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A morphometric and genetic framework for the genus *Gazella* de Blainville, 1816 (Ruminantia: Bovidae) with special focus on Arabian and Levantine mountain gazelles

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Gazella is one of the most species-rich genera within horned ruminants. Despite overall similarity in body size and morphology, gazelles show variability in coloration and horn morphology. Unfortunately, however, species differentiation based on these characters, or on discrete skull characters, is very difficult due to high intraspecific variability. Furthermore, most species have fragmented and allopatric distributions, so that species boundaries were hard to define in the past. Mitochondrial DNA sequences have proven useful for investigating gazelle taxonomy in recent years, but especially for old museum material, i.e. type specimens, destructive sampling is often impossible. We provide a comprehensive morphometric framework for the genus *Gazella* based on linear skull measurements reconciled with results from molecular phylogenetic analysis based on the largest dataset available so far. In particular for males, the skull morphology shows interspecific differences concurrent with DNA data and provides a reliable tool for species identification. Based on morphometric data we synonymize *G. karamii* with *G. marica*, and confirm the identification of the *G. arabica* and *G. a. rueppelli* type skulls from analyses of mitochondrial DNA sequences.

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INTRODUCTION

Gazella de Blainville, 1816 is a remarkably uniform and easily recognizable genus, but also one of the most species-rich within horned ruminants (Bovidae). Its geographical range spans northern Africa, Arabia, the Middle East, India, China, and Mongolia. Gazelles are adapted to arid conditions and inhabit steppe, semi-desert, and desert regions. Adult males are territorial, at least during the breeding season, while females and their offspring form small groups. Migration in response to seasonal changes is common in some species. The total number of species listed, up to 23, varies considerably from author to author, depending on the preferred species concept. About ten extant (and recently extinct) species are currently accepted among most taxonomists (Table 1; Grubb, 2005; IUCN, 2013; but see also Groves & Grubb, 2011). Several fossil species are furthermore assigned to this genus, but these are not included in our study. Phenotypic differences in coloration and horn shape (e.g. Groves, 1988, 1996, 1997) have been used to define *Gazella* (sub)species. However, these traits have long been perceived as being 'subject to great individual variation' (Brooke, 1873: 537). Distinct morphological features defining each species are rare. One reason for this could be that some species with a large geographical range include several allopatric populations that show slight differences in their morphology. These are usually regarded as subspecies, but in their recent book, Groves & Grubb (2011) treat them as separate species. They split *G. bennettii* Sykes, 1831, *G. gazella* Pallas, 1766, and *G. subgutturosa* Guldenstaedt, 1778 into numerous species (Table 1), using predominantly coloration, horn morphology, and nasal bone shape as diagnostic characters. But most of these species are based on very few individuals (in the case of *G. karamii* on a single specimen), so it is difficult or even impossible to assess intraspecific variation. Moreover, the current geographical range of most gazelle species has been restricted by human hunting to a few scattered, allopatric populations (Thouless *et al*., 1991, 1997; UNEP/CMS, 1999; Mallon & Kingswood, 2001; Karami, Hemami & Groves, 2002; Beudels *et al*., 2006). This hampers studies on possible hybridization or local adaptation phenomena, which could uncover a potential morphological continuum between the supposed distinct morphologies of the different subspecies.

Molecular studies provide tools for reconstructing past population structures and help to identify species. In the last decade, several such studies have been conducted on gazelles: Wacher *et al*. (2011) and Hassanin *et al*. (2012) have confirmed that *G. marica* Thomas, 1897 and *G. subgutturosa* are two separate species; and Wronski *et al*. (2010) and Lerp *et al*. (2013) found two reciprocally monophyletic clades within *G. gazella* that could be treated as distinct species. Lerp *et al*. (2011) showed that there is no evidence for geographical subspecies within *G. dorcas* Linnaeus, 1758, except that *G. saudiya* Carruthers and Schwartz, 1936, *G. d. pelzelni* Kohl, 1886, and *G. d. massaesyla* Cabrera, 1928 might form distinct (sub)species as they seem to comprise monophyletic groups within *G. dorcas* (Hammond *et al*., 2000; Lerp *et al*., 2011; Godinho *et al*., 2012). To evaluate the new taxonomy by Groves & Grubb (2011) and to reconstruct the phylogeny of the genus *Gazella*, studies like these are needed for other gazelle species as well. However, a continuous sampling throughout the historical distribution of several gazelle species is impossible, because many populations are known from at most a few skins or skulls remaining in museum collections. In these cases, molecular methods can be expensive and time-consuming with no guarantee of amplification success.

The gazelle species with probably the most confusing taxonomic history is *Gazella arabica*. It first appeared in 1827 in a very unusual species description: H. Lichtenstein, director of the Zoologisches Museum der Königlichen Universität zu Berlin (today the Museum für Naturkunde Berlin), published a series of booklets in which he described recent museum acquisitions for a non-specialist audience. His second booklet includes the first mentioning of a new gazelle species, *Antilope arabica* Lichtenstein, 1827. It does not specifically designate a type, but gives a few measurements of one male and one female specimen, referring to a more detailed manuscript by Hemprich and Ehrenberg, the collectors of the *G. arabica* type material. That manuscript was published one year later in Latin (Hemprich & Ehrenberg, 1828). It includes measurements of four individuals of *Antilope arabica*, two males, one female, and a juvenile, collected in 1825 in different locations along the Arabian coast. Both documents (Lichtenstein, 1827; Hemprich & Ehrenberg, 1828) do not include any catalogue numbers for the type material. In later publications (Neumann, 1906; Groves, 1983) a maximum of three specimens constituting the *G. arabica* type material is mentioned: one male (ZMB_MAM_2115, skull and skin), one female (ZMB_MAM_2108, skull and skin), and one juvenile (ZMB_MAM_2109, the skin only is listed, but a mandible is also present). According to the letters of Hemprich and Ehrenberg (compiled by Stresemann, 1954 and mentioned in Groves, 1983) two individuals of *G. arabica* were collected on the Sinai peninsula, and one individual on the Farasan Archipelago, about 40 km offshore in south-western Saudi Arabia. It was presumed that the two individuals collected in Sinai are the female and juvenile, as their catalogue numbers are consecutive and they possibly are

Table 1. *Gazella* species taxonomy in recent publications; species are separated by solid lines

Grubb (2005)	IUCN (2013)	Groves & Grubb (2011)	This study
G. leptoceros	*G. leptoceros*	*G. leptoceros*	*G. leptoceros*
G. cuvieri	*G. cuvieri*	*G. cuvieri*	*G. cuvieri*
G. subgutturosa	*G. subgutturosa*	*G. subgutturosa*	*G. subgutturosa*
		G. yarkandensis	
		G. gracilicornis	
	G. s. marica	*G. marica*	*G. marica*
G. spekei	*G. spekei*	*G. spekei*	*G. spekei*
G. dorcas	*G. dorcas*	*G. dorcas*	*G. dorcas*
		G. pelzelni	
G. saudiya	†*G. saudiya*	*G. saudiya*	*G. d. saudiya*
G. arabica	*G. arabica*	*G. arabica*	*G. arabica*
	†*G. bilkis*	*G. bilkis*	
G. erlangeri		*G. erlangeri*	
		G. acaciae	
		G. cora	
		G. dareshurii	
		G. muscatensis	
G. gazella	*G. gazella*	*G. gazella*	*G. gazella*
		G. karamii	(belongs to *G. marica*)
G. bennettii	*G. bennettii*	*G. bennettii*	*G. bennettii*
		G. christii	
		G. fuscifrons	
		G. shikarii	
		G. salinarum	

mother and fawn (Neumann, 1906; Groves, 1983). Neumann (1906) designated the male individual the lectotype of *G. arabica* and erected a new subspecies *G. a. rueppelli* based on the female individual.

Unfortunately, for the male specimen most of the measurements (e.g. total length from head to tail, lengths of head, ear, and tail) in Hemprich & Ehrenberg (1828) do not match the measurements in Lichtenstein (1827). The horn measurements of the male type skull given by Lichtenstein are identical to our own measurements of ZMB_MAM_2115 (28.9 cm, assuming that 1 inch = 2.53 cm). Measurements given

by Hemprich & Ehrenberg (1828) for the two male specimens are smaller (26.8 and 24.0 cm, respectively). It could be that Hemprich and Ehrenberg took measurements of some specimens in Arabia and sent another specimen to the museum in Berlin from the numerous gazelles they shot during their expedition (Hemprich & Ehrenberg, 1828).

Bärmann *et al*. (2013) recently used mitochondrial DNA to investigate the phylogenetic position of the male *G. arabica* lectotype ZMB_MAM_2115. They found that the skin and skull of the supposed lectotype individual derive from two individuals belonging to two different phylogenetic groups. The skin belongs to the Arabian mountain gazelles *G. arabica*, while the skull comes from an individual from the Levantine form of mountain gazelles, *G. gazella*. As the skin was the only specimen named when the lectotype of *G. arabica* was erected (Neumann, 1906), the authors recommend it being the lectotype specimen of *G. arabica*.

The horn measurements of the adult females in the two publications are similar (15 cm in Hemprich & Ehrenberg, 15.2 cm in Lichtenstein). However, both differ from our own measurements (18.4 cm) taken from the putative female type skull ZMB_MAM_2108, so we have some doubt about the identity of this skull.

This example shows that species identification in museums is often insufficient, especially when the geographical origin of specimens is not given in detail (e.g. 'North Africa'). Therefore, the first aim of our study is to provide a tool for species identification based on morphometric data. Skull measurements have repeatedly been used in the past to differentiate gazelle species (Gentry, 1964; Lange, 1972; Rostron, 1972; Groves, 1983, 1996, 1997; Thouless & al Basri, 1991; Karami *et al*., 2002). Only one study included all gazelle species (Lange, 1972), but with different species and subspecies concepts compared with our study. Studies by Rostron (1972) and Groves (1983, 1996, 1997) used multivariate statistics, but specimens were grouped a priori into the different (sub)species under investigation. Hence, our study is the first to use multivariate statistics including all species currently recognized by the IUCN (see Table 1). We use linear measurements instead of three-dimensional landmark analysis to enable any person to collect data on gazelle skulls and use our reference data for species identification.

We further attempt to identify other specimens assigned to *G. arabica*, using skull measurements and mitochondrial DNA sequences, and, by comparing skulls from Arabian and Levantine mountain gazelles, we analyse if and how *G. arabica* is morphometrically different from *G. gazella*.

MATERIAL AND METHODS

Species concept

We follow de Queiroz (2007) in defining a species as a group of connected populations that evolves separately from other such groups. Many different criteria can be applied for identifying species, e.g. ecological differentiation, reproductive isolation, morphological differentiation, or monophyly in molecular phylogenetic analyses. The more of these lines of evidence speak in favour of the separation of two groups, the better supported is the hypothesis that they are different species.

In the case of mountain gazelles, we found that the morphometric data provide an additional line of evidence that favours separation of *G. gazella* (Levantine mountain gazelles) and *G. arabica* (Arabian mountain gazelles), as other authors have suggested (Wronski *et al*., 2010; Lerp *et al*., 2012; Bärmann *et al*., 2013). Similarly, we consider *G. marica* to be distinct from *G. subgutturosa* (Wacher *et al*., 2011). In the case of *G. dorcas* and *G. saudiya*, we found no evidence that they are distinct groups in the morphometric analyses. We therefore classified *G. d. saudiya* as the Arabian subspecies of *G. dorcas*, although we kept the two taxa separate in the analyses. For all other species we followed the taxonomy used by the IUCN (2013). Figure 1 shows the geographical origins of specimens in our study (not including captive individuals).

