Environ Biol Fish (2018) 101:1053–1065
https://doi.org/10.1007/s10641-018-0758-7

The morphological diversity of *Garra barreimiae* [Teleostei: Cyprinidae]

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Received: 15 September 2017 / Accepted: 2 April 2018 / Published online: 14 April 2018
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Abstract *Garra barreimiae* (Fowler and Steinitz, Bull Res Counc Isr 5B:262–289, 1956) is a freshwater fish that lives throughout the Hajar Mountains of Oman and the United Arab Emirates. Previously, four different genetic clades (West clade, Central Clade, North clade and East clade) have been identified within this species. This study observes morphological differences between these clades using morphometrics and meristics as well as micro CT imaging, further strengthening the assumption that the taxon of *G. barreimiae* should be restricted to one of the genetic clades detected. Although many morphometric and meristic characteristics are highly variable within the clades, the West clade fits the original description of *G. barreimiae* and is distinguishable by its higher number of fifteen to seventeen gill rakers on the lower limb of the first gill arch (compared to the other clades with ten to fourteen gill rakers), its seven branched pelvic fin rays (in contrary to the other clades with eight branched pelvic fin rays) and its number of vertebrae, with typically nineteen precaudal and one intermediate vertebrae, compared to eighteen precaudal and two intermediate vertebrae in the Central and North clade and seventeen precaudal and two intermediate vertebrae in the East clade.

Keywords *Garra barreimiae* · Labeonini · Micro CT · Morphometrics · Oman · Hajar Mountains

Introduction

Labeonine cyprinids of the genus *Garra* (Hamilton, 1822) are widespread in tropical and subtropical Asia, Africa and the Middle East (Menon 1964; Yang and

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Mayden 2010). Following species of *Garra* occur on the Arabian Peninsula: *Garra buettikeri* (Krupp 1983), *Garra dunsirei* (Banister 1987), *Garra lautior* (Banister 1987), *Garra longipinnis* (Banister and Clarke 1977), *Garra mamshuqa* (Krupp 1983), *Garra sahilia* (Krupp 1983), *Garra smarti* (Krupp and Budd 2009), *Garra tibanica* (Trewavas, 1941) and *Garra barreimiae* (Fowler and Steinitz 1956) (Freyhof et al. 2015). Only recently *Garra barreimiae gallagheri* was raised to species level and *Garra sindhi* has been described from the Dhofar region in Oman (Lyon et al. 2016). New *Garra* species have also been described from areas adjacent to the Arabian Peninsula, including *Garra jordanica* (Hamidan et al. 2014), *Garra mondica* (Sayyadzadeh et al. 2015), *Garra lorestanensis* (Mousavi-Sabet and Eagderi 2016), *Garra tashanensis* (Mousavi-Sabet et al. 2016) and *Garra amirhosseini* (Esmaeili et al. 2016), demonstrating, that more undescribed species might be found in the area. This is especially true for the Arabian Peninsula, where most freshwater fish now live in isolated refuges. These fish live in very dry and often unstable habitats only inhabited by a restricted number of fish species which have adapted to massive changes in water level, temperature and therefore oxygen content as well as availability of food (Krupp 1988).

Garra barreimiae is one of these species having a very fragmented distribution area with many small and isolated populations. The species is widespread in Northern Oman and the United Arab Emirates (Banister and Clarke 1977; Krupp 1983; Freyhof et al. 2015). Fowler and Steinitz (1956) described *G. barreimiae* from Buraimi (also Al Buraimi, Baraimi, Barreimi) in Oman. Later, two additional subspecies were described: *Garra barreimiae shawkahensis* (Banister and Clarke 1977) from the United Arab Emirates and *Garra barreimiae gallagheri* (Krupp 1988) from Wadi Bani Khalid in Oman. In 1980 Dunsire and Gallagher reported a subterranean population of *G. barreimiae* from the Al Hoota Cave close to Nizwa (Banister 1984). This troglomorphic population seems to be of recent origin less than one million years ago (Kruckenhauser et al. 2011), however occasional gene flow between the cave population and the surface population seems to take place (Kirchner et al. 2017). Although many populations of *G. barreimiae* are very isolated from each other and inhabit different drainage systems around the Hajar Mountains (Kruckenhauser et al. 2011; Freyhof et al. 2015), in former times during intermittent pluvial intervals, the river network was much denser, so populations might have been more connected. Kruckenhauser et al. (2011) were the first to use molecular genetic methods to assess genetic differentiation among populations of *G. barreimiae*. On the basis of DNA sequences of the mitochondrial control region (mt CR) two well differentiated clades were found, one in the surroundings of the Al Hoota cave, with a separated subclade which consists of individuals from Wadi Bani Khalid (= *G. gallagheri*), and one in the northeast of the Hajar Mountains. Subsequent genetic analysis (Kirchner et al. 2017) distinguished at least four different clades with p-distances between 6% and 10%, as measured in a section of the mt *cytochrome c oxidase subunit 1* gene (*COI*). These clades are geographically separated but their distribution does not reflect the distribution of the subspecies previously described. Furthermore, including *G. rufa* into the tree revealed that *G. barreimiae* in the sense of Kruckenhauser et al. (2011) might not be monophyletic. With the newly discovered and strikingly different mt clades, which may indicate cryptic species, the morphological characteristics of these clades ought to be examined.

Water extraction in Oman has increased drastically in the last three decades (Al-Rawahi et al. 2014) which poses a threat to isolated fish populations. *G. barreimiae* is widely distributed throughout the Northern Oman, but since many populations are isolated and potentially vulnerable to water extraction (Freyhof et al. 2015) it appears even more urgent to study potentially cryptic species within *G. barreimiae*.

This study is part of a broader phylogenetic analysis of the *G. barreimiae* complex and aims to reveal whether morphological differences can be found between previously determined genetic clades using morphometrics and meristics as well as micro CT imaging, which is used for the first time to compare the osteology within *G. barreimiae*.

