boebel O, Rae CD, Garzoli S, Lutjeharms J, Richardson P, Rossby T, et al. Float experiment studies interocean exchanges at the tip of Africa. Eos Trans AGU. 1998;79(1):1–8. https://doi.org/10.1029/98EO00001

51. United States National Ocean Service. How fast is the Gulf Stream? [webpage on the Internet]. No date [cited 2022 Feb 23]. Available from: https://oceanservice.noaa.gov/facts/gulfstreamspeed.html

52. Moodley R, Nthontho M, Chowdhury S, Chowdhury SP. A technical and economic analysis of energy extraction from the Agulhas current on the east coast of South Africa. In: Proceedings of IEEE Power and Energy Society General Meeting; 2012 July 22–26; San Diego, CA, USA. IEEE; 2012. p. 1–8. https://doi.org/10.1109/PESGM.2012.6344793

53. Bryden HL, Beal LM, Duncan LM. Structure and transport of the Agulhas Current and its temporal variability. J Oceanogr. 2005;61:479–492. https://doi.org/10.1007/s10872-005-0057-8

54. Intergovernmental Oceanographic Commission (IOC). The Gulf Stream. Paris: IOC; 2006. Available from: https://unesdoc.unesco.org/ark:/48223/pf0000148252

55. Roelf W. Total stops S. Africa offshore drilling after mechanical failure. Reuters. 2014 November 04. Available from: https://www.reuters.com/article/total-safrica-idUSL6N0SU1MQ20141104

56. Bloomberg. The massive engineering problems drilling for oil in the Agulhas current. Daily Maverick. 2019 March 26. Available from: https://www.dailymaverick.co.za/article/2019-03-26-the-massive-engineering-problems-drilling-for-oil-in-the-agulhas-current/

57. Mallory JK. Abnormal waves in the south-east coast of South Africa. Int Hydrogr Rev. 1974;51:99–129.

58. Lavrenov I The wave energy concentration at the Agulhas current of South Africa. Nat Hazards. 1998;17:117–127. https://doi.org/10.1023/A:1007978326982

59. Liu PC, Machuchon KR, Wu CH. Exploring rogue waves from observations in South Indian Ocean. Actes Colloques-IFREMER. 2004;39:1–10.

60. Quilfena Y, Yurovskayab M, Chaprona B, Ardhuina F. Storm waves focusing and steepening in the Agulhas current: Satellite observations and modelling. Rem Sens Environ. 2018;216:561–571. https://doi.org/10.1016/j.rse.2018.07.020

61. Ponce de León S, Soares CG. Extreme waves in the Agulhas Current region inferred from SAR wave spectra and the SWAN Model. Mar Sci Eng. 2021;9:153. https://doi.org/10.3390/jmse9020153

62. Hansteen OE, Jostad HP, Tjelta TI. Observed platform response to a 'monster' wave. In: Myrvoll F, editor. Field measurements in geomechanics: Proceedings of the Sixth International Symposium on Field Measurements in Geomechanics; 2003 September 23–26; Oslo, Norway. Boca Raton, FL: CRC Press; 2003. p. 73.

63. Vallero DA, Letcher TM, editors. Unraveling environmental disasters. Boston, MA: Elsevier; 2013. p. 131–162. https://doi.org/10.1016/B978-0-12-397026-8.00006-9

64. Laconde T. Fugitive emissions: A blind spot in the fight against climate change. INIS-FR—20-1019 [document on the Internet]. c2019 [cited 2022 Feb 23]. Available from: https://www.climate-chance.org/wp-content/uploads/2019/03/new-fugitive-emissions-a-blind-spot-in-the-fight-against-climate-change.pdf

65. Yacovitch TI, Daube C, Herndon SC. Methane emissions from offshore oil and gas platforms in the Gulf of Mexico. Environ Sci Technol. 2020;54(6):3530–3538. https://doi.org/10.1021/acs.est.9b07148

66. McMichael AJ, Campbell-Lendrum DH, Corvalán CF, Ebi KL, Githeko AK, et al. Climate change and human health – risks and responses. Geneva: World Health Organization; 2003. Available from: http://www.who.int/globalchange/publications/climchange.pdf

67. Singh JA. Why human health and health ethics must be central to climate change deliberations. PLoS Med. 2012;9, e1001229. https://doi.org/10.1371/journal.pmed.1001229

68. Intergovernmental Panel on Climate Change (IPCC). The physical science basis [document on the Internet]. c2021 [cited 2022 Feb 23]. Available from: https://www.ipcc.ch/report/ar6/wg1/downloads/report/IPCC_AR6_WGI_Full_Report_smaller.pdf

69. Intergovernmental Panel on Climate Change (IPCC). An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty [document on the Internet]. c2018 [cited 2022 Feb 23]. Available from: https://www.ipcc.ch/site/assets/upload\s/sites/2/2019/06/SR15_Full_Report_Low_Res.pdf

70. Tong D, Zhang Q, Zheng Y, Caldeira K, Shearer C, Hong C, et al. Committed emissions from existing energy infrastructure jeopardize 1.5 °C climate target. Nature. 2019;572:373–377. https://doi.org/10.1038/s41586-019-1364-3

71. Welsby D, Price J, Pye S, Ekins P. Unextractable fossil fuels in a 1.5 °C world. Nature. 2021;597:230–234. https://doi.org/10.1038/s41586-021-03821-8

72. South Africa's Low Emission Development Strategy 2050 [document on the Internet]. c2020 [cited 2022 Feb 23]. Available from: https://unfccc.int/sites/default/files/resource/South%20Africa%27s%20Low%20Emission%20Development%20Strategy.pdf

73. South Africa: First Nationally Determined Contribution Under the Paris Agreement [document on the Internet]. c2021 [cited 2022 Feb 23]. Available from: https://www4.unfccc.int/sites/ndcstaging/PublishedDocuments/South%20Africa%20First/South%20Africa%20updated%20first%20NDC%20September%202021.pdf

74. Publication of draft Upstream Petroleum Resources Development Bill for public comment. Government Gazette No. 42931; 2019. Available from: https://www.gov.za/sites/default/files/gcis_document/201912/42931gon1706.pdf

75. Border Deep Sea Angling Association and Others vs. Minister of Mineral Resources and Energy and Others. Case No. 3865/2021 in the High Court of South Africa Eastern Cape Division, Makhanda/Grahamstown; 3 November 2021. Available from: https://cer.org.za/wp-content/uploads/2021/12/Judgment-Border-Deep-Sea-Angling-Association-v-Minister-of-Mineral-Resources-and-....pdf

76. Sustaining the Wild Coast NPC and Others vs. Minister of Mineral Resources and Energy and Others. Case No. 3491/2021 in the High Court of South Africa Eastern Cape Division, Makhanda/Grahamstown; 28 December 2021. Available from: https://cer.org.za/wp-content/uploads/2021/12/SWC-v-Shell-Wild-Coast-Seismic-Blasting-Interdict-28.12.2021.pdf

77. Christian John Adams & Others v Minister of Mineral Resources and Energy & Others. Case No. 1306/22 in the High Court of South Africa Western Cape Division, Cape Town; 1 March 2022. Available from: https://cer.org.za/wp-content/uploads/2022/03/Adams-and-Others-v-Minister-of-Mineral-Resources-and-Energy-and-Others-ZAWCHC-24.pdf

78. South African Department of Mineral Resources and Energy (DMRE). Gas master plan 2022: Base case report [document on the Internet]. c2021 [cited 2022 Feb 23]. Available from: http://www.energy.gov.za/files/media/explained/Gas_Master_Plan_Basecase_Report.pdf

79. Mantashe G. Developments in the upstream petroleum industry [media release]. c2021 [cited 2022 Feb 23]. Available from: https://www.gov.za/speeches/media-statement-developments-upstream-petroleum-industry-9-dec-2021-0000

80. Davis R. Gwede Mantashe vs academia and activism (and the media, and the world). Daily Maverick. 2022 January 25. Available from: https://www.dailymaverick.co.za/article/2022-01-25-gwede-mantashe-vs-academia-and-activism-and-the-media-and-the-world/

81. Convention on the Conservation of Migratory Species of Wild Animals. Date enacted: 1979-06-23. In force: 1983-11-01. Available from: https://www.cms.int/en/convention-text

82. South African Department of Forestry, Fisheries, and the Environment (DFFE). Cabinet approves a representative network of Marine Protected Areas in the South African exclusive zone [media release]. c2018 [cited 2022 Feb 23]. Available from: https://www.dffe.gov.za/mediarelease/cabinetapproves_representativenetworkofMPAs#:~:text=The%20Department%20of%20Environmental%20Affairs,(EEZ)%20to%205%25

83. Convention on the Conservation of Migratory Species of Wild Animals (CMS). Adverse impacts of anthropogenic noise on cetaceans and other migratory species. Bonn: CMS; 2017. Available from: https://www.cms.int/en/document/adverse-impacts-anthropogenic-noise-cetaceans-and-other-migratory-species-0

84. Environmental Impact Assessment Regulations. Passed in accordance with the National Environmental Act 107 of 1998. Government Notice R982 in Government Gazette 38282, Transitional Arrangements [document on the Internet]. c2014 [cited 2022 Feb 23]. Available from: https://www.dffe.gov.za/sites/default/files/legislations/nema_eia2014regulations_g38282_0.pdf

85. Global Caron Atlas. Fossil fuel emissions [webpage on the Internet]. No date [cited 2022 Feb 23]. Available from: http://www.globalcarbonatlas.org/en/CO2-emissions

86. Department of Mineral Resources and Energy, South Africa. Integrated Resource Plan (IRP 2019) [document on the Internet]. c2019 [cited 2022 Feb 23]. Available from: http://www.energy.gov.za/IRP/2019/IRP-2019.pdf

87. South African Petroleum Industry Association (SAPIA). The economic contribution of the downstream oil industry to South Africa in 2019. Executive summary presentation [document on the Internet]. c2021 [cited 2022 Feb 23]. Available from: https://www.sapia.org.za/portals/0/MediaStatements/SAPIA_EIA_Exec%20Summary.pdf

88. International Institute for Sustainable Development. South Africa's energy fiscal policies: An inventory of subsidies, taxes, and policies impacting the energy transition [document on the Internet]. c2022 [cited 2022 Feb 23]. Available from: https://www.iisd.org/system/files/2022-01/south-africa-energy-subsidies.pdf

89. Climate Policy Initiative. Understanding the impact of a low carbon transition on South Africa [document on the Internet]. c2019 [cited 2022 Feb 23]. Available from: https://www.climatepolicyinitiative.org/wp-content/uploads/2019/03/CPI-Energy-Finance-Understanding-the-impact-of-a-low-carbon-transition-on-South-Africa-March-2019.pdf

90. Presidential Climate Commission, South Africa. Recommendations on South Africa's draft updated Nationally Determined Contribution (NDC) [document on the Internet]. c2021 [cited 2022 Feb 23]. Available from: https://a9322a19-efe3-4459-9a6c-ab806fededa3.filesusr.com/ugd/1eb85a_896d0493b6284743b2ff3986b36be622.pdf

91. Republic of South Africa. South Africa: First Nationally Determined Contribution under the Paris Agreement – Updated September 2021 [document on the Internet]. c2021 [cited 2022 Feb 23]. Available from: https://www4.unfccc.int/sites/ndcstaging/PublishedDocuments/South%20Africa%20First/South%20Africa%20updated%20first%20NDC%20September%202021.pdf

92. Ward DS, Mahowald NM. Contributions of developed and developing countries to global climate forcing and surface temperature change. Environ Res Lett. 2014;9(7):4008. https://doi.org/10.1088/1748-9326/9/7/074008

93. Wei T, Dong WJ, Yan Q, Chou JM, Yang ZY, Tian D. Developed and developing world contributions to climate system change based on carbon dioxide, methane and nitrous oxide emissions. Adv Atmos Sci. 2016;33(5):632–643. https://doi.org/10.1007/s00376-015-5141-4

94. United Nations Framework Convention on Climate Change. Climate finance in the negotiations [document on the Internet]. No date [cited 2022 Feb 23]. Available from: https://unfccc.int/topics/climate-finance/the-big-picture/climate-finance-in-the-negotiations

95. European Commission. France, Germany, UK, US and EU launch ground-breaking International Just Energy Transition Partnership with South Africa [webpage on the Internet]. c2021 [cited 2022 Feb 23]. Available from: https://ec.europa.eu/commission/presscorner/detail/en/ip_21_5768

96. International Energy Agency (IEA). Net zero by 2050. A roadmap for the global energy sector. Flagship report. Paris: IEA; 2021. Available from: https://www.iea.org/reports/net-zero-by-2050

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Research and the meaning of 'public interest' in POPIA

Significance:
'Public interest' is an important concept in POPIA. However, the way in which it has been interpreted by the Information Regulator is subject to criticism. A better interpretation is suggested.

The term 'public interest' is used 16 times in the *Protection of Personal Information Act 4 of 2013* (POPIA). Understanding the concept is important for research. This is because the public interest is relevant when considering a research exception for allowing the processing of special personal information (section 27(1)(d)(i)), and a research exemption from the conditions for processing personal information (section 37(1)(a)). But what exactly does public interest mean?

In the absence of a definition in POPIA itself, the Information Regulator proffered the following 'basic formulation' of public interest in a recent Guidance Note[1]:

> *Public interest is a wide and diverse concept that cannot, and should not, be limited in its scope and application. The definition of what constitutes public interest varies across jurisdictions and should be assessed on a case-by-case basis. In its very basic formulation public interest is the notion that an action or process or outcome widely and generally benefits the public at large (as opposed to a few or a single entity or person) and should be accepted or pursued in the spirit of equality and justice.*

As I show here, this basic formulation of public interest is misaligned with the way in which public interest has been interpreted by South African courts and hence is in need of revision. Furthermore, it would assist the research community if the basic formulation could be expanded by the Code of Conduct for Research (the Code) that the Academy of Science of South Africa (ASSAf) is currently developing.[2] To inform ASSAf's Code development process, this article provides a concise analysis of the principles concerning public interest that have crystallised in our law.

In specific contexts, such as where it forms part of the statutory mandate of a public body, the meaning of public interest may become complex and contentious, as illustrated by the *Rail Commuter Action Group* series of cases.[3-5] However, in this article I confine the analysis to general principles relevant to research in the context of POPIA.

The basic formulation reconsidered

Similar to the Information Regulator's Guidance Note, the South African courts have called public interest a 'broad and uncertain'[6] concept that does not permit a clear, precise and comprehensive definition.[6-8] Yet a good general understanding of the concept can be developed by considering the ways in which the courts have interpreted and applied the concept. At a general level, public interest has been interpreted as benefiting the public[4,8], promoting the general welfare of the public[3], or better serving the public[6,7]. Although there may be differences in nuance between these choices of wording, for present purposes they effectively have the same meaning. Accordingly, the Information Regulator's use of the word 'benefit' in its basic formulation of public interest is appropriate and aligned with our law.

However, this is where the alignment between the Information Regulator's understanding of public interest and the actual meaning of public interest as expounded by South African courts stops. Importantly, the Information Regulator's interpretation of the 'public' as meaning the 'public at large' is not necessarily true. The courts have made it clear that the 'public' concerned need *not* be widely representative of the general public – it can be a smaller community within the broader national community.[6,8,9]

In this context it is also important to note that the public interest does not depend on each member of the relevant community or 'public' directly benefiting. As succinctly held by the High Court[10]:

> [A] *scheme is 'in the public interest' if it is to the general interest of the community that it should be carried out, even if it directly benefits only a section or class or portion of the community.*

While private interests and the public interest can overlap (or be in conflict), the public interest is not merely an aggregate of private interests. The word 'general' in the quote above indicates that something broader than the private interests of individuals is envisaged.[7] Accordingly, although direct benefits may befall only a section of the community, the relevant consideration is whether the community (the relevant 'public') would *qua* collective reap (indirect) benefit. This can be illustrated by three examples from case law: (1) introducing competition in a regulated market (rather than a monopoly) in Mookgopong (previously Naboomspruit) was held to be in the public interest[8], even though there is direct benefit for the individual new competitor that is granted a licence; (2) a rebuilding scheme to answer the demand among persons of a certain profession for office space in downtown Johannesburg was held to be *prima facie* in the public interest[10], even though there is direct benefit for the individual landlord; and (3) the principle of open justice (court proceedings being open to the public, rather than held in secret) was held to be in the public interest[11], even though there is direct benefit for the media outlets that are reporting on the cases. It is worth noting that while the relevant 'public' in example (3) was the South African public at large, in example

(1) the 'public' was a small town, and in example (2) it was a class of persons defined by their common profession.

From examples (1) and (2) it should also be clear that 'the spirit of equality and justice' is a novel criterion invented by the Information Regulator without any basis in law. Although equality and justice are certainly core values of our common law and our constitutional dispensation, these values are simply not criteria for public interest. The focus on these two values also raises the question: Why elevate equality and justice above other constitutional values such as openness and individual freedom? While something that undermines equality or justice (or openness, or freedom, *et cetera*) will likely not be in the public interest, as illustrated by examples (1) and (2), there are many examples of things that the courts held to be in the public interest that do not actively promote or protect any of these values.

Given my analysis above, I suggest that the basic formulation for being in the public interest ought to be whether something *benefits the people of South Africa qua collective, or benefits a group of people in South Africa qua collective.* Such benefit may be indirect, and any direct benefit to one or more individuals does not detract from the indirect benefit to the relevant public. Also, benefit to the relevant public is sufficient to constitute public interest; it need not be shown that 'the spirit of equality and justice' or any other value of our law is being promoted or protected.

Application

I now consider the application of the revised basic formulation of public interest to exceptions and exemptions for research in terms of POPIA.

Section 27(1)(d)(i) (exceptions to the prohibition on processing of special personal information) entails a simple public interest test: either the research study in question serves the public interest or not. Accordingly, the revised basic formulation should be used as a general guideline. It would further help the research community if examples of research studies that would be deemed to be in the public interest could be provided by the Code. It may also be good if the Code makes it clear that the public interest requirement need not be satisfied by the research study itself, but may be satisfied by the envisioned application of the study's results, the resource investment made during the study, the employment created, or any other downstream benefits.

Health research is a special case.[12] Consider that all health research must be approved by a health research ethics committee and that these committees have a statutory duty to ensure that health research will 'promote health, contribute to the prevention of communicable or non-communicable diseases or disability or result in cures for communicable or non-communicable diseases'[13]. The outcome is that *all* health research conducted in South Africa is *prima facie* in the public interest. To deny this would amount to a denial that the system of ethics review in South Africa is robust and as a general rule functions well. Although there have been some exceptions, there is no evidence in the public domain to cast general doubt on the system of ethics review in South Africa.

In contrast with section 27(1)(d)(i), section 37(1)(a) (exemptions from the conditions for processing of personal information) assumes that research is in the public interest (see section 37(2)(e)) and focuses on the importance of the public interest served. This implies a potential hierarchy among different instances of the public interest. The idea that some instances of the public interest (or simply 'public interests') may be more important than others is also found in case law. For example, the court held, with reference to English case law, that one of two competing public interests is of a 'high order'.[14] Section 37(1)(a) requires that 'the public interest in the processing *outweighs, to a substantial degree*, any interference with the privacy of the data subject that could result from such processing' (my emphasis). Accordingly, while some instances of the public interest may substantially outweigh the data subject's privacy rights, some may not. Clearly, section 37(1)(a) entails a *balancing exercise*, where the inevitable question is how to allocate weight to the conflicting interests.

I suggest that this balancing exercise should be informed by the Constitution *qua* the ultimate normative guide in South African law.[15] The Code can play an important role in facilitating the balancing exercise by applying constitutional rights and values to the *research* context and identifying factors that should be considered. Given that the Code aims to be inclusive of all academic disciplines, it should provide for a diverse range of factors, which gives researchers from all disciplines a fair opportunity to demonstrate the importance of their intended research.

It bears repetition that equality and justice – important as they are – are not the only constitutional values, and that values such as openness, dignity, freedom, and *ubuntu* should not be sidelined.

Concluding note

The guidance provided by the Information Regulator should be well informed and aligned with the law of the land. Although the Information Regulator has wide-ranging powers, it cannot create law. Similarly, it cannot limit the rights bestowed by POPIA through a restrictive interpretation of the meaning of public interest. Members of the South African research community have the right, where relevant, to seek a research exception to allow the processing of special personal information (section 27(1)(d)(i)), and to seek a research exemption from the conditions for processing personal information (section 37(1)(a)) provided for in POPIA. When exercising this right, the research institution (or individual researcher) is entitled to rely on the broader, more inclusive meaning of public interest that is found in South African law, rather than on the description of public interest provided by the Information Regulator in its Guidance Note.[1] Neither the Guidance Note nor the Code can override the judicial view of what public interest is. At best, these documents can explain and elaborate on the judicial view of public opinion. To avoid confusion, the Guidance Note should be amended in line with the revised basic formulation of being in the public interest that was developed in this article.

Furthermore, this article provides basic pointers regarding the application of public interest to research in the context of POPIA. Building on these pointers, the Code should, at a minimum: (1) distinguish between instances of research that are in the public interest from those that are not; and (2) where research is deemed to be in the public interest, allocate weight to the public interest being served and the privacy interest that is to be limited. Both (1) and (2) should be done in a meaningful, predictable, and reproducible manner.

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Competing interests

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References

1. Information Regulator. Guidance note on [the] Processing of Special Personal Information. c2021 [cited 2021 Sep 07]. Available from: https://www.justice.gov.za/inforeg/docs/InfoRegSA-GuidanceNote-Processing-SpecialPersonalInformation-20210628.pdf
2. Adams R, Veldsman S, Ramsay M, Soodyall H. Drafting a Code of Conduct for Research under the Protection of Personal Information Act No. 4 of 2013 (with corrigendum). S Afr J Sci. 2021;117(5/6), Art. #10935. https://doi.org/10.17159/sajs.2021/10935
3. Rail Commuter Action Group v Transnet Ltd t/a Metrorail (No 1) 2003 (5) SA 518 (C).
4. Transnet Ltd t/a Metrorail v Rail Commuter Action Group 2003 (6) SA 349 (SCA).
5. Rail Commuters Action Group v Transnet Ltd t/a Metrorail 2005 (2) SA 359 (CC).

6. Asko Beleggings v Voorsitter van die Drankraad NO 1997 (2) SA 57 (NC).
7. Leicester Properties (Pty) Ltd v Farran 1976 (1) SA 492 (D).
8. CJW Marketing CC v Limpopo Provincial Liquor Board [2008] ZAGPHC 403.
9. Maharaj v Chairman, Liquor Board 1997 (1) SA 273 (N).
10. Clinical Centre (Pty) Ltd v Holdgates Motor Co (Pty) Ltd 1948 (4) SA 480 (W).
11. Centre for Child Law v Media 24 Limited [2019] ZACC 46, 2020 (4) SA 319 (CC).
12. Thaldar DW, Townsend BA. Exempting health research from the consent provisions of POPIA. PER. 2021;24:1–32. https://doi.org/10.17159/1727-3781/2021/v24i0a10420
13. National Health Act 61 of 2003, South Africa.
14. Financial Mail (Pty) Ltd v Sage Holdings Ltd 1993 (2) SA 451 (AD).
15. Carmichele v Minister of Safety and Security 2001 (4) SA 938 (CC).

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Why research institutions should indemnify researchers against POPIA civil liability

Significance:

In the research context, a 'responsible party' as contemplated in terms of POPIA is typically the research institution as well as the individual researcher involved. Given the potential civil liability that individual researchers could face, we suggest that the Code of Conduct for Research should place a duty on research institutions to indemnify their researchers from civil liability. While this measure will limit individual researchers' personal financial risk in the extra-institutional legal sphere, it will in no way shield individual researchers from intra-institutional accountability and disciplinary action. Accordingly, we suggest that this measure strikes a fair balance.

Introduction and background

The research community eagerly awaits the publication of a draft Code of Conduct for Research (the Code) in terms of the *Protection of Personal Information Act* (POPIA).[1] The Code should provide researchers with practical guidance and animate the provisions of POPIA which are largely principles-based. With predominantly principles-based legislation, as opposed to a strictly rules-based approach, there is often room to interpret certain principles – depending on how the various provisions and concepts are phrased. Although a Code cannot re-define concepts (this would be *ultra vires*), it will play a particularly important role in providing guidance on how terms are to be applied and understood, especially in the context of scientific research.

One of the essential foundational definitions in POPIA – as a general concept, and in the context of duties and potential liability – relates to the person that controls and directs the processing of personal information: a 'responsible party'.[1] Accordingly, the purpose of this Commentary is to analyse this concept and outline certain areas in relation thereto that the Code ought to provide clarity and guidance on.

The definition of a 'responsible party'

POPIA, in section 1 thereof, defines 'responsible party' as 'a public or private body or any other person which, alone or in conjunction with others, determines the purpose of and means for processing personal information'[1].

Importantly, the definition does not restrict a responsible party to juristic persons (the research institutions under whose auspices the research is being conducted). The words 'any other person'[1] includes any natural person. The definition is certainly wide enough to encompass both an individual researcher, and the research institution as a responsible party. Further, the phrase 'alone or in conjunction with others'[1] indicates that more than one person may be considered a responsible party. In addition, if one considers POPIA's definition of 'operator', which is 'a person who processes personal information for a responsible party in terms of a contract or mandate, without coming under the direct authority of that party'[1], an employee is specifically excluded from the ambit thereof, while the definition of 'responsible party' makes no such exclusion. An employee is by definition a person who is 'under the direct authority' of an employer. Therefore, an employee cannot be defined as an 'operator', but may well be considered a 'responsible party' if in fact the employee 'determines the purpose of and means for processing personal information'[1] (as per the definition of 'responsible party'). With this being the case, it is clear from the definition of 'responsible party' that multiple persons may, depending on the circumstances, jointly qualify as responsible parties for a single act of processing personal information.

How does one determine who the responsible party will be in each situation? This is a factual question that ought to be determined on a case-by-case basis. In the research context, typically, there will always be at least two responsible parties. Firstly, the research institution, acting through its organs, such as a research unit or an individual researcher, that determines the purpose of and means for processing personal information, and, secondly, a natural person in the form of the researcher who, as an employee of the institution, determines the purpose of and means for processing personal information. While it is conceivable that a researcher who does independent research may be the sole responsible party, researchers are typically employed or contracted by research institutions, in which case the research institution is likely to be the joint responsible party. As a result, research institutions must appoint an information officer, and that person must ensure that the organisation has a policy on how employees should implement the conditions of lawful processing set out in POPIA, and conduct training and monitoring.[2] Researchers employed or contracted by the institution must therefore take decisions on data protection in consultation with their institution's information officer and in compliance with all applicable policies. While employees generally act as an agent only, employees are not exonerated from personal liability in all circumstances. It is well established in South African law that in cases of wrongful breach of a duty of care or criminal misconduct, personal liability may follow. Therefore, we regard it as prudent for individual researchers to regard themselves as potentially joint responsible parties in line with the definition in POPIA, and to act accordingly.

In many instances, particularly in larger organisations, there will be multiple joint responsible parties. In some research projects, it is possible that the principal investigator, as well as the co-investigators or even technicians may determine the purpose of or means for processing personal information, hence making them all responsible parties. Furthermore, in the context of research consortia, all the research institutions may qualify as responsible parties. The decisive consideration is determining, objectively, who decides the purpose of or means for processing

personal information. As pointed out above, there is no hard-and-fast rule here – it is a determination that must be made on a case-by-case basis.

Consequences for a responsible party of non-compliance with POPIA

Whenever there is non-compliance with the provisions of POPIA, data subjects (research participants) will have recourse against the non-complaint responsible party or parties – i.e. the individual researchers and/or research institutions involved. Accordingly, we provide a brief overview of the consequences of non-compliance. We also make recommendations, where appropriate, of what the Code should do to protect individual researchers, who are more vulnerable to lawsuits than research institutions.

POPIA's enforcement mechanisms

In the event of non-compliance with POPIA, data subjects (research participants) can lay a complaint with the Information Regulator. Chapter 10 of POPIA regulates enforcement, and sections 73 and 74 provide that where there has been 'interference with the protection of the personal information of a data subject'[1], the data subject may make a complaint, in writing, in the prescribed manner and form, to the Information Regulator. In terms of section 76, the Regulator is required to conduct a pre-investigation, act as conciliator where appropriate, decide on whether a full investigation is required, and, where necessary, refer the matter to its Enforcement Committee. The Regulator is given wide and expansive powers in Chapter 10 and may summon and enforce the appearance of witnesses, administer oaths, receive evidence, conduct interviews, apply to a judge or magistrate for a search and seizure warrant, and enter and search any premises occupied by a responsible party (where a warrant is granted).

Where a breach of POPIA leads to an investigation and ultimately a referral to the Regulator's Enforcement Committee, section 93 provides that the Enforcement Committee may make any recommendation to the Regulator against the responsible party or an information officer of a responsible party. These recommendations, as set out in section 95, will include an order for the responsible party and/or information officer to take certain steps within a period specified, or to refrain from taking such steps. A responsible party may, in terms of section 97, appeal any decision of the Regulator to a High Court to set aside or vary any order.

Thwarting POPIA's enforcement mechanisms by, for example, obstructing the Regulator, or failing to comply with an enforcement notice, could lead to criminal prosecution and administrative fines. The sanctions are potentially severe, with fines up to ZAR10 million, and prison sentences for a period not exceeding 10 years.

Private remedies for data subjects

The thrust of section 99 is that a data subject, or the Information Regulator on the data subject's behalf, may initiate civil action to claim damages against a responsible party where the responsible party has breached a provision of POPIA (for example, a breach of the conditions for the lawful processing of personal information) or for breach of the provisions of a code of conduct for research approved and issued by the Regulator in terms of section 60. Generally, in South African law, plaintiffs in civil actions for damages caused by wrongful acts (called 'delicts' in South Africa and 'torts' in the USA and UK) must prove that the defendants acted with fault, which is either intent or negligence. However, the new *sui generis* delictual action created by POPIA explicitly excludes the requirement of fault. As such, responsible parties can be held delictually liable by data subjects even if the responsible parties did not act intentionally or negligently. This is referred to as 'no-fault liability' or 'strict liability', and other examples exist in South African law, such as (strict) product liability of a manufacturer under the *Consumer Protection Act*, and the (strict) liability of the owner of a domesticated animal for damage caused by such animal in terms of the ancient Roman *actio de pauperie*. Strict liability clearly benefits plaintiffs in delictual actions, as it significantly lessens their evidentiary burden. In the research context, research participants only need to prove that their personal data were unlawfully processed by one or more researchers (and vicariously by their research institutions) and that they have suffered damages as a result. The mental state (intention or lack thereof) of the researcher or researchers involved is not relevant.

In delictual actions, plaintiffs are entitled to choose their defendant or defendants from a group of potential wrongdoers.[3] To use the language employed by the Durban High Court in *Parekh v Shah Jehan Cinemas (Pty) Ltd,* the plaintiff may 'select his target'.[4] In the research context, this means that research participants whose personal information has been processed unlawfully and who intend to sue in terms of section 99 have the right to select their target from *all the persons who qualify as responsible parties* in terms of POPIA's definition of responsible party (and who fulfil the other criteria of section 99).

The most likely scenario would be that potential plaintiffs would cite the research institution and the researchers involved as defendants. In the alternative, for whatever strategic reason, the plaintiffs may choose to cite only the research institution or only one of the researchers involved – this is an election the plaintiffs are free to make in their own discretion.

While research institutions would have resources to defend themselves against legal action, individual researchers are unlikely to have the resources to do so. Accordingly, we recommend that the Code should place a duty on research institutions to, on condition that the research project has been approved by the research institution's ethics committees, indemnify the researchers in their employ against section 99 claims. This indemnification should be stated in ethics clearance letters. There should also be a procedure provided for in the Code for researchers who are sued in terms of section 99 to notify the relevant research institution and for the research institution to immediately intervene as a further defendant in the action and cover the costs of the legal defence of itself and its employees. In the event that a researcher who is sued in terms of section 99 notifies the research institution, but the research institution fails to intervene, the researcher can force the research institution to become a co-defendant based on the indemnification statement in the ethics clearance letter. This would be accomplished by serving a third-party notice on the research institution in terms of Rule 13 of the Uniform Rules of Court.

To ensure that the indemnification is not misinterpreted by researchers as a free pass to ignore POPIA subsequent to ethics clearance, institutions can require researchers, when filing applications for ethics clearance, to specifically declare that they (a) know the data protection requirements of POPIA and (b) will uphold such requirements. In the event of non-compliance with POPIA by a researcher, the research institution can investigate and take disciplinary action against such researcher. Although such a declaration is important, it should be integrated within the context of a more comprehensive POPIA-compliance awareness and training programme by a research institution. Institutions should ensure that their researchers know how to fully comply with POPIA, and that although the institution would shield them from personal financial risk in the extra-institutional legal sphere – the indemnification that we propose – this would in no way shield individual researchers from intra-institutional accountability and disciplinary action.

Joint liability?

As we set out above, it is possible that in any given research situation, there may be *more than one* responsible party. How does POPIA deal with this? In simple terms, it does not. It may be interpreted as meaning that where there are multiple responsible parties[5]:

> *they are jointly and severally liable for any processing which is carried out jointly (that is to say, where both the purposes and means of processing are shared), but are individually liable for any processing which is carried out separately for their own purposes, and by their own means.*

Joint and several liability, which applies to delictual wrongdoers under the common law, entails that each party can be held liable for the whole

of the damages. This is wider than 'joint' liability where multiple parties are each only liable for a proportionate share of a joint debt.[6]

However, where there is partial overlap of either the purposes or means of processing, the position is less clear. In its ordinary meaning, the phrase 'in conjunction with' used in the definition of responsible party refers to 'the situation in which events or conditions combine or happen together'[7]. It thus includes, but is somewhat wider than, the adjective 'joint' or 'jointly', which means 'belonging to or shared between two or more people'[8]. Thus again, each case would have to be dealt with on its own facts, but the point of departure in our view is that it is only where parties did act jointly in the latter sense that there can be joint and several liability. This accords with the view expressed by the Court of Justice of the European Union in two cases (albeit decided in the context of online services rather than research) that responsibility as joint controllers does not imply 'equal' responsibility.[9] The level of responsibility would be determined in accordance with the individual circumstances of each case.[9,10]

It is worth noting that POPIA's definition of 'responsible party' is drawn from Article 2(d) of the Data Protection Directive[11], which is in all material respects identical to Article 4(7) of the General Data Protection Regulation[12] (GDPR) (although the European data protection regime uses the term 'controller' rather than 'responsible party', the definitions are similar and the principles the same). One caveat in this regard: although there are striking similarities between POPIA and the GDPR, Article 26 of the GDPR makes specific provision for joint responsibility, enjoining parties to 'determine their respective responsibilities for compliance'[12]. POPIA does not have a similar provision, and it is hoped that the Code will suggest – on a similar basis to Article 26 – that responsible parties make suitable arrangements and make same available to the data subject. However, it should be noted that even if joint-responsible parties conclude an agreement regarding their respective responsibilities for compliance with POPIA, this will not be binding on the data subject, who can still select their target in the event of section 99 civil liability litigation – joint-responsible parties may well have indemnity provisions between themselves in the underlying agreement, but these are not binding on the data subject, who will still in theory have a choice of whom to litigate against.

Conclusion

The Code should clarify, using practical examples, who qualifies as a 'responsible party'. That said, POPIA does not provide that codes of conduct can limit the rights of data subjects in any way. In light of our analysis of plaintiffs' right to select their target in delictual actions from the entire pool of wrongdoers, it means that the Code cannot define 'responsible party' narrower than in POPIA itself. Stated differently, the Code cannot prescribe to data subjects who to sue and who not to sue. This is the prerogative of the data subjects. What the Code can and should do, is to arrange for research institutions to indemnify individual researchers. Furthermore, to ensure that researchers who work in consortia do not just assume that somebody else will take responsibility for POPIA compliance, the Code should provide guidance regarding suitable POPIA compliance arrangements between research consortium partners.

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Competing interests

We have no competing interests to declare.

References

1. Protection of Personal Information Act 4 of 2013, South Africa.
2. Information Regulator. Guidance note on Information Officers and Deputy Information Officers, 1 April 2021, South Africa.
3. Myeni v Organisation Undoing Tax Abuse NPC (15996/2017) [2019] ZAGPPHC 565. Available from: http://www.saflii.org/za/cases/ZAGPPHC/2019/565.html#_ftnref32
4. Parekh v Shah Jehan Cinemas (Pty) Ltd 1982 (3) SA 618 (D) at 622E.
5. Donnelly D. Privacy by (re)design: A comparative study of the protection of personal information in the mobile applications ecosystem under United States, European Union and South African law [thesis]. Durban: University of KwaZulu-Natal; 2020.
6. De Pass v The Colonial Govt (1886) 4 SC 283 at 390 per De Villiers CJ.
7. Cambridge English Dictionary [online]. Cambridge: Cambridge University Press; 2021. Conjunction [cited 2020 Mar 30]. Available from: https://dictionary.cambridge.org/dictionary/english/conjunction
8. Cambridge English Dictionary [online]. Cambridge: Cambridge University Press; 2021. Joint [cited 2020 Mar 30]. Available from: https://dictionary.cambridge.org/dictionary/english/joint
9. Wirtschaftsakademie Schleswig-Holstein (C-210/16) ECLI:EU:C:2018:388 para 43.
10. Fashion ID GmbH & Co. KG v Verbraucherzentrale NRW eV (C-40/17) ECLI:EU:C:2019:629 para 75–76.
11. Data Protection Directive 95/46/EC, European Union.
12. General Data Protection Regulation (EU) 2016/679, European Union.

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What we say and what we do: The perils of ethical consensus

Significance:

A new book argues that South Africa could better fight poverty and inequality if the country recognised that caring for others is a duty. It is argued here that the problem is not that we do not all agree on the need for care – it is that we disagree on what that means. A more equal country is possible, not if we all claim to support the same principles, but if we acknowledge our differences and seek compromises between them.

Do South African women die violent deaths because the country lacks morality? Or because a complex set of social and psychological factors make violence against women more likely?

Does the country continue to live with high levels of poverty and inequality because its elite have an inappropriate ethic? Or because everyone agrees that this is a moral problem but the meaning they attach to the ethical words they use differs and a combination of past and current realities means that power resides with those who are happy to allow it to continue as they pay lip service to its moral repugnance?

These questions are raised by a chapter in the Human Sciences Research Council's latest *State of the Nation* compilation[1] which, as the title suggests, seeks to assemble a range of scholarly articles on the current state of South African society. The chapter[2] is written by the three editors of the 2021 volume and seeks to set the direction of the entire collection – the 18 chapters by a variety of authors on topics ranging from foreign policy to film-making were clearly meant to reflect the concerns argued in this chapter. The hope was not realised: the authors of many of the chapters do not examine their subject matter through the lens proposed by the editors. This is predictable: attempts to persuade contributors to collections to address a particular theme repeatedly fail as authors insist on discussing what interests them. But it does convey what the editors understand the present state of the nation to be. It also raises issues which are crucial to debates on the country's current realities.

In search of an ethic

In essence, the authors argue that the state should become the vehicle of an 'ethics of care' which they ground in feminist theory and the anti-racist work of David Theo Goldberg:

> *When decision-making is based on the values enshrined in the constitution – in which an ethical stance is inherent – then we will begin to realise the practical effects of a just and caring society.*[2(p.15)]

This concern for the ethical is central to what they hoped the collection would stress – the volume is entitled *Ethics, Politics, Inequality: New Directions* and some of the contributions place ethics at the centre of their concern.

It is easy to see what prompted this interest in ethics. Corruption has been an abiding national concern for a decade. The country is still living through a pandemic in which concern for the ill and vulnerable often seemed to be trumped by pressures to 'keep the economy open'.[3] The government remains convinced that economic health can be achieved by addressing the concerns of private investors rather than giving priority to poverty and inequality.[4] Media and politicians insisted that the only issue of importance to the recent local government election campaign was 'service delivery', a term which reduces democratic government to a technical arrangement much like that between a customer and a company rather than an ethical relationship between representatives and citizens.[5]

Less topically, but no less importantly, one of the many hangovers from pre-1994 South Africa which remains central to the national self-image is a celebration of conspicuous consumption: the media and other instruments of socialisation proclaim that ownership of consumer goods is the test of human worth – a message which has percolated deep into the society's consciousness: the last census reported that more South Africans owned television sets than owned refrigerators.[6] The governing elite did not invent this but it has done little to dispel it because its chief concern has not been to challenge the privilege a minority enjoyed under apartheid but to seek to ensure that everyone enjoys it. Thus, while former president Thabo Mbeki famously devoted a Mandela lecture to decrying the acquisitiveness of a new elite[7], the policies of the government over which he presided encouraged precisely this.

But to understand this concern is not to accept it. An 'ethics of care' sounds attractive, but, on closer scrutiny, will not produce the change the society needs.

Problems and pitfalls

First, states – and the political elites who govern them – do not exist in a vacuum. They may influence prevailing ethics, but are also influenced by them.[8]

The ethics of the South African state largely reflect that of the society's elite, the opinion formers who reflect and shape what the society is expected to value. We cannot consider the state's ethic – or lack of one – in isolation from the voices which frame the national debate. To name an example, we cannot understand the ethics of the state's response to COVID-19 unless we recognise that it reflects, and is shaped by, the concerns of the roughly one-third of citizens who enjoy some access to the national debate.[4] To argue for an ethic of care in the state

without addressing the values and understandings of the interests which influence the state's ethic is to address the symptom, not the cause.

Second, the claim that poverty and inequality and their attendant ills are products of an inappropriate ethic or no ethic at all is curious as the authors acknowledge that such an ethic does exist 'inherently' in the Constitution. There is surely no one within the state – or among elites outside it – who openly rejects the Constitution and the values which underpin it. And yet poverty and inequality persist, as do corruption, violence, and racial and gender bigotry. The problem is not the ethics that key social actors acknowledge, but that, in good or bad faith, they choose to interpret a common stated ethic in ways which leave inequity untouched or worsen it.

In February 2021, when he presented what was to be his last budget, then Minister of Finance Tito Mboweni indignantly rejected claims that it was a vehicle for 'austerity' – he said it allowed for substantial spending on social services.[9] So fervently did he feel about this that he began arguing his point before the criticism began. When it did begin, critics pointed out that, 'each of the 250 pages in the Treasury's Budget Review' stressed the need to reduce government spending[10] – that this was indeed an austerity budget. It was, among other features, the first budget in a long time to cut social grants in real terms, reducing the lifeline on which many who live in poverty depend.

What is important for our purposes is why Mr Mboweni felt any need to hotly deny that his was an austerity budget. Many Ministers of Finance in many parts of the world proclaim their budgets to be exercises in austerity, insisting that spending less on social needs will rescue their economies. In those countries – and in this one – austerity budgets are usually greeted with fervent praise from business analysts and the media. Mboweni had been appointed at a time when public spending was, in the view of mainstream economists, close to ruinous and so he had a plausible reason to acknowledge the real nature of his budget. He insisted that it was not an austerity budget because proclaiming that the poor will need to go without is ethically unacceptable to the state and to the governing party. As the example illustrates, this does not necessarily mean that they do give priority to fighting poverty. But they know that they would be flouting the prevailing ethical consensus if they acknowledged that this is not what they were doing.

Mboweni's fealty to an ethic his Treasury did not feel bound by in practice is hardly unique – it is ubiquitous in the public debate. It has become common to insist that any proposal a lobby, political party or commentator dislikes will 'hit the poor hardest' and that their favoured reforms would do the opposite. This makes it inevitable that policies which would benefit people in poverty are denounced as harbingers of greater penury and those which would worsen poverty and inequality are justified as boons to the poor. To quote but one example, one theme in repeated assaults on social grants is that they 'create dependency'. All the evidence points in the opposite direction – grants are, in the main, used to boost local economies and make it easier for people not to be dependent.[11] But the prevailing ethic ensures that insisting that people in poverty should get less to sustain an economy from which others benefit disproportionately is unacceptable. Those who believe that they should pay a price insist, therefore, that they are motivated by concern for the poor – lip service to the ethic justifies policy stances which are likely to deepen poverty and inequality.

The problem is not the absence of an ethic but that its presence enables anyone who seeks respectability to proclaim a concern they do not share. Supposed ethical agreement which obscures damaging difference is not new – it was identified in 1995 by the scholar and activist Harold Wolpe in a critique of the Reconstruction and Development Programme White Paper which was to prove his last published article before his passing.[12] The White Paper, issued by the Government of National Unity which comprised the African National Congress, National Party and Inkatha Freedom Party, contained a preface by then President Mandela asserting that the 'interdependence of reconstruction and development and growth' was now widely accepted by all the country's interests.[12] Wolpe[12] argued that this premature announcement of a consensus hid deep divisions between both the parties in the government and the society's economic actors: '…the tensions cannot be eliminated by fiat of the RDP'.

At the time, Wolpe's critique was confirmed by the fact that just about all social actors who wanted to promote schemes ranging from tougher policing through to personal or company profit insisted solemnly that what they wanted would advance the aims of the RDP. Since then, it has been confirmed by repeated government summits on economics and development which began during the Mbeki presidency in the early 2000s and have been partly revived by the Ramaphosa administration. These events tend to end with interests committing themselves to common goals none have any intention of pursuing. They sign because they do not wish to be seen to be defying the regnant moral consensus.

Neither the RDP White Paper nor the outcome of the summits were explicitly about generating a common ethic. But, like the ethic the authors see in the Constitution, it is 'inherent'. Mandela's formulation claimed consensus on an 'ethic of care' which held that addressing poverty was at least as important as economic growth. The summit agreements required that parties promise to look beyond their own interest and acknowledge those of their 'social partners'. But in both cases, persuading potential opponents to endorse an ethic is the easy part. The more difficult, but much more important, task, is to ensure that they interpret it in a way which reduces poverty and inequality and ensures that care is, as the authors put it, 'corporeal', that it assists real flesh-and-blood human beings, remains elusive.

The most obvious example of the dangers of a proclaimed ethical consensus which hides deep difference is the mainstream debate's treatment of race since 1994. Apartheid was so decisively discredited that no one who seeks even minimal respectability would admit to supporting it or desiring its return. And yet racial attitudes which implicitly or explicitly endorse white supremacy as they proclaim their opposition to apartheid are ubiquitous: measures aimed at undoing the effects of minority rule are thus labelled 'a form of apartheid'.[13] The supposed ethical consensus hides deeply unethical commitments to racial domination.

A similar problem faces the quest for gender equality. It has become equally unacceptable to insist that men are superior to women. But this has not ended male supremacist action and attitudes. The disjuncture has arguably grown. Violence against women, described by the oddly technical term borrowed from international agencies Gender Based Violence, has been the subject of campaigns actively promoted by the current President.[14] Initiatives by men exhorting men to respect women have become fairly common – but not as common as continued violence against women. The professions of equality may be sincere. But they illustrate the limits of a shared ethic which seems unable to change behaviour.

The authors are aware of the problem: they insist that an 'ethic of care' is 'a duty and a set of practices designed to alter, modify or repair dimensions of inequality'. But who decides what practices really do ameliorate inequality? In a constitutional democracy, different interpretations are not only allowed but celebrated. People who interpret the ethic by insisting that an end to social grants and trade union bargaining are essential to the fight against inequality have as much right to be heard as those who believe they would worsen it. In a society in which everyone agrees that care is appropriate but no one agrees on how that care should be expressed, the stress on an ethic simply continues what we have now.

Third, a stress on the need for an ethic can encourage moralising which substitutes for attempts to seek solutions. Violence against women is again an example. Whenever a new example emerges of a woman murdered or maimed, often by an intimate partner, the public debate is awash with people denouncing the act. But these responses have taken on a ritualistic form and do far more to proclaim the ethics of the speaker than they do to protect women. The moralising also rarely acknowledges that men who murder women are unlikely to stop because of public campaigns which stress the ethic of care. More effective than ethical piety would be campaigns for concrete, specified, measures which would reduce violence and support victims. It is open to serious question

whether femicide and male violence directed at women is the product of an ethical failure rather than the same reality which is fuelling white nationalism in the USA and Europe: the belief by dominant groups that their dominance is under threat and that the dominated must be shown who ought really to be in charge.[15] The remedy would then surely be not new ethics but new power relations.

Conclusion: Recognising difference

The notion that South Africa may be redeemed by an ethics of care is more likely to entrench than to threaten poverty and inequality.

A key reason is that, since 1994, a spurious moral consensus has reigned in which stated support for equality often entrenches inequality. If the idea of an 'ethics of care' were to take hold, we can confidently expect it to be embraced loudly by all political parties, most citizens' organisations and much of the media – and by advertising agencies and marketing departments. It would obscure the need for new power relations in a cloud of goodwill.

The road towards a more equitable society lies not in another attempt to unite all behind another stated ethic. It lies, rather, in its polar opposite – the acknowledgment of difference, in ethics and the interests they often express, and a willingness to negotiate these differences, an approach which this author has sought to develop in a recently published book.[16] Negotiation is impossible as long as racists, patriarchs and xenophobes hide behind the rhetorical fig leaf which an 'ethic of care' would provide. Holders of privilege would need to be coaxed out of their current shelter behind an apparently common ethic and pressed to negotiate a path which would acknowledge some of their core interests but would also reduce privilege.

The result would not be a utopia in which everyone subscribed in word and deed to the same ethic of care. Attempts to create this inevitably justify coercion as measures to end poverty and inequality. But it would be a richer, more equal, society precisely because it would have recognised its ethical divides and would have found ways of negotiating them in ways which would allow new voices to be heard and new power to be created.

The way out of the impasse lies not in a new quest for apparent ethical sameness, but in the more rewarding but difficult task of living with difference which does not impose domination.

References

1. Bohler-Muller N, Soudien C, Reddy V, editors. Ethics, politics, inequality: New directions: State of the Nation. Cape Town: HSRC Press; 2021.
2. Bohler-Muller N, Soudien C, Reddy V. An ethico-political approach to poverty and inequality: Embodying care and corporeal citizenship. In: Bohler-Muller N, Soudien C, Reddy V, editors. Ethics, politics, inequality: New directions: State of the Nation. Cape Town: HSRC Press; 2021. p. 1–25.
3. Friedman S. One virus, two countries: What Covid-19 tells us about South Africa. Johannesburg: Wits University Press; 2021. https://doi.org/10.18772/12021117434
4. National Treasury. Economic transformation, inclusive growth, and competitiveness: Towards an Economic Strategy for South Africa [document on the Internet]. c2019 [cited 2021 Dec 01]. Available from: http://www.treasury.gov.za/comm_media/press/2019/Towards%20an%20Economic%20Strategy%20for%20SA.pdf
5. Pithouse R. The service delivery myth. South African Civil Society Information Service. 27 January 2011. Available from: https://sacsis.org.za/site/article/610.1
6. SAPA. Census: More TVs than fridges in SA homes. IOL. 30 October 2012. Available from: https://www.iol.co.za/news/south-africa/census-more-tvs-than-fridges-in-sa-homes-1414075
7. Thabo Mbeki Nelson Mandela Memorial Lecture by President Thabo Mbeki: University of Witwatersrand, July 29, 2006. Available from: http://www.dirco.gov.za/docs/speeches/2006/mbek0729.htm
8. Jessop B. State theory: Putting the capitalist state in its place. State College, PA: Pennsylvania State University Press; 1990.
9. Omarjee L. 'Austerity'? Mboweni slams critics, citing balancing act between grants and fiscal stability. Fin24. 25 February 2021. Available from: https://www.news24.com/fin24/economy/austerity-mboweni-slams-critics-citing-balancing-act-between-grants-and-fiscal-stability-20210225
10. Gqubule D. Mboweni resorts to austerity denialism in the face of overwhelming evidence. Business Day. 22 March 2021. Available from: https://www.businesslive.co.za/bd/opinion/columnists/2021-03-22-duma-gqubule-mboweni-resorts-to-austerity-denialism-in-the-face-of-overwhelming-evidence/
11. Kelly G. We need to change how we think (and talk) about social grants. GroundUp. 7 October 2013. Available from: https://www.groundup.org.za/article/we-need-change-how-we-think-and-talk-about-social-grants/
12. Wolpe H. The uneven transition from apartheid in South Africa. Transformation. 1995;27:88–101.
13. Affirmative action 'a new form of apartheid'. Mail and Guardian. 6 March 2007. Available from: https://mg.co.za/article/2007-03-06-affirmative-action-a-new-form-of-apartheid
14. President Cyril Ramaphosa: Dialogue on Gender-Based Violence and Femicide, 25 November 2020. Available from: https://www.gov.za/speeches/dialogue-mark-16-days-activism-26-nov-2020-0000
15. Peterson R. Understanding ethnic violence: Fear, hatred, resentment in twentieth century Eastern Europe. Cambridge: Cambridge University Press; 2002. https://doi.org/10.1017/CBO9780511840661
16. Friedman S. Prisoners of the past: South African democracy and the legacy of minority rule. Johannesburg: Wits University Press; 2021. https://doi.org/10.18772/12021066840

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Rusty gold in Nigeria: Untapped advances in nanotechnology

Significance:

Reasons for the slow pace of nanotechnology research in Nigeria, Africa's most populous country, are explored, given Nigeria's huge human and natural endowments, and solutions are proffered to address the seemingly lagging outlook of the country in nanotechnology. This Commentary is relevant to all critical stakeholders at national, regional and international levels to mobilise efforts to advance the course of nanotechnology in Nigeria and beyond.

This Commentary was born of the author's experience as a leading researcher in nanobiotechnology in Nigeria, having published more than 70 articles on nanotechnology since 2015, a textbook titled *Microbial Nanobiotechnology: Principles and Applications*[1], and as head of a multidisciplinary nanotechnology research group in Nigeria. The Commentary draws on several types of data on nanotechnology R&D in Nigeria including demographic, economic (GDP and budgetary allocation) and scientometric.

Education and its funding in Nigeria

Nigeria is the most populous African nation, with an estimated population of 206 million in 2020[2] and a growth rate of about 2.6%; its population is expected to surpass that of the USA by 2050. Nigeria also has the largest GDP in Africa, estimated at USD432.294 billion in 2020.[3] As a major oil-producing nation, crude oil and gas exports account for about 10% of Nigeria's GDP, 65% of government revenue and about 88% of the earnings from export activities.[4] These data indicate that Nigeria is not substantially earning from the export of finished goods and is also not a knowledge-based economy. However, the country is endowed with vast natural resources, arable land and a youthful population (more than 62% of Nigerians are under 25 years) that can stimulate industrial growth. Because of the large youthful population, Nigeria is expected to commit huge resources to education, but this is not the case. Nigeria's budget steadily increased from NGN5.07 trillion (USD12.32 billion) in 2015 to NGN13.59 trillion (USD33.02 billion) in 2021, whereas the budgetary allocation to education reduced gradually from 7.38% in 2017 to 5.8% in 2021. Generally, the allocation to education has oscillated between 4.83% and 9.94% since 2010[5] (Figure 1).

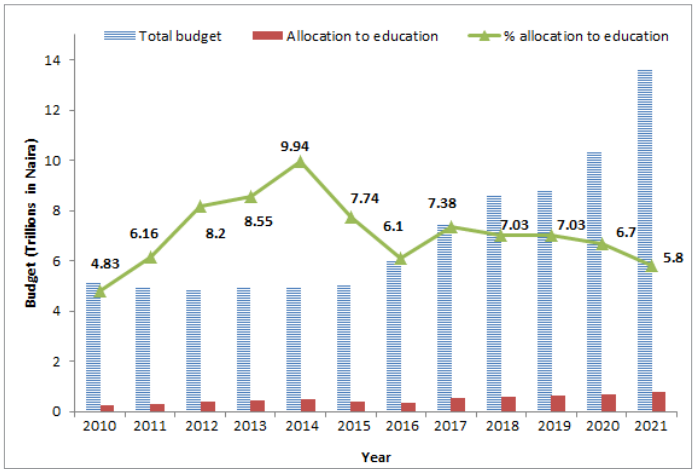

Figure 1: Nigeria's total budget, 2010–2021, and the allocation to education in trillions naira. The line graph and numbers indicate the percentage allocation of the budget to education during the period.

Aside from the federal budgetary allocation, there are state allocations to education, which often follow similar patterns to those of central government. For tertiary education, which covers colleges of education, mono/polytechnics and universities, the Tertiary Education Trust Fund (TETFund), an intervention agency, provides funds for physical infrastructure, acquisition of equipment, and training of personnel at public tertiary institutions in the country. Since 1993, TETFund has independently managed an education tax of 2% of assessable profit imposed on all companies in Nigeria. TETFund has remained the major source of provision of facilities for teaching and research in tertiary institutions in Nigeria in over two decades. However, the impact of its intervention has reduced on a yearly basis as the number of benefitting institutions has grown through their establishment by state and federal governments. For instance, there were only 37 public universities in Nigeria in 1993, but this figure has risen to 101 in 2021.[6] Between 2009 and 2013, TETFund made interventions totalling about NGN2.065 trillion (USD5.018 billion) to public universities in Nigeria.[7]

Development of science and technology policy in Nigeria

Science and technology policy in Nigeria has been re-engineered with the enactment of a science, technology and innovation (ST&I) policy in 2012 geared towards realisation of the full potentials of ST&I for the nation's development.[8] This policy is in addition to the earlier establishment of the National Agency for Science and Engineering Infrastructure (NASENI) in 1992 among several agencies to promote ST&I in Nigeria and to move the nation towards a knowledge-based economy. Nigeria is a major contributor of knowledge in science in Africa as documented in various studies[9-11], but this is yet to materialise in translational output as seen, for example, in South Africa. Both national policy on ST&I and NASENI have identified nanotechnology[12] as a critical input towards realisation of the goals of ST&I. The country set forth to engage in nanotechnology research since 2006 through the National Nanotechnology Initiative programme that was spearheaded by NASENI through organisation of a workshop on the production of nanoparticles, involvement in the activities of the African Materials Research Society and designation of one of its constituent laboratories (the Engineering Materials Development Institute, Akure), as the coordinating centre on nanotechnology research. But how successful has this journey of 15 years of nanotechnology pursuit been? The answer is mixed.

Outlook of nanotechnology research in Nigeria in comparison with South Africa

Some authors have described Nigeria's participation in nanotechnology as dormant – characterised by a non-existent budgetary allocation for nanotechnology and lack of a national policy on nanotechnology.[13] With the lack of advanced equipment to study nanomaterials, the nation's engagement can only be seen in limited research activities by scholars in some universities and occasional organisation of conferences on nanotechnology. Research groups at the University of Nigeria, Nsukka (http://nanotechunn.com/new/) and Ladoke Akintola University of Technology, Ogbomoso (https://lautechnanotech.com/) take the lead, having organised four and five conferences, respectively. Coincidentally, these two universities are the major contributors to knowledge in nanotechnology research among public-funded universities in Nigeria.[14]

Recent data obtained from Scopus shows that Nigeria contributed only 508 articles on nanotechnology in a decade (2010–2019), whereas South Africa and Egypt published 2282 and 4604 articles, respectively, in the same period. Abodunde and Jegede[9] reported a poor outlook for Nigeria in nanotechnology publishing in their report. It is noteworthy that the South African Nanotechnology Strategy commenced in 2006 and the country has made giant strides since then. The Strategy provided a framework for the funding of nanotechnology research and establishment of centres for characterisation of nanomaterials as well human capital development, and it was followed with the 10-year plan on nanoscience and nanotechnology that was released in 2010. These efforts, among others, have propelled South Africa to 23 patents on nanotechnology issued by the US Patent and Trademark Office (2016–2020), development of nanotechnology standards and production of nano-based products.[15] Mintek is a successful nano-based company in South Africa that has produced and marketed gold, nanospheres, and nanorods, including polyethylene glycol coated gold nanoparticles and polyethylene glycol–biotin gold nanoparticles, to the research community and industry.[16]

To date, Nigeria is yet to develop a nanotechnology standard or have a patent on nanotechnology issued by the US Patent and Trademark Office. There is also no national strategy on the exploitation of nanotechnology within the research and development landscape of Nigeria. Thus, Nigerian researchers are limited to synthesis of nanomaterials and composites, along with the evaluation of their basic activities, which have not been transformed into products in the market. In contrast, South African researchers have used nanotechnology to reach milestones in nanomedicine, sensors, magnetism, catalysis, phosphors and optics, electronics, energy, nanobiotechnology, water research and communicable diseases.[16,17] Unlike Nigeria which lacks specific budget for nanotechnology, South Africa invested about USD77.5 million on nanotechnology R&D between 2005 and 2012[18] and additionally committed USD28.6 million to her national nanotechnology equipment programme to support nanotechnology infrastructure as of 2015[19], thereby laying a very solid foundation for the country in the pursuit of nanotechnology enterprise by universities, research councils and even private entities.

Is nanotechnology a worthwhile venture to be pursued by a developing nation?

Nanotechnology is the gold mine of the future as nanotechnology products and services could contribute USD3 trillion by 2030, produce 6 million jobs and a share of more than 10% of the global GDP.[19] Yet Nigeria with her vast human and natural resources is not tapping into this bourgeoning nano-inspired economy – nanotechnology remains a dusty gold in Nigeria at present. Although effort is being made by the Federal Ministry of Science, Technology and Innovation to put in place a national policy on nanotechnology R&D in the country, this policy also is yet to be realised. Therefore, there is no national stimulus for nanotechnology growth and development – be it policy or funding. By comparison, in 2020, TETFund committed about USD29.161 million to establish 12 centres of excellence in fields such as agriculture, food science, ecology, renewable energy, public governance, arid research, medical science and computational intelligence; such commitment has not been extended to nanotechnology, which is a key thematic area in Nigeria's ST&I. Meanwhile, the quartet of nanotechnology, biotechnology, informatics and cognitive science has been recognised as a convergent discipline that will address global challenges and opportunities[20] and drive the Fourth Industrial Revolution and beyond. Thus, it is imperative that Nigeria, as a developing nation, responds to the new ST&I landscape in the world by investing in nanotechnology along with biotechnology and informatics to stimulate convergence science and technology.

Lessons for Nigeria from South Africa and international collaboration to fast-track nanotechnology R&D

The success story of South Africa so far in nanotechnology R&D provides lessons for Nigeria to move up the ladder in nanotechnology. Nigeria was the most collaborating nation in Africa with South Africa on nanotechnology, with these collaborations accounting for 5.2% (585) of the publications of South Africa in the Web of Science during 2000–2019.[17] Several notable researchers in nanotechnology in Nigeria have footprints in South Africa, either through training or collaboration[14]; these experiences can benefit Nigeria in developing its roadmap for nanotechnology. The national policy on nanotechnology that is long overdue must be enacted without further delay, and should address the aggressive and sustainable funding of nanotechnology initiatives in the country through improved budgetary allocation to education, science, technology and innovation. This policy should not be limited to support for nanotechnology research, a nanotechnology equipment programme, human resource development, and establishment of centres of excellence in nanotechnology research and innovation, but also should establish the framework for public engagement and a tripartite partnership of academia with government and the private sector.