Specimens and samples investigated

For the morphometric analyses, 171 skulls (Supporting Information Appendix S1) were measured in the Museum für Naturkunde, Berlin (MfN, the abbreviation ZMB for Zoologisches Museum Berlin is used in the catalogue), the Natural History Museum, London (BMNH), the University Museum of Zoology, Cambridge (UMZC), the Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt (SNF), and the King Khalid Wildlife Research Center, Saudi Arabia (KKWRC). This includes the type skulls of *G. arabica*, *G. arabica rueppelli*, *G. bennettii*, *G. isabella*, *G. karamii*, *G. littoralis*, *G. loderi*, *G. marica*, *G. merilli*, *G. osiris*, and *G. saudiya*.

The molecular phylogenetic analysis focuses on the skins ZMB_MAM_2108, 2109, and 2115, and skulls ZMB_MAM_2108 and 2115 collected from 1824–1825 by Hemprich and Ehrenberg and housed at the MfN Berlin. Additionally, further museum materials assigned to *G. arabica* available at the MfN (adult skin ZMB_MAM_66104) and the Zoologische Staatssammlung München (juvenile limb skeleton ZSM AM/1063, Wagner & Zuccarini, 1839), and six living individuals (*G. dorcas*, *G. arabica*, *G. marica*) held captive at the Al Wabra Wildlife Preservation

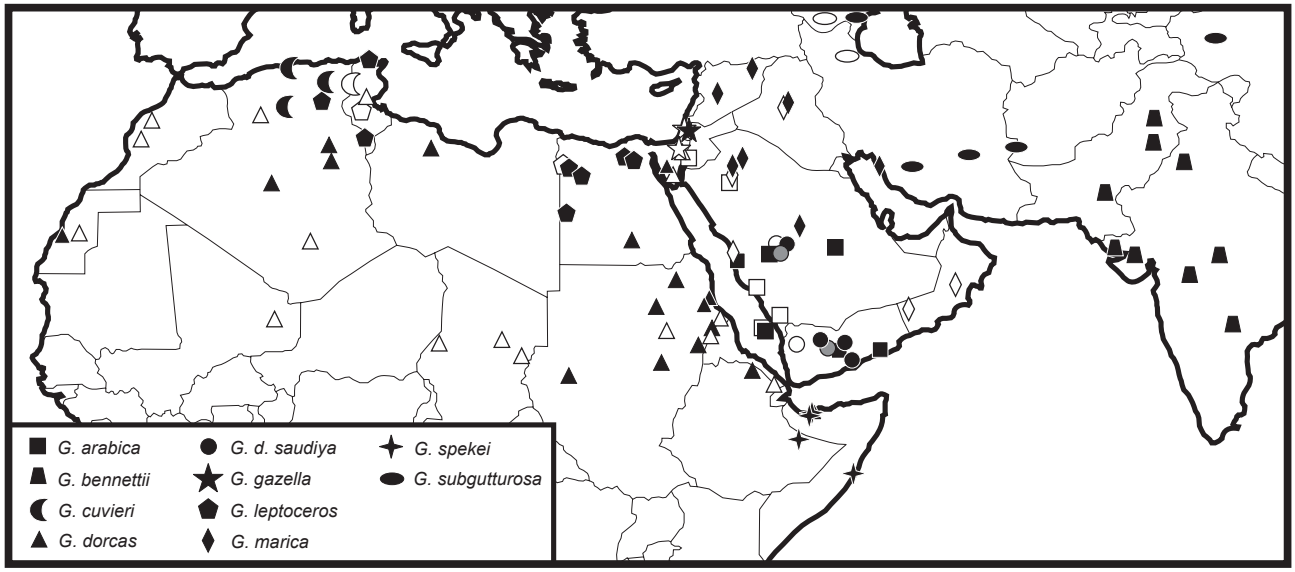

Figure 1. Geographical origin of gazelle specimens included in this study. Black icons, specimens sampled for morphometrical data; white icons, specimens sampled for DNA data; grey icons, specimens sampled for morphometrical and DNA data. The geographical origins of specimens sampled for DNA data were taken from the original publications of the sequences (Hammond *et al*., 2001; Wronski *et al*., 2010; Zachos *et al*., 2010; Lerp *et al*., 2011; Wacher *et al*., 2011) or the specimen information on GenBank. The geographical range of *G. subgutturosa* extends further to the east into China and Mongolia.

Table 2. Origin of gazelle samples used in the molecular analysis

Specimen	Species	Coll.	Age	Origin	Sample taken
*ZMB-MAM-2115 skull	*G. gazella*	MfN	Adult	1824, Syria?	Tissue within braincase
*ZMB-MAM-2115 skin	*G. arabica*	MfN	Adult	1824, Arabia?	Skin
ZMB-MAM-2108 skull	*G. dorcas*	MfN	Adult	1822–1824	Tissue within nasal cavity
ZMB-MAM-2108 skin	*G. gazella*	MfN	Adult	1824, Syria?	Skin
ZMB-MAM-2109	*G. arabica*	MfN	Juvenile	1825, Arabia?	Skin
ZMB-MAM-66104	*G. gazella*	MfN	Adult	1911, Berseba	Skin
ZSM AM/1063	*G. arabica*	ZSM	Juvenile	1836, near Akaba	Sesamoid
AWWP 9879	*G. arabica*	AWWP		Captive	Blood
AWWP 9923	*G. arabica*	AWWP		Captive	Blood
AWWP 9634	*G. dorcas*	AWWP		Captive	Blood
AWWP 8882	*G. dorcas*	AWWP		Captive	Blood
AWWP 6681	*G. marica*	AWWP		Captive	Blood
AWWP 8961	*G. marica*	AWWP		Captive	Blood

AWWP, Al Wabra Wildlife Preservation, Qatar; MfN, Museum für Naturkunde, Berlin; ZSM, Zoologische Staatssammlung, München. The sequences from the male *G. arabica* type specimens (marked with *) were already published in Bärmann *et al*. (2013).

(Qatar) were sampled for molecular data (Table 2). Additional sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/GenBank/; for accession numbers see Appendix S2).

Morphometric data

The core data set consists of measurements from 132 skulls, taken by E.V.B., in the collections of the MfN, BMNH, and UMZC, and includes *G. bennettii* (16 specimens), *G. cuvieri* (5), *G. dorcas* (31), *G. gazella* (8), *G. leptoceros* (14), *G. marica* (10), *G. d. saudiya* (10), *G. spekei* Blyth, 1863 (11), *G. subgutturosa* (15), and 12 specimens labelled *G. arabica* or *G. gazella arabica* from the Arabian Peninsula. Figure 1 gives an overview of the geographical origins of the specimens (for more details see Appendix S1). For every skull up to 50 measurements were taken (Fig. 2, Table 3).

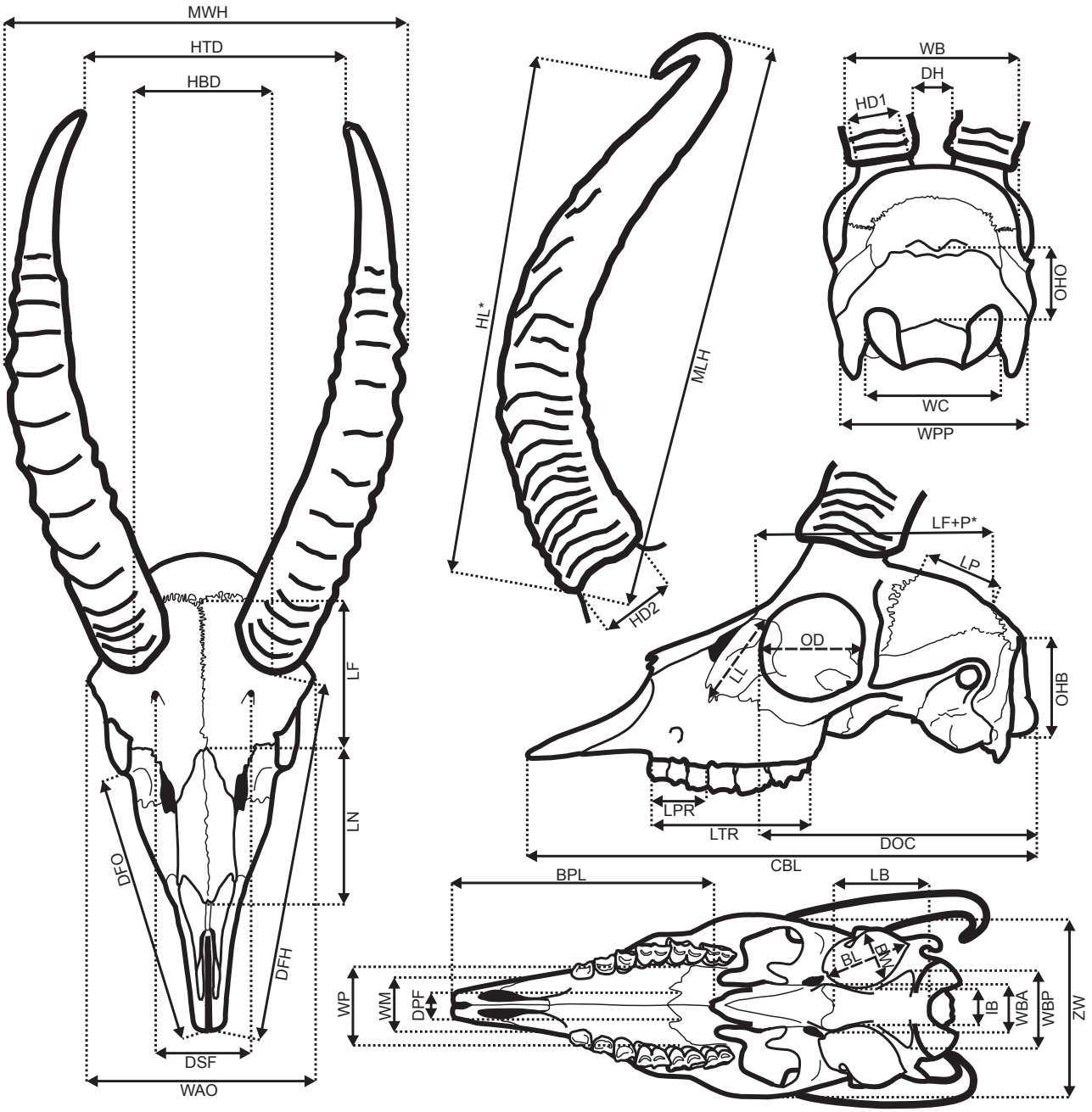

Figure 2. Skull measurements used in this study. Abbreviations correlate with descriptions in Table 3.

The core data set is complemented with data from 23 *G. arabica* from the captive population at KKWRC, and 14 gazelles from the Farasan Islands measured by T.W. These specimens are housed in the collection of the KKWRC in Saudi Arabia. A further two specimens were measured by H.L. in the collection of the Senckenberg Forschungsinstitut und Naturmuseum Frankfurt (SNF). Both, a male and a female, were collected in Sinai (1827) as *G. dorcas* var. *arabica* (Brooke, 1873) and were thought to belong to *G. arabica rueppelli* by Neumann (1906). They are currently classified as *G. dorcas* in the SNF catalogue. Analyses with these additional specimens were conducted separately, excluding some variables that seemed affected by measurement inconsistencies, e.g. antero-posterior horn diameter (DH2), length of frontal plus parietal bone (LF+P1, LF+P2), and the maximum width of the horns (MWH) in the analyses with captive *G. arabica* and Farasan Island gazelles.