Material and methods

Eighty-seven specimens of *G. barreimiae*, collected at 20 localities in Oman (Fig. 1; Table 1), were included in the morphological analysis. Specimens were captured with fishing nets, immediately anesthetized with clove oil and fixed in 80% ethanol. The individuals, which are part of the collection of the Natural History Museum Vienna (NHMV), represent four mitochondrial (mt)

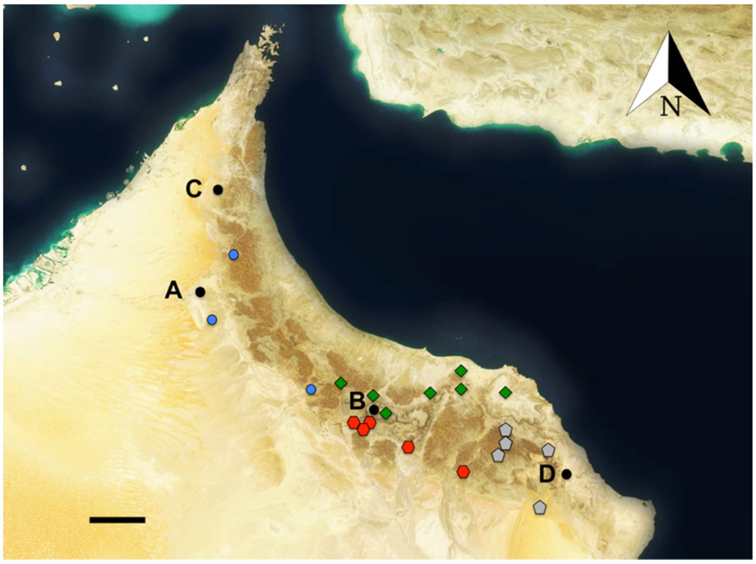


Fig. 1 Sampling sites of specimens used in morphological analysis and sites of original descriptions across the Al Hajar Mountains, Oman. West clade: blue; Central clade: red; North clade: green; East clade: grey; A: Buraimi, type locality of *Garra barreimiae* (Fowler and Steinitz 1956); B: Wadi Sahtan; assigned to *Garra barreimiae barreimiae* by Banister and Clarke (1977); C: Wadi Shawkah, type locality of *Garra barreimiae shawkahensis* (Banister and Clarke 1977); D: Wadi Bani Khalid; type locality of *Garra gallagheri* (Krupp 1988), not included in this study. Scale bar: 50 km

clades (Kruckenhauser et al. 2011; Kirchner et al. 2017): West clade ($n = 19$), Central clade ($n = 25$), North clade (n = 19) and East clade ($n = 24$).

Table 1 Sampling sites for each clade with corresponding coordinates as decimal degrees [longitude, latitude] and number of morphologically examined specimens (n)

Clade	Sampling Site	Coordinates	n
West clade	Al Hayyal	[23.43, 56.74]	7
	Al Juwayf	[24.54, 56.10]	7
	Hafeet	[23.99, 55.84]	5
Central clade	Al Hamra	[23.11, 57.28]	1
	Birkat al Mawz	[22.92, 57.66]	2
	Lizq	[22.71, 58.17]	4
	Misfat al Abriyeen	[23.14, 57.31]	10
	Wadi an Nakhar	[23.17, 57.20]	8
North clade	Wadi al Mayh	[23.40, 58.52]	4
	Wadi Hammam	[23.37, 57.82]	4
	Wadi Hat	[23.18, 57.41]	1
	Wadi Khawd	[23.57, 58.11]	5
	Wadi Mansah	[23.40, 58.11]	1
	Wadi Sahtan	[23.38, 57.30]	2
	Wadi Shkar	[23.45, 57.06]	2
East clade	Al Hajir	[22.98, 58.50]	7
	Wadi Dayn	[22.89, 58.42]	2
	Wadi Khabbah 1	[22.91, 58.87]	7
	Wadi Khabbah 2	[23.11, 58.50]	6
	Mintirib	[22.43, 58.79]	2

Twenty-five measurements (Table 2) were taken at least two times with a digital calliper from point to point to the nearest 0.1 mm. Methods for counts and measurements largely followed Armbruster (2012). Due to the condition of preserved specimens (crooked fixation, mutilated fins, cuts in the ventral region) not all measurements and counts could be taken from all specimens. Measurements were described in percent of standard length unless stated otherwise. Measurements were tested for normality (Shapiro Wilk's test) and equality of variance (Levene's Test) before conducting One-way ANOVA. Kruskal-Wallis test was used to test for significant differences in non-parametric data. Tukey's honest significant difference test was used as post-hoc test after ANOVA. Principal component analysis (PCA) and linear discriminant analysis (LDA) were conducted using the following characters, which were chosen because of their parametric nature and significant differences between the mt clades: anal fin - caudal fin, head length, head width (in % of head length), internostral distance (in % of head length), orbit diameter (in % of head length), snout length (in % of head length), pectoral fin - pelvic fin, pelvic fin - anal fin and prepelvic length. All tests and analyses were conducted using Past3 (Hammer et al. 2001). Violin plots were constructed using Rstudio version 1.0.136 and the R package ggplot2 (R Development Core Team 2015; Wickham 2009).

Meristic counts of gill rakers of the lower limb of the first gill arch (ceratobranchial), simple and branched dorsal fin rays, anal fin rays, pectoral fin rays, pelvic fin rays, caudal fin rays as well as lateral line scales and

