Export permits

Please note an export permit must be linked to an object or site that has to be created on SAHRIS! If the object/site you want to work on has not been created yet, you would need to do so. Thanks!

The proposal should include (you can fill these in below):

- a list of participants (name, affiliation, phone no, email addresses) and how they are involved;
- the name and address of the facility, including address, it is being scanned at;
- name and address of the museum/university department that currently hosts the object;
- names of the responsible person(s) during transport and while the fossil is at the facility;
- the period/time frame during which the fossil(s) will be outside the country;
- detailed information on the fossil(s), especially as it is a "unique" specimen;
- detailed information on the research project behind it & methodology including expected outcomes (i.e., the reason for export);
- the written confirmation of the institution that currently hosts the object that the object may be used as proposed and be returned in good condition;
- should there be any damage/destructive analysis (e.g., coating for higher resolution) undertaken, this needs to be stated in detail;
- Statement why this study cannot be done in South Africa.

Applicant (name and affiliation): Dr Bernhard Zipfel

Applied for (principal researcher): Nomawethu Hlazo

Participants with affiliations, email addresses, phone numbers (& their role):

1) Professor Rebecca R. Ackermann,
   University of Cape Town
   Human Evolution Research Institute
   Becky.ackermann@uct.ac.za
   Beattie Building,
   University Avenue,
   University of Cape Town,
   Woolsack Drive,
   Rondebosch,
   Cape Town, 7700
   012 650 2353

Role: Supervisor

2) Enrico Capellilni
   Natural History Museum of Denmark
   University of Copenhagen
   Øster Voldgade 5-7
   1350 København K
   Denmark
   ecappellini@snm.ku.dk
   +45 35 32 13 38

Role: Technical Supervisor, palaeoproteomicvs specialist

The material will be **hand-carried/exported** to **Natural History Museum of Denmark** in **SAHRA dependent** (month, year) by **Nomawethu Hlazo** (name of person responsible for transport) and brought back by **Nomawethu Hlazo** (leave blank if same person as above).
Enrico Capellini_(name) will be involved with the **transport/scanning/general handling** (e.g., transport/scanning) of objects and the proteomic analysis of the specimens.

Institution incl. address that currently hosts the object
Beattie Building,
University Avenue,
University of Cape Town,
Woolsack Drive,
Rondebosch,
Cape Town, 7700

Facility incl. address at which the experiment will be done:
Natural History Museum of Denmark
University of Copenhagen
Øster Voldgade 5-7
1350 København K
Denmark

Table of objects or upload file: Uploaded

Time frame:
Transport to **Natural History Museum of Denmark** (facility): **SAHRA dependent**(date)
Return date: **Within a year** (date)

Aim/rationale:

The genus *Paranthropus* is made up of three morphologically similar species: *P. aethiopicus* and *P. boisei* from east Africa, and *P. robustus* from South Africa (Wood and Schroer, 2017). The first specimens of *Paranthropus* – *P. robustus* – were recovered at Kromdraai B in South Africa in 1938, just over a decade after the discovery of *Australopithecus* (Broom, 1938; Kimbel, 2006). Since those initial discoveries more than 200 *Paranthropus* fossil specimens have been found in South African sites such as Gondolin, Drimolen, Swartkrans and Coopers cave (Kimbel, 2006; Wood, 2010). Specimens include crania, post crania and teeth fragments (Kimbel, 2006; Wood, 2010). Over the decades other *Paranthropus* specimens have also been discovered throughout East Africa from the species *P. boisei* (previously known as "*Zinjanthropus boisei*"), discovered by Mary Leakey (Wood, 2005; Wood, 2010; Wood and Schroer, 2017), and *P. aethiopicus* (Walker et al., 1986; Walker and Leakey, 1988).

The ultimate approach to understanding *Paranthropus* phylogeny would require genetic analysis based on ancient biomolecule sequencing. Knowing the direction and pace of evolutionary change driving human evolution is critical to understanding how selective forces shaped our ancestors. Exactly 20 years ago, the discovery that ancient DNA (aDNA) could be retrieved from remains of extinct humans opened a new epoch of ground breaking results that completely changed the way we see the past of our species (Capellini et al., 2014). However, even using the most sophisticated technology currently available, massive ancient aDNA sequencing is unlikely to be suitable for genetic characterization of *Paranthropus boisei*, whose fossil remains are too old at 1.4 to 2.3 million years (Ma) old (Kimbel, 2006; Wood, 2010). However, it is possible that palaeoproteomics could recover sufficient ancient DNA and provide insight into *Paranthropus* phylogeny. Proteins are the direct product of gene expression and

consolidated evidence reproducibly demonstrates that ancient protein stability outperforms aDNA survival (Welker et al., 2015). the ultimate goal of this research is to understand the diversification of *Paranthropus* though protein analyses – i.e. overcoming the limits of aDNA by extracting and sequencing *P. robustus*, followed by *P. boisei* protein residues from dental enamel. Teeth are among the most frequent findings in the fossil record, and approximately 2% of the mass of mature dental enamel represents protein residues trapped during amelogenesis and enamel maturation, making this material ideal for such analyses. These protein analyses will be conducted on the most damaged and therefore morphologically (relatively) less informative tooth fragments. Collaborators of the Ackermann lab, based at the Natural History Museum of Denmark and the Novo Nordisk Foundation Center of Protein Research (group leaders: Enrico Cappellini and Jesper V. Olsen), are pioneers with extended experience in high-resolution, high-sensitivity mass spectrometry-based ancient protein sequencing (Capellini et al., 2014).