Table 3. Skull measurements used in this study

Abbreviation in Figure 2	Description
BL	Bulla length
BPL	Basi-palatal length – length of the palate along the midline
BW	Bulla width
CBL	Condylo-basal length
DFH	Distance front to horns
DFO	Distance front to orbit
DH	Distance between horns pedicles
DOC	Distance orbit to condyle (measured parallel to tooth row)
DPF	Distance between palatal foramina
DSF	Distance between supra-orbital foramina
HBD	Horn base distance (distance of the anteriormost parts of the pedicles)
HD1	Horn pedicle diameter 1 (medio-lateral)
HD2	Horn pedicle diameter 2 (antero-posterior)
HL 1, 2*	Horn length, distance between the base of the horn sheath and the horn tip
HTD	Horn tip distance
IB	Inter-bullae distance
LF+P 1, 2*	Length of frontal+parietal
LB	Length of basioccipital (at sagittal plane)
LF	Length of frontal
LL	Length of lacrimal (maximum length of facial part)
LN	Length of nasal
LP	Length of parietal
LPR	Length of premolar row (measured at alveoli)
LTR	Length of tooth row (measured at alveoli)
MLH	Maximum length of horn sheath
MWH	Maximum width of horns sheaths
OD	Orbit diameter (parallel to tooth row)
OHB	Occipital height, braincase complete
OHO	Occipital height, occiput only (dorsal of foramen magnum)
WAO	Width across orbits (maximum width of frontals)
WB	Width of braincase
WBA	Width of basioccipital anterior
WBP	Width of basioccipital posterior
WC	Width of condyle
WM	Width of maxilla (not premaxilla), measured at the midpoint of the diastema
WP	Width of palate, measured at level of palatal foramina
WPP	Width across paroccipital processes
ZW	Zygomatic width (behind orbits)

*Measured with callipers (1) and measuring tape (2).

Prior to analysis, measurements were $\log_{10}$-transformed. Horn measurements were used from one side of the skull only to avoid overweighting of these variables. Usually the measurements from the right side were used, except when the right horn showed damage or was missing. The raw measurement data is available at Dryad (Bärmann *et al*., 2013).

Principal component analysis

To obtain an ordination of the specimens as a function of their size and morphology, several principal component analyses (PCA) were carried out with IBM SPSS statistics 19, including different combinations of variables and varying numbers of specimens (Table 4). The first analysis (PCA 1) aimed at including the maximum number of specimens possible. Several skulls show damage, usually at the snout tip or the skull base due to the way they have been mounted on wooden plates, so that many variables had to be excluded from this first analysis. To maximize the number of included variables in the subsequent analyses, the missing values for every specimen were filled in with the average values of

Table 4. Principal component analyses; selection of specimens and variables

PCA	Sex	No. of variables		No. of taxa	No. of specimens
1	M/F	13	Variables available for all specimens from the core data set (excluding very incomplete ones)	10	127
2	M	40	All variables; missing values are filled with species average*	10	82
3	F	40	All variables; missing values are filled with species average*	7	43
4	M	30	Variables with good correlation†; missing values are filled with species average‡	10	70
5	F	22	Variables with good correlation and available for all species§; missing values are filled with species average‡	10	44
6	M	26	Same as PCA4, including captive *G. arabica*	10	87
7	F	18	Same as PCA5, including captive *G. arabica*	10	49
8	M	27	Similar to PCA4; only variables available for *G. arabica* type	10	71
9	M	27	Similar to PCA8, including gazelles from the Farasan Islands	11	74
10	F	18	Similar to PCA5, including gazelles from the Farasan Islands	11	48

M, male; F, female. No. of variables, number of linear measurements, for details see Tables 6–8, Appendix S3–S4 and S6–S7. No. of taxa, number of discernible groups, corresponding with names in Table 1.
*The type skulls of *G. arabica* and *G. a. rueppelli* were not included.
†See Table 7.
‡Specimens with > 4 missing values are excluded (but all *G. cuvieri* specimens were included).
§See Table 8.

all other specimens belonging to the same sex and species. This complemented data set was used for identifying the most important variables in separate analyses of males (PCA 2) and females (PCA 3). Only those variables that showed high factor loading, i.e. > 0.7 for the first component, and/or > 0.5 for the second, and/or > 0.4 for the third component (see Appendix S3–S4), and therefore have a strong impact on maximizing the distance between the specimens, were selected for subsequent analyses. The scores of the specimens in the first PCA factors were used to set them out in bivariate plots (Figs 3–4, 6–7).

Discriminant analysis

Discriminant analyses (DA) were used to evaluate the ability of the sets of metric variables to differentiate between the species, using various combinations of variables and specimens (Table 5). In contrast to PCA, discriminant analysis requires a priori definition of the group identity of each specimen. The predictive capacity of the data set was tested with cross-validation analyses, where each specimen is classified using a model derived from all other specimens. Squared Mahalanobis distances (based on the probability density distribution of the data) of the respective specimen to every group centroid (centre of mass) are calculated, and the group with the shortest distance is chosen as the most probable group containing the specimen. Furthermore, discriminant analysis was used to classify the uncertain cases, i.e. the specimens from the *G. arabica* type material and the two specimens from the SNF, according to the model derived. The scores of the specimens in the discriminant canonical functions were used to set them out in bivariate plots (Figs 5–7). The complete set of discriminant functions (DA 2 and 6) and values of the group centroids are provided in Appendix S5–S6.

DNA extraction

Dry tissue from museum specimens was used for DNA extraction with the Qiagen (Hilden) blood & tissue kit according to the manufacturer's instructions, with a prolonged initial incubation step with proteinase K (up to 24 h). Samples consisted of several pieces of tissue, each 2–5 mm^3 in volume, taken from the skin close to the hooves and the inside of the nasal cavity. For the DNA extraction of ZSM AM/1063 we used a sesamoid bone and followed the method described in Rohland & Hofreiter (2007). Blood samples of living specimens were fixated on Whatman FTA elute cards. DNA extraction followed the manufacturer's instructions.

PCR and sequencing

A fraction of the mitochondrial cytochrome *b* gene (*CYTB*), and control region (CR) were amplified using primers from Bärmann *et al*. (2013). The 12S rRNA gene (*12S*) was amplified using the primers U1230, L1946, L2226 (from Ropiquet & Hassanin,

Table 5. Discriminant analyses; selection of specimens and variables

DA	Sex	No. of variables		No. of taxa	No. of specimens	Correct classification (%)	
						Original data	Cross-validation
1	M/F	13	Variables available for all specimens (excluding very incomplete ones)	10	125	90.2	75.6
2	M	30	Variables with good correlation*; missing values are filled with species average†	10	70	100	88.6
3	M	26	Similar to DA2 including captive *G. arabica*	10	86	100	96.5
4	M	27	Similar to DA2; only variables available for *G. arabica* type	10	71	100	88.6
5	M	23	Similar to DA3; only variables available for *G. arabica* type	10	87	98.8	95.3
6	F	22	Variables with good correlation and available for all species‡; missing values are filled with species average†	10	44	100	67.4
7	F	18	Similar to DA6, including captive *G. arabica*	10	49	97.9	68.8
8	M	23	Similar to DA3; only variables available for male SNF specimen	10	87	100	93.0
9	F	17	Similar to DA7; only variables available for female SNF specimen	10	50	97.9	64.6

*See Table 7.
†Specimens with > 4 missing values are excluded (but all *G. cuvieri* specimens were included).
‡See Table 8.

2004), and A850 (from Gatesy & Arctander, 2000). PCR followed a standard protocol, as described in Bärmann *et al*. (2013). For cycle sequencing of forward and reverse strands we used the BigDye3.1 Terminator-Mix (Applied Biosciences) in 10 µL final volume and 30 cycles. Sequences were manually improved using Sequence Scanner 1 (Applied Biosystems, 2005); fragments were assembled and aligned manually using Mesquite 2.5 (Maddison & Maddison, 2009). Complementary sequences from GenBank (42 for *CYTB*, 27 for CR, 13 for *12S*; see Appendix S2) were selected with a focus on individuals that were sampled for several markers.

Phylogenetic analysis

Phylogenies were reconstructed using MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003) with default priors (dirichlet distributions for base frequencies as well as substitution rates, exponential branch length priors), using an HKY+G model for every partition (models were selected using likelihood ratio tests in MrModeltest 2.3; Nylander, 2004). Separate analyses for every marker were run for 4 000 000 generations (10 000 000 for the combined analysis), sampling every 1000th generation. For every analysis we used two independent runs with one cold chain and three heated chains each (temp = 0.15, 0.05 for the combined analysis). A standard deviation of split frequencies ≤ 0.01 was used as an indicator for convergence. Burn-in was set to 0.5. Parameter convergence and mixing were monitored with Tracer v. 1.5 (Rambaut & Drummond, 2007). The nexus file for the combined analysis is available as Appendix S7.

RESULTS

Preliminary studies and mislabelled specimens

During the preliminary analyses (results not shown) it became clear that the identification of several skulls was doubtful. One of them – a female zoo animal (ZMB_MAM_105467) labelled *G. leptoceros* – was subsequently excluded from the analyses. Another female (BMNH 32.7.6.55), a putative *G. spekei*, was consistently classified as *G. marica* in the cross-validation of the discriminant analyses. As the geographical provenance of this specimen was unknown, it was also excluded from the analyses. One male specimen (BMNH 35.7.24.2) labelled '*G. pelzelni*' (south-eastern subspecies of *G. dorcas*) from Berbera, Somalia, was consistently classified as *G. spekei* in cross-validation. The geographical origin of this animal renders this classification very probable, so it was treated as *G. spekei* in all subsequent analyses. The changed classification of this animal affected the cross-validation classification of other specimens and improved the discrimination between *G. dorcas* and *G. spekei*.

Table 6. PCA 1, all specimens

Variables	Factor loadings in each component				Extraction communalities
	C1	C2	C3	C4	
WP	**0.756**	0.066	**–0.519**	0.083	0.852
LL	**0.717**	0.251	**–0.479**	–0.050	0.810
OD	0.697	0.182	**–0.455**	0.186	0.760
WB	**0.841**	0.161	–0.199	0.135	0.790
DSF	0.518	**0.533**	0.067	**0.547**	0.857
LF	**0.806**	0.087	0.166	**–0.348**	0.806
LP	0.651	–0.064	**0.551**	–0.130	0.748
L F + P1	**0.908**	0.021	0.303	–0.266	0.987
L F + P2	**0.872**	0.032	0.388	–0.279	0.990
DH	–0.320	**0.868**	0.290	0.087	0.946
HD1	0.362	**–0.761**	0.248	**0.453**	0.976
HD2	0.376	**–0.791**	0.206	**0.416**	0.982
HBD	0.010	**0.724**	**0.464**	**0.406**	0.905
Eigenvalues	5.58	2.91	1.73	1.20	
% of Variance	42.91	22.34	13.29	9.22	

Factor loadings for the first four principal components (C1–C4) and extraction communalities for the 13 included variables; abbreviations correspond to Table 3 and Figure 2. For each component the variables showing high factor loadings are highlighted in bold.

Another case of changed taxonomic affiliation was the type and only specimen of *G. karamii* Groves, 1993 (ZMB_MAM_41400). This skull never clustered with *G. gazella* or *G. bennettii*, the two species that had been proposed as close relatives of *G. karamii* by Groves (1993) and Groves & Grubb (2011), respectively. Instead, the specimen was very close to *G. marica* in every PCA, and classified as such by all discriminant analyses, with a distance to the group centroid that was well within the range of other *G. marica* specimens. Therefore, we synonymized *G. karamii* Groves, 1993 with *G. marica* Thomas, 1897.