Table 2 Morphometric data of the four mitochondrial (mt) clades

	West clade				Central clade				North clade				East clade			
	mean	min	max	SD	mean	min	max	SD	mean	min	max	SD	mean	min	max	SD
SL (mm)	40.1	28.3	54.7	8.8	35.5	26.0	50.9	6.9	41.5	27.5	61.8	9.0	35.4	25.4	50.9	6.8
% SL																
Anal fin - Anus	4.8	3.4	6.4	0.9	4.2	3.4	5.0	0.5	5.2	3.8	6.5	0.7	5.6	4.4	6.6	0.6
Anal fin - Caudal fin	21.6	19.0	25.0	1.7	20.2	17.6	22.3	1.1	20.9	18.8	24.4	1.3	20.9	19.0	23.6	1.3
Anal fin height	15.9	14.4	17.9	0.9	16.2	14.3	17.7	0.8	16.7	14.5	19.4	1.2	16.0	13.9	18.7	1.3
Body depth	23.0	20.9	25.7	1.7	24.8	21.7	28.7	1.6	25.2	21.4	29.2	2.1	22.6	19.9	24.9	1.4
Caudal peduncle depth	12.1	11.1	13.1	0.7	12.4	11.4	13.2	0.5	13.1	11.8	13.8	0.6	12.4	11.7	13.0	0.3
Caudal peduncle length	15.1	12.9	17.5	1.4	13.8	11.9	15.6	0.8	14.6	12.3	16.9	1.1	14.1	12.6	16.3	1.0
Dorsal fin height	21.3	19.9	23.7	1.2	20.6	19.0	22.9	1.1	22.2	20.3	28.0	1.6	21.9	20.2	25.6	1.3
Head depth	16.6	14.8	19.0	1.0	17.8	16.0	19.5	0.9	17.4	16.1	18.8	0.9	16.9	15.7	18.4	0.8
Head length	23.9	21.2	27.2	1.4	25.3	22.0	27.1	1.2	24.2	21.9	27.5	1.5	24.8	21.7	27.3	1.3
Head width	18.8	16.6	21.4	1.2	19.5	16.2	21.9	1.4	18.7	16.7	21.0	1.3	18.0	16.4	19.4	0.9
Internostral distance	7.2	5.8	8.4	0.6	7.8	6.9	9.0	0.5	7.7	6.4	8.6	0.5	7.8	6.9	8.5	0.4
Interorbital width	11.0	9.9	11.9	0.7	12.7	11.9	14.3	0.6	11.5	10.6	12.4	0.6	12.1	11.2	13.0	0.4
Pectoral fin height	20.2	18.7	23.7	1.2	20.8	17.9	24.8	1.8	20.2	17.2	22.8	1.4	19.7	17.4	21.9	1.0
Pectoral fin - Pelvic fin	33.0	30.2	35.1	1.3	30.9	27.5	33.8	1.6	31.7	28.6	33.8	1.4	31.3	29.4	34.1	1.4
Postorbital length	9.23	7.8	10.6	0.8	10.2	8.6	12.0	0.8	9.1	8.0	10.5	0.6	9.1	7.8	11.1	0.8
Postdorsal length	35.8	32.0	38.4	1.8	34.8	31.6	37.8	1.4	35.9	33.4	38.5	1.7	35.1	32.2	39.0	1.6
Preanal length	78.6	76.0	81.1	1.5	79.3	77.4	81.3	1.0	78.6	75.3	81.2	1.4	79.0	76.0	81.0	1.2
Predorsal length	51.6	49.5	54.5	1.4	51.9	50.5	54.2	1.1	50.9	49.0	53.6	1.2	50.8	47.7	53.3	1.4
Prepelvic length	56.0	52.2	59.2	2.0	55.6	52.9	58.2	1.4	54.6	52.2	58.5	1.4	54.7	51.4	57.5	1.4
Pelvic fin - Anal fin	22.5	20.3	25.1	1.6	23.3	20.5	25.1	1.2	23.6	22.1	26.4	1.2	24.0	19.7	26.8	1.6
Pelvic fin - Caudal fin	44.2	39.9	48.5	1.9	43.9	40.7	46.4	1.3	45.0	39.9	47.5	1.8	45.0	41.3	49.0	1.6
Pelvic fin height	16.6	15.2	18.8	1.0	16.8	15.2	19.2	1.0	16.9	15.0	19.8	1.2	16.8	14.5	18.4	1.0
Snout length	10.5	8.2	12.2	1.0	10.3	9.0	12.1	1.0	10.8	9.7	12.5	0.8	10.6	9.2	12.7	1.0
% HL																
Head depth	69.4	62.4	74.8	3.1	70.3	62.4	74.7	2.9	72.4	63.2	78.2	4.0	68.1	63.6	78.6	3.4
Head width	78.7	70.0	90.2	5.4	77.2	64.8	89.9	5.2	77.6	70.3	84.2	4.0	72.8	65.4	79.6	3.2
Internostral distance	30.2	27.3	33.7	1.6	30.8	27.0	34.8	1.7	31.7	27.2	34.7	1.9	31.4	26.9	34.1	1.7
Interorbital width	46.4	41.7	50.2	2.9	50.1	46.3	54.9	2.3	48.0	38.5	55.7	4.4	49.0	44.0	54.0	2.6
Orbit diameter	20.8	19.5	23.5	1.1	21.4	18.9	25.0	1.6	22.1	19.4	25.0	1.6	22.7	20.4	24.7	1.3
Postorbital length	38.7	33.6	43.9	2.8	40.4	36.3	46.6	2.6	38.0	32.7	43.2	3.4	36.6	34.1	41.5	1.9
Snout length	44.1	38.1	49.0	3.2	40.6	35.1	46.3	3.4	44.7	36.9	48.5	2.2	42.9	36.9	48.5	3.0

numbers of scale rows were taken. Vertebrae were counted as precaudal, intermediate and caudal vertebrae, without including the urostyle. Precaudal vertebrae have no closed haemal arches and caudal vertebrae have a joined parapophyses forming an arch. The parapophyses of the ultimate precaudal vertebrae may be joined by a bony bridge, forming a narrow arch, but their ends are not joined (Ahnelt and Duchkowitsch 2004). In this case these vertebrae were counted as intermediate vertebrae. Since the posterior two rays of the dorsal fin and the anal fin are branches of the same ray, they were counted as 1½. Unbranched dorsal fin rays and unbranched anal fin rays could only be counted via micro CT images.

For micro CT scanning, 30 individuals were used (West clade: $n = 7$; Central clade: $n = 7$; North clade: $n = 8$; East clade: $n = 8$). The fish were mounted vertically in plastic tubes containing 70% ethanol. The diameter of sample holder tubes was 12 mm. For each tube two fish were mounted in opposite direction. Micro CT scans were acquired using a Scanco μCT35 (SCANCO Medical AG, Brüttisellen, Switzerland) at 70 kVp source voltage and 114 μA intensity. Projections were recorded with 420 ms integration time (camera binning = 2) and an angular increment of 0.36°. Reconstructed slices measured 1024 × 1024 pixel, and isotropic voxel resolution of reconstructed volumes was 12 μm. Image processing and analysis was done using Fiji (Schindelin et al. 2012; Fiji ImageJ version 2.0.0) and Amira 6 (FEI Visualization Sciences Group, Mérignac Cédex, France).

