Export/sampling 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):
Stephany Potze, Plio-Pleistocene Palaeontology Section, Ditsong National Museum of Natural History, 432 Paul Kruger Street, South Africa, 0002; stephany.potze@gmail.co. or potze@ditsong.org.za

Applied for (principal researcher):

Justin W. Adams, PhD

Participants with affiliations, email addresses, phone numbers (& their role):
1) Justin W. Adams, PhD; Department of Anatomy and Developmental Biology, Monash University, Melbourne, VIC Australia 3800; justin.adams@monash.edu; +61 03 9902 4280
Role: Senior supervisor of all sampling, data analysis and interpretation
2) Georgia Zadow, PhD Candidate; Department of Anatomy and Developmental Biology, Monash University, Melbourne, VIC Australia 3800; georgia.zadow@monash.edu; +61 03 9902 4280
Role: PhD student assisting in sampling, data analysis and interpretation
3) Renaud Joannes-Boyau, PhD; Department of GeoSciences, Southern Cross University, Lismore NSW Australia 2480; renaud.joannes-boyau@scu.edu.au; +61 02 6620 3271
Role: Secondary researcher supervising analysis of samples and interpretation

The material will be **hand-carried** to the Department of Anatomy and Developmental Biology, Monash University (then subsequently to the Department of GeoSciences, Southern Cross University in August 2016 by Justin W. Adams, PhD and any residuals will be brought back by Justin W. Adams, PhD
Ms. Georgia Zadow and Dr. Renaud Joannes-Boyau will be involved with the sampling of objects.

Institution incl. address that currently hosts the object: Plio-Pleistocene Palaeontology Section, Ditsong National Museum of Natural History, 432 Paul Kruger Street, Pretoria, 0002.

Facility incl. address at which the experiment will be done:
Department of GeoSciences, Southern Cross University, Lismore NSW Australia 2480

Table of objects or upload file:

Site	Specimen Number	Species
Coopers	CO101, 135, 112, 117 108B, 105B, 121, COA554, COB104	*Papio angusticeps*
Kromdraai	KB5228A, 5268, 230, KA165/2340, 163/2338, KA166A, KA179/2342	*Papio angusticeps*
Haasgat	HGT 1, 4, 250, 861	*Papio angusticeps*

Site including age at which object was found:

See table above; sites include Cooper's A and B, Kromdraai A and B, and Haasgat. All sites are dated to approximately the early Pleistocene (~2-1.5 Ma).

Time frame:
Transport to Monash University in August 2016; to Southern Cross University in November 2016.
Return date: June 2017

Aim/rationale:

This project would sample dental specimens of the extinct primate *Papio angusticeps* from the Ditsong National Museum of Natural History Plio-Pleistocene Section collections for stable carbon and oxygen isotope analysis, as well as electron-spin resonance and trace element analysis.

This sampling request arises from my prior research on the stable isotope palaeoecology of faunas from Haasgat that were undertaken with the support of CGS for destructive sampling and specimen exportation (Adams et al., 2013). The results of these analyses indicated significant palaeodietary diversity within the taxon, necessitating a comprehensive assessment of dietary composition in the species using further stable isotope sampling and trace element analysis (see below). Because this sampling can only be undertaken overseas (given our desire to use the minimally invasive and essentially non-destructive laser ablation facilities at Southern Cross University in Australia), we also propose simultaneous ESR assessment of dental elements which will assist in establishing the depositional age of these largely *ex situ* primate specimens.

To summarise the contents of this proposal:

1. All specimens are derived from the early Pleistocene fossil localities (Haasgat, Cooper's, Kromdraai) and were removed by a number of researchers over the past seven decades. All specimens are formally accessioned and as described by Freedman (1965) and Brain (1981); excepting the newly recovered HGT specimens which have been accessioned and catalogue but have primary descriptions that are currently in development (Adams et al., in prep.).

2. Specimens would be exported to myself (Dr. Justin W. Adams) at Monash University in Melbourne Australia. These would be hand-carried in individually labelled and sealed bags/sample tubes from CGS to Monash University. For lab analysis, these specimens would be then hand-carried by myself to the lab facilities run by Dr. Renaud Joannes-Boyau at Southern Cross University (Special Research Centre, GeoScience) in New South Wales, Australia.

3. The exported specimens consist of enamel flakes, partial molars, and some complete molars of the extinct primate species *Papio angusticeps*. We have identified specimens that represent minimal amounts of fossil material to leave the collections for sampling.