The establishment of a centre of excellence in nanotechnology should be based on empirical data on the productivity of scholars and institutions in the field, so that there will be a return on investments made in nanotechnology. This principle was effectively explored by South Africa. Nigeria could also benefit from the advances made by trading partners such as Brazil, China, India, Japan, USA and the European Union through science diplomacy within the channels of bilateral and multilateral cooperation via training of experts and donations of nanotechnology infrastructure. As a leading nation in the South, developed nations, active players in nanotechnology, and development partners should assist Nigeria to bridge another widening gap in nanotechnology pursuits between the North and the South to arrest the widening nanotechnology divide.

At the regional level, the African Union, Economic Community of West African States, and African Development Bank should also set agendas for nanotechnology research among member states for the development of Africa. Similarly, the African Academy of Sciences and other national African academies should set agendas for African nations on nanotechnology by 2025–2030. For instance, the African Materials Research Society in conjunction with the UN Economic Commission for Africa offered a platform on 3–4 May 2021 to discuss 'Nanotechnology for Transformation of African Development: Looking Towards a Sustainable African Future' and seeks to mentor young African scientists in nanotechnology through its nanotechnology research and innovation bootcamp.[21]

Through these interventions, Nigeria can tap into the rapidly developing nano-economy and improve its economic indices through mobility towards a knowledge-based economy. In addition, nanotechnology can be deployed to address a myriad of problems that confront the nation, such as physical and food insecurity, water and sanitation, environmental degradation, inadequate healthcare facilities and an energy crisis. The aforementioned can serve as the focal points of national initiatives in nanotechnology. Thus, nanotechnology becomes a complementary tool to achieving some Sustainable Development Goals (SDGs) in the country; particularly SDG 1 (No poverty), SDG 2 (Zero hunger), SDG 3 (Good health and wellbeing), SDG 6 (Clean water and sanitation), SDG 7 (Affordable and clean energy), and SDG 9 (Industry and innovation). Nigeria cannot afford to further watch the rusty gold of nanotechnology; it must advance in the field of nanotechnology for the good of the country, and the time to act is now.

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Competing interests

I have no competing interests to declare.

References

1. Lateef A, Gueguim-Kana EB, Dasgupta N, Ranjan S. Microbial nanobiotechnology: Principles and applications. Singapore: Springer Nature; 2021. https://doi.org/10.1007/978-981-33-4777-9
2. World Bank. Population, total – Nigeria [webpage on the Internet]. c2020 [cited 2021 Jul 23]. Available from: https://data.worldbank.org/indicator/SP.POP.TOTL?locations=NG
3. World Bank. GDP (current US$) – Nigeria [webpage on the Internet]. c2020 [cited 2021 Jul 23]. Available from: https://data.worldbank.org/indicator/NY.GDP.MKTP.CD?locations=NG
4. KPMG. Nigerian oil and gas update [webpage on the Internet]. c2019 [cited 2021 Jul 23]. Available from: https://home.kpmg/ng/en/home/insights/2019/04/Nigerian-Oil-and-Gas-Update.html
5. Udo ES, Abner IP, Inim V, Akpan EJ. SARS-CoV-2 Pandemic on the Nigerian educational system. Int J Manag. 2020;11:626–635. https://doi.org/10.34218/IJM.11.10.2020.059
6. National Universities Commission [homepage on the Internet]. No date [cited 2021 Jul 23]. Available from: https://www.nuc.edu.ng
7. TETFund. Allocation [webpage on the Internet]. c2021 [cited 2021 Jul 23]. Available from: https://tetfundserver.com/index.php/publications/allocation/
8. Elegbede JA, Lateef A. Green nanotechnology in Nigeria: The research landscape, challenges and prospects. Ann Sci Technol. 2019;4:6–38. https://doi.org/10.2478/ast-2019-0008
9. Abodunde O, Jegede O. R&D Productivity for science, technology and innovation policy development in Nigeria: A scientometric analysis of academic literature. Afr J Sci Technol Innov Dev. 2020;12:787–795. https://doi.org/10.1080/20421338.2020.1718364
10. Lateef A, Ogunkunle ATJ, Adigun GO. Google Scholar citation in retrospect: Visibility and contributions of African scholars. COLLNET J Scientometrics Inf Manag. 2016;10:219–236. http://dx.doi.org/10.1080/09737766.2016.1213966
11. Sooryamoorthy R. The production of science in Africa: An analysis of publications in the science disciplines, 2000–2015. Scientometics. 2018;115:317–349. https://doi.org/10.1007/s11192-018-2675-0
12. National Agency for Science and Engineering Infrastructure. Nanotechnology [webpage on the Internet]. c2019 [cited 2021 Jul 23]. Available from: https://naseni.org/intervention-areas/nanotechnology
13. Ezema IC, Ogbobe PO, Omah AD. Initiatives and strategies for development of nanotechnology in nations: A lesson for Africa and other least developed countries. Nanoscale Res Lett. 2014;9:133. https://doi.org/10.1186/1556-276X-9-133
14. Lateef A, Azeez MA, Suaibu OB, Adigun GO. A decade of nanotechnology research in Nigeria (2010–2020): A scientometric analysis. J Nanopart Res. 2021;23:211. https://doi.org/10.1007/s11051-021-05322-1
15. StatNano. Nanotechnology patents in USPTO (Patent) [webpage on the Internet]. c2021 [cited 2021 Jul 23]. Available from: https://statnano.com/report/s103
16. Dube A, Ebrahim N. The nanomedicine landscape of South Africa. Nanotechnol Rev. 2017;6:339–344. https://doi.org/10.1515/ntrev-2016-0108
17. Masara B, Van der Poll JA, Maaza M. A nanotechnology-foresight perspective of South Africa. J Nanoparticle Res. 2021;23:92. https://doi.org/10.1007/s11051-021-05193-6
18. Molapisi JJ. Nanotechnology development in South Africa. In: International Symposium on Assessing the Economic Impact of Nanotechnology; 2012 March 27–28; Washington DC, USA. Available from: https://www.nano.gov/sites/default/files/molapisi.pdf
19. Roco MC. Overview: Affirmation of nanotechnology between 2000 and 2030. In: Mensah TO, Wang B, Bothun G, Winter J, Davis V, editors. Nanotechnology commercialization: Manufacturing processes and products. Hoboken, NJ: John Wiley & Sons; 2017. p. 1–23. https://doi.org/10.1002/9781119371762.ch1
20. Roco MC. Principles of convergence in nature and society and their application: from nanoscale, digits, and logic steps to global progress. J Nanoparticle Res. 2020;22:321. https://doi.org/10.1007/s11051-020-05032-0
21. African Materials Research Society. Events [webpage on the Internet]. No date [cited 2021 Jul 28]. Available from: https://africanmrs.net/events/

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Research contract relationship between a large industry partner and South African universities

We propose and evaluate a contractual structuring instrument (in the form of a Framework Research Agreement) in support of research collaboration partnerships between universities and large industry, specifically for the case of large industry in the South African context. This study includes one large South African originated industry (as the pioneer of concluding Framework Research Agreements with several universities) and multiple South African universities, and stretches over several decades. This study was done within the broader context of the challenges and benefits experienced by both industry and university in the academic engagement sphere of industry–university collaboration. By providing insight from both industry and university perspectives, factors impacting on academic engagement (with specific emphasis on the legislative framework, contractual aspects, institutional research contract practices and institutional risks), are considered and discussed. Recommendations are made for improved industry–university collaboration by sharing experiences from the industry and universities on challenges faced, managing the expectations and proposing mechanisms to support constructive research collaboration through a mutually beneficial contractual framework instrument.

Significance:

- A novel Framework Research Agreement as a contractual instrument was developed and pioneered by the industry partner with several South African universities, to create a transparent framework based on fair contracting and determinable remuneration principles.
- This study specifically highlights the need for a contractual instrument, in which the intention is to build a long-term contractual relationship to support industry–university collaboration and academic engagement within the existing South African legislative framework.
- It further draws attention to research contract management practices and contractual aspects, which until now have been largely ignored in industry–university collaboration and academic engagement frameworks of this kind.

Introduction

Industry–university collaboration and academic engagement

It is widely acknowledged that industry–university collaboration (IUC) is to the benefit of both industry and universities, but also poses substantial challenges.[1-4] When considering IUC, available studies highlight the characteristics and motivations for industry and university, with foci ranging from gender, seniority, and scientific fields to geographical locations and funding.[5] Several frameworks to improve IUC have been proposed with particular attention to the consideration of the institutional context and national policy to support commercialisation of intellectual property (IP) and the academic engagement aspects.[6]

Most recently, Awasthy et al.[7] proposed an improved IUC framework which focuses on aspects such as different views, perspectives, motivations, and needs, held by universities and industry, the need to understand the various forms of interactions, and the importance of effective collaborations. In addition, they note that an efficient communication strategy between all stakeholders and leadership must be in place, and that the nature of the collaboration will depend on the creation and establishment of basic partnership characteristics, including management and encouragement of the collaboration, and adopting a joint strategy. Another relevant aspect is for IP concerns to be addressed appropriately.[7]

For this specific study we focus on academic engagement, and further distinguish between academic engagement and commercialisation.

Academic engagement is defined by Perkmann et al.[6] as 'knowledge related collaboration by academic researchers with non-academic organisations [and] include formal activities such as collaborative research, contract research, and consulting, [as well as] informal activities like providing ad hoc advice and networking…'. In addition, they noted that academic engagement involves more than financial benefits, and often includes in-kind benefits for research such as access to equipment, data/material, or input into ideas. Apart from serving academic goals, such as generating publications, it also serves the non-academic partners (such as industry) by offering expertise to create new ideas, solve problems, and provide input on novel application of the industry's expertise.[6]

On the other hand, commercialisation involves 'the patenting and licensing of inventions as well as academic entrepreneurship'[6]. Commercialisation within the context of this study is aligned with the activities usually undertaken by Technology Transfer Offices which focus on the '…generation of patents and the creation of spin-off firms stemming from research projects'[4]. Whether intentional or not, academic engagement can lead to commercialisation.[6]

It is acknowledged that universities' income derived via academic engagement is significantly higher (in high multiple) than income derived through commercialisation. Despite this, less attention is paid to the 'underpinning of academic engagement' theory, as well as to 'the role of the institutional environment and national policies' in contrast to commercialisation.[6] It is notable that academic engagement is considered by many companies to be significantly more valuable than commercialisation.[6] It is further noted that to use commercialisation (patents and spin-off firms) as indicators of collaborative research at universities, largely ignores the economic and productive context within which universities operate and 'may be detrimental to the strengthening of emerging trends that are oriented towards softer collaborative experiences and other forms of knowledge transfer'[4].

Further to the above-mentioned shortcomings in existing literature, there is very limited, if any, literature that guides industry or universities, in the contractual structuring of an appropriate contractual instrument, as well as very limited guidance on improving research contract management practices at universities within the context of academic engagement and IUC.

We therefore specifically argue the need for a contractual instrument, in which the intention is to build a long-term contractual relationship to support IUC and academic engagement within the existing South African legislative framework. It further draws attention to research contract management practices and contractual aspects, which until now have been largely ignored in IUC and academic engagement frameworks of this kind.

In this article we bring a better understanding to the external and internal landscape in the South African context that will impact the contractual structuring, in the hope that it will strengthen IUC through mutually beneficial academic engagement.

The South African context

Globally, national policies tend to inform IUC significantly (for example, one of the most recent global governmental initiatives was the creation of the European Innovation Council in 2021 which was designed to support high potential and breakthrough technologies as a result of IUC).[5] However, apart from studies on the role of national policies supporting academic engagement in countries such as the United States of America and European countries, information on other geographical areas is limited.[6] Although this article is intended to discuss the contractual relationship in terms of academic engagement, brief reference to some of the South African national policies is made here to provide context.

The importance of IUC is underlined in the White Paper on Science, Technology, and Innovation, March 2019[8], and the National Development Plan[9]. In addition, in 2018, the Human Resource Development Council undertook a workshop on behalf of the South African Department of Higher Education and Training, with stakeholders from universities, government, and industry, where partnerships amongst these organisations were examined.[10] From these partnerships, knowledge sharing, technology transfer and commercialisation of research, analysing and influencing government policies and legislative frameworks, among others, are listed as success factors emanating from IUC. The lack of funding for research and the mismatch in expectations of research output were given as challenges for IUC.

There are South African government initiatives to support and encourage IUC, some of which are briefly mentioned in this article. If the deliberate strategic focus from an industry partner is to support research with the 'intent to derive income', it may qualify for the South African Research and Development (R&D) Tax incentive.[11] With recent changes in the Skills Development Element (Code 300) of the BEE Codes of Good Practice (published on 31 May 2019 and effective as of 1 December 2019), industry has an opportunity to claim BEE points on its score card by funding black students at universities.[12] Another incentive programme from the national Department of Trade, Industry and Competition, is the Technology for Human Resources and Industry Programme (THRIP), which intends to boost industry through support of R&D and skills development by incentivising industry to work closer with universities and science councils.[13]

The above initiatives and regulatory/policy documents, support the R&D landscape and underpin the importance of partnerships, with IUC as one form. However, the lack of funding will remain a challenge, as is evident from the most recent South African National Survey of Research and Experimental Development (R&D) (2017/2018).[14] South Africa's gross domestic expenditure on research and experimental development (GERD) amounted to ZAR38.725 billion in 2017/2018, an 8.5% nominal increase from the previous financial year. The two major contributors of funding towards R&D activities were government (46.7% of total investment) and the industry sector (41.5%). However, a steady decline in funding from industry, from 57% in 2006/2007, was also reported.[14]

From an IUC perspective, the most prevalent legislation to consider is South Africa's *Intellectual Property Rights from Publicly Financed Research and Development Act 51 of 2008* (the IPR Act)[15], which came into effect in August 2010.

For this study we focused on a large South African originated company with an extensive international footprint in over 30 countries in the mining, exploration and production of oil and gas, and a focus on feedstock supply activities.[16] As this company has a range of formalised agreements with several South African universities, the scope covers perspectives from industry and universities.

Research methodology

Figure 1 illustrates the research methodology used for the purpose of this study, and each step is further described below.

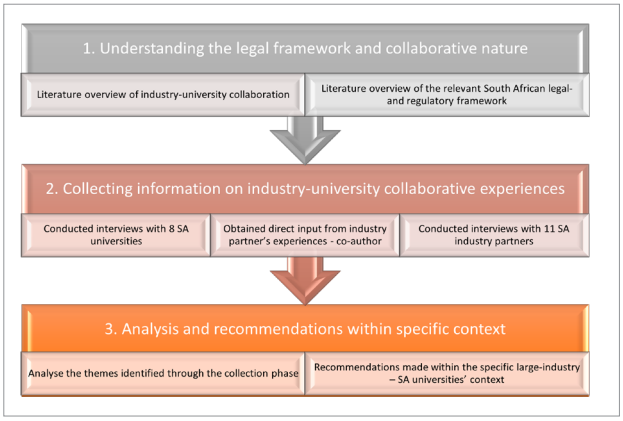

Figure 1: Research methodology undertaken in this study.

Understanding the legal framework and collaborative nature

A literature study on IUC, academic engagement as well as the legislative and regulatory frameworks relevant to IUC in South Africa, were used to identify themes for further analysis and provided the context of this study as explained in the introduction. As industry and universities are subject to several legislative frameworks, specific consideration was given to those that had a direct bearing on structuring research partnerships.

Collecting information on IUC experiences

Ethics approval (#14565) for this study was obtained from Stellenbosch University's Research Ethics Committee for Social, Behavioural and Education. Informed consent was obtained from the participants and the Principles of the Declaration of Helsinki were adhered to. The interview data are confidential and are therefore not available. The interview questionnaires are provided in the supplementary material.

Interviews were conducted with eight South African universities (from April 2020 to May 2021) that included high, medium and low research-intensive universities. The most pressing themes and findings have been described in detail for seven universities.[17] Those themes most

prevalent to this study were further explored and discussed and an eighth university was included to broaden the study. The findings were confirmed by this additional university, and therefore should be read as the collective views from eight universities.

South African universities were classified as high, medium, or low research-intensive universities as presented in Figure 2.

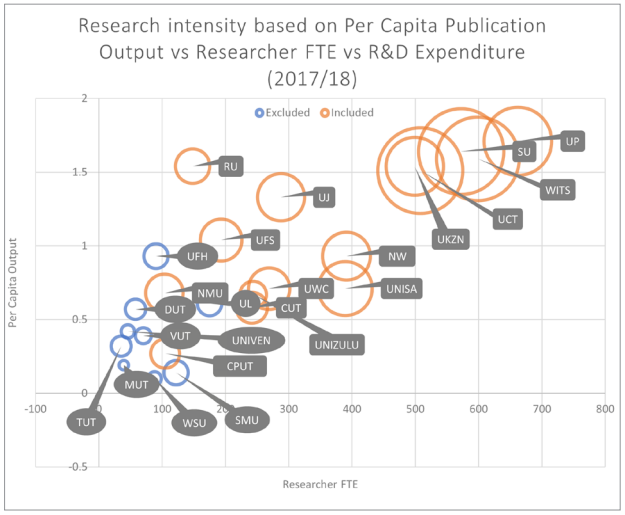

Figure 2: Classification of South African universities according to research intensity, with the highest research-intensive universities in the top right quadrant and the lowest in the bottom left quadrant. The point of the arrow meets the centre of the bubble for each university. Universities represented by blue bubbles were excluded from the sample.

The graph in Figure 2 is based on the South African National Survey of Research and Experimental Development (R&D) 2017/2018[14(p.82)], where the R&D expenditure per university (size of the bubble) and the full-time equivalent (FTE) per researcher (*x*-axis) were plotted against the per capita research publication output (*y*-axis), in line with the Report on the Evaluation of the 2018 Universities' Research Output[18]. The universities in the top right quadrant are the highest research-intensive universities, and those in the bottom left quadrant are the least research-intensive universities, based on the chosen criteria. The nine universities excluded from the sample group had a reported R&D income of less than ZAR200 000 for the 2017/2018 year, and are marked in blue. The universities that participated in this study were spread across the range of universities represented in the graph in orange. Interviews were conducted with senior management responsible for management of all research-related contracts at 53% of these universities marked in orange, to ensure that a broad perspective, across all levels of research intensity, was presented.

Internal views from stakeholders within the industry partner (represented by one of the co-authors, C.J.H.N., with input from senior management within the company, and permission from executive management), formed an integral part of this study, as this industry partner has current formalised partnerships with nine South African universities. This provides a valuable perspective of challenges in establishing collaborations within the complexity of the national legislative framework.

Interviews with executives from 11 other South African industry partners, with an interest in IUC, were conducted during the period April–June 2021, to obtain a more comprehensive perspective from them to corraborate the views of this specific large industry partner.

Analysis and recommendations within specific context

Themes for IUC, with specific reference to the contractual aspects with the intent on long-term research collaboration, were identified, analysed and discussed. Recommendations are made to enable and improve IUC.

Understanding the legislative framework leading to the Framework Research Agreement

Legislative framework

Industry and universities are required to operate in a complex legislative and governance framework. From an IUC perspective, the most prevalent legislation to take into consideration is the IPR Act.[15] When the IPR Act commenced in August 2010, from both our experience and corroboration through interviews, both industry and universities found it challenging to fully understand the impact thereof on research contract negotiations, and a significant mind-shift was required by all parties to redesign collaborative efforts that had already been ongoing for at least three decades.

The purpose of the IPR Act is

> *...to provide for more effective utilisation of intellectual property emanating from publicly financed research and development; to establish the National Intellectual Property Management Office (NIPMO) and the Intellectual Property Fund; to provide for the establishment of offices of technology transfer at institutions; and to provide for matters connected therewith.*[19]

NIPMO is mandated under the legislative framework, to ensure that

> *intellectual property from publicly financed research and development is identified, protected, utilised and commercialised for the benefit of the people of the Republic, whether it be for social, economic, military or any other benefit.*[19]

Once a research project is fully funded (on 'full cost') by an industry partner, the IPR Act does not apply, and the parties are free to negotiate the terms of the IP transaction. However, if the full cost principle is not applied, the transaction will be subject to the IPR Act as it is deemed to be subsidised by public funding. The IPR Act explains what is deemed as full cost which is based on the General Acceptable Accounting Principles.[15]

Since the commencement of the IPR Act, both industry and universities have been faced with uncertainties in the interpretation of the IPR Act which impacted contractual negotiations.[20] However, NIPMO was very supportive in addressing the challenges in application of the IPR Act. Several of these challenges were addressed over years, such as clarification on the definition of R&D, calculation of full cost, and granting of licences when research was not funded on a full-cost basis.[19]

Universities' perspectives

Costing and pricing practices

From our experience and as evidenced from the interviews, an immediate challenge faced by universities was to address the costing and pricing practices. A legislative requirement to agree to specific IP transactions (such as licensing of IP to industry, or assignment of IP ownership to industry), required that universities had to ensure that all direct and indirect costs were fully paid by industry. Calculating the direct cost of projects was less challenging than determining the indirect cost. South African universities cooperated over several years to define a generally accepted methodology in determining indirect cost, as approved by NIPMO.[21]

Specific requirements in terms of costing and pricing practices must be established and implemented at universities for compliance purposes. During interviews with the eight participating universities, it became clear that the high research-intensive universities already had established costing and pricing practices (including an indirect cost recovery policy) prior to the commencement of the IPR Act in 2010, and the challenge for these universities was mostly amending the calculation methodology and implementation of indirect cost policies for IPR Act compliance. For the lower to medium research-intensive universities, no established

or informal costing and pricing practices were in place prior to the IPR Act, and no indirect cost recovery was applied, which caused far more challenges for these universities to ensure IPR Act compliance.[17]

Dedicated research contracts function

It was evident from interviews that high research-intensive universities had dedicated research contracts functions/offices and technology transfer expertise available (and the appropriate mandates) to review, negotiate and manage research contracts. Consequently, these universities had a more accurate insight into all research contracts, which enhanced the ability to understand, assess and mitigate potential risks associated with the research contracts portfolio.[17]

In contrast, the lower research-intensive universities had no dedicated legal function to review research contracts, and this function was either managed by the central legal services or external law firms. These universities established Technology Transfer Offices (TTOs) in response to the IPR Act, but with their mandates limited to verification of IP clauses for IPR Act compliance. These universities were frustrated to manage research contracts in this manner, arguing that, the said central or external legal services functions had limited understanding of the research conducted or the relevant legislative, regulatory, ethical, post-contractual, and funder compliance aspects related to the research; insufficient involvement of research directorates in research contract management; the TTO's mandates were not conducive to improving research contract management, as their limited roles to verify IP clauses against IPR Act compliance excluded full contextual review of the research contract transaction; there was unavailability of accurate insight into the whole research contract portfolio, with the said legal functions only capturing information important for work-flow management, and not for appropriate research contract management; and there was a lack of costing and pricing practices, including severe problems in implementing full-cost and indirect cost policies, thus entailing a risk of non-compliance of the IPR Act.[17]

In fact, some of the medium research-intensive universities were moving towards establishing a dedicated research contracts function/office and TTO (or a combination) with appropriate mandates to support the research contract management functions. Like the lower research-intensive universities, TTOs were established in response to the IPR Act. These universities were making good strides towards improved insight into research contracts which would assist in improved risk management. Some medium research-intensive universities were still struggling with these aspects and faced similar challenges. These universities were all experiencing challenges in establishing costing and pricing practices and only research contracts subject to full cost in terms of the IPR Act were tested and costed on full cost.[17]

For some universities (especially lower and medium research-intensive universities), it was more challenging to negotiate research collaboration agreements with industry partners, and appropriately manage them through the contract lifetime, due to a lack of dedicated research contract functions and appropriate mandates, accurate insight into the research contract portfolio as well as the lack in established costing and pricing practices (especially for IPR Act compliance purposes).

Since commencement of the IPR Act, universities had been experiencing pressure to become more focused on IP commercialisation. However, valuation of IP created as such remained a challenge, especially where it formed part of a larger technology pipeline and core technology of an industry. Universities might also experience push-back from the industry when the value of the IP potentially emanating from the research, is under discussion at the start of a project. The broader context of what the industry offers to universities in terms of funding the research, as well as providing valuable confidential and sensitive information as background IP to universities in conducting research, must be duly considered.

Industry partner perspective

Considerations and negotiations

Contract research collaboration at universities is one of various options available when outsourcing research projects. Factors taken into consideration when allocating projects to universities include expert knowledge, cost, technology reward and ease of securing IP exclusivity. Although IP exclusivity (in the context of IUC) has been impacted by the IPR Act, this has not deterred the industry partner from collaborating with universities and allocating research projects.

The industry partner firstly formalised a research policy aimed at establishing IP principles that would be fair and reasonable to universities and, based on this policy, commenced negotiations with universities with which it had collaborated in the past (as these universities had the scientific expertise, as required), i.e. primarily high and medium research-intensive universities.

Negotiations on behalf of the universities were led by a few medium and high research-intensive universities. The other universities eventually accepted similar terms, albeit with a few differences depending on each university's research policy. As negotiations commenced prior to the implementation of the IPR Act, the primary challenge was to establish fair principles that would be consistent with the IPR Act, once effective. Evident throughout this process was the limited number of dedicated legal resources (also at some high research-intensive universities) that could provide meaningful contributions during negotiations.

Framework Research Agreements

This resulted in the conclusion of the Framework Research Agreements (FRAs) from 2008 onwards, within which sub-agreements for specific projects could be negotiated with minimal effort. FRAs provided a mechanism to pursue projects within the scope of and subject to the IPR Act, together with other benefits such as uniformity of agreements and establishment of fair contractual principles. Previously, separate agreements were negotiated for each research project, which was a cumbersome process.

Upon allocation of a research project, the IP terms are classified by the industry partner as either low, medium, or high risk. The risk categories are based upon the risk to the industry partner that its competitive advantage may be compromised if any foreground IP, as may be generated as a result of a project, is owned, used or commercially exploited by any entity other than the industry partner. If, for example, the foreground IP relates to or falls within the industry partner's core technology area, the project will be classified as high risk. In some instances, FRA do not provide for a high and/or medium risk allocation.

For low-risk projects, the university owns foreground IP generated, together with exploiting rights, including granting of non-exclusive licences for exploitation to third parties. The industry partner has a right to acquire a non-exclusive, royalty-bearing licence for commercial exploitation, negotiated on reasonable terms.

For high-risk projects, the industry partner owns the foreground IP against payment of a fee determined on a full-cost basis. The industry partner has the right to exploit the IP and obtain registered protection therefor. The university retains the right to use the IP for further research purposes. Reference is also made to serendipitous IP, i.e. other IP generated under the sub-agreement that falls outside the parties' collaboration field under the agreement, but within the scope of the industry partner's key operational activities. In these instances, the industry partner retains the right to exploit the IP and obtain registered protection. Apart from the right to use the IP for further research purposes, the university also has the right to a sole licence to commercially exploit and sub-licence the IP in applications outside the industry partner's key operational activities. In the event that serendipitous IP is commercialised by the industry partner, it will make an additional payment to the university, the amount of which will be negotiated by the parties on reasonable terms, but to a maximum limit.

In practice, some of these principles (e.g. mechanisms for handling serendipitous IP) are seldom applied. The benefit of FRAs is, however, that all foreseeable IP scenarios are covered irrespective of the nature of any sub-agreement, for beneficial application if foreground IP is generated.

Projects allocated to South African universities are generally classified as either low or high risk, but in most instances are low risk. Consequently, foreground IP generated under a project is generally university owned. While these projects involve the disclosure of confidential information of the industry partner to universities, such disclosures are carefully managed, as there are risks associated with disclosures – specifically to students for purposes of dissertations, theses and conference proceedings. Projects that are classified as high risk and which may entail disclosure of strategic information to the university are the exception to the norm and occur only where IP exclusivity is important to the industry partner, and a university has proven expertise in a certain field. The industry partner and the universities agreed not to build in a co-ownership option (as provided for by the IPR Act, under certain conditions) into FRAs, due to the complexities of managing jointly owned IP and potentially having to negotiate commercialisation or licence rights at a later stage, but rather to allocate IP ownership to one of the contracting parties on commencement of projects and completion of all payment obligations.

While the industry partner's strategic partnerships on important research projects with specific high and medium research-intensive universities have continued, the focus was also on building of capacity at universities, to maintain high academic standards and delivery of graduates for future employment. This explains why most projects allocated were classified as low risk, with limited emphasis on foreground IP ownership. Collaboration with lower research-intensive universities has been limited and the Open Call for Sasol Research Grant Proposals is an initiative to create a mechanism to enable the industry partner to collaborate with some of these universities.[22]

Balancing needs and expectations

For meaningful IUC, both parties' needs and expectations should be considered and balanced, and reasonable arrangements should follow.

The industry partner understands that a specific research or technological innovation or outcome cannot always be guaranteed due to the nature of experimental academic research where postgraduate students are involved. However, the benefits of social investment in postgraduate students and access to the research results, pose sufficient benefits to furthering this collaboration.

The opportunity remains for the industry partner to contract a specific university with the needed expertise on high-risk research projects where the outcomes of the research are related to strategic purposes or core technology. However, commercialisation and implementation of new innovations come with high risk and high costs for industry, and it remains a challenge for industry if some universities overvalue their innovation contribution to a commercial application. For this reason, it was important for the industry partner to negotiate limits to fees payable to universities in the case of high-risk projects as described above, even in the event in which serendipitous IP is generated by the university.

In the industry partner's experience, universities are still inexperienced when it comes to commercialisation of innovation and underestimate the risks and cost of implementation of new innovations as commercial solutions. This results in universities overestimating the value of the front-end innovation, which can complicate IP negotiations. It is notable that the 11 other industry partners interviewed shared this concern in terms of universities' overestimation of IP value and technology readiness levels.

On the other hand, universities need funding for postgraduate research projects and the opportunity to conduct applied research on a specific industry problem to enable a context to work on alternative solutions. Building of prototypes for proof of concept is a very expensive component of experimental research and generally supported by industry funds. Knowledge sharing between academics and industry R&D professionals benefits IUC, ensuring that postgraduate students gain valuable experience and are better equipped for industry once they have completed their studies. Funding is generally utilised to support students through bursaries, purchase equipment, obtain access to expensive industry standard software, present results at conferences, and fund postdoctoral fellows, to strengthen the research outputs of the university.

Universities and industry in general experience a disconnect in expectations when an industry partner requires a specific innovation or technological solution to a problem, whilst universities focus on the academic research which might, or might not, lead to a solution to the problem. However, it is notable that this specific industry partner embraces the importance of supporting universities to 'enhance world class research, teaching and innovation at universities …. where the industry partner has an operational footprint'[22]. This is an important element for building truly collaborative partnerships.

Specific challenges faced through IUC

This section gives attention to some challenges faced through IUC which are more project specific and should be appropriately considered at the project development stage to mitigate risk.

Compliance to contractual obligations

As determined through the interviews, the lower and medium research-intensive universities (except for one), have no accurate overview of research contracts conducted by researchers, and therefore also have no ability to ensure that researchers keep to the contractual deliverables as specified in the contracts. This poses a risk of contractual breach for universities.[17]

In a university context, critical to the success of implementing a FRA with sub-agreements, is a dedicated research contract function with an accurate overview of all contracts, to ensure that researchers are appropriately guided on the contractual terms applicable to their projects, which include aspects such as publication rights, IP management, confidential information management, adherence to deliverable schedules, and invoicing. Without a dedicated research contract function to filter out and communicate the contractual obligations for the researchers, the risk for contractual breach is much higher.

The industry partner follows a similar approach, with the roles fulfilled by its Technology Contracting department, a centre of expertise within its legal function, and the company's Research and Technology function. The latter function is responsible for the disclosure of confidential information to universities, management of the use of such information for dissertations and publications, recording of IP or foreground IP generated, and approval of funds payable as per the project schedule. While this arrangement generally works well, more controls are required with regard to the disclosure of information to universities.

Confidential information

Disclosing information

In the context of this specific scope, the norm is that in most instances confidential information is shared by the industry partner with the university; however, the contractual clauses are reciprocal. Sharing of confidential information has the potential to cause significant risks for both parties if the practical implications of the contractual requirements are not appropriately considered and addressed.

When entering into an agreement, the legal entities (namely the industry partner and the university) will be liable in the event of any breach. Within any large organisation, ensuring that employees who receive such confidential information understand the impact of non-adherence to the confidentiality obligations, poses significant risks. In the university context, the additional risk is that students will receive confidential industry information to utilise as part of their postgraduate research projects. Students are enrolled at universities and are not university employees, and therefore act in their own capacity but also under the

auspices of the university. The university should ensure that appropriate mechanisms for the treatment of confidential information, including aspects such as information security and obtaining approval from the industry partner for use of confidential information in publications, are in place and are communicated to researchers and students. This requirement also applies to external examiners. For this purpose, in addition to the normal confidentiality undertakings between the contracting parties, individual undertakings of confidentiality should be obtained from individual recipients of confidential information.

Confidentiality periods

Due to the challenges experienced by universities to police their confidentiality obligations for excessive periods, a practical solution to overcome this issue was negotiated under the FRA. The term of the recipient's confidentiality obligations will depend on the nature of confidential information disclosed, e.g. in the case of low-risk projects, a confidentiality term of 2–10 years may apply, whereas in the case of a high-risk project, a term of 15 years generally applies.

Consequently, information to be disclosed under a low-risk project may in certain instances be of limited value to the university but will generally enable a university to use such information for purposes of dissertations and publications. In this regard, confidential information is classified as either disclosed information or derived information. The industry partner requires that dissertations and publications be verified by the university and industry partner to ensure that no disclosed information is included. Derived information is treated as foreground IP, which may be generated by either of the parties. Such information may be included in the dissertation or publication, provided that where industry partner derived information is included, the dissertation or publication will be delayed for a certain period, allowing the industry partner to file a patent application.

Main findings and recommendations

Main findings

The IPR Act undoubtedly had an impact on the contractual considerations for IUC where academic engagement is concerned.

Some universities are facing more severe challenges to support academic engagement through contracting processes, due to institutional aspects that need improvement (such as dedicated research contract functions, appropriate mandates, an oversight of all research-related contracts, and improved costing and pricing practices).

By understanding the needs, motivations and expectations of both industry and university, academic engagement can be supported by applying fair and transparent contracting principles within an IUC framework. An effective framework is dependent on the negotiation of contractual terms governing future collaboration, which take cognisance of all foreseeable IP scenarios that may occur.

FRAs between the industry partner and the respective universities resulted from joint negotiations with various universities. This not only ensured that similar principles could be negotiated with all participating universities but is an example of how innovative principles can be developed by sharing best practices. Going forward it would be in the interest of universities to share best contracting practices with each other from time to time.

The development and implementation of an FRA to support a long-term IUC through academic engagement is a powerful instrument in contractual structuring.

Recommendations

A FRA which makes provision for various risk levels of projects according to the IP ownership and exploitation options, as well as confidentiality terms associated with these risk levels, is recommended when a long-term IUC is envisioned. Negotiations for a FRA may, however, take substantially longer than those for separate research projects, as all possible scenarios and risks must be carefully considered within the legislative framework, as well as the research strategies and policies of both parties. On the other hand, any requirement to negotiate a research agreement for a specific project for which there is no governing FRA is also challenging. Within this context, and within the experience of both industry and universities, formalising a FRA to govern long-term relationships is indeed contributing to a more effective IUC framework.

It should be ensured that negotiations are not conducted by the legal teams without appropriate consultation with researchers from the respective parties. During FRA negotiations, the industry partner occasionally experienced misplaced distrust from the negotiation teams of a few universities mainly due to differences in IP philosophies. This has been addressed by means of constructive and continued dialogue to ensure that research endeavours are not negatively affected.

A remaining cause for concern is the ability of universities to have an accurate overview of research sub-agreements and ensure that contractual obligations, especially in terms of IP and confidentiality management, are communicated to researchers and students and formally addressed. Appropriate costing and pricing practices at universities are critical to ensure that the IP transactions are compliant in terms of the IPR Act. We made specific recommendations to universities for improvement in this regard.[17]

A user-friendly risk assessment document should be provided to researchers by the research contracts office and should highlight the most pertinent contractual obligations to which researchers and students must adhere (i.e. publication requirements, disclosure of creation of new IP, treatment of confidential information, etc.). Due consideration must be given to signing of confidentiality agreements between the university and the students, external collaborators, and examiners. The practical aspect to safeguarding of confidential information is also essential.

Several industry partners (national and international) and universities have based IUC agreements on the principles of this FRA, which could therefore be considered as pioneering for IUC frameworks in South Africa.

In summary, Figure 3 presents an example of a flow chart for consideration, which may assist with the contractual structuring of a formal IUC research agreement.

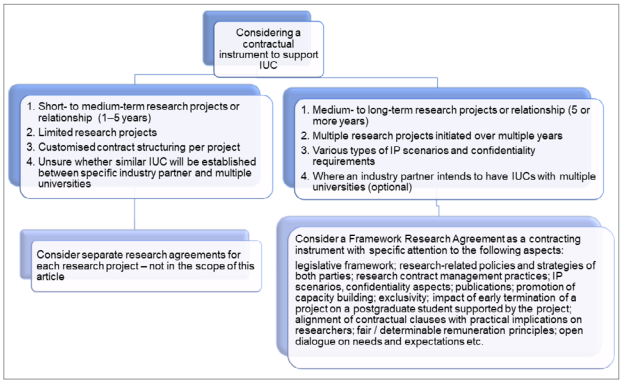

Figure 3: Aspects to consider when structuring an industry–university collaboration (IUC) research agreement.

Further work is needed on rethinking the current national drive towards commercialisation where significant funding is channeled to TTOs via NIPMO, whilst very limited, if any, government support is provided to universities to establish or grow a research contracts function to support academic engagement, even though it has been shown that industry deems academic engagement to be significantly more valuable than commercialisation activities.

Competing interests

We have no competing interests to declare.

Authors' contributions

C.M.: Conceptualisation; methodology; data collection; data analysis; validation and data curation; writing of the initial draft without industry

context; writing revisions; project leadership and management. C.J.H.N.: Conceptualisation; methodology; industry data collection and analysis; industry consultation and approval of the article; writing the industry context in the initial draft; writing revisions. C.S.L.S.: Student supervision; provided guidance on the design of the methodology; data collection (interviews with universities and the 11 industry partners and the literature study); data analysis; validation; writing – provided input through several rounds of revisions.

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References

1. Scandura A. University-industry collaboration and firms' R&D effort. Res Policy. 2016;45:1907–1922. https://doi.org/10.1016/j.respol.2016.06.009
2. Wright M, Clarysse B, Lockett A, Knockaert M. Mid-range universities' linkages with industry: Knowledge types and the role of intermediaries. Res Policy. 2008;37(8):1205–1223. https://doi.org/10.1016/j.respol.2008.04.021
3. Slotte V, Tynjälä P. Industry-university collaboration for continuing professional development. J Educ Work. 2003;16(4):445–464. https://doi.org/10.1080/13639080320000930 58
4. Ramos-Vielba I, Fernández-Esquinas M. Beneath the tip of the iceberg: Exploring the multiple forms of university-industry linkages. High Educ. 2012;64(2):237–265. https://doi.org/10.1007/s10734-011-9491-2
5. Perkmann M, Salandra R, Tartari V, McKelvey M, Hughes A. Academic engagement: A review of the literature 2011-2019. Res Policy. 2021;50(1), Art. #101114. https://doi.org/10.1016/j.respol.2020.104114
6. Perkmann M, Tartari V, McKelvey M, Autio E, Broström A, D'Este P, et al. Academic engagement and commercialisation: A review of the literature on university-industry relations. Res Policy. 2013;42(2):423–442. https://doi.org/10.1016/j.respol.2012.09.007
7. Awasthy R, Flint S, Sankarnarayana R, Jones RL. A framework to improve university-industry collaboration. J Ind Univ Collab. 2020;2(1):49–62. https://doi.org/10.1108/JIUC-09-2019-0016
8. South African Department of Science and Technology (DST). White Paper on Science Technology and Innovation. Pretoria: DST; 2019.
9. South African Department of Science and Technology (DST). National Development Plan 2030: Our future – make it work. Pretoria: DST; 2012.
10. Human Resource Development Council of South Africa. Research on the nature and extent of Post-School Education Institutions and Industry Partnerships. Pretoria: Human Resource Development Council of South Africa; 2018.
11. South African Revenue Service (SARS). Research and development (R&D) incentive [webpage on the Internet]. c2006 [cited 2021 Oct 26]. Available from: https://www.sars.gov.za/ClientSegments/Businesses/My-Bus-and-Tax/Pages/Research-and-Development-Incentive.aspx
12. Wilkes T. New BEE amendments allow you to recognise bursaries for your BEE Scorecard [webpage on the Internet]. c2019 [2020 Nov 14]. Available from: https://www.tathamwilkes.co.za/NewsResources/NewsArticle.aspx?ArticleID=2998
13. South African Department of Trade, Industry and Competition. Innovation and Technology Funding instruments: The Technology and Human Resources for Industry Programme (THRIP) [webpage on the Internet]. No date [cited 2021 Oct 24]. Available from: http://www.thedtic.gov.za/financial-and-non-financial-support/incentives/thrip/
14. South African Department of Science and Technology (DST). South African National Survey of Research and Experimental Development. Statistical report 2017/18. Pretoria: DST; 2019.
15. Intellectual Property Rights from Publicly Financed Research and Development Act 51 of 2008, South Africa.
16. Sasol. Sasol company profile – overview [webpage on the Internet]. No date [cited 2021 Oct 26]. Available from: https://www.sasol.com/about-sasol/company-profile/overview
17. Malherbe C, Schutte C. Moving beyond the challenges and seizing the opportunities: A study of South African universities' efforts to protect their research and innovation offerings during the COVID-19 pandemic. S Afr J High Educ. 2021;35(5):138–162. https://dx.doi.org/10.20853/35-5-4248
18. South African Department of Higher Education and Training (DHET). Report on the evaluation of the 2018 universities' research output [document on the Internet]. c2020 [cited 2020 Apr 20]. Available from: https://www.up.ac.za/media/shared/1/2020/May%202020/report-on-the-evaluation-of-the-2018-universities-research-output_april_2020.doc.zp189504.pdf
19. National Intellectual Property Management Office (NIPMO). About us [webpage on the Internet]. No date [cited 2021 Oct 26]. Available from: https://nipmo.dst.gov.za/about
20. Brand A, Dean OH. Intellectual property rights flowing from universities: An analysis of the impact of the current South African legal framework on international research collaboration. J S Afr Law. 2018;2018(3):475–506.
21. National Intellectual Property Management Office (NIPMO). Guideline 5.1 of 2019: Guidance for determining the full cost of research and development as per the Intellectual Property Rights from Publicly Financed Research and Development Act [document on the Internet]. c2019 [cited 2021 Oct 26]. Available from: https://nipmo.dst.gov.za/uploads/files/Guideline-5.1-of-2019_FC_FINAL-6-Aug-2019.pdf
22. Sasol. Open call for Sasol Research Grant proposals. SASOL; 2018.

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Determining safe retirement withdrawal rates using forward-looking distributions

An important topic for retirees is determining how much they can safely withdraw from their retirement savings: draw too much from their retirement fund and risk outliving their retirement savings, or draw too little and live below their means. For retirees to decide on the appropriate withdrawal rate, retirees need to have the tools available to decide on their spending rates. There are many factors that influence withdrawal rates, such as initial wealth, asset allocations, age, life expectancy, and risk tolerances. The topic of safe withdrawal rates aims to optimise spending rates while minimising the risk of running out of retirement savings. The focus of this study was on using forward-looking moments of the risk-neutral and real-world asset distributions in determining safe withdrawal rates for South African retirees. The use of forward-looking information, typically derived from traded derivative securities (rather than historical data), is essential in optimising safe withdrawal rates for retirees. In particular, we extracted the forward-looking risk-neutral and real-world distributions from option prices on the South African Top 40 index, and used the moments of the distributions as a signal in a simple tactical asset allocation framework. That is, when we expect the growth asset to decrease in value, we hold cash (or short the asset) and, alternatively, when we expect the growth asset to increase in value, we hold the growth asset for the period. Using this approach, we found that we can sustain withdrawal rates of up to 7% compared to the commonly quoted 4% safe withdrawal rate obtained by historical simulations.

Significance:

- Through this paper, we aim to create further awareness on safe retirement spending rates. It is important that retirees are guided through this process with the correct knowledge of the risk and return of asset classes.
- Using forward-looking information allows for a more realistic modelling of portfolio returns, which allows for the possibility of better modelling of safe withdrawal rates.
- We show that using the moments of the forward-looking distributions in a simple tactical asset allocation framework yielded superior portfolio returns to a fixed asset allocation structure.

Introduction

Studies on safe retirement spending rates typically draw information from historical data.[1-5] A prime example of such a study is the commonly quoted '4% safe withdrawal rate' published in Cooley et al.[1], where the authors used historical data over the period 1926 to 1995 to assess safe spending rates for retirees. The assumptions made in these studies are that the statistical properties of historical returns remain stable over time. However, it is known that historical (backward-looking) returns do not necessary predict future returns. Furthermore, in these studies, the authors assume a fixed asset allocation and a constant spending rate. Both assumptions are heavily criticised in the literature.[5-7] People nowadays are living longer and face a further 20 to 30 years of life with substantial probability after retirement.[8-10] Van Appel et al.[5] demonstrated in an empirical study, using historical data, that for higher spending rates, a higher allocation in growth assets is needed. Even then, the portfolio is unlikely to be successful over a 30-year period (representing a typical post-retirement investment cycle). Ideally, a retiree would like to draw as much as possible, with a low probability of depleting the fund before their duration of life, or 30 years.

An important part of modelling is to generate scenarios for financial practitioners. In particular, it is important that these scenarios are as close as possible to the true representation of what could happen. In extension of the work presented by Cooley et al.[1,2], Bengen[3], and Maré[4], the focus of this study was to improve the modelling of safe retirement spending rates by using forward-looking information rather than historical information. Many large financial institutions regularly estimate forward-looking distributions from option prices in order to gain insights into the weights investors place on different future asset prices.[11,12] Therefore, modelling retirement withdrawal rates using forward-looking information should provide a more realistic assessment of safe withdrawal rates. The main advantage of using forward-looking information is that it allows for the implementation of a tactical asset allocation framework instead of a fixed asset allocation structure that is used in the literature. This allows the portfolio to potentially achieve higher asset returns, which increases the success rates of retirement portfolios. In particular, we would like to be invested in the risky (or growth) asset when the return is expected to be favourable to the portfolio. To assess when returns will be favourable, forward-looking information should be used rather than historical data. We thereby show that models based on historical data do not provide a true representation of modelling retirement portfolio success rates optimally.

Methodology

Typically, safe retirement withdrawal rates are analysed by using models based on historical data and Monte Carlo simulation.[1-6,13,14] In particular, Scott[13] studied the impact that a portfolio's rate of return has on safe withdrawal

rates, and found that increasing the rate of return, by increasing the equity allocation, in the retirement portfolio drastically increases safe withdrawal rates. However, this comes with higher variability in returns (or risk).[5] Therefore, the aim of this section is to use forward-looking information in a tactical asset allocation framework to maximise portfolio returns and reduce the variability in returns.

Extracting forward-looking return distributions

A European-style call option is a contract that gives the holder the right (but not the obligation) to purchase a prescribed asset from the writer, for a prescribed price at a prescribed time, in the future. The price of the contract is determined by its expected future pay-off, under the risk-neutral measure, discounted by the risk-free interest rate:

$$C(T,K) = e^{-rT}E^{\mathbb{Q}}[(S_T - K)^+] = e^{-rT}\int_K^\infty (x-K) f_{S_T}^{\mathbb{Q}}(x)dx, \quad \text{Equation 1}$$

where T denotes the time to expiry (tenor), K the predetermined asset price (strike price), r the risk-free interest rate, S_T the asset price at expiry and $f_{S_T}^{\mathbb{Q}}(x)$ the risk-neutral probability density function of the future asset price. These options are typically traded on a number of official exchanges. Because the option pay-off extends out in time, option prices capture some market sentiment.[15-20] This is known as forward-looking information.

The forward-looking risk-neutral probability density function, $f_{S_T}^{\mathbb{Q}}(x)$, is easily extracted from market option prices by taking the second partial derivative with respect to the strike of a European call option as follows (see, Breeden and Litzenberger[21]):

$$f_{S_T}(x) = e^{rT}\frac{\partial^2 C(T,K)}{\partial K^2}\Big|_{K=x}. \quad \text{Equation 2}$$

However, option price data are normally sparse and noisy, especially in South Africa. Therefore, to estimate the forward-looking risk-neutral density function in Equation 2, one typically first needs to interpolate and extrapolate call option prices over a dense strike range.[22-25] It is practically more desirable to interpolate and extrapolate over the implied volatilities rather than the call option prices, where the implied volatilities can be obtained by the Black–Scholes option pricing formula.[18] That is, the Black–Scholes option pricing formula is simply used to move between prices and implied volatilities. In this paper, we used the stochastic volatility inspired model, proposed by Gatheral[24], to interpolate and extrapolate the implied volatility over a 50–150% moneyness range[25]. Thereafter, we numerically approximated the risk-neutral distribution by taking the second difference along the interpolate and extrapolate call option price at tenor T.[11,18,26]

Consequently, the moments of the distribution have become powerful in analysing and forecasting future returns.[15,18,27] The risk-neutral distribution differs from the real-world distribution in that the risk-neutral distribution's expected return is the risk-free rate as investors are risk-neutral under this measure. However, investors are typically risk-averse and therefore require a premium for taking on the risk. That is, the risk-neutral measure is the real-world measure with the risk premium removed. For forecasting future asset returns, the real-world measure is, therefore, preferred.[12,28] The risk premium is not directly observed and, therefore, obtaining the real-world measure normally involves additional assumptions on a utility function of terminal wealth and using historical data.[18,29-31]

Recently, Ross[32] proposed the recovery theorem, which is an alternative method of extracting a real-world distribution from the risk-neutral matrix of a Markovian state variable, i.e.:

$$Q(t,x) = e^{rt}\frac{\partial^2 C(t,K)}{\partial K^2}\Big|_{K=x}, t = 1,2,\dots,m, \quad \text{Equation 3}$$

where t represents the option tenor. In this study, we numerically discretise Equation 3 over n=51 return states, in total spanning the moneyness range 50–150%, which is placed every 2% symmetrically around the moneyness of 100% to obtain the (m x n) state price matrix, S.[32] This is done by numerically integrating Equation 3 over the discrete grid for each of the 51 states.[33] In essence, the discretised $S(t,n)$ represents the price of an Arrow–Debreu security that agrees to pay one unit of currency if state j is reached at time t and zero in all other states.

In contrast, the recovery theorem does not make use of historical returns, but rather makes assumptions about market restrictions.[32] This makes the recovery theorem a desirable candidate for extracting the real-world probabilities, particularly for new assets on the market, where large historical data sets do not exist. Under the assumption that transition state prices are time-homogeneous, the transition probability, $P_{i,j}$), of moving from state i to state j in one period is given as:

$$P_{i,j} = \mathbb{P}(S_{t+1} = j | S_t = i), t = 1,2\dots,m-1, \quad \text{Equation 4}$$

which can be estimated by solving the linear system of equations (see, for example, Ross[32], Audrino et al.[15], Van Appel and Maré[25]):

$$S_{t+1}^{\mathsf{T}} = S_t^{\mathsf{T}}P,\ t = 1,2,\dots,m-1, \quad \text{Equation 5}$$

where P denotes the one-period risk-neutral transition probability matrix. Intuitively, P represents the richer set of probabilities of moving from all hypothetical initial states to all hypothetical future states, where Equation 3 only represents the probabilities of moving from the single known current state to all future states.[34] Assuming no arbitrage, irreducibility of the transition matrix P, and that the pricing matrix is generated by a transition independent kernel, then under Ross's framework there exists a unique positive pricing kernel defined as the ratio price per unit probability:

$$\Psi_{i,j} = \frac{P_{i,j}}{f_{i,j}}, \quad \text{Equation 6}$$

where represents the pricing kernel, and the real-world transition probability of moving from state to state in one period. In essence, Ross[32] then estimates the two unknowns, namely the real-world probabilities and pricing kernel in Equation 6 using the Perron–Frobenius theorem.

To test the usability and practicality of the real-world distribution obtained by the recovery theorem in determining safe withdrawal rates, we next use the forward-looking forecasted moments in a simple tactical asset allocation framework to obtain higher returns than a model based purely on historical data with a fixed asset allocation. Furthermore, we also consider a hedging strategy by buying and selling put and call options, respectively.

Tactical asset allocation

In this section, we use the extracted forward-looking risk-neutral and real-world return distributions to forecast movements in the underlying asset returns. We extracted the forward-looking risk-neutral and real-world distributions, at the start of each month, from market-observed option prices quoted on the FTSE/JSE Top 40 index (Top40) over the period August 1996 to January 2018 (sourced from the South African FTSE/JSE), giving a total of 259 forecast months (or 259 one-month forecast distributions). The Top40 index was used as it is a key market factor in South Africa and, along with the exchange-traded derivatives on this asset, is one of the most liquid in the South African market. Furthermore, the duration of the sample (i.e. 21.5 years) would typically embrace at least three South African business cycles.[35] Thereafter, we use the extracted risk-neutral and real-world moments in a simple tactical asset allocation framework to obtain higher returns for the portfolio.

Investors are normally in search of higher returns, skewness and kurtosis, and lower volatility. Therefore, as outlined in Audrino et al.[15] and Flint and Maré[16], we carried out a simple tactical asset allocation, where we hold the Top40 for the full month when the forecasted moments (mean, skewness, and kurtosis) are higher than the previous month's forecast, or when the forecasted volatility is lower than the previous month's forecast. Because trading occurs once a month, there are a limited number of trades, resulting in negligible transactional costs

(transactional costs are normally around 2 basis points). In particular, we found that trading costs decreased yearly returns on average by no more than 0.2%. For completeness, all results based on the tactical asset allocation framework reported in this paper include transaction costs.

Lastly, we also considered a fixed asset allocation framework by incorporating a hedging strategy, where instead of selling the Top40 in the tactical asset allocation framework, we protect the portfolio against large losses by buying put options. This strategy involves purchasing put options with 6% out the money (OTM) strike with a 30% participation rate. Because put options are expensive, we offset the cost by selling call options with 1% OTM strike with a 30% participation rate. Because we use observed market-quoted prices, these parameter values were chosen to yield stable and desirable results. That is, our index portfolio value evolves as follows:

$$\begin{aligned}\pi(t) = {} & p^{(put)} \times \pi(t-1) \times \max\left(M^{(put)} - R_t, 0\right) \\ & - p^{(call)} \times \pi(t-1) \times \max\left(R_t - M^{(call)}, 0\right) \\ & + \pi(t-1)\left[1 - P\left(t, M^{(put)}\right) \times p^{(put)} + C\left(t, M^{(call)}\right) \times p^{(call)}\right] \times R_t,\end{aligned} \qquad \text{Equation 7}$$

where $\pi(t)$ represents the portfolio value at time t, $p^{(\cdot)}$ represents the participation rates, $M^{(\cdot)}$ the moneyness rate, R_t the asset return, and $P(t,M^{(put)})$ the market-quoted put option price.

The cumulative portfolio value over the period August 1996 to January 2018 for the simple tactical asset allocation framework using the moments of the risk-neutral and real-world distributions is shown in Figure 1a and Figure 1b, respectively, if one unit of currency was invested in the Top40 in August 1996.

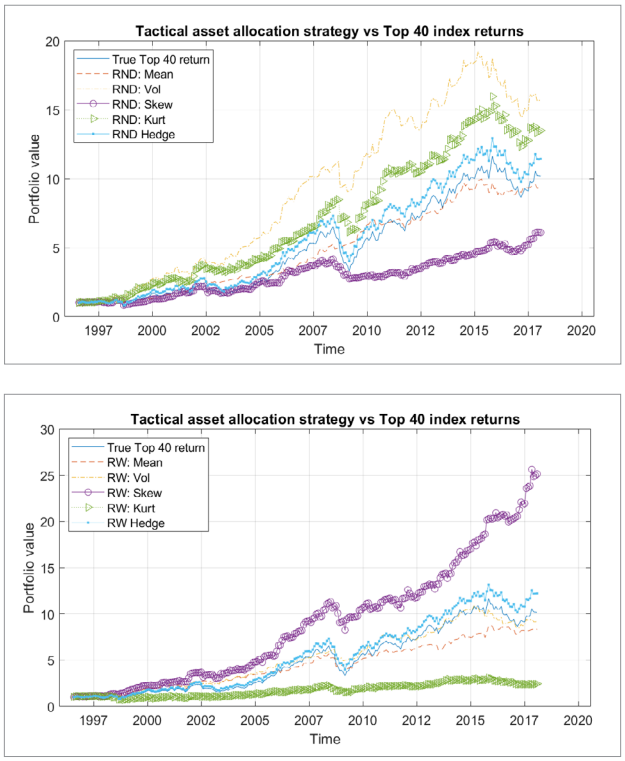


Figure 1: Tactical asset allocation with withdrawals returns using (a) the risk-neutral moments vs the Top 40 index returns and (b) the real-world moments vs the Top 40 index returns.

Figure 1a shows the portfolio using the forecasted risk-neutral volatility in returns as the signal in the tactical asset allocation framework yielded the best results. The trading strategy using the risk-neutral kurtosis also outperformed the Top40, while the trading strategy using the risk-neutral mean return yielded similar results to the Top40. The skewness yielded similar results to around 2005, but thereafter yielded poor results. In Figure 1b, the real-world skewness in the simple tactical asset allocation framework yielded the best results, while the volatility and mean yielded similar returns to the Top40, and the kurtosis yielded poor results. Furthermore, Figure 1 shows that the tactical asset allocation based on the real-world skewness significantly outperformed the risk-neutral moments. In both the risk-neutral and real-world settings, the hedging strategy (with a fixed asset allocation as described above) involving the buying and selling of put and call options did not perform as well as the simple tactical asset allocation method. However, the hedging strategy did outperform the Top40 throughout the duration of the study.

In Table 1, we show some descriptive statistics of the annualised returns using the volatility in the risk-neutral tactical asset allocation framework (RND), and the skewness in the real-world tactical asset allocation framework (RW). We also consider the tactical asset allocation of combining both signals from the risk-neutral volatility and real-world skewness (RW & RND). That is, we hold the asset when the forecasted risk-neutral volatility is lower than the previous month's forecast and the forecasted real-world skewness is higher than the previous month's forecast. The hedging strategy, based on the risk-neutral volatility (RND Hedge) and real-world skewness (RW Hedge), is also shown in Table 1.

Table 1: Descriptive statistics

	Mean	Volatility	Sharpe ratio	Skewness	Kurtosis
Top 40	13.62%	19.90%	0.23	-0.28	5.75
RND	14.85%	14.63%	0.40	0.62	8.23
RW	17.13%	13.01%	0.63	0.39	6.16
RW & RND	15.73%	8.74%	0.77	2.35	12.79
RND Hedge	13.86%	18.32%	0.27	-0.03	5.02
RW Hedge	14.12%	17.89%	0.29	-0.16	4.25

The tactical asset allocation strategy involving the real-world skewness yielded the highest mean return over the sample period with a low variation in returns. The strategy involving the combination of the real-world and risk-neutral moments yielded the lowest variation in returns with a high expected return over our sample period. Low variation in returns, in conjunction with high expected returns, is obviously a desirable property for investment managers.

In Table 2, we show the number of trades carried out in each tactical asset allocation strategy shown in Table 1 over the total of 259 forecast months (or 21.5 years).

Table 2: Number of trades

	RND Vol	RW Skew	RND Vol & RW Skew
Number of trades	139	167	111

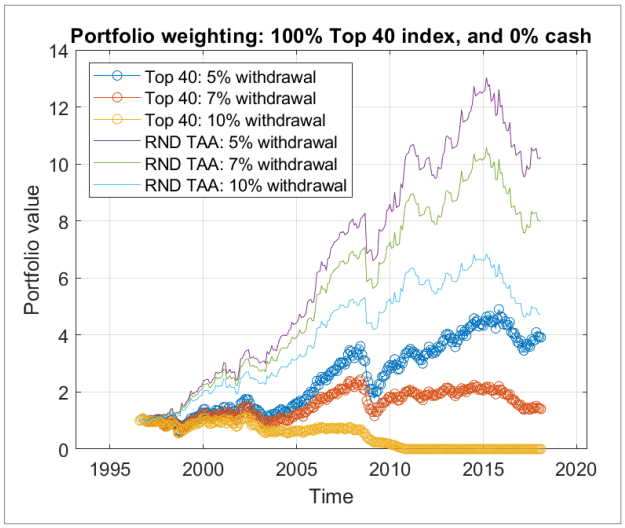
In the next section, we examine safe withdrawal rates in a forward-looking environment using the tactical asset allocation framework.

Results

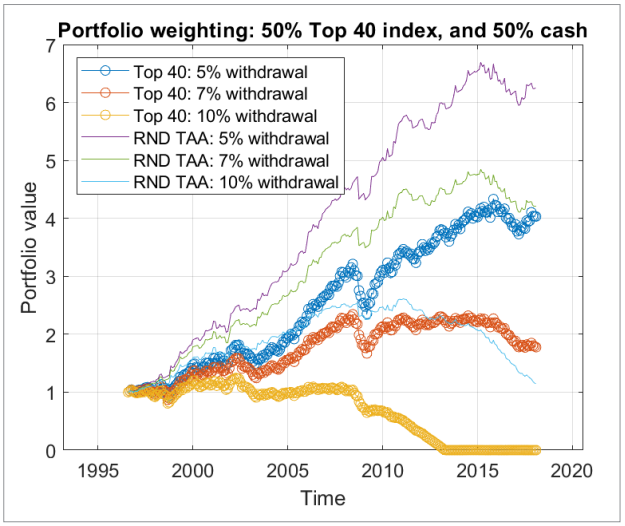
In this section, we assume that a person retired on 1 August 1996 with one unit in retirement savings. The retiree needs to decide how much to withdraw from the retirement fund; draw too much and carry the risk of running out of money, or draw too little and carry the risk of a compromised living standard. Therefore, in this section, we study the life expectancy of a basic retirement portfolio with three commonly used withdrawal rates used in the literature, namely 5%, 7%, and 10% per year of the initial portfolio size. Furthermore, these withdrawals will be adjusted monthly according to inflation rates and historical cash returns – which have been sourced form Firer and McLeod[36], Firer and Staunton[37], and I-Net – are used in the portfolio.