Separation of the sexes

The first principal component analysis (PCA 1) including all specimens of the core data-set (13 variables, see Table 6) showed an almost complete separation of males and females with components 1 and 2 (C1, C2; Fig. 3B). Males are generally larger (C1, Table 6) and have more robust horns (C2, Table 6). Therefore, sexes were treated separately in the following analyses. The only male specimen that was placed among the females is a very young *G. dorcas*. It was excluded from the subsequent analyses. Discriminant analysis confirmed the marked separation of the sexes (Fig. 3A), with 98.4% of the original cases classified correctly and a cross-validation success of 95.3%.

Components 3 and 4 showed separation among species (Fig. 3C). C3 contrasted the parietal length and horn base distance with palate width, lacrimal length and orbit diameter and separates *G. subgutturosa*, *G. marica* and *G. leptoceros* from *G. gazella*, *G. arabica*, *G. d. saudiya* and *G. dorcas*. C4 has high positive factor loadings (Table 6) for horn diameter, distance between the horns (referred to as 'horn distance' in the following text) and the distance between the supra-orbital foramina, and high negative factor loading for frontal length. It separated, for example, *G. marica* from *G. leptoceros*, and *G. d. saudiya* from *G. gazella* and *G. arabica*. The male *G. arabica* type skull was placed close to *G. d. saudiya*; the female type clustered with *G. dorcas*. Both these groupings are confirmed by DA 1 (not shown).

Separation of the species

With the optimized variable set used for males in PCA 4 (Table 7), it is possible to distinguish between nearly all ten taxa (Fig. 4A, B). The only exception is *G. dorcas* which shows considerable overlap with *G. d. saudiya* and *G. spekei*. Five main components (C) are retained: as usual, C1 is mainly a size component and clearly separates the larger species, *G. cuvieri* and *G. subgutturosa*, from the smaller ones (Fig. 4A). C2 predominantly contrasts the shape of the skull roof (length of frontal and parietal, as well as horn distance) with horn length and width. It separates *G. leptoceros* and *G. marica*, both characterized by relatively large horns and short parietals, from the remaining species (Fig. 4A). C3 shows high

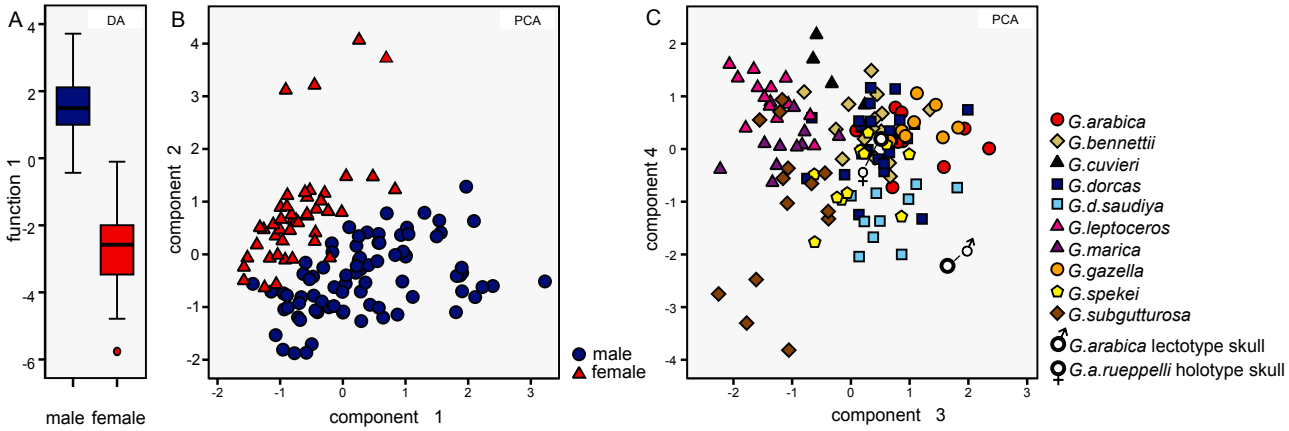

Figure 3. A, separation of the sexes in discriminant analysis. B, C, bi-variate plots of specimen scores for main components in PCA 1 including all specimens and 13 variables. B, separation of males and females by components 1 and 2; C, separation of the species by components 3 and 4.

positive factor loadings for horn distance and horn length, as opposed to high negative loadings for the antero-posterior horn diameter and horn width. It separates *G. subgutturosa*, *G. marica*, and some individuals of *G. dorcas* (with more anterior-posteriorly curved horns) from the straight-horned *G. cuvieri*, *G. bennettii*, and *G. leptoceros* (Fig. 4B). C4 is also influenced by horn distance and horn curvature, but here in particular by the lateral curvature with in-turned horn tips that characterizes *G. marica*, *G. subgutturosa*, *G. arabica*, and many specimens of *G. dorcas* (not shown). C5, which clearly separates *G. gazella* from *G. arabica* (Fig. 4B), shows positive factor loadings for variables associated with braincase shape, i.e. parietal length, basioccipital width, interbullae distance, occipital height, and horn distance, and negative factor loadings for tooth-row length and lacrimal length.

In females the differentiation between the species was not as pronounced as in males (Fig. 4C, D). Based on the optimized variable set for females, PCA 5 (Table 8) resulted in four main components: C1 mainly reflects the overall size of the skull, but has negative factor loadings for horn length and horn diameter. It separates the large sized *G. subgutturosa* and *G. cuvieri* from the other species (Fig. 4C). C2 shows high positive factor loadings for the distance between the palatal foramina, contrasted with high negative loadings for brain case length and horn distance. It clearly separates *G. leptoceros*, *G. marica*, and *G. cuvieri*, all characterized by a long distance between the palatal foramina, from the other species (Fig. 4C). C3 is largely influenced by braincase curvature, horn diameter, and horn distance, but it does not show any clear species separation (Fig. 4D). C4, however, was largely influenced by horn distance contrasted with negative factor loadings for several braincase variables and clearly separated *G. dorcas* from *G. d. saudiya* (Fig. 4D).

Differences between *G. gazella* and *G. arabica*

Focusing on specimens measured by E.V.B., for which most data were available, there was no overall size difference between males of the two groups (CBL = 179.6 ± 6.4 mm in *G. arabica*; 180 ± 4.6 mm in *G. gazella*), and no marked difference in horn lengths (HL1 = 223 ± 18.6 mm in *G. arabica*; 233.4 ± 9.7 mm in *G. gazella*). However, in PCA 4, several components, i.e. C1, C4, and C5, show separation of *G. gazella* and *G. arabica* (Fig. 4). *Gazella arabica* has on average less robust horns, i.e. a smaller horn diameter, and more in-turned horn tips. The brain case also differs between the two groups: it is longer in *G. gazella* (LF+P2 = 107.6 ± 3.1 mm) than in *G. arabica* (101.1 ± 5.6 mm), but higher in *G. arabica* (OHO = 26.8 ± 2.2 mm) than in *G. gazella* (21.4 ± 1.3 mm). These differences are significant (*t*-tests, Appendix S8), but sample sizes are small.

In PCA 6 the captive and wild *G. arabica* specimens together formed a distinct group for C1 and C2, although some of the wild specimens were placed relatively close to *G. dorcas*. C4, influenced mainly by horn distance, almost completely separated the *G. arabica* specimens measured by T.W. from those measured by E.V.B. (data not shown).

A direct comparison of females of *G. gazella* and *G. arabica* is not possible, as only one female *G. gazella* was included in our study. Furthermore, in PCA 7 the putatively wild *G. arabica* specimen BMNH 46.259 is morphologically most similar to *G. dorcas* and does not cluster with the captive *G. arabica* specimens from KKWRC (Fig. 7A, B).

Table 7. PCA 4, males

Variables	Factor loadings in each component					Extraction communalities
	C1	C2	C3	C4	C5	
WP	**0.906**	−0.166	−0.068	0.101	−0.013	0.863
LL	**0.836**	−0.058	−0.075	0.033	**−0.319**	0.810
OD	**0.790**	−0.154	0.035	0.012	0.041	0.650
WB	**0.892**	0.052	−0.120	0.142	0.109	0.845
LP	0.429	**0.718**	−0.088	−0.189	**0.241**	0.802
L F + P1	**0.745**	**0.542**	−0.105	−0.139	0.109	0.891
L F + P2	0.680	**0.598**	−0.052	−0.201	0.122	0.878
DH	−0.214	**0.551**	**0.655**	**0.383**	−0.002	0.926
HD1	**0.812**	0.094	−0.206	0.079	0.020	0.717
HD2	0.650	−0.024	**−0.460**	−0.009	**0.227**	0.686
HBD	0.114	**0.546**	**0.628**	**0.440**	−0.065	0.904
LTR	**0.838**	0.056	−0.203	0.182	**−0.326**	0.885
LPR	**0.768**	0.090	−0.087	0.276	**−0.336**	0.794
HL1	0.577	**−0.609**	**0.420**	−0.272	−0.072	0.959
HL2	0.643	**−0.630**	0.162	−0.233	−0.056	0.893
MLH	0.580	**−0.609**	**0.415**	−0.273	−0.066	0.958
WM	**0.818**	0.149	0.010	0.148	−0.102	0.724
DFO	**0.945**	0.060	0.065	−0.032	−0.159	0.927
DFH	**0.909**	0.079	0.105	−0.091	−0.119	0.865
CBL	**0.936**	0.130	−0.053	−0.032	−0.189	0.932
WBA	**0.790**	−0.072	−0.087	0.156	**0.309**	0.756
WPP	**0.869**	−0.087	0.115	0.053	0.088	0.786
OHO	**0.828**	0.143	0.311	−0.243	0.135	0.880
OHB	**0.763**	0.089	0.345	−0.239	**0.245**	0.826
DOC	**0.851**	0.218	−0.124	−0.078	−0.125	0.808
IB	**0.750**	−0.169	0.048	0.143	**0.403**	0.775
HTD	0.062	**−0.592**	0.292	**0.458**	**0.252**	0.713
MWH	0.247	**−0.523**	**−0.410**	**0.448**	0.130	0.719
ZW	**0.932**	−0.148	0.036	0.099	−0.049	0.904
WAO	**0.945**	−0.040	−0.010	0.111	0.076	0.913
Eigenvalues	16.68	3.8	2.08	1.42	1.02	
% of Variance	55.59	12.66	6.92	4.74	3.41	

Factor loadings for the first five principal components and extraction communalities for the 30 variables selected for species separation. For each component the variables showing high factor loadings are highlighted in bold.

Discriminant analyses

A discriminant analysis based on all specimens and a much reduced set of variables (DA 1, Table 5) already provides a good separation of the ten gazelle taxa. In total, 90.2% of the cases were identified correctly; in cross-validation 75.6% of the cases were correctly classified.

Discriminant analyses exclusively based on males (DA 2–5) show an even better separation of the species, with > 98% of the original cases classified correctly and a cross-validation success of more than 88% (Table 5). DA 2 examined male specimens (Fig. 5A) and showed a cross-validation success of 88.6%. Among the misclassifications there were specimens of *G. arabica* placed in *G. dorcas* (1); *G. bennettii* placed in *G. arabica* (1); *G. dorcas* placed in *G. arabica* (1), *G. leptoceros* (1), *G. spekei* (1), or *G. d. saudiya* (1); and *G. subgutturosa* placed in *G. marica* (1). A cross-validation success of 100% was achieved for *G. cuvieri*, *G. gazella*, *G. leptoceros*, *G. marica*, *G. d. saudiya*, and *G. spekei*. When the captive *G. arabica* specimens were included (DA 3) the cross-validation success was slightly higher (96.5%), as only three specimens were misidentified: one *G. gazella* was placed in *G. arabica*, and two *G. dorcas* were placed in *G. d. saudiya* (Appendix S9).