4. The request for exportation from South Africa is based on the ability to undertake simultaneous laser-ablation isotopic, trace element, and ESR analysis within the facilities at Southern Cross University. **No such single lab facilities exist in South Africa, minimising risk to the specimens in transit between facilities.** The laser ablation facilities available through SCU represent the most advanced, and minimally invasive, technology to acquire data from these fossils that are nearly impossible to locate in South Africa; and as a collaborating researcher, **Dr. Joannes-Boyau can offer at-cost analysis which is the only financially-feasible method to undertake this level of research**. Furthermore, Dr. Joannes-Boyau operates **the only ESR lab in the world that is calibrated to deal with teeth of this time period**, and is the only researcher who has published experience in all three analysis methods.

5. As we are taking advantage of laser ablation technology in most cases, such that every relatively complete and potentially measurable specimen detailed below, once sampled, will be returned effectively intact (with only microscopic sampling marks imposed) into the collections and will be available for researchers to continue to study. Enamel flakes derived from specimens will be destroyed during analysis, but these lack independent morphological value and have been derived from naturally damaged teeth that were already compromised.

Under this proposal fossils *Papio angusticeps* specimens from the Haasgat, Cooper's and Kromdraai deposits will be subjected to carbon and oxygen isotope analysis, trace element analysis, and electron-spin resonance analysis. I have previously conducted stable isotope analysis for paleoecological reconstruction and taphonomic analysis with the Gondolin GD 2 assemblage (Adams, 2012) and Haasgat HGD assemblage (Adams et al., 2013) through exportation permits secured previously through CGS. This latter study highlighted that the Haasgat *P. angusticeps* specimens exhibit an intraspecific dietary range beyond even the notably diverse *Parapapio broomi* from Sterkfontein Member 4 (Adams et al., 2013). The direction of this dietary diversity is significant, with both a bimodal distribution and an individual displaying a heavy C_3 value consistent with a closed forest-dwelling primate. This has led Adams et al. (2013) to propose three potential hypotheses: 1) *P. angusticeps* was dietarily flexible as it adapted to the changing ecosystems of the early Pleistocene; 2) the sample is possibly temporally mixed and reflects *P. angusticeps* populations sampled at different time periods; 3) contrary to McKee and Keyser (1994) more than a single papionin species that occupied different ecological niches occur in the HGD assemblage. This proposal would allow for the testing of these hypotheses and resolve the underlying questions about *P. angusticeps* palaeodietary diversity.

In this project we will couple stable isotope sampling with trace elemental and isotopic mapping, which represents a paradigm shift in the investigation of past populations dietary habits. In this project each tooth will be firstly mapped for trace elements analyses before being investigated with the Multicollector for isotopic ratios. The elemental maps are used to better understand the diffusion and adsorption pattern of each elements, and allow to account for diagenetic processes using particular rare earth elements such as cerium or europium, while element such as barium (Ba) and strontium (Sr) are used to describe the dietary pattern of *P. angusticeps* as has been previously done in hominin populations (Balter et al., 2012; Austin et al., 2013). To complete the work, maps of calcium and strontium isotopes ratio will be obtain on the Multicollector ICPMS, allowing to better understand the diet variations, potential migration pattern and the trophic level of each specimen.

Finally, this export permit would support the sampling of *P. angusticeps* specimens for electron-spin resonance – in fact, this would represent the first application of new electron-spin resonance (ESR) dating methods developed and applied by Dr. Joannes-Boyau on fossil primates remains from South Africa. This will allow us to establish absolute ages for these primate specimens, essential for providing temporal context for the dietary data we have gathered through these other methods and establishing whether any dietary variability in Haasgat *P. angusticeps* specimens reflects evolutionary changes in populations over time.

Stable Isotope and Trace Element Analysis

The relative proportions of chemical isotopes have been widely used to examine changes in global and local changes in climate over time (e.g. Pagani et al. 1999). An equally important application of isotopic relationships in paleontological analysis has been those methods which sample fossil specimens for stable carbon, nitrogen and oxygen isotopes and trace elements to reconstruct modern and paleodiets and, thereby, paleohabitat usage and life-history traits (Lee-Thorp et al., 2000; Richards et al., 2000; Sponheimer et al., 2003; Grün et al., 2008; Austin et al., 2013; Price et al., 2015; Moffat et al., in press). The most commonly used isotopes for paleodietary studies among African terrestrial mammals are carbon. Nitrogen isotope studies are best suited for examining marine ecosystems, and oxygen isotopes derived from extant and fossil organisms are still under investigation (Sponheimer and Lee-Thorp, 2001). Trace elements like calcium, strontium and barium can inform on a number of life history parameters like population movements and major dietary shifts like weaning (Balter et al., 2012; Austin et al., 2013).