Safe withdrawal rates

In Figures 2, 3, and 4, we clearly see that the tactical asset allocation framework using the moments obtained from the forward-looking distributions outperformed the fixed asset allocation for the duration of the period under study. Figure 2 shows the accumulated portfolio value for two different asset allocations (see Figures 2a and 2b), with the risk-neutral volatility used in the tactical asset allocation framework described in the section above. Similarly, Figures 3 and 4 show the accumulated portfolio values using the real-world skewness and the combination of the real-world skewness and risk-neutral volatility in the tactical asset allocation framework, respectively. Combining the signal from the risk-neutral volatility and the real-world skewness yielded superior fund prospects for higher withdrawal rates (Figure 4). Although this strategy does not yield the highest mean return over the sample period (Table 1), it has the least variation in returns. Scott et al.[6] and Waring and Siegel[7] criticised the notion of withdrawing a fixed real amount from an inherently volatile portfolio. This is known as sequence risk. Therefore, reducing the variation in returns is vitally important in determining safe retirement withdrawal rates. This is particularly evident in Figure 4, where a high withdrawal rate of 10% yielded higher portfolio prospects than using only the real-world skewness.

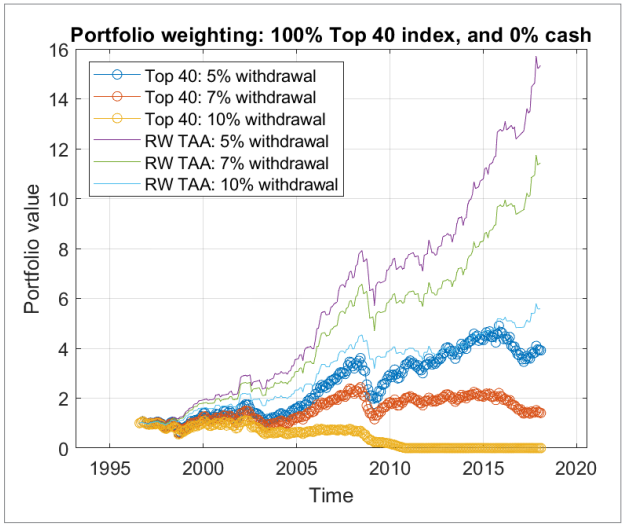


(a) 100% Equity

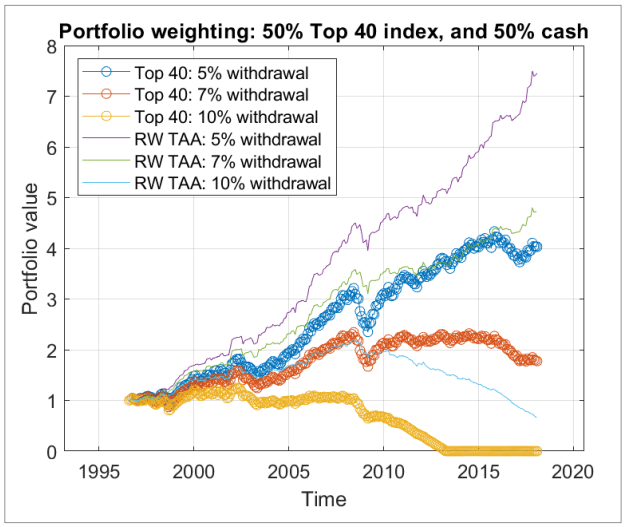


(b) 50% Equity, 50% Cash

Figure 2: The accumulated portfolio value for a fixed asset allocation vs the risk-neutral tactical asset allocation (RND TAA) framework with withdrawal rates of {5%, 7%, 10%} returns.

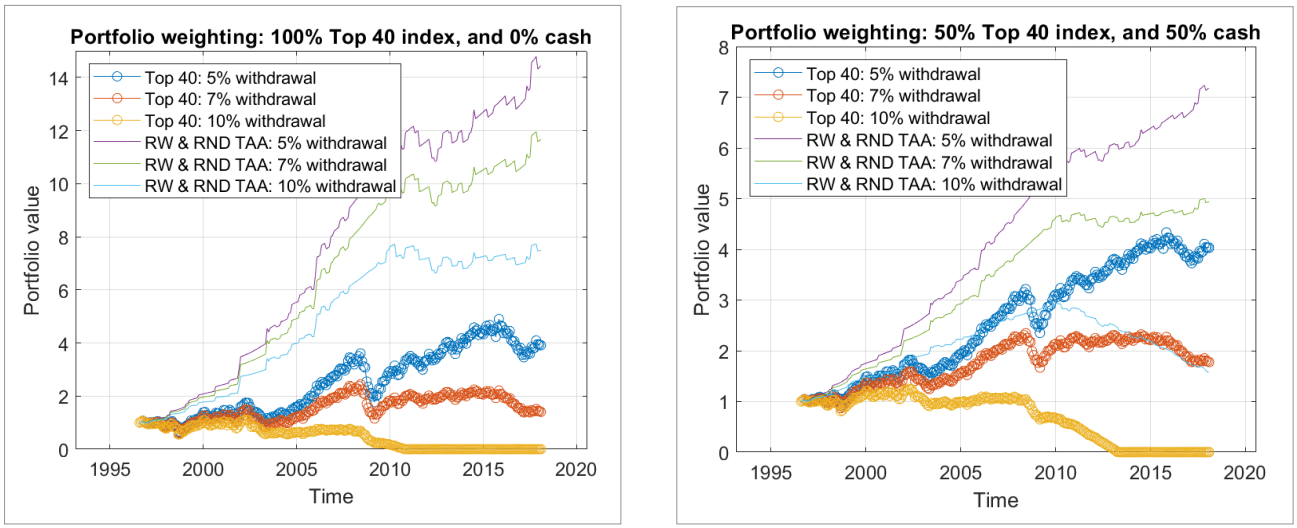


(a) 100% Equity



(b) 50% Equity, 50% Cash

Figure 3: The accumulated portfolio value for a fixed asset allocation vs the real-world tactical asset allocation (RW TAA) framework with withdrawal rates of {5%, 7%, 10%} returns.



(a) 100% Equity

(b) 50% Equity, 50% Cash

Figure 4: The accumulated portfolio value for a fixed asset allocation vs the real-world and risk-neutral tactical asset allocation (RW & RND TAA) framework with withdrawal rates of {5%, 7%, 10%} returns.

Next, we assess the robustness of the tactical asset allocation framework in determining safe retirement withdrawal rates. In particular, the robust analysis is carried out to determine how much, if any, of the improvement above the commonly quoted 4% safe withdrawal rate is attributed to using forward-looking information, rather than the different market or time periods used in this study.

Robust analysis

To assess the robustness of the forward-looking distributions in modelling safe withdrawal rates, we carried out a random sampling study. We randomly selected, with replacement, a month from the sample period and used the equity, bonds, and cash returns to generate a one-month sample path. In the tactical asset allocation framework, we also used the previous month's forecasted moments of the randomly selected month to determine the portfolio asset allocation. We then continued to randomly sample from the period to simulate a 30-year period. We, therefore, simulated the evolution of the portfolio over a 30-year period and constructed 10 000 such sample paths. This approach maintains the correlation structure between the assets, as we are using the true observed returns for the selected month for all assets. In Table 3, we show the success rates based on the fixed asset allocation versus the tactical asset allocation framework using the real-world skewness. We found similar results in our sample to the commonly quoted 4% safe withdrawal rate using historical (backward-looking) returns. However, by using forward-looking information in a tactical asset allocation framework, we were able to show significantly improved safe withdrawal rates. Table 4 shows the fugit of the retirement portfolio. In this study, the fugit is defined as the expected duration of the portfolio given that the portfolio fails before the predefined 30-year duration.

Table 3: Success rates

	Withdrawal rate						
Asset allocation	**4%**	**5%**	**6%**	**7%**	**8%**	**9%**	**10%**
Fixed asset allocation							
100% Stocks	79%	66%	52%	40%	30%	21%	14%
75% Stocks / 25% Bonds	89%	77%	61%	45%	32%	20%	13%
50% Stocks / 50% Bonds	96%	86%	70%	50%	31%	18%	10%
25% Stocks / 75% Bonds	99%	92%	76%	54%	29%	13%	5%
Real-world tactical asset allocation							
100% Stocks	100%	98%	95%	86%	72%	59%	41%
75% Stocks / 25% Bonds	100%	99%	96%	87%	71%	52%	31%
50% Stocks / 50% Bonds	100%	100%	97%	85%	63%	37%	17%
25% Stocks / 75% Bonds	100%	99%	93%	73%	44%	19%	6%

Table 4: Fugit

	Withdrawal rate						
Asset allocation	**4%**	**5%**	**6%**	**7%**	**8%**	**9%**	**10%**
Fixed asset allocation							
100% Stocks	256	239	223	207	193	178	165
75% Stocks / 25% Bonds	276	259	243	226	210	190	175
50% Stocks / 50% Bonds	296	281	262	247	226	204	184
25% Stocks / 75% Bonds	308	297	281	263	240	213	188
Real-world tactical asset allocation							
100% Stocks	294	283	268	257	242	226	208
75% Stocks / 25% Bonds	322	308	296	276	258	239	218
50% Stocks / 50% Bonds	272	313	304	288	268	245	217
25% Stocks / 75% Bonds	340	314	303	285	262	235	205

It is evident from Table 3 and Table 4 that the simple tactical asset allocation framework, using the forward-looking real-world skewness, yielded superior success and fugit rates to the strategy that involved the fixed asset allocation. As the tactical asset allocation is based on either holding, or selling, the growth asset for a one-month period, the strategy is most prominent with a large growth asset allocation. These results illustrate that one can possibly achieve high portfolio success rates when making use of forward-looking information in portfolio management.

Conclusion

In this study, we used forward-looking information, extracted from observed market-quoted derivative prices, to determine safe retirement withdrawal rates. In particular, we extracted the forward-looking risk-neutral and real-world return distribution functions, and used the distribution moments as a signal in a simple tactical asset allocation framework. We found that using forward-looking information in a tactical asset allocation framework yielded higher portfolio returns with a lower variation in returns compared to the portfolio with a fixed asset allocation.

Many large financial firms frequently extract forward-looking information from derivative securities to infer market sentiment. Therefore, using a forward-looking modelling approach provided a more market consistent analysis of safe retirement withdrawal rates. We found that the portfolio based on the forward-looking real-world skewness in a tactical asset allocation framework supported safe withdrawal rates of up to 7% per annum (inflation adjusted). This strategy obtained similar success rates to the previously quoted 4% safe withdrawal rate determined from the fixed asset allocation based on historical returns. Thus, the performance of the real-world moments, used as a signal in a tactical asset allocation, allows for the possibility of higher withdrawal rates with high success rates. This confirms the usefulness of using forward-looking real-world moments in the management of retirement portfolios to improve the modelling of safe retirement withdrawal rates.

Competing interests

We have no competing interests to declare.

Authors' contributions

V.v.A.: Conceptualisation, methodology, data analysis, writing – the initial draft. E.M.: Conceptualisation, writing – the initial draft, student supervision, project leadership.

References

1. Cooley P, Hubbard C, Walz D. Retirement savings: Choosing a withdrawal rate that is sustainable. Journal of the American Association of Individual Investors. 1998;10(1):40–50.
2. Cooley P, Hubbard C, Walz D. Sustainable withdrawal rates from your retirement portfolio. Financ Counsel Plan. 1999;20(2):16–21.
3. Bengen W. Determining withdrawal rates using historical data. J Financ Plan. 1994;7(1):171–180.
4. Maré E. Safe spending rates for South African retirees. S Afr J Sci. 2016;112(1/2), Art. #a0138. https://doi.org/10.17159/sajs.2016/a0138
5. Van Appel V, Maré E, Van Niekerk AJ. Quantitative guidelines for retiring (more safely) in South Africa. S Afr Actuar J. 2021;21(1), Art. #4. https://hdl.handle.net/10520/ejc-actu_v21_n1_a4
6. Scott J, Sharpe W, Watson J. The 4% rule – at what price? J Invest Manag. 2009;7(3):31–48.
7. Waring MB, Siegel LB. The only spending rule article you will ever need. Financial Anal J. 2015;71(1):91–107. https://doi.org/10.2469/faj.v71.n1.2
8. World Health Organization (WHO). Global health observatory data repository [webpage on the Internet]. c2020 [cited 2021 Nov 14]. https://apps.who.int/gho/data/view.main.SDG2016LEXv?lang=en
9. Purdy M. The first person to live to 150 has already been born. Innovation Hub. 2015 August 8. Available from: https://www.pri.org/stories/2015-08-08/first-person-live-150-has-already-been-born-it-you
10. Ennart H. Åldrandets gåta [The mystery of aging]. Stockholm: Ordfront; 2012. Swedish.
11. Shimko DC. Bounds of probability. Risk (Concord, NH). 1993;6(4):33–37.
12. De Vincent-Humphreys R, Noss J. Estimating probability distributions of future asset prices: Empirical transformations from option-implied risk-neutral to real-world density functions. Bank of England Working Paper no. 455. SSRN; 2012. https://doi.org/10.2139/ssrn.2093397
13. Scott MC. Assessing your portfolio allocation from a retiree's point of view. Journal of the American Association of Individual Investors. 1996;May:16-19.
14. Abuizam R. A risk-based model for retirement planning. J Bus Econ Res. 2009;7(6):31–43. https://doi.org/10.19030/jber.v7i6.2304
15. Audrino F, Huitema R, Ludwig M. An empirical analysis of the Ross recovery theorem. SSRN; 2014. http://dx.doi.org/10.2139/ssrn.2433170
16. Flint E, Maré E. Estimating option-implied distributions in illiquid markets and implementing the Ross recovery theorem. S Afr Actuar J. 2017;17(1):1–28. https://doi.org/10.4314/saaj.v17i1.1

17. Van Appel V, Maré E. The recovery theorem with application to risk management. S Afr Stat J. 2020;54(1):65–91. https://doi.org/10.37920/sasj.2020.54.1.5

18. Christoffersen P, Jacobs K, Chang BY. Forecasting with option-implied information. In: Elliott G, Timmermann A, editors. Handbook of economic forecasting. Elsevier; 2013. p. 581–656. https://doi.org/10.1016/B978-0-444-53683-9.00010-4

19. Hollstein F, Prokopczuk M, Tharann B, Simen CW. Predicting the equity market with option-implied variables. Eur J Financ. 2019;25(10):937–965. https://doi.org/10.1080/1351847X.2018.1556176

20. Dillschneider Y, Maurer R. Functional Ross recovery: Theoretical results and empirical tests. J Econ Dyn Control. 2019;108, Art. #103750. https://doi.org/10.1016/j.jedc.2019.103750

21. Breeden DT, Litzenberger RH. Prices of state-contingent claims implicit in option prices. J Bus. 1978;51(4):621–651. Available from: https://www.jstor.org/stable/2352653

22. Malz AM. A simple and reliable way to compute option-based risk-neutral distributions. Federal Reserve Bank of New York Staff Report 677. SSRN; 2014. https://doi.org/10.2139/ssrn.2449692

23. Aït-Sahalia Y, Lo AW. Nonparametric estimation of state-price densities implicit in financial asset prices. J Financ. 1998;53(2):499–547. https://doi.org/10.1111/0022-1082.215228

24. Gatheral J. A parsimonious arbitrage-free implied volatility parametrization with application to the valuation of volatility derivatives. Presentation at: Global Derivatives and Risk Management; 2004 May 26; Madrid, Spain.

25. Van Appel V, Maré E. The Ross recovery theorem with a regularised multivariate Markov chain. ORiON. 2018;34(2):133–155. https://doi.org/10.5784/34-2-594

26. Figlewski S. Estimating the implied risk neutral density for the US market portfolio. In: Bollerslev T, Russell JR, Watson M, editors. Volatility and time series econometrics: Essays in honor of Robert F. Engle. Oxford: Oxford University Press; 2010. p. 323–353. https://doi.org/10.1093/acprof:oso/9780199549498.003.0015

27. Bollerslev T, Tauchen G, Zhou H. Expected stock returns and variance risk premia. Rev Financ Stud. 2009;22(11):4463–4492. https://doi.org/10.1093/rfs/hhp008

28. Shackleton MB, Taylor SJ, Yu P. A multi-horizon comparison of density forecasts for the S&P 500 using index returns and option prices. J Bank Financ. 2010;34(11):2678–2693. https://doi.org/10.1016/j.jbankfin.2010.05.006

29. Aït-Sahalia Y, Lo AW. Nonparametric risk management and implied risk aversion. J Econometrics. 2000;94(1–2):9–51. https://doi.org/10.1016/S0304-4076(99)00016-0

30. Bliss RR, Panigirtzoglou N. Option-implied risk aversion estimates. J Financ. 2004;59(1):407–446. https://doi.org/10.1111/j.1540-6261.2004.00637.x

31. Hansen LP, Renault E. Pricing kernels and stochastic discount factors. In: Cont R, editor. Encyclopedia of quantitative finance. Chichester: John Wiley & Sons; 2010; p. 1418–1427.

32. Ross S. The recovery theorem. J Financ. 2015;70(2):615–648. https://doi.org/10.1111/jofi.12092

33. Chakraborty S. Generating discrete analogues of continuous probability distributions – A survey of methods and constructions. J Stat Distrib. 2015;2(6):1–30. https://doi.org/10.1186/s40488-015-0028-6

34. Cuesdeanu H, Jackwerth JC. The pricing kernel puzzle: Survey and outlook. Ann Finance. 2018;14:289–329. https://doi.org/10.1007/s10436-017-0317-9

35. Thomson D, Van Vuuren G. Forecasting the South African business cycle using Fourier analysis. Int Bus Econ Res J. 2016;15(4):175–192. https://doi.org/10.19030/iber.v15i4.9755

36. Firer C, McLeod H. Equities, bonds, cash and inflation: Historical performance in South Africa 1925 to 1998. Invest Anal J. 1999;28(50):7–28. https://doi.org/10.1080/10293523.1999.11082398

37. Firer C, Staunton M. 102 Years of South African financial market history. Invest Anal J. 2002;31(56):57–65. https://doi.org/10.1080/10293523.2002.11082442

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Malaria risk and receptivity: Continuing development of insecticide resistance in the major malaria vector *Anopheles arabiensis* in northern KwaZulu-Natal, South Africa

Malaria incidence in South Africa is highest in the three endemic provinces: KwaZulu-Natal, Mpumalanga and Limpopo. The contribution to malaria transmission by several mosquito species, variation in their resting behaviours and low levels of insecticide resistance makes it necessary to periodically monitor *Anopheles* species assemblages and resistance phenotypes in vector populations. The aim of this study was therefore to assess *Anopheles* species assemblage in northern KwaZulu-Natal and to collect insecticide susceptibility data for *An. arabiensis*, the primary vector of malaria in that province. *Anopheles* specimens were collected from Mamfene, Jozini, northern KwaZulu-Natal from November 2019 to April 2021. Progeny of wild-collected *An. arabiensis* females were used for standard insecticide susceptibility tests and synergist bioassays. *Anopheles arabiensis* contributed 85.6% (n=11 062) of the total catches. Samples for subsequent insecticide susceptibility bioassays were selected from 212 *An. arabiensis* families. These showed low-level resistance to DDT, permethrin, deltamethrin, and bendiocarb, as well as full susceptibility to pirimiphos-methyl. Synergist bioassays using piperonyl butoxide and triphenyl phosphate suggest oxygenase-based pyrethroid and esterase-mediated sequestration of bendiocarb. These low levels of resistance are unlikely to be operationally significant at present. It is concluded that northern KwaZulu-Natal Province remains receptive to malaria transmission despite ongoing control and elimination interventions. This is due to the perennial presence of the major vector *An. arabiensis* and other secondary vector species. The continued detection of low-frequency insecticide resistance phenotypes in *An. arabiensis* is cause for concern and requires periodic monitoring for changes in resistance frequency and intensity.

Significance:

- Insecticide resistance in the major malaria vector *Anopheles arabiensis* in northern KwaZulu-Natal Province is cause for concern in terms of resistance management and ongoing vector control leading toward malaria elimination.
- Despite ongoing control interventions, northern KwaZulu-Natal remains receptive to malaria owing to the perennial presence of several *Anopheles* vector species.

Introduction

South Africa's malaria-endemic provinces are KwaZulu-Natal, Mpumalanga, Limpopo and, to a far lesser extent, the North West. The incidence of locally acquired malaria is generally highest in those regions bordering southern Mozambique, eSwatini, Zimbabwe, and Botswana. Malaria vector control in the context of scaling up toward elimination is conducted annually in affected districts/municipalities in all of these provinces with the exception of the North West Province (as the incidence is extremely low). The primary methods of control include indoor residual spraying (IRS) of specially formulated insecticides, and larval source management.[1]

The human malarias are transmitted by *Anopheles* mosquitoes. To date, five *Anopheles* species have been directly implicated in the transmission of the malarial parasite *Plasmodium falciparum* in South Africa; these are the major vectors *Anopheles funestus* Giles, and *An. arabiensis* Patton, and the secondary vectors *Anopheles merus*, *Anopheles vaneedeni* and *Anopheles parensis*.[2-5] Populations of *An. arabiensis*[3,6,7], *An. merus*[3,8] and *An. parensis*[5,9,10] may include indoor- and outdoor-resting components; female *An. funestus* have a strong but not exclusive tendency to rest indoors[2,9,10] and *An. vaneedeni* tend to rest outdoors[11]. By targeting indoor-resting *Anopheles* mosquitoes, IRS-based vector control has reduced malaria incidence in South Africa to a point where elimination (i.e. zero locally acquired malaria cases) is a feasible prospect.[12] Yet despite the pro-active implementation of vector control/elimination operations year-on-year, local transmission persists at low levels in several districts and municipalities across the endemic provinces. This persistence can be attributed to several factors, one of which is the occurrence of outdoor-resting vector mosquitoes that are far less vulnerable to IRS. Another critical factor is the development of resistance to insecticides.

High-intensity resistance to pyrethroid insecticides was first recorded in southern African populations of *An. funestus* in 1999.[13] This phenotype caused substantial control failure in South Africa during the malaria epidemic of 1996–2000. The re-introduction of DDT for malaria vector control in South Africa in 2000, played a crucial role in substantially reducing incidence because the pyrethroid-resistant *An. funestus* populations retained full susceptibility to DDT.[14,15] Current control operations in South Africa include the concurrent use of deltamethrin (pyrethroid) and DDT in a mosaic approach designed to manage insecticide resistance in *An. funestus* and maintain

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control efficacy. This resistance management strategy is, however, under constant review because low-level resistance to pyrethroid, DDT, and carbamate insecticides has since been recorded in *An. arabiensis* populations in northern KwaZulu-Natal Province.[16] Although these phenotypes have been detected in *An. arabiensis*, they have been of low intensity and frequency and are therefore not considered to be operationally significant at present.[17]

The contribution to malaria transmission by several vector species and variation in their resting behaviours makes it necessary to periodically monitor *Anopheles* species assemblages in endemic areas, especially in terms of malaria risk and receptivity. Additionally, and given that low-level resistance is likely to increase in intensity and frequency under selection pressure imposed by insecticide use, it is necessary to periodically monitor for resistance phenotypes in vector populations. The aim of this study was therefore to assess *Anopheles* species assemblage in northern KwaZulu-Natal Province and to collect insecticide susceptibility data for *An. arabiensis*, the primary vector of malaria there.

Materials and methods

Anopheles mosquito specimens were collected from Mamfene in the Jozini municipality of northern KwaZulu-Natal Province. Collections were made from three sites: Section 2 (S 27°24'14.2"; E 32°12'41.8"), Section 8 (S 27°27'34.3"; E 32°10'43.7"), and Section 9 (S 27°23'50.5"; E 32°12'20.1"). Collections took place from November 2019 to April 2021. Adult female mosquitoes were collected from permanently stationed clay pots. A total of 56 clay pots were deployed up to and including 12 October 2020, following which 116 were deployed in Sections 2 (n=39), 8 (n=37), and 9 (n=40) (Figure 1). Each pot was sampled twice per week (i.e. 8 times/month) during the surveillance period. Mosquitoes were also sampled from disused vehicle tyres (n=6) and drums (n=1) from Section 9, modified plastic buckets from Section 2 (n=3), carbon dioxide baited net traps on two occasions in each of the three sections, and direct aspiration of mosquitoes resting at cattle kraals, in a few instances.

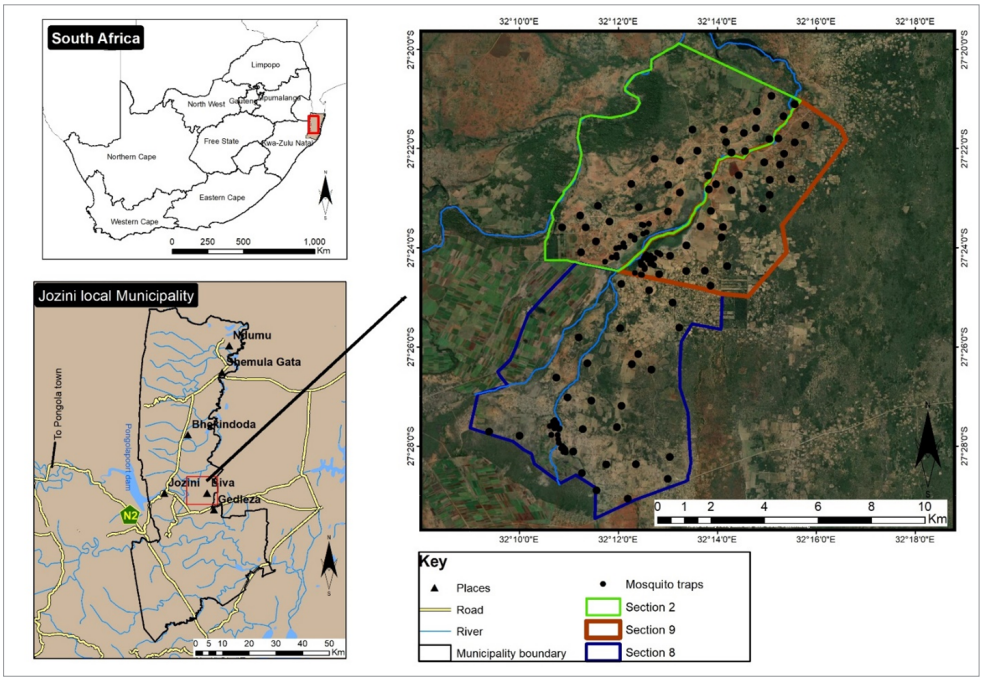

Figure 1: Map of Jozini municipality, northern KwaZulu–Natal Province, South Africa, showing sampling points from which anopheline mosquitoes were collected monthly between November 2019 and April 2021.

Specimens were identified to species, species complex or group in the field using dichotomous keys.[11] Live female mosquitoes identified as members of the *An. gambiae* complex were used to establish isofemale lines in the Botha De Meillon insectary, National Institute for Communicable Diseases, Johannesburg. After the first egg batch, the female adult was killed and preserved for subsequent identification by a standardised multiplex polymerase chain reaction.[18] Based on these species identifications, *An. arabiensis* F_1 larvae were pooled according to collection site. Larvae were reared according to standard procedures[19], i.e. rearing at 25 °C (±2 °C) and 80% relative humidity (±5%) with a 12:12 hour photoperiod and 30-min dawn/dusk cycles. The F_1 adults were maintained with ad libitum access to 10% sucrose until used for insecticide susceptibility assays.[20] These were performed on non-blood fed adults aged 3–5 days. F_1 *An. arabiensis* adults were assayed against 4% DDT, 0.75% permethrin, 0.05% deltamethrin, 0.1% bendiocarb, and 0.25% pirimiphos-methyl.[20]

Synergist bioassays were performed using synergist-impregnated papers that were produced in-house. Papers were impregnated with 4% piperonyl butoxide (PBO), a cytochrome P450 synergist, or 20% triphenyl phosphate (TPP), a general esterase synergist (Sigma Aldrich St. Louis, MO, USA). Treatment samples were exposed to one of the synergists for 60 min followed by exposure to an insecticide for 60 min (either 0.75% permethrin or 0.1% bendiocarb) according to the World Health Organization (WHO) standardised protocol.[20] Control samples were exposed to insecticide only, or to untreated papers. Adults were allowed access to 10% sucrose, ad libitum, and mortality was scored 24-h post-exposure.

Table 1: *Anopheles* mosquitoes sampled from Mamfene, northern KwaZulu-Natal, South Africa, between November 2019 and April 2021, stratified by section, year of collection, season and species

	Year of collection and total number of anophelines collected, N (relative abundance %/variable)			Total (% of total collected)
	Nov – Dec 2019	Jan – Dec 2020	Jan – April 2021	
Section of collection				
Section 2	94 (5.7%)	1282 (78.3%)	262 (16.0%)	**1638 (12.7%)**
Section 8	141 (7.9%)	1334 (75.0%)	303 (17.1%)	**1778 (13.8%)**
Section 9	497 (5.2%)	6848 (72.1%)	2158 (22.7%)	**9503 (73.5%)**
Total	**732 (5.7%)**	**9464 (73.3%)**	**2723 (21.1%)**	**12919**
Collection method used				
Carbon dioxide baited tent	0	33 (94.3%)	2 (5.8%)	**35 (0.3%)**
Clay pot	408 (5.4%)	5534 (73.4%)	1594 (21.2%)	**7536 (58.3%)**
Miscellaneous	50 (7.0%)	487 (68.0%)	179 (25.0%)	**716 (5.5%)**
Modified buckets	30 (9.4%)	276 (86.2%)	14 (4.4%)	**320 (2.5%)**
Disused tyres	244 (5.7%)	3134 (72.7%)	934 (21.6%)	**4312 (33.4%)**
Season of collection				
Autumn	0	2367 (65.0%)	1273 (35.0%)	**3640 (28.2%)**
Spring	576 (17.8%)	2651 (82.2%)	0	**3227(25.0%)**
Summer	156 (4.1%)	2208 (57.9%)	1450 (38.0%)	**3814 (29.5%)**
Winter	0	2238 (100%)	0	**2238 (17.3%)**
Species collected				
An. arabiensis	570 (5.2%)	8559 (77.4%)	1933 (17.4%)	**11062 (85.6%)**
An. coustani	4 (7.8%)	36 (70.6%)	11 (21.6%)	**51 (4.0%)**
An. demeilloni	0	9 (100%)	0	**9 (0.1%)**
An. leesoni	1 (5.8%)	8 (47.1%)	8 (47.1%)	**17 (0.1%)**
An. maculipalpis	0	9 (69.2%)	4 (30.8%)	**13 (0.1%)**
An. marshallii group	12 (25.0%)	36 (75.0%)	0	**48 (0.4%)**
An. merus	9 (7.1%)	102 (80.3%)	16 (12.6%)	**127 (1.0%)**
An. parensis	1 (0.2%)	81 (13.0%)	539 (86.8%)	**621 (4.8%)**
An. pharoensis	0	9 (75.0%)	3 (25.0%)	**12 (0.1%)**
An. pretoriensis	5 (12.5%)	33 (82.5%)	2 (5.0%)	**40 (0.3%)**
An. quadriannulatus	1 (50.0%)	1 (50.0%)	0	**2 (0.01%)**
An. rivulorum	0	19 (24.4%)	59 (75.6%)	**78 (0.6%)**
An. rufipes	18 (7.9%)	181 (79.4%)	29 (12.7%)	**228 (1.8%)**
An. squamosus	0	1 (50.0%)	1 (50.0%)	**2 (0.01%)**
An. vaneedeni	3 (3.3%)	43 (47.3%)	45 (49.4%)	**91 (0.7%)**
An. ziemanni	0	1 (100%)	0	**1 (0.01%)**
Not identified to species	108 (20.9%)	336 (65.0%)	73 (14.1%)	**517 (4.0%)**

Data were tested for normality using a Shapiro–Wilk test.[21] As the data were not normally distributed, a Kruskal–Wallis one-way analysis of variance (ANOVA) was used to determine differences between final mortality means.[22] For two-sample tests, a Mann–Whitney U-test was performed.[23]

Ethical approval

The Faculty of Health Sciences Research Ethics Committee of the University of the Witwatersrand (CR 20200218-10/ AREC-101210-002) and KwaZulu-Natal Health Research and Knowledge Management (KZ_202003_016) granted ethical approval. All household owners gave verbal consent to sample mosquitoes from their households.

Results

Species assemblage

In total, 12 919 anophelines were collected during the sampling period. Of these, 5.6% (n=732) were collected between November and December 2019, 73.3% (n=9464) were collected in 2020, and 21.1% (n=2723) were collected between January and April 2021 (Table 1). Most specimens (73.6%; n=9503) were collected from Section 9, while Section 2 was the least productive (n=1638). The largest number of mosquitoes was collected in summer (29.5%, n=3814) and the least in the winter months (17.3%, n=2238). Stratification of mosquito collections by method shows that clay pots (58.3%; n=7536) were the most productive, most likely because they were used more intensively than the other methods, and carbon dioxide baited net traps were the least productive (10.3%, n=35).

In total, 16 *Anopheles* species were collected over the sampling period. These included three members from the *An. gambiae* complex (*An. arabiensis*, *An. merus,* and *An. quadriannulatus*), two members from the *An. funestus* subgroup (*An. vaneedeni* and *An. parensis*), and one member each from the *An. minimus* subgroup (*An. leesoni*) and the *An. rivulorum* subgroup (*An. rivulorum* s.s). Stratification of species collected by section showed that some species were limited in their geographical range – *An. maculipalpis* was limited to Sections 2 and 9 while *An. quadriannulatus* was exclusively sampled from Section 9, and *An. squamosus* and *An. ziemanni* were limited to Sections 2 and 8, respectively. A total of 521 specimens could not be identified to species.

Anopheles arabiensis was the predominant species collected, contributing 85.6% (n=11 062) of the total. The population density of *An. arabiensis* (number caught/trap/month) shows a cyclical pattern with no discernible trend (Figure 2). Overall, Section 9 had the highest mean *An. arabiensis* density of 3.1/trap/month compared to that of Section 2 (1.1/trap/month) and Section 8 (0.9/trap/month). There were major peaks in *An. arabiensis* density in January 2020 (8.5 mosquitoes/trap/month), May and June 2020 (8.1 mosquitoes/trap/month) and September 2020 (5.1 mosquitoes/trap/month), all of which occurred in Section 9. The lowest mean number of mosquitoes caught per trap occurred in January 2021. No collections were conducted during April 2020 owing to COVID-19 restrictions.

Insecticide susceptibility tests

A total of 212 *An. Arabiensis* families were used for WHO susceptibility studies. According to the standardised method of interpreting insecticide susceptibility data[20], female and male *An. Arabiensis* F_1 samples showed signs of resistance to DDT, deltamethrin, permethrin and bendiocarb, and full susceptibility to pirimiphos-methyl. These results were consistent across all three sections of Mamfene (Table 2 and Figure 3). There was no significant difference in DDT-induced mortality (Kruskal–Wallis ANOVA: p=0.63, $F_{(2,37)}$=0.47, X^2=0.97), deltamethrin-induced mortality (p=0.64, $F_{(2,35)}$=0.45, X^2=4.47) or bendiocarb-induced mortality (p=0.10, $F_{(2,33)}$=2.43, X^2=4.47) between the Mamfene sections. There was, however, a significant difference in permethrin-induced mortality (p<0.01, $F_{(2,25)}$=6.34, X^2=8.89), with Section 2 showing the lowest mortality, followed by Sections 9 and 8 (Figure 3).

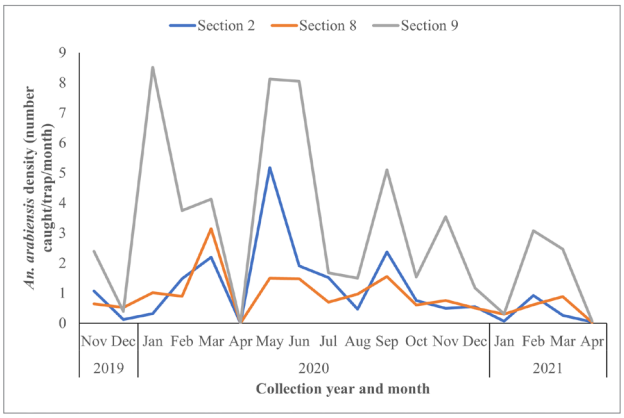

Figure 2: Mean number of *Anopheles arabiensis* collected per clay pot/month from Mamfene, northern KwaZulu-Natal, South Africa, November 2019 to April 2021, stratified by section.

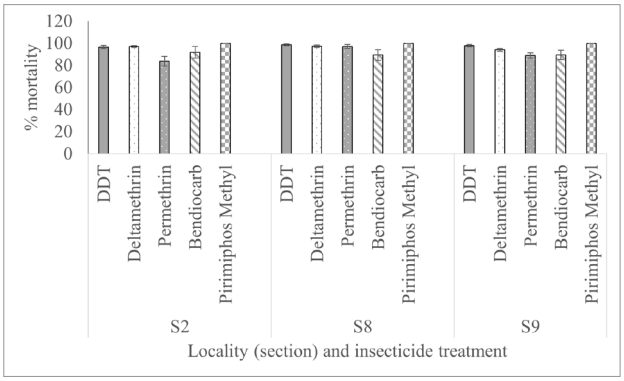

Figure 3: Insecticide susceptibilities of F1 *Anopheles arabiensis* derived from wild-collected material from Mamfene, northern KwaZulu-Natal Province, South Africa, 2019–2021. Mean mortalities (%) and standard errors are shown for each insecticide by Mamfene section (S2, S8, and S9).

Pre-exposure to the P450 synergist PBO caused a significant increase in permethrin-induced mortality in Sections 2 and 9, but not in bendiocarb-induced mortality in any of the sections. Pre-exposure to the general esterase synergist TPP also caused a significant increase in permethrin-induced mortality in all sections, and in bendiocarb-induced mortality in Sections 8 and 9 (Table 3). Median permethrin-induced mortality was significantly higher after PBO treatment (Mann–Whitney U=3, p=0.01, two-tailed) as well as after TPP treatment (Mann–Whitney U=0, p<0.01, two-tailed). PBO treatment did not result in a significant difference in bendiocarb-induced mortality (Mann–Whitney U=6, p=0.24, two-tailed). TPP treatment did, however, result in a significant increase in bendiocarb-induced mortality (Mann–Whitney U=2, p=0.03, two-tailed).

Discussion

Malaria vector surveillance in an elimination setting is specifically designed to collect information on a set of essential indicators – the most important being susceptibility to insecticides in those *Anopheles* populations implicated in disease transmission. Also important, therefore, are data on *Anopheles* species assemblages that can be used to assess malaria risk and receptivity, and to indicate which populations need to be prioritised for insecticide susceptibility assessments. This study presents a comprehensive survey of anopheline mosquitoes in northern KwaZulu-Natal Province and the most recent data on insecticide resistance in *An. arabiensis*, the primary vector of malaria there.

During the sampling period, 16 *Anopheles* species were collected. This level of diversity is comparable to a similar cross-seasonal anopheline survey conducted in the northern Kruger National Park where 9

Anopheles species were collected[24], and in the Limpopo Province where 20 species were collected[25]. *Anopheles arabiensis* was the most abundant member of the *An. gambiae* complex while *An. parensis* predominated in collections of the *An. funestus* group. The high density of *An. arabiensis* observed in this survey tallies with previous studies conducted between 2014 and 2015.[3] However, there was a notable difference in seasonal distribution between this study and a previous survey. The data presented here show higher numbers of *An. arabiensis* sampled during the winter months compared to the previous survey.[3] This could be due to uninterrupted mosquito surveillance throughout the year, although surveillance was scaled down during winter and no sampling was conducted in April 2020 due to COVID-19 restrictions.

Table 2: Combined insecticide susceptibilities of male and female F_1 *Anopheles arabiensis* derived from wild-collected material from three sections of Mamfene, northern KwaZulu-Natal Province, South Africa, 2019–2021. Mean percentage mortalities (%), standard errors (s.e.) and sample sizes (*n*) are given by sex.

Insecticide (class)	Number tested		24-h post-exposure % mortality ± s.e.		Susceptibility level
	Females	Males	Females	Males	
Pirimiphos-methyl (TP)	103	105	100 ± 0	100 ± 0	S
DDT (OC)	102	103	96.38 ± 1.55	98.07 ± 1.11	R
Deltamethrin (PYII)	121	113	92.6 ± 0.48	98.61 ± 0.39	R
Permethrin (PYI)	101	106	80.93 ± 4.33	94.52 ± 2.28	R
Bendiocarb (Carb)	117	114	72.1 ± 3.83	73.79 ± 6.21	R

TP, triphosphate; OC, organochlorine; PYII, pyrethroid class II; PYI, pyrethroid class I; Carb, carbamate; R, resistant; S, susceptible

Table 3: Insecticide (permethrin and bendiocarb) susceptibilities of F1 *Anopheles arabiensis* with or without pre-exposure to the P450 synergist piperonyl butoxide (PBO) or the general esterase synergist triphenyl phosphate (TPP). Mean mortalities (%), standard errors (s.e.) and sample sizes (*n*) are given by Mamfene section (S2, S8, and S9), northern KwaZulu-Natal Province, South Africa, 2019–2021

Treatment	S2	S8	S9
	% mortality ± s.e. (*n*)	% mortality± s.e. (*n*)	% mortality ± s.e. (*n*)
Permethrin	81.5 ± 1.76 (106)	95.24 ± 2.58 (112)	82.25 ± 1.82 (143)
Permethrin+PBO	98.96 ± 1.04 (100)	95.9 ± 1.36 (128)	96.36 ± 3.64 (122)
Permethrin+TPP	92.61 ± 3.08 (86)	98.68 ± 1.32 (91)	100 ± 0 (126)
Bendiocarb	91.88 ± 8.54 (113)	89.38 ± 0.53 (107)	84.67 ± 1.54 (130)
Bendiocarb+PBO	95.35 ± 2.69 (96)	95.48 ± 1.87 (93)	90.51 ± 9.48 (103)
Bendiocarb+TPP	100 ± 0 (81)	100 ± 0 (100)	96,74 ± 3.26 (101)

An interesting observation was the difference in trap productivity. Clay pots collected relatively high numbers of mosquitoes, re-emphasising their effectiveness as an *Anopheles* collection method. It is also notable that despite having access to only six disused tyres, these tyres collected over a third of the total collection, showing their potential as a sampling tool.

The perennial presence of the major vector *An. arabiensis* in northern KwaZulu-Natal indicates a high level of risk and receptivity to malaria. This receptivity is reinforced by the presence of secondary vectors such as *An. vaneedeni*, *An. parensis,* and *An. merus*, as well as several other *Anopheles* species that may also contribute to transmission, although none of the other species listed here have been directly implicated in malaria transmission in South Africa. Despite this high level of receptivity, malaria incidence in northern KwaZulu-Natal Province is currently very low, because of a scarcity of *Plasmodium* parasites for transmission, as a result of the IRS-based vector control programme and a well-developed case management system that includes active case detection in response to incidences of local transmission.

The continued presence of *An. arabiensis* in northern KwaZulu-Natal Province, despite a long history of IRS, may be attributable to the variable resting and feeding behaviours recorded for this species.[6,7,26] Female *An. arabiensis* will take blood meals from humans, livestock animals (especially cattle), and game animals such as buffalo. An important indicator of variability is also rooted in the methods used to collect samples of this species. Although *An. arabiensis* has been collected indoors (and outdoors) at other localities such as Tanzania[26], Ethiopia[6] and Malawi[7], all of the *Plasmodium*-infective *An. arabiensis* specimens collected in South Africa to date were found in outdoor-placed traps.[3,4] We do not know whether these specimens acquired their human blood meals indoors or outdoors, but their inclination to rest outdoors presumably made them substantially less susceptible to the insecticide deposits on sprayed walls indoors. Anecdotal evidence gathered over the last decade and based on periodic indoor searches in northern KwaZulu-Natal Province, shows that the IRS programme is particularly effective at controlling indoor-resting *Anopheles* mosquitoes because they are seldom, if ever, found inside sprayed houses in northern KwaZulu-Natal.

Evidence of ongoing resistance to several classes of insecticides in *An. arabiensis* in northern KwaZulu-Natal Province is of concern. The frequencies of resistance are, however, low. Previous analysis shows that the pyrethroid-resistant phenotypes inherent in this population are of low intensity and are, therefore, highly unlikely to be operationally significant.[17,20] These data also importantly show full susceptibility to pirimiphos-methyl, an insecticide that, along with DDT, is also indicated for use against pyrethroid-resistant *An. funestus* in southern Africa.[27-30]

An assessment of resistance mechanisms can yield important information on where cross-resistances between insecticide classes are likely, and on how quickly resistance might develop to high levels in an affected vector population under selection pressure. The synergist data given here suggests that cytochrome P450s (oxygenases) and general esterases are at least partially responsible for the pyrethroid- and carbamate-resistant phenotypes, although these data need to be interpreted with caution. This is because of the low-resistance frequencies recorded and the fact that enzyme synergists will always enhance the toxicity of insecticides, even in non-resistant mosquitoes. Nevertheless, resistance mechanisms based on enzyme-mediated detoxification have the potential to reach high levels of intensity that can lead to control failure. This includes the high-intensity pyrethroid resistance in *An. funestus* that has previously undermined vector control in South Africa and Mozambique.[2,13,16,31] Pyrethroid resistance in southern African populations of *An. funestus* is primarily based on P450 metabolism[32,33], bolstered by increased production of glutathione-S-transferases that likely protect against the oxidative damage caused by pyrethroid insecticides[34], and thickened cuticles that reduce the rate of insecticide absorption[35].

Conclusion

The northern regions of KwaZulu-Natal Province remain receptive to malaria transmission despite ongoing control and elimination interventions. This receptivity is due to the perennial presence of the major vector *An. arabiensis* and other secondary vector species whose populations include outdoor-resting components that are less susceptible to control by indoor residual spraying. The continued detection of low-frequency insecticide resistance phenotypes in *An. arabiensis* is cause for concern, and it is recommended that populations of this and other vector species be periodically monitored for changes in resistance frequency and intensity going forward.

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Competing interests

We have no competing interests to declare.

Authors' contributions

G.M. conceived and supervised the study, contributed to data analysis and reviewed the first and subsequent drafts of the manuscript. S.V.O. designed and performed the laboratory component of the study, analysed the data, and reviewed all manuscript versions. L.N.L. and Y.D.M. coordinated laboratory activities and reviewed the final draft of the manuscript. T.T.M. participated in field data collection, mapped study sites and provided comments in the final version of the manuscript. N.M. and D.M.D. led field data collection and provided comments in the final version of the manuscript. M.Z., F.M., B.D.L., J.Z., A.B. and A.M. generated laboratory-based data. M.K. participated in field activities and provided comments on the final version of the manuscript. B.D.B. drafted the manuscript and critically revised the final draft. All authors read and approved the manuscript.

References

1. South African Department of Health. Malaria elimination strategic plan for South Africa 2019–2023 [document on the Internet]. c2019 [cited 2021 Jun 10]. Available from: https://www.nicd.ac.za/wp-content/uploads/2019/10/MALARIA-ELIMINATION-STRATEGIC-PLAN-FOR-SOUTH-AFRICA-2019-2023-MALARIA-ELIMINATION-STRATEGIC-PLAN-2019-2023.pdf
2. Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J, Coetzee M. *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Med Vet Entomol. 2000;14(2):181–189. https://doi.org/10.1046/j.1365-2915.2000.00234.x
3. Dandalo LC, Brooke BD, Munhenga G, Lobb LN, Zikhali J, Ngxongo SP, et al. Population dynamics and *Plasmodium falciparum* (Haemosporida: Plasmodiidae) infectivity rates for the malaria vector *Anopheles arabiensis* (Diptera: Culicidae) at Mamfene, KwaZulu-Natal, South Africa. J Med Entomol. 2017;54(6):1758–1766. https://doi.org/10.1093/jme/tjx169
4. Burke A, Dandalo L, Munhenga G, Dahan-Moss Y, Mbokazi F, Ngxongo S, et al. A new malaria vector mosquito in South Africa. Sci Rep. 2017;7:43779. https://doi.org/10.1038/srep43779
5. Burke A, Dahan-Moss Y, Duncan F, Qwabe B, Coetzee M, Koekemoer L, et al. *Anopheles parensis* contributes to residual malaria transmission in South Africa. Malar J. 2019;18(1):257. https://doi.org/10.1186/s12936-019-2889-5
6. Ameneshewa B, Service MW. Resting habits of *Anopheles arabiensis* in the Awash river valley of Ethiopia. Ann Trop Med Parasitol. 1996;90(5):515–521. https://doi.org/10.1080/00034983.1996.11813077
7. Mburu MM, Zembere K, Mzilahowa T, Terlouw AD, Malenga T, Van den Berg H, et al. Impact of cattle on the abundance of indoor and outdoor resting malaria vectors in southern Malawi. Malar J. 2021;20(1):353. https://doi.org/10.1186/s12936-021-03885-x
8. Aranda C, Aponte JJ, Saute F, Casimiro S, Pinto J, Sousa C, et al. Entomological characteristics of malaria transmission in Manhica, a rural area in southern Mozambique. J Med Entomol. 2005;42(2):180–186. https://doi.org/10.1093/jmedent/42.2.180
9. Mouatcho JC, Hargreaves K, Koekemoer LL, Brooke BD, Oliver SV, Hunt RH, et al. Indoor collections of the *Anopheles funestus* group (Diptera: Culicidae) in sprayed houses in northern KwaZulu-Natal, South Africa. Malar J. 2007;6:30. https://doi.org/10.1186/1475-2875-6-30
10. Kamau L, Koekemoer LL, Hunt RH, Coetzee M. *Anopheles parensis*: The main member of the *Anopheles funestus* species group found resting inside human dwellings in Mwea area of central Kenya toward the end of the rainy season. J Am Mosq Control Assoc. 2003;19(2):130–133.
11. Gillies MT, Coetzee M. A supplement to the Anophelinae of Africa south of the Sahara. Johannesburg: South African Institute for Medical Research; 1987.
12. Raman J, Morris N, Frean J, Brooke B, Blumberg L, Kruger P, et al. Reviewing South Africa's malaria elimination strategy (2012-2018): Progress, challenges and priorities. Malar J. 2016;15(1):438. https://doi.org/10.1186/s12936-016-1497-x
13. Brooke BD, Kloke G, Hunt RH, Koekemoer LL, Temu EA, Taylor ME, et al. Bioassay and biochemical analyses of insecticide resistance in southern African *Anopheles funestus* (Diptera: Culicidae). Bull Entomol Res. 2001;91(4):265–272. https://doi.org/10.1079/ber2001108
14. Brooke B, Koekemoer L, Kruger P, Urbach J, Misiani E, Coetzee M. Malaria vector control in South Africa. S Afr Med J. 2013;103(10 Pt 2):784–788. https://doi.org/10.7196/samj.7447
15. Coetzee M, Kruger P, Hunt RH, Durrheim DN, Urbach J, Hansford CF. Malaria in South Africa: 110 years of learning to control the disease. S Afr Med J. 2013;103(10 Pt 2):770–778. https://doi.org/10.7196/samj.7446
16. Brooke BD, Robertson L, Kaiser ML, Raswiswi E, Munhenga G, Venter N, et al. Insecticide resistance in the malaria vector *Anopheles arabiensis* in Mamfene, KwaZulu-Natal. S Afr J Sci. 2015;111(11/12), Art. #2015-0261. http://dx.doi.org/10.17159/sajs.2015/20150261
17. Venter N, Oliver SV, Muleba M, Davies C, Hunt RH, Koekemoer LL, et al. Benchmarking insecticide resistance intensity bioassays for *Anopheles* malaria vector species against resistance phenotypes of known epidemiological significance. Parasit Vectors. 2017;10(1):198. https://doi.org/10.1186/s13071-017-2134-4

18. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am J Trop Med Hyg. 1993;49(4):520–529. https://doi.org/10.4269/ajtmh.1993.49.520

19. Hunt RH, Brooke BD, Pillay C, Koekemoer LL, Coetzee M. Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector *Anopheles funestus*. Med Vet Entomol. 2005;19(3):271–275. https://doi.org/10.1111/j.1365-2915.2005.00574.x

20. World Health Organization (WHO). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2nd ed. Geneva: WHO; 2016. Available from: https://apps.who.int/iris/handle/10665/250677?search-result=true&query=Test+procedures+for+insecticide+resistance+monitoring+in+malaria+vector+mosquitoes&scope=&rpp=10&sort_by=score&order=desc

21. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965;52:591–611. https://doi.org/10.1093/biomet/52.3-4.591

22. Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. J Am Stat Assoc. 1952;47(260):583–621. https://doi.org/10.1080/01621459.1952.10483441

23. Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. Ann Math Stat. 1947;18(1):50–60. https://doi.org/10.1214/aoms/1177730491

24. Munhenga G, Brooke BD, Spillings B, Essop L, Hunt RH, Midzi S, et al. Field study site selection, species abundance and monthly distribution of anopheline mosquitoes in the northern Kruger National Park, South Africa. Malar J. 2014;13:27. https://doi.org/10.1186/1475-2875-13-27

25. Braack L, Bornman R, Kruger T, Dahan-Moss Y, Gilbert A, Kaiser M, et al. Malaria vectors and vector surveillance in Limpopo Province (South Africa): 1927 to 2018. Int J Environ Res Public Health. 2020;17(11):4125. https://doi.org/10.3390/ijerph17114125

26. Charlwood JD, Kessy E, Yohannes K, Protopopoff N, Rowland M, LeClair C. Studies on the resting behaviour and host choice of *Anopheles gambiae* and *An. arabiensis* from Muleba, Tanzania. Med Vet Entomol. 2018;32(3):263–270. https://doi.org/10.1111/mve.12299

27. Hunt R, Edwardes M, Coetzee M. Pyrethroid resistance in southern African *Anopheles funestus* extends to Likoma Island in Lake Malawi. Parasit Vectors. 2010;3:122. https://doi.org/10.1186/1756-3305-3-122

28. Sande S, Zimba M, Chinwada P, Masendu HT, Mazando S, Makuwaza A. The emergence of insecticide resistance in the major malaria vector *Anopheles funestus* (Diptera: Culicidae) from sentinel sites in Mutare and Mutasa Districts, Zimbabwe. Malar J. 2015;14:466. https://doi.org/10.1186/s12936-015-0993-8

29. Chanda J, Saili K, Phiri F, Stevenson JC, Mwenda M, Chishimba S, et al. Pyrethroid and carbamate resistance in *Anopheles funestus* Giles along Lake Kariba in Southern Zambia. Am J Trop Med Hyg. 2020;103(2_Suppl):90–97. https://doi.org/10.4269/ajtmh.19-0664

30. Wagman JM, Varela K, Zulliger R, Saifodine A, Muthoni R, Magesa S, et al. Reduced exposure to malaria vectors following indoor residual spraying of pirimiphos-methyl in a high-burden district of rural Mozambique with high ownership of long-lasting insecticidal nets: Entomological surveillance results from a cluster-randomized trial. Malar J. 2021;20(1):54. https://doi.org/10.1186/s12936-021-03583-8

31. Riveron JM, Huijben S, Tchapga W, Tchouakui M, Wondji MJ, Tchoupo M, et al. Escalation of pyrethroid resistance in the malaria vector *Anopheles funestus* induces a loss of efficacy of piperonyl butoxide-based insecticide-treated nets in Mozambique. J Infect Dis. 2019;220(3):467–475. https://doi.org/10.1093/infdis/jiz139

32. Amenya DA, Naguran R, Lo TC, Ranson H, Spillings BL, Wood OR, et al. Overexpression of a cytochrome P450 (CYP6P9) in a major African malaria vector, *Anopheles funestus*, resistant to pyrethroids. Insect Mol Biol. 2008;17(1):19–25. https://doi.org/10.1111/j.1365-2583.2008.00776.x

33. Riveron JM, Ibrahim SS, Chanda E, Mzilahowa T, Cuamba N, Irving H, et al. The highly polymorphic CYP6M7 cytochrome P450 gene partners with the directionally selected CYP6P9a and CYP6P9b genes to expand the pyrethroid resistance front in the malaria vector *Anopheles funestus* in *Anopheles arabiensis* Africa. BMC Genomics. 2014;15(1):817. https://doi.org/10.1186/1471-2164-15-817

34. Oliver SV, Brooke BD. The role of oxidative stress in the longevity and insecticide resistance phenotype of the major malaria vectors and *Anopheles funestus*. PLoS ONE. 2016;11(3), e0151049. https://doi.org/10.1371/journal.pone.0151049

35. Wood O, Hanrahan S, Coetzee M, Koekemoer L, Brooke B. Cuticle thickening associated with pyrethroid resistance in the major malaria vector *Anopheles funestus*. Parasit Vectors. 2010;3:67. https://doi.org/10.1186/1756-3305-3-67

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Impact of land-use changes on ant communities and the retention of ecosystem services in Rashad District, Southern Kordofan, Sudan

The ecological consequences of biodiversity loss are usually the reduction of ecosystem functions. These responses, however, differ depending on the type of land-use change and the ecological setting. We investigated the impact of land-use type and ecosystem functions on the ant assemblage of Rashad District, Sudan. We analysed the effects of three different land uses (soy monoculture, pasture and organic production of vegetables) on the ant community by assessing ant composition in 176 different locations. The collection sites were conventional soy monoculture, pastures, organic agriculture, and native vegetation such as Campo, Kubos, and forests. We recorded 264 ant species on the soil surface of the Rashad District, where 342 to 354 species were thought to exist. Pastures and organic agriculture areas have 61% and 56% of the native myrmecofauna, respectively, while conventional soy monoculture areas are home to only 17% of native ant species. Forest areas present a unique community, and soy monoculture areas have the strongest pattern of biotic homogenisation. We also detected that rare species (of low frequency) were the chief promoters of richness in the Rashad District, and the most threatened with local extinction, due to their low density and low occurrence in agrosystems. Overall, we found that agricultural expansion reduces ant diversity, particularly in soybean crops, and can affect ecosystem functions. To mitigate the reduction in the ant assemblage, we recommend the conservation of multiple natural habitats.

Significance:

- Agricultural land conversion and climate change play a major role in shaping tropical landscapes, but the direct and indirect links to biodiversity and species community composition remain poorly understood.
- Ant richness is correlated with biomass, demonstrating that the effects on ecosystem function are dependent on the particularities of each assessed function (such as resource type), the types of land uses, and the abundance of ants in the region.
- Land-use effects on ant diversity were strongly scale dependent.
- The highest ant diversity occurred in soy monoculture areas.

Introduction

Conservation efforts have primarily focused on plant and vertebrate groups. Norris et al.[1], for example, determined the planet's 'hotspots' using only vascular plants and vertebrates. Although these groups are the most well known in terms of described species, they account for a small proportion of biodiversity.[2] Insects, which account for the majority of animal biodiversity, have received little attention in conservation studies, possibly due to the large number of species and/or the difficulty in identifying these species, which are frequently unnamed.[2]

Insects play a significant role in ecosystems through influencing the composition and size of populations of plants, herbivores, predators and detritivores.[3,4] Insect communities are influenced by fire[5,6], vegetation heterogeneity[7], seasonality[8], fragmentation[9], exotic species, and the effects of change in land use, more especially in recent decades, among other factors[10,11].

Land conversion to agrosystems has been viewed as detrimental to biodiversity conservation.[12]. The agricultural systems are derived from conversion of complex natural ecosystems to simplified ecosystems through intensive use of machinery, chemical and biological inputs, as well as improved and changed crops.[10,13,14] These agrosystems are a major cause of biodiversity loss because of fragmentation and destruction of native vegetation.[15] However, the effects of land-use change on native fauna depend on the size of divergence of pre- and post-land-use conversion ecological conditions.[16] There are thus agricultural systems that have a high impact on native fauna and flora, and other types of low-impact agricultural systems, which can play a key role in species conservation[17], as the landscapes under low-impact agriculture can harbour much of the world's biodiversity[14]. According to Gareng[13], before 1970, land use in the Rashad District was primarily cattle production over natural pastures. Since then, there has been an intense expansion of mechanised agricultural output to export soy and corn. Due to agricultural expansion, an estimated 40% to 80% of the natural Rashad area has been converted to agrosystems during the last five decades. However, these estimates are highly variable due to difficulties in discriminating in satellite images of areas of native vegetation and pasture areas.[13]

Recent research indicates that shifting land use to simpler and more intensively managed systems, such as soybean or corn plantations, reduces ant richness.[10,18] Less-intensive agrosystems, such as pasture, may have a species richness comparable to that of natural areas[18], demonstrating that agricultural production areas are used for conservation of at least part of the biodiversity and/or are managed to reduce species loss. However,

the presence of many native species in some types of agriculture does not guarantee the integral conservation of biodiversity. According to Cingolani et al.[19], the number of species in a region (gamma diversity) is determined by the number of species in a location (alpha diversity) plus the exchange of species with other locations (beta diversity). It is critical to understand how diversity is distributed in the landscape and to determine conservation actions.

Rubene et al.[20] developed a method for deconstructing beta diversity or species exchange between sites into two distinct processes, which they refer to as species replacement or species loss. The beta diversity increases as a result of species exchange between sites (by different species) or because of a difference in the number of species due to nesting. This is nothing more than a pattern in which the community with fewer species is a subset of the communities with more species.[21,22]

According to Ebenman and Jonsson[23], as the structure of the vegetation became more complex, the number of ant species increased, beginning with a gradient from footpath to Rashad. However, preserving only these complex habitats (with more species) far from ensures the full conservation of ant species, because the exchange of species between Rashad vegetation types accounts for 50% of the richness (beta diversity), and different areas of the same physiognomy account for 25%.[23] These studies were limited to a few areas, and the diversity patterns, as well as the ability of these species to maintain themselves in different environments altered by humans, have yet to be evaluated. The aim of this study was to determine how changes in land use affect the ant community and its ecological functions. Furthermore, we aimed to determine how the conversion of native vegetation affects the richness and composition of ant species based on the type of land use. We evaluated soy monoculture, pasture and organic agriculture in terms of patterns of local diversity, exchange of species between places, and regional diversity.