For females the discriminant analyses (DA 6 and 7) also show accurate classification with the original cases (> 97%), although they have a much lower cross-validation success (67–69%, see Table 5). In

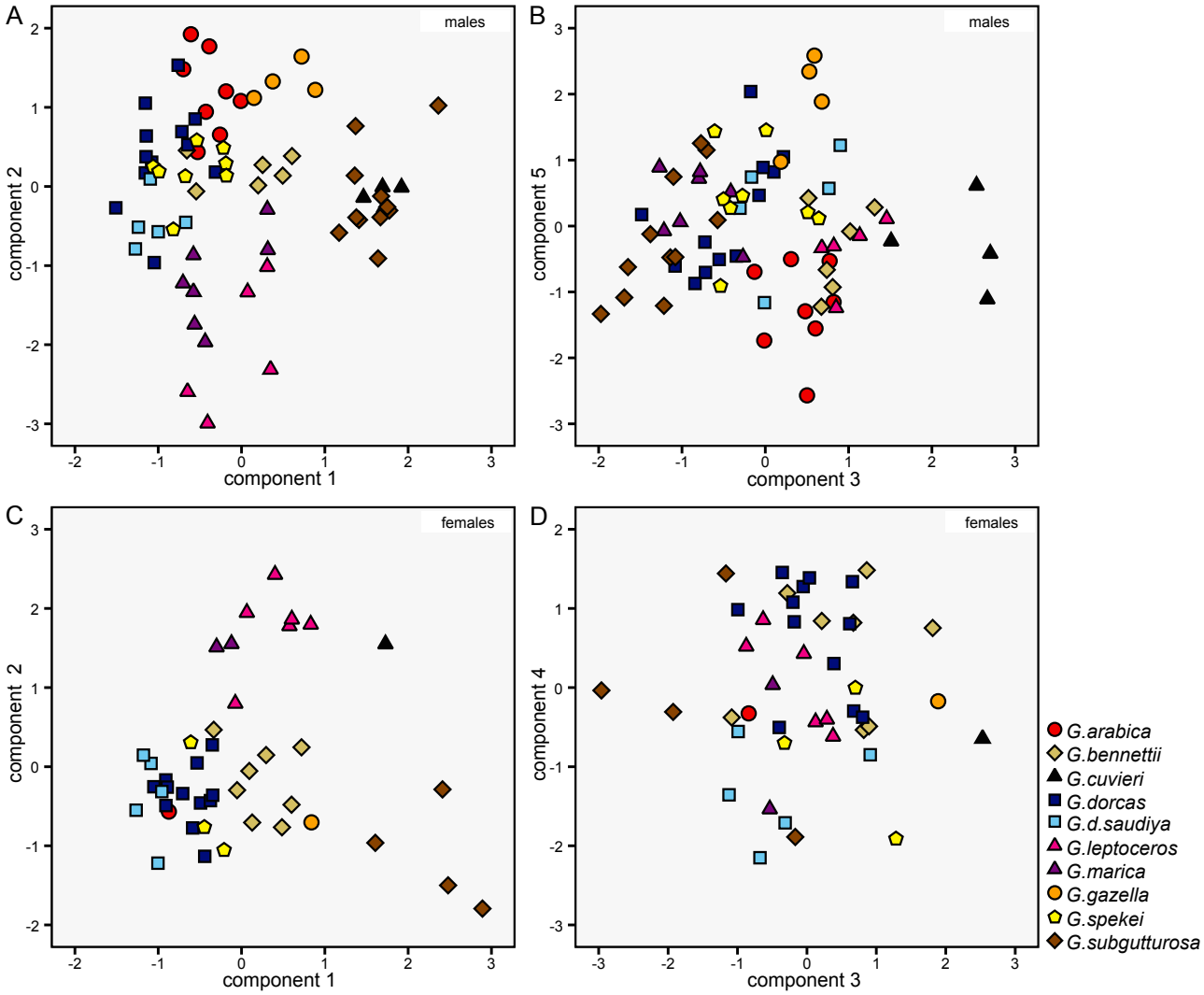

Figure 4. Bi-variate plots of specimen scores for main components in PCAs with optimized variable sets. A, B, males, PCA 4; C, D, females, PCA 5.

DA 6 (Fig. 5B) there were only three taxa where all members were identified correctly: *G. d. saudiya*, *G. marica*, and the hornless *G. subgutturosa*. In DA 7, which included the captive *G. arabica* females (Fig. 7C, D), the wild *G. arabica* (BMNH 46.259) was classified as *G. dorcas* with the original data. In the cross-validation, misclassification was highest for *G. spekei* where all three specimens were misclassified as either *G. arabica* or *G. dorcas*. Five out of six *G. arabica* were placed in other groups, i.e. in *G. bennettii*, *G. dorcas*, *G. gazella*, *G. marica*, and *G. spekei*; three out of 12 *G. dorcas* females were classified as *G. spekei* or *G. d. saudiya*; one *G. bennettii* was grouped with *G. arabica*; and one *G. leptoceros* was placed in *G. bennettii*. A cross-validation success of 100% was achieved for *G. marica*, *G. d. saudiya*, and *G. subgutturosa*.

The unstandardized canonical discriminant function coefficients and functions at group centroids for DA 2 (males) and DA 6 (females) are provided in Appendix S5–S6. They can be used to identify other specimens according to their skull measurements.

SPECIES IDENTIFICATION FOR *G. ARABICA* TYPE MATERIAL AND SNF SPECIMENS

To classify the skull assigned by Neumann (1906) as the *G. arabica* lectotype (ZMB_MAM_2115), PCA 8, PCA 9, and DA 4 were conducted including only the variables available for this particular specimen. In PCA 8 the first three components place the type skull close to *G. gazella* (Fig. 6A, B), but C4, mostly reflecting horn measurements, places the specimen among

Table 8. PCA 5, females

Variables	Factor loadings in each component				Extraction communalities
	C1	C2	C3	C4	
WP	**0.814**	0.431	−0.013	−0.056	0.851
LL	**0.892**	0.149	−0.026	−0.014	0.818
OD	**0.736**	0.191	0.343	0.164	0.723
LP	0.007	**−0.529**	**0.583**	−0.100	0.630
L F + P1	0.488	**−0.644**	**0.422**	**−0.336**	0.943
L F + P2	0.352	**−0.741**	**0.409**	**−0.317**	0.941
DH	0.686	**−0.503**	−0.072	**0.474**	0.953
HD1	**−0.775**	0.369	**0.489**	−0.002	0.976
HD2	**−0.775**	0.377	**0.486**	−0.004	0.980
HBD	0.350	−0.282	**0.468**	**0.728**	0.951
LTR	**0.859**	0.213	−0.114	−0.052	0.799
LPR	**0.826**	0.198	−0.239	−0.062	0.782
HL1	**−0.746**	0.447	**0.476**	0.014	0.983
HL2	**−0.748**	0.446	**0.473**	0.015	0.983
LN	**0.822**	0.233	0.016	0.092	0.739
WM	**0.730**	0.267	0.036	−0.082	0.612
BPL	**0.909**	0.074	0.155	−0.139	0.875
DFO	**0.896**	0.081	0.195	−0.099	0.857
DFH	**0.826**	0.070	0.355	−0.084	0.820
IPD	0.653	**0.559**	0.127	−0.072	0.760
ZW	**0.849**	0.281	0.137	0.024	0.819
WAO	**0.876**	0.125	0.260	0.047	0.852
Eigenvalues	12.13	3.13	2.32	1.08	
% of Variance	55.12	14.24	10.53	4.89	

Factor loadings for the first four principal components and extraction communalities for the 22 variables selected for species separation. For each component the variables showing high factor loadings are highlighted in bold.

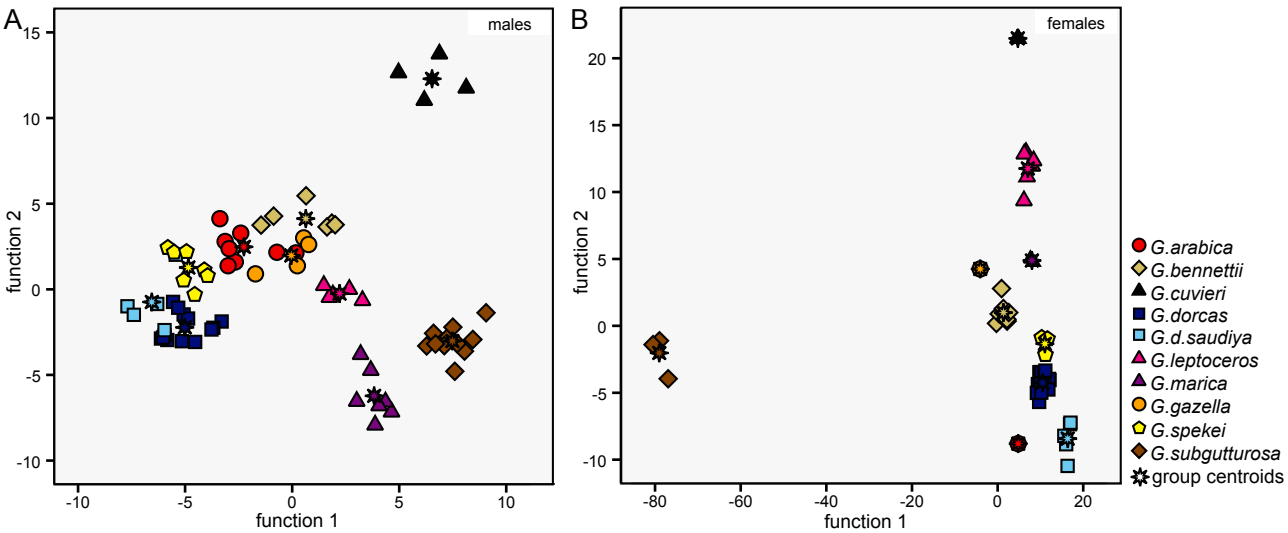

Figure 5. Bi-variate plots of specimen scores for discriminant functions. A, males, DA 2; B, females, DA 6.