Hypothesis and Aims of Research: To further explore the possible drivers of niche differentiation and phylogenetic relationships through ancient protein sequencing. If successful, palaeoproteomics will be a breakthrough for early hominin studies, and will demonstrate that it is possible to: (i) sequence protein residues older than 1 Ma from hominin dental enamel, (ii) measure evolutionary changes in amino acid sequences, and (iii) reconstruct *Paranthropus* evolutionary history.

Methodology (short):

Palaeoproteomics

Proteins are the direct product of gene expression. The goal here is to retrieve genetic information from *Paranthropus* fossils, by extracting and sequencing ancient protein residues from dental enamel. Consolidated evidence reproducibly demonstrates that ancient protein stability outperforms aDNA survival (Cleland, et al., 2016). Recently a very short, just 18 amino acids, stretch of a 3.8 Ma old eggshell protein was retrieved (Demarchi et al., 2016), and not so long ago, sequencing of dinosaur proteins ~80 Ma old from exceptionally preserved fossils was convincingly confirmed (Schroeter et al., 2017). These cases, however, still present significant limits.

To make recovery of deep time genetic information routine, a simple procedure to inexpensively obtain extended and reliable protein sequence coverage, from dental enamel was recently discovered (Cappellini lab, *pers comm*). Unconventionally**,** for "bottom-up" proteomics, samples will *not* be digested with trypsin, assuming the prolonged proteolytic action of time already took care of this step. High-resolution, high-sensitivity mass spectrometry (MS) is used to retrieve a population of partially-overlapping, unspecifically-cleaved peptides that will be "mapped" to extant hominin enamel protein reference sequences. The reconstructed archaic sequences will then be aligned and compared with homolog sequences from extant hominins, Neanderthal and Denisovans, using conventional phylogeny procedures.

To test feasibility and yield of palaeoproteomic investigation of dental enamel samples collected from the sites and strata returning *P. robustus* samples, exclusively non-hominin mammalian teeth will initially be processed. For the materials housed at the Ditsong Museum, I am requesting faunal (n=4) and primate teeth (n=2), which were carefully selected with the help of the curator Mirriam Tawane. The specimens I wish to sample are: SK 3059, SK 2232, SK 2693, SK 3099, SK 455and KB 3227 B. These faunal and primate species are quite common in the sites and are not holotypes or rare finds, nor are the teeth of morphological significance. These specimens are found in Swartkrans from Members 1-2, where Paranthropine material has also been found. Furthermore, KB 3227 B was discovered in Kromdraai B, where the genus *Paranthropus* was first discovered. The minimum amount of starting material required for each sample is 100-300 mg. **Should the contemporary mammalian set of samples**

prove protein recovery from this material is possible, it will then be decided whether to proceed onto the analysis of *P. robustus* dental enamel.

Confirmation/permit by museum (Attached?):

Damage/destructive analysis? (if yes, explain in detail):
From each specimen, approximately 100 mg of dental enamel will be removed and powdered to extract ancient protein residues.

Statement why this study cannot be done in South Africa:
Dental enamel proteome sequencing from fossil remains older than 1 Ma is an approach so far successfully achieved only at the University of Copenhagen by the palaeoproteomics group led by Entico Cappellini (https://www.biorxiv.org/content/early/2018/09/10/407692). No other research group so far demonstrated to have the expertise to apply this approach.

Potential Impacts: This research will provide greater insight into the fossils that we continuously find in the African context. Deep-time palaeoproteomics will enable: (a) unprecedented access to genetic evidence from epochs still considered impossible to routinely access by biomolecular investigation, and (b) molecular-based investigation of major human evolutionary processes so far intractable for molecular phylogenetics. If successful, this component of my larger PhD project (which also includes morphological analyses – not described here) would revolutionize analyses of hominin materials of this age. However, to keep project risk manageable, this high risk/high gain component of the project will remain "sandboxed" and, although fully integrated, independent from the more established, and methodologically consolidated, morphometric one.

Beyond this, the proposed training in paleoproteomics at the University of Copenhagen will have a significant impact on my future career possibilities. My competence will be expanded in at least three areas: (1) enhancement of proteomics skills to state-of-the-art standards, (2) comprehensive training-through-research in proteomics data analysis at SNM and (3) improvement in networking abilities. With a highly international team of more than fifty scientists at SNM, as well as a high turnover of visiting scientists, international guest lecturers and collaborations, there will be plenty of opportunities for open discussions, cross-fertilisation of ideas and for international networking for my future career. This interdisciplinary nature is the keystone of this collaborative research project. The collaboration with SNM provides an ideal framework to communicate the main findings of the research and to harness the public fascination with proteomics applied to paleoanthropological fossils. SNM has well-structured Public Outreach Departments that will help me, as the applicant, to achieve the greatest possible visibility for my results.