The basis for reconstructing paleodiets from the proportion of carbon isotopes in bone apatite and enamel carbonate is based on the differential pathways of photosynthesis among plants (see summaries in Lee-Thorp et al., 1989; Lajtha and Marshall, 1994). Different plants vary in their $^{13}C/^{12}C$ ratio values (expressed as values of $\delta^{13}C$ per thousand relative to the PDB standard) based on which of the three photosynthetic pathways a given plant species follows: C_3, C_4, or CAM. C_3 plants, which include trees, bushes, shrubs, and herbs, produce a three-carbon sugar product at the end of the photosynthetic process. Because the process occurs near the outer cell wall, more ^{13}C can transpire across the cell wall, leading to the sugar product having reduced ^{13}C isotope content. Plants that follow a C_4 photosynthetic process, including tropical grasses and sedges, are more efficient at absorbing CO_2 than are C_3 plants. Because the production of sugars occurs further from the outer cell wall, the transpiration of ^{13}C out of the cell is more limited and as a result, more ^{13}C is bound within the resulting sugars. CAM plants, which include succulents, have the ability to swap between the two photosynthetic pathways based on local environmental conditions, and generally exhibit intermediate $\delta^{13}C$ values.

While both fossilized bones and teeth can be sampled for isotopic values, studies have shown that enamel is less affected by chemical diagensis during fossilization due to its greater inorganic content, density and crystallinity (Sponheimer and Lee-Thorp, 1999). Sampling of enamel carbonate from modern fauna has shown that primary herbivores inherit the $\delta^{13}C$ values of the plant species they consume, i.e. a primary grazer will have $\delta^{13}C$ values between +2‰ and -2‰, while a primary browser will have $\delta^{13}C$ values between -10‰ and -16‰ (and mixed-feeders will have values which fall in-between these two extremes; Lee-Thorp et al., 1989; Sponheimer et al., 2003). As one moves up trophic levels within ecosystems, carnivores will inherit the isotopic values of the prey items they consume. Studies of Plio-Pleistocene faunal samples, both at the level of primary herbivores and higher trophic levels, have consistently shown that diagensis during fossilization does not alter the $\delta^{13}C$ values such that primary browsers or grazers cannot be readily distinguished (Sponheimer et al., 1999; Lee-Thorp et al., 2000). Similarly, trace elements are absorbed by organisms based on both geographic factors (e.g., levels of elements in groundwater) and dietary inputs (e.g., maternal feeding vs. landscape feeding) and as recorded in tooth enamel are insensitive to significant diagenesis.

In summation, direct isotopic and trace element sampling of fossil *P. angusticeps* specimens from these sites are critical for accurate paleobiological interpretation of the species. As has been demonstrated in several past paleodietary analyses, the diets of extinct organisms regularly violate the assumption of taxonomic uniformity (e.g. that the diets of living and ancestral conspecifics are the same) or vary from the diets reconstructed solely through morphological comparisons (Sponheimer et al., 1999; Harris and Cerling, 2002; Schubert et al., 2006). Thus, isotopic study (or other methods such as mesowear analysis; see Schubert et al., 2006) are the only direct, unambiguous methods for directly assessing the palaeodiet and addressing the specific hypotheses that we raised above (Adams et al., 2013).

Electron-Spin Resonance

The use of ESR has been both variable and debated within the South African fossil deposits (see Herries et al., 2009 for discussion of prior dates). This non-destructive technique investigates unpaired electrons within materials (in this case, tooth enamel) that, given an accurate dose rate calculation, can be used to establish the direct depositional age of the specimen (see Grün, 1989). This obviously represents a substantial advance over the relative dating methods that are typically applied, but developing the technology to apply the method at the South African cave systems has required substantial development. The modern phase of ESR applications is being driven by Dr. Joannes-Boyau (Joannes-Boyau, 2013), who as a collaborator in this project will provide the first direct dating of these *P. angusticeps* specimens; an essential source of data to evaluate our palaeodietary hypotheses about the species.

Methodology (short):

A table of specimens to be sampled is provided below. Specimens from these assemblages were selected so as to represent distinct individuals (e.g. samples from only right lower m3s, etc.) and specimens with naturally-fragmented enamel or partial tooth crowns that minimise any impact of sampling on subsequent analysis of the *P. angusticeps* samples.

Site	Specimen Number	Species
Coopers	CO101, 135, 112, 117 108B, 105B, 121, COA554, COB104	*Papio angusticeps*
Kromdraai	KB5228A, 5268, 230, KA165/2340, 163/2338, KA166A, KA179/2342	*Papio angusticeps*
Haasgat	HGT 1, 4, 250, 861	*Papio angusticeps*

All isotopic samples analysed here will be from enamel carbonate (CO_3^{2-}), which is a substitution in apatite for phosphate (PO_4^{3-}), rather than by extracting samples from fossilized bone elements. Because of the prismatic, crystalline structure of enamel, enamel is a more closed system than bone to diagenesis, such that there are fewer substitutions during fossilization in enamel than in bone.