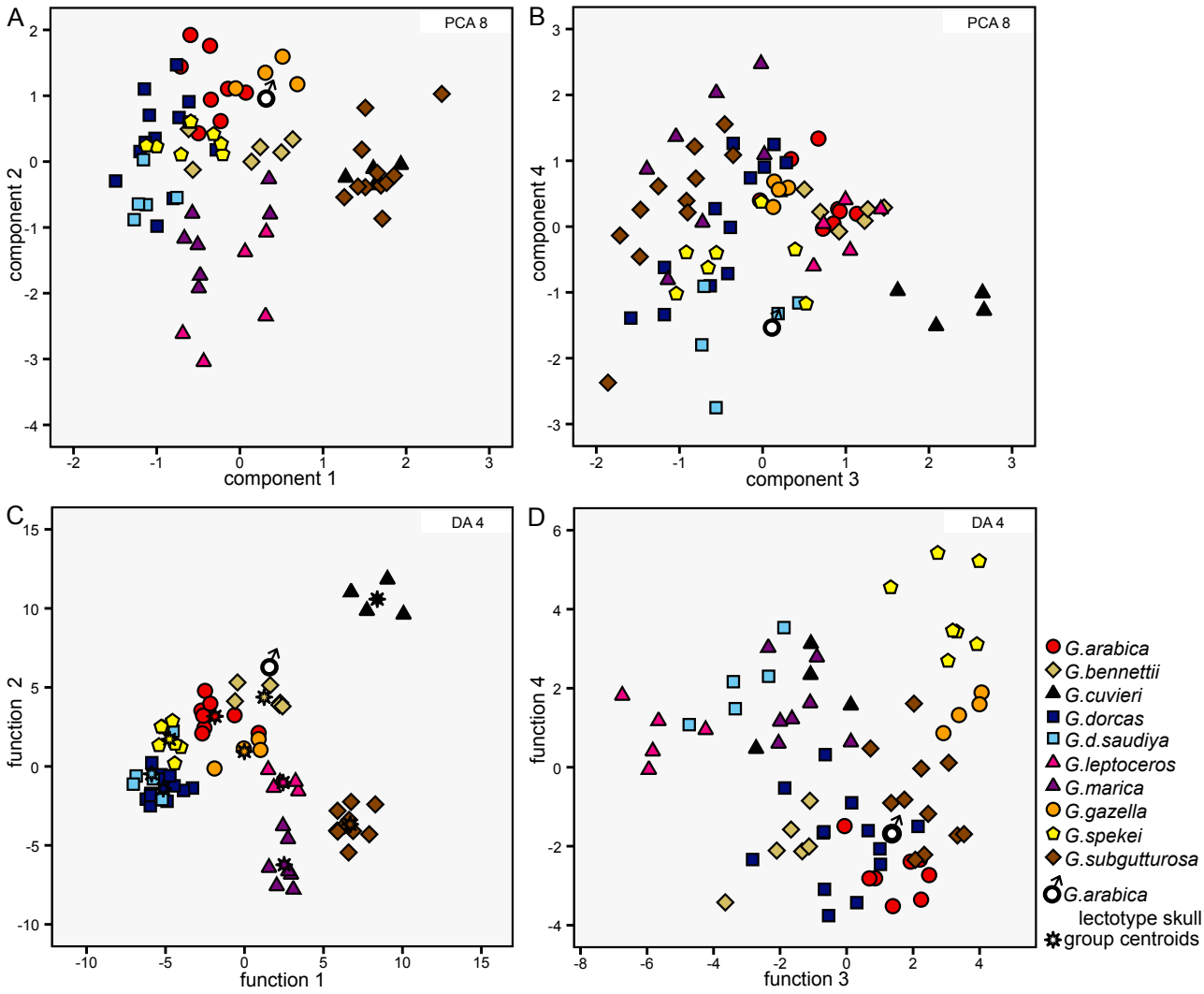

Figure 6. Classification of the male *Gazella arabica* type skull ZMB_MAM_2115. A, B, bi-variate plots of specimen scores for main components in PCA 8; C, D, bi-variate plots of specimen scores for discriminant functions in DA 4.

G. d. saudiya (Fig. 6B). In PCA 10 we included specimens from the Farasan Islands, as the *G. arabica* type skull was supposed to have been collected there. The Farasan males are distinctly smaller than the mainland gazelles, as shown by the first principal component in PCA 9 (Appendix S10A, B). All other components show no marked difference between the males of *G. gazella*, *G. arabica*, and '*G. g. farasani*'. The type of *G. arabica* does not cluster with the Farasan gazelles.

The discriminant analysis (DA 4) assigned the *G. arabica* type skull to *G. bennettii* (Fig. 6C, D). Its distance to the group centroid of *G. bennettii* [squared Mahalanobis distance (SMD = 108.86)] is significantly smaller ($P < 0.01$) than the distance to the centroid of *G. arabica* (SMD = 124.24). However, the specimen is relatively far from the group centroid of *G. bennettii*, more than six times the distance of all other specimens to their respective centroids. Including the captive *G. arabica* specimens (DA 5) does not change the classification of the *G. arabica* type (data not shown).

The female *G. arabica rueppelli* type skull ZMB_MAM_2108 is placed close to *G. dorcas* and *G. spekei* in the PCAs with the core data set (PCA 5, not shown) and including the captive *G. arabica* specimens (PCA 7, Fig. 7A, B). This placement did not change when specimens from Farasan were included (PCA 10, Appendix S10C, D). The Farasan gazelles are clearly separated from the mainland gazelles by their smaller size (component 1) and their very short horns (component 2).

The DAs find an ambiguous placement of the *G. arabica rueppelli* type skull as well: in DA 6 it is assigned to either *G. spekei* (SMD = 42.28) or *G. d.*

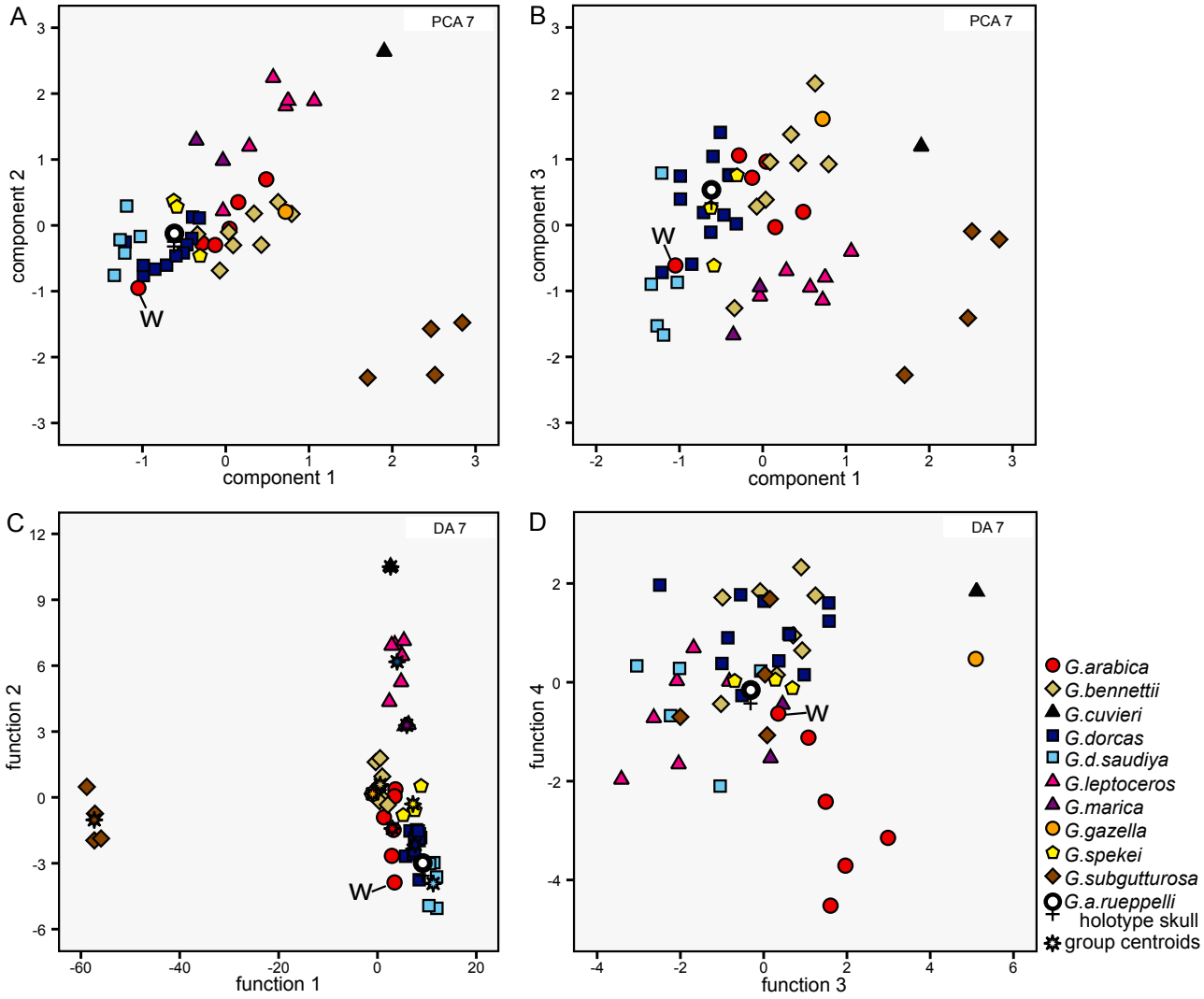

Figure 7. Classification of the female *Gazella arabica rueppelli* type skull ZMB_MAM_2108. A, B, bi-variate plots of specimen scores for main components in PCA 7; C, D, bi-variate plots of specimen scores for discriminant functions in DA 7. The wild *G. arabica* specimen is marked with 'W'.

saudiya (SMD = 42.64) (data not shown); in DA 7 including the captive *G. arabica* it is assigned to either *G. dorcas* (SMD = 6.85) or *G. d. saudiya* (SMD = 14.03) (Fig. 7C, D).

The two specimens housed at the SNF are classified in DA 8 (males) and DA 9 (females) (Appendix S11). The male specimen is assigned to *G. dorcas* (DA 8; SMD = 34.71); the distance to the centroid of *G. spekei* (SMD = 68.71) is significantly longer ($P < 0.001$). For the female the preferred group is also *G. dorcas*, with an SMD of 51.97 (DA 9). The distance to *G. spekei* (SMD = 80.71) is significantly longer ($P < 0.001$).

Phylogenetic analysis

For most museum specimens only the mitochondrial CR was successfully sequenced (Appendix S2). The phylogenetic analysis (Fig. 8) placed two specimens in *G. arabica*: the juvenile skin from the original type material, ZMB_MAM_2109, and the juvenile limb skeleton from Munich, ZSM AM/1063. The skin of the female type of *G. arabica rueppelli*, ZMB_MAM_2108, and the other skin from Berlin, ZMB_MAM_66104, were both placed in *G. gazella*, the same clade that also includes the male *G. arabica* type skull ZMB_MAM_2115. For the skin ZMB_MAM_2108 the sequence is identical to the sequence of the ZMB_MAM_2115 skull. The putative *G. a. rueppelli* type skull, ZMB_MAM_2108, however, was nested within *G. dorcas* (Fig. 8).