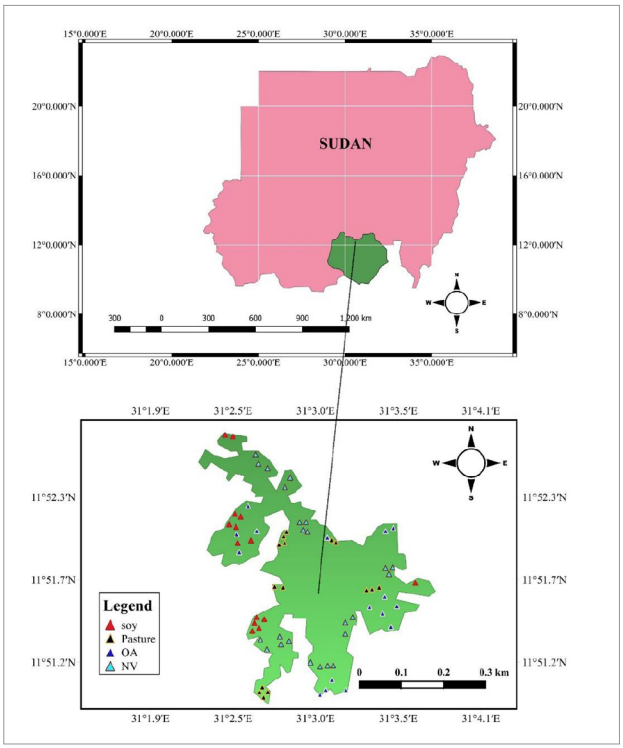
Materials and methods

Study area

This study was conducted in the Rashad District from February to June 2019 and February to May 2020. The study area was 7872 km^2, and was located in the centre of the Kordofan State between latitudes 10° and 13° N and between longitudes 29° and 33° E) (Figure 1). The study site included areas such as pasture, soy monoculture and organic agriculture, as well as areas of native vegetation (see Supplementary table 1). In total, 69 sites were sampled: 14 conventional soy monoculture sites, 15 pastures, 16 organic agriculture areas (14 vegetable plantations and 2 organic soy plantations, OA-15 and OA-16) and 24 native vegetation areas. From the 24 areas of native vegetation, we chose 7 rural formations, 8 Kubos areas (old-growth forests), and 9 forest formations to collect the three most common phytophysiognomies. The annual rainfall of the study area is between 500 mm and 800 mm. The majority of the basement complex in the area is overlaid with Nubian sandstone. Vegetation cover in the Rashad area consists of legumes dominated by the *Acacia* genus annual grasses, and shrubs. The vegetation, on the other hand, varies with rainfall patterns and soil structure. According to Hassan and Ibrahim[24], the total population in the study area is estimated to be 240 000 persons. There are two main livelihood groups in the study area, including minor livestock holders and agriculturalists.

Sample design

We sampled three 20-m-long transects in each location (sample plot) (Figure 2a). Each sample plot was made up of a grid of 12 pitfall traps, which were arranged in three rows with four traps each, with a 2-m spacing between them (Figure 2b). The pitfall traps used were 200-mL plastic cups filled up to 1/3 with water and detergent, and were active in the field for 24 h. There were 12 pitfalls per plot, 36 per location, and 2484 in 69 locations (Figure 2c).



Habitats: OA, organic agriculture; soy, soy monoculture; NV, Native vegetation

Figure 1: Map of the study area showing distribution of sampling sites.

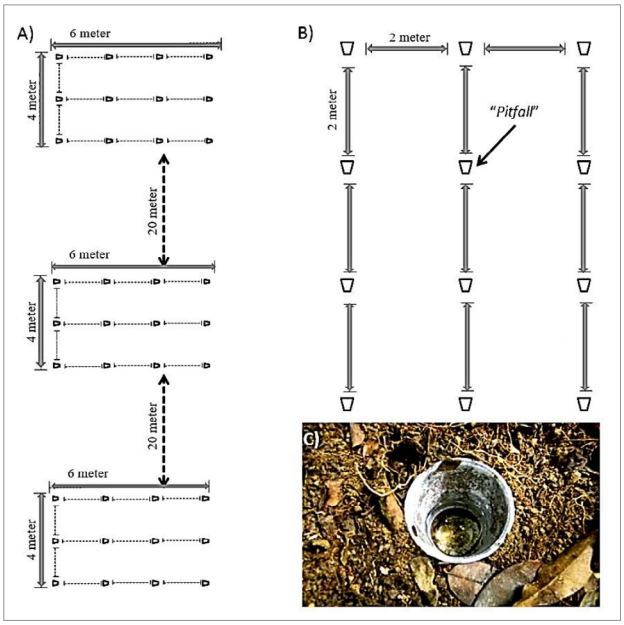


Figure 2: Method used to collect ants. (A) Distribution of sample plots in each location. (B) Distribution of pitfalls in each sample plot. (C) Photograph of a pitfall in the field.

Before sorting, the contents of each trap were sieved (in a fine mesh to reduce the loss of tiny invertebrates) and preserved in a container filled with 75% alcohol. We combined all 12 pitfalls from each sample plot into a single composite sample to shorten the screening time. As a result, each locality has three sub-samples, each of which was screened for Formicidae. Specimen processing and identification included keys used for genus and species level identification, comparison with voucher specimens, and comparison with type images available at AntWeb and AntWiki. To reduce the impact of the weather on the sampling, we

collected data on days when rain was not forecast. Furthermore, we sampled two to three locations at the same time (Supplementary table 1), diluting any climatic effects amongst the many categories investigated.

Data analysis

We compared the richness of ant species between native vegetation environments and different agricultural systems in order to estimate the potential loss of species due to conversion of native areas into agricultural systems. We plotted species accumulation curves and estimated the richness using Jack1 and Chao2 to determine how much of the expected total number of ant species was sampled and whether the richness per habitat type differed between systems. EstimateS 9.1.0[25] was used for all of the analyses. To determine whether changes in land use affected the richness of ants in the locality (alpha diversity), an ANOVA with a Tukey post-hoc test[26] was used, with the habitat factor being divided into 'native vegetation', 'pasture', 'soy monoculture', and 'organic agriculture'.

Species composition was analysed between habitats presence-absence matrix by location (176 species x 69 locations). The first analysis was a PERMANOVA, with 999 permutations, using the adonis function of the vegan R package[27] (R Development Core Team 2016). We used this analysis because it is the most robust for unbalanced data.[28] However, as the PERMANOVA analysis cannot determine which habitats differ from one another, non-metric multidimensional scaling was performed[29] using the metaMDS function of the vegan package[27], grouping the collection points by their similarities. Indicator value (IndVal) analysis was used to show associated species and a particular habitat with a significance value.[30] The interval function of the labdsv package was used for this final analysis.[29] Aside from difference in species occurrence, we did a functional group analysis, adapting the classification proposed by Divieso et al.[31] The ant species were classified into:

1. Arboreal – species that nest in tree and shrub vegetation
2. Attini – a tribe that includes all ants that grow on fungus
3. Camponotini – a tribe that includes all species of the genus *Camponotus*
4. Correction – a group of migratory species that forage in large columns (many individuals, one next to the other), and are incredibly greedy, with records of predation on small rodents
5. Specialist – a group of species that have particular morphological or ecological characteristics (in general, their recording frequency is low, with most predators being specialists of a specific taxon)
6. Generalist – a group that includes species with great ecological and omnivorous plasticity, composed of most species of the genera *Pheidole*, *Solenopsis*, and *Brachymyrmex*, in addition to exotic species
7. Dominant generalist – a group composed of three omnivorous species with characteristic large recruitment of individuals to monopolise the resource found in relation to other competitors
8. Opportunistic – a group capable of foraging, which forages in the warmer periods and monopolises the food source in the face of competitors
9. Large predator – large species, some of which are specialist predators

Following classification, we assessed the frequency of occurrence of each functional group in different habitats. For that, we analysed generalised linear models using the glm function of R.[32] In this analysis, we counted the number of records per location and recorded the number of plots including each species within each location.

To assess the richness of ant species, an additive partition diversity analysis was used at three hierarchical levels: sample plot, location, and habitat type. The sum of alpha diversity (average number of species in the sample plot) plus each of the components of beta diversity (number of different species between locations and habitat types) must equal the total diversity of ant species collected (i.e. 176). We created a null model to see if any factor was influencing ant diversity by changing the values of alpha and beta diversity more than what would be expected at random. We created this null model by randomising the data in the field 2000 times, and from these randomisations, generating a pattern that we defined as random. After this step, we compared the pattern found in the field, with the pattern considered at random; if the probability of the pattern found in the field had a less than 5% chance of having occurred due to chance, we considered this pattern to be different, and thus influenced by some factor.[33] For this type of analysis, the adipart function of the vegan package was used.[27]

Beta diversity (β) is divided into two components that are distinct ecological processes: nesting (βnes) and species exchange (βsim).[21] We calculated β diversity pairwise between the localities with Sorensen (βsor) and Simpson (βsim) indexes. We calculated βsor by taking into account the species identity and incorporating information from both the turnover and species nesting. βsim was calculated from the probability of capturing a specific species as a function of the total species (regardless of their identity), and calculating only the species exchange. Therefore, the difference between the calculated indices provides the beta diversity value due to nesting[21]:

$$\beta nes = \beta sor - \beta sim$$

To detect differences between agrosystems and native vegetation, we made comparisons between the values of βsor, then between those of βsim and between those of βnes, using T-tests.[21] We made the same comparison between habitats (soybean, pasture, organic agriculture, field, savanna, and forest), but using the Kruskal–Wallis non-parametric test with Kruskal.Nemenyi post-hoc test of the package PMCMR.[34] In this Kruskal–Wallis test, equal values within the database were ranked randomly to avoid nodes (ties) in the analysis. Then, using the Mantel analysis in the vegan package, compare the dissimilarity matrix generated by the beta. A multifunction and a distance matrix between the locations were used to determine whether the distance between the points affects the species exchange. Where the result was significant, we performed a Pearson correlation to determine the direction and strength of the relationship.

Results

Diversity of ant assemblages

In total, 176 ant species were recorded, accounting for 74% to 78% of the species estimated on the soil surface for the Rashad area using the diversity estimators Jack1 (342 species) and Chao2 (354 species). An average of 3965 ants (in 58 genera and 8 subfamilies) were recorded from the sites (Supplementary table 1). Myrmicinae was the most diverse subfamily (101 species), and Tetramorium was the most diverse genus (27 species) (occurring in 97% of the locations). The subfamily Formicinae was the second most diverse (34 species), and had the second most diverse genus, *Crematogaster* (10 species). Genera *Cataulacus*, *Monomorium* and *Pheidole* also are notable for having between 17 and 10 species, all belonging to the subfamily Myrmicinae, except for *Brachymyrmex* of the subfamily Formicinae.

Ant species richness in different land-use classes

Species richness at the site scale was highest in soy monoculture areas (83%), followed by organic farming areas (44%), and pastures (39%) (Figure 3). These values are comparable with the values calculated using the Jack1 wealth estimator, that is, 40% for pasture, 47% for organic agriculture areas and 82% for soy monoculture (Figure 4).

The analysis of local wealth, which corresponds to the average number of species collected in each of the 69 locations, also reveals that there is a difference between areas of native vegetation and organic agriculture systems. Soy monoculture areas had fewer species than organic agriculture systems and pastures ($F_{3,65}=66.96$; $p<0.001$), which had the same number of species (Figure 5). Areas of native vegetation had more species than all managed areas combined.

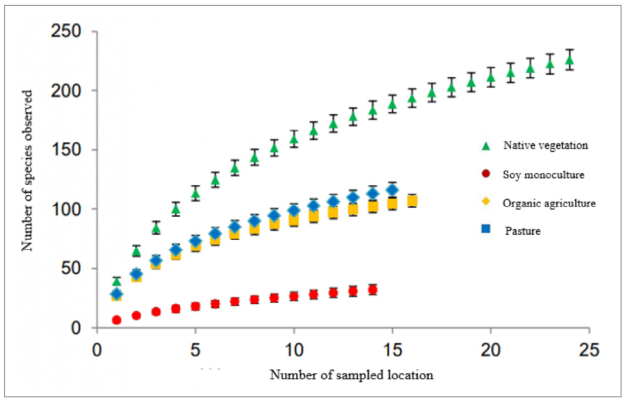


Figure 3: Accumulated number of ant species by sampled locations with the respective standard deviation based on data collected between May 2019 and August 2020 in the Rashad District.

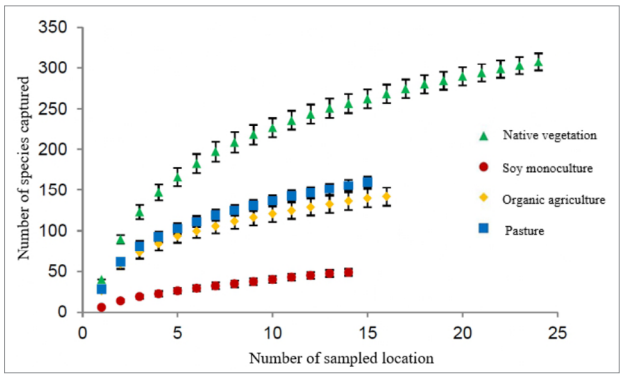


Figure 4: Estimated number of ant species by number of sampled locations using the Jack1 estimation with the respective standard deviation based on collections made between June 2019 and August 2020 in the Rashad District.

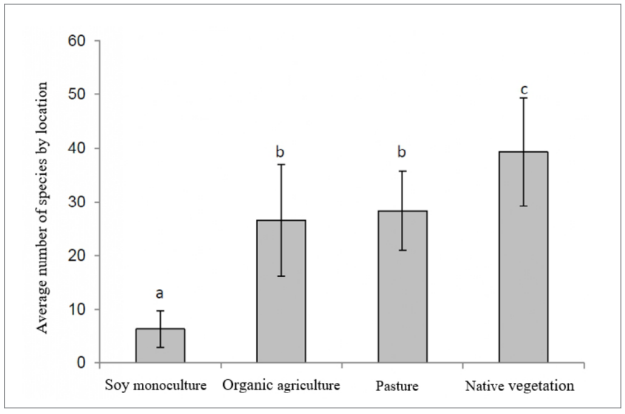


Figure 5: The average number of ant species per location, collected in different habitat types in the Rashad District between June 2019 and August 2020 using a pitfall trap. The bars represent the standard deviation and different letters indicate significant differences ($p<0.05$).

We found that the presence of rare species influenced both the richness and composition of species in native vegetation areas (Figure 6 and Figure 7). We classified rare species as those that occurred only once in a specific type of organic agriculture and native vegetation phytophysiognomy, and unique species as those that appeared only once throughout the study. Unique species accounted for 30% of the species found in both agrosystems and native vegetation areas (Figure 6). However, areas of native vegetation had more unique species than did organic agriculture systems (Figure 7). It is also worth noting that species with a high frequency of occurrence were sampled, that are capable of surviving both in areas of native vegetation and in organic agriculture, but as the species' frequency decreased, they showed a preference or exclusivity for a specific type of habitat.

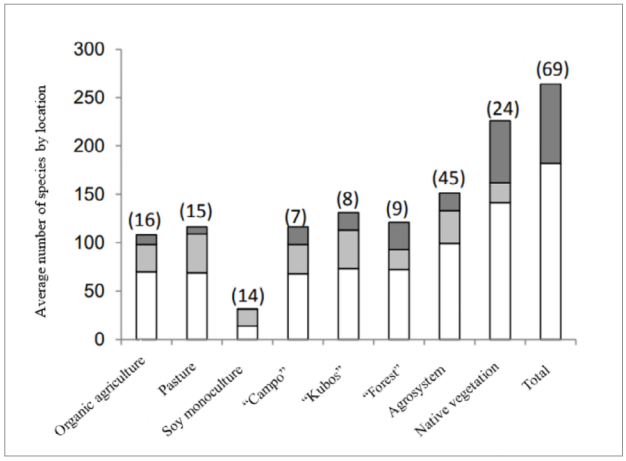


Figure 6: Occurrence of common, rare and unique species collected in the Rashad District between June 2019 and August 2020 with pitfall traps in different types of habitats. Numbers in parentheses indicate the number of sampled locations in each habitat. White bars indicate species considered typical, that is, that occurred more than once within the habitat; light grey bars indicate rare species that occurred only once in the habitat but that occurred in other habitats; and dark grey bars indicate species that occurred only once in the entire study. 'Campo' refers to rural formations, 'Kubos' refers to old-growth forests, and 'Forest' refers to forest formations.

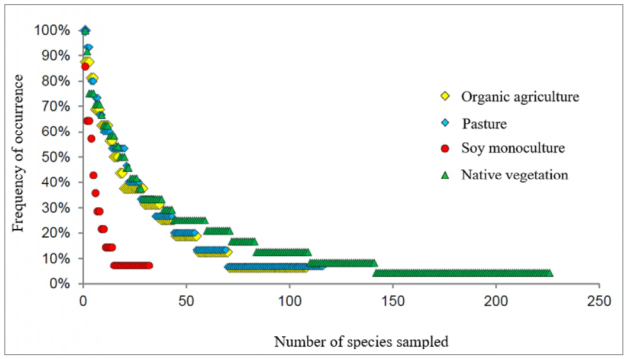


Figure 7: Frequency of occurrence of species collected in the Rashad District between June 2019 and August 2020 using pitfall traps in different habitat types. The species were ordered in descending order, according to their frequency of occurrence in the 69 sampled locations.

The PERMANOVA analysis revealed a significant difference in species composition ($F_{3,68}=10.697$; $p<0.001$). The ranking (Figure 8) demonstrates that, in addition to richness, there are differences between the species composition of native vegetation systems and that of organic agriculture systems. There is a visible gradient of change, with soy monoculture and forest areas having distinct communities (different from all others). Furthermore, two additional groups formed Rashad and the countryside, with two natural phytophysiognomies that are much more similar to each other than between forest areas. Pasture and organic agriculture areas also have a similar community.

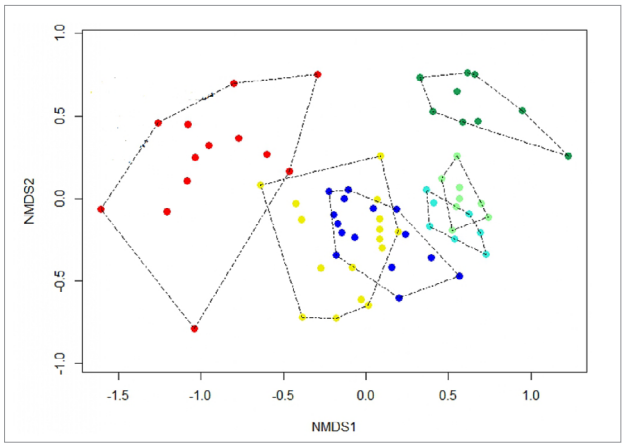


Figure 8: Non-metric multidimensional scaling (NMDS) based on the presence and absence matrix approximates the locations with the composition of ants most similar to each other. Forest (green), soy monoculture (red), pasture (blue), Kubos (light green), Campo (cyan), and organic agriculture (yellow) species.

We found further evidence of the change in the pattern of distribution and abundance of species in the results obtained from the Indval analysis, which detected 76 species with significant indication values, ranging from 1% to 75% (Supplementary table 2). For example, *Pheidole andrieui* has a 59% chance of occurring in a soybean monoculture area, *Pheidole termitophila* has a 61% chance of occurring in organic farming areas, and *Dorylus aethiopicus* includes a 46% chance of occurring in pastures. It is important to note that the number of indicator species in native phytophysiognomies is almost three and a half times higher than that in agricultural areas.

It is also obvious that the conversion of native areas to agricultural systems had an impact on functional groups or the type of land use (Figure 9). Of the several functional groups, 'Correction' was the only group that showed no significant difference in occurrence between the different habitats; however, this is a nomadic and rare group. The 'Dominant generalists' were the only group for which there was a higher number of records in organic agriculture and pasture when compared to native vegetation. Native vegetation areas had higher numbers than agricultural systems for all other groups except 'Generalists' and 'Opportunists'.

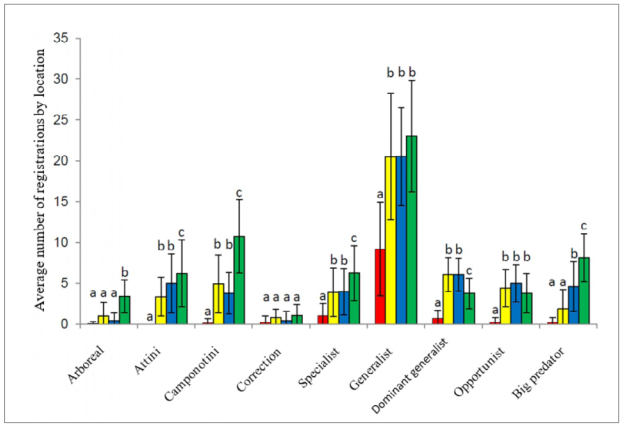


Figure 9: The average number of records per location of ants belonging to different functional groups, collected between June 2019 and August 2020 in the Rashad District using pitfall traps. Error bars indicate standard deviations and different letters indicate significant differences ($p<0.05$) between habitats.

When we analysed additive diversity partition (Figure 10) with all hierarchical systems and phytophysiognomies, we observed that the alpha richness (α1) is greater than expected (5.8%). The exchange of species between the plots of each location is 29% lower than expected, and the exchange of species between locations within the same system or phytophysiognomy was 14% lower than expected. The exchange of species between the different systems and phytophysiognomies (β3) had the greatest importance for the pattern of diversity observed, being 15.9% greater than expected at random.

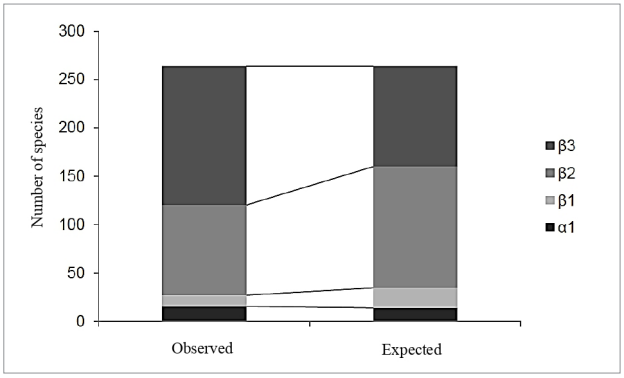


Figure 10: Analysis of additive partition of ant diversity in the Rashad District. α1 is the average number of species in the plots. β1 is the exchange of species between plots within the exact location. β2 is the exchange of species between locations belonging to the same type of agroecosystem or phytophysiognomy. β3 is the exchange of species between different types of agroecosystems and phytophysiognomies. For all observed values, the difference was significant ($p<0.001$) compared to the expected model.

We also performed additive partition of diversity analyses for each system or phytophysiognomy (Supplementary figure 1). The additive partition patterns of diversity are similar, regardless of the system or phytophysiognomy. All of them differed from what we expected at random, withthe alpha diversity (richness within each plot) about 2.3% higher than expected. Beta1 diversity, or the difference in species between pitfall plots within each location, is 4.1% lower than expected, indicating homogeneity on this scale. The exchange of species between locations is 4.1% higher than expected; beta diversity (βsor) may group two distinct processes: the exchange of species (turnover) and/or nesting (loss of species). The analysis of multiplicative diversity separated these two factors, revealing that the majority of the diversity is due to the exchange of species (βyes), with little influence from nesting (βnes) (Figure 11). However, nesting for agrosystems is three times that of native areas, indicating greater homogenisation in agricultural areas. In addition, Mantel's analysis between the level of dissimilarity and the distance between the points shows that, only for the agrosystems, the greater the distance, the greater the exchange of species (Figure 12).

Discussion

Effects on richness

The findings show that converting native areas of the Rashad District into agrosystems resulted in the loss of local diversity, with the magnitude of this loss determined by the type of agrosystem used, as soy monocultures caused a greater loss of diversity than pastures or organic agriculture. Ribeiro et al.[35] use the term 'divergence from ecological conditions' to explain why some land uses have a greater impact on biodiversity than others. This term attempts to portray that the lower the expected diversity is, the more different the post-conversion condition is from the original vegetation simplifying or increasing the intensity of system management. These findings are consistent with those found in the scientific literature; several studies with various taxa show that conversion of natural habitats and intensification of agrosystems leads to species reduction and changes in composition, as well as changes in the structure and functioning of the ecosystem.[36-38] Among the several

examples in the literature of how conversion of native areas for food production affected the local fauna, perhaps the best studied is that of coffee production in Latin America, according to which a reduction in the diversity of ants was noted in a review by Eldridge et al.[39] However, the magnitude of this reduction was dependent on the type of land management (monoculture of coffee or coffee in the shade of native trees), in addition to considering ecological services.[14,40] Similar patterns of reduction in ant diversity or richness in planting systems were also found in the Amazon[41], Argentina[42,43], Australia[44], Africa[45], and China[46]. Despite the fact that the loss of species due to conversion of native vegetation into agrosystems is a well-known global occurrence, the magnitude of this effect and its interaction with the different agrosystems remain unknown. The reduction in diversity observed in the current study is greater than that described by Bremer and Farley[10] and Moranz et al.[11]

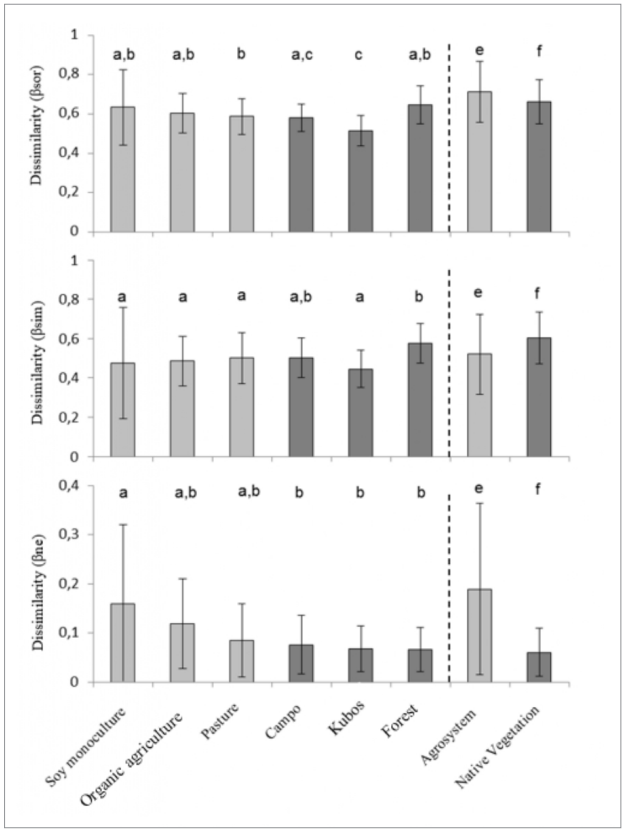


Figure 11: Values of beta diversity based on data of presence and absence of ant species collected using pitfall traps between June 2019 and August 2020 in the Rashad District. βsor represents the sum of βsim and βnes.

This work depicts that soy monocultures – with their application of pesticides, ploughing and harrowing the soil, off-season periods without live soil cover and pre-planting desiccation – are forms of management that make these fields environments that are difficult for pest insects to survive in (which is the main reason for some of these management activities, such as application of pesticides and pre-planting desiccation), but consequently make it a difficult environment for insects to survive in. Many authors have used the term 'intensification of land use' to describe this process, which is a way of characterising the management of these areas as a cause of the different riches found in these environments, regardless of the crop planted.[12]

Pastures are not managed, and thus have a greater diversity of ant species than soybean fields (as previously described by Bremer and Farley[10]), as grazing has little effect on ant diversity.[47] The most important effect in these areas is the indirect effect of occupation of exotic grass species, which outcompete native grasses and reduce floral and faunal diversity.[48]

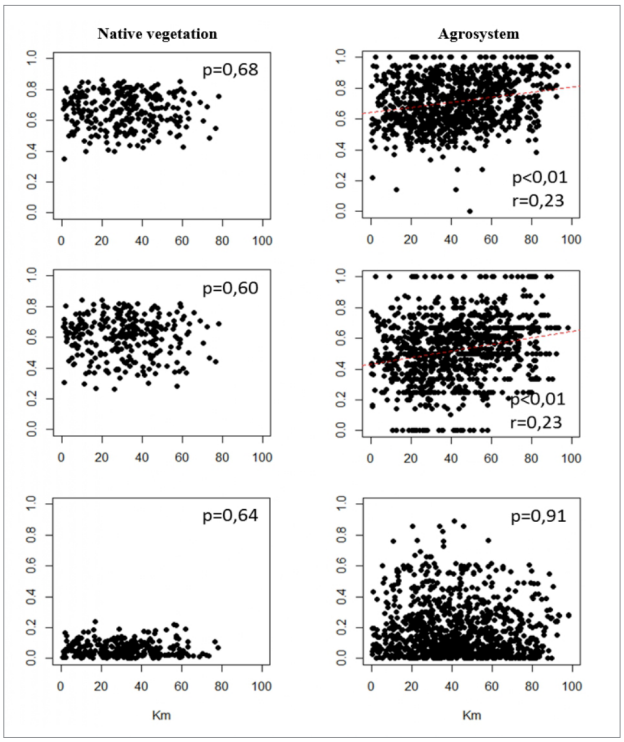


Figure 12: Relationship between ant fauna dissimilarity (βsor, βsim, and βnes) and the distance between the locations of native vegetation and agrosystems. Pearson's correlation (r) and the significance computed using Mantel's analysis are displayed within the graphs. The points correspond to the comparison values between the sampling locations.

Organic agriculture areas are as diverse as grazing areas, suggesting that pesticides, as well as management techniques, reduce diversity in conventional organic agriculture areas.[49] In addition to the restriction in using pesticides, organic areas have a high level of local heterogeneity, planting of flowers to provide an alternative resource for predators, and year-round irrigation to ensure that there is always green matter, and herbivores and other organisms that can serve as a source of food for ants (Eisawi, personal observation). Furthermore, unlike what is found in soy monocultures, organic agriculture areas have bands of land with perennial plants and trees where the soil is not disturbed, and which act as a refuge for ants that nest in the soil or in the trees themselves.[10]

Effects on composition

In terms of composition, the species that persist in organic agriculture were distinguishable by being generalists, recruiting to monopolise available resources in the field and/or exhibiting dominant behaviour, such as the genera *Pheidole* and *Solenopsis*.[11] Other common species in the studied agrosystems are opportunistic ants with high temperature tolerance, such as those of the genera *Dorymyrmex* and *Linepithema*.[50,51] In addition to these, there are tiny generalist ants (~1.5 mm) such as *Brachymyrmex* and the so-called 'thief ants', also of the genus *Solenopsis*, which occur in both natural and disturbed environments, including residences in urban areas.

Arboreal ants, large predators, specialists and the Attini and Camponotini tribes suffered a reduction in their occurrence with conversion of native areas into organic agriculture systems (Figure 8). This reduction is due to both environmental changes and, with ants belonging to the Attini tribe, to direct combat (that is, management to reduce the population). It is also clear that the type of land use affects the occurrence of certain functional groups of ants to varying degrees of magnitude. Soy planting had the most negative effects on almost all functional groups, whereas organic agriculture and pasture had intermediate effects. However, we associate the significant difference found in the composition of

ants between agrosystems and native vegetation with rare species or those that occurred only once. As shown in Figure 6, areas of native vegetation have 3.5 times more unique species (sampled only once) than agrosystems, despite the fact that the sampling effort in these areas was nearly half that of agrosystems. These rare species show some aspect of morphology, ecology or specialised behaviour.[52,53] Mohammed et al.[54] demonstrated that the Rashad ant fauna is characterised by a large proportion of rare ants (45% of the species occur in less than 5% of the samples), compared to the Australian savannah (where 27% of the species are rare). This makes the advance of agrosystems over native areas even more worrying, because rare species are the most vulnerable to extinction due to their low density and limited ability to persist in disturbed environments.[55]

Many studies have discussed the factors that can limit the colonisation of an agricultural area, and most seem to assume a limiting factor is the nesting place, which can be, in addition to the soil, litter, branches fallen on the soil, and the stratum.[56,57] Conservationists should emphasise the change in composition caused by the collected native phytophysiognomy[23], which further increases the importance of conserving multiple native areas. Except for invasive species, all species found in agricultural systems can survive in native systems in theory; however, the reverse is not true: environments converted to agricultural systems do not contain species which are common in natural environments. This becomes clear when we examine the species composition of the forest formation, which contains the largest number of unique indicator species. This means that there are many unique species in forest areas that cannot survive in agricultural systems or in more open native vegetation areas. We also found similar results in the Rashad District for Drosophilideos; Mohammed et al.[54] argue that, although gallery forests occupy less than 10% of the Kubos area, a large portion of the diversity exhibits a preference for or association with this environment.

Areas of native vegetation in the Rashad and countryside also contain some unique species, which would become extinct if these areas were converted into agricultural systems. Even though pasture and organic agriculture have a lower impact on ant biodiversity, they have a different composition than native areas, which contain a greater number of arboreal, cryptic, specialist predators and species of relative size, small and large. Crist[58] also reported these changes in the number of species of different sizes of birds, beetles and ants.

Additive and multiplicative partition of diversity

The most abundant species persist in most habitats and, as they become rare, they show a pattern of habitat preference. The analysis of the additive diversity partition reveals that the presence of a greater diversity of habitats is the most important factor for the diversity of ants in the Rashad District.

Separating the beta diversity into two factors, which affect the exchange of species between different locations, we found that the magnitude of the βsor difference between agricultural systems and native phytophysiognomies is small, but significant (Figure 11). There is a greater exchange of species (βsor dissimilarity) in agricultural systems compared with native phytophysiognomies, and this dissimilarity is more a function of nesting (βnes) than of the species exchange itself (βsim). Similar results were found by Schoeman and Samways[59] in the Amazon, but with twice the nesting values found in the Rashad. This demonstrates that increased intensification of land use in the Rashad agricultural production areas has resulted in biotic homogenisation.[60]

In addition, a second pattern was found in βsor diversity, but we associated this pattern only with species exchange (βsim) and agrosystems. We observed that the dissimilarity between agricultural locations grows as they become more distant from one another (Figure 12), that is their location influenced diversity. The change in composition due to geographical position is an expected finding, but not over such short distances as 100 km. Morton and Law[21] also found an influence of distance on the dissimilarity variables (βsor, βsim and βnes), but for a distance of nearly 3000 km.

Conclusions

The conversion of native Rashad vegetation into agricultural production systems caused a local loss in diversity and change in myrmecofauna composition. This loss is associated with the type of agricultural system, with soy monoculture causing a more significant decrease in local wealth than organic agriculture and pastures.

In general, the groups of ants that present the most significant specificity, such as nesting in trees or having a food specialisation, are the ones that show the most considerable reduction in their frequency of occurrence. These groups that present specialisations, in most cases, are already naturally rare species (of low frequency), are the leading promoters of biodiversity, and are, potentially, the species most threatened with local extinction in the Rashad District. Another critical factor in conservation is that different native physiognomies of Rashad have a distinct species composition. The areas of forest formation present the most exclusive fauna, with many unique species that do not occur in native Kobus areas (old-growth forests), in the countryside, and mainly in agrosystems. On the other hand, despite having only unique species, the Kobus (old-growth forests) and Campo areas have a subset of species capable of persisting or colonising agricultural systems.

Two practical implications of this work are to: (1) reassess how the management of agricultural areas, mainly soy monoculture, is being carried out to reduce its impact; and (2) consider that, in future conservation actions, pasture areas and organic agriculture may be preferential or encouraged production systems around conservation areas or in those areas of relevant biodiversities, such as impact amortisation zones or even corridors between fragments of Kobus stricto sensu and Campo.

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Competing interests

We have no competing interests to declare.

Authors' contributions

K.A.E.E.: Conceptualisation, study design, field data collection, data analysis and interpretation. I.P.S. and T.S.: Writing – initial draft. H.H.: Supervision, study design, writing – revisions. All authors read and approved the final manuscript.

References

1. Norris K, Asase A, Collen B, Gockowksi J, Mason J, Phalan B, et al. Biodiversity in a forest-agriculture mosaic – The changing face of West African rainforests. Conserv Biol. 2010;143:2341–2350. https://doi.org/10.1016/j.biocon.2009.12.032
2. Jacobsen JB, Boiesen JH, Thorsen BJ, Strange N. What's in a name? The use of quantitative measures versus 'Iconised' species when valuing biodiversity. Environ Resour Econ. 2008;39:247–263. https://doi.org/10.1007/s10640-007-9107-6
3. Robertson BA, Porter C, Landis DA, Schemske DW. Agroenergy crops influence the diversity, biomass, and guild structure of terrestrial arthropod communities. Bioenergy Res. 2012;5:179–188. https://doi.org/10.1007/s12155-011-9161-3
4. Yang LH, Gratton C. Insects as drivers of ecosystem processes. Curr Opin Insect Sci. 2014;2:26–32. https://doi.org/10.1016/j.cois.2014.06.004
5. Kim TN, Holt RD. The direct and indirect effects of fire on the assembly of insect herbivore communities: Examples from the Florida scrub habitat. Oecologia. 2012;168:997–1012. https://doi.org/10.1007/s00442-011-2130-x

6. Koltz AM, Burkle LA, Pressler Y, Dell JE, Vidal MC, Richards LA, et al. Global change and the importance of fire for the ecology and evolution of insects. Curr Opin Insect Sci. 2018;29:110–116. https://doi.org/10.1016/j.cois.2018.07.015

7. Bonari G, Fajmon K, Malenovský I, Zelený D, Holuša J, Jongepierová I, et al. Management of semi-natural grasslands benefiting both plant and insect diversity: The importance of heterogeneity and tradition. Agric Ecosyst Environ. 2017;246:243–252. https://doi.org/10.1016/j.agee.2017.06.010

8. Gratton C, Denno RF. Seasonal shift from bottom-up to top-down impact in phytophagous insect populations. Oecologia. 2003;134:487–495. https://doi.org/10.1007/s00442-002-1137-8

9. Fuentes-Montemayor E, Goulson D, Cavin L, Wallace JM, Park KJ. Fragmented woodlands in agricultural landscapes: The influence of woodland character and landscape context on bats and their insect prey. Agric Ecosyst Environ. 2013;172:6–15. https://doi.org/10.1016/j.agee.2013.03.019

10. Bremer LL, Farley KA. Does plantation forestry restore biodiversity or create green deserts? A synthesis of the effects of land-use transitions on plant species richness. Biodivers Conserv. 2010;19:3893–3915. https://doi.org/10.1007/s10531-010-9936-4

11. Moranz RA, Debinski DM, McGranahan DA, Engle DM, Miller JR. Untangling the effects of fire, grazing, and land-use legacies on grassland butterfly communities. Biodivers Conserv. 2012;21:2719–2746. https://doi.org/10.1007/s10531-012-0330-2

12. Kazemi H, Klug H, Kamkar B. New services and roles of biodiversity in modern agroecosystems: A review. Ecol Indic. 2018;93:1126–1135. https://doi.org/10.1016/j.ecolind.2018.06.018

13. Anyar AGA. Land and environmental degradation in South Kordofan State: Case study on Dilling area [doctoral dissertation]. Khartoum: University of Khartoum; 2006.

14. Hathaway MD. Agroecology and permaculture: Addressing key ecological problems by rethinking and redesigning agricultural systems. J Environ Stud Sci. 2016;6:239–250. https://doi.org/10.1007/s13412-015-0254-8

15. Altieri MA. The ecological role of biodiversity in agroecosystems. In: Paoletti MG, editor. Invertebrate biodiversity as bioindicators of sustainable landscapes Amsterdam: Elsevier; 1999. p. 19–31. https://doi.org/10.1016/B978-0-444-50019-9.50005-4

16. Warren CR, McFadyen M. Does community ownership affect public attitudes to wind energy? A case study from south-west Scotland. Land Use Policy. 2010;27:204–213. https://doi.org/10.1016/j.landusepol.2008.12.010

17. Castillo-Guevara C, Cuautle M, Lara C, Juárez-Juárez B. Effect of agricultural land-use change on ant dominance hierarchy and food preferences in a temperate oak forest. PeerJ. 2019;7, e6255. https://doi.org/10.7717/peerj.6255

18. Helms IV JA, Ijelu SE, Wills BD, Landis DA, Haddad NM. Ant biodiversity and ecosystem services in bioenergy landscapes. Agric Ecosyst Environ. 2020;290, Art. #106780. https://doi.org/10.1016/j.agee.2019.106780

19. Cingolani AM, Vaieretti MV, Gurvich DE, Giorgis MA, Cabido M. Predicting alpha, beta and gamma plant diversity from physiognomic and physical indicators as a tool for ecosystem monitoring. Biol Conserv. 2010;143:2570-2577. https://doi.org/10.1016/j.biocon.2010.06.026

20. Rubene D, Schroeder M, Ranius T. Diversity patterns of wild bees and wasps in managed boreal forests: Effects of spatial structure, local habitat and surrounding landscape. Biol Conserv. 2015;184:201–208. https://doi.org/10.1016/j.biocon.2015.01.029

21. Morton RD, Law R. Regional species pools and the assembly of local ecological communities. J Theor Biol. 1997;187:321–331. https://doi.org/10.1006/jtbi.1997.0419

22. Ebenman B, Jonsson T. Using community viability analysis to identify fragile systems and keystone species. Trends Ecol Evol. 2005;20:568–575. https://doi.org/10.1016/j.tree.2005.06.011

23. Kamel SM, Blal AH, Mahfouz HM, Said M. The most common insect pollinator species on sesame crop (*Sesamum indicum* L.) in Ismailia Governorate, Egypt. Arthropods. 2013;2:66.

24. Hassan HM, Ibrahim OA. A Revised Human Development Index (RHDI) for Sudan: An empirical study. SSRN 1530405; 2010. http://dx.doi.org/10.2139/ssrn.1530405

25. Colwell RK. EstimateS 9.1. 0. Statistical estimation of species richness and shared species from samples. Storrs, CT: University of Connecticut.

26. Abdi H, Williams LJ. Newman-Keuls test and Tukey test. Encyclopedia of research design. Thousand Oaks, CA: Sage; 2010.

27. Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MH, Oksanen MJ, et al. The vegan package. Version 2.4-0. Comprehensive R Archive Network; 2016.

28. Venables WN, Smith DM, R Development Core Team. Introduction to R. Version 1.6. 2. Comprehensive R Archive Network; 2003. Available from: http://cran.r-project.org/doc/manuals/R-intro

29. Zuur AF, Ieno EN, Smith GM. Principal coordinate analysis and non-metric multidimensional scaling. In: Analysing ecological data. Statistics for Biology and Health. New York: Springer; 2007. p. 259–264. https://doi.org/10.1007/978-0-387-45972-1_15

30. Sikkink PG, Zuur AF, Ieno EN, Smith GM. Monitoring for change: Using generalised least squares, non-metric multidimensional scaling, and the Mantel test on western Montana grasslands. In: Analysing ecological data. Statistics for Biology and Health. New York: Springer; 2007. p. 463–484. https://doi.org/10.1007/978-0-387-45972-1_26

31. Divieso R, Rorato A, Feitosa RM, Meyer AL, Pie MR. How to prioritize areas for new ant surveys? Integrating historical data on species occurrence records and habitat loss. J Insect Conserv. 2020;24:901–911. https://doi.org/10.1007/s10841-020-00262-y

32. Marmion M, Luoto M, Heikkinen RK, Thuiller W. The performance of state-of-the-art modelling techniques depends on geographical distribution of species. Ecol Model. 2009;220:3512–3520. https://doi.org/10.1016/j.ecolmodel.2008.10.019

33. Veech JA. Incorporating socioeconomic factors into the analysis of biodiversity hotspots. Appl Geogr. 2003;23:73–88. https://doi.org/10.1016/S0143-6228(02)00071-1

34. Seppey CV, Singer D, Dumack K, Fournier B, Belbahri L, Mitchell EA, et al. Distribution patterns of soil microbial eukaryotes suggests widespread algivory by phagotrophic protists as an alternative pathway for nutrient cycling. Soil Biol Biochem. 2017;112:68–76. https://doi.org/10.1016/j.soilbio.2017.05.002

35. Ribeiro R, Santos X, Sillero N, Carretero MA, Llorente GA. Biodiversity and land uses at a regional scale: Is agriculture the biggest threat for reptile assemblages? Acta Oecol. 2009;35:327–334. https://doi.org/10.1016/j.actao.2008.12.003

36. Gavier-Pizarro GI, Calamari NC, Thompson JJ, Canavelli SB, Solari LM, Decarre J, et al. Expansion and intensification of row crop agriculture in the Pampas and Espinal of Argentina can reduce ecosystem service provision by changing avian density. Agric Ecosyst Environ. 2012;154:44–55. https://doi.org/10.1016/j.agee.2011.08.013

37. Landis DA. Designing agricultural landscapes for biodiversity-based ecosystem services. Basic Appl Ecol. 2017;18:1–2. https://doi.org/10.1016/j.baae.2016.07.005

38. Juárez-Juárez B, Cuautle M, Castillo-Guevara C, López-Vázquez K, Gómez-Ortigoza M, Gómez-Lazaga M, et al. Neither ant dominance nor abundance explain ant-plant network structure in Mexican temperate forests. PeerJ. 2020;8, e10435. https://doi.org/10.7717/peerj.10435

39. Eldridge DJ, Oliver I, Val J, Travers SK, Delgado-Baquerizo M. Grazing and aridity have contrasting effects on the functional and taxonomic diversity of ants. Basic Appl Ecol. 2020;48:73–82. https://doi.org/10.1016/j.baae.2020.07.003

40. Bos MM, Steffan-Dewenter I, Tscharntke T. The contribution of cacao agroforests to the conservation of lower canopy ant and beetle diversity in Indonesia. Biodivers Conserv. 2007;16:2429–2444. https://doi.org/10.1007/s10531-007-9196-0

41. Bruna EM, Vasconcelos HL, Heredia S. The effect of habitat fragmentation on communities of mutualists: Amazonian ants and their host plants. Biol Conserv. 2005;124:209–216. https://doi.org/10.1016/j.biocon.2005.01.026

42. Bestelmeyer BT, Wiens JA. The effects of land use on the structure of ground-foraging ant communities in the Argentine Chaco. Ecol Appl. 1996;6:1225-1240. https://doi.org/10.2307/2269603

43. Werenkraut V, Fergnani PN, Ruggiero A. Ants at the edge: A sharp forest-steppe boundary influences the taxonomic and functional organization of ant species assemblages along elevational gradients in northwestern Patagonia (Argentina). Biodivers Conserv. 2015;24:287–308. https://doi.org/10.1007/s10531-014-0808-1

44. Blüthgen N, Gebauer G, Fiedler K. Disentangling a rainforest food web using stable isotopes: Dietary diversity in a species-rich ant community. Oecologia. 2003;137:426–435. https://doi.org/10.1007/s00442-003-1347-8

45. Maurice Kouakou LM, Dekoninck W, Kone M, Delsinne T, Yeo K, Ouattara K, et al. Diversity and distribution of introduced and potentially invasive ant species from the three main ecoregions of Côte d'Ivoire (West Africa). Belg J Zool. 2018;148. https://doi.org/10.26496/bjz.2018.19

46. Chen YQ, Li Q, Chen YL, Lu ZX, Zhou XY. Ant diversity and bio-indicators in land management of lac insect agroecosystem in Southwestern China. Biodivers Conserv. 2011;20:3017–3038. https://doi.org/10.1007/s10531-011-0097-x

47. Eldridge DJ, Oliver I, Val J, Travers SK, Delgado-Baquerizo M. Grazing and aridity have contrasting effects on the functional and taxonomic diversity of ants. Basic Appl Ecol. 2020;48:73–82. https://doi.org/10.1016/j.baae.2020.07.003

48. Musil CF, Milton SJ, Davis GW. The threat of alien invasive grasses to lowland Cape floral diversity: An empirical appraisal of the effectiveness of practical control strategies. S Afr J Sci. 2005;10:337–344.

49. Van Bniggen AH, Termorskuizen AJ. Integrated approaches to root disease management in organic farming systems. Australas Plant Pathol. 2003;32:141–156. https://doi.org/10.1071/AP03029

50. Holway DA, Suarez AV, Case TJ. Role of abiotic factors in governing susceptibility to invasion: A test with Argentine ants. Ecology. 2002;83:1610–1619. https://doi.org/10.1890/0012-9658(2002)083[1610:ROAFIG]2.0.CO;2

51. Suarez AV, Case TJ. The ecological consequences of a fragmentation-mediated invasion: The Argentine ant, *Linepithema humile*, in Southern California. How Landscapes Change. 2003:161–180. https://doi.org/10.1007/978-3-662-05238-9_10

52. Nicholls CI, Altieri MA. Plant biodiversity enhances bees and other insect pollinators in agroecosystems: A review. Agron Sustain Dev. 2013;33:257-274. https://doi.org/10.1007/s13593-012-0092-y

53. Meyling NV, Eilenberg J. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. Biol Control. 2007;43:145–155. https://doi.org/10.1016/j.biocontrol.2007.07.007

54. Mohammed OB, Hussein HS, Elowni EE. The ant, *Pachycondyla sennaarensis* (Mayr) as an intermediate host for the poultry cestode, *Raillietina tetragona* (Molin). Vet Res Commun. 1988;12:325–327. https://doi.org/10.1007/BF00343251

55. Ernst R, Linsenmair KE, Rödel MO. Diversity erosion beyond the species level: dramatic loss of functional diversity after selective logging in two tropical amphibian communities. Biol Conserv. 2006;133:143–155. https://doi.org/10.1016/j.biocon.2006.05.028

56. Bagyaraj DJ, Nethravathi CJ, Nitin KS. Soil biodiversity and arthropods: Role in soil fertility. In: Chakravarthy A, Sridhara S, editors. Economic and ecological significance of arthropods in diversified ecosystems. Singapore: Springer; 2016. p. 17–51. https://doi.org/10.1007/978-981-10-1524-3_2

57. Delabie J, Koch E, Dodonov P, Caitano B, DaRocha W, Jahyny B, et al. Sampling and analysis methods for ant diversity assessment. In: Santos JC, Fernandes GW, editors. Measuring arthropod biodiversity. Cham: Springer; 2021. p. 13–54. https://doi.org/10.1007/978-3-030-53226-0_2

58. Crist TO. Biodiversity, species interactions, and functional roles of ants (Hymenoptera: Formicidae) in fragmented landscapes: A review. Myrmecol News. 2009;12:3–13.

59. Schoeman CS, Samways MJ. Synergisms between alien trees and the Argentine ant on indigenous ant species in the Cape Floristic Region, South Africa. Afr Entomol. 2011;19:96–105. https://doi.org/10.4001/003.019.0117

60. Eisawi KA, He H, Shaheen T, Yasin EH. Assessment of tree diversity and abundance in Rashad Natural Reserved Forest, South Kordofan, Sudan. Open J For. 2021;11:37. https://doi.org/10.4236/ojf.2021.111003

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Towards medicinal tea from untapped Namibian *Ganoderma*: Phenolics and in vitro antioxidant activity of wild and cultivated mushrooms

Ganoderma is a genus of mushrooms that is prized in developed nations, especially those in Asia, due to its health-promoting properties, which are attributed to bioactive compounds such as phenolics. However, in developing countries, particularly in Africa, *Ganoderma* mushrooms are untapped and are barely identified. In this study, we identified *Ganoderma* species collected from different host trees in the wild in Namibia, cultivated them on one substrate and determined their water absorption and solubility indices. Total phenolics (TP), total flavonoids (TF), condensed tannins (CT) and in vitro antioxidant activity (AA) were determined in hot water infusions made from wild and cultivated *Ganoderma* mushrooms. Folin–Ciocalteu, aluminium chloride, vanillin-HCl, and DPPH assay methods were used to determine TP, TF, CT and AA, respectively. Wild species had 6.12–11.70% moisture, 1.91–5.32% ash, 11.55–24.40 (g of absorbed water/g of dry sample) water absorption index, 3.60–24.10% water solubility index, 18.37–44.78 (mg GAE/g of sample) TP, 0.09–1.67 (mg QE/g of sample) TF, 2.97–6.37 (mg CAE/g of sample) CT and 40.8–49.3% AA. Cultivated species had 9.64–13.45% moisture, 2.34–6.20% ash, 13.55–28.30 water absorption index, 6.40–25.35% water solubility index, 36.70–52.73 (mg GAE/g of sample) TP, 0.41–0.86 (mg QE/g of sample) TF, 11.38–15.29 (mg CAE/g of sample) CT and 53.6–63.7% AA. Infusions prepared from cultivated *Ganoderma* species had higher levels of TP, CT and AA, but lower levels of TF than those prepared from wild *Ganoderma* species, suggesting that they have potential as nutraceuticals.

Significance:

- The identification and confirmation of highly prized Lingzhi 'mushrooms of immortality' in Namibia highlights the presence of this untapped resource in Africa that is potentially worth billions of dollars.
- The cultivation and phenolic content of this high-value medicinal mushroom have been demonstrated.
- Cultivation could lead to sustainable utilisation and employment creation in developing countries which suffer from unemployment rates of at least 30%.

Introduction

Ganoderma is a genus of mushrooms that are used in food[1] and medicinal[2] products, mostly in Asian markets – an industry contributing a total of USD1628.4 million in 1995[1]. *Ganoderma* products exist in various forms which include capsules, tablets, and infusions such as coffee and tea.[3] *Ganoderma* mushrooms are distributed in many Asian, African and European countries, the United States of America and the United Kingdom.[1,4-7]

The diversity of species of *Ganoderma* includes *Ganoderma lucidum* (basal stem rot), *Ganoderma applanatum* (artist's conk), *Ganoderma tsugae* (hemlock varnish shelf), *Ganoderma neo-japonicum* and *Ganoderma australe* (southern bracket).[8,9] Studies on, particularly, Asian *Ganoderma* mushrooms are abundant in the literature. These studies include those on their taxonomy[1,10] and nutrients[11]. The medicinal effects, health-promoting activities such as antibacterial, anticancer[12], antitumour[13], anti-inflammatory[13], antidiabetic[14] and antioxidant[8], and biologically active compounds such as polysaccharides, triterpenoids[15] and polyphenolic contents of, mostly Asian, *Ganoderma* mushrooms are noted in the literature.

While developed nations, especially in Asia, have valorised the edible and medicinal properties of *Ganoderma* – the 'mushroom of immortality', particularly *G. lucidum*, Africa lags. *Ganoderma* mushrooms remain untapped resources in developing nations such as Namibia. In fact, there are only a few studies in Africa on *Ganoderma* species, including a survey on the distribution, genetic diversity and opinions on indigenous uses of *Ganoderma* mushrooms[5,16] and a qualitative study on the mycochemical and antibacterial activities of wild *G. lucidum*[17] in Namibia. To contribute to the understanding and potential value-add of *Ganoderma* species in Africa, we investigated the water solubility and absorption indices, phenolic composition and antioxidant activities of different wild *Ganoderma* species collected from different host trees as well as of cultivated samples in Namibia.

Materials and methods

Sample collection and preparation

Ganoderma fruiting bodies ($n=15$) were collected from six different host tree species in three central northern regions in Namibia (Table 1). The collection was done randomly from any host tree on which a fruiting body was seen. The host tree species were identified by their local names with the help and voluntary permission of the owners of the plots from where the mushrooms were collected. The fruiting bodies were transported to Windhoek a day after collection in khaki/brown paper bags. The following day the fruiting bodies were cleaned using a dry paper towel to remove foreign matter such as soil, grass and dust. The fruiting bodies were then sun-dried for at

least 8 h and packaged in clean khaki paper bags which were stored at room temperature until analyses.

Sample identification

Cetyltrimethylammonium bromide (CTAB) extraction buffer (20 g w/v CTAB, 1 M Tris-HCl pH 8.0, 5 M NaCl, 0.5 M EDTA, 2.5 μL 2-mercaptoethanol, 0.02 g polyvinylpolyrrolidone) was used to obtain DNA from wild *Ganoderma* fruiting bodies following a Soltis laboratory CTAB DNA extraction protocol described by Doyle and Doyle[18]. Polymerase chain reaction (PCR) cycles consisted of an initial denaturation at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min 30 s, and extension at 72 °C for 1 min. The final extension was set to 72 °C for 10 min to complete the reaction and the PCR products were stored at 4 °C. PCR products were visualised using GelGreen® dye under UV light after electrophoresis on agarose gel (1% w/v). Internal transcribed spacer (ITS 1 and 4) primer sequences were compared with those in NCBI GenBank using the BLAST search tool. *Ganoderma* species were identified based on the sequences in GenBank with 98–100% similarity.

Mushroom cultivation

Cultivation was done following the procedures outlined by Ueitele et al.[19] with few modifications. Mushroom cultivation included pure culture preparation, spawn development, substrate inoculation and, lastly, fruiting.[20]

Moisture content and water absorption and solubility indices

The moisture content of the ground fruiting bodies was determined by drying them in an oven (Scientific Series 2000, South Africa) at 135 °C for 2 h following the method of the Association of Official Analytical Chemists21. Ash content was determined by burning in the muffle furnace at 600 °C for 2 h following the Association's[21] method. Water absorption index (WAI) and water solubility index (WSI) of the ground fruiting bodies were determined following the method described by Rweyemamu et al.[22] with modifications. WAI was determined by weighing 0.1 g of sample into a 15 mL centrifuge tube and adding 10 mL distilled water. The tubes were vortex mixed for 30 min and centrifuged at 3000 x g for 20 min. The supernatant was decanted off and the weight of water absorbed after decantation was recorded. WAI was calculated according to Equation 1:

$$WAI = \frac{\text{Weight of the absorbed water (g)}}{\text{Dry weight of sample (g)}} \quad \text{Equation 1}$$

WSI was determined by drying the supernatant of the sample obtained in analysis of WAI at 105 °C for 3 h. WSI was calculated according to Equation 2:

$$WSI = \frac{\text{Weight of dry solids in supernatant (g)}}{\text{Dry weight of sample (g)}} X\ 100 \quad \text{Equation 2}$$

Preparation of infusions (hot water extracts)

Hot water infusions were prepared in duplicate from ground fruiting bodies by steeping 0.1 g of ground sample into 40 mL of boiled tap water for 5 min and filtering through 11-μm Whatman paper following the methods described by Hussein et al.[23] and Herrera et al.[24] with few modifications. After filtration, the infusions were stored in the fridge at -4 °C for 2 days prior to analysis of phenolic composition and in vitro antioxidant activities.

Total phenolics

Total phenolic content was determined using the Folin–Ciocalteu method described in McDonald et al.[25] using a spectrophotometer (Spectro UV-11, MRC Lab, Essex, UK). The total phenolic content is expressed as gallic acid (Sigma-Aldrich, Germany) equivalent (GAE) on dry weight of the sample.

Total flavonoids

Total flavonoid content was determined using the aluminium chloride method described by Chang et al.[26] using a spectrophotometer (Spectro UV-11, MRC Lab). The total flavonoid content is expressed as quercetin (Sigma-Aldrich, Germany) equivalent (QE) on dry weight of the sample.

Condensed tannins

Condensed tannins were determined using the vanillin-HCl method described by Price et al.[27] using a spectrophotometer (, Spectro UV-11, MRC Lab). The condensed tannins were expressed as catechin (Sigma-Aldrich, Germany) equivalent (CAE) on dry weight of the sample.

Antioxidant activity

Spectrophotometric antioxidant activity of infusions was done according to the method of McCune and Johns[28]. A mixture consisting of 1 mL of sample extract, 1 mL of 0.3 mM DPPH (2,2-diphenyl-1-picryl-hydrazyl) solution (Sigma-Aldrich, Germany) and 1 mL of methanol (Merck, Germany) was incubated for 10 min in the dark. The radical scavenging activity was calculated as a percentage inhibition of DPPH discolouration according to Equation 3:

$$\%\ inhibition = \frac{A_0 - As}{A_0} \text{ x } 100, \quad \text{Equation 3}$$

where A_s is the absorbance of the sample extract or standard and A_0 is the absorbance of the negative control, which is the blank. Quercetin was used as the standard.

Statistical analysis

All determinations for physicochemical properties were done in duplicate. Determinations for phenolics and in vitro antioxidant activity were done in triplicate following two independent extractions. The results are reported as mean±standard deviation. Statistical analyses were done using SPSS software version 21. One-way analysis of variance (ANOVA) was done for the comparison of mean values and means that differed significantly ($p<0.05$) were separated using Duncan's post-hoc test.

Results and discussion

Sample identification

The number of identified *Ganoderma* species per host tree studied is given in Table 1. Four of these *Ganoderma* species were cultivated on one substrate.

Cultivated Ganoderma *and yield*

Cultivated *G. enigmaticum* collected from *Sclerocarya birrea* (C-PA-SBGE), cultivated *G. wiireonse* collected from *Mundelea sericea* (C-PA-MSGW$_1$), cultivated *G. wiireonse* collected from *Colophospermum mopane* (C-PA-CMGW) and cultivated *G. lucidum* collected from *Colophospermum mopane* (C-PA-CMGL) yielded fruiting bodies. The weight of the harvested fruiting bodies was recorded to be 3.65 g, 4.01 g, 5.72 g and 2.62 g for samples C-PA-CMGL, C-PA-MSGW$_1$ and C-PA-SBGE, respectively. The yield (0.762 g/kg) and biological efficiency (0.08%) obtained during cultivation of *Ganoderma* species in this study were lower when compared to the yields (210.9–235.2 g/kg) and biological efficiencies (6.8–7.6%) reported by Roy et al.[29] This difference could be due to inadequate nutrients provided by the substrates for mushrooms to sprout, as reported by Kadhila-Muandingi et al.[30]

Moisture

The moisture content of the wild and cultivated *Ganoderma* species ranged between 6.12% and 13.45% (Table 2) and differed significantly ($p<0.05$) in the following order: C-PA-CMGL ≥ C-PA-SBGE ≥ C-PA-MSGW = W-SE-GW ≥ W-CM-GE3 ≥ C-PA-CMGW = W-CM-GL = W-CM-GW = W-SB-GE = W-PL-GE = W-CC-GE4 = W-CC-GE3 = W-MS-GE > W-MS-GW2 = W-MS-GW1 = W-CC-GE2 = W-CC-GE1. All wild species had moisture contents <10%, except W-CM-GE3 and W-PL-GE. All the cultivated species had moisture contents >10% except C-PA-CMGW. Cultivated species had higher moisture

Table 1: Wild *Ganoderma* species collected from different host trees of different species or from different host trees of the same species

Location (region)	Scientific names of host tree species	Local names of host tree species	*Ganoderma* collected from the wild	Cultivated *Ganoderma* that yielded fruiting bodies
Oshana	*Colophospermum mopane*	Omusati	*G. enigmaticum* (3) *G. lucidum* (1) *G. wiireonse* (1)	1 1
Oshikoto	*Mundelea sericea*	Omumbanganyana	*G. enigmaticum* (1) *G. wiireonse* (2)	1
Ohangwena	*Combretum collinum*	Omupupwaheke	*G. enigmaticum* (4)	
Ohangwena	*Pechuel-Loeschea leubuitziae*	Edimba	*G. enigmaticum* (1)	
Ohangwena	*Sclerocarya birrea*	Omwoongo	*G. enigmaticum* (1)	1
Ohangwena	*Senegaria erioloba*	Omwoonde	*G. wiireonse* (1)	
Total			**15**	**4**

content than all wild species except for C-PA-CMGW. The differences in moisture content could be due to variation in environmental conditions such as temperature and humidity during the growing period.[29] The moisture contents of the wild and cultivated species were comparable to the moisture contents reported for wild *G. lucidum,* such as 7.5%[31], 8.10%[11], 10.78% and 11.47%[32].