The use of trace elemental and isotopic mapping corresponds to a paradigm shift in the investigation of past populations dietary habits. Laser ablation Ablation Inductively Coupled Plasma Mass Spectrometer (LA-ICP-MS) provided a breakthrough in micro-scale analyses and has subsequently been used to measure trace elements and isotopes, continuously along teeth profiles (Grün et al., 2008; Austin et al., 2013). The system comprised a NW213 New Wave laser unit from ESI® firing at 213nm and coupled to an Agilent 7700x ICP-MS quadrupole for trace elements analyses. Isotopic mapping will be conducted on a NW193 laser from ESI® (higher wave length 193nm) coupled to a Thermo Neptune Plus Multicollector ICPMS allowing to separate elements with a mass very close to each other.

Any complete teeth or large enamel portions are sectioned with a high-precision wire saw offering a flat freshly exposed surface of enamel and dentine. This allows accurate measurements with the laser, as well as the ability to investigate the enamel growth band using SEM scanning electron microscope analyses. The elemental composition is obtained by ablating sequentially parallel tracks covering the entire samples. The maps are then reconstructed by merging all the parallel ablation tracks together. This technique offers several advantages including: (i) providing spatially resolved (10–100 mm scale) elemental distributions; (ii) high-precision and highly sensitive technique for micro-scale variations and (iii) combined enamel growth bands record to provide a chronological understanding of the intake of trace elements and isotopes. Sectioned tooth samples will then be refit, restoring the specimen and leaving a barely visible indication of the sampling.

Each tooth will be firstly mapped with the quadrupole for trace elements analyses before being investigated with the Multicollector for isotopic ratios. The elemental maps are used to better understand the diffusion and adsorption pattern of each elements, and allow to account for diagenetic processes using particular rare earth elements (REE) such as cerium, or europium, while element such as barium (Ba) and strontium (Sr) are used to describe the dietary pattern of the primate specimens as well as reconstructing breastfeeding sequence (Austin et al., 2013; Balter et al., 2012). To complete the work, maps of calcium and strontium isotopes ratio will be obtain on the Neptune Plus Multicollector ICPMS, allowing to better understand the diet variations, potential migration pattern and the trophic level of each specimen. ESR methods will follow those described in detail in Joannes-Boyau (2013).

Samples from the fossil specimens will need to be exported to the Southern Cross University GeoSciences Isotope Facility, Department of GeoSciences, New South Wales to be analyzed by Dr Renaud Joannes-Boyau. Pending permission and the granting of an export permit by SAHRA, samples will either be transported directly to Dr Renaud Joannes-Boyau in my hand luggage. Samples will be stored in a secure facility at the Department of Anatomy and Developmental Biology at Monash University for any periods of time other than when stored at the Department of GeoSciences at Southern Cross University, Lismore Road, NSW prior to being analysed.

Results gained by the isotopic analysis of these *P. angusticeps* specimens will be used to reconstruct the paleodiets of the species as well as the larger paleoenvironment of the Haasgat region at the time the fossils were deposited (assisted by the chronological data derived from ESR sampling). When coupled with isotope values gained from newly sampled Haasgat HGD samples from the Council for Geosciences we will be able to offer a comprehensive interpretation of palaeodietary variability in the species across South Africa in the early Pleistocene. Our results will be formatted for publication in early 2017 and submitted for review in either the Journal of Human Evolution, a Quaternary science journal (e.g. Quaternary Science Reviews; Palaeogeography, Palaeoclimatology, Palaeoecology) or potentially through an open-access wide-audience publication format (e.g., PLoS One, PeerJ).

Confirmation/permit by museum: Attached official letter from Ditsong Museum Acting Director, Dr Shaw Badenhorst

Damage/destructive analysis? (if yes, explain in detail) Yes.

Please see the description of the methodology above regarding the nature of the analysis and how this presents a controlled, invasive approach that utilizes largely isolated fragments to provide data. All remaining portions after sampling will be returned to the collections.

Statement why this study cannot be done in South Africa:

The request for exportation from South Africa is based on the ability to undertake simultaneous laser-ablation isotopic, trace element, and ESR analysis within the facilities at Southern Cross University. **No such single lab facilities exist in South Africa, minimising risk to the specimens in transit between facilities.** The laser ablation facilities available through SCU represent the most advanced, and minimally invasive, technology to acquire data from these fossils that are nearly impossible to locate in South Africa; and as a collaborating researcher, **Dr. Joannes-Boyau can offer at-cost analysis which is the only financially-feasible method to undertake this level of research**. Furthermore, Dr. Joannes-Boyau operates **the only ESR lab in the world that is calibrated to deal with teeth of this time period**, and is the only researcher who has published experience in all three analysis methods.