The combined analysis from the three mitochondrial regions (Fig. 8) shows a basal split into a 'Goitered gazelle clade' [posterior probability (PP) = 1]

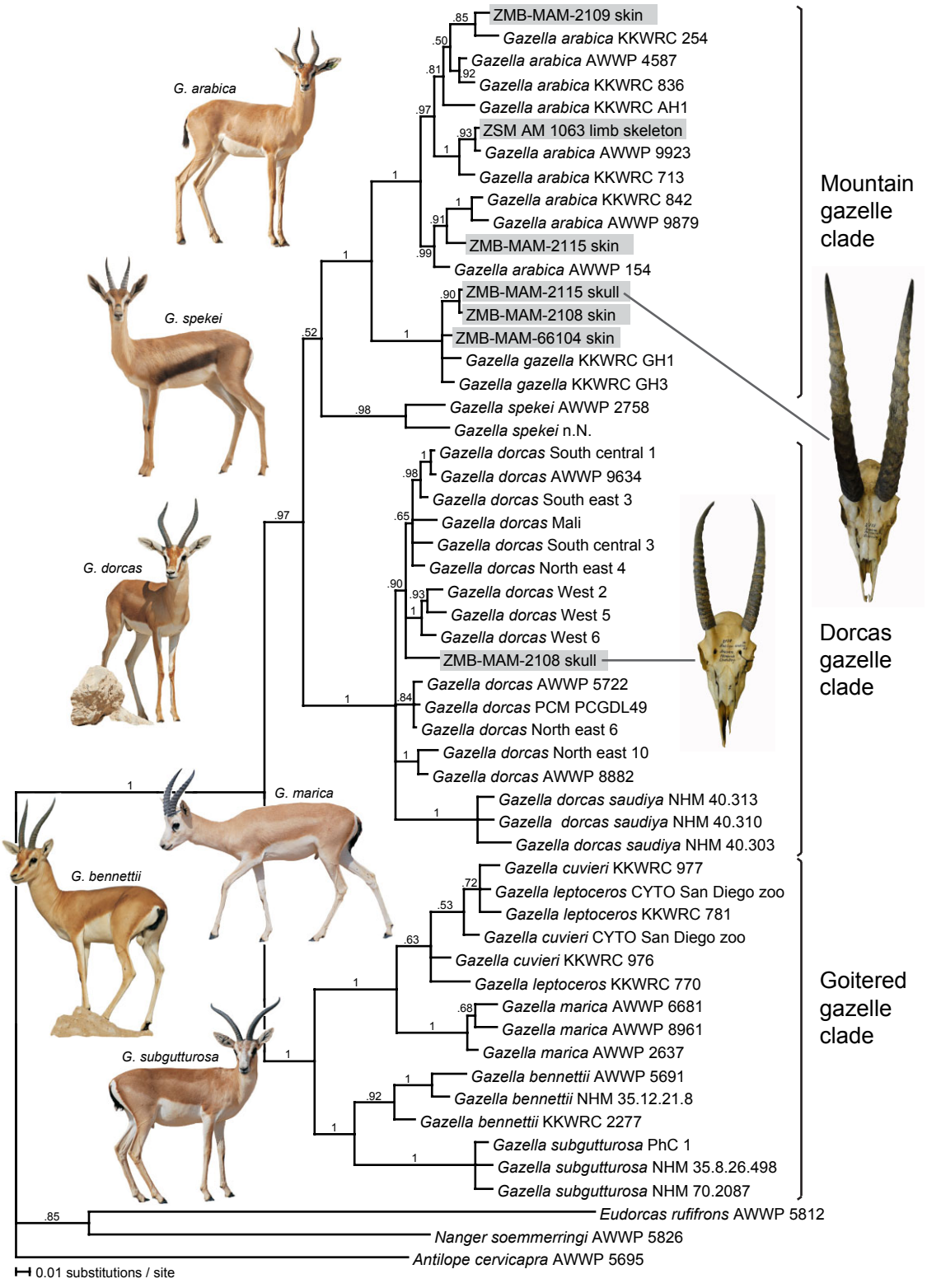

Figure 8. Phylogeny of the genus *Gazella*. Consensus tree of 5000 post burn-in trees from Bayesian analysis of mitochondrial control region, cytochrome b and 12S rRNA gene sequences. Branch labels are posterior probabilities. Museum specimens labelled '*G. arabica*' are highlighted with grey background. For GenBank accession numbers see Appendix S2. Photographs of living animals courtesy of S. Hammer (http://awwp.alwabra.com, photo gallery).

and a clade comprising Dorcas gazelles, Speke's gazelles and mountain gazelles (PP = 0.97). The relationships within the Goitered gazelle clade are well resolved, showing a sister-group relationship of *G. subgutturosa* + *G. bennettii* (PP = 1) and *G. marica* + *G. cuvieri* + *G. leptoceros* (PP = 1). *Gazella leptoceros* and *G. cuvieri* are not resolved as separate species. Within the 'mountain gazelle clade' (PP = 1), two reciprocally monophyletic lineages are present: *G. arabica* and *G. gazella*, both supported with PP = 1. Within the 'Dorcas gazelle clade' (PP = 1), *G. dorcas* and *G. d. saudiya* are not resolved as sister-taxa; instead, there is a basal polytomy with a monophyletic clade *G. d. saudiya* and several *G. dorcas* groups. The relationship of *G. spekei* as being the sister-species of the 'mountain gazelle clade' is insufficiently supported (PP = 0.52).

DISCUSSION

Usability of skull measurements in species differentiation

Our results show that most gazelle species are well characterized and easily identified by their skull dimensions. Even though DA 1 did not include horn length, horn width, or curvature, and males and females were all analysed together, the 13 variables (Table 6) were able to distinguish between the groups fairly accurately. This suggests that skull dimensions are as useful as horn measurements for differentiating between species.

Refined analyses, conducted separately for males and females, revealed that the most problematic species is *G. dorcas*. Dorcas gazelles have a large geographical range across northern Africa (Fig. 1). They are extremely variable in coloration, leading to the description of several subspecies: for example, *isabella* Gray, 1846, *pelzelni* Kohl, 1886, *osiris* Blaine, 1913, *massaesyla* Cabrera, 1928, and *beccarii* De Beaux, 1931. However, low genetic differentiation among the different groups of Dorcas gazelles, with samples stemming from sites as distant as Mali and the Sinai, may be indicative of high ongoing gene flow because of migration or a recent range expansion (Lerp *et al*., 2011). The morphometric data as well show no morphological differences for *G. dorcas* skulls from eastern and western Africa, or between sub-Saharan individuals and individuals from northern populations (data not shown). However, not all subspecies of *G. dorcas* were adequately represented in our study; for example, we could not include *G. d. massaesyla*. Hence, additional analyses with more specimens might show further differentiation that is not detectable with the current data set. Within *G. dorcas* substantial variability in horn morphology is observable which might have caused the misclassification of 11–42% of the specimens in various cross-validation analyses. Furthermore, the geographical range of *G. dorcas* is large and partly overlaps with that of *G. leptoceros*, *G. spekei*, and *G. gazella*. Therefore, misidentifications in the field should be taken into account. This was already demonstrated by the previously mentioned specimen BMNH 35.7.24.2 that appears to have been mislabelled. In the cross-validation analyses, four females and five males of *G. dorcas* were misclassified. Fortunately their geographical provenances were known, and in most cases misidentification was unlikely, as the proposed species identity was not congruent with the geographical origin of the specimen. However, one male specimen (BMNH 74.711) originated from Berbera, Somalia, could be a misidentified *G. spekei*. A discriminant analysis with changed group membership of this specimen slightly increased the overall cross-validation success from 88.6 to 91.4% (data not shown).

The use of skull measurements for species identification seems to be more difficult for females than males. Females are usually smaller than males and inter-specific differences are less pronounced, especially in horn characteristics. Furthermore, females are often under-represented in zoological collections as they do not make impressive trophies. Therefore, the overall number of female specimens included in our study was very small, especially for *G. cuvieri*, *G. gazella*, and wild *G. arabica* with only one specimen each. It is therefore not surprising that a discriminant analysis excluding these three species had a much higher cross-validation success, increasing from 67.4 to 75% and from 68.8 to 73.9% when captive *G. arabica* were included (data not shown).

In general, the identification of specimens with unknown identity and geographical provenance should be possible using skull measurements. Expansion of the data set with well-documented specimens from other collections will further improve its diagnostic capacity. The DAs allow for assigning new specimens to the respective groups and for testing the accuracy of our analyses. Even for incomplete specimens, a discriminant analysis with the remaining variables might provide helpful information, and will allow species identification in many cases, especially when combined with data on geographical origin of the specimen.

Similarity of closely related species

In several cases closely related species had most similar skull dimensions, i.e. they are close to each other in PCA. In phylogenetic analyses *G. d. saudiya*

and *G. dorcas* are usually resolved as closely related (Hammond *et al*., 2001; Lerp *et al*., 2011; Wacher *et al*., 2011) with *G. d. saudiya* being the Arabian form of *G. dorcas*. In PCA 4 and 6 for males, no single component (C1–5) separates the two groups. For females (PCA 5 and 7), *G. dorcas* and *G. d. saudiya* are separated by C1 and C4, as *G. d. saudiya* is smaller and has relatively longer horns than *G. dorcas* and because the horn distance in *G. d. saudiya* is shorter. In the DAs the cross-validations showed one male and one female *G. dorcas* that were misclassified as *G. d. saudiya*. It will probably be impossible to find an answer to the question about the species status of *G. d. saudiya*, as the taxon is fully extinct (Thouless *et al*., 1991; Habibi & Williamson, 1997). With the current data set, however, we suggest classifying *G. d. saudiya* as the Arabian subspecies of *G. dorcas*.

Further examples for similarity in closely related species are *G. marica* and *G. leptoceros*. At least the first two components of each analysis for males and females, respectively, do not differentiate between the two species. The cross-validations of the DAs, however, only revealed one case of misidentification, i.e. one female *G. leptoceros* is placed in *G. marica* (DA 6). Closely related to *G. leptoceros* and *G. marica* is *G. cuvieri*, which is larger than the other two taxa and formed a well-separated group in all morphometric analyses. This is not surprising as *G. cuvieri* is a highly specialized gazelle living in mountainous habitat. It is easily identifiable by skull measurements and the relatively dark coat colour. Nevertheless, complete mitochondrial DNA sequences neither support a monophyletic *G. cuvieri* nor support a monophyletic *G. leptoceros*; instead they form a paraphyletic assembly at the base of a monophyletic *G. marica* (Hassanin *et al*., 2012). The clade comprising *G. marica*, *G. leptoceros*, and *G. cuveri* was estimated to date back to the Middle Pleistocene by Hassanin *et al*. (2012). We therefore hypothesize that *G. cuvieri* evolved in a relatively short time with positive selection for its current morphology, whereas a signal for speciation on neutral markers, i.e. mitochondrial DNA, is not yet observable.

Morphological differences between *G. arabica* and *G. gazella*

The separation of *G. gazella* and *G. arabica* that was found in mitochondrial sequences (Wronski *et al*., 2010) and nuclear microsatellite markers (Lerp *et al*., 2013) was confirmed by the morphometric analyses, at least for males. The clear separation of the two groups by several principal components and the fact that we observed no case of misclassification in the discriminant analysis with all variables support the proposal of Wronski *et al*. (2010) to recognize each as a valid species.

For females the picture is more complicated. The one *G. arabica* female initially included in our study (BMNH 46.259) was collected in 1946 in Thuwal, a village in western Saudi Arabia. Its skull morphology is much more similar to *G. dorcas* than to the one female *G. gazella* specimen from Jerusalem (Fig. 4C, D). However, the six captive *G. arabica* females from KKWRC do not show a close similarity to the putative wild specimen, but are closer to *G. gazella* in PCA 7 (Fig. 7A, B). This could have several causes. First, there might be a difference in the way the measurements were taken, as the captive specimens were all measured by T.W. We tried to address this problem by excluding a few variables that seemed to be affected by measurement inconsistencies, but subtle differences are probably not detectable by comparing raw measurements. Secondly, captive breeding of the animals could have an impact on the morphology; for example, they could be larger because of increased food availability, or have a unique morphology due to out-breeding depression. A third possibility is that the putative wild *G. arabica* specimen actually belongs to *G. d. saudiya* or *G. dorcas*. Dorcas gazelles do not naturally inhabit Saudi Arabia, but gazelles were long held in captivity and frequently escaped into the wild (T. Wronski, pers. comm.). In any case, additional wild specimens of female *G. arabica* are needed to solve this contradiction.