Morphometrics

Morphometric measurements of the preserved specimens of *G. barreimiae* are given for each clade in Table 2. All measurements show overlaps between all of the mt clades of *G. barreimiae*, although many measurements show significant differences (Table 3). A probability value of less than 0.05 of the statistical tests was considered significant. The following measurements are not normally distributed and are thus not suitable for ANOVA, Tukey's HSD test and PCA: body depth (West clade), dorsal fin height (North clade), head depth in % of head length (East clade and Central clade), predorsal length (Central clade), pectoral fin length (West clade). The following measurements have no equality of variance and are thus not suitable for ANOVA, Tukey's HSD and PCA: pectoral fin length, caudal peduncle length, interorbital width in % of head length, anal fin - anus, postorbital length in % of head length, caudal peduncle depth. Parametric measurements which show significant differences between the clades in ANOVA are plotted in Fig. 2.

Results for Levene's test, ANOVA and Kruskal-Wallis test are given in the appendix. Results for Tukeys HSD test are shown in Table 3 based on the results of ANOVA.

In the PCA (Fig. 3) PC1 explains 51.65% of the variance and PC2 explains 19.61% of the variance. Ellipses with 75% tolerance are used in the plot. The four different clades are widely overlapping. In the LDA Axis 1 (Fig. 4) explains 53.19% of the variance. Axis 2 explains 41.16% of the variance. The four clades are overlapping but also differentiated to some extent, especially the West clade and the Central clade. 75.86% (63.22% Jackknife tested) of all specimens are correctly classified in the confusion matrix.

Meristics

Vertebral numbers, positions of dorsal fin and anal fin pterygophores, pharyngeal teeth and unbranched dorsal and anal fin rays were counted using micro CT. Other structures were counted using light microscopy. Meristics were summarized in Table 4. Meristics of gill rakers, vertebrae and branched pelvic fin rays can be used to distinguish the West clade from the other clades. Intermediate vertebral numbers: one in West clade (Fig. 5) versus mostly two in the other clades (Fig. 6), mostly i,7 pelvic fin rays in the West clade and i,8 in the

Table 3 Tukey's honest significant difference test of parametric morphometric measurements of the four clades depicts which clades differ significantly from one another in specific measurements

West Central	West North	West East	Central East	Central North	North East
AFCDF 0.0069	INTN%HL 0.0152	HW%HL 0.0005	BD 0.0006	HL 0.0266	BD 0.0002
HL 0.0036	OD%HL 0.0159	OD%HL 0.0003	HW%HL 0.0125	SNL%HL 0.0003	HW%HL 0.0049
PCTPV 0.0002	PCTPV 0.0217	PCTPV 0.002	OD%HL 0.0158		
SNL%HL 0.0016	PRPV 0.019	PRPV 0.038			
	PVFAF 0.0464	PVFAF 0.0045			

AFCDF: anal fin - caudal fin; BD: body depth; HL: head length; HW%HL: head width in % of head length; INTN%HL: internostral distance in % of head length; OD%HL: orbit diameter in % of head length; PCTPV: pectoral fin - pelvic fin; PRPV: prepelvic length; PVFAF: pelvic fin - anal fin; SNL%HL: snout length in % of head length

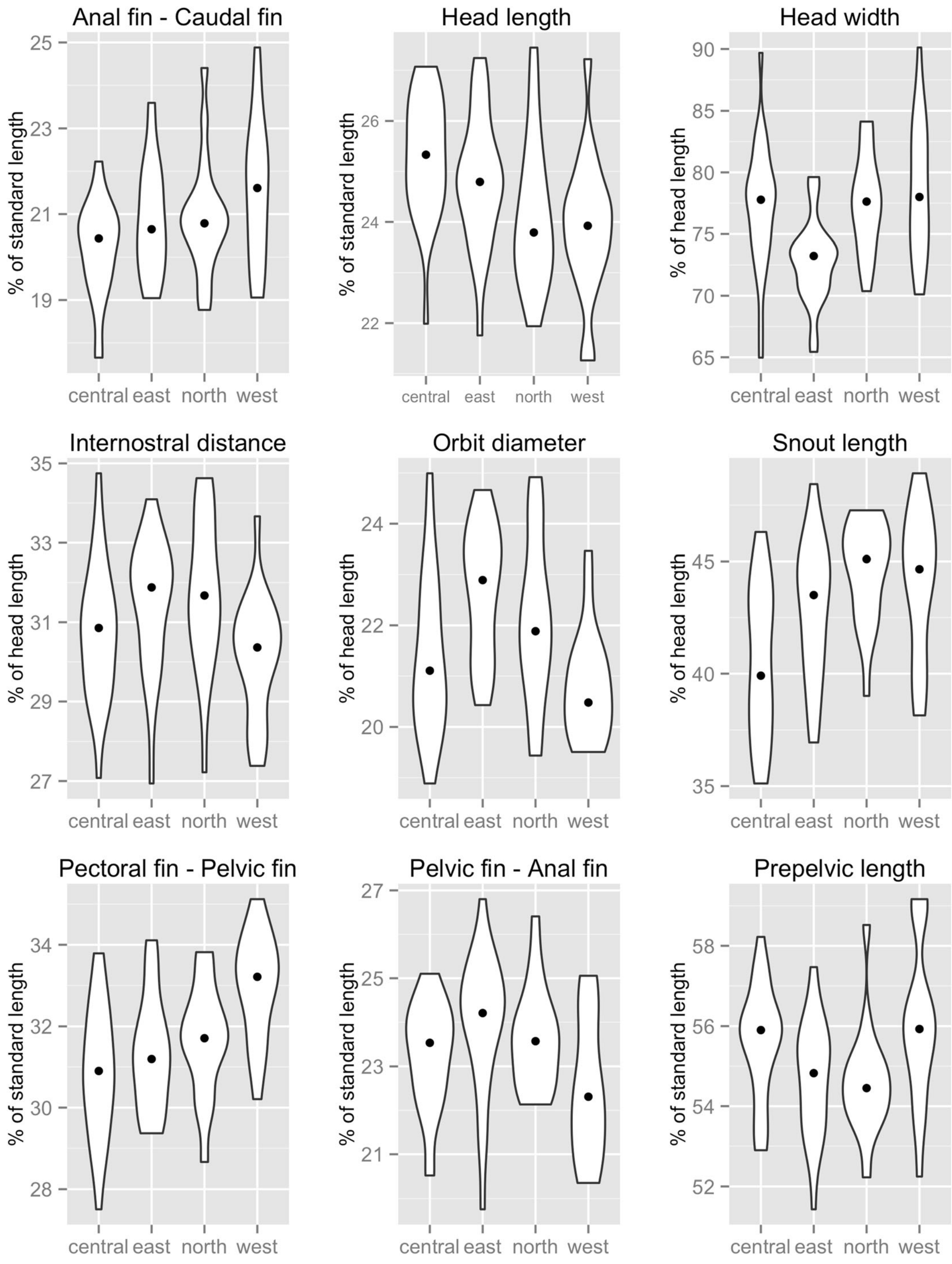


Fig. 2 Violin plots based on nine parametric measurements which show significant differences between the four mt clades (x-axis) in One-way ANOVA. The median is shown in each plot as a black dot

other clades (Fig. 7). Typically, there are 17 precaudal vertebrae in the East clade, 18 in the Central clade and North clade and 19 in the West clade, although there are overlaps. There are 15 to 17 gill rakers on the lower limb of the first gill arch in the West clade, clearly separating it from the other clades with 12 to 14 in the Central clade and 10 to 14 in the North clade and East clade. Across all clades the following traits are quite stable: Dorsal fin