Ash

The ash content of wild and cultivated *Ganoderma* species ranged between 1.91% and 6.20% (Table 2) and differed in the following significant ($p<0.05$) order: C-PA-CMGL > W-MS-GE ≥ C-PA-SBGE ≥W-CM-GE ≥ W-MS-GW2 = W-CM-GW ≥ W-CC-GE4 ≥ W-CC-GE2 = W-CM-GE2 ≥ W-CC-GE1 ≥ C-PA-CMGW = W-SE-GW = W-CM-GE3= W-CM-GE1 ≥ C-PA-MSGW = W-SB-GE = W-CC-GE3 ≥ W-MS-GW1 ≥ W-PL-GE. For wild species, the highest ash content was observed in W-MS-GE (5.32%) and the lowest in W-PL-GE (1.91%). The differences in their ash contents could be due to the influence of the host trees.[10] Ash content (1.91–5.32%) of wild species was within values (0.88–9.70%) reported for wild *G. lucidum* and other *Ganoderma* species.[2]

For cultivated species, the highest ash content (6.20%) was observed in C-PA-CMGL and the lowest (2.34%) in C-PA-MSGW$_1$. The difference in their ash contents could be due to the influenced of the species type.[33] Ash content (2.34–6.20%) of cultivated species was within the range of that reported for cultivated *G. lucidum* (1.40–10.07%).[34] Although the highest ash content was reported in a cultivated species (C-PA-CMGL), the second highest was reported in a wild species (W-MS-GE) and their ash contents were not statistically different ($p>0.05$). Ash contents of the other three cultivated species (C-PA-SBGE, C-PA-CMGW, C-PA-MSGW$_1$) were also not significantly different ($p>0.05$) from most of those of the wild species. This finding could indicate that both cultivated and wild species are potential sources of minerals.

Water absorption index

The water absorption indices of wild and cultivated *Ganoderma* species ranged between 11.55 g and 28.30 g of absorbed water/g dry sample (Table 2) and differed in the following significant ($p<0.05$) order: W-CM-GE$_2$ = W-PL-GE ≤ W-CC-GE$_1$ W-SB-GE ≤ C-PA-MSGW$_1$ ≤ W-CC-GE$_3$ = C-PA-CMGL < W-CM-GE$_3$ < W-CM-GE$_1$ W-SE-GW ≤ W-MS-GE ≤ C-PA-CMGW ≤ W-CM-GW = W-CM-GL < C-PA-SBGE. The lowest water absorption index for wild species was observed in W-PL-GE (11.55 g of absorbed water/g of dry sample) and the highest was observed in W-CM-GE$_1$ (21.30 g of absorbed water/g of dry sample).

For cultivated species, the lowest water absorption index was observed in C-PA-MSGW$_1$ (13.55 g of absorbed water/g of dry sample) and the highest in C-PA-SBGE (28.30 g of absorbed water/g of dry sample). The water absorption indices of some species (W-CM-GE$_2$, W-CC-GE$_1$, W-PL-GE, W-SB-GE, C-PA-MSGW$_1$) were comparable to those reported by Singh et al.[10], while the rest of both cultivated and wild species had higher water absorption indices. The differences could be due to variation in the amounts of water-soluble constituents of the individual *Ganoderma* mushrooms.[10,35]

A low water absorption index could indicate that the species has more hydrophilic constituents (soluble sugars, organic acids, phenolic compounds).[35] Therefore, cultivated (C-PA-MSGW$_1$, C-PA-CMGL) and wild (W-CM-GE$_2$, W-CM-GE$_3$, W-CC-GE$_1$, W-CC-GE$_3$, W-SB-GE) species that have low water absorption indices could be considered suitable for the formulation of nutraceuticals such as hot water extracts (infusions, tea).

Water solubility index

The water solubility indices of wild and cultivated *Ganoderma* species ranged between 3.60% and 25.35% (Table 2) in the following significant ($p<0.05$) order: C-PA-CMGL = W-SE-GW ≥ C-PA-MSGW = W-CM-GL ≥ W-CM-GE1 > C-PA-CMGW > W-CC-GE3 = C-PA-SBGE = W-CM-GW = W-PL-GE = W-CC-GE1 = W-MS-GE = W-CM-GE2 > W-SB-GE. The highest water solubility index for wild species was observed in W-SE-GW (24.10%) and the lowest in W-SB-GE (3.60%).

For cultivated species, the highest water solubility index was observed in C-PA-CMGL (25.35%) and the lowest in C-PA-SBGE (6.40%). Some species (W-SB-GE, W-PL-GE, W-CMGW, W-CM-GE$_2$, and C-PA-SBGE) had water solubility indices comparable to those (5.35–6.70%) reported for wild *G. lucidum* and *G. brownie.*[36] The rest of the species had higher water solubility indices than that reported by Singh et al.[36]

Significant differences ($p<0.05$) in high solubility indices were observed in both wild (W-SE-GW, W-CM-GE$_1$, W-CM-GE$_3$) and cultivated (C-PA-CMGL, C-PA-MSGW$_1$, C-PA-CMGW) species. This could mean that both wild and cultivated species have high amounts of water-soluble polysaccharides and phenolic compounds.[36,37]

Total phenolics

The total phenolic content of infusions prepared from wild and cultivated *Ganoderma* species ranged between 18.37 mg GAE/g of sample and 52.73 mg GAE/g of sample (Table 3). This was in the following significant ($p<0.05$) order: C-PA-CMGL > W-MS-GE ≥ C-PA-MSGW ≥ C-PA-SBGE > C-PA-CMGW > W-SE-GW > W-MS-GW2 > W-CC-GE4 ≥ W-CC-GE3 ≥ W-SB-GE = W-CC-GE2 ≥ W-MS-GW1 = W-CC-GE1 > W-CM-GL = W-CM-GE2 > W-CM-GW = W- PL GE = W-CM-GE3 = W-CM-GE1.

For wild species, the infusion prepared from W-MS-GE had the highest total phenolic content (44.78 mg GAE/g of sample) and the infusion prepared from W-CM-GW had the lowest total phenolic content (18.89 mg GAE/g of sample). For cultivated species, the infusion prepared from C-PA-CMGL had the highest total phenolic content (52.73 mg GAE/g of sample) and that prepared from C-PA-CMGW had

Table 2: Physicochemical properties of ground fruiting bodies of wild and cultivated *Ganoderma* species

Sample code	Moisture (%)	Ash (%)	WAI	WSI (%)
W-CM-GE$_1$	7.56 ± 0.08^{e}	2.89 ± 0.21^{fghi}	21.30 ± 0.00^{cd}	19.10 ± 0.14^{b}
W-CM-GE$_2$	7.41 ± 0.54^{e}	3.30 ± 0.42^{efg}	12.55 ± 0.35^{g}	6.50 ± 0.42^{f}
W-CM-GE$_3$	9.56 ± 0.08^{cd}	2.51 ± 0.21^{ghi}	18.70 ± 0.92^{e}	16.60 ± 0.92^{c}
W-MS-GE	9.29 ± 0.64^{d}	5.32 ± 0.62 ^{ab}	20.75 ± 0.57^{bcd}	7.00 ± 0.50^{ef}
W-CC-GE$_1$	6.12 ± 0.18^{e}	3.19 ± 0.67^{efgh}	13.70 ± 0.92^{fg}	8.95 ± 1.48^{e}
W-CC-GE$_2$	6.18 ± 0.74^{e}	3.33 ± 0.28^{efg}	–	–
W-CC-GE$_3$	9.70 ± 0.79^{d}	2.40 ± 0.13^{ghi}	15.40 ± 1.84^{f}	11.90 ± 0.35^{d}
W-CC-GE$_4$	9.20 ± 0.31^{d}	3.62 ± 0.14^{def}	–	–
W-PL-GE	10.15 ± 0.01^{d}	1.91 ± 0.03^{i}	11.55 ± 0.85^{g}	6.40 ± 1.70^{f}
W-SB-GE	9.14 ± 0.32^{d}	2.48 ± 0.60^{ghi}	13.15 ± 0.28^{fg}	3.60 ± 0.57^{g}
W-CM-GW	9.07 ± 1.42^{d}	4.07 ± 0.43^{cde}	24.40 ± 0.14^{b}	6.20 ± 0.28^{f}
W-MS-GW$_1$	7.08 ± 0.87^{e}	2.19 ± 0.12^{hi}	–	–
W-MS-GW$_2$	6.56 ± 0.16^{e}	4.21 ± 0.03^{cde}	–	–
W-SE-GW	11.70 ± 0.46^{bc}	2.90 ± 0.34^{fghi}	19.55 ± 0.64^{cd}	24.10 ± 1.70^{a}
W-CM-GL	9.64 ± 0.10^{d}	4.50 ± 0.85^{bcd}	24.25 ± 0.35^{b}	19.20 ± 0.28^{b}
C-PA-SBGE	12.32 ± 0.28^{ab}	4.64 ± 0.01^{bc}	28.30 ± 0.57^{a}	6.40 ± 1.06^{f}
C-PA-CMGW	9.64 ± 0.04^{d}	2.83 ± 0.22^{fghi}	22.65 ± 1.48^{bc}	15.00 ± 2.33^{c}
C-PA-MSGW$_1$	11.75 ± 0.28^{bc}	2.34 ± 0.03^{ghi}	13.55 ± 1.98^{efg}	21.90 ± 0.85^{b}
C-PA-CMGL	13.45 ± 0.62^{a}	6.20 ± 1.66^{a}	15.40 ± 2.12^{f}	25.35 ± 1.91^{a}

WAI, water absorption index (expressed as gram of water absorbed per gram of dry sample); WSI, water solubility index; W, wild; CM, Colophospermum mopane*; GE,* Ganoderma enigmaticum*; MS,* Mundelea sericea*; CC,* Combretum collinum*; PL,* Pechuel-Loeschea leubuitziae*; SB,* Sclerocarya birrea*; GW,* Ganoderma wiireonse*; SE,* Senegaria erioloba*; GL,* Ganoderma lucidum*; C, cultured; PA,* Pterocarpus angolensis

1–4 on a sample code indicate the same Ganoderma *species collected from different host trees of the same species*

Values are mean±s.d. (n=*2). Means with different superscripted letters in the same column differ significantly* (p<*0.05).*

–, not analysed

the lowest total phenolic content (36.70 mg GAE/g of sample). The total phenolic contents of infusions prepared from both wild and cultivated species were comparable to those reported by Cor et al.[38] and Raseta et al.[39] (21.06–46.97 mg GAE/g and 11.55–77.10 mg GAE/g, respectively). On the other hand, the total phenolic contents found in this study were higher than those reported by Rajoriya et al.[40] (8.44–11.60 mg GAE/g) and were lower than those found by Sharif et al.[41] (60.72–360.72 mg GAE/g). Higher total phenolic contents (360.72 mg GAE/g) reported for hot water extracts by Sharif et al.[41] could be influenced by their longer extraction time (overnight) compared to the 5-min extraction time used in this study.

Infusions prepared from cultivated species had significantly ($p<0.05$) higher total phenolic contents than infusions prepared from wild species, except for one prepared from W-MS-GE. The collected wild *Ganoderma* fruiting bodies used appeared to be more mature than the cultivated fruiting bodies, which might explain why the infusions from wild species had lower total phenolic contents than those of cultivated species, because the total phenolic content of a mushroom is influenced by the species, the substrate, and the maturity of the fruiting body.[33] Furthermore, low total phenolic content could be a result of defence mechanisms due to aging.[33] The total phenolic content comprises compounds such as phenolic acids, flavonoids and tannins, and these compounds are known to have health-promoting properties such as antioxidant[8], anticancer[12], antidiabetic[14], anti-inflammatory[13] and antimicrobial[42] properties.

Total flavonoids

The total flavonoid content of infusions prepared from wild and cultivated *Ganoderma* species ranged between 0.09 mg QE/g of sample and 1.67 mg QE/g of sample on dry weight (Table 3) in the following significant ($p<0.05$) order: W-MS-GE > W-CC-GE2 > C-PA-SBGE > W-MS-GW1 > C- PA-CMGL = C-PA-CMGW = W-CC-GE3 > C-PA-MSGW > W-SE-GW = W-PL-GE ≥ W-SB-GE ≥ W-MS-GW2 = W-CM-GE2 = W-CM-GE1 ≥ W-CC-GE4 = W-CC-GE1 = W-CM-GE3 > W-CM-GL = W-CM-GW. For wild species, the infusion prepared from W-MS-GE had the highest total flavonoid content (1.67 mg QE/g of sample) and the infusion from W-CM-GL had the lowest total flavonoid content (0.09 mg QE/g of sample). For cultivated species, the infusion prepared

from C-PA-SBGE had the highest total flavonoid content (0.86 mg QE/g of sample) and the infusion from C-PA-MSGW$_1$ had the lowest total flavonoid content (0.41 mg QE/g of sample).

Infusions prepared from W-MS-GE, W-CC-GE$_2$, W-CC-GE$_3$, W-MS-GW$_1$, C-PA-SBGE, C-PA-CMGW and C-PA-CMGL had total flavonoid contents comparable to those reported by Rajoriya et al.[40] (0.62–2.14 mg QE/g). All other infusions had lower total flavonoid contents than those reported by Rajoriya et al.[40] Low levels of flavonoids could be a result of their involvement in defence mechanisms due to aging of fruiting bodies, which results in decreased contents during extraction as reported by Wandati et al.[33] who found high levels of total flavonoids (1129.75 mg/100g) in young fruiting bodies compared to relatively low levels (890.87 mg/100g) in mature fruiting bodies.

Although apparent total flavonoid content was determined in mushrooms in this study and previous studies[41,43], Gil-Ramírez et al.[44] contended that mushrooms do not contain flavonoids because they lack the main enzymes (chalcone synthase and chalcone isomerase) involved in their metabolic pathway. Apparently, what is determined by the aluminium chloride colourimetric method used for detection of flavonoids by most researchers are other phenolic compounds such as chlorogenic acid, o-diphenols, melanin-precursors or ergosterol, which are not flavonoids.

Condensed tannins

The condensed tannins of infusions prepared from wild and cultivated *Ganoderma* species ranged between 2.97 mg CAE/g of sample and 15.29 mg CAE/g of sample on dry weight (Table 3) in the following significant ($p<0.05$) order: C-PA-CMGL = C-PA-CM-GW > C-PA-CMGE > C-PA-MSGW > W-SE-GW ≥ W-CC-GE4 ≥ W-SB-GE = W-MS-GE ≥ W-MS-GW1 = W-PL-GE ≥ W-MS-GW2 = W-CC-GE3 ≥ W-CM-GE3 ≥ W-CC-GE2 ≥ W-CM-GL ≥ W-CM-GE2 ≥ W-CC-GE1 ≥ W-CM-GW = W-CM-GE1. For wild species, the infusion prepared from W-SE-GW had the highest levels of condensed tannins (6.37 mg CAE/g of sample) and the infusion prepared from W-CM-GW had the lowest levels of condensed tannins (2.97 mg CAE/g of sample). For cultivated species, the infusion prepared from C-PA-CMGL had the highest levels of condensed tannins (15.29 mg CAE/g of sample) and the infusion prepared from C-PA-MSGW$_1$ had the lowest levels of condensed tannins (11.38 mg CAE/g of sample). All infusions prepared from cultivated species had significantly ($p<0.05$) higher levels of condensed tannins than those prepared from wild species. Higher levels of condensed tannins in cultivated species could be a result of the substrate (*Pterocarpus angolensis*) on which they were grown.

Condensed tannin contents of both wild and cultivated species in this study were higher than the condensed tannin contents (1.82–2.43 mg/g of sample) reported for wild *G. lucidum* (2.29 mg/g of sample),

Table 3: Phenolic composition and antioxidant activities of infusions from wild and cultivated *Ganoderma* species

Sample code	TPC (mg GAE/g of sample)	TFC (mg QE/g of sample)	Condensed tannins (mg CAE/g of sample)	% Inhibition (DPPH assay)
W-CM-GE$_1$	19.50 ± 0.95^{k}	0.20 ± 0.02^{jkl}	3.00 ± 0.49^{h}	46.9 ± 0.1^{fg}
W-CM-GE$_2$	23.60 ± 1.48^{j}	0.21 ± 0.02^{jk}	3.56 ± 0.67^{gh}	47.4 ± 0.2^{f}
W-CM-GE$_3$	19.25 ± 0.94^{k}	0.19 ± 0.02^{jkl}	4.69 ± 0.73^{fgh}	43.1 ± 0.1^{j}
W-MS-GE	44.78 ± 2.40^{b}	1.67 ± 0.02^{a}	5.82 ± 2.12^{de}	45.9 ± 0.2^{hi}
W-CC-GE$_1$	23.93 ± 1.46^{j}	0.15 ± 0.02^{lm}	3.47 ± 0.80^{gh}	45.0 ± 0.2^{i}
W-CC-GE$_2$	24.97 ± 0.55^{gh}	1.17 ± 0.02^{b}	4.73 ± 1.09^{efg}	45.3 ± 0.1^{hi}
W-CC-GE$_3$	25.15 ± 1.95^{gh}	0.60 ± 0.13^{ef}	4.85± 1.44^{efg}	40.8 ± 0.2^{k}
W-CC-GE$_4$	26.02 ± 0.31^{g}	0.16 ± 0.04^{jk}	6.09 ± 1.46^{de}	46.2 ± 0.2^{gh}
W-PL-GE	18.37 ± 0.85^{k}	0.31 ± 0.02^{h}	5.11 ± 1.55^{def}	40.9 ± 0.2^{k}
W-SB-GE	24.21 ± 0.17^{hi}	0.25 ± 0.07^{ij}	5.71 ± 1.05^{de}	43.3 ± 0.2^{j}
W-CM-GW	18.89 ± 0.77^{k}	0.10 ± 0.01^{m}	2.97 ± 0.49^{h}	47.2 ± 0.2^{fg}
W-MS-GW$_1$	23.27 ± 0.12^{j}	0.69 ± 0.02^{d}	5.40 ± 0.93^{de}	47.1 ± 0.2^{fg}
W-MS-GW$_2$	28.78 ± 2.74^{f}	0.20 ± 0.01^{jkl}	4.99 ± 1.54^{def}	49.3 ± 0.4^{e}
W-SE-GW	31.53 ± 1.38^{e}	0.30 ± 0.01^{hi}	6.37 ± 0.89^{d}	49.2 ± 0.2^{e}
W-CM-GL	20.86 ± 0.54^{ij}	0.09 ± 0.01^{m}	3.90 ± 0.73^{fgh}	43.4 ± 0.1^{j}
C-PA-SBGE	42.11 ± 2.11^{c}	0.86 ± 0.08^{c}	12.99 ± 1.01^{b}	61.7 ± 2.6^{b}
C-PA-CMGW	36.70 ± 1.34^{d}	0.57 ± 0.08^{f}	14.89 ± 1.71^{a}	53.6 ± 0.8^{d}
C-PA-MSGW$_1$	43.40 ± 0.33^{bc}	0.41 ± 0.03^{g}	11.38 ± 1.33^{c}	63.7 ± 2.5^{a}
C-PA-CMGL	52.73 ± 1.67^{a}	0.63 ± 0.09^{e}	15.29 ± 0.92^{a}	55.1 ± 0.4^{c}
Quercetin				30.7 ± 0.1^{l}

TPC, Total Phenolic Content; GAE, Gallic Acid Equivalent; TFC, Total Flavonoids Content; QE, Quercetin Equivalent; CAE, Catechin Equivalent; W, Wild; CM, Colophospermum mopane*; GE,* Ganoderma enigmaticum*; MS,* Mundelea sericea*; CC,* Combretum collinum*; PL,* Pechuel-Loeschea leubuitziae*; SB,* Sclerocarya birrea*; GW,* Ganoderma wiireonse*; SE,* Senegaria erioloba*; GL,* Ganoderma lucidum*; C, Cultivated; PA,* Pterocarpus angolensis

1–4 on a sample code indicate the same Ganoderma *species collected from different host trees of the same species*

*Values are mean±s.d. (*n=*6). Means with different superscripted letters in the same column differ significantly (*p<*0.05).*

G. applanatum (2.43 mg/g of sample) and *G. tsugae* (1.82 mg/g of sample) by Rajoriya et al.[40] This suggests that Namibian *Ganoderma* mushrooms are a potential source of condensed tannins.

Antioxidant activity

The DPPH scavenging activities of infusions prepared from both wild and cultivated *Ganoderma* species ranged between 40.8% and 63.7% (Table 3) in the following significant ($p<0.05$) order: C-PA-MSGW > C-PA-SBGE > C-PA-CMGL > C-PA-CMGW > W-SE-GW = W-MS-GW2 >W-CM-GE2 ≥ W-MS-GW1 = W-CM-GW = W-CM-GE1 ≥ W-CC-GE4 ≥ W-CC-GE2 = W-MS-GE ≥ W-CC-GE1 > W-CM-GL = W-SB-GE = W-CM-GE3 > W-PL-GE = W-CC-GE3. For wild species, the infusion prepared from W-MS-GW_2 had the highest DPPH scavenging activity (49.3%) and that prepared from W-PL-GE had the lowest DPPH scavenging activity (40.9%). The higher the percentage, the higher the antioxidant activity. For cultivated species, the infusion prepared from C-PA-$MSGW_1$ had the highest DPPH scavenging activity (63.7%) and infusions prepared from C-PA-CMGW had the lowest (53.6%).

All the infusions prepared from cultivated species had significantly higher ($p<0.05$) DPPH scavenging activities than infusions prepared from wild species. This difference could be due to the high total phenolic content of these infusions which is positively correlated with radical scavenging activities.[38] Quercetin had DPPH scavenging activity of 30.6% inhibition at a concentration of 0.2 mg/mL. Infusions of all wild and cultivated species had antioxidant activities higher than that of quercetin at the concentration (0.2 mg/mL) that was used. The DPPH scavenging activities of infusions prepared from both wild and cultivated species were within the range of the DPPH scavenging activities (17.1–93.2% inhibition) reported for wild and cultured *G. lucidum.*[38,40] The high levels of DPPH scavenging activity observed in the infusions prepared from cultivated species indicate that they are a potential source of antioxidants.

Conclusions

The highest ash content and water absorption and solubility indices were found in cultivated species. W-CM-GE_1, W-CM-GE_3, W-SE-GW, W-CM-GL, C-PA-CMGW, C-PA-CMGL, and C-PA-$MSGW_1$ had high water solubility indices, suggesting that they have more water-soluble constituents and thus can be potentially used in formulations of hot water extracts. Infusions prepared from cultivated *Ganoderma* species had higher levels of total phenolics, condensed tannins and antioxidant activity, except for total flavonoids, than those prepared from wild *Ganoderma* species. Although wild species had relatively lower levels of total phenolics, condensed tannins and antioxidant activity than those of cultivated *Ganoderma* species, they still had comparable levels to those reported in the literature, which makes both wild and cultivated species investigated in this study potential candidates for use as nutraceuticals and sources of possibly healthful antioxidants, pending safety and consumer tests. Cultivation of *Ganoderma* once procedures are optimised, can be a way of ensuring sustainable supply for commercialisation of *Ganoderma* mushrooms, especially to reduce the levels of unemployment in Africa.

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Competing interests

We have no competing interests to declare.

Authors' contributions

K.K.N.H.: Data collection; writing – initial draft; data analysis. I.S.E.U.: Conceptualisation; methodology; validation; student supervision; writing – revisions. N.P.K.: Conceptualisation; methodology; student supervision; writing – revisions. W.E.: Methodology; validation; student supervision; writing – revisions. K.K.M.N.: Conceptualisation; student supervision; writing – revisions; project leadership.

References

1. Hapuarachchi KK, Wen TC, Deng CY, Kang JC, Hyde KD. Mycosphere essays 1: Taxonomic confusion in the *Ganoderma lucidum* species complex. Mycosphere. 2015;6:542–559. https://doi.org/10.5943/mycosphere/6/5/4
2. Obodai M, Mensah DLN, Fernandes A, Kortei NK, Dzomeku M, Teegarden M, et al. Chemical characterization and antioxidant potential of wild *Ganoderma* species from Ghana. Molecules. 2017;22:196. https://doi.org/10.3390/molecules22020196
3. Yang J, Chen Y, Leong N, Zhao J, Duan J, Tang Y, et al. Quality evaluation of different products derived from *Ganoderma*. J Med Plant Res. 2012;6:1969-1974. https://doi.org/10.5897/JMPR11.1668
4. Kim HK, Shim MY, Seo GS, Kim HG. Comparison of characteristics of *Ganoderma lucidum* according to geographical origins (III): Classification between species of genus *Ganoderma* using Dikaryon-Monokaryon mating. Mycobiology. 2002;30:61–64. https://doi.org/10.4489/MYCO.2002.30.2.061
5. Kadhila-Muandingi NP. The distribution, genetic diversity and uses of *Ganoderma* mushrooms in Oshana and Ohangwena regions of northern Namibia [master's dissertation]. Windhoek: University of Namibia; 2010.
6. Coetzee MPA, Marincowitz S, Muthelo VG, Wingfield MJ. *Ganoderma* species, including new taxa associated with root rot of the iconic *Jacaranda mimosifolia* in Pretoria, South Africa. IMA Fungus. 2015;6:249–256. https://doi.org/10.5598/imafungus.2015.06.01.16
7. Yalcin OU, Sarikurkcu C, Cengiz M, Gungor H, Zeljković SC. *Ganoderma carnosum* and *Ganoderma pfeifferi*: Metal concentration, phenolic content, and biological activity. Mycologia. 2020;112:1–8. https://doi.org/10.1080/00275514.2019.1689748
8. Kozarski M, Klaus A, Niksic M, Vrvic MM, Todorovic N, Jakovljevic D, et al. Antioxidative activities and chemical characterization of polysaccharide extracts from the widely used mushrooms *Ganoderma applanatum*, *Ganoderma lucidum*, *Lentinus edodes* and *Trametes versicolor*. J Food Compost Anal. 2012;26:144–153. https://doi.org/10.1016/j.jfca.2012.02.004
9. Luangharn T, Karunarathna SC, Khan S, Xu JC, Mortimer PE, Hyde KD. Antibacterial activity, optimal culture conditions and cultivation of the medicinal *Ganoderma australe*, new to Thailand. Mycosphere. 2017;8:1108-1123. https://doi.org/10.5943/mycosphere/8/8/11
10. Singh R, Dhingra GS, Shri R. A comparative study of taxonomy, physicochemical parameters, and chemical constituents of *Ganoderma lucidum* and *G. philippii* from Uttarakhand, India. Turk J Bot. 2014;38:186-196. https://doi.org/10.3906/bot-1302-39
11. Abdalla RR, Ahmed AI, Abdalla AI, Abdelmaboud OA, Khiery NTMA, Elriah N, et al. Some wild edible and medicinal mushroom species at Khartoum and Sinnar States-Sudan. J Microb Biochem Technol. 2016;8:6.
12. Fathima AT, Reenaa M. Anticancer and antibacterial activity of *Ganoderma lucidum*. Int J Curr Microbiol Appl Sci. 2016;5:891–909. https://doi.org/10.20546/ijcmas.2016.510.097
13. Joseph S, Sabulal B, George V, Antony KR, Janardhanan KK. Antitumor and anti-inflammatory activities of polysaccharides isolated from *Ganoderma lucidum*. Acta Pharmaceut. 2011;61:335–342. https://doi.org/10.2478/v10007-011-0030-6
14. Ma H, Hsieh J, Chen S. Anti-diabetic effects of *Ganoderma lucidum*. Phytochemistry. 2015;114:109–113. https://doi.org/10.1016/j.phytochem.2015.02.017
15. Xu T, Beelman RB. The bioactive compounds in medicinal mushrooms have potential protective effects against neurodegenerative diseases. Adv Food Technol Nutr Sci Open J. 2015;1:62–66. https://dx.doi.org/10.17140/AFTNSOJ-1-110
16. Ekandjo LK. Genetic diversity of *Ganoderma* species in the north-eastern parts of Namibia [master's dissertation]. Windhoek: University of Namibia; 2012.
17. Shikongo LT. Analysis of the mycochemical components of the indigenous Namibian *Ganoderma* mushrooms [master's dissertation]. Windhoek: University of Namibia; 2012.
18. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull. 1987;19:11–15.

19. Ueitele ISE, Chimwamurombe P, Kadhila-Muandingi NP. Optimisation of indigenous *Ganoderma lucidum* productivity under cultivation in Namibia. Int Sci Tech J Namibia. 2014;3:35-41.

20. Hamwenye KKN. Identification and cultivation of *Ganoderma* mushroom species in Namibia and the physicochemical properties, phenolics composition and in vitro antioxidant activity of their infusions [master's dissertation]. Windhoek: University of Namibia; 2020.

21. Association of Official Analytical Chemists (AOAC). Official methods of analysis, 18th ed. Washington DC: AOAC; 2005.

22. Rweyemamu LMP, Yusuph A, Mrema GD. Physical properties of extruded snacks enriched with soybean and moringa leaf powder. Afr J Food Sci Tech. 2015;6:28–34.

23. Hussein AMS, Shedeed NA, Abdel-Kalek HH, El-Din MHAS. Antioxidative, antibacterial and antifungal activities of tea infusions from berry leaves, Carob and Doum. Polish J Food Nutr Sci. 2011;61:201–209. https://doi.org/10.2478/v10222-011-0022-8

24. Herrera T, Aguilera Y, Rebollo-Hernanz M, Bravo E, Benítez V, Martínez-Sáez N, et al. Teas and herbal infusions as sources of melatonin and other bioactive non-nutrient components. LWT-Food Sci Technol. 2018;89:65–73. https://doi.org/10.1016/j.lwt.2017.10.031

25. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73:73–84. https://doi.org/10.1016/S0308-8146(00)00288-0

26. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178–182. https://doi.org/10.38212/2224-6614.2748

27. Price ML, Van Scoyoc S, Butler LG. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J Agric Food Chem. 1978;26:1214-1218. https://doi.org/10.1021/jf60219a031

28. McCune LM, Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North America bored forest. J Ethnopharmacol. 2002;82:197–205. https://doi.org/10.1016/s0378-8741(02)00180-0

29. Roy S, Jahan MAA, Das KK, Munshi SK, Noor R. Artificial cultivation of *Ganoderma lucidum* (Reishi medicinal mushroom) using different sawdusts as substrates. Am J Biosci. 2015;3:178–182. https://doi.org/10.11648/j.ajbio.20150305.13

30. Kadhila-Muandingi NP, Mubiana FS, Halueendo KL. Mushroom cultivation: A beginner's guide. 2nd ed. Windhoek: University of Namibia; 2012.

31. Ogbe AO, Ditse U, Echeonwu I, Ajodoh K, Atawodi SE, Abdu PA. Potential of a wild medicinal mushroom, *Ganoderma* sp. as feed supplement in chicken diet: Effect on performance and health of pullets. Int J Poult Sci. 2009;8:1052–1057. https://doi.org/10.3923/ijps.2009.1052.1057

32. Slynko NM, Blinov AG, Babenko VN, Mihailova SV, Bannikova SV, Shekhovtsov SV, et al. Phylogenetic and biochemical analysis of the Reishi mushroom (*Ganoderma lucidum*) populations from Altai. Ann Appl Microbiol Biotechnol J. 2017;1:1004. https://doi.org/10.36876/aamb.1004

33. Wandati TW, Kenji GM, Onguso JM. Phytochemicals in edible wild mushrooms from selected areas in Kenya. J Food Res. 2013;2:137–144. https://doi.org/10.5539/jfr.v2n3p137

34. Zhou Q, Yang W, Lin J, Guo L. Optimization of medium pH, growth media compositions and analysis of nutritional components of *Ganoderma lucidum* in submerged culture fermentation. Eur J Med Plants. 2014;6:17–25. https://doi.org/10.9734/EJMP/2015/14828

35. Stojkovic DS, Barros L, Calhelha RC, Glamoclija J, Ciri A, Van Griensven LJLD, et al. A detailed comparative study between chemical and bioactive properties of *Ganoderma lucidum* from different origins. Int J Food Sci Nutr. 2014;65:42–47. https://doi.org/10.3109/09637486.2013.832173

36. Singh R, Singh AP, Dhingra GS, Shri R. Taxonomy, physicochemical evaluation and chemical investigation of *Ganoderma applanatum* and *G. brownie*. Int J Adv Res. 2014;2:702–711.

37. Zhu Y, Tan ATL. Discrimination of wild-grown and cultivated *Ganoderma lucidum* by fourier transform infrared spectroscopy and chemometric methods. Am J Anal Chem. 2015;6:480–491. https://doi.org/10.4236/ajac.2015.65047

38. Cor D, Botic T, Knez Z, Gregori A, Pohleven F. The effects of different solvents on bioactive metabolites and 'in vitro' antioxidant and anti-acetylcholinesterase activity of *Ganoderma lucidum* fruiting body and Primordia extracts. Maced J Chem Chem Eng. 2017;36:129–141. https://doi.org/10.20450/mjcce.2017.1054

39. Raseta MJ, Vrbaski SN, Boskovic EV, Popovic MR, Mimica-Dukic NM, Karaman MA. Comparison of antioxidant capacities of two *Ganoderma lucidum* strains of different geographical origins. Matica Srpska J Nat Sci. 2017;133:209–219. https://doi.org/10.2298/ZMSPN1733209R

40. Rajoriya A, Tripathy SS, Gupta N. *In vitro* antioxidant activity of selected *Ganoderma* species found in Odisha, India. Int J Trop Plant Res. 2015;2:72-77.

41. Sharif S, Shahid M, Mushtaq M, Akram S, Rashid A. Wild mushrooms: A potential source of nutritional and antioxidant attributes with acceptable toxicity. Prev Nutr Food Sci. 2017;22:124–130.

42. Dharmaraj K, Kuberan T, Sivasankari R. Studies on antimicrobial activities in *Ganoderma lucidum, Fomes Fomentarius* and *Ganoderma tsugae*. J. Sci. 2015;5:116–123.

43. Islam T, Yu X, Xu B. Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China. LWT-Food Sci Technol. 2016;72:423–431. https://doi.org/10.1016/j.lwt.2016.05.005

44. Gil-Ramírez A, Pavo-Caballero C, Baeza E, Baenas N, Garcia-Viguera C, Marín FR, et al. Mushrooms do not contain flavonoids. J Funct Foods. 2016;25:1-13. https://doi.org/10.1016/j.jff.2016.05.005

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Antioxidant activity of the bioactive compounds from the edible fruits and leaves of *Ficus sur* Forssk. (Moraceae)

Ficus sur Forssk. (Moraceae) is a medicinal plant species found in Africa and the leaves are used in traditional medicine as a blood builder to boost iron levels for the treatment of anaemia, skin disorders and sexually transmitted diseases. In this study, a phytochemical investigation was conducted on *F. sur* and the antioxidant properties of the isolates and extracts were evaluated. The major secondary metabolites that were isolated from the fruits and leaves were the triterpenoid (lupeol), sterol (β-sitosterol), phaeophytin (phaeophytin a) and flavonoid (epicatechin). The findings reveal significantly higher ($p<0.05$) antioxidant activity for the methanol extract of the fruits (IC_{50} 9.06 μg/mL), which may be attributed to the higher phenolic content and presence of epicatechin. The results show the species to be rich in pharmacologically active compounds that are documented to exhibit haematinic effects, stimulate reconstruction and cell proliferation in skin, and inhibit the growth and proliferation of pathogenic agents of sexually transmitted infections. This study therefore validates the ethnomedicinal use of the plant, and its consumption could have a profound influence on nutrition and health, especially amongst indigenous people of Africa.

Significance:

- In South Africa, the use of indigenous plants for food and medicine, especially by rural populations, has increased due to availability and accessibility.
- This study highlights the benefits of the edible fruits of *Ficus sur* as a nutraceutical.
- *Ficus sur* is shown to contain biomolecules with well-known therapeutic value, which lends scientific credence and validity to its ethnomedicinal use.

Introduction

Oxidative stress is a major risk factor leading to a variety of chronic and degenerative disorders such as cardiovascular and neurodegenerative diseases, aging and cancer.[1,2] Plant-based antioxidants are well known for their anticancer, anti-inflammatory and anti-aging properties.[3] These activities are largely attributed to the presence of compounds such as flavonoids, tannins, steroids, coumarins and pentacyclic triterpenes.[4]

The genus *Ficus* (Moraceae) has more than 850 species growing all over the world, of which 36 are indigenous to southern Africa (Namibia, Botswana, Zimbabwe, Mozambique south of the Zambezi River and South Africa), 25 of which are indigenous to South Africa.[5] Although a wide variety of compounds including phenolics, flavonoids, alkaloids, coumarins and sterols have reportedly been isolated from the genus, members of this genus are particularly known for their high content of triterpenoids.[6-9]

Ficus sur Forssk. is commonly referred to as the Cape fig, broom cluster fig, bush fig or Malabar tree. It is a large spreading tree, usually about 12 m high, but reaches 25–30 m in some areas. The fruits of the plant are edible. The fruit is oblong-ovoid in shape and usually 15–40 mm across the length. When ripe, the fruit is orange-red, soft with many seeds, and is readily consumed by indigenous people of Africa. Fruits are reported to contain sterols, triterpenoids, flavonoids, glycosides, tannins and carbohydrates.[8] The root and bark decoctions from the plant are used in traditional medicine to treat a variety of ailments including pulmonary tuberculosis, influenza and skin diseases.[10] Fresh leaves of the plant are also used as a blood builder for boosting iron levels and to treat diarrhoea, anaemia and sexually transmitted diseases.[11] Previously, we reported on the antioxidant activity of *Ficus burtt-davyi* Hutch, the cytotoxicity of the bioactive compounds from *F. burtt-davyi* as well as the nutritional and the toxicological assessment of heavy metals in edible fruits of *Ficus sur* Forssk.[12-14] In this study, we report on the isolation and identification of the bioactive compounds from the fruits and leaves of *F. sur*. Additionally, we report on the antioxidant activity of selected crude extracts and isolated compounds from this plant species.

Materials and methods

General experimental procedures

Ultraviolet (UV) spectra were obtained on a Hewlett Packard UV-3600 spectrophotometer. Infrared (IR) spectra were recorded using a Perkin-Elmer Universal ATR spectrometer. The ^{1}H, ^{13}C and two-dimensional nuclear magnetic resonance (2D-NMR) spectra were recorded in deuterated $CDCl_3$ and DMSO (Merck, Darmstadt, Germany) using a 400-MHz spectrometer (Avance III, Bruker, Rheinstetten, Germany). High-resolution mass spectra were recorded using a time-of-flight mass spectrometer (LCT Premier TOF-MS, Waters Micro-mass, Milford, MA, USA). Column chromatography was performed with Merck silica gel 60 (0.040–0.063 mm) and Sephadex LH-20 (25–100 μm bead size, Sigma-Aldrich, Germany). Thin-layer chromatography was performed on Merck 20 × 20 cm silica gel 60, F_{254} aluminium sheets. The spots were analysed under UV (254 nm and 366 nm), visualised using 10% H_2SO_4 in MeOH, followed by heating. Solvents (analytical grade) and other chemicals used were supplied by either Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA) chemical companies.

Plant materials

The plant was collected in August 2015 from the University of KwaZulu-Natal, Westville Campus, Durban, South Africa. The identity was confirmed by one of the authors (H.B). A voucher specimen (Ogunlaja, 2) of the plant was deposited at the WARD herbarium, School of Life Sciences, University of KwaZulu-Natal, South Africa.

Extraction and isolation

Dried, powdered fruits (800 g) and leaves (950 g) were subjected to sequential extraction with *n*-hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) by continuous shaking on an orbital shaker for 48 h at room temperature for each solvent. All extracts were concentrated by evaporation under vacuum at controlled temperatures, dried and stored in a refrigerator at 4 °C until analysed. The crude DCM extract of fruits (9.19 g) was subjected to column chromatography using 100% Hex that was stepwise increased by 10% to 100% EtOAc at a flow rate of approximately 50 mL/min, collecting eight 100-mL fractions for each eluent step. Fractions 20–23 were combined and further purified with Hex:EtOAc (1:1) to afford compound **1** (150.5 mg). From the same combined fractions, compound **2** was eluted with Hex:EtOAc (8:2), and re-crystallised in MeOH to give a white powder.

The MeOH extract of fruits (13.7 g) was subjected to partitioning with an equal volume of EtOAc and DCM. The EtOAc fraction was dried with anhydrous Na_2SO_4, and the resultant concentrated extract subjected to column chromatography with fractions 9–12 yielding yellow crystals of compound **3** (41.0 mg). The Hex extract of leaves (14.07 g) was separated similarly to the DCM extract of fruits and yielded compound **1** (105 mg) with Hex:EtOAc (8:2). Similarly, the crude EtOAc extract of leaves (10.44 g) was subjected to column chromatography with Hex:EtOAc step gradient (with 10% increments every 100 mL). Compound **4** (41.87 mg) was eluted with Hex:EtOAc (8:2) as a dark green amorphous solid. The other extracts from the fruits and leaves did not yield compounds that could be elucidated.

Ferric reducing antioxidant power assay

The total reducing power of the MeOH extracts (fruits and leaves) and isolated compounds from *F. sur* was determined according to the ferric (Fe^{3+}) reducing antioxidant power (FRAP) method as described by Behera et al.[15] with some modifications. Various concentrations (7.5-500 μg/mL) in MeOH were mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 0.1% potassium ferricyanide and the mixture was incubated at 50 °C for 30 min. After the addition of 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 1008 *g* for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm. In this assay, the Fe^{3+}/ferricyanide complex is reduced to the ferrous form (Fe^{2+}), and the test solution colour changes from yellow to pale green or blue, depending on the reducing power of the antioxidant. MeOH without reagents was used as a negative control while ascorbic acid and butylated hydroxyanisole (E320) (BHA) with the same concentrations were used as positive controls. All procedures were performed in triplicate.

DPPH radical scavenging activity assay

The antioxidant activity of the extracts (fruits and leaves) and isolated compounds was measured in terms of radical scavenging ability, using the DPPH method as described by Ahmad et al.[16] with some modifications. Various concentrations (7.5–500 μg/mL) of extracts and isolated compounds (150 μL) made from stock solutions (10 μg/mL) were mixed with 2850 μL MeOH solution containing DPPH radicals. The mixture was then vortexed and incubated for 30 min at room temperature. Thereafter, the absorbance was measured at 517 nm against MeOH as a blank using a UV-Vis spectrophotometer.

The scavenging activity was evidenced by a change in colour from purple to yellow, due to proton transfer to the $DPPH^{\bullet}$ free radical by a scavenger which was further measured by the decrease in absorbance at 517 nm using a Shimadzu UV–Vis spectrophotometer. Ascorbic acid and BHA were used as standards and the procedure was done in triplicate. The difference in absorbance between the test sample and the negative control (DPPH + MeOH) was expressed as percentage inhibition. The percentage free radical scavenging activity was calculated according to the following equation:

$$\% \text{ scavenging activity} = \% \text{ inhibition} = [(A_o - A_{sample}/ A_{0)} \times 100]$$

where A_o = absorbance of the negative control and A_{sample} = absorbance of sample.

Statistical analyses

The experimental results were expressed as mean±standard deviation (s.d.) of three replicates and IC_{50} values were calculated by linear regression. The data were subjected to one-way analysis of variance (ANOVA) to determine significant differences between means ($p<0.05$). Tukey's test was used for post-hoc analyses. All the statistical tests were performed using GraphPad Prism 6.0.

Results and discussion

The DCM extract from the fruits of *F. sur* afforded two compounds, compounds **1** and **2** (Figure 1), which were identified as β-sitosterol and lupeol, respectively.[17,18] β-sitosterol was also isolated from the leaves. Previously, these compounds were isolated from the edible fruits of *Harpephyllum caffrum*.[19] β-sitosterol and lupeol have also been isolated from figs such as *F. racemosa.*[20]

The MeOH extract of the fruits yielded compound **3** (Figure 1). The ^{1}H-NMR spectrum for compound **3** showed characteristic resonances for flavonoids at δ_H 6.88 (H-2', d, J=1.60 Hz), δ_H 6.65 (H-5', d, J=8.2 Hz) and δ_H 6.64 (H-6', dd, J=1.60, 8.2 Hz) from the B-ring catechol moiety as well as at δ_H 5.88 (H-6, d, J=2.24 Hz) and δ_H 5.70 (H-8, J=2.24 Hz) from the meta-coupled protons of the A-ring resorcinol moiety. The isomers catechin and epicatechin may be differentiated by the chemical shift of C-2 in the ^{13}C-NMR spectrum, which is approximately δ_c 78.0 for epicatechin and δ_c 82.2 for catechin, and by correlations between H-2 and H-3 in the COSY experiment, which is strong for catechin and weak for epicatechin because of the difference in the dihedral angle.[21] Based on the resonance for C-2 at δ_c 78.0, a weak H-2/H-3 correlation in the COSY experiment, ^{1}H-NMR, ^{13}C-NMR, and data in the literature[22,23], compound **3** was identified as epicatechin. This identification was further confirmed by gas chromatography–mass spectrometry data, IR and UV-Vis spectroscopy. Epicatechin has previously been isolated from other *Ficus* species.[22-24]

The EtOAc extract from the leaves afforded compound 4 (Figure 1), which was a dark green amorphous pigment (phaeophytin a). The spectral data for compound 4 compared well with our data on phaeophytin a which was previously isolated from *F. burtt-davyi*[12], confirming compound 4 to be phaeophytin a. Similarly, *F. carica* has been reported to contain phaeophytin a.[8] The spectral assignments for compounds 1–4 are indicated in the supplementary material.

The functional components in food are widely assessed by their antioxidant capacity, hence, in this study, the extracts of fruits and leaves and isolated compounds were evaluated for their in vitro antioxidant potential using the FRAP and DPPH assays, relative to the positive controls (ascorbic acid and BHA). Except for epicatechin and the MeOH extracts from fruits and leaves, other isolated compounds and extracts showed very weak antioxidant activity for both assays and are therefore omitted from the results.

Both the DPPH and FRAP assays showed the antioxidant activity of the extracts and tested compounds to be concentration-dependent (Figures 2 and 3). The MeOH extracts of the fruits displayed significantly higher ($p<0.05$) radical scavenging activity than other extracts, especially at higher concentrations (50–500 μg/mL), and the difference was not significant ($p>0.05$) with the standard antioxidants in some cases (250 and 500 μg/mL).

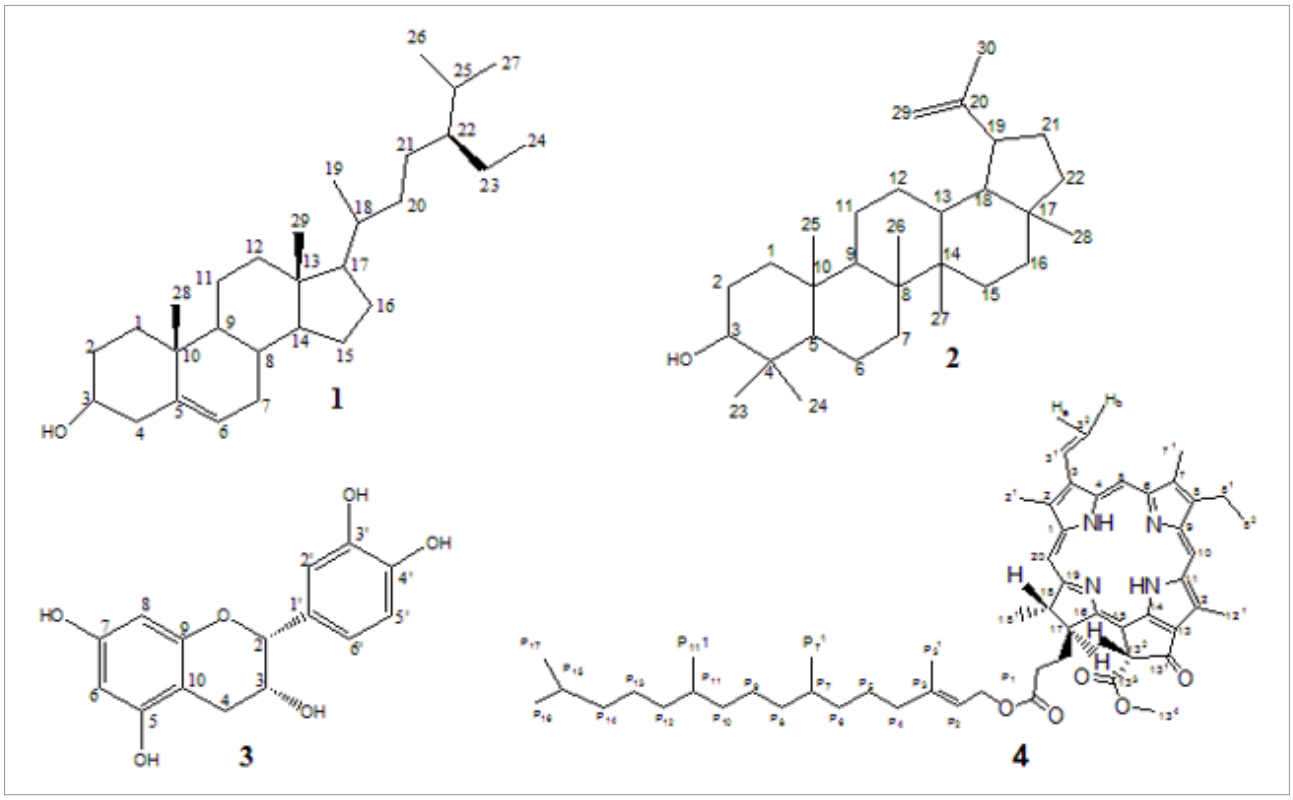

Figure 1: Chemical structures of compounds **1** (β-sitosterol), **2** (lupeol), **3** (epicatechin) and **4** (phaeophytin a) isolated from *Ficus sur.*

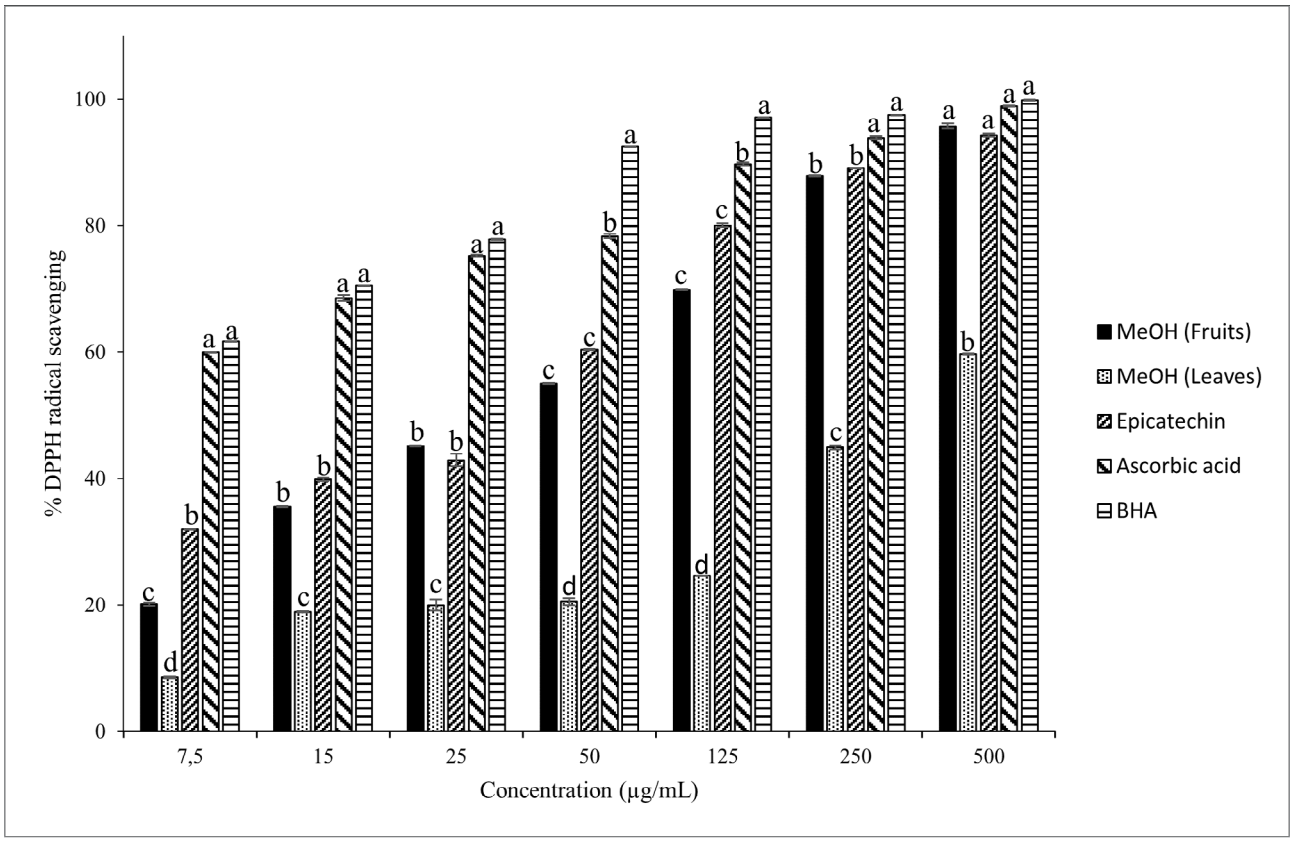

Figure 2: DPPH radical scavenging activity (%) of MeOH extracts of *Ficus sur* (fruits and leaves), epicatechin and positive controls (ascorbic acid and butylated hydroxyanisole (E320) (BHA)). Different letters over the bars indicate significantly different concentrations (Tukey's HSD multiple range post-hoc test, $p<0.05$).

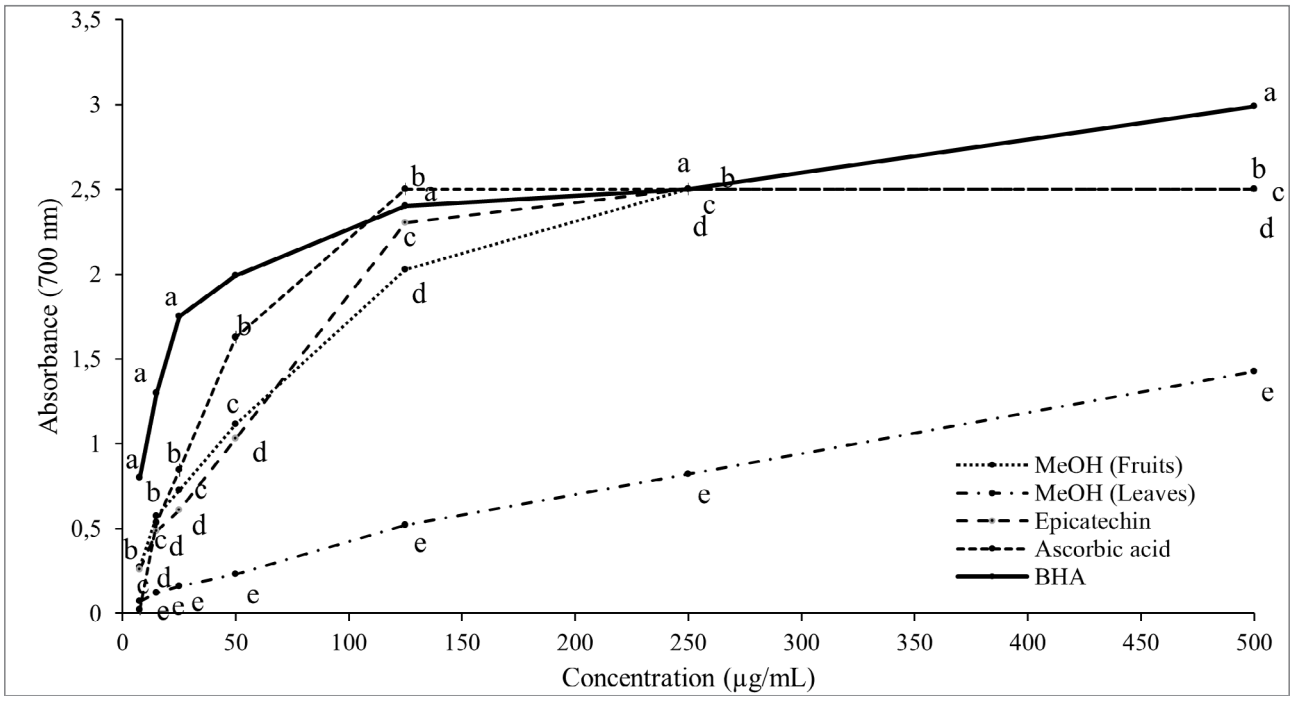

Figure 3: Reducing power of the MeOH extract of *Ficus sur* (fruits and leaves), epicatechin, ascorbic acid and butylated hydroxyanisole (E320) (BHA). Values with different letters for a given concentration of each extract and compound are significantly different from each other (Tukey's HSD multiple range post-hoc test, $p<0.05$).

Similarly, the results produced by the FRAP assay showed the reducing power of the MeOH extract of fruits to be comparable to that of the positive controls (Figure 3). The antioxidant activity of the MeOH extract of leaves was significantly lower than the other test samples. The DPPH radical scavenging activity was found to be in the order of BHA > ascorbic acid > MeOH (fruits) > epicatechin > MeOH (leaves). The ferric reducing antioxidant power was found to be in the order of BHA > ascorbic acid > epicatechin > MeOH (fruits) > MeOH (leaves).

The results that include BHA ($IC_{50}=1.93\pm0.11$ µg/mL) and ascorbic acid ($IC_{50}=2.03\pm0.01$ µg/mL) as the controls, showed that the antioxidant activity of the extract of the fruits ($IC_{50}=9.06\pm2.21$ µg/mL) was comparable to that of the controls, which was significantly higher ($p<0.05$) than that of the leaves ($IC_{50}=369.19\pm12.04$ µg/mL). As polyphenolic compounds, epicatechin and catechin can act as antioxidants via a free radical scavenging mechanism with the formation of the less reactive flavonoid phenoxyl radical. The high antioxidant potential of flavonoids may be explained by their ability to donate a hydrogen atom from their hydroxyl group and thereby scavenge the free radicals as well as to chelate certain metals, such as iron. Data from this study and our previous report[12] suggest that the antioxidant activity of the MeOH extract of the stem bark of *F. burtt-davyi* and MeOH extracts of fruits of *F. sur* is due to the presence of catechin and epicatechin, respectively.

Previous studies on grape seed extracts, which are rich in catechins, have shown increased absorption of haemic iron on the apical side with blockage of transepithelial transporter inhibition of intestinal absorption of non-haemic iron, thereby indicating the benefits of the extract in treating anaemia.[25,26] Another study that investigated the haematinic effect of the ethanol extract of *F. sur* leaves on diethyl nitrosamine-induced haemolytic anaemia in Wistar rats demonstrated its amelioration on haematoxicity by increasing blood haemoglobin or stimulating blood cell formation.[27] The authors indicated that the haematinic effect of the antioxidant molecules present in the plant extract could explain its traditional use as a blood booster. Triterpenes, such as lupeol, are widely used to treat skin burns and various skin ailments as studies have demonstrated that the skin is stimulated to reconstruction and cell proliferation under lupeol treatment.[28,29] A review on the therapeutic and clinical effects of medicinal plants and their phytocompounds that inhibit the growth and proliferation of pathogenic agents of sexually transmitted infections revealed that the most important phytocompounds involved in reducing sexually transmitted infections are flavonoids (quercetin, myricetin and luteolin), alkaloids, terpenoids (lupeol) and sterols (β-sitosterol).[30]

In our study, we isolated and identified the secondary metabolites present in the extracts of *F. sur* and propose that its use as a blood booster and for the treatment of anaemia is mostly due to the presence of the major flavonol, epicatechin; the treatment of skin diseases is mostly due to the presence of lupeol and the treatment of sexually transmitted infections could be due to synergistic effects of the flavonoid, sterol and triterpene (epicatechin, β-sitosterol and lupeol, respectively).

Conclusion

The phytochemical investigation shows *F. sur* fruits to be rich in β-sitosterol, lupeol and epicatechin and leaves to be rich in phaeaophytin a and β-sitosterol. The methanol extracts of the fruits showed significant antioxidant activity. This study highlights the nutraceutical potential of the edible fruits of *F. sur,* emphasising its importance in South Africa, where reliance on wild foods and traditional medicine is on the rise due to availability and accessibility. This study also lends scientific credence and validity to the ethnomedicinal use of the plant as a blood booster and for the treatment of anaemia, skin disorders and sexually transmitted diseases.

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Competing interests

We have no competing interests to declare.

Authors' contributions

O.O.O.: Methodology, data collection, sample analysis, data analysis, validation, writing - the initial draft, writing – revisions. R.M.: Conceptualisation, student supervision, project leadership, project management, writing – revisions. H.B.: Conceptualisation, student

supervision, data collection. S.B.J.: Conceptualisation, student supervision, funding acquisition.