Morphometric analyses of the *G. arabica* and *G. a. rueppelli* type skulls

In their molecular characterization of the *G. arabica* type material, Bärmann *et al*. (2013) assigned the skull ZMB_MAM_2115 to *G. gazella* on the basis of mitochondrial CR sequences. The morphometric analyses only partly support this result (Fig. 6): in PCA 8, the first three components, together accounting for approx. 76% of the variability of the data set, place the specimen in *G. gazella*. However, C4 (accounting for 5% of the variability) shows high similarity with *G. d. saudiya* and *G. cuvieri*. PCA 9, including the gazelles from Farasan – from where the *G. arabica* type skull was said to originate – did not uncover close similarity between them and the *G. arabica* type skull (Appendix S10A, B). The discriminant analysis (DA 4) assigned the skull to the Indian species *G. bennettii*. Perhaps Groves (1983) was right and the specimen indeed harbours pathological deformations. Another possibility is a hybrid origin, which can also affect skull proportions to a considerable degree (Ackermann *et al*., 2010). Hybridization is known to occur in captive gazelles (Rebholz & Harley, 1997; Hammond *et al*., 2001), and

as the origin of the specimen is not known, this cannot be ruled out.

The female skull ZMB_MAM_2108 was assigned to *G. dorcas* or *G. d. saudiya* in DA 7 (Fig. 7), whereas the analysis excluding the captive *G. arabica* gazelles (DA 6) favoured a placement either in *G. d. saudiya* or *G. spekei*. Both *G. spekei* and *G. d. saudiya* show considerable morphological overlap with *G. dorcas*, but do not inhabit the Sinai Peninsula where the specimen is supposed to have originated. *Gazella arabica* was not indicated as the taxon for the female type skull in any of the discriminant analyses.

The two specimens from the SNF, classified as *G. arabica rueppelli* by Neumann (1906) and listed as *G. dorcas* in the museum catalogue, are grouped with *G. dorcas*. Both specimens are comparatively far away from the group centroid, possibly because of measurement inconsistencies. These results for the three specimens assigned to *G. arabica rueppelli* by Neumann (1906) confirm the findings of Groves (1983) who synonymized *G. arabica rueppelli* with *G. dorcas* based on skull measurements (but see Results below).

Identification of '*G. arabica*' specimens using mitochondrial DNA sequences

The mitochondrial CR was suitable for identifying old gazelle museum specimens. Although sometimes only a short sequence was obtained (234 bp), it was sufficiently diagnostic for assigning the specimens to their respective species. The results of the morphometric analysis for the putative female type skull of *G. arabica rueppelli* ZMB_MAM_2108, placing it in *G. dorcas*, are confirmed by the molecular analyses (Fig. 8). However, the corresponding skin ZMB_MAM_2108 is placed within *G. gazella*. If the female skull ZMB_MAM_2108 is the original skull collected by Hemprich and Ehrenberg, there was – as in the case of the male type (Bärmann *et al*., 2013) – a mistake in assigning skull and skin to the same individual. Another possibility is that the original female type skull was accidentally substituted by a *G. dorcas* skull in later years. The difference in skull measurements between the original species description (horn length: 6 inches = 15.2 cm) and the actual specimen (18.4 cm) is striking. Hemprich and Ehrenberg did collect six *G. dorcas* females during their expedition (Museum für Naturkunde Berlin, Historische Bild- und Schriftgutsammlungen, SI, Hemprich & Ehrenberg, Blatt 76), so confusion is possible. Neumann (1906) described only the skin ZMB_MAM_2108 when he erected the subspecies *G. arabica rueppelli*. Therefore, we suggest excluding the skull from the type, according to the ICZN Code (Chapter 16 Article 73.1.5). Consequently, *G. arabica rueppelli* would be a junior synonym of *G. gazella* Pallas, 1766 and not of *G. dorcas* as suggested by Groves (1983).

Of the other '*G. arabica*' specimens, the skin ZMB_MAM_66104 was assigned to *G. gazella* based on several DNA markers. This specimen originated from Berseba (Be'er Sheva) in Israel, which is well within the known geographical range of *G. gazella*. The two other specimens – the juvenile skin ZMB_MAM_2109 and the juvenile limb skeleton ZSM-Am-1063 – belong to the same taxon as the *G. arabica* lectotype skin (ZMB_MAM_2115). ZSM-Am-1063 was collected near Aqaba in Jordan, thus in the north-western range of *G. arabica* (Mendelssohn, Groves & Shalom, 1997; Wronski *et al*., 2010). ZMB_MAM_2109 is part of the original *G. arabica* material collected by Hemprich and Ehrenberg and was assumed to have originated from Sinai. However, we have some concerns about this assumption. The collectors themselves did not give any detailed information on the geographical origin of the specimens in their species description (Hemprich & Ehrenberg, 1828). The idea that the female ZMB_MAM_2108 and the juvenile ZMB_MAM_2109 were collected in Sinai is based on letters that Hemprich and Ehrenberg wrote during the expedition (Stresemann, 1954), and in which they report to have shot two specimens in Sinai and one on Farasan. Despite the two 'individuals' having consecutive catalogue numbers, the fact that ZMB_MAM_2108 is composed of a *G. dorcas* skull and a *G. gazella* skin and that ZMB_MAM_2109 belongs to *G. arabica* falsifies the assumption that they were mother and fawn.

We checked the original lists of specimens that were shipped to Berlin by Hemprich and Ehrenberg from 1823 to 1826 (Museum für Naturkunde Berlin, Historische Bild- und Schriftgutsammlungen, SI, Hemprich & Ehrenberg, Blatt 113, 126, 182, 188, 189). Three shipments contained specimens that the collectors referred to as *Antilope arabica*: one male skull arrived with the eighth shipment in May 1824 (specimens collected in Arabia and Egypt in 1823); two skins and one skeleton (sex not specified) arrived with the ninth shipment in April 1825 (specimens collected in Arabia and Syria in 1824); and one adult and one juvenile individual (objects not specified) arrived with the tenth shipment in April 1826 (specimens collected in Arabia and Abyssinia in 1825).

The first skull that was sent with the eighth shipment was probably lost, although it might be one of the specimens measured by Hemprich and Ehrenberg in Arabia. The two skins from the ninth shipment could be the skins ZMB_MAM_2115 (probably from Arabia) and ZMB_MAM_2108 (probably from Syria). The skull ZMB_MAM_2115 (probably also from Syria)

could be the skull belonging to the skeleton from the same shipment, which would account for the erroneous assumption that it belongs to the male skin. The postcranial skeleton is probably lost, as it was never mentioned again. The juvenile specimen sent with the tenth shipment must be the skin and mandible ZMB_MAM_2109 (probably from Arabia/Farasan). The adult from the same shipment might be the skull ZMB_MAM_2108 (probably from Abyssinia), or the original specimen was lost and erroneously replaced by ZMB_MAM_2108.

CONCLUSIONS

This study provides a guide for identifying museum skull specimens of *Gazella* based on linear measurements. For males, in particular, the morphometric analyses accurately differentiate between the taxa that were resolved as monophyletic groups in molecular phylogenetic analyses. One shortcoming of the study is that although we have complete species overlap between the morphometric and the molecular analyses, only a handful of specimens were sampled for both types of data. Museum specimens are not often sequenced, and samples for molecular analyses are usually taken from living specimens for which the skull is not (or at least not immediately) available. DNA sequences from old museum specimens, preferably the holotypes, would be useful for evaluating the overlap of molecular and morphological species differentiation found in our study. In addition, captive specimens that were sequenced for at least one mitochondrial marker could be included in the morphological data set when the respective animals die. This would be especially interesting for investigating the status of the numerous described subspecies of *G. arabica* (i.e. *acaciae*, *cora*, *muscatensis*).

We can clearly separate *G. gazella* and *G. arabica* based on morphological and genetic data. This agrees with the findings of Lerp *et al*. (2013) who found no signs for recent gene flow in nuclear microsatellite markers. Therefore, species status should be assigned to these two clades.

The only case of non-agreement between molecular and morphometric data is the case of *G. leptoceros* and *G. cuvieri*. The two species form clearly separate clusters in the morphometric analyses, but are not resolved as monophyletic in phylogenetic analyses using mitochondrial markers.

The ambiguous phylogenetic position of *G. spekei*, as more closely related to *G. gazella*, or *G. dorcas*, or both, is reflected in the intermediate position of this species in the morphometric analyses. In these two cases, and for investigating the status of the subspecies of *G. arabica*, further research including nuclear markers is needed to investigate phylogenetic relationships and taxonomic classification of gazelles.

The holotype of *G. arabica rueppelli* was found to consist of specimens from two different species: a *G. dorcas* skull (confirmed by two molecular markers and morphometric analysis) and a *G. gazella* skin (from CR sequence). According to the ICZN code, one of the specimens should be excluded from the type. We recommend excluding the skull, as its horn length casts doubt on the identity of this specimen. Therefore, *G. a. rueppelli* is a junior synonym of *G. gazella*.

We further synonymize *G. karamii* with *G. marica* based on morphometric similarities. However, as the skin of the *G. karamii* type (ZMB_MAM_41400) is unusually dark compared with *G. marica*, we plan to gain molecular sequences from the type skull and skin of *G. karamii* in the near future to evaluate this hypothesis of synonymy.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. Geographical origin of gazelle specimens used in the morphometric analyses. *Molecular data available for this specimen; specimens highlighted in bold are holotypes. BMNH, Natural History Museum, London; KKWRC, King Khalid Wildlife Research Center, Saudi Arabia; SNF, Senckenberg Forschungsinstitut und Naturmuseum, Frankfurt; UMZC, University Museum of Zoology Cambridge; ZMB, Museum für Naturkunde, Berlin. The origin is given as written on the label; modern names of the location and the state they are located in are written in parentheses. Geographical coordinates were taken from online maps, mostly from http://www.getamap.net.

Appendix S2. GenBank accession numbers; *new sequences from this study. AWWP, Al Wabra Wildlife Preservation; BMNH, Natural History Museum London; KKWRC, King Khalid Wildlife Research Center; PCM, Powell Cotton Museum; ZMB, Museum für Naturkunde Berlin.

Appendix S3. PCA 2, males. Factor loadings and extraction communalities for all variables; abbreviations correspond to Table 3 and Figure 2. For each component the variables showing high factor loadings are highlighted in grey.

Appendix S4. PCA 3, females. Factor loadings for the first seven prinicipal components and extraction communalities for all variables. [1]Excluded as all females have MLH = HL1.

Appendix S5. Discriminant analysis 2, males. (a) Unstandardized canonical discriminant function coefficients. (b) Functions at group centroids.

Appendix S6. Discriminant analysis 6, females. (a) Unstandardized canonical discriminant function coefficients. (b) Functions at group centroids.

Appendix S7. Combined analysis, Nexus file incl. matrix and commands for MrBayes.

Appendix S8. Comparison of mean intraspecific variance in skull measurements between *G. gazella* and *G. arabica*.

Appendix S9. Bi-variate plots of specimen scores for discriminant functions for DA 3: males including captive *G. arabica*.

Appendix S10. Classification of the *G. arabica* type material in PCA including specimens from Farasan. A, B, bi-variate plots of specimen scores for main components in PCA 9 including ZMB_MAM_2115 (type skull of *G. arabica*); C, D, bi-variate plots of specimen scores for main components in PCA 10 including ZMB_MAM_2108 (type skull of *G. arabica rueppelli*).

Appendix S11. Classification of the specimens from SNF. A, B, male (SNF 15863), bi-variate plots of specimen scores for discriminant functions in DA 8; C, D, female (SNF 15963), bi-variate plots of specimen scores for discriminant functions in DA 9.