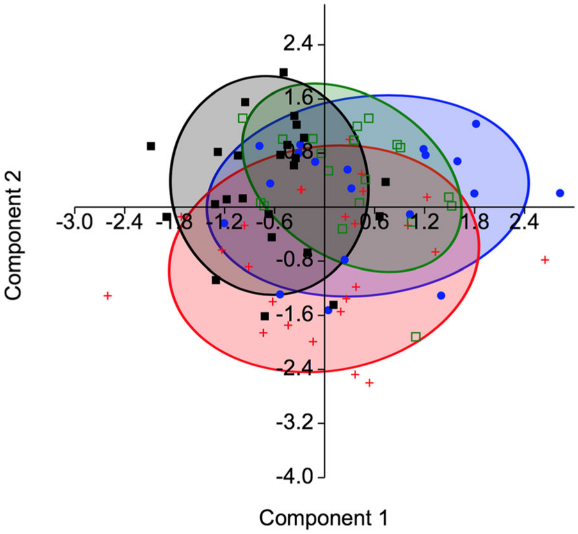


Fig. 3 PCA of significant parametric measurements with ellipses indicating 75% tolerance. PC1 explains 51.65% of the variance and PC2 explains 19.61% of the variance. West clade: blue, Central clade: red, North clade: green, East clade: black

rays of all clades are iii,7½ and anal fin rays are iii,5½. Individual cases of two unbranched anal fin rays as well as 4½ branched anal fin rays occur. The first two pectoral fin rays of *Garra* are usually unbranched (Lundberg and Marsh 1976; Stiassny and Getahun 2007). In *G. barreimiae,* however, one or two unbranched fin rays are present, but one unbranched pectoral fin ray is the common case. The number of branched rays is very

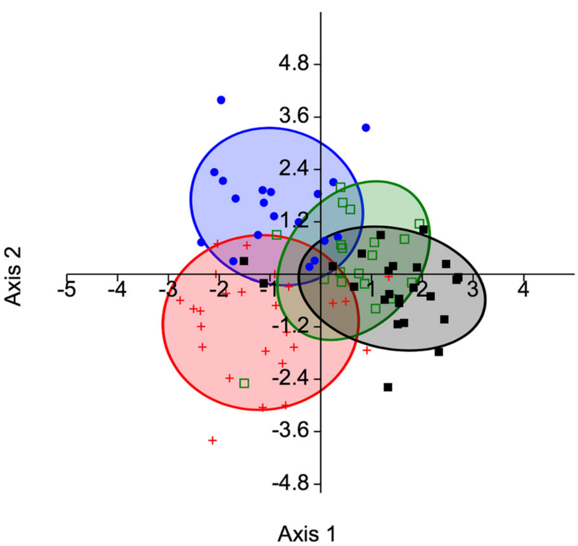


Fig. 4 LDA of significant parametric measurements with ellipses indicating 75% tolerance. Axis 1 explains 53.19% of the variance. Axis 2 explains 41.16% of the variance. West clade: blue, Central clade: red, North clade: green, East clade: black

variable. The first unbranched dorsal fin ray and anal fin ray as well as the final branched pectoral fin rays often are extremely small and thus hard to detect. The caudal fin usually has 10 upper and nine lower principal fin rays (equivalent to 9 + 8 branched caudal fin rays). Gill rakers are small, sometimes only knob-like, very fragile and present on the lower limb (ceratobranchial), but sometimes missing on the upper limb (epibranchial). There are 12 or 13 predorsal scales with individual cases of 11 or 14 predorsal scales, however the pattern very often is irregular. There are four scales above the lateral line and three scales below the lateral line. Individual cases of five scales above the lateral line and four scales below the lateral line occur. All fish have four barbels. Pharyngeal teeth are in three rows: typically 2.4.5–5.4.2. Teeth numbers of 1.2.5, 1.3.5 or 2.3.5 occur.

Discussion

With the introduction of molecular genetic methods into phylogenetic questions, distinct lineages in *G. barreimiae* have been detected recently (Kirchner et al. 2017). Our results show that at least some of these lineages are differentiated by morphological characters. All specimens of this study have originally been caught for genetic analyses only. Therefore, the samples sometimes did not represent the full size spectrum of a population and contained many small individuals. However, this study is based on the largest number of morphologically examined specimens of the *G. barreimiae* species complex to this point. The morphometric work that had been previously performed on *G. barreimiae* yielded contradicting results, which could be interpreted as even higher morphological variability than observed in the present study. Yet they could also be explained by different techniques of measurement and counting. As an example, the number of dorsal fin rays was stated as iii,7 by Fowler and Steinitz (1956), iii,6 by Menon (1964), iv,6 or 8 by Banister and Clarke (1977) and iv,6–8 by Krupp (1983). As demonstrated in the results, all specimens in this study had iii,$7_{1/2}$ dorsal fin rays. Especially the first unbranched dorsal fin ray often is rather small and could be observed in greater detail using micro CT.