References

1. Kao C, Jesuthasan AC, Bishop KS, Glucina MP, Ferguson LR. Anti-cancer activities of *Ganoderma lucidum*: Active ingredients and pathways. Functional Foods Health Dis. 2013;3(2):48–65. https://doi.org/10.31989/ffhd.v3i2.65
2. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr. 2004;44(4):275–295. https://doi.org/10.1080/10408690490468489
3. Mayne ST. Antioxidant nutrients and chronic disease: Use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr. 2003;133(3):933S–940S. https://doi.org/10.1093/jn/133.3.933S
4. Ragasa CY, Tsai PW, Shen CC. Terpenoids and sterols from the endemic and endangered Philippine trees, *Ficus pseudopalma* and *Ficus ulmifolia*. Philipp J Sci. 2009;138(2):205–209.
5. Burrows JE, Burrows S. Figs of southern & south-central Africa. Pretoria: Umdaus Press; 2003.
6. Chang MS, Yang YC, Kuo YC, Kuo YH, Chang C, Chen CM, et al. Furocoumarin glycosides from the leaves of *Ficus ruficaulis* Merr. var. antaoensis. J Nat Prod. 2005;68(1):11–13. https://doi.org/10.1021/np0401056
7. Chiang YM, Kuo YH. Novel triterpenoids from the aerial roots of *Ficus microcarpa*. J Organic Chem. 2002;67(22):7656–7661. https://doi.org/10.1021/jo020262e
8. Lansky EP, Paavilainen HM. Figs: The genus *Ficus*. Boca Raton, FL: CRC Press; 2011.
9. Lee TH, Kuo YC, Wang GJ, Kuo YH, Chang CI, Lu CK, et al. Five new phenolics from the roots of *Ficus beecheyana*. J Nat Prod. 2002;65(10):1497–1500. https://doi.org/10.1021/np020154n
10. Eldeen IM, Elgorashi EE, Van Staden J. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. J Ethnopharmacol. 2005;102(3):457–464. https://doi.org/10.1016/j.jep.2005.08.049
11. Yakubu OE, Nwodo OFC, Udeh SMC, Abdulrahman M. The effects of aqueous and ethanolic extracts of *Vitex doniana* leaf on postprandial blood sugar concentration in Wistar rats. Int J Biochem Res Rev. 2016;11:1–7. https://doi.org/10.9734/IJBCRR/2016/12893
12. Ogunlaja OO, Moodley R, Baijnath H, Jonnalagadda SB. Chemical constituents and in vitro antioxidant activity of crude extracts and compounds from leaves and stem bark of *Ficus burtt-davyi.* Acta Poloniae Pharmaceutica. 2016;73(6):1593–1600.
13. Ogunlaja OO, Moodley R, Baijnath H, Jonnalagadda SB. Nutritional evaluation, bioaccumulation and toxicological assessment of heavy metals in edible fruits of *Ficus sur* Forssk (Moraceae). J Environ Sci Health B. 2017;52(2):84–91. https://doi.org/10.1080/03601234.2016.1239974
14. Ogunlaja OO, Moodley R, Singh M, Baijnath H, Jonnalagadda SB. Cytotoxic activity of the bioactive principles from *Ficusburtt-davyi*. J Environ Sci Health B. 2018;53(4):261–275. https://doi.org/10.1080/03601234.2017.1410385
15. Behera BC, Verma N, Sonone A, Makhija U. Determination of antioxidative potential of lichen *Usneaghattensis in vitro*. LWT-Food Sci Technol. 2006;39(1):80–85. https://doi.org/10.1016/j.lwt.2004.11.007
16. Ahmad R, Hashim HM, Noor ZM, Ismail NH, Salim F, Lajis NH, et al. Antioxidant and antidiabetic potential of Malaysian *Uncaria*. Res J Med Plant. 2011;5(5):587–595. https://doi.org/10.3923/rjmp.2011.587.595
17. Venkata SP, Indra P. Isolation of stigmasterol and β-sitosterol from the dichloromethane extract of *Rubus suavissimus*. Int Curr Pharm J. 2012;1:239–242. https://doi.org/10.3329/icpj.v1i9.11613
18. Mahato SB, Kundu AP. ^{13}C NMR spectra of pentacyclic triterpenoids – a compilation and some salient features. Phytochemistry. 1994;37(6):1517-1575. https://doi.org/10.1016/S0031-9422(00)89569-2
19. Moodley R, Koorbanally NA, Shahidul Islam MD, Jonnalagadda SB. Structure and antioxidant activity of phenolic compounds isolated from the edible fruits and stem bark of *Harpephyllum caffrum*. J Environ Sci Health B. 2014;49(12):938–944. https://doi.org/10.1080/03601234.2014.951578
20. Deshmukh TA, Yadav BV, Badole SL, Bodhankar SL, Dhaneshwar SR. Antihyperglycaemic activity of petroleum ether extract of *Ficus racemosa* fruits in alloxan induced diabetic mice. Pharmacol Online. 2007;2:504–515.
21. Es-Safi NE, Guyot S, Ducrot PH. NMR, ESI/MS, and MALDI-TOF/MS analysis of pear juice polymeric proanthocyanidins with potent free radical scavenging activity. J Agric Food Chem. 2006;54(19):6969–6977. https://doi.org/10.1021/jf061090f
22. Van Kiem P, Cuong NX, Nhiem NX, Thu VK, Ban NK, Van Minh C, et al. Antioxidant activity of a new C-glycosylflavone from the leaves of *Ficus microcarpa*. Bioorg Med Chem Lett. 2011;21(2):633–637. https://doi.org/10.1016/j.bmcl.2010.12.025
23. Ragab EA, Mohammed AE, Abbass HS, Kotb SI. A new flavan-3-ol dimer from *Ficus sprague*ana leaves and its cytotoxic activity. Pharmacognosy Magazine. 2013;9(34):144–148. https://doi.org/10.4103/0973-1296.111274
24. Awolola GV, Koorbanally NA, Chenia H, Shode FO, Baijnath H. Antibacterial and anti-biofilm activity of flavonoids and triterpenes isolated from the extracts of *Ficus sansibarica* Warb. subsp. *sansibarica* (Moraceae) extracts. Afr J Tradit Complement Altern Med. 2014;11(3):124–131. https://doi.org/10.4314/ajtcam.v11i3.19
25. Ma Q, Kim EY, Han O. Bioactive dietary polyphenols decrease heme iron absorption by decreasing basolateral iron release in human intestinal Caco-2 cells. J Nutr. 2010;140:1117–1121. https://doi.org/10.3945/jn.109.117499
26. Kim EY, Ham SK, Shigenaga MK, Han O. Bioactive dietary polyphenolic compounds reduce nonheme iron transport across human intestinal cell monolayers. J Nutr. 2008;138:1647–1651. https://doi.org/10.1093/jn/138.9.1647
27. Yakubu OE, Ojogbane E, Abu MS, Shaibu CO, Ayegba WE. Haematinic effects of ethanol extract of *Ficus sur* leaves on diethylnitrosamine-induced toxicity in Wistar rats. J Pharmacol Toxicol. 2020;15:16–21. https://doi.org/10.3923/jpt.2020.16.21
28. Xu F, Huang X, Wu H, Wang X. Beneficial health effects of lupeonone triterpene: A review. Biomed Pharmacother. 2018;103:198–203. https://doi.org/10.1016/j.biopha.2018.04.019
29. Naaimi D. Lupeol stimulates the production of high-quality type I collagen in human skin through HSP47 induction. J Am Acad Dermatol. 2008;58:AB62. https://doi.org/10.1016/j.jaad.2007.10.282
30. Nazer M, Abbaszadeh S, Darvishi M, Kheirollahi A, Shahsavari S, Moghadasi M. The most important herbs used in the treatment of sexually transmitted infections in traditional medicine. Sudan J Med Sci. 2019;14(2):41–64. https://doi.org/10.18502/sjms.v14i2.4691

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Tiger nut (*Cyperus esculentus*): Nutrient profiling using HPLC and UV-spectroscopic techniques

Food insecurity and undernourishment constitute a major challenge in Africa and the world at large. To meet key nutritional targets and tackle the menace of undernourishment, we need to exploit available but underutilised food crops. A common underutilised food crop with the potential to improve daily nutrition is tiger nut. This potential is evidenced in the number of essential amino acids detected, which constitute 74.425% of the entire amino acids detected, in addition to important minerals and vitamins. The nutritional composition of the yellow variety of tiger nut (*Cyperus esculentus*) was determined using the standard methods of high-performance liquid chromatography and UV-spectroscopy. Ten amino acids were identified and quantified, including six essential amino acids, of which valine had the highest concentration (67.59 μg/100 g), followed by leucine (3.019 μg/100 g), phenylalanine (1.767 μg/100 g), lysine (0.946 μg/100 g), histidine (1.048 μg/100 g) and tryptophan (0.055 μg/100 g). The other amino acids were proline (24.124 μg/100 g), cysteine (1.269 μg/100 g), glycine (0.024 μg/100 g), and glutamine (0.022 μg/100 g). Monosaccharides detected were ribose (41.76%), glucose (21.52%), sedoheptulose (17.94%), fructose (4.566%), rhamnose (1.78%) and mannose (1.58%), whilst disaccharides detected were sucrose (87.66%) and maltose (11.39%). Mineral concentrations were K 144.80 ± 1.10 mg/100 g, Ca 94.39 ± 0.02 mg/100 g, Na 83.92 ± 0.04 mg/100 g, Fe 19.36 ± 0.54 mg/100 g, Mg 17.63±0.13 mg/100 g, Cu 13.28±0.05 mg/100 g and Zn 5.18±0.01 mg/100 g Vitamins A, B_2, C and E were detected and quantified as 53.93±1.03, 7.61±1.20, 31.70±1.25 and 128.75±0.74 μg/100 g, respectively. The chemical and nutritional properties of the yellow variety of tiger nut suggest that it is rich in essential amino acids, minerals, and some vitamins. Hence, it should be recommended to persons with nutritional deficiencies as it is cheap and available all year round.

Significance:

- The nutritional composition of the yellow tiger nut will assist in meeting the recommended daily intake of essential amino acids, monosaccharides, disaccharides, minerals, and vitamins, thus contributing towards solving the challenge of food insecurity and malnutrition, particularly in the African sub-region.
- The rich concentration of these nutrients could be harnessed in the biofortification of food materials known to be deficient in one nutrient or another.
- These important attributes of tiger nut, if harnessed, will add value to this underutilised crop and enhance the economic livelihood of the local farmers.

Introduction

As the global population grows, it is important to improve the food base to avoid food crises and their attendant effects. This improvement could be achieved by developing existing feedstocks that are currently underutilised. Some common underutilised food crops include African yam bean, baobab[1] and tiger nut[2].

Tiger nut (*Cyperus esculentus* L.) is an edible sweet nut-like tuber that is most common in Spain, Nigeria, Senegal, Ghana, and Chile.[3] It has three varieties: yellow, brown and black. The black variety is common in Ghana.[4] It has many identities, as it is being cultivated both as a livestock food and for human consumption, and is eaten raw or baked.[5] It is an underutilised crop with high potential for development, which sometimes is considered a weed.[6] In West Africa, the tubers are an essential part of people's diets, because they are cheap, readily available all year round, and have nutritional benefits.[7]

Tiger nut has been reported to have some medicinal effects, such as activation of blood circulation, and prevention of heart disease and thrombosis.[8] Other health benefits that have been attributed to tiger nut include reduction in the risk of colon cancer[7], relief of indigestion due to its fibre content, benefit to diabetics, reduction in cholesterol, and stimulant effects[5].

Three major products are derived from *C. esculentus,* namely tiger nut oil, tiger nut flour, and tiger nut drink.[9] The flour is used as a flavouring agent in ice cream and to produce biscuits and other bakery products[10], and the oil is used in making soap[11]. The main importance of tiger nuts in the food industry is the production of tiger nut milk as a beverage, and a dairy alternative. It is preferred by people with underlying conditions such as lactose intolerance and diabetes mellitus, because of the belief that it does not contain lactose and glucose. Similarly, it is believed to be rich in polyphenolic compounds[12], which are essential antioxidants necessary in the management and treatment of several ailments.

We undertook a comprehensive analysis of the amino acid, monosaccharide, disaccharide, mineral, and vitamin components of the yellow variety of *C. esculentus*.

Materials and methods

Collection of samples

A 2-kg quantity of fresh tiger nut was purchased from a modern market in Nsukka, Enugu State, Nigeria. Defective tubers were discarded, and the healthy ones were washed thoroughly in clean running water and used for the study. The sample was milled using an electric blender to produce a paste-like sample. The sample was stored at 4 °C for further analysis.

Determination of amino acids, monosaccharides and disaccharides

Amino acids, monosaccharides and disaccharides in the sample were quantified using high-performance liquid chromatography with diode-array detection (HPLC-DAD).[13,14]

Determination of minerals

The concentrations of the essential minerals calcium, magnesium, potassium, sodium, iron, copper, and zinc were determined using the spectrophotometric method of the Association of Official Analytical Chemists.[15]

Determination of vitamins

The vitamin A, C and E contents of the sample were determined using the spectrophotometric method described previously.[16,17]

Statistical analysis

The experiment was carried out in triplicate and results were analysed using Statistical Package for Social Sciences (SPSS) version 20.0; significant difference was established at $p<0.05$.

Results

Amino acids

The amino acid contents of tiger nut (yellow variety) are shown in Table 1. Ten amino acids were identified and quantified, including six essential amino acids. Of the essential amino acids, valine had the highest concentration (67.59 μg/100 g), followed by leucine (3.019 μg/100 g), phenylalanine (1.767 μg/100 g), histidine (1.048 μg/100 g), lysine (0.946 μg/100 g), and tryptophan (0.055 μg/100 g). The other amino acids detected were proline, cysteine, glycine, and glutamine, with concentrations of 24.124 μg/100 g, 1.269 μg/100 g, 0.024 μg/100 g, and 0.022 μg/100 g, respectively. The essential amino acids constituted 74.425% of the entire amino acids detected, making the yellow variety of tiger nut a good source of amino acids for humans. Thus, the high content of essential amino acids of the yellow variety of tiger nut should be harnessed and utilised for human benefit. The presence of these amino acids suggests why it is used in the production of different types of drinks such as kunu[18], and for fortification of other food products including biscuits, bread, and cookies[19,20]. Its use in food fortification would in turn improve the market value of the crop.

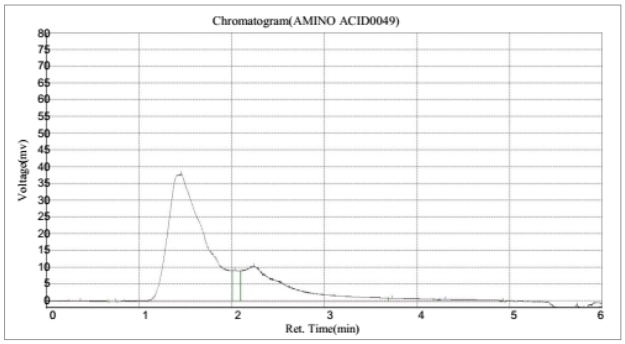

Figure 1: High-performance liquid chromatogram of tiger nut amino acids.

Table 1: Amino acid content of yellow variety of tiger nut

Amino acid	Concentration (μg/100g)
Glycine	0.024
Valine	67.590
Leucine	3.019
Proline	24.124
Phenylalanine	1.767
Cysteine	1.269
Glutamine	0.022
Lysine	0.946
Histidine	1.048
Tryptophan	0.055

Monosaccharides

Ribose was the monosaccharide present in the yellow variety of tiger nut in the highest concentration at 41.76% (Table 2). The concentration of glucose was 21.525%, followed by sedoheptulose, fructose, rhamnose, and mannose at 17.946%, 4.566%, 1.787%, and 1.582%, respectively. These results show that the yellow variety of tiger nut is rich in monosaccharides, which makes it a good source of energy. Although glucose and the other six carbon sugars were present, 41.76% of the monosaccharide content was made up of ribose, an important sugar for the biosynthesis of ATP and other high energy compounds. The low concentrations of the six carbon sugars suggests that tiger nut may not pose a threat or aggravate diabetic-related complications.

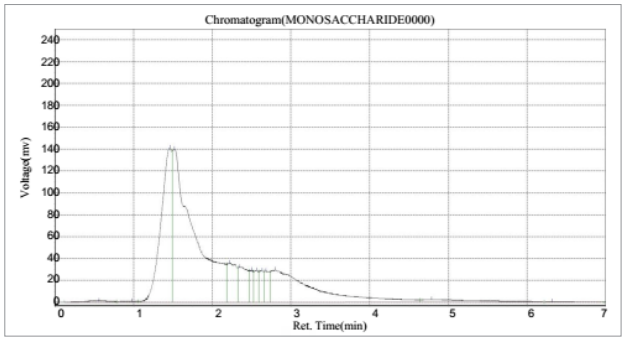

Figure 2: High-performance liquid chromatogram of yellow variety of tiger nut monosaccharide.

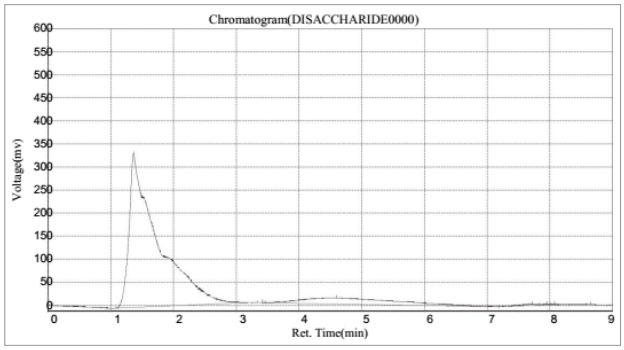

Figure 3: High-performance liquid chromatogram of yellow variety of tiger nut disaccharide.

Table 2: Monosaccharide content of yellow variety of tiger nut

Monosaccharide	Concentration (%)
Glucose	21.525
Ribose	41.764
Fructose	4.566
Mannose	1.582
Rhamnose	1.787
Sedoheptulose	17.946

Table 3: Disaccharide content of yellow variety of tiger nut

Disaccharide	Concentration (%)
Sucrose	87.66
Maltose	11.39

Disaccharides

The disaccharides detected were sucrose (87.66%) and maltose (11.39%). This makes the yellow variety of tiger nut a good source of energy and of precursors for other important biochemical molecules. Sanchez-Zapata et al.[21] reported a sucrose concentration of 13.03%, which is low when compared to the 87.66% obtained in this study. Conversely, a concentration of 130.4 g/kg of sucrose was obtained by Oguike et al.[22] for the yellow variety of tiger nut.

Minerals

The mineral contents of tiger nut are reported in Table 4. The concentration of K was 144.80±1.10 mg/100 g, Ca was 94.39±0.02 mg/100 g, and Na was 83.92±0.04 mg/100 g. Fe, Mg, Cu and Zn were detected at concentrations of 19.36±0.54 mg/100 g, 17.63±0.13 mg/100 g, 13.28±0.05 mg/100 g and 5.18±0.01 mg/100 g, respectively. Micronutrients are an essential part of human nutrition, as they support and strengthen body tissues and organs. These elements also aid or are important for biochemical reactions in the body. Thus, tiger nut could provide the daily requirements of these minerals. They could be sourced from different origins at different concentrations. Among the minerals studied, K and Ca concentrations were highest, and Zn was the lowest (Table 4). Reports from previous researchers show high concentrations of K (110.70, 194 and 216 mg/100 g) and C (185, 152 and 84 mg/100 g) in the yellow variety of tiger nut.[23-25] The concentration of K obtained (144.80 mg/100 g) is high compared to the 110.70 mg/100 g reported by Nina et al.[23] This difference could be attributed to the many factors that influence the plant, such as site of cultivation, period of cultivation and system of cultivation. Tiger nut is an ideal food for pregnant women to consume as it will supplement the K requirement for both the mother and the developing baby.

Na is an important physiological electrolyte that controls responses to stimuli. Na was detected in high concentration (83.92 mg/100 g) (Table 4). Nina et al.[23] reported a higher concentration (100.5 mg/100 g) of Na than that obtained in this study. Obasi and Ugwu[24] obtained a higher concentration of Na than the 83.92 mg/100 g obtained in this study.

The concentration of Cu in the yellow variety of tiger nut was 13.28 mg/100 g; this concentration is high when compared to the concentration reported by previous researchers.[23,25,26] The concentration of Zn was 5.18 mg/100 g (Table 4), which is higher than the 0.01 mg/100 g reported by Oladele and Aina[25]. Similarly, Bado et al.[26] reported a Zn concentration of 2.34 mg/100 g in the yellow variety of tiger nut, which is lower than the 5.18 mg/100 g obtained in this study. Contrarily, Chukwu et al.[27] obtained a higher concentration of Zn (34.77 mg/100 g) in their work on tiger nut. This suggests that the yellow variety of tiger nut contains varying concentrations of Zn. The variations in the concentration of Zn could be attributed to the source of tiger nut harvest.

It is evident that tiger nut is rich in minerals, making it an important nutritional supplement to augment products deficient in these minerals. Also, due to its natural form, it could be recommended for individuals with mineral deficiency diseases and people with special needs for minerals, such as pregnant women and older persons. It is also a very cheap source of these minerals and is available all year round.

Table 4: Mineral content of yellow variety of tiger nut

Mineral	Concentration (mg/100g)
Calcium (Ca)	94.39±0.02
Magnesium (Mg)	17.63±0.13
Potassium (K)	144.80±1.10
Sodium (Na)	83.92±0.04
Iron (Fe)	19.36±0.54
Copper (Cu)	13.28±0.05
Zinc (Zn)	5.18±0.01

Table 5: Vitamin content of yellow variety of tiger nut

Vitamin	Concentration (μg/100g)
Vitamin A	53.93±1.03
Vitamin B2	7.61±1.20
Vitamin C	31.70±1.25
Vitamin E	128.75±0.74

Vitamins

Vitamins C, A, E, and B_2 were detected in the yellow variety of tiger nut in the concentrations of 128.75 μg/100 g, 53.93 μg/100 g, 31.70 μg/100 g, and 7.61 μg/100 g, respectively. Vitamins are important components of our diets. They are essential for the proper functioning of the tissues, organs, and systems. The presence of vitamin E suggests that the consumption of tiger nut could support healthy skin, retard aging and improve fertility. The presence of vitamin A in the sample suggests that the consumption of tiger nut would support sight as well as increase the dietary antioxidant quotient. Suleiman et al.[28] also reported vitamins A and C in the yellow variety of tiger nut at concentrations of 0.87 mg/100 g and 30.70 mg/100 g, respectively. The consumption of tiger nut could assist in meeting the recommended daily intake of vitamin C in children.[29] The presence of vitamin C, as an antioxidant, would aid in the prevention or retrogression of diseases caused by pro-oxidants such as cancer.[30]

Conclusion

We studied the nutritional composition of the yellow variety of tiger nut, that is, the amino acid, monosaccharide, disaccharide, mineral, and vitamin contents. The data obtained show the presence of ten amino acids including six essential amino acids, as well as the monosaccharides glucose, ribose, fructose, mannose, rhamnose and sedoheptulose. The yellow variety of tiger nut is also rich in minerals such as K, Ca, Na and vitamins A, C and E, which are important for the proper growth and development of the body. The rich concentration of these nutrients should be harnessed in the biofortification of food materials known to be deficient in one nutrient or another. Similarly,

these important attributes of tiger nut, if harnessed, will add value to this underutilised crop, improve the nutritional status of consumers, provide food security and enhance livelihoods of the population.

Competing interests

We have no competing interests to declare.

Authors' contributions

K.O.O.: Conceptualisation and resources, project administration, writing – original draft, supervision. J.N.O. Methodology, data curation. B.O.E.: Formal analysis, investigation, writing – review, editing. S.O.O.E.: Conceptualisation and resources, supervision, writing – review, editing.

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References

1. Adegboyega TT, Abberton MT, AbdelGadir AH, Dianda M, Maziya-Dixon B, Oyatomi OA, et al. Evaluation of nutritional and antinutritional properties of African yam bean (*Sphenostylis stenocarpa* (Hochst ex. A. Rich.) Harms.) seeds. J Food Qual. 2020;2020, Art. #6569420 https://doi.org/10.1155/2020/6569420
2. Bamishaiye EI, Bamishaiye OM. Tiger nut. As a plant, its derivatives, and benefits. Afr J Food Agric Nutr Dev. 2011;11(5):5157–5170. https://doi.org/10.4314/ajfand.v11i5.70443
3. De Castro O, Gargiulo R, Del Guacchio E, Caputo P, De Luca P. A molecular survey concerning the origin of *Cyperus esculentus* (Cyperaceae, Poales): Two sides of the same coin (weed vs. crop). Ann Bot. 2015;115:733–745. https://doi.org/10.1093/aob/mcv001
4. Asante FA, Oduro I, Ellis WO, Saalia FK. Effects of planting period and site on the chemical composition and milk acceptability of tigernut (*Cyperus esculentus L.*) tubers in Ghana. Am J Food Nutr. 2014;2(3):49–54. https://doi.org/10.12691/ajfn-2-3-3
5. Bamishaiye EI, Muhammad NO, Bamishaiye OM. Assessment of biological value of tiger nut (*Cyperus esculentus*) tuber oil meal-based diet in rats. Ann Bio Res. 2010;1(4):274–280.
6. Roselló-Soto E, Garcia C, Fessard A, Barba FJ, Munekata PES, Lorenzo JM, Remize F. Nutritional and microbiological quality of tiger nut tubers (*Cyperus esculentus*), derived plant-based and lactic fermented beverages. Fermentation. 2018;5(1):3–13. https://doi.org/10.3390/fermentation5010003
7. Adejuyitan JA, Otunola ET, Akande EA, Bolarinwa IF, Oladokun FM. Some physicochemical properties of flour obtained from fermentation of tiger nut (*Cyperus esculentus)* sourced from a market in Ogbomoso, Nigeria. Afr J Food Sci. 2009;3(2):51–55. https://doi.org/10.5897/AJFS.9000269
8. Chukwuma ER, Obioma N, Ononogbu IC. The phytochemical composition and some biochemical effects of Nigerian tigernut (*Cyperus esculentus L.*) tuber. Pak J Nutr. 2010;9(7):709–715. https://doi.org/10.3923/pjn.2010.709.715
9. Maduka N, Ire FS. Studies on tigernut-ogi incorporated into basal feed as a potential animal growth enhancer using Wistar albino rats as experimental animal. J Adv Microbiol. 2018;8(2):1–15. https://doi.org/10.9734/jamb/2018/38662
10. Arafat SM, Gaafar AM, Basuny AM, Nassef SL. Chufa tubers (*Cyperus esculentus*): As a new source of food. World Appl Sci J. 2009;7(2):151–156.
11. Adejuyitan JA. Tiger nut processing: Its food uses and health benefits. Am J Food Tech. 2011;6(3):197–201. https://doi.org/10.3923/ajft.2011.197.201
12. Adebayo SF, Arinola SO. Effect of germination on the nutrient and antioxidant properties of tigernut (*Cyperus esculentus*). J Biol Agric Healthc. 2017;7(18):88–94.
13. Kambhampati S, Li J, Evans BS. Accurate and efficient amino acid analysis for protein quantification using hydrophilic interaction chromatography coupled tandem mass spectrometry. Plant Methods. 2019;15:46. https://doi.org/10.1186/s13007-019-0430-z
14. Petkova TR, Nadezhda BA, Pascal MA, Denev PP. HPLC analysis of mono- and disaccharides in food products. In: Proceedings of Food Science, Engineering and Technology Conference; 2013 October 18–19; Plovdiv, Bulgaria. Scientific Works. 2013;LX:761–765.
15. Association of Official Analytical Chemists (AOAC). Official methods of analysis. 18th ed. Washington DC: AOAC; 2010.
16. Rahman MM, Islam MS, Begun SA. A simple UV spectrophotometric method for the determination of vitamin C content in various fruits and vegetables at Sylhet Area in Bangladesh. J Biol Sci. 2006;6:388–392. https://doi.org/10.3923/jbs.2006.388.392
17. Rutkowski M, Grzegorczyk K. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. Acta Sci Pol Technol Aliment. 2007;6:17–28.
18. Belewu MA, Abodunrin OA. Preparation of kunu from unexploited rich food source: Tiger nut (*Cyperus esculentus*). World J Dairy Food Sci. 2006;7(1):109–111. https://doi.org/10.3923/pjn.2008.109.111
19. Ukwuru MU, Ibeneme CL, Agbo GI. New product development from tigernut (*Cyperus esculentus*) and their sensory, proximate and microbiological evaluation. Pak J Nutr. 2011;10(2):101–105. https://doi.org/10.3923/pjn.2011.101.105
20. Okudu HO, Ogubuike LA. Evaluation of chemical composition of candy developed from tigernut (*Cyperus esculentus*) milk. Afr J Food Sci Tech. 2016;7(1):27–31. https://doi.org/10.14303/ajfst.2016.006
21. Sánchez-Zapata E, Fernández-Lopez J, Pérez-Alvarez JA. Tigernut (*Cyperus esculentus)* commercialization: Health aspects, composition, properties, and food applications. Compr Rev Food Sci Food Saf. 2012;11:366–377. https://doi.org/10.1111/j.1541-4337.2012.00190.x
22. Oguike MA, Aboaja CU, Herbert U. Influence of tigernut (*Cyperus esculentus* L.) on the semen characteristics and testicular parameters of rabbits. Bull Anim Health Prod Afr. 2008;56:67–73. https://doi.org/10.4314/bahpa.v56i1.32829
23. Nina GC, Ogori AF, Ukeyima M, Lukas H, Miroslava C, Eleonora O, et al. Proximate, mineral, and functional properties of tiger nut flour extracted from different tiger nuts cultivars. J Microbiol Biotech Food Sci. 2019;9(3):653–656. https://doi.org/10.15414/jmbfs.2019/20.9.3.653-656
24. Obasi NE, Ugwu VC. Quality characteristics of candies produced from tiger nuts tubers (*Cyperus esculentus*) and melon seeds (*Colocynthis citrullus* L.) milk blend. Glob J Sci Front Res Agric Vet. 2015;15(2):1–13.
25. Oladele AK, Aina JO. Chemical composition and functional properties of flour produced from two varieties of tiger nut (*Cyperus esculentus*). Afr J Biotech. 2007;6(21):2473–2476. https://doi.org/10.5897/ajb2007.000-2391
26. Bado S, Bazongo P, Son G, Kyaw MT, Forster BP, Nlelen S, et al. Physicochemical characteristics and composition of three morphotypes of *Cyperus esculentus* tubers and tuber oils. J Anal Methods Chem. 2015;2015, Art. #673547. https://doi.org/10.1155/2015/673547
27. Chukwu MO, Ibiam OF, Okoi A. Studies on the fungi and phytochemical and proximate composition of dry and fresh tigernuts (*Cyperus esculentus* L.) Int Res J Biotech. 2013;4(1):11–14.
28. Suleiman MS, Olajide JE, Omale JA, Abbah OC, Ejembi DO. Proximate composition, mineral and some vitamin contents of tiger nut (*Cyperus esculentus*). Clin Invest (Lond.). 2018;8(4):161–165. https://doi.org/10.4172/clinical-investigation.1000143
29. FAO/WHO/UNU. Human vitamin and mineral requirements (Recommended Dietary Intakes): Report of a joint food and agricultural organization. Rome/Tokyo: World Health Organization / United Nations University; 2002.
30. Wardlaw GM, Kessel MW. Calcium in food. In: Perspective in nutrition. 5th ed. New York: McGraw Hill; 2002. p. 410–411.

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Effects of browse legume species addition on nutritional composition, fermentation characteristics and aerobic stability of *Opuntia* cladodes silage

Forage legumes are commonly used as an absorbent additive in high-moisture silages. Thus this study was carried out to assess the nutritive value, fermentation characteristics and aerobic stability of *Opuntia*–legume browse mixed silages. Five browse legume species (*Leucaena leucocephala, Acacia mellifera, Searsia lancea, Prosopis velutina,* and *Grewia flava*) were mixed with *Opuntia* cladodes. The silage mixture was formulated at a ratio of 60 *Opuntia* cladodes: 40 leguminous browse species and ensiled in polythene bags and kept in a laboratory for 42 days to determine chemical composition and fermentation characteristics. Silage samples were also subjected to an aerobic stability test. One-way analysis of variance in a completely randomised design was used to analyse the data. The pH values for silages made from *Opuntia* cladodes with *L. leucocephala*, *A. mellifera* and *G. flava* were lower than 4.8, which is considered an indicator of good-quality silage. The water-soluble carbohydrates content of silages made with *Opuntia* cladodes and *S. lancea* and *G. flava* was within the range of 8–12 g/kg dry matter, which is sufficient for good fermentation. The highest CO_2 production, which signifies poor aerobic stability, was recorded for the control silage (*Opuntia*) compared to all *Opuntia*–legume mixed silage treatments. The addition of legume browse leaf-meal to *Opuntia* cladodes improved nutritive value, fermentation characteristics, and silage quality. Therefore, despite some limitations, *Opuntia*–legume browse silages, particularly *Opuntia–G. flava* and *Opuntia–L. leucocephala,* proved to be beneficial for livestock, as they meet the nutritional requirement of a ruminant.

Significance:

This study underlines the importance of co-ensiling *Opuntia* cladodes and high protein legume browse hay to offer an alternative feeding strategy for ruminant livestock and ensure sustainable provision of high-quality feed during dry periods.

Introduction

The problem associated with ruminant productivity in semi-arid areas is the scarcity of feed.[1] This scarcity is due to seasonal changes that influence feed quantity and quality. The cost of conventional feed is high and this negatively impacts on the reliability and sustainability of the industry due to increased mortality rates resulting in decreased production.[2] Therefore, alternative strategies to conserve forage material for utilisation during periods of feed scarcity, such as during droughts and winter, need further exploration.[3]

Opuntia is a genus of flowering plants in the cactus family (Family: Cactaceae) and is commonly known as the prickly pear. *Opuntia* species are found in most arid and semi-arid regions and have the capacity to grow in extremely harsh environments.[4] Consequently, the species can be considered as an alternative feed source for winter livestock during droughts. However, *Opuntia* cladodes (or flattened, photosynthetic shoots) have a high moisture content and are low in crude protein[5], which means that they are not easily ensiled. Utilisation of shrubs or trees as possible feeds for ruminant animals has become progressively more essential in several tropic and subtropic regions around the world.[6,7] Fodder trees and shrubs have high potential as feed sources for wildlife and domestic livestock as they can be incorporated successfully into various animal production systems as alternative feed resources for livestock.

Studies have revealed that protein supplementation enhances the nutritional value of *Opuntia*-based diets offered to ruminants to improve their productivity.[8] Browse legumes are generally higher in nitrogen composition[9,10] but with variable digestibility rates depending on the composition of the bioactive compounds[11,12]. Co-ensiling *Opuntia* cladodes and high-protein legume browse hay offers an alternative feeding strategy to ensure sustainable provision of high-quality feed throughout the year, including during winter and dry periods.[13] Therefore, the current study was carried out to assess the nutritive value, fermentation characteristics and aerobic stability of *Opuntia*–legume browse mixed silages.

Materials and methods

Study site

This experiment was conducted at the Agricultural Research Farm of North West University (Mafikeng Campus), which is located approximately 7 km from Mmabatho (latitude: 25°47'27''S and longitude 25°37'18''E) in Mahikeng region in the North West Province of South Africa. The study area is categorised as semi-arid with an average annual rainfall of 300 mm. The area of Mahikeng is characterised by fairly high temperatures in summer (27–38 °C)

and cold in winter (11–18 °C). The area is composed of a mixture of bushveld and grassveld under dry Highveld grassland vegetation.[14]

Sample collection

Fresh *Opuntia* cladodes (Roedtan variety) were cut on the Shalom farm close to Mahikeng town in the North West Province. The *Opuntia* cladodes collected were cut into smaller pieces (20–30 mm) with a sharp machete (Lasher-302). *Senegalia mellifera*, *Searsia lancea*, *Prosopis velutina* and *Grewia flava* leaves were harvested from Mahikeng, North West Province and *Leucaena leucocephala* leaves were collected from the Nkangala District, Dr James Moroka local municipality, Mpumalanga Province, South Africa. The harvested area in Mpumalanga Province consists of a flat landscape with loamy-sandy soil. The average temperature varies between 25 °C in summer and 12 °C in winter. Annual rainfall ranges from 400 mm to 800 mm. The area receives rainfall mainly in summer.

After collection, the harvested leaves were air-dried on a plastic sheet in a shaded area for 7 days, then ground into various leaf-meals using a Wiley mill (Philadelphia, PA, USA) to pass through a 1-mm sieve.

Sample preparation

Each of the prepared leaf-meals was then mixed with fresh cut *Opuntia* cladodes prior to ensilage. The silage mixture comprised 60% fresh *Opuntia* cladodes and 40% browse legume leaf-meal on a weight/weight basis and was ensiled in polythene bags. In addition, 2% of molasses (Kalori 3000) was applied to the mixture in order to enhance silage fermentation. For each treatment, five replicates were ensiled. The silage was incubated in a rodent-free laboratory room for 42 days, after which a representative sample was drawn from all the treatments for pH analysis. The representative samples from every treatment were also ground using a Wiley mill (Philadelphia, PA, USA) to pass through a 3-mm sieve and were further analysed for silage nutritional composition.

Nutritional composition and fermentation characteristics analyses

The dry matter (DM) content of the silages was determined after drying samples in an oven at 105 °C to a constant mass. Organic matter was determined by igniting the samples at 600 °C overnight. The nitrogen (N) composition of the samples was determined using the technique of the Association of Official Analytical Chemists.[15] Acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were evaluated according to the procedure of Van Soest et al.[16] The atomic absorption spectrophotometer (AAS-Buck 205) was used to analyse the mineral composition (calcium (Ca) and magnesium (Mg)).[17] Phosphorus composition of the silages was assessed calorimetrically.[17] The determination of pH was done using a digital pH meter (Hanna HI-2211). Water-soluble carbohydrate (WSC) content was determined by the phenol-sulfuric acid technique.[18] Lactic acid in the silage was determined as described by the Lepper methodology.[19] The formula applied to estimate total digestible nutrients (TDN) was 82.38 – (0.7515 x ADF) as given by Bath and Marble[20]. Estimated potential daily dry matter intake (DMI) was calculated according to Mertens[21] using the following formula:

$$DMI = \frac{1.2 \times bodyweight}{NDF\%}, \quad \text{Equation 1}$$

where

$$NDF\% = \frac{(W3-(W1\times C1))\times 100}{W2}, \quad \text{Equation 2}$$

and W1 is the bag tare weight, W2 is the sample weight, W3 is the final bag weight and C is the correction factor.

Aerobic stability determination

An aerobic stability test was performed on silage samples on day 42 by drawing a representative 500-g silage sample from all the polythene bags and placing them into plastic jars, covering the jars using a double layer of cheesecloth and storing them at an ambient temperature of about 28 °C. Production of carbon dioxide (CO_2)[22], pH and yeast and moulds was determined after the 7-day aerobic exposure as described by the International Dairy Federation[23] technique.

Statistical analysis

All data generated were analysed by an analysis of variance using the general linear model procedure of SAS[24] for a completely randomised design. The following model was used:

$$Yij = \mu + Di\,(i = 6) + Eij, \quad \text{Equation 3}$$

where Yij = response variable ij; μ = overall mean; Di = dietary treatment effect (i=6; control, *L. leucocephala*, *A. mellifera, S. lancea, P. velutina* and *G. flava*); and Eij = random error.

For parameters with significant difference, multiple comparisons of treatment means were conducted by applying the probability of difference (pdiff) alternative of the general linear model procedures of SAS.[24] Correlation (linear regression) was used to determine the degree of association amongst the variables:

$$Y = aX + b, \quad \text{Equation 4}$$

where Y is the dependent variable, a is constant, X is the independent variable and b is the intercept.

Results

Nutritional composition before ensiling

The results of the nutritional composition analyses of cladodes and leaves from *L. leucocephala*, *A. mellifera*, *S. lancea*, *G. flava* and *P. velutina* are presented in Table 1. The DM content of various legume browse species ranged from 955.23 g/kg DM to 972.94 g/kg DM. *L. leucocephala* and *A. mellifera* had similar ($p>0.05$) DM contents compared to that of *P. velutina*. Between the species, crude protein content from legume browse species ranged from 39.13 g/kg DM in *Opuntia* to 364.70 g/kg DM in *G. flava*. *P. velutina* had the highest neutral (483.67 g/kg DM) and acid detergent fibre (347.20 g/kg DM) than any other legume browse species. *S. lancea* had the highest acid detergent lignin (253.70 g/kg DM) as compared to *Opuntia* (88.63 g/kg DM).

Nutritional composition after ensiling

Data on nutritional composition of the six silages are displayed in Table 2. The results indicate that *Opuntia–L. leucocephala* (OLL; 421.77 g/kg, 932.67 g/kg DM) and *Opuntia–A. mellifera* (OAM; 404.00 g/kg, 927.00 g/kg DM) silages had higher dry matter and organic matter, respectively, than other silages. The crude protein content was significantly different ($p<0.05$), being highest in *Opuntia–G. flava* (OGF) and lowest in *Opuntia* alone. Ether extracts of the silages also differed significantly ($p<0.05$), with the lowest ether extract level observed in OAM and the highest in *Opuntia* alone (control). There were significant differences in ash contents of the silages. The highest ash content was recorded for *Opuntia–P. velutina* (OPV; 147.73 g/kg DM) followed by OLL (106.69 g/kg DM), and the lowest were from OLL (67.33 g/kg DM) and OAM (73.00 g/kg DM). The highest NDF and ADF contents were recorded for *Opuntia* alone and the lowest for OAM. Calcium contents of the silages were significantly different ($p<0.05$), with the lowest recorded for OPV and the highest for *Opuntia* alone. The highest phosphorus level was recorded for OGF while the lowest level was for *Opuntia* alone.

The highest total digestible nutrient content was recorded for *Opuntia–S. lancea* (OSL), followed by OAM and OLL, and the lowest was recorded for *Opuntia* alone (Figure 1).

Table 1: Nutritional composition (g/kg dry matter) of *Opuntia* and legume browse species

Species	Dry matter	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin
Opuntia	957.5^{b}	39.1^{f}	297.1^{d}	181.3^{e}	88.6^{f}
Leucaena leucocephala	972.9^{a}	275.1^{b}	236.0^{f}	178.7^{e}	192.6^{b}
Acacia mellifera	971.3^{a}	170.2^{c}	424.6^{b}	245.4^{d}	106.6^{e}
Searsia lancea	966.7^{ab}	98.8^{f}	386.8^{c}	340.6^{b}	253.7^{a}
Prosopis velutina	955.2^{c}	137.7^{d}	483.7^{a}	347.2^{a}	176.0^{c}
Grewia flava	963.9^{abc}	364.7^{a}	255.5^{e}	248.2^{c}	157.8^{d}
p-value	0.0124	<0.0001	<0.0001	<0.0001	<0.0001
Standard error	3.29	0.28	1.37	0.84	0.32

Means within the same column with different superscript letters differ significantly (p<0.05).

Table 2: Nutritional composition (g/kg dry matter, unless otherwise stated) of the silages used in the experiment

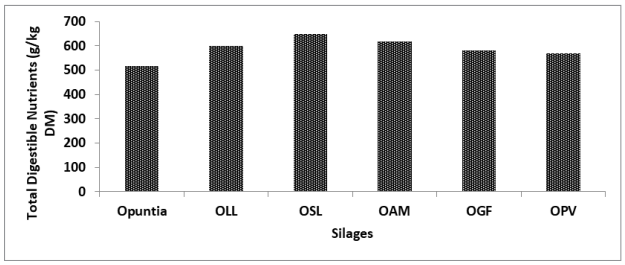
Species	Dry matter	Organic matter	Ash	Crude protein	Ether extract	Neutral detergent fibre	Acid detergent fibre	Ca%	P%	Mg%
Opuntia	251.3^{c}	852.3^{e}	124.7^{b}	50.0^{f}	85.5^{a}	624.5^{a}	406.6^{a}	3.83^{a}	0.25^{f}	1.19^{a}
Opuntia–Leucaena leucocephala	421.8^{a}	932.7^{a}	67.3^{e}	106.7^{b}	24.2^{d}	544.2^{d}	296.5^{d}	1.67^{c}	0.99^{b}	0.89^{c}
Opuntia–Acacia mellifera	404.0^{a}	927.0^{a}	73.0^{e}	64.4^{e}	13.6^{e}	533.7^{e}	272.3^{e}	1.39^{d}	0.87^{c}	0.86^{c}
Opuntia–Searsia lancea	310.3^{b}	887.6^{c}	112.4^{c}	92.5^{d}	44.1^{b}	567.3^{b}	234.6^{f}	0.98^{e}	0.67^{e}	0.69^{e}
Opuntia–Prosopis velutina	291.1^{bc}	875.3^{d}	147.7^{a}	100.5^{c}	37.4^{c}	558.8^{c}	337.3^{b}	0.93^{e}	0.77^{d}	0.76^{e}
Opuntia–Grewia flava	281.1^{bc}	897.8^{b}	102.2^{d}	134.5^{a}	48.4^{b}	518.6^{f}	321.7^{c}	1.84^{b}	1.11^{a}	1.04^{b}
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Standard error	1.40	2.07	2.07	1.93	1.98	0.08	0.16	0.03	0.02	0.01

Means within the same column with different superscript letters differ significantly (p<0.05).

Table 3: Fermentation characteristics and aerobic stability of the silages used in the experiment

Parameter	Silages						*p*-value	Standard error
	Opuntia	*Opuntia–Leucaena leucocephala*	*Opuntia–Acacia mellifera*	*Opuntia–Searsia lancea*	*Opuntia–Prosopis velutina*	*Opuntia–Grewia flava*		
Fermentation characteristics								
pH	6.59^{a}	4.66^{de}	4.34^{e}	5.48^{c}	5.97^{b}	4.75^{d}	<0.0001	0.117
Lactic acid (g/kg dry matter)	0.23^{d}	15.07^{a}	12.00^{b}	2.43^{c}	0.50^{d}	2.50^{c}	<0.0001	0.612
Water-soluble carbohydrates (g/kg dry matter)	7.49^{d}	11.28^{a}	11.12^{ab}	8.66^{e}	8.39^{c}	10.79^{b}	<0.0001	0.115
Yeast and moulds (log cfu/g)	6.03^{a}	2.73^{c}	1.33^{d}	1.73^{d}	3.83^{b}	1.00^{d}	<0.0001	3.075
Aerobic stability								
pH	8.82^{a}	6.71^{d}	6.17^{e}	7.21^{c}	7.80^{b}	6.17^{e}	<0.0001	0.129
CO_2 (g/kg dry matter)	32.28^{a}	2.38^{c}	9.00^{bc}	11.57^{b}	5.37^{bc}	1.92^{c}	<0.0001	2.933

Means within the same column with different superscript letters differ significantly (p<0.05).



OLL, Opuntia–Leucaena leucocephala*; OSL,* Opuntia–Searsia lancea*; OAM,* Opuntia–Acacia mellifera*; OGF,* Opuntia–Grewia flava*; OPV,* Opuntia–Prosopis velutina

Figure 1: Effect of silages on total digestible nutrients (g/kg dry matter).

Fermentation characteristics and aerobic stability

The silages had significant differences in pH, lactic acid, WSC, and yeast and moulds (Table 3). The pH levels varied significantly, with the highest pH recorded for *Opuntia* alone and the lowest for OAM. The highest WSCs were recorded in OLL, OAM and OGF silages compared to OPV, OSL and the control (*Opuntia* cladodes alone). Addition of browse legume leaf-meal into *Opuntia* cladodes resulted in improved lactic acid composition of the resultant silages. The pH level of silages subjected to the aerobic stability test varied significantly, with the highest pH level recorded in *Opuntia* alone and the lowest in OGF and OAM. CO_2 production was highest in *Opuntia* ensiled alone than in any of the experimental silages.

Negative relationships were observed between DMI and NDF, and DMI and Ca composition of silages, whereas positive relationships were observed between DMI and crude protein, and DMI and WSC of the silages (Table 4).

Discussion

The nutritive value of forages for livestock feeding depends on the balance between the nutritive components of the plants, the digestibility of such nutrients and the quality of the nutrient ingested by the animal.[25] In order to minimise the cost and the time involved in *in vivo* forage evaluation, laboratory procedures have been developed. According to Ligouri et al.[26], *Opuntia* have very low dry matter content. However, the results from the current study showed that *Opuntia* silage had higher dry matter content than the 90.7 g/kg reported by Inácio et al.[27] for *Opuntia* as the average. The increase in the dry matter content of the silages is associated with the addition of browse species.[28] This suggests that incorporation of legume browse and *Opuntia* cladodes can result in production of silage with moderate ruminal degradability and without laxative effects, and ultimately improve animal production. *Opuntia* cladodes ensiled alone had lower crude protein composition but was within the range of 3.66–8.08% DM observed by Inácio et al.[27] and Mciteka[29]. As a general rule, browse species such as *L. leucocephala*, *A. mellifera*, *P. velutina* and *G. flava* which have crude protein within the range 11–13% are capable of supplying adequate protein for maintenance and growth.[6,7]

The silage formulated using *G. flava* with *Opuntia* (OGF) legume leaves had the highest crude protein (134.47 g/kg DM) of all the silages and also had higher than the average for cereal silages (110 g/kg DM).[30] According to the current findings, the lowest organic matter (852.27 g/kg DM) was observed for *Opuntia* silage (control). These findings are similar to the organic matter content of different *Opuntia* cladode varieties reported in the literature.[31,32] *Opuntia*, *L. leucocephala* and *G. flava* had low neutral detergent fibre contents which fell within the range of 20–35% reported by Nsubuga et al.[33] for a variety of browse species, and these species will be more digestible than *A. mellifera*, *S. lancea* and *P. velutina* which have more than 35% NDF. Silage pH ranged between 4.34 and 6.59. The pH values for silages made from *Opuntia* cladodes with *L. leucocephala*, *A. mellifera* and *G. flava* were lower than 4.8, which, according to Cürek and Özen[13], is considered an indicator of good quality silage. The lower the pH level, the higher are the soluble sugars in the silage ingredients.[34] The pH values of the *Opuntia*–legume browse mixed silages were higher than those reported by Nogueira et al.[35] of 3.8 and 4.2. The legumes generally have resistance to pH reduction in silages due to their high buffering capacity mainly from the presence of cations.

Water-soluble carbohydrates are considered vital for growth of lactic acid bacteria for enhanced fermentation.[36] The WSC contents of all silages except *Opuntia* alone were within the range of 8–12 g/kg DM, and thus sufficient for good fermentation.[37] The concentrations of lactic acid in silages in the current study were lower than those observed by Cürek and Özen[13] and da Silva Brito et al.[38] who obtained *Opuntia* silages with average lactic acid contents of 25.90 g/kg and 9.03 g/kg DM, respectively. Achieving an increase in the content of lactic acid is the most reliable indicator for the success of a microbial additive in improving silage quality. The addition of legume browse (except *P. velutina*) to *Opuntia* at ensiling increased ($p<0.05$) the lactic acid concentration. Yeasts are the main silage degrading microorganisms, especially after aerobic exposure of the material, promoting marked losses of DM throughout the ensiling process. These microorganisms cannot grow in the silages used in the current study, as the pH is above 3.5, because they can use lactic acid as a substrate when in anaerobiosis, thus increasing the pH in the silages, which may favour the emergence of undesirable fermentations.[39]

The greater CO_2 concentration recorded in *Opuntia* (control) ensiled alone indicates microbial metabolic activity, which may prompt silage temperature to rise, leading to deterioration of silage quality.[22,40] The aerobic stability of the *Opuntia* silage was affected by browse legume addition. Although the addition of browse legumes to *Opuntia* silage resulted in higher residual sugar and lactic acid compared to the control, the differences in these fermentation indices affected the aerobic stability of the silage. The relationship between NDF, crude protein and WSC versus DMI was established. The relationship between NDF content and DMI was negative because this component is less digestible than non-fibrous components such as protein. In the current investigation, the regression coefficient value was 0.99 between NDF and DMI, which is

Table 4: Regression equations predicting dry matter intake from chemical composition and fermentation+characteristics of silages

Factor	Y-variable	Formula	R^2	*p*-value
Crude protein (CP)	Dry matter intake	Y = 0.142CP + 84.109	0.5015	0.0100
Neutral detergent fibre (NDF)	Dry matter intake	Y = -0.165NDF + 189.296	0.9964	<0.0001
Acid detergent fibre (ADF)	Dry matter intake	Y = -0.057ADF + 114.871	0.3026	0.0639
Ca	Dry matter intake	Y = -3.716Ca + 103.719	0.4229	0.0220
Mg	Dry matter intake	Y = -10.771Mg + 106.869	0.1087	0.2952
Water-soluble carbohydrate (WSC)	Dry matter intake	Y = 3.197WSC + 66.379	0.7315	0.0004
Lactic acid (LA)	Dry matter intake	Y = -6.781LA + 135.993	0.2006	0.1442
Total digestible nutrients (TDN)	Dry matter intake	Y = 0.757TDN + 52.461	0.3026	0.0639

higher than the range reported by Coleman and Moore[41]. Water-soluble carbohydrates are, however, often associated with dry matter intake.[42]

Conclusion and recommendations

The addition of browse legume species had an effect on the nutritional composition, fermentation characteristics and aerobic stability of *Opuntia* cladode silage. Ensiling *Opuntia*-*G. flava* and *Opuntia*–*L. leucocephala* can make good-quality silage and small-scale farmers can use these silages as feed for ruminant livestock during dry seasons. Further studies are needed to explore the influence of bacterial inoculants and fibrolytic enzymes on fermentation characteristics and quality of *Opuntia*–legume silage and also to determine *Opuntia*–legume silage effect on animal performance. Further *in vivo* trials are required to assess the productivity of animals fed these silages.

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Competing interests

We have no competing interests to declare.

Authors' contributions

G.M., H.K.M., C.K.L. and A.N.S.: Conceptualisation; methodology. G.M.: Data collection, sample analysis, data curation. H.K.M., O.H. and K.E.R.: Data analysis. H.K.M., C.K.L., A.N.S., K.E.R.: Validation. G.M., K.E.R., O.H. and H.K.M.: Writing – the initial draft, writing – revisions. H.K.M., C.K.L. and A.N.S.: Student supervision. H.K.M.: Project leadership. All authors read and approved the manuscript.

References

1. Hatab A, Cavinato MER, Lagerkvist CJ. Urbanization, livestock systems and food security in developing countries: A systematic review of the literature. Food Secur. 2019;11:279–299. https://doi.org/10.1007/s12571-019-00906-1
2. Mnisi CM, Mlambo V. Influence of harvesting site on chemical composition and potential protein value of *Acacia erioloba*, *A. nilotica* and *Ziziphus mucronata* leaves for ruminants. J Anim Physiol Anim Nutr. 2016;101:994–1003. http://dx.doi.org/10.1111/jpn.12535
3. Bernardes TF, Daniel JL, Adesogan AT, McAllister TA, Drouin P, Nussio LG, et al. Silage review: Unique challenges of silages made in hot and cold regions. J Dairy Sci. 2018;101:4001–4019. https://doi.org/10.3168/jds.2017-13703
4. Makkar HPS. Cactus as a fodder and beyond. Broadening Horizons. Feedipedia. Rome: Animal Production and Health Division, FAO; 2017. p. 1–6.
5. Rodrigues AM, Pitacas FI, Reis MG, Blasco M. Nutritional value of *Opuntia ficus-indica* cladodes from Portuguese ecotypes. Bul J Agric Sci. 2016;22:40–45.
6. Ravhuhali KE, Mlambo V, Beyene TS, Palamuleni LG. Effects of soil type on density of trees and nutritive value of tree leaves in selected communal areas of South Africa. S Afr J Anim Sci. 2020;50:88–98. https://doi.org/10.4314/sajas.v50i1.10
7. Mokoboki HK, Sebola AN, Ravhuhali KE, Nhlane L. Chemical composition, *in vitro* ruminal dry matter degradability and dry matter intake of some selected browse plants. Cog Food Agric. 2019;5, Art. #1587811. https://doi.org/10.1080/23311932.2019.1587811
8. Todaro M, Alabiso M, Di Grigoli A, Scatassa ML, Cardamone C, Mancuso I, et al. Prickly pear by-product in the feeding of livestock ruminants: Preliminary investigation. Animals. 2020;10:949. https://doi.org/10.3390/ani10060949
9. Muir JP, Santos MVF, Cunha MV. Value of endemic legumes for livestock production on Caantiga rangelands. Revista Brasileira de Ciéncias Agrárias. 2019;14, e5648. https://doi.org/10.5039/agraria.v14i2a5648
10. Mudzengi CP, Murwira A, Zengeya FM, Murungweni C. Screening key browse species in a semi-arid rangeland. Cog Food Agric. 2017;3:1285854 https://doi.org/10.1080/23311932.2017.1285854
11. Mlambo V, Marume U, Gajana SC. Utility of the browser's behavioral and physiological strategies in coping with dietary tannins: Are exogenous tannin-inactivating treatments necessary? S Afr J Anim Sci. 2015;45:441–451. https://doi.org/10.4314/sajas.v45i5.1
12. Huang Q, Liu X, Zhao G, Hu T, Wang Y. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Anim Nutr. 2018;4:137–150. https://doi.org/10.1016/j.aninu.2017.09.004
13. Cürek M, Özen N. Feed value of cactus and cactus silage. Turk J Vet Anim Sci. 2004;28:633–639.
14. Mucina L, Rutherford MC. The vegetation of South Africa, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute; 2006.
15. Horwitz W, Association of Official Analytical Chemists (AOAC). Official methods of analysis of AOAC International. Volume 1. 17th ed. Gaithersburg, MD: AOAC International; 2000.
16. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991;74:3583–3597. https://doi.org/10.3168/jds.s0022-0302(91)78551-2
17. Association of Official Analytical Chemists (AOAC). Official methods of analysis. 12th ed. Washington DC: AOAC; 1990. p. 1094.
18. Masuko T, Minami A, Iwasaki N, Majima T, Nishimura SI, Lee YC. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. Anal Biochem. 2005;339:69–72. https://doi.org/10.1016/j.ab.2004.12.001
19. Akyıldız AR. Analyses of feed handbook. Ankara: Faculty of Agriculture Publications, University of Ankara; 1984. p. 236.
20. Bath DL, Marble VL. Testing alfalfa for its feed value. Leaflet 21457. Oakland, CA: University of California Cooperative Extension; 1989.
21. Mertens DR. Physical and chemical characteristics of fiber affecting dairy cow performance. In: Proceedings of the 2002 Cornell Nutrition Conference for Feed Manufacture; 2002 October 23–25; East Syracuse, NY, USA. Ithaca, NY: Cornell University; 2002. p. 125–144.
22. Ashbell G, Weinberg ZG, Azriel A, Hen Y, Horey B. A simple system to study the aerobic deterioration of silages. Canad Agric Eng.1991;33:391–393.
23. International Dairy Federation (IDF). International Standard 94B. Milk and milk products – enumeration of yeasts and moulds. Colony count technique at 25 ^{0}C. Brussels: IDF; 1990.
24. SAS. Statistical Analysis Systems user's guide. Version 9.1. Cary, NC: SAS Institute Inc.; 2010.
25. Dong L, Li B, Diao Q. Effects of dietary forage proportion on feed intake, growth performance, nutrient digestibility, and enteric methane emissions of Holstein heifers at various growth stages. Animals. 2019;9:725. http://dx.doi.org/10.3390/ani9100725
26. Ligouri G, Inglese P, Sortino G, Inglese G. Dry matter accumulation and seasonal partitioning in mature *Opuntia ficus-indica* (L.) Mill. fruiting trees. Ital J Agron. 2014;9:44–47. https://doi.org/10.4081/ija.2014.537
27. Inácio JG, da Conceição MG, Dos Santos DC, de Oliveira JC, Chagas JC, de Oliveira Moraes GS, et al. Nutritional and performance viability of cactus *Opuntia*-based diets with different concentrate levels for Girolando lactating dairy cows. Asian-Australas J Anim Sci. 2020;33:35–43. https://doi.org/10.5713/ajas.18.0916
28. Mbatha KR, Bakare AG. Browse silage as potential feed for captive wild ungulates in southern Africa: A review. Anim Nutr. 2018;4:1–10. https://doi.org/10.1016/j.aninu.2017.12.003
29. Mciteka H. Fermentation characteristics and nutritional value of *Opuntia ficus-indica* var. *fusicaulis* cladode silage [Msc thesis]. Bloemfontein: University of the Free State; 2008.
30. Nefzaoui A, Inglese P, Belay T. Improved utilization of cactus pear for food, feed, soil and water conservation and other products in Africa. In: Proceedings of International Workshop; 2009 October 19–21; Mekelle, Ethiopia. Ethiopia: Publisher unknown; 2010. p. 224.
31. Zeeman DC. Evaluation of sun-dried *Opuntia ficus-indica* var. Algerian cladodes in sheep diets [thesis]. Bloemfontein: University of the Free State; 2005

32. Mokoboki HK, Sebola AN. Chemical composition and feed intake of *Opuntia* cladodes varieties offered to goats. J Anim Plant Sci. 2017;32:5096–5103.

33. Nsubuga D, Nampazira DK, Masembe C, Muwanika VB. Nutritional properties of some browse species used as goat feed in pastoral dry lands, Uganda. Agroforest Syst. 2020;94:933–940. https://doi.org/10.1007/s10457-019-00452-x

34. Gusha J, Ngongoni NT, Halimani TE. Nutritional composition and effective degradability of four forage trees grown for protein supplementation. J Anim Feed Res. 2013;3:170–175.

35. Nogueira MS, Santos EM, Araújo GGL. Ensilagem de Palma forrageira [Forage palm silage]. In: Santos EM, Parente HN, Oliveira JS, Parente MOM, editors. Ensilagem de plantas forrageiras para o Semiárido [Forage plant silage for the semiarid region]. São Luís: EDUFMA; 2016. p. 249. Portuguese.

36. McDonald P, Henderson N, Heron S. The biochemistry of silage. 2nd ed. Marlow: Chalcombe Publications; 1991.

37. Wilkinson JM. Silage. In: Wilkinson JM, editor. Analysis and clinical assessment of silage. Marlow: Chalcombe Publications; 2005. p. 198–208.

38. da Silva Brito GS, Santos EM, de Araújo GG, de Oliveira JS, de Moura Zanine A, Perazzo AF, et al. Mixed silages of cactus pear and gliricidia: Chemical composition, fermentation characteristics, microbial population and aerobic stability. Sci Rep. 2020;10:1–3. https://doi.org/10.1038/s41598-020-63905-9

39. Muck RE. Silage microbiology and its control through additives. Revista Bras de Zootec. 2010;39:183–191. https://doi.org/10.1590/s1516-35982010001300021

40. Woolford MK. The detrimental effects of air on silage. J Appl Bacteriol. 1990;68:101–116. https://doi.org/10.1111/j.1365-2672.1990.tb02554.x

41. Coleman S, Moore JE. Variability in relationships among forage intake, digestibility, NDF, and ADF. J Anim Sci. 2002;85:94.

42. Huntington GB, Burns JC. Afternoon harvest increases readily fermentable carbohydrate concentration and voluntary intake of gamagrass and switchgrass baleage by beef steers. J Anim Sci. 2007;85:276–284. https://doi.org/10.2527/jas.2006-370

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Isotopic profiling of natural uranium mined from northern Nigeria for nuclear forensic application

Four mined samples of natural uranium from northern Nigeria were studied through inductively coupled plasma mass spectrometry, at the Environmental Analytical Chemistry Laboratory, University of the Witwatersrand, Johannesburg. The samples were characterised for lead, thorium and uranium isotopic concentrations, isotopic ratios and age. The objective was to obtain nuclear forensic fingerprints as baseline data to add to the Nigerian National Nuclear Forensic Library. Results showed significant variation in the isotopic concentrations of lead, thorium and uranium across the mines. Isotopic ratios of $^{238}U/^{235}U$, $^{235}U/^{238}U$ and $^{234}U/^{238}U$ across the sample of 137.881 ± 0.007, $7.253 \times 10^{-03} \pm 2.05 \times 10^{-04}$ and $5.540 \times 10^{-05} \pm 4.08 \times 10^{-07}$ were found to be consistent with the natural values. The age of natural uranium is comparable to the age of earth. Uranium, lead, and thorium isotopic concentrations and ratios, as well as the age of the samples characterised, provide an isotopic profile that can be used for nuclear forensic application.