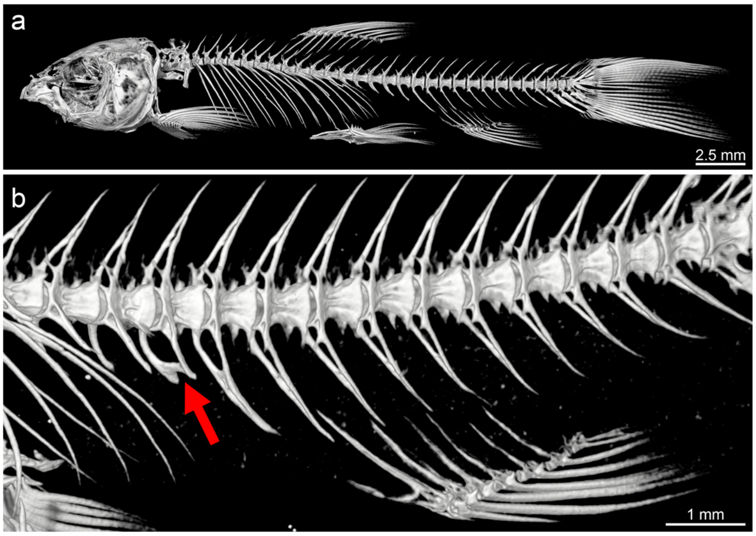
Banister & Clarke divided *G. barreimiae* into *G. b. shawkahensis* (from one locality in Wadi Shawkah) and *G. b. barreimiae* (from all other mentioned localities and collections) based on a different number of gill rakers on the lower limb of the first gill arch (*G. b. barreimiae*:

Table 4 Meristic counts and associated number of specimens per clade. Dorsal fin pterygophore insertion refers to the precaudal vertebrae number anterior to the first major dorsal fin pterygophore. Anal fin pterygophore insertion refers to the caudal vertebrae number anterior to the first major anal fin pterygophore

	Count	West	Central	North	East
Precaudal vertebrae	17	–	–	2	7
	18	1	6	5	–
	19	6	1	1	–
Intermediate vertebrae	1	7	1	1	2
	2	–	6	6	5
	3	–	–	1	–
Caudal vertebrae	12	1	1	1	–
	13	5	4	6	4
	14	1	1	1	3
Total vertebrae	31	–	–	–	1
	32	1	1	1	4
	33	6	4	7	2
	34	–	1	–	–
Dorsal fin pterygophore insertion	10	–	3	4	6
	11	6	4	4	1
	12	1	–	–	–
Anal fin pterygophore insertion	1	2	1	5	1
	2	5	6	2	5
	3	–	–	1	1
Lateral line scales	30	–	6	6	2
	31	11	16	11	13
	32	6	3	2	8
	33	2	–	–	–
Lateral scales on caudal fin base	1	–	1	–	–
	2	11	23	8	17
	3	8	1	11	6
Total lateral scale count	32	–	6	–	2
	33	6	16	13	7
	34	8	3	5	14
	35	5	–	1	–
Scales between anus and anal fin	1	–	2	1	3
	2	17	20	10	11
	3	2	3	8	7
	4	–	–	–	1
Scales between anus and pelvic fin	4	2	–	–	2
	5	4	1	5	3
	6	11	16	10	16
	7	1	8	3	2
Postdorsal scales	13	–	1	–	6
	14	4	3	2	4
	15	7	8	14	10
	16	2	5	2	1
Circumpeduncular scales	12	7	1	12	11
	13	1	–	3	4
	14	9	8	3	7
	15	2	8	–	2
	16	–	8	1	–
Breast scales	yes	12	18	11	10
	no	7	7	8	14
Gill rakers on the lower limb of the first gill arch	10	–	–	3	2
	11	–	–	3	6
	12	–	12	3	5
	13	–	6	6	4
	14	–	2	2	5
	15	7	–	–	–
	16	3	–	–	–
	17	1	–	–	–
Branched pelvic fin rays	7	18	3	4	4
	8	1	22	15	19
Unbranched pectoral fin rays	1	16	24	15	18
	2	3	1	4	4
Branched pectoral fin rays	11	–	1	–	4
	12	3	3	1	4
	13	6	15	8	9
	14	5	3	6	5
	15	5	3	4	–
Tubercles	yes	13	11	11	13
	no	6	14	8	11

11–14; *G. b. shawkahensis*: 15–18) and a different mean prepelvic distance (*G. b. barreimiae:* 53.9%; *G. b. shawkahensis*: 56.2%). They also described *G. longipinnis* from the Saiq region based on eight specimens with strikingly long paired fins (Banister and Clarke 1977) like the pectoral fins with 23.3–32.9%. Saiq is within the region of this study's Central clade and Hamidan et al. (2014) identified fish from that area as *G.* cf. *longipinnis*. The pectoral fin is variable in the Central clade with a range of 17.9–24.8%, thus some specimens would fit the lower range values of the original species description of *G. longipinnis*, however the mean height of the pectoral fins is not longer in the Central clade than in the others. Krupp (1983) published a species key for Arabian *Garra* in which *G. b. barreimiae* should have less than fifteen gill rakers on

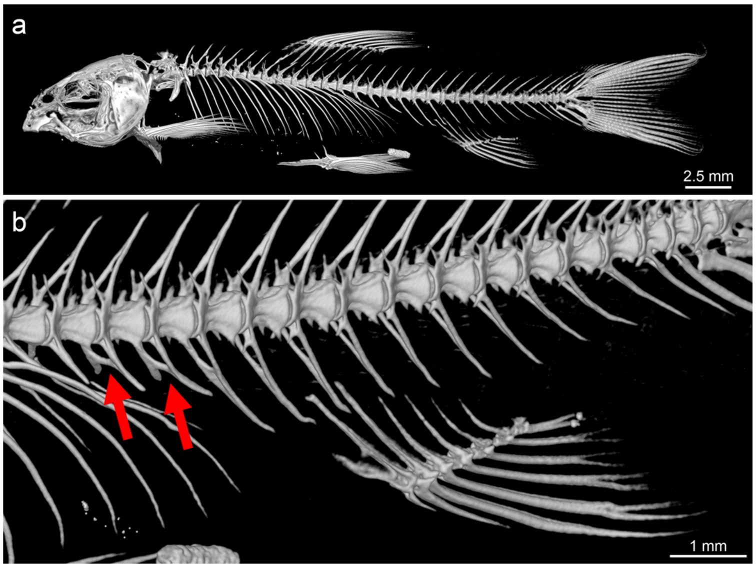
Fig. 5 Micro CT image of selected specimens of the West clade: all examined West clade specimens have one intermediate vertebra (indicated with red arrow). The upper image (**a**) shows the whole skeleton, the lower image (**b**) represents a detailed view on the intermediate vertebra, caudal vertebra and the anal fin



the lower limb of the first gill arch and *G. b. shawkahensis* should have more than fourteen gill rakers, which was the substantial morphological difference between the two subspecies.

In this study, the mean prepelvic distance is the largest in the West clade with 56.0% (Fig. 2, Table 2) and thus in fact very close to that reported by Banister and Clarke (1977) (56.2%) for *G. b. shawkahensis*. In addition, all West clade specimens have more than 14 gill rakers on the lower limb of the first gill arch, further fitting the description of *G. b. shawkahensis* of Banister and Clarke as well as that of Krupp (1983). One might be tempted to expand the known range of *G. b. shawkahensis* by our three West clade sampling sites, but things may be more complicated. Fowler and Steinitz did not measure the prepelvic distance and did not count gill rakers in their original description of *G. barreimiae.* Banister and Clarke did not take measurements of specimens from Buraimi, but considered them to belong to the same taxon as specimens from Wadi Sahtan, a locality that clearly belongs to another genetic clade (North clade), as well as fishes from undisclosed regions of Oman. In an analysis of mt sequences (Kirchner et al. 2017) fishes from Wadi Shawkah did cluster together with specimens from the West clade (Hafeet, Hayyal and Al Juwayf).

Fig. 6 Micro CT image of an East clade specimen. The Central, North, or East clade usually have two intermediate vertebrae (indicated by red arrows). The upper image (**a**) shows the whole skeleton, the lower image (**b**) shows details of the intermediate vertebrae, caudal vertebrae and the anal fin



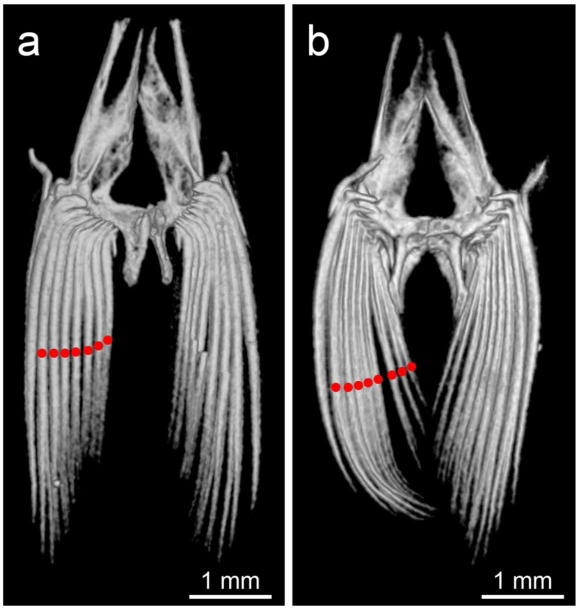


Fig. 7 Variations of pelvic fin ray numbers. West clade specimens (**a**) typically have i,7 pelvic fin rays, while the other clades (**b**), with a Central clade specimen depicted, usually have i,8 pelvic fin rays. Branched fin rays are marked by red dots

Furthermore, all sampling sites from the West clade and the original description sites of *G. barreimiae* and *G. b. shawkahensis* are found in the same region, the western slopes of the Al Hajar Mountains (Fig. 1). In the Catalog of Fishes *G. b. shawkahensis* is considered to be a synonym of *G. barreimiae* (Eschmeyer et al. 2017). During two collection trips of the Natural History Museum Vienna, the original description site of *G. barreimiae*, Buraimi, was visited, but no fish could be found. Type specimens have not been examined in this study, thus comparisons refer exclusively to literature. However, it is very likely that Fowler & Steinitz' *G. barreimiae,* Banister & Clark's *G. b. shawkahensis* and this study's specimens from the sampling sites Hafeet, Hayyal and Al Juwayf belong to the same taxon. The West clade fits the original description of *G. barreimiae* best, both concerning distribution and morphology. Whether the classification of *G. b. shawkahensis* as subspecies is justified, remains open at the current stage of knowledge.

The sampling locations of the mt clades Central and East have not been mentioned in any valid species description. Individuals belonging to the North clade have at least partially falsely been grouped together with individuals of *G. b. barreimiae* in the past. All morphometric measurements overlap between the clades and are very variable within the clades (Fig. 2, Table 2). The clades are not separated in the PCA (Fig. 3), although in the LDA (Fig. 4) the West clade, Central clade and East clade are distinguishable and most specimens can be correctly classified based on the same measurements in the confusion matrix. A noteworthy difference is the pelvic fin ray count with seven branched rays in most of the West clade specimens and eight branched rays in most specimens of the other clades (Table 4; Fig. 7). The West clade also has generally nineteen precaudal and one intermediate vertebrae, both of which are very uncommon in the other clades. All East clade specimens have seventeen precaudal vertebrae, a feature apart from that only found in two of the North clade specimens.

Conclusion

Besides the number of gill rakers, which distinctly separates the West clade from the other clades, no clearly well-defined morphological character distinguishing the clades could be identified with the methods applied. Several morphometric measurements however, display significant differences between the clades. Measurements of head structures are particularly conspicuous in all clades and therefore should be considered as primary targets of more precise morphological methods (e.g. geometric morphometrics). Besides a few overlaps, the numbers of precaudal and intermediate vertebrae as well as those of branched pelvic fin rays indicate that the West clade is morphologically distinct from the other clades. The original descriptions of *G. barreimiae* and *G. b. shawkahensis* correspond to the West clade both geographically and morphologically. According to the concept of Integrative Taxonomy multiple disciplines should be used for species delimitation with morphology and methods revealing nuclear genetic information always being applied (Schlick-Steiner et al. 2010). Thus, concerning the Central clade, North clade and East clade, the question whether they represent separate taxa has to be addressed combining genetic and morphological data.

Acknowledgements Open access funding provided by University of Vienna. We thank Oliver Macek, Julia Schindelar, Barbara Tautscher and Lorena Toscano for their continued support in the lab, as well as Robert Illek and Lukas Plan for helping with the collection of samples. We want to express our gratitude to the Ministry of Environment and Climate Affairs of Oman and the Austrian Economy Chambers for their cooperation, support and sampling permits.