Significance:

- Given the abundant deposits of natural uranium in Africa and the consequent potential for nuclear insecurity, determining the isotopic profiles and signatures of natural uranium is important for application in nuclear forensics.
- Isotopic concentrations of ^{232}Th, ^{238}U, ^{235}U and ^{234}U from the respective sampling sites differed significantly, thereby providing characteristic isotopic profiles.

Introduction

Nuclear forensics operates on the premise that some measurable parameters in nuclear materials provide signatures according to their geological origin for identification purpose.[1-3] 'Nuclear forensics is the examination of nuclear or other radioactive material, or of evidence that is contaminated with radionuclides, in the context of legal proceedings under international or national law related to nuclear security. The analysis of nuclear or other radioactive material seeks to identify what the materials are, how, when, and where the materials were made, and what their intended uses were.'[2,4] Successful studies have been reported across the globe on many samples of materials that are nuclear and radioactive in nature, yielding signatures such as uranium isotopic compositions and ratios, and impurities of elements such as lanthanides (rare earth elements), lead (Pb), strontium (Sr), sulfur (S) and neodymium (Nd).[5-9] However, data on Africa – a continent that is well endowed with natural uranium – are still scarce; hence, the present study is pertinent.[10]

Natural uranium, which is radioactive, is mined and processed to obtain uranium ore concentrate or yellowcake, which is further transformed into nuclear fuel elements, used in research reactors and power reactors for electricity generation.[2,11] The database of the International Atomic Energy Agency on unlawful trafficking[12] includes many events of peddling in inferior nuclear materials containing natural uranium. For instance, in recent years, 400 of 3068 incidents involving depleted, natural or low-enriched uranium, were included in the Incident and Trafficking Database of the International Atomic Energy Agency, through the application of nuclear forensic science.[2-4,12] Keegan et al.[13] reported a small glass jar labelled 'Gamma Source' discovered by police in a clandestine drug laboratory in Australia, 2009. After extensive nuclear forensic analysis of the material, Keegan et al.[13] found it to be most likely from Mary Kathleen, a defunct Australian uranium mine. Nuclear forensic science is used to characterise various uranium samples and obtain nuclear fingerprints to enable the tracing of their origins.

The isotope system of uranium is special: of its 25 known isotopes which have a mass range of 217–241u[6], only ^{238}U ($t_{1/2} = 4.468 \times 10^{9}$ y; 99.274%), ^{235}U ($t_{1/2} = 7.038 \times 10^{8}$ y; 0.7204%) and ^{234}U ($t_{1/2} = 2.455 \times 10^{5}$ y; 0.00548%) are naturally occurring at significant concentrations.[7,14,15] The decay series nuclides of uranium and thorium have characteristics and chemical affinities applicable to a wide range of processes.[1,4,16] They have half-lives that are proportional to their equilibrium abundances, while the short-lived progenies exhibit low concentrations with very great isotope ratios.[16] For instance, the concentrations of ^{232}Th and ^{238}U are available in ppb to ppm while those of ^{234}U and ^{236}U can be as low as ppt for ores of granitic origin.[2,17,18]

The natural compositions, concentrations and ratios of uranium, thorium and lead isotopes have proved to be viable signatures in nuclear forensics.[3,8] In addition, when the isotopic ratios $^{234}U/^{238}U$ and $^{235}U/^{238}U$ are simultaneously analysed, nuclear and radioactive materials that contain uranium from various geological origins can be distinguished.[19-21] Until very recently, there was an assumption that the isotopic ratios $^{235}U/^{238}U$ and $^{234}U/^{238}U$ of terrestrial materials are constant (7.25×10^{-5} and 5.48×10^{-7}, respectively). However, using modern analytical procedures, the ratios now show variations in different geological materials of 1.3%.[2,7,13,22] Recent nuclear forensic investigations on natural uranium and uranium ore concentrate samples showed that uranium isotopic compositions, isotopic ratios and age, among other parameters, are key nuclear forensic signatures that can be used to identify materials.[3,10,23] Bopp et al.[24] studied uranium samples from both low- and high-temperature deposits and concluded that during the low-temperature redox transition, $^{238}U/^{235}U$ fractionation occurred, which is the phase transition of isotopes from a lighter to heavier isotope, which results in higher ratio values. Brennecka et al.[7], on the other hand, studied $^{235}U/^{238}U$ ratios of uranium ore concentrate samples in order to relate observed

variations in $^{238}U/^{235}U$ to the U mineralisation mechanism in the main ore. They found that $^{235}U/^{238}U$ fractionation occurred following redox transformation of uranium from U(VI) to U(IV) at low temperature, giving uranium ore concentrates originating from low-temperature redox type deposits a unique $^{235}U/^{238}U$ ratio.

We used a modern analytical (destructive) technique, inductively coupled plasma mass spectrometry (ICP-MS), to measure and establish the isotopic profiles of samples of natural uranium from the northern part of Nigeria. Our aim was to obtain baseline data for application in the area of nuclear forensic science.

Materials and methods

Study site and investigated samples

Four open pit samples of natural uranium in rock form were investigated. The vein-type, high-temperature magmatic, granite-related uranium deposit[25,26] samples were collected from the northern part of Nigeria (Figure 1). The samples were collected according to the uranium ore sample collection standard at the following uranium mining sites: Riruwai (a pyrochlore in peralkaline granite uranium of average grade 540 ppm), Mika (two locations – Mika-I and Mika-II – of pitchblende-rhyolite mineralisation of grade 215 ppm) and Michika (brecciated, silicified and mylonitised rocks of average grade 2000 ppm). The sample sites were chosen based on their geology and mineralisation, following previous prospecting of uranium in the sampling locations.[27-33] About 2 kg of natural uranium rock samples, obtained by means of chisel and mallet, from each pit mine site, were placed in a zip-locked plastic bag to avoid contamination. The samples were collected between 11 February and 3 March 2018, at a depth ranging from 1.3 m to 5.9 m. The collected samples were then kept in a safe location for a short time and transferred to the Environmental Analytical Chemistry Laboratory (University of the Witwatersrand, Johannesburg, South Africa) for measurement and analysis.

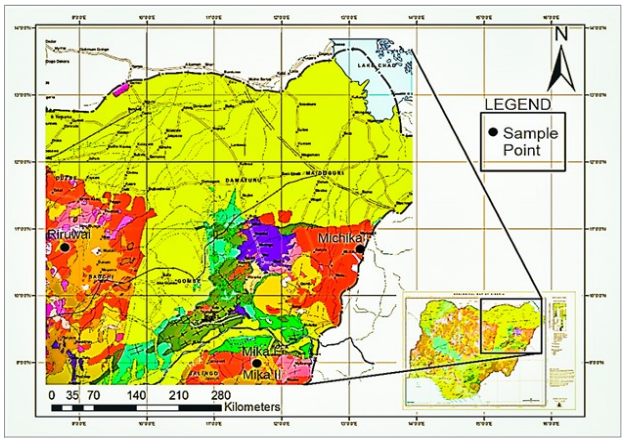

Figure 1: Map of Nigeria showing the uranium mining sites where samples for this work were collected.

Instrumentation and measurement

Sample preparation for ICP-MS

The ICP-MS analytical method requires that samples for measurement be presented in liquid form or as an aerosol matrix. The rock crusher and milling devices located at the Geosciences Laboratory (University of the Witwatersrand), were used to process the natural uranium samples into a soluble fine powder.

A digital microwave digestive device was used to dissolve the milled natural uranium samples under conditions of high pressure and temperature. Microwave preparation of samples under such conditions presents benefits such as complete sample dissolution using hydrofluoric and nitric acids. It also improves detection limits due to the use of a lower sample to solution ratio and prevents volatilisation of certain elements through use of high pressure.

A quick test for the presence of silica was done, as silica can form precipitate during sample preparation and tends to absorb the elements, making it difficult for their detection by ICP-MS. Silica can also affect the nebuliser, by making it difficult for the sample to get into the plasma for the process of isotope measurement. The milled samples (~0.5 g each) were placed in Teflon heating containers. Concentrated solutions of HNO_3 and HCl in volumes of 1.0 mL and 4.0 mL, respectively, were added followed by H_2O_2 in drops to a volume of 0.5 mL. After sample pre-digestion, each container was tightly sealed and placed on a rotor in the microwave. The microwave unit was set to a method ASTM D4 309 (half-scale): 4 vessels, 9.0 mL sample per vessel with a temperature of 180 °C and 90% (10.13 kPa) pressure then powered-on for a maximum period of 50 min. The cooled digested sample solutions appeared to be very clear without silica precipitate, implying that silicates were not present in the samples in a quantity that required further action using hydrofluoric acid to remove the silicate.

ICP-MS analysis

An ICP-MS device (Agilent 7700, Agilent Technologies, Inc., Santa Clara, CA, USA), was used to analyse the samples. Table 1 presents the summary of the set-up parameters for optimised equipment usage.

Table 1: Set-up parameters for the inductively coupled plasma mass spectrometry device used in natural uranium sample analysis

Parameter	Value
Radiofrequency forward and reflected power	1550 W; 14 W
Plasma mode and gas flow	Normal, robust; 1000 mL/min
Flow rate of auxiliary gas	1000 mL/min
Flow of carrier gas and pressure	1000 mL/min; 740.5 kPa
Sampling depth	8 mm
Cones	Nickel
Cell gas	Argon
Spray chamber temperature	2.0 °C
Type of detector value	Dual mode
Integration time	100 μs
Replicate per sample	2
Mode	Collision mode

Both the prepared and blank samples, the calibration standards (5 ppb, 20 ppb, 50 ppb, 100 ppb, 500 ppb, and 1000 ppb) with samples for quality control (20 ppb and 100 ppb) were all put into the auto-sampler for ICP-MS analysis. The tubes of the device were cleaned using a nitric acid solution to avoid washing of residual memory, usually done before placement of samples in the device. The tubing cleaning process was repeated after each sample measurement, to avoid cross-contamination. The process is automatic and computerised. The control was performed on the computer system via installed software (ICP-MS MassHunter Workstation for running Agilent 7700 series). The analysis produced results for various isotopes, which were then analysed further. Additional details of the experimental analysis can be found in John et al.[33]

Results

Uranium isotopic concentration

Concentrations of ^{238}U and ^{232}Th, which are the most stable and abundant of the isotopes, were obtained through ICP-MS analysis. As the isotopic

Table 2: Measured and determined isotopic concentrations of thorium and uranium

Sample location	^{232}Th (ppm)	^{238}U (ppm)	^{235}U (ppm)	^{234}U ($\times10^{-05}$) (ppm)
Riruwai	5.410 ± 0.318	1.318 ± 0.049	0.009 ± 0.0001	0.053 ± 2.0×10^{-08}
Mika-I	0.055 ± 0.006	0.084 ± 0.003	0.001 ± 1.9×10^{-05}	0.0034 ± 1.0×10^{-09}
Mika-II	0.159 ± 0.003	73.965 ± 1.371	0.536 ± 0.009	2.972 ± 5.5×10^{-07}
Michika	0.061 ± 0.002	7.854 ± 0.293	0.057 ± 0.002	0.316 ± 1.2×10^{-07}

concentration of uranium in nature is constant (the relative percentages being 99.2745%, 0.7204% and 0.00548% for ^{238}U, ^{235}U and ^{234}U, respectively), isotopes ^{235}U and ^{234}U were determined by Equation 1[15,34]:

$$C_{235_U} = \frac{0.7200}{99.2745} \times C_{238_U}, \qquad \text{Equation 1}$$

where and are the respective concentrations of isotopes ^{235}U and ^{238}U. In the same way, the value for isotope ^{234}U was obtained where isotope ^{235}U was replaced by ^{234}U alongside the value of its percentage composition.

A summary of the results of uranium and thorium concentrations for the various locations is presented in Table 2. From these results, the concentration range of ^{232}Th for the entire samples, 0.055±0.006 to 5.410±0.318 ppm, was below the average crustal concentration range for ^{232}Th, which is 8–12 ppm.[35]

Two concentrations obtained for the uranium isotopes were lower than the average crustal concentration (2–3 ppm ^{238}U): Riruwai (1.318±0.049 ppm) and Mika-I (0.084±0.003 ppm). However, the concentration of uranium isotopes from Mika-II (73.965±1.371 ppm) was far higher than the world average, while that for Michika (7.854±0.293 ppm) was close to the world average.

^{235}U and ^{234}U concentration ranges across the sample locations were 0.001±1.9×10^{-5} ppm to 0.536±0.009 ppm and 3.384×10^{-8}±1.0×10^{-9} ppm to 2.972×10^{-5}±5.5×10^{-7} ppm, respectively. Lower and higher concentrations obtained were both from Mika-I and Mika-II. Possible factors contributing to errors in values reported in this study could be sample inhomogeneity, uncertainty calculations with respect to uranium isotope composition, and uncertainty of weighing. However, due to the method used – ICP-MS involving isotope dilution or calibration curve – uncertainty was reduced to the lowest possible.

Figure 2 presents the variable isotopic concentration of radiogenic lead in this study. Its presence in samples in differing amounts from both primordial and radiogenic lead depends on geological age and uranium/thorium content. The Riruwai sample had the highest concentration of radiogenic lead while the Michika sample had the lowest. A range of 0.004±0.001 ppm to 4.447±0.322 ppm was found for all the samples. These measured lead isotope values were further used to determine the age of the samples.

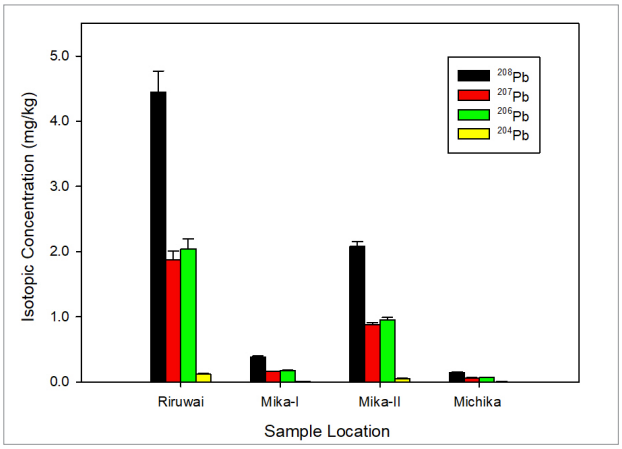

Figure 2: Lead (Pb) isotopic concentration (mg/kg or ppm) of samples obtained from the four study sites.

Isotopic concentration ratio

The natural isotopic ratio and variation of uranium and thorium is a characteristic profile used to trace the origin of a uranium-bearing material.[2,13] The isotopic ratios considered in this study were ^{238}U/^{235}U and ^{235}U/^{234}U together with ^{235}U/^{238}U and ^{234}U/^{238}U. The ^{232}Th/^{238}U isotopic ratio was also determined.

The isotopic ratios ^{238}U/^{235}U, ^{235}U/^{234}U, ^{235}U/^{238}U together with ^{234}U/^{238}U were determined from concentration values (ppm) measured by ICP-MS and are presented in Table 3. The ^{238}U/^{235}U isotopic ratio obtained in this study is 137.881±0.007 and it shows no significant variation across the sample locations, but equals the constant value for natural uranium under the solar system, which is 137.88.[22,36,37] The ^{235}U/^{234}U isotopic ratio yielded a value of 130.911±0.290, and also shows no significant variation across the samples, but is comparable with that reported by Brennecka et al.[7] within the world's range value of 83.63–164.17, from different geochemical environments. The standard value for the isotopic ratios ^{235}U/^{238}U and ^{234}U/^{238}U and their variability are well known as 7.253×10^{-3} and 5.502×10^{-5}, respectively.[6,38] The values determined in this study are 7.253×10^{-3}±2.05×10^{-4} and 5.540×10^{-5}±4.08×10^{-7}, which are identical to the standard values, although there was no significant variation across the sample locations. The data on uranium isotopic ratios ^{238}U/^{235}U and ^{235}U/^{234}U from this study were compared with those from other parts of the world, as illustrated in Figure 3a and 3b; they were found to be consistent and comparable with the reported values.[7,13,38,39]

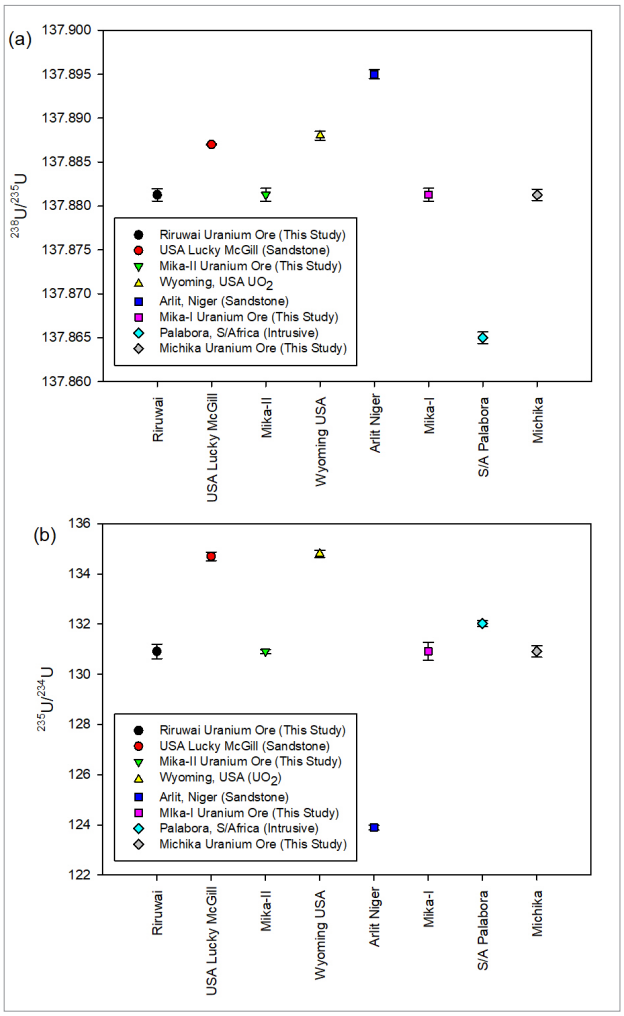

Figure 3: Comparison of uranium isotopic ratios (a) $^{238}U/^{235}U$ and (b) $^{235}U/^{234}U$ from our study sites and other parts of the world.

Uranium age determination

Daughter-parent isotopic ratios ($^{206}Pb/^{238}U$, $^{207}Pb/^{235}U$ and Pb-Pb) were used to determine the age of natural uranium samples in units of mega-annum (Ma). Table 4 presents values of the ratios $^{206}Pb/^{238}U$, $^{207}Pb/^{235}U$ and average age determined. Table 4 shows variable ranges of 0.008±0.002 Ma to 2.095±0.375 Ma for $^{206}Pb/^{238}U$, 0.0105±0.003 Ma to 2.653±0.271 Ma for $^{207}Pb/^{235}U$ and 29.4±0.009 Ma to 4280 ± 0.046 Ma for average age. The equation for the law of basic radioactive decay and its derivative, Equation 2, were used to determine the age of natural uranium material[40-42]:

$$t = \frac{\left(1 - \frac{R}{K}\right)}{\beta}, \qquad \text{Equation 2}$$

where t is the time or age of $^{206}Pb/^{238}U$ and $^{207}Pb/^{235}U$, R is the isotopic ratio of daughter-parent ($^{206}Pb/^{238}U$ and $^{207}Pb/^{235}U$), β is the factor that is composed of decay constants of the daughter-parent nuclei and K is the isotopic decay ratio .

Table 4: U-Pb isotopic ratio and average age of measured natural uranium samples

Sample location	$^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$	Age (Ma)
Riruwai	1.552 ± 0.332	1.962 ± 0.076	3550 ± 0.021
Mika-I	2.095 ± 0.375	2.653 ± 0.271	4280 ± 0.046
Mika-II	0.013 ± 0.027	0.016 ± 0.026	49.3 ± 0.005
Michika	0.008 ± 0.002	0.0105 ± 0.003	29.4 ± 0.009

From the results, Mika-I has an age of 4280±0.046 Ma, as for younger granite, while Michika has an age of 29.4±0.009 Ma and is the youngest, yielding an age difference of 4250.6±0.038 Ma. The mean age of all the samples was 1978.2±0.019 Ma. The age of natural uranium is a reflection of the presence of radiogenic lead: $^{206}Pb/^{238}U$ isotopic ratio and age of material are significantly correlated, with a correlation coefficient of 0.997 and a p-value of 0.00286. In addition, Figure 4a shows a regression curve fit with $R^2 = 0.9998$, implying that there was a significant correlation between the variables, which was not by chance. Hence, the variation in the age of the investigated natural uranium samples with half-lives of $t_{½} \approx 4500$ Ma of ^{238}U and $t_{½} \approx 704$ Ma of ^{235}U, shows that some fractionation occurred over the period. In addition, a negative correlation was observed between uranium concentration and age of the material. Mika-I and Mika-II samples showed an age difference of 4230.7±0.062 Ma due to the differences in mineralisation, geochemical formation, homogeneity of rock and concentration from the strong correlation of U-Pb in materials as well as possible error from determination of the isotopic ratio. In addition, Figure 4b–d shows the U-Pb discordia and Pb-Pb isochron for the four investigated samples.

Discussion

Characterisation of natural uranium to obtain isotopic profiles and signatures for nuclear forensic application in support of nuclear security, safeguards and non-proliferation, is increasingly becoming important and of global interest.[2] Given the abundant deposits of natural uranium in Africa and the consequent potential for nuclear insecurity, providing isotopic profiles and signatures of natural uranium, especially its nuclear content, isotopic ratio and age, has application in nuclear forensics, by providing a database for reference.

Table 3: Uranium concentration isotopic ratios of the natural uranium samples

Sample location	$^{238}U/^{235}U$	$^{235}U/^{234}U$	$^{235}U/^{238}U$	$^{234}U/^{238}U$
Riruwai	137.881 ± 0.007	130.911 ± 0.290	$7.253\times10^{-03} \pm 2.05\times10^{-04}$	$5.540\times10^{-05} \pm 4.08\times10^{-07}$
Mika-I	137.881 ± 0.075	130.911 ± 0.086	$7.253\times10^{-03} \pm 6.33\times10^{-04}$	$5.540\times10^{-05} \pm 3.33\times10^{-07}$
Mika-II	137.881 ± 0.008	130.911 ± 0.360	$7.253\times10^{-03} \pm 6.56\times10^{-04}$	$5.540\times10^{-05} \pm 4.01\times10^{-07}$
Michika	137.881 ± 0.006	130.911 ± 0.228	$7.253\times10^{-03} \pm 6.83\times10^{-04}$	$5.540\times10^{-05} \pm 4.15\times10^{-07}$

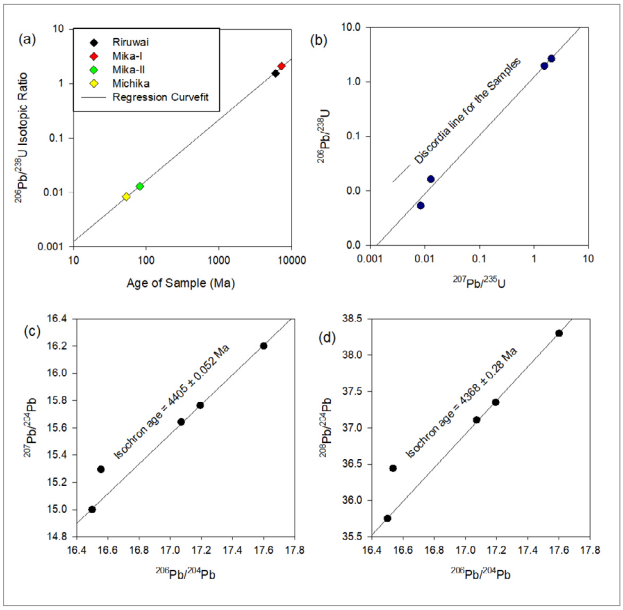

Figure 4: (a) A regression curve fit for the correlation of the $^{206}Pb/^{238}U$ isotopic ratio with determined age of the natural uranium samples. (b) U-Pb discordia graph for the parent-daughter isotopic ratios ($^{206}Pb/^{238}U$ and $^{207}Pb/^{235}U$) of the samples. (c) Pb-Pb isochron diagram (for the $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ isotopic ratios) defining an isochron of 4405±0.052 Ma for the four samples of natural uranium from northern Nigeria. (d) Pb-Pb isochron diagram (for the $^{208}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ isotopic ratios) defining an isochron of 4368±0.027 Ma for the four samples of natural uranium from northern Nigeria.

The concentration ranges of uranium and thorium at various sites were below the crustal values, except at Mika-II and Michika. This, therefore, forms the basis for identifying the samples as having concentration values lower than the average crustal concentration in ppm. The high and low concentration values of the radioactive elements (uranium and thorium) in natural uranium samples measured are comparable with those reported previously.[8,34,36] Only small amounts of ^{204}Pb were present in all the samples, as shown in Figure 2 – an indication of minerals rich in uranium/thorium.

Comparison of the data in Table 3 and Figure 3 for the uranium isotopic ratios $^{238}U/^{235}U$ and $^{235}U/^{234}U$ for the four samples gave a correlation coefficient of -0.775 and *p*-value of 0.225, implying that there is no significant relationship between any pair of the ratios. There was no significant difference between the various sample locations. Isotope concentration ratio of $^{235}U/^{238}U$ together with $^{234}U/^{238}U$ showed no significant variation across the various mines, with a correlation coefficient of -0.829 and *p*-value of 0.171 implying that there is no significant relationship between any pair of the isotope ratios. Thus, the invariant uranium isotopic ratios determined in this study can serve as supplementary information to characterise the natural uranium found in the study area.

In the decay series U-Th-Pb, analysis of daughter-parent isotopic ratios of $^{206}Pb/^{238}U$, $^{207}Pb/^{235}U$ and $^{232}Th/^{208}Pb$ alongside the Pb-Pb isochron is useful in determining the fingerprint and age of uranium-bearing material. The parameters were measured and determined in this study. The actual $^{235}U/^{238}U$, $^{206}Pb/^{238}U$ and $^{232}Th/^{208}Pb$ ratios present at the time of formation of the geological structure of the ore when uranium and thorium were fastened into the mineral deposit, indicate the presence of radiogenic isotopic vector. Hence, some geological age information of the deposit can be determined through isotopic composition of the decay-generated lead. The isotope system of $^{206}Pb/^{238}U$, $^{207}Pb/^{235}U$ and Pb-Pb were used in this study, considering the decay system $^{238}U \rightarrow {}^{206}Pb$, $^{235}U \rightarrow {}^{207}Pb$ and $^{232}Th \rightarrow {}^{208}Pb$ plays a key role in long- and short-range chronometers. Ages of the samples in this study are comparable to the age of the earth, 4543 Ma[43], determined through radioactive decay chronometry and other related studies[44,45]. The Pb-Pb isochron (Figure 4c and 4d) presents a compatible age value to that of the earth, which correlates well with other U-Pb methods used. The highest age (of the Mika-I sample) was less than the age of the earth by 263 Ma, while the lowest age (of Michika) differed by 4513.6 Ma. The age variation, therefore, presents a unique signature for the sample in this study, which is relevant for nuclear forensic applications.

Conclusion

Based on the results of the investigation, isotopic concentrations of ^{232}Th, ^{238}U, ^{235}U and ^{234}U for the respective mine sites differ significantly, thereby forming characteristic isotopic profiles of the samples. The isotopic ratios $^{206}Pb/^{238}U$ and $^{207}Pb/^{235}U$ show distinct variation across the mines and hence form part of the fingerprint. Other isotopic concentration ratios $^{238}U/^{235}U$, $^{235}U/^{234}U$, $^{235}U/^{238}U$ and $^{234}U/^{238}U$ were invariant for the respective mines. Perhaps, partly because concentrations of ^{235}U and ^{234}U isotopes were determined from ^{238}U and were not measured directly, ICP-MS is not precise enough to measure the small differences in the isotopic ratios, thereby yielding concentration values that produced no significant variations. However, their values are identical and comparable to global standards and other related works, thus providing supplementary isotopic profile information to characterise the materials.

Using the isotopic ratios $^{206}Pb/^{238}U$, $^{206}Pb/^{238}U$ and Pb-Pb isochron, ages of the samples were determined and showed significant variation for the mining sites under investigation. Sample age was, therefore, a unique isotopic profile for nuclear forensic application. The very good correlation observed between isotopic ratio $^{206}Pb/^{238}U$ and age shows the occurrence of fractionate directly related to vein-type granite mineralogy. Therefore, the samples studied can be identified by their ^{232}Th, ^{238}U, ^{235}U and ^{234}U isotopic concentrations and ratios $^{206}Pb/^{238}U$, $^{207}Pb/^{235}U$, as well as age.

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Competing interests

We have no competing interests to declare.

Authors' contributions

S.O.O.J.: Data collection, data analysis, writing. I.T.U.: Conceptualisation, methodology, student supervision.

References

1. Schwerdt IJ, Brenkmann A, Martinson S, Albrecht BD, Heffernan S, Klosterman MR, et al. Nuclear proliferomics: A new field of study to identify signatures of nuclear materials as demonstrated on alpha-UO_3. Talanta. 2018;186:433–444. https://doi.org/10.1016/j.talanta.2018.04.092
2. Kristo MJ, Gaffney AM, Marks N, Knight K, Cassata WS, Hutcheon ID. Nuclear forensic science: Analysis of nuclear material out of regulatory control. Annu Rev Earth Planet Sci. 2016;44:555–579. https://doi.org/10.1146/annurevearth-060115-012309
3. Mayer K, Wallenius M, Lützenkirchen K, Galy J, Varga Z, Erdmann N, et al. Nuclear forensics: A methodology applicable to nuclear security and to non-proliferation. J Phys Conf Ser. 2011;312(062003):1–9. https://doi.org/10.1088/1742-6596/312/6/062003
4. International Atomic Energy Agency (IAEA). Nuclear forensics support. IAEA Nuclear Security Series No. 2-G (Rev. 1). Vienna: IAEA; 2019.
5. Varga Z, Mayer K, Bonamici CE, Hubert A, Hutcheon I, Kinman W, et al. Validation of reference materials for uranium radiochronometry in the frame of nuclear forensic investigations. Appl Radiat Isotopes. 2015;102:81–86. http://dx.doi.org/10.1016/j.apradiso.2015.05.005
6. Krajkó J, Varga Z, Yalcintas E, Wallenius E, Mayer K. Application of neodymium isotope ratio measurements for the origin assessment of uranium ore concentrates. Talanta. 2014;129:499–504. http://dx.doi.org/10.1016/j.talanta.2014.06.02

7. Brennecka GA, Borg LE, Hutcheon ID, Sharp MA, Anbar AD. Natural variations in uranium isotope ratios of uranium ore concentrates: Understanding the $^{238}U/^{235}U$ fractionation mechanism. Earth Planet Sci Lett. 2010;291:228–233. http://doi.org/10.1016/j.epsl.2010.01.023

8. Švedkauskaite-LeGore J. Development and validation of a method for origin determination of uranium-bearing material [PhD thesis report JRC-ITU-TN-2008/25]. Karlsruhe: European Union Joint Research Council, Institute of Transuranium Elements; 2008. Available from: https://publications.jrc.ec.europa.eu/repository/handle/JRC44987

9. Wallenius M, Mayer K, Ray I. Nuclear forensic investigations: Two case studies. Forensic Sci Int. 2006;156:55–62. https://doi.org/10.1016/j.forsciint.2014.12.029

10. Mathuthu M, Khumalo N. Developing nuclear forensics signatures and national nuclear forensics libraries for the African continent: A case review for South Africa. Int J Appl Sci Res Rev. 2017;4:1–3. https://doi.org/10.21767/23949988.100052

11. Keatley AC, Martin PG, Hallam KR, Payton OD, Awbery R, Carvalho F, et al. Source identification of uranium-containing materials at mine legacy sites in Portugal. J Environ Radioact. 2018;183:102–111. https://doi.org/10.1016/j.jenvrad.2017.12.009

12. IAEA Incident and Trafficking Database (ITDB). Incidents of nuclear and other radioactive material out of regulatory control [document on the Internet]. c2016 [cited 2019 Nov 04]. Available from: http://wwwns.iaea.org/downloads/security/itdb-fact-sheet.pdf

13. Keegan E, Kristo MJ, Colella M, Robel M, Williams R, Lindvall R, et al. Nuclear forensic analysis of an unknown uranium ore concentrate sample seized in a criminal investigation in Australia. Forensic Sci Int. 2014;240:111–121. https://doi.org/10.1016/j.forsciint.2014.04.004

14. Moody KJ, Hutcheon ID, Grant PM. Nuclear forensic analysis. 2nd ed. Boca Raton, FL: CRC Press; 2014.

15. Firestone RB, Shirley VS, Chu SYF, Balgin CM, Zipkin J. Table of isotopes. CD ROM 8th ed. Version 1. Hoboken, NJ: Wiley-Interscience; 1996.

16. Scott SR, Sims KWW, Reagan MK, Ball L, Schwieters JB, Bouman C, et al. The application of abundance sensitivity filters to precise and accurate measurement of uranium series nuclides by plasma mass spectrometry. Int J Mass Spect. 2019;435:321–332. https://doi.org/10.1016/j.ijms.2018.11.011

17. Hutcheon I, Kristo M, Knight K. Non-proliferation nuclear forensics. Short course series #43 LLNLCONF-679869. Winnipeg: Mineralogical Association of Canada; 2015.

18. Maxwell O, Wagiran H, Ibrahim N, Lee SK, Sabri S. Comparison of activity concentration of ^{238}U, ^{232}Th and ^{40}K indifferent layers of subsurface structures in DeiDei and Kubwa, Abuja, north central Nigeria. Radiat Phys Chem. 2013;91:70–80. http://dx.doi.org/10.1016/j.radphyschem.2013.05.006

19. Spano TL, Simonetti A, Balboni E, Dorais C, Burns PC. Trace element and U isotope analysis of uraninite and ore concentrate: Applications for nuclear forensic investigations. Appl Geochem. 2017;84:277–285. http://dx.doi.org/10.1016/j.apgeochem.2017.07.003

20. Švedkauskaite-LeGore J, Mayer K, Millet S, Nicholl A, Rasmussen G, Baltrunas D. Investigation of the isotopic composition of lead and of trace elements concentrations in natural uranium materials as a signature in nuclear forensics. Radiochim Acta. 2007;95:601–605. https://doi.org/10.1524/ract.2007.95.10.601

21. Richer S, Alonso A, De Bolle W, Wallum R, Taylor PDP. Isotopic "fingerprint" for natural uranium ore samples. Int J Mass Spectrom. 1999;193:9–14. https://doi.org/10.1016/S1387-3806(99)00102-5

22. Weyer S, Anbar AD, Gerdes A, Gordon GW, Algeo TJ, Boyle EA. Natural fractionation of $^{238}U/^{235}U$. Geochim Cosmochim Acta. 2008;72:345–359. https://doi.org/10.1016/j.gca.2007.11.012

23. Mishra S, Sahoo SK, Chaudhury P, Pradeepkumar KS. Measurement and validation of uranium isotope ratio in uranium ore for isotopic fingerprinting. Radiat Prot Environ. 2017;40:3–8. https://doi.org/10.4103/rpe.RPE_36_16

24. Bopp CJ, Lundstrom CC, Johnson TM, Glessner JJ. Variations in $^{238}U/^{235}U$ in uranium ore deposits: Isotopic signatures of the U reduction process? Geology. 2009;37:611–614. https://doi.org/10.1130/G25550A.1

25. Bute SI. Uranium ore deposits in northeastern Nigeria: Geology and prospect. Continent J Earth Sci. 2013;8:21–28.

26. Tsang H, Akhtar S, Saif-ur-Rehman, Wu QF, Lee I, Sahir N, Yang XY. The uranium prospects in Mika Region, Northeastern Nigeria. Open J Geol. 2018;8:1043–1055. https://doi.org/10.4236/ojg.2018.811063

27. Martin RF, Bowen P. Per-aluminous granite produced by rock fluid interaction in the Riruwai non-orogenic ring complex, Nigeria: Mineralogical evidence. Can Mineral. 1981;19:65–82.

28. Olasehinde A, Ashano EC, Singh GP. Analysis of magnetic anomaly over the Riruwai Younger granite ring complex: A geodynamic implication. Continent J Earth Sci. 2012;7:9–18.

29. Nigeria Geological Survey Agency. Report on the integrated geophysical investigation of suspected base metal deposits in Kaffo part of Riruwai [document on the Internet]. No date [cited 2019 Dec 18]. Available from: https://ngsa.gov.ng/author/ng-mod/page/3/

30. Oruonye ED, Ahmed MY. Assessment of environmental effect of abandoned uranium mine site in Mika village of Taraba State Nigeria. Int J Geog Geol. 2017;6(4):70–78. https://doi.org/10.18488/journal.10.2017.64.70.78

31. Haruna AI, Ameh DP, Mohammed AA, Umar US. Uranium mineralisation in Gubrunde Horst, Upper Benue Trough, North-East, Nigeria. J Geosci Geomat. 2017;5(3):136–146. https://doi.org/10.12691/jgg-5-3-5

32. Suh CE, Dada SS. Mesostructural and microstructural evidences for a two stage tectono-metallogenetic model for the uranium deposit at Mika, northeastern Nigeria: A research note. Nonrenewable Resour. 1998;7:75–77.

33. John SOO, Usman IT, Akpa TC, Ibrahim U. Rare earth elements in uranium ore for nuclear forensic application. IOP Conf Ser Earth Environ Sci. 2021;655, Art. #012075. https://doi.org/10.1088/1755-1315/655/1/012075

34. Almeida GM, Campos SSS, Gennari RF, Souza SO. Determination of the concentration of radionuclides in soil and water next the uranium mine of Caetité-ba. International Nuclear Atlantic Conference – INAC 2011; 2011 October 24–28; Belo Horizonte, Minas Gerais, Brazil. Associação Brasileira De Energia Nuclear; 2011.

35. Nicolet JP, Erdi-Krausz G. Guidelines for radioelement mapping using gamma ray spectrometry data. IAEA-TECDOC-1363. Vienna: International Atomic Energy Agency; 2003.

36. Tamborini G. SIMS analysis of uranium and actinides in microparticles of different origin. Microchim Acta. 2004;145:237–245. http://doi.org/10.1007/s00604-003-0160-8

37. Stirling CH, Anderson MB, Potter E-K, Halliday A. Low-temperature isotopic fractionation of uranium. Earth Planet Sci Lett. 2007;264:208–225. https://doi.org/10.1016/j.epsl.2007.09.019

38. Varga Z, Wallenius M, Mayer K, Mappen M. Analysis of uranium ore concentrates for origin assessment. Proc Radiochem Acta. 2011;1:1–4. http://doi.org/10.1524/rcpr.2011.0004

39. Fujii Y, Nomura M, Onitsuka H, Takeda K. Anomalous isotope fractionation in uranium enrichment process. J Nucl Sci Technol. 1989;26:1061–1064. https://doi.org/10.1080/18811248.1989.9734427

40. Morgenstern A, Apostolidis C, Mayer K. Age determination of highly enriched uranium: Separation and analysis of ^{231}Pa. Anal Chem. 2002;74(21):5513-5516. https://doi.org/10.1021/ac0203948

41. Robin KH. Depleted uranium, natural uranium and other naturally occurring radioactive elements in Hawaiian environments. A report prepared for National Defence Centre for Environmental Excellence. Unpublished report; 2008.

42. Stanley FE. A beginner's guide to uranium chronometry in nuclear forensics and safeguards. J Anal At Spectrom. 2012;27:1821–1830. https://doi.org/10.1039/c2ja30182b

43. Dalrymple GB. Age of the earth. Redwood City, CA: Stanford University Press; 1991.

44. Connelly JN, Bollard J, Bizzarro M. Pb-Pb chronometry and the early solar system. Geochim Cosmochim Acta. 2017;201:345–363. http://dx.doi.org/10.1016/j.gca.2016.10.044

45. Merle RE, Nemchin AA, Whitehouse MJ, Snape JF, Kenny GG, Bellucci JJ, et al. Pb-Pb ages and initial isotopic composition of lunar meteorites: NWA 773 clan, NWA 4734, and Dhofar 287. Meteor Planet Sci. 2020;55(8):1808-1832. https://doi.org/10.1111/maps.13547

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Shape analysis of the StW 578 calotte from Jacovec Cavern, Gauteng (South Africa)

The fossiliferous deposits within the lower-lying Jacovec Cavern in the locality of Sterkfontein yielded valuable hominin remains, including the StW 578 specimen. Because StW 578 mainly preserves the calotte, the taxonomic status of this specimen has been a matter of discussion. Within this context, here we employed high-resolution microtomography and a landmark-free registration method to explore taxonomically diagnostic features in the external surface of the StW 578 calotte. Our comparative sample included adult humans and common chimpanzees as well as one *Australopithecus africanus* specimen (Sts 5). We partially restored the StW 578 calotte digitally and compared it to extant specimens and Sts 5 using a landmark-free registration based on smooth and invertible surface deformation. Our comparative shape analysis reveals morphological differences with extant humans, especially in the frontal bones, and with extant chimpanzees, as well as intriguing specificities in the morphology of the StW 578 parietal bones. Lastly, our study suggests morphological proximity between StW 578 and Sts 5. Given the intimate relationship between the brain and the braincase, as well as the integration of the hominin face and neurocranium, we suggest that cranial vault shape differences between StW 578 and extant humans, if confirmed by further analyses, could be either explained by differences in brain surface morphology or in the face. Besides providing additional information about the morphology of the Jacovec calotte that will be useful in future taxonomic discussion, this study introduces a new protocol for the landmark-free analysis of fossil hominin cranial shape.

Significance:

- We provide further information on the enigmatic fossil specimen StW 578.
- We introduce a new approach for the morphological study of fossil hominin crania.
- We highlight morphological similarities between StW 578 and 'Mrs Ples'.

Introduction

The palaeocave infills of the Sterkfontein Caves, which lie 50 km northwest of Johannesburg (South Africa), are well known for having yielded iconic fossil specimens, such as Sts 5 ('Mrs Ples') and StW 573 ('Little Foot').[1] Fossiliferous deposits within the lower-lying Jacovec Cavern have provided additional hominin remains that are of particular interest; prominent among them is the StW 578 cranium.[2] StW 578 was discovered in 1995 partly in situ in 'Orange' sediments (Lo27 coordinates: Y, -73582.548; X, 2878771.407; Z, 1450.909) that are composed of partially calcified breccia exposed in the roof of the eastern part of the Jacovec Cavern[2], and partly in collapsed breccia beneath. Because of the conflicting geomorphological scenarios of the infilling of the lower chambers in Sterkfontein[2,3-7], determining a geological age for StW 578 is particularly challenging[2,8-11]. Absolute dating using cosmogenic nuclide burial methods originally provided an age of 4.02±0.27 Ma for the cranium.[2]

Together with the 3.67-million-year-old skeleton of StW 573, StW 578 represents some of the oldest evidence of human evolution in southern Africa.[12] Because of its late Pliocene age, the StW 578 calotte (i.e. the top part of the cranium) is of great interest for reconstructing early hominin evolution. Late Pliocene fossiliferous deposits in the Jacovec Cavern coincide with an important radiation of the genus *Australopithecus*, with the emergence of *Australopithecus afarensis*, *Australopithecus deyiremeda*, *Australopithecus bahrelgahzali* and the 'Burtele Foot'.[13] For instance, previous studies of the postcranial assemblage from the Jacovec Cavern revealed an interesting mosaic of features, with the femur having a very long neck relative to a small head but a round-sectioned shaft, and with a clavicle (StW 606) that has morphological similarities to extant chimpanzees.[2,14] Accordingly, StW 578 has the potential to contribute to our understanding of morphological variation and taxonomic diversity within *Australopithecus* in the late Pliocene.

Because the Jacovec cranium mainly preserves part of the calotte (Figure 1), along with fragments of the face and dentition, and a temporal bone (see Partridge et al.[2]; all initially cleaned and reconstructed by RJ Clarke), the taxonomic status of this specimen has been a matter of discussion. Indeed, in the original description, the authors refrained from attributing StW 578 to any species.[2] Study of the cranial vault thickness and composition revealed close affinities with the *Australopithecus* specimens from Sterkfontein Member 4.[8] However, Partridge et al. noticed that:

> *posterior to this fossa the tympanic plate slopes strongly posteriorly as it does in* Pan*, and in this respect differs from all other* Australopithecus *temporals from Sterkfontein Member 4, which resemble more closely the human, vertically inclined tympanic plate.*[2(p.609)]

The dental and facial morphology and the presence of a metopic ridge on the frontal convinced Clarke and Kuman[12] to classify this specimen as *Australopithecus africanus* rather than *Australopithecus prometheus*, teeth of which also occur in the Jacovec sample.

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Given the significance of this specimen and ongoing debates, we virtually reconstructed the StW 578 calotte and compared its shape to those of extant humans, extant chimpanzees, and *Australopithecus africanus* (as represented by Sts 5). Besides introducing a new protocol for the shape analysis of fossil hominin crania, we also provide new insights into the morphology of the Jacovec specimen.

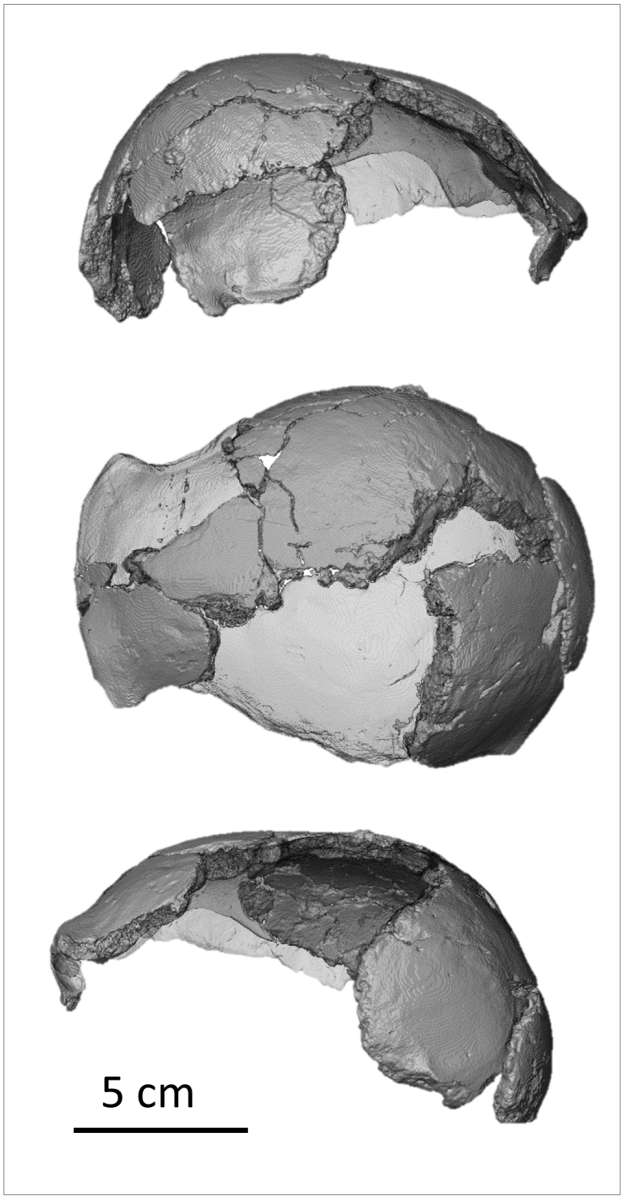

Figure 1: Virtual rendering of the StW 578 calotte in lateral right (top), superior (middle) and lateral left (bottom) views. Plaster used to physically reconstruct the cranium is in light grey and transparent.

Material and methods

Comparative material

Our comparative sample of extant specimens comprised mixed-sex samples of non-pathological adult humans (*Homo sapiens*, $n=6$) and common chimpanzees (*Pan troglodytes*, $n=6$). Extant human samples were obtained from the Pretoria Bone Collection of the University of Pretoria[15] while extant chimpanzee samples were obtained from the Royal Museum for Central Africa in Tervuren (Belgium). Additionally, we included the *Australopithecus africanus* specimen Sts 5 that comes from Sterkfontein Member 4, dated to about 2.8–2.4 Ma based on faunal assemblages.[16-20] Sts 5 is currently housed in the Ditsong National Museum of Natural History (South Africa). Parts of the outer bone table of Sts 5 remained in the breccia during the initial preparation by Broom[17,21], but this does not alter the overall shape of the cranial vault.

Scanning and pre-processing step

StW 578 and Sts 5 were scanned at the microfocus X-ray tomography facility of the Palaeosciences Centre at the University of the Witwatersrand, in Johannesburg (South Africa), at a spatial resolution of 66.6 μm and 75.0 μm (isotropic voxel size), respectively. A 3D model of StW 578 is available on MorphoSource[22]. Extant humans and

chimpanzees have been similarly scanned at the South African Nuclear Energy Corporation in Pelindaba, South Africa, and at the Centre for X-ray Tomography of Ghent University (UGCT) in Ghent, Belgium, with a resolution ranging from 70.0 μm to 102.3 μm.

As a pre-processing step, all of the crania were oriented the same way with the opisthocranion and glabella aligned on a single transverse plane, and a new image stack reflecting the standardised orientation was generated using Avizo v9.0[23] (FEI Visualization Sciences Group Inc., https://www.fei.com/software/amira-avizo/)[8]. The same transverse plane was used to virtually cut the crania so that the superior part of the braincase (that is preserved in StW 578) could be separated from the rest of the cranium and studied. Finally, because we were interested in the external shape of the cranium only, we virtually isolated the external surface from the inner surface.

Reconstruction of StW 578

Before virtually reconstructing the StW 578 calotte, we removed the plaster using segmentation tools in Avizo v9.0 (STEP1, Figure 2A). We fitted a surface through StW 578 using the tool 'Patch' in Rhinoceros 3D v6.0[24] (STEP2, Figure 2B). The resulting surface (Figure 2B) was opened in Avizo v9.0 and the parts of the new surface that filled in the gaps between bones were manually extracted (Figure 2C) and merged with the initial surface using the tool 'Flatten visible layer' in Meshlab[25] (STEP3, Figures 2D,E). This new surface was mirrored to reconstruct missing regions in the opposite side (Figure 2F). Finally, STEP2 and STEP3 were repeated (Figure 2G,H). Inferior portions of the parietal and frontal bones were still missing at this stage (Figure 2E). Because these regions cannot be reconstructed using existing bones, we preferred not to estimate them and thus systematically removed the homologous regions in all comparative specimens (see below).

Similarly, we removed the plaster in Sts 5 and the missing regions were virtually filled in by applying STEP2 and STEP3.

Shape analysis

We compared StW 578 to extant specimens and Sts 5 by using landmark-free registration based on smooth and invertible surface deformation.[26-30] This approach has been previously applied to a number of craniodental and postcranial structures, including endocasts[27,29], enamel-dentine junctions[28,31], vertebrae[32] and bony labyrinths[33] and comparisons between the landmark-based and landmark-free approaches have revealed the ability of the latter approach for capturing geometric details and for statistical determination of geometric correspondence[34]. Because the StW 578 calotte is partial, instead of using the whole surface to align specimens as in previous studies[27-29], we used four landmarks, i.e. glabella, opisthocranion, and two landmarks positioned on the external surfaces of the right and left parietal bones at the intersection of (1) a coronal plane positioned at three-quarters of the maximum length of the calotte and of (2) the transverse plane that was used for isolating the top of the calotte. Surfaces were aligned in position, orientation and scale using one surface that was randomly selected as a reference and by using the tool 'Landmark Surface Warp' (method: Rigid + Uniform Scale) in Avizo v9.0 which is based on the iterative closest point algorithm that minimises the root mean square distance between the points of each specimen to corresponding points on the reference.[35] A template was deformed to extant specimens using Deformetrica v4.[26,36,37] The extant specimens and Sts 5 were deformed to StW 578. Based on this computation, the parts of the frontal and parietal bones that are missing in StW 578 (Figure 2E) were systematically removed from the comparative extant and fossil specimens using an automated method.[29,30,36] We then deformed another template to the extant comparative surfaces generated after this step and a global mean shape as well as taxon-specific mean shapes (*Homo sapiens* and *Pan troglodytes*, each $n=6$) were generated. Finally, the global mean shape and taxon mean shapes were deformed to StW 578 and Sts 5. The resulting 3D deformation fields that integrate local orientation and the amplitude of the deformation were analysed using principal component analysis (PCA) (Figure 3). Displacements from the taxon mean shapes to StW 578 and from Sts 5 to StW 578 were rendered by colour maps.

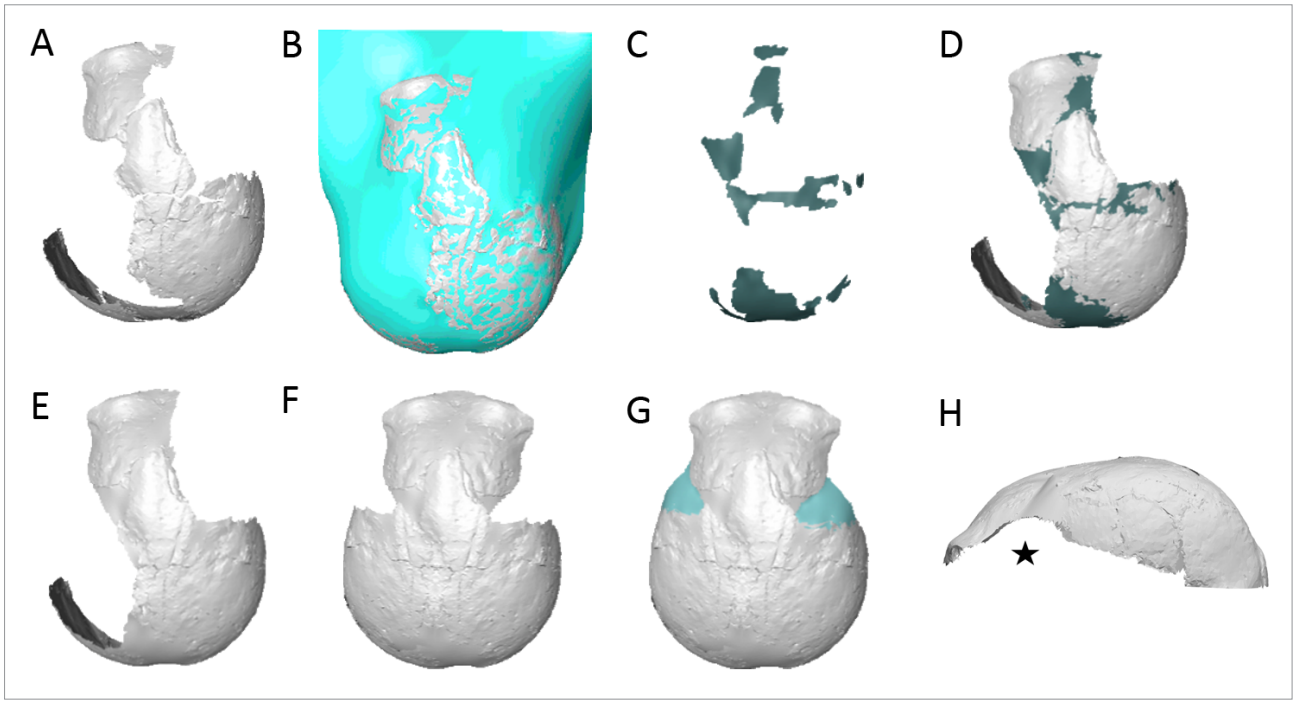

Figure 2: Successive steps for the virtual reconstruction of the StW 578 cranium. The initial surface (A) has been wrapped using a patch (B) and the gaps reconstructed (C) and merged with the initial surface (D). To the end result of the first iteration (E), a mirror was applied (F) and the overall process repeated (G). The resulting surface is lacking lateral portions of the frontal and parietal bones (H) indicated by a star.

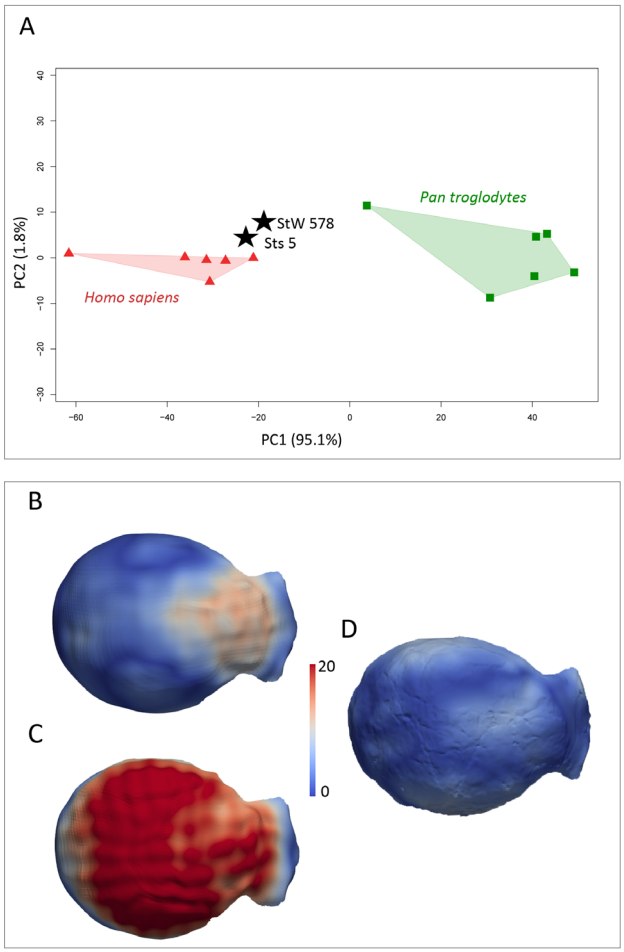

Figure 3: Principal component (PC) analysis of the deformation-based shape comparisons of the StW 578 and Sts 5 calottes and of the extant human and chimpanzee calottes (A). Comparative maps of morphological deformations from the taxon-specific mean shapes computed for extant humans (B) and extant chimpanzees (C) to StW 578 and from Sts 5 to StW 578 (D) in superior view. The colour bar represents the number of deformations.

To estimate potential bias induced by our reconstruction of StW 578 (Figure 2) in the shape analysis, we computed a second set of deformations using the original surface of the StW 578 calotte (i.e. not reconstructed, neither physically nor virtually). The regions that correspond to the missing bone in StW 578 were automatically removed from the extant specimens by using the results of the first set of deformations.[29,36] We then computed a PCA using the deformation fields generated by this second set of deformation (Figure 4).

Results

Figure 3A shows the PCA of the deformation-based shape comparison of StW 578 and extant and fossil comparative specimens. The two extant comparative groups are well discriminated along PC1, which accounts for 95.0% of the variation. Chimpanzees plot in positive space while extant humans plot in negative space. Along PC1, both StW 578 and Sts 5 plot close to each other and closer to the extant human cluster in negative space. Along PC2, which represents 1.8% of the variation, the two comparative groups and the fossil specimens mostly overlap.

The nature and extent of the differences between StW 578 and the comparative specimens were investigated using topological mapping of interspecific variation (Figure 3B–D). Compared with extant humans, the lateral part of the parietal bones in StW 578 is slightly more elevated as indicated by the white spots on both sides of the cranial vault in Figure 3B. On the contrary, the frontal bones in StW 578 are more flattened than in extant humans (in which the rounded forehead contributes to the globular aspect of the overall cranium) as emphasised by the large pink to red area in the colour maps. Compared with the extant chimpanzees, the parietal and frontal bones of the StW 578 calotte are significantly more elevated, which is shown in Figure 3C by the cranial vault being nearly entirely red. The shapes of the external surfaces of the tops of the calottes of StW 578 and Sts 5 are closely similar (Figure 3D), with only minimal differences in the superior part of the frontal bone and the lateral surface of the parietal bones.

Finally, we investigated how our virtual reconstruction of StW 578 may influence our shape analysis by applying the same protocol to the bones preserved in the original specimen and comparing the morphology to the extant groups. If we consider the results of the deformation-based shape comparison of the regions of the calotte that are preserved in StW 578 and artificially isolated in the extant comparative sample (Figure 4), StW 578 plots closer to the extant human cluster along PC1 that represents 93.2% of the variation. Here again, StW 578, extant humans and extant chimpanzees overlap along PC2 that represents 2.0% of the variation. We thus obtain similar results as in the first set of deformation that includes our reconstruction of the calotte, which tends to suggest that our reconstruction does not bias the result of the shape analysis.

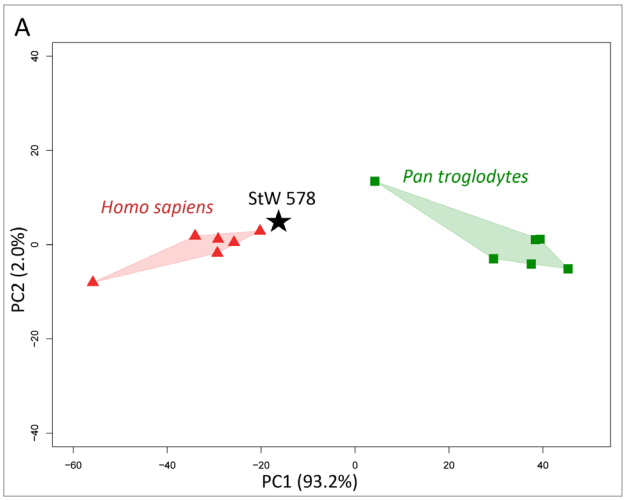

Figure 4: Principal component (PC) analysis of the deformation-based shape comparisons of the unreconstructed upper part of the StW 578 calotte and of the extant human and chimpanzee calottes, focusing on bones originally preserved in StW 578 and artificially isolated in the comparative sample.