Appendix

Table 5 Results of Levene's test for homogeneity of variance, ANOVA and Kruskal-Wallis test for each morphometric measurement. In the case of non-parametric data, results of the Kruskal-Wallis test are preferable over ANOVA. In this case results of the Kruskal-Wallis test are written in bold letters. A probability value of less than 0.05 is considered significant

	Levene's test	ANOVA	Kruskal-Wallis test
Anal fin - Anus	0.0012	2.460E-10	3.652E-08
Anal fin - Caudal fin	0.2325	0.0125	0.0430
Anal fin height	0.0881	0.0808	0.1639
Body depth	0.6192	1.263E-06	1.445E-05
Caudal peduncle depth	0.0035	1.649E-06	9.943E-05
Caudal peduncle length	0.0170	0.0006	0.0033
Dorsal fin height	0.9175	0.0003	0.0002
Head depth (% of HL)	0.4949	0.0007	0.0005
Head length	0.8436	0.0023	0.0013
Head width (% of HL)	0.1002	0.0002	0.0002
Internostral dist. (% of HL)	0.8597	0.0196	0.0114
Interorbital width (% of HL)	0.0367	0.0012	0.0032
Orbit diameter (% of HL)	0.2250	0.0001	0.0002
Pectoral fin height	0.0154	0.0625	0.1778
Pectoral fin - Pelvic fin	0.7127	0.0001	0.0030
Postorbital length (% of HL)	0.0040	4.836E-05	0.0001
Postdorsal length	0.3254	0.0539	0.0777
Preanal length	0.1987	0.1715	0.1955
Predorsal length	0.7126	0.0079	0.0335
Prepelvic length	0.3664	0.0105	0.0036
Pelvic fin - Anal fin	0.4907	0.0063	0.0180
Pelvic fin - Caudal fin	0.6056	0.0895	0.1220
Pelvic fin height	0.8987	0.8523	0.7766
Snout length (% of HL)	0.1126	9.016E-05	0.0003

Table 6 Confusion matrix of LDA. 75.86% of specimens were correctly classified based on significant parametric measurements. Rows show the given groups, columns show the predicted groups

	West	Central	North	East	Total
West	15	0	4	0	19
Central	2	19	1	3	25
North	2	1	12	4	19
East	1	1	2	20	24
Total	20	21	19	27	87

Table 7 Confusion matrix of LDA. After Jackknife resampling 63.22% of specimens were correctly classified based on significant parametric measurements. Rows show the given groups, columns show the predicted groups

	West	Central	North	East	Total
West	12	1	6	0	19
Central	2	18	2	3	25
North	2	1	9	7	19
East	1	1	6	16	24
Total	17	21	23	26	87

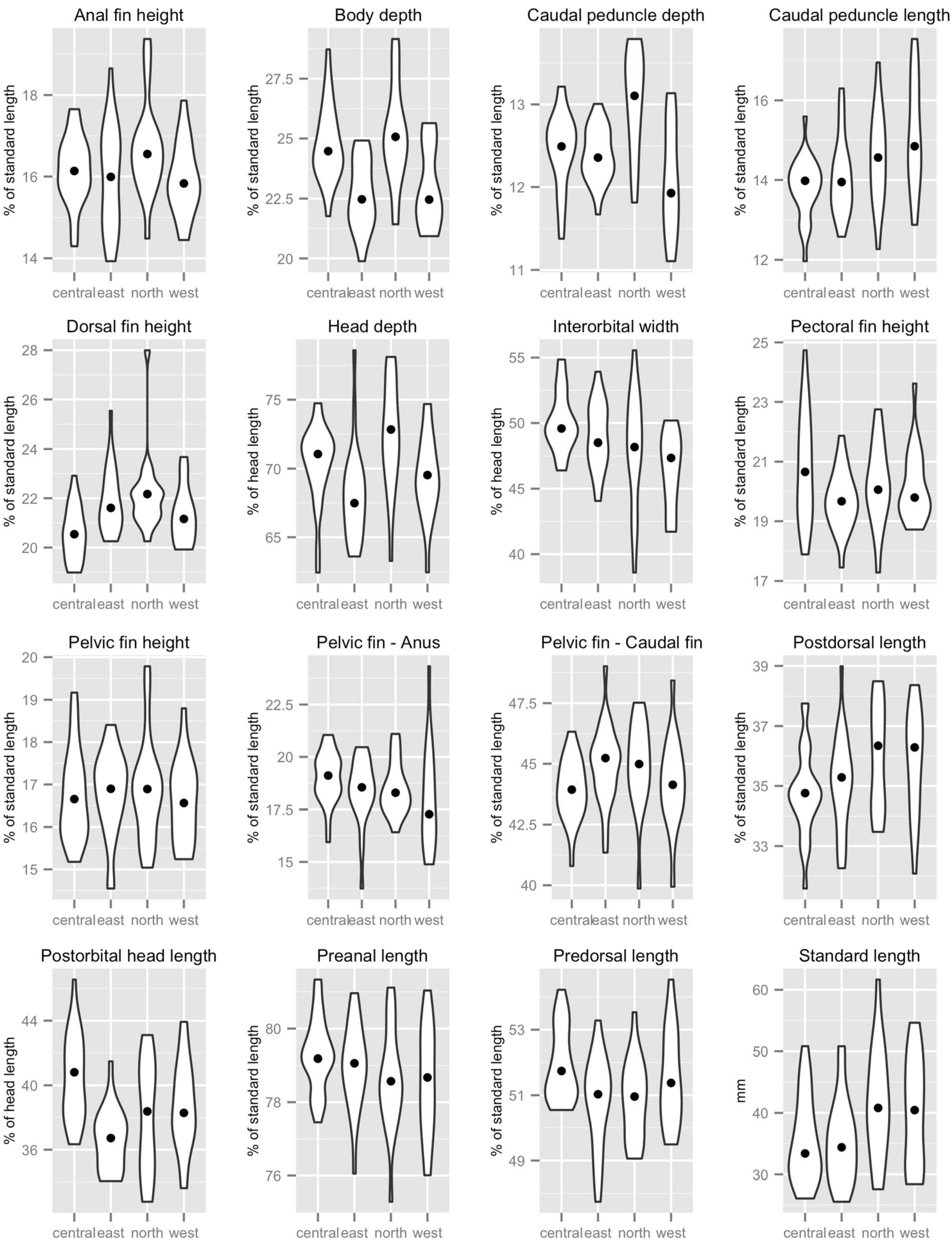


Fig. 8 Violin plots of non-parametric measurements of the four mt clades (in % of standard length and % of head length) and absolute measurements of the standard length. The median is shown in each plot as a black dot

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