Discussion

Because of its fragmentary nature, the taxonomic and phylogenetic status of the StW 578 calotte remains debatable, although it has been recently assigned to *A. africanus* by Clarke and Kuman[12]. Within the limits of our sample, our comparative shape analysis reveals morphological differences with the cranial vault of extant humans, mainly located in the frontal bones and on the lateral surface of the parietal bones, and more dramatically with the cranial vault of extant chimpanzees. As such, our study further clarifies the polarity (i.e. derived or ancestral) of the cranial features identified in StW 578.[2]

The hominin cranium is highly integrated.[38] Frontal bone morphology reflects the integration of the bone structures of the upper face and anterior neurocranium.[39] Within this context, the marked differences identified in the frontal bones of StW 578 and extant humans by our comparative shape analysis might reflect the primitive morphology of the upper face in *Australopithecus*. Indeed, the hominin face has experienced dramatic changes through time, with the earliest changes affecting primarily the canines and supraorbital regions (potentially associated to social interactions) and later modifications related to the masticatory apparatus and a transition from tough and hard to soft or pre-processed food.[40] On the other hand, within the frame of the hypothesis

of modularity of the hominin face and neurocranium, we might consider the possibility that differences in the frontal bone could be the result of neutral evolution.[41,42] Alternatively, this result might be explained by the influence of the frontal and temporal lobes on the morphology of the frontal bones.[43] However, because significant reorganisation of the frontal and temporal lobes occurred in later hominins[44,45], this hypothesis is unlikely. Lastly, it is noteworthy that the extant human frontal bones are characterised by the presence of two bosses which are absent in fossil hominins.[46] Nonetheless, these results are preliminary and will need to be further supported by future analyses including a large extant human sample.

The assessment of the cranial vault thickness variation in StW 578 demonstrated particularly thick parietal bones, which differs from the pattern of cranial thickness reported for extant humans and extant chimpanzees (see Fig. 8 in Beaudet et al.[8]). The growth and development of the neurocranial bones is closely related to the growth and development of the brain (within the frame of the functional matrix hypothesis[47]). Accordingly, we might hypothesise that differences in brain development and growth between StW 578 and extant humans could explain differences in the external cranial shape. Alternatively, it has been hypothesised that cranial vault bones may adapt to empty space and become thicker.[48] Consequently, thickened bone in the parietal region[8] and the resulting shape of the external surface of the braincase in StW 578 (this study) could be related to the morphology of the brain, and the lack of parietal expansion that is specifically found in modern human brains.[49]

Finally, our study reveals close affinities of the shape of the external surface of the calotte of StW 578 and the *Australopithecus africanus* specimen Sts 5. At this stage, no conclusions on the taxonomic status of StW 578 could be drawn, as our sample, besides being relatively small, did not include any representatives of the taxon *Australopithecus prometheus*.[12] However, unlike what is seen in the tympanic plate[2], our study of the external morphology of the StW 578 calotte does not reveal any substantial differences with another *Australopithecus* specimen, Sts 5, nor distinct proximity with extant chimpanzees. However, given the fact that the present preliminary results derive from a new protocol, our results should be carefully considered and future analyses should help clarify this question.

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Competing interests

We have no competing interests to declare.

Authors' contributions

Conceptualisation: A.B.; methodology: A.B., J.D.; data collection – excavations: R.J.C., D.S., J.L.H., T.R.P.; data collection – scanning: L.V.H., L.B.; K.J.C.; data analysis: A.B., K.C.; writing – the initial draft: A.B.; writing – revisions: A.B., D.S., R.J.C., K.J.C., J.D., L.V.H., L.B., J.L.H., T.R.P.; funding acquisition: A.B., D.S.

References

1. Stratford DJ. A review of the geomorphological context and stratigraphy of the Sterkfontein Caves, South Africa. In: Klimchouk A, Palmer AN, De Waele J, Auler AS, Audra P, editors. Hypogene karst regions and caves of the world. Cham: Springer; 2017. p. 879–891. https://doi.org/10.1007/978-3-319-53348-3
2. Partridge TC, Granger DE, Caffee MW, Clarke RJ. Lower Pliocene hominid remains from Sterkfontein. Science. 2003;300(5619):607–612. https://doi.org/10.1126/science.1081651
3. Partridge TC. Re-appraisal of lithostratigraphy of Sterkfontein hominid site. Nature. 1978;275:282–287. https://doi.org/10.1038/275282a0
4. Wilkinson MJ. Geomorphic perspectives on the Sterkfontein australopithecine breccias. J Archaeol Sci. 1983;10(6):515–529. https://doi.org/10.1016/0305-4403(83)90034-1
5. Wilkinson MJ. Lower-lying and possibly older fossiliferous deposits at Sterkfontein. In: Tobias PV, editor. Hominid evolution: Past, present and future. New York: Alan R. Liss; 1985. p. 165–170. https://doi.org/10.1525/aa.1987.89.1.02a00770
6. Partridge TC, Watt IB. The stratigraphy of the Sterkfontein hominid deposit and its relationship to the underground cave system. Palaeontol Africana. 1991;28:35–40.
7. Pickering TR, Kramers JD. Re-appraisal of the stratigraphy and determination of new U-Pb dates for the Sterkfontein hominid site, South Africa. J Hum Evol. 2010;59(1):70–86. https://doi.org/10.1016/j.jhevol.2010.03.014
8. Beaudet A, Carlson KJ, Clarke RJ, De Beer F, Dhaene J, Heaton J, et al. Cranial vault thickness variation and inner structural organization in the StW 578 hominin cranium from Jacovec Cavern, South Africa. J Hum Evol. 2018;121:204–220. https://doi.org/10.1016/j.jhevol.2018.04.004
9. Bruxelles L, Clarke RJ, Maire R, Ortega R, Stratford D. Stratigraphic analysis of the Sterkfontein StW 573 *Australopithecus* skeleton and implications for its age. J Hum Evol. 2014;70:36–48. https://doi.org/10.1016/j.jhevol.2014.02.014
10. Bruxelles L, Stratford DJ, Maire R, Pickering TR, Heaton JL, Beaudet A, et al. A multiscale stratigraphic investigation of the context of StW 573 'Little foot' and Member 2, Sterkfontein caves, South Africa. J Hum Evol. 2019;133:78-98. https://doi.org/10.1016/j.jhevol.2019.05.008
11. Stratford DJ, Grab S, Pickering TR. The stratigraphy and formation history of fossil-and artefact-bearing sediments in the Milner Hall, Sterkfontein Cave, South Africa: New interpretations and implications for palaeoanthropology and archaeology. J Afr Earth Sci. 2014;96:155–167. https://doi.org/10.1016/j.jafrearsci.2014.04.002
12. Clarke RJ, Kuman K. The skull of StW 573, a 3.67 Ma *Australopithecus prometheus* skeleton from Sterkfontein Caves, South Africa. J Hum Evol. 2019;134:102634. https://doi.org/10.1016/j.jhevol.2019.06.005
13. Wood B, Doherty D, Boyle E. Hominin taxic diversity. In: Oxford Research Encyclopedias: Anthropology. Oxford University Press; 2020. https://doi.org/10.1093/acrefore/9780190854584.013.194
14. Pickering TR, Heaton JL, Clarke RJ, Stratford D, Heile AJ. Hominin lower limb bones from Sterkfontein Caves, South Africa (1998–2003 excavations). S Afr J Sci. 2021;117(1/2), Art. #6758. https://doi.org/10.17159/sajs.2021/6758
15. L'Abbé EN, Loots M, Meiring JH. The Pretoria Bone Collection: A modern South African skeletal sample. Homo. 2005;56(2):197–205. https://doi.org/10.1016/j.jchb.2004.10.004

16. Broom R. Discovery of a new skull of the South African ape-man, *Plesianthropus*. Nature. 1947;159(4046):672. https://doi.org/10.1038/159672a0

17. Broom R, Robinson JT, Schepers GWH. Sterkfontein ape-man, *Plesianthropus*. Transv Mus Mem. 1950;4(4065):1–117. https://doi.org/10.1038/160430b0

18. Vrba ES. Early hominids in southern Africa: Updated observations on chronological and ecological background. In: Tobias PV, editor. Hominid evolution: Past, present and future. New York: Alan R. Liss; 1958. p. 195-200. https://doi.org/10.1525/aa.1987.89.1.02a00770

19. Delson E. Chronology of South African australopiths site units. In: Grine FE, editor. Evolutionary history of the 'robust' australopithecines. New York: Aldine de Gruyter; 1988. p. 317–324. https://doi.org/10.4324/9780203792667

20. McKee JK, Thackeray JF, Berger LR. Faunal assemblage seriation of Southern African Pliocene and Pleistocene fossil deposits. Am J Phys Anthropol. 1995;96(3):235–250. https://doi.org/10.1002/ajpa.1330960303

21. Potze S, Thackeray JF. Temporal lines and open sutures revealed on cranial bone adhering to matrix associated with Sts 5 ("Mrs Ples"), Sterkfontein, South Africa. J Hum Evol. 2010;58(6):533–535. https://doi.org/10.1016/j.jhevol.2009.11.005

22. Beaudet A. Media 000405957: Calotte [Mesh] [CT] [data set]. In: Morphosource. Available from: https://www.morphosource.org/concern/media/000405957?locale=en

23. Avizo v9.0. Hillsboro, OR: FEI Company; 2015.

24. Rhinoceros 3D v6.0. Seattle, WA: Robert McNeel & Associates; 2010. Available from: https://www.rhino3d.com/

25. Cignoni P, Corsini M, Ranzuglia G. MeshLab: An open-source 3D mesh processing system. ERCIM News. 2008;73:47–48.

26. Durrleman S, Pennec X, Trouvé A, Ayache N, Braga J. Comparison of the endocranial ontogenies between chimpanzees and bonobos via temporal regression and spatiotemporal registration. J Hum Evol. 2012;62(1):74–88. https://doi.org/10.1016/j.jhevol.2011.10.004

27. Beaudet A, Dumoncel J, De Beer F, Duployer B, Durrleman S, Gilissen E, et al. Morphoarchitectural variation in South African fossil cercopithecoid endocasts. J Hum Evol. 2016;101:65–78. https://doi.org/10.1016/j.jhevol.2016.09.003

28. Beaudet A, Dumoncel J, Thackeray JF, Bruxelles L, Duployer B, Tenailleau C, et al. Upper third molar internal structural organization and semicircular canal morphology in Plio-Pleistocene South African cercopithecoids. J Hum Evol. 2016;95:104–120. https://doi.org/10.1016/j.jhevol.2016.04.004

29. Beaudet A, Dumoncel J, De Beer F, Durrleman S, Gilissen E, Oettlé A, et al. The endocranial shape of *Australopithecus africanus*: Surface analysis of the endocasts of Sts 5 and Sts 60. J Anat. 2018;232(2):296–303. https://doi.org/10.1111/joa.12745

30. Beaudet A, Holloway R, Benazzi S. A comparative study of the endocasts of OH 5 and SK 1585: Implications for the paleoneurology of eastern and southern African *Paranthropus*. J Hum Evol. 2021;156:103010. https://doi.org/10.1016/j.jhevol.2021.103010

31. Pan L, Dumoncel J, Mazurier A, Zanolli C. Hominin diversity in East Asia during the Middle Pleistocene: A premolar endostructural perspective. J Hum Evol. 2020;148:102888. https://doi.org/10.1016/j.jhevol.2020.102888

32. Beaudet A, Clarke RJ, Heaton JL, Pickering TR, Carlson KJ, Crompton R, et al. The atlas of StW 573 and the late emergence of human-like head mobility and brain metabolism. Sci Rep. 2020;10:4285. https://doi.org/10.1038/s41598-020-60837-2

33. Urciuoli A, Zanolli C, Almécija S, Beaudet A, Dumoncel J, Morimoto N, et al. Reassessment of the phylogenetic relationships of the late Miocene apes *Hispanopithecus* and *Rudapithecus* based on vestibular morphology. Proc Natl Acad Sci USA. 2021;118, e2015215118. https://doi.org/10.1073/pnas.2015215118

34. Braga J, Zimmer V, Dumoncel J, Samir C, De Beer F, Zanolli C, et al. Efficacy of diffeomorphic surface matching and 3D geometric morphometrics for taxonomic discrimination of Early Pleistocene hominin mandibular molars. J Hum Evol. 2019;130:21–35. https://doi.org/0.1016/j.jhevol.2019.01.009

35. Besl, PJ, McKay ND. A method for registration of 3-D shapes. IEE Trans Pattern Anal. 1992;14:239e256

36. Dumoncel J, Subsol G, Durrleman S, Jessel J-P, Beaudet A, Braga J. How to build an average model when samples are variably incomplete? Application to fossil data. In: IEEE Conference on Computer Vision and Pattern Recognition Workshops; 2016 June 27–30; Las Vegas, NV, USA. IEEE; 2016. p. 541–548. https://doi.org/10.1109/CVPRW.2016.74

37. Deformetric v4.0. 2014. https://www.deformetrica.org/

38. Lieberman DE. The evolution of the human head. Cambridge, MA: Harvard University Press; 2011. https://doi.org/10.2307/j.ctvjnrtmh

39. Ravosa MJ. Interspecific perspective on mechanical and nonmechanical models of primate circumorbital morphology. Am J Phys Anthropol. 1991;86(3):369–396. https://doi.org/10.1002/ajpa.1330860305

40. Lacruz RS, Stringer CB, Kimbel WH, Wood B, Harvati K, O'Higgins P, et al. The evolutionary history of the human face. Nat Ecol Evol. 2019;3(5):726–736. https://doi.org/10.1038/s41559-019-0865-7

41. Harvati K, Weaver TD. Human cranial anatomy and the differential preservation of population history and climate signatures. Anat Rec A Discov Mol Cell Evol Biol. 2006;288(12):1225–1233. https://doi.org/10.1002/ar.a.20395

42. Schroeder L, Roseman CC, Cheverud JM, Ackermann RR. Characterizing the evolutionary path(s) to early *Homo*. PLoS ONE. 2014;9(12), e114307. https://doi.org/10.1371/journal.pone.0114307

43. Pereira-Pedro AS, Masters M, Bruner E. Shape analysis of spatial relationships between orbito-ocular and endocranial structures in modern humans and fossil hominids. J Anat. 2017;231(6):947–960. https://doi.org/10.1111/joa.12693

44. Beaudet A, Bruner E. A frontal lobe surface analysis in three archaic African human fossils: OH 9, Buia, and Bodo. C R Palevol. 2017;16:499–507. https://doi.org/10.1016/j.crpv.2016.12.002

45. Ponce de León MS, Bienvenu T, Marom A, Engel S, Tafforeau P, Alatorre Warren JL, et al. The primitive brain of early *Homo*. Science. 2021;372(6538):165-171. https://doi.org/10.1126/science.aaz0032

46. Cameron D, Patnaik R, Sahni A. The phylogenetic significance of the Middle Pleistocene Narmada hominin cranium from Central India. Int J Osteoarchaeol. 2004;14(6):419–447. https://doi.org/10.1002/oa.725

47. Moss ML, Young RW. A functional approach to craniology. Am J Phys Anthropol. 1960;18(4):281–292. https://doi.org/10.1002/ajpa.1330180406

48. Anzelmo M, Ventrice F, Barbeito-Andres J, Pucciarelli HM, Sardi ML. Ontogenetic changes in cranial vault thickness in a modern sample of *Homo sapiens*. Am J Hum Biol. 2015;27(4):475–485. https://doi.org/10.1002/ajhb.22673

49. Bruner E, Athreya S, de la Cuetara JM, Marks T. Geometric variation of the frontal squama in the genus *Homo*: Frontal bulging and the origin of modern human morphology. Am J Phys Anthropol. 2013;150:313–323. https://doi.org/10.1002/ajpa.22202

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Mandibular ramus morphology and species identification in *Australopithecus sediba*

The site of Malapa, South Africa, has produced fossil evidence from multiple individuals of *Australopithecus sediba* including the partial skeletons designated as MH1 (holotype) and MH2 (paratype). A recent article in this Journal presented the hypothesis that MH1 and MH2 are not one species but instead represent two different genera: *Australopithecus* and *Homo*, respectively. Here we briefly evaluate this claim. We review the evidence from across the skeleton that demonstrates that MH1 and MH2 represent a single species, and we highlight other fossil samples that show the same pattern of mandibular ramus variation as observed in MH1 and MH2. The evidence shows that there is no reason to separate MH1 and MH2 into different species or genera based upon mandibular ramus morphology. This case illustrates how misleading small fragments of anatomy can be, why researchers should not use such fragments particularly for species and genus-level diagnoses, and why it is essential to use all available evidence.

Significance:

This study shows that the mandibular variation that is present in fossils from Malapa attributed to *Australopithecus sediba* has parallels in both *Australopithecus africanus* and in *Homo*. This helps to demonstrate that mandibular form is not sufficient to provide evidence of species diagnosis, but also that the development and adaptations to diet in *Au. sediba* were overlapping with those present in other related species of hominins.

The MH1 and MH2 skeletons are among the most complete known for *Australopithecus*, dating to approximately 1.977 million years ago.[1,2] The preserved elements of each skeleton include portions of upper and lower limb, thorax, pelvis, mandible, dentition and, for MH1, the face and cranial vault.[1,3] These remains are among the most studied of any early hominin specimens. Excavation at Malapa has recovered substantial evidence of the burial position of each skeleton, including joints found in articulation or in close anatomical proximity, with all recovered parts showing a low degree of post-mortem dispersion.[4] Additional context comes from the different ontogenetic stages and biological sex of the two skeletons. MH2 is adult and MH1 is juvenile with postcranial and dental elements consistent with a maturational age of between 9 and 11 years when compared to a chimpanzee maturational pattern.[5] The pelvic remains of MH1 and MH2 are closely similar in size, and similar in most aspects of morphology, but differ in features related to sex, suggesting female sex for MH2 and male sex for MH1.[6,7] The slightly larger size of MH1 in many dental and postcranial measurements is consistent with this sex difference. The metric differences between MH1 and MH2 are consistently slight in comparison to the variation observed within other hominin fossil samples that represent single species, within living humans, and within species of other living great apes. The mandibles of both skeletons preserve most of their mandibular dentition, and the teeth of both individuals are very similar in size and morphology.[8]

Not only are these two skeletons very similar to each other across all aspects of their anatomy, together they exhibit the same differences from other hominin species. The lateral plantar process of the MH2 calcaneus has a dorsal position not observed in any other fossil hominin except for the immature calcaneal apophysis of MH1.[9] Both MH1 and MH2 share a more human-like parasagittal orientation of the ilium, contrasting not only with other *Australopithecus* pelves but also with pelvic material from *Homo erectus* and *Homo naledi*.[7] Both the MH1 and MH2 proximal femora exhibit superoinferiorly short femoral neck diameters compared to other fossils attributed to *Australopithecus*.[10] Both MH1 and MH2 share small molar size and small mandibular corpus area compared to samples of other species of *Australopithecus*.[1,11] Both MH1 and MH2 have similar dental microwear textures, and both have near-identical enamel carbon-13 values that are far below any other contemporary sample of *Australopithecus, Paranthropus*, or *Homo*.[12] All of this evidence strongly supports the diagnosis of MH1 and MH2 as belonging to the same species, *Au. sediba*.

Rak et al.[13] claim that MH1 and MH2 belong to different genera. In their assessment, MH2 shares a generalised mandibular ramus form with modern humans, chimpanzees, bonobos, and orangutans, which they infer to be ancestral in hominins. In their description, MH1 shares a derived ramus morphology with *Australopithecus afarensis* and *Paranthropus robustus*. In their opinion, these differences are best explained by the hypothesis that MH1 is *Australopithecus* and MH2 is *Homo*.

But Rak et al.[13] include only 12 fossil mandibles for comparison in addition to MH1 and MH2 in their analysis. Only five of these are used to represent the entire variation exhibited by the genus *Homo*, while three represent *Australopithecus afarensis* and three represent *Paranthropus robustus*. This tiny number omits many well-known mandibular fossils of *Australopithecus* and *Homo*. Previous work has shown the extensive variation in ramus form within *Homo,* including both variation within species and populations, as well as overlap between them.[14,15] In this short comment we cannot reiterate this extensive work, but we can bring attention to a few critical omissions.

One critical omission is the geographically contiguous species *Australopithecus africanus*. Rak et al.[13] claim that a fragment of the Sts 7 mandible embedded in breccia appears to resemble the MH1 morphology, but otherwise do not comment on this key species. We illustrate two additional mandibles in Figure 1. The holotype, from Taung, preserves the right ramus cemented to the calvaria in anatomical position. The coronoid process is of similar height to the condyle and the form of the mandibular notch is similar to that of SK 63, a mandible of similar ontogenetic

age attributed to *P. robustus*. This is intermediate in morphology between MH1 and MH2 (Figure 1). The Sterkfontein Member 4 hominin Sts 52 has a well-preserved right mandibular ramus, with slight damage to the superior margin of the coronoid process. The outline of the preserved mandibular notch and anterior ramus border are very similar to MH2 (Figure 1). The break to the coronoid process is likewise similar, and a reasonable reconstruction yields a similar coronoid height in Sts 52 as in MH2. The Taung fossil is thought to date to between 3.0 and 2.6 million years old while Sts 52 dates to between 2.6 and 2.0 million years ago.[16]

Another critical omission is the sample of well-known mandibular ramus fossils attributed to *Homo*. In Figure 1, we picture four mandibles of *Homo* that were not examined by Rak et al.[13] One pair includes Tighènif 2 and 3 from Tighènif, Algeria[17,18], thought to date to the later Early Pleistocene between 1.4 million and 900 000 years ago[19,20]. The other pair includes AT-950 and AT-605 from Sima de los Huesos, Spain, which date to approximately 430 000 years ago.[21-23] Both pairs exhibit the same pattern of mandibular ramus variation as the two Malapa mandibles. Tighènif 3 and AT-605 both have tall, hook-shaped coronoid processes that extend higher than the condyle, posteriorly positioned mandibular notches, and anterior ramus margins that ascend smoothly with no incurvation – all features found in MH1 that Rak et al.[13] suggest are diagnostic of *Australopithecus*. In contrast, Tighènif 2 and AT-950 share the anatomy seen in MH2, with low coronoid height, mandibular notch position near the midpoint of the ramus, and an anterior ramus profile with an incurving base. These are the features of MH2 that Rak et al.[13] suggest are diagnostic of *Homo*.

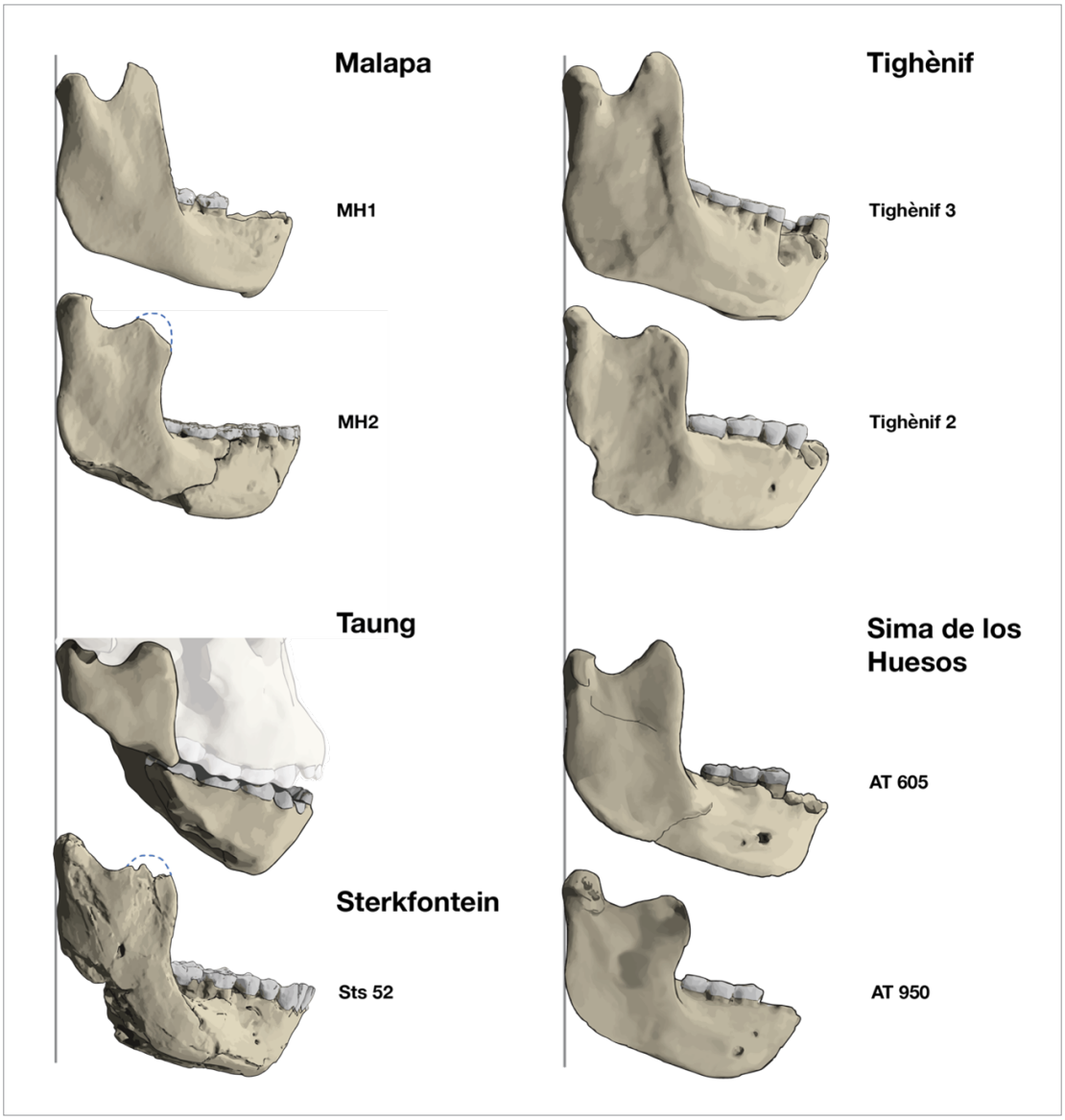

Figure 1: Mandibles of *Australopithecus sediba*, *Australopithecus africanus,* and *Homo*. The difference between MH1 and MH2 noted by Rak et al.[13] is shown at upper left, with the coronoid process of MH1 in a higher position than the condyle, while the coronoid process of MH2 is lower than the condyle and has a marked incurvation of the anterior ramus border. The same anatomical contrast is manifested in each of the other three samples shown. At lower left, Taung and Sts 52 represent *Au. africanus*. Taung has coronoid and condyloid processes of similar height, intermediate between the MH1 and MH2 morphology, while Sts 52 is similar in coronoid height and profile to MH2. At upper right, Tighènif 3 has a similar ramus morphology to MH1, while Tighènif 2 is similar to MH2. At lower right, AT 605 has a high and posteriorly curving coronoid process like MH1, while AT 950 is more similar to MH2. All mandibles are pictured following the procedure described by Rak et al.[13], including rotation to a position where the posterior ramus border is vertical, and scaling to equal ramus width. The Tighènif and Sima de los Huesos mandibles are shown horizontally mirrored to allow their better-preserved left anatomy to be compared with the Malapa mandibles. The Taung right mandibular ramus is cemented to the calvaria; this is shown in occlusion with the separate mandible fragment to demonstrate the preserved mandibular anatomy.

Rak et al.[13] claim to show that 'the differences [between MH1 and MH2] are beyond what is expected in a trait's normal range of distribution in a given population' (p. 2). The three other samples that we show in Figure 1 disprove this assertion. Each sample has the mandibular ramus variation manifested at Malapa. The morphological features that Rak et al.[13] describe as derived exclusively in *Australopithecus* actually occur widely across *Homo*. The form that Rak et al.[13] describe as characteristic of *Homo* occurs not only within *Au. sediba,* but also within *Au. africanus*. The observation that hominin samples vary in these features is consistent with the results of Ritzman et al.[24], who found that the morphological distance of mandibular ramus form between MH1 and MH2 is no greater than between randomly chosen pairs within modern humans, *Pan*, and *Gorilla*. The widespread variation of these ramus features makes them unsuitable to be used for taxonomic diagnosis on their own.

Palaeoanthropologists have grappled with the boundary between *Homo* and *Australopithecus* for more than 70 years. The fragmentary condition of fossils attributed to 'early *Homo*' is one reason for a lack of consensus about the definition of the genus. Samples that preserve more complete skeletal material, including Malapa, demonstrate that features usually found in later *Homo* do not assort with each other in the ways that researchers once predicted based upon fragmentary remains. Human evolution was not tidy. Hominin skeletons including MH1 and MH2 are anatomical mosaics that do not always fit a simple evolutionary tree. We are far from alone in recognising the homoplasy in the hominin fossil record and the way that it can confuse phylogenetic placement of fossils.[25,26] The field must meet these challenges by considering all relevant data. A small sample cannot be better evidence than a large sample. A small fragment of anatomy cannot be better evidence than a skeleton.

Competing interests

We have no competing interests to declare.

Authors' contributions

Both authors conceived of the study, contributed data, wrote the manuscript, and edited the manuscript.

References

1. Berger LR, De Ruiter DJ, Churchill SE, Schmid P, Carlson KJ, Dirks PH, et al. *Australopithecus sediba*: A new species of *Homo*-like australopith from South Africa. Science. 2010;328(5975):195–204. https://doi.org/10.1126/science.1184944
2. Pickering R, Dirks PH, Jinnah Z, De Ruiter DJ, Churchill SE, Herries AI, et al. *Australopithecus sediba* at 1.977 Ma and implications for the origins of the genus *Homo*. Science. 2011;333(6048):1421–1423. https://doi.org/10.1126/science.1203697
3. Williams SA, DeSilva JM, De Ruiter DJ. *Australopithecus sediba* Malapa at 10: Introduction to the Special Issue on *Australopithecus sediba*. PaleoAnthropology. 2018:49–55.
4. Val A, Dirks PH, Backwell LR, d'Errico F, Berger LR. Taphonomic analysis of the faunal assemblage associated with the hominins (*Australopithecus sediba*) from the Early Pleistocene cave deposits of Malapa, South Africa. PLoS ONE. 2015;10(6), e0126904. https://doi.org/10.1371/journal.pone.0126904
5. Cameron N, Bogin B, Bolter D, Berger LR. The postcranial skeletal maturation of *Australopithecus sediba*. Am J Phys Anthropol. 2017;163(3):633–640. https://doi.org/10.1002/ajpa.23234
6. Kibii JM, Churchill SE, Schmid P, Carlson KJ, Reed ND, De Ruiter DJ, et al. A partial pelvis of *Australopithecus sediba*. Science. 2011;333(6048):1407-1411. https://doi.org/10.1126/science.1202521
7. Churchill SE, Kibii J, Schmid P, Reed ND, Berger LR. The pelvis of *Australopithecus sediba*. PaleoAnthropology. 2018;2018:334–356.
8. Irish JD, Guatelli-Steinberg D, Legge SS, De Ruiter DJ, Berger LR. Dental morphology and the phylogenetic "place" of *Australopithecus sediba*. Science. 2013;340(6129), Art. #1233062. https://doi.org/10.1126/science.1233062
9. Zipfel B, DeSilva JM, Kidd RS, Carlson KJ, Churchill SE, Berger LR. The foot and ankle of *Australopithecus sediba*. Science. 2011;333(6048):1417-1420. https://doi.org/10.1126/science.1202703
10. DeSilva JM, Carlson KJ, Claxton AG, Harcourt-Smith WE, McNutt EJ, Sylvester AD, et al. The anatomy of the lower limb skeleton of *Australopithecus sediba*. PaleoAnthropology. 2018;2018:357–405.
11. De Ruiter DJ, DeWitt TJ, Carlson KB, Brophy JK, Schroeder L, Ackermann RR, et al. Mandibular remains support taxonomic validity of *Australopithecus sediba*. Science. 2013;340(6129), Art. #1232997. https://doi.org/10.1126/science.1232997
12. Henry AG, Ungar PS, Passey BH, Sponheimer M, Rossouw L, Bamford M, et al. The diet of *Australopithecus sediba*. Nature. 2012;487(7405):90–93. https://doi.org/10.1038/nature11185
13. Rak Y, Geffen E, Hylander W, Ginzburg A, Been E. One hominin taxon or two at Malapa Cave? Implications for the origins of *Homo*. S Afr J Sci. 2021;117(5-6), Art. #8747. https://doi.org/10.17159/sajs.2021/8747
14. Wolpoff MH, Frayer DW. Unique ramus anatomy for Neandertals? Am J Phys Anthropol. 2005;128(2):245–251. https://doi.org/10.1002/ajpa.10432
15. Terhune CE, Robinson CA, Ritzman TB. Ontogenetic variation in the mandibular ramus of great apes and humans. J Morphol. 2014;275(6):661–677. https://doi.org/10.1002/jmor.20246
16. Herries AI, Pickering R, Adams JW, Curnoe D, Warr G, Latham AG, et al. A multi-disciplinary perspective on the age of *Australopithecus* in southern Africa. In: Reed K, Fleagle J, Leakey RF, editors. The paleobiology of *Australopithecus.* Dordrecht: Springer; 2013. p. 21–40. https://doi.org/10.1007/978-94-007-5919-0_3
17. Arambourg C. Le gisement de Ternifine et l'Atlanthropus [The Ternifine deposit and the *Atlanthropus*]. Bulletin de la Société préhistorique de France. 1955;52(Fasc. 1/2):94–95. French. https://doi.org/10.3406/bspf.1955.3159
18. Arambourg C. Le gisement de Ternifine, t. 1. Deuxième partie: l'*Atlanthropus mauritanien* [The Ternifine deposit, t. 1. Second part: *Atlanthropus mauritanicus]*. Archives de l'Institut de Paléontologie humaine, Mémoire. 1963;1963(32):37–141. French.
19. Geraads D. Pleistocene Carnivora (Mammalia) from Tighennif (Ternifine), Algeria. Geobios. 2016;49(6):445–458. https://doi.org/10.1016/j.geobios.2016.09.001
20. Pickford M. The fossil Suidae (Mammalia, Artiodactyla) from Ternifine (Tighènif) Algeria. Münchner Geowissenschafltiche Abhandlungen. 2020;50:1–67.
21. Rosas A. Seventeen new mandibular specimens from the Atapuerca/Ibeas Middle Pleistocene Hominids sample (1985-1992). J Hum Evol. 1995;28(6):533–559. https://doi.org/10.1006/jhev.1995.1041
22. Rosas A, Bastir M, Alarcón JA. Tempo and mode in the Neandertal evolutionary lineage: A structuralist approach to mandible variation. Quat Sci Rev. 2019;217:62–75. https://doi.org/10.1016/j.quascirev.2019.02.025
23. Demuro M, Arnold LJ, Aranburu A, Sala N, Arsuaga JL. New bracketing luminescence ages constrain the Sima de los Huesos hominin fossils (Atapuerca, Spain) to MIS 12. J Hum Evol. 2019;131:76–95. https://doi.org/10.1016/j.jhevol.2018.12.003
24. Ritzman TB, Terhune CE, Gunz P, Robinson CA. Mandibular ramus shape of *Australopithecus sediba* suggests a single variable species. J Hum Evol. 2016;100:54–64. https://doi.org/10.1016/j.jhevol.2016.09.002
25. Wood B, Harrison T. The evolutionary context of the first hominins. Nature. 2011;470(7334):347–352. https://doi.org/10.1038/nature09709
26. Dembo M, Radovčić D, Garvin HM, Laird MF, Schroeder L, Scott JE, et al. The evolutionary relationships and age of *Homo naledi*: An assessment using dated Bayesian phylogenetic methods. J Hum Evol. 2016;97:17–26. https://doi.org/10.1016/j.jhevol.2016.04.008

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Morphological variation in the distal phalanges of the springbok, *Antidorcas marsupialis* (Zimmermann, 1780) (Mammalia: Bovidae)

A comparative study of distal phalanges belonging to adult springbok individuals shows distinctive morphological differences between the subspecies *Antidorcas marsupialis marsupialis* and *Antidorcas marsupialis hofmeyri*, most notably reflected by significant lengthening of the sole of the latter. Results were derived from comparative osteomorphological techniques, using standard anatomical nomenclature for descriptions and parametric statistics for measurements and dimensions. The configuration in *A. m. hofmeyri* proved useful for distinguishing between the two subspecies. The findings suggest that the osteometrical differences observed in the distal phalanges relate to different habitats occupied by the species.

Significance:

Infraspecific morphological variation exhibited by distal phalanges in *Antidorcas marsupialis* is significant. The results lay the groundwork for further testing of relationships between functional morphology of foot bones and substrate in bovids.

Introduction

This study was undertaken after cursory examination revealed observable morphological variation in the distal phalanges of the springbok, *Antidorcas marsupialis*. Springbok are plains-dwelling animals, adapted to arid regions as well as open grasslands; they are confined largely to the inland plateau and the western coastal regions of southern Africa (Figure 1).[1-4] The species is the sole living representative of the genus *Antidorcas* and the only antelopine species found in southern Africa today. It belongs to a group of antelopes that evolved from a single cluster of gazelline-like antelopes that eventually separated from the genus *Gazella* during the Pliocene in East Africa to evolve further in isolation in southern Africa.[5-8] A long history of intensive farming activities in South Africa severely depleted wildlife, but following the proclamation of several national parks since the beginning of the last century, ungulates were re-introduced to these reserves in an attempt to approximate the original wildlife composition in those areas.[9,10] Today, the existing populations of springbok in the Republic of South Africa are commercially managed through introduction into nature reserves and game farms resulting in the reoccupation of most of the springbok's historical range.[11,12] Although the species is continuously distributed, differences in body size, size of the postcranial skeleton and other external characteristics have been shown to vary according to locality.[13-15] Body mass was highly correlated with winter dietary protein, demonstrating significant differences between the nominate subspecies *Antidorcas marsupialis marsupialis* and two larger northern groups.[16] Three subspecies are recognised: the nominate subspecies *Antidorcas marsupialis marsupialis* or southern springbok, found in the central interior of South Africa and south of the lower Orange River; *Antidorcas marsupialis angolensis*, a northwestern springbok group confined mainly to the western parts of southern Angola, the Kaokoveld and the northern Namib Desert; and the Kalahari springbok, *Antidorcas marsupialis hofmeyri* (Thomas, 1926), which is generally restricted to southern Namibia, southern and western Botswana, and the adjacent parts of the Northern Cape in South Africa north of the Orange River.[17,18] Current opinion on the taxonomic status of *A. marsupialis* varies, with recent classifications also raising all three subspecies to species rank.[19] The general taxonomy in this study followed Groves' description of the springbok as a polytypic species, based on the geographical division of three subspecies.[20]

Materials and methods

Testing variance in size and morphology in the distal phalanx of the subspecies *A. m. marsupialis* and *A. m. hofmeyri* was based on a sample comprising 19 *marsupialis* and 16 *hofmeyri* individuals from both sexes. Measurements for the 19 *marsupialis* individuals were obtained from a comparative osteomorphological study of *Antidorcas marsupialis* and grey rhebok, *Pelea capreolus* (Forster, 1790) published in 1992.[15] The 16 *hofmeyri* individuals come from a single springbok population, culled several years ago in the Kalahari Gemsbok National Park and curated at the Florisbad Quaternary Research Station near Bloemfontein in the Free State Province, South Africa (Supplementary table 1). The *hofmeyri* specimens were measured with a slide caliper to the nearest 0.1 mm. Osteomorphological descriptions follow the nomenclature proposed by the International Committee on Veterinary Gross Anatomical Nomenclature.[21] Dimensions included greatest diagonal length of the sole (DLS), greatest height in the region of the extensor process (processus extensorius, HP), length of the dorsal surface (Ld) and greatest breadth of the proximal articular surface (facies articularis proximalis, BFp)[22] (Figure 2, Supplementary table 2). The dimensions were statistically tested for subspecies and sex-based effects using the parametric Student's *t*-test (Table 1).

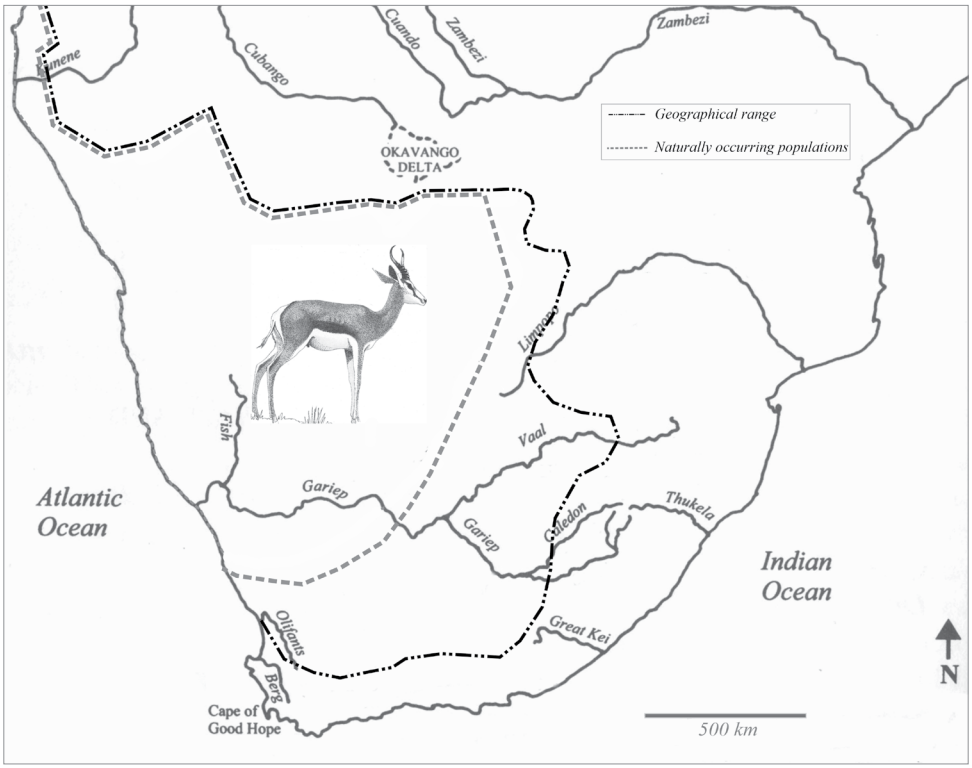
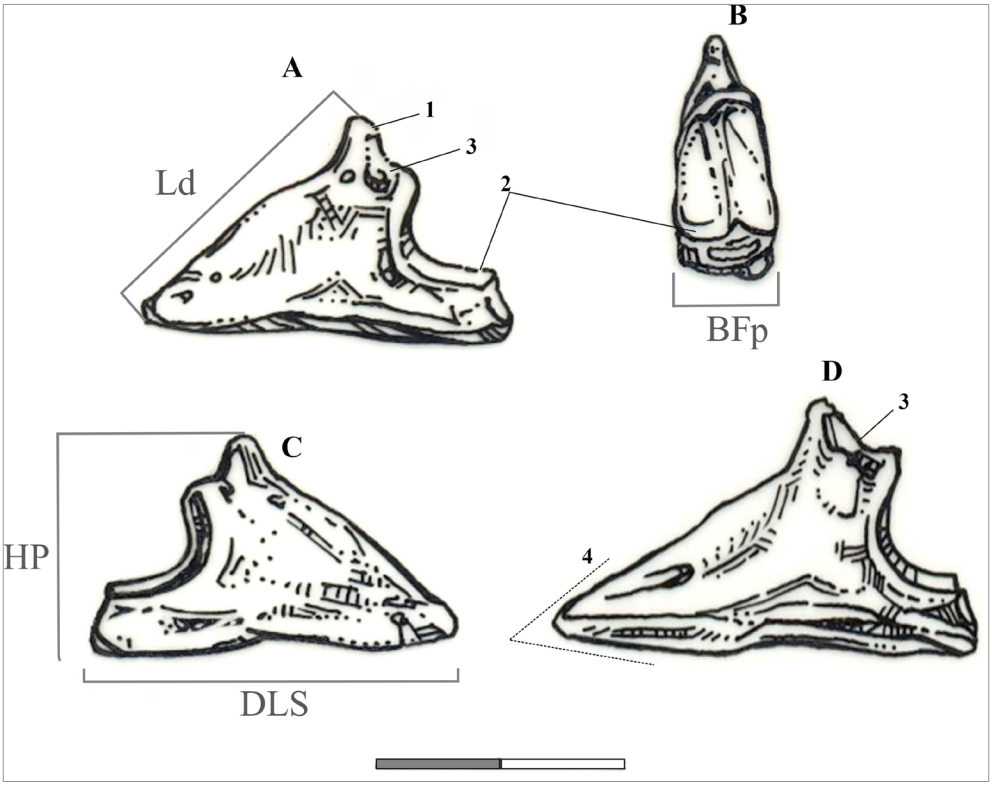


Figure 1: Present-day geographical range and present-day range of naturally occurring populations of *Antidorcas marsupialis* according to Smithers[2] and Skinner and Louw[3].



Illustrations: Estie Rossouw, with the light coming from the top left-hand corner
Scale = each scale bar represents 10 mm

Figure 2: (A) *A. m. marsupialis*, p. distalis manus, axial view; Ld = length of dorsal surface. (B) *A. m. marsupialis*, p. distalis manus, proximal view; BFp = (greatest) breadth of the proximal articular surface. (C) *A. m. marsupialis*, p. distalis manus, abaxial view; HP = height in the region of the extensor process and DLS = (greatest) diagonal length of sole. (D) *A. m. hofmeyri*, p. distalis manus, axial view; 1= extensor process, 2 = horizontal and posteriorly extended proximal articular surface, 3 = dorsal to palmar (manus) / dorsal to plantar (pedis) angle.

Table 1: Comparison of means between groups for sex-based and subspecies effects using *t*-tests

Variable	Mean *marsupialis M*	Mean *marsupialis F*	*p*-value	*n marsupialis F*	*n marsupialis M*	s.d. m*arsupialis M*	s.d. m*arsupialis F*
DLS	28.20	27.39	0.072738	15	22	0.84	1.55
Ld	23.53	22.89	0.081820	15	22	0.72	1.26
HP	17.58	16.65	**0.009391**	15	22	0.79	1.12
BFp	8.34	7.89	**0.027606**	15	22	0.41	0.67
	Mean *hofmeyri M*	**Mean *hofmeyri F***	***p*-value**	***n hofmeyri M***	***n hofmeyri F***	***s.d. hofmeyri M***	**s.d. *hofmeyri F***
DLS	36.24	33.68	**0.000000**	20	11	1.00	1.03
Ld	31.25	28.36	**0.000000**	20	11	1.09	1.07
HP	19.82	18.43	**0.000062**	20	11	0.89	0.55
BFp	9.20	8.86	**0.004870**	20	11	0.33	0.20
	Mean *marsupialis*	**Mean *hofmeyri***	***p*-value**	***n marsupialis***	***n hofmeyri***	**s.d. *marsupialis***	**s.d. *hofmeyri***
DLS	27.72	35.33	**0.000000**	37	31	1.36	1.59
Ld	23.15	30.22	**0.000000**	37	31	1.11	1.76
HP	17.03	19.32	**0.000000**	37	31	1.09	1.03
BFp	8.07	9.08	**0.000000**	37	31	0.62	0.33
Ratio DLS/Ld	1.20	1.17	**0.000150**	37	31	0.03	0.02
Ratio DLS/BFp	1.59	1.62	**0.009385**	37	31	0.04	0.02
Ratio DLS/HP	1.63	1.83	**0.000000**	37	31	0.08	0.08

DLS, (greatest) diagonal length of sole; Ld, length of dorsal surface; HP, height in the region of the extensor process; BFp, (greatest) breadth of the proximal articular surface

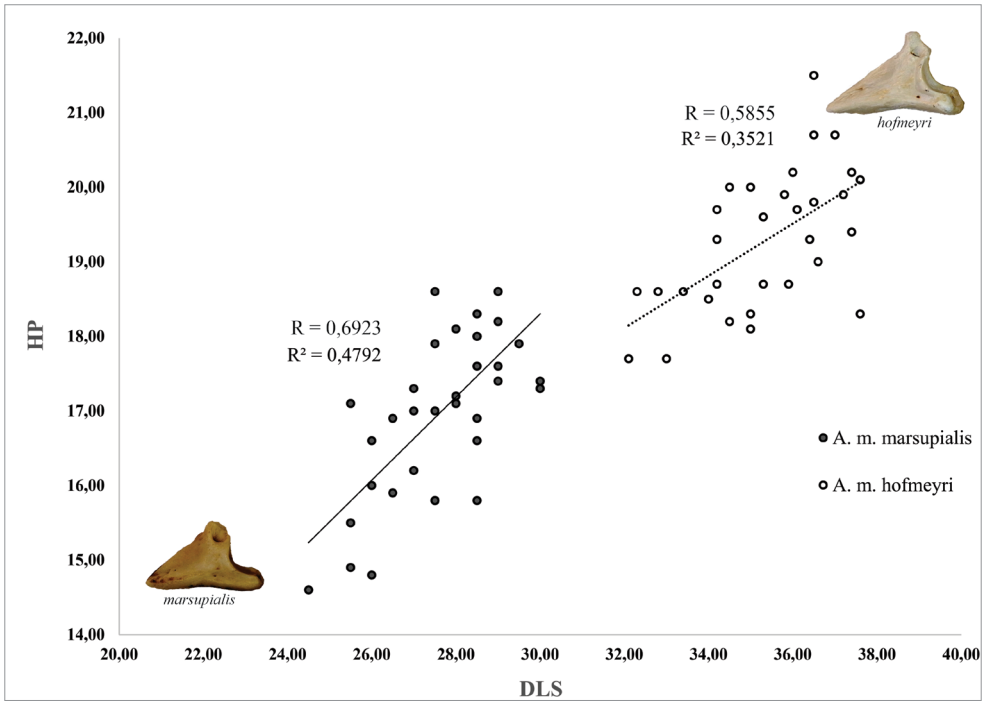
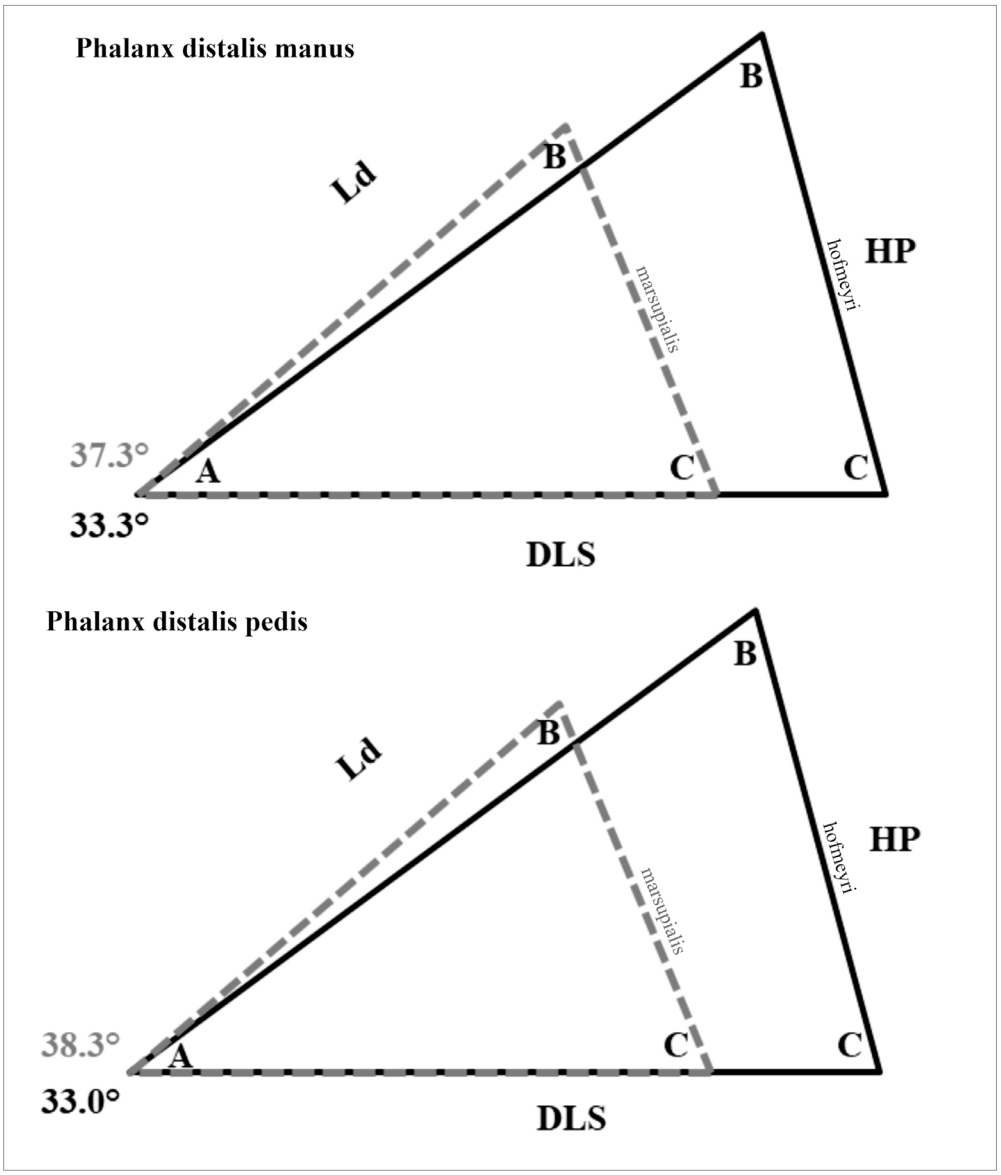


Figure 3: Bivariate plot of variables DLS ((greatest) diagonal length of sole) and HP (height in the region of the extensor process) measured for *Antidorcas marsupialis*. Smaller *A. m. marsupialis* was clearly differentiated from larger *A. m. hofmeyri*, with no overlap in values.



Ld, length of dorsal surface; HP, height in the region of the extensor process; DLS, (greatest) diagonal length of sole

Figure 4: Diagrammatic representation of distal phalanges manus (A) and pedis (B) in both subspecies. Trigonometric formulae for solution of oblique triangles were based on means for DLS, Ld and HP and calculated for dorsal to palmar / plantar surface (AB to AC), dorsal to proximal surface (AB to BC) and the proximal to palmar / plantar surface (BC to BA).

Results

The shape of the distal phalanx in the springbok is typical of the family Bovidae, resembling a triangular-shaped and mediolaterally flattened body (Figure 2). Both subspecies exhibited a prominent extensor process (Figure 2, Attr. 1), situated proximally on the dorsal surface of the body and above a posteriorly extended proximal articular surface that ends in a well-developed flexor tubercle (Figure 2, Attr. 2). The phalanges also revealed a characteristically large nutrient foramen on the axial side at the base of the extensor process (Figure 2, Attr. 3). Compared to *A. m. marsupialis*, the distal phalanx in *A. m. hofmeyri* appeared to be distally elongated, resulting in a comparably more peg-like appearance (Figure 2, Attr. 4). This morphological trait remained noteworthy in overall proportion when compared metrically to that of *A. m. marsupialis.* As expected, variables DLS, Ld, HP and BFp were larger in *A. m. hofmeyri* for both sexes with *A. m. marsupialis* and *A. m. hofmeyri* falling into two discrete and non-overlapping clusters (Figure 3). This also applied to the log-transformed values. Even though sex-based variability varied from significant in *A. m. hofmeyri* to not in *A. m. marsupialis*, it had no effect on subspecies, which were significant in all the dimensions ($n=66$, $p<0.05$). Disproportionate lengthening along the anteroposterior axis of the corpus was indicated by significantly higher DLS:HP ($p<0.001$) and DLS:BFp ($p=0.009$) ratios for *A.m. hofmeyri* (Supplementary figure 1). The results were supported by simple trigonometrical analysis, based on mean values of dimensions DLS, Ld and HP, showing comparatively lower dorsal to palmar (manus) and dorsal to plantar (pedis) angles in *A. m. hofmeyri* (Figure 4, Supplementary table 3).

Concluding remarks

Findings from this study indicate that configuration of the distal phalanx in *A.m. hofmeyri* is at variance with that of the nominate subspecies and that the measurements, and the indices based on them, proved useful for distinguishing between the distal phalanges of the two subspecies. In this case, a significant increase in the DLS:HP ratio as merely a function of increased body size in *A.m. hofmeyri* is not consistent with the assumption that two subspecies of different size, but sharing a common morphology, will also have the dimensions of their distal phalanges in the same ratio.

Selection pressures that operate within a specific environment have been linked to the functional expression of postcranial characteristics in modern African bovids, e.g. broad-level correlations between bovid postcrania and open or closed habitats.[23-26] While *Antidorcas marsupialis* is a plains-living species, the osteometrical differences observed in the distal phalanges relate to the different habitats that the species occupy. Being generally restricted to southern Namibia, southern and western Botswana and the adjacent parts of the Northern Cape Province north of the Orange River, the natural habitat of *A. m. hofmeyri* is underlain by a distinctive and homogeneous substrate, made up of thick surface sands of different ages that were established during periods of widespread aridity.[27] Deposits vary from extensive areas covered by linear dunes to gently undulating sand sheets that extend from southern Angola and western Zambia in the north to the Orange River in the south.[28-30] With the lower Orange River acting as a natural barrier, it is readily perceived that the distinct morphology exhibited by *A. m. hofmeyri*'s distal phalanges, could have resulted from an allopatric isolation event sometime in the past, following continual locomotion on unconsolidated dune and sand sheet accumulations in the region. Further investigations, using a larger, more inclusive data set, will be required to test these assumptions, but for now, it is postulated that in addition to dietary effects[16], abiotic conditions like substrate may also be a driver of morphological alteration within the polytypic springbok.

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Competing interests

I have no competing interests to declare.

References

1. Nagy KA, Knight MH. Energy, water, and food use by springbok antelope (*Antidorcas marsupialis*) in the Kalahari Desert. J Mammal. 1994;75(4):860-872. https://doi.org/10.2307/1382468
2. Smithers RHN. The mammals of the southern African subregion. Pretoria: University of Pretoria; 1983.
3. Skinner JD, Louw GN. The springbok: *Antidorcas marsupialis* (Zimmermann, 1780). Transv Mus Monogr. 1996:10.
4. Bigalke RC. Observations on the behaviour and feeding habits of the springbok, *Antidorcas marsupialis*. Zool Afr. 1972;7(1):333–359. https://doi.org/10.1080/00445096.1972.11447448
5. Vrba ES. Two species of *Antidorcas* Sundevall at Swartkrans (Mammalia: Bovidae). Ann Transv Mus. 1973;28:287–351.
6. Hendey QB, Hendey H. New Quaternary fossil sites near Swartklip, Cape Province. Ann S Afr Mus. 1968;52:43–78.
7. Gentry AW. Bovidae. In: Werdelin L, Sanders WJ, editors. Cenozoic mammals of Africa. Berkeley, CA: University of California Press; 2010. p. 741–796. https://doi.org/10.1525/california/9780520257214.003.0038
8. De Ruiter DJ. Revised faunal lists for Members 1–3 of Swartkrans, South Africa. Ann Transv Mus. 2003;40:29–41.
9. Penzhorn BL. A summary of the re-introduction of ungulates into South African national parks (to 31 December 1970). Koedoe. 1971;14:145–159. https://doi.org/10.4102/koedoe.v14i1.725
10. De Graaf G, Penzhorn BL. The re-introduction of springbok *Antidorcas marsupialis* into South African National Parks – a documentation. Koedoe, 1976;19:75–82. https://doi.org/10.4102/koedoe.v19i1.1184
11. Bigalke RC. Springbok management – what's new? Pelea. 1994;13:23–29.
12. Du Plessis S. The past and present geographical distribution of the Perissodactyla and Artiodactyla in southern Africa [MSc thesis]. Pretoria: University of Pretoria; 1969.
13. Robinson TJ. A comparative study of the three subspecies of springbok, *Antidorcas marsupialis marsupialis* (Zimmermann, 1780), *A. m. hofmeyri* (Thomas, 1926) and *A. m. angolensis* (Blaine, 1922) [MSc thesis]. Pretoria: University of Pretoria; 1975.
14. Bigalke RC, Hartl BG, Berry MPS, Van Hensbergen HJ. Population genetics of the springbok *Antidorcas marsupialis* – a preliminary study. Acta Theriol 1993;38(2):103–111. https://doi.org/10.4098/AT.arch.93-45
15. Peters J, Brink JS. Comparative postcranial osteomorphology and osteometry of springbok, *Antidorcas marsupialis* (Zimmerman, 1780) and grey rhebok, *Pelea capreolus* (Forster, 1790) (Mammalia: Bovidae). Nav Nas Mus Bfn. 1992;8(4):161–207.
16. Robinson TJ. Influence of a nutritional parameter on the size differences of the three springbok subspecies. S Afr J Zool. 1979;14:13–15.
17. Bigalke RC. The springbok. Nat Hist NY. 1966;75(6):20–25.
18. Meester JAJ, Rautenbach IL, Dippenaar NJ, Baker CM. Classification of southern African mammals. Transv Mus Monogr 5. Pretoria: Transvaal Museum; 1986.
19. Groves C, Grubb P. Ungulate taxonomy. Baltimore, MD: The Johns Hopkins University Press; 2011.
20. Groves CP. Subspecies and clines in the springbok (*Antidorcas*). Z Saugetierkd. 1981;46:189–197.
21. International Committee on Veterinary Gross Anatomical Nomenclature, World Association of Veterinary Anatomists. Nomina Anatomica Veterinaria. 6th ed. Hannover/Ghent/Columbia, MO/Rio de Janeiro: Editorial Committee; 2017.
22. Von den Driesch A. A guide to the measurement of animal bones from archaeological sites. Peabody Museum Bulletin 1. Cambridge, MA: Peabody Museum of Archaeology and Ethnology, Harvard University; 1976.
23. Kappelman J, Plummer IT, Bishop L, Duncan A, Appleton S. Bovids as indicators of Plio-Pleistocene paleoenvironments in east Africa. J Hum Evol. 1997;32:229–256. https://doi.org/10.1006/jhev.1996.0105
24. DeGusta D, Vrba E. Methods for inferring paleohabitats from the functional morphology of bovid phalanges. J Archeol Sci. 2005;32:1099–1113. https://doi.org/10.1016/j.jas.2005.02.010
25. DeGusta D, Vrba E. Methods for inferring paleohabitats from discrete traits of the bovid postcranial skeleton. J Archeol Sci. 2005;32:1115–1123. https://doi.org/10.1016/j.jas.2005.02.011
26. Schellhorn R, Pfretzschner H. Analyzing ungulate long bones as a tool for habitat reconstruction. Mammal Res. 2015;60:195–205. https://doi.org/10.1007/s13364-015-0218-0
27. Grove AT. Landforms and climatic change in the Kalahari and Ngamiland. Geogr J. 1969;134:194–208. https://doi.org/10.2307/1792436
28. Lancaster N. Palaeoenvironmental implications of fixed dune systems in southern Africa. Palaeogeogr Palaeoclimatol Palaeoecol. 1981;33:327–346. https://doi.org/10.1016/0031-0182(81)90025-0
29. Bullard JE, Thomas DSG, Wiggs GFS, Livingstone I. Analysis of linear sand dune morphological variability, southwestern Kalahari Desert. Geomorphology. 1995;11:189–203. https://doi.org/10.1016/0169-555X(94)00061-U
30. Thomas DSG, Knight M, Wiggs GFS. Remobilization of southern African desert dune systems by twenty-first century global warming. Nature. 2005;435(7046):1218. https://doi.org/10.1038/nature03717