



universität  
wien

# MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Morphological diversity and variation of a nuclear marker sequence in the endemic Arabian barb *Garra barreimiae* [Teleostei: Cyprinidae]“

verfasst von / submitted by

Arthur Pichler, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of  
Master of Science (MSc)

Wien, 2016 / Vienna 2016

Studienkennzahl lt. Studienblatt /  
degree programme code as it appears on  
the student record sheet:

A 066 829

Studienrichtung lt. Studienblatt /  
degree programme as it appears on  
the student record sheet:

Masterstudium Evolutionsbiologie

Betreut von / Supervisor:

Priv.Do. Dr. Elisabeth Haring

# Contents

|                                           |           |
|-------------------------------------------|-----------|
| <b>1. INTRODUCTION</b>                    | <b>3</b>  |
| 1.1 TAXONOMY                              | 3         |
| 1.2 ORIGIN AND DISPERSION                 | 3         |
| 1.3 <i>GARRA BARREIMIAE</i>               | 6         |
| 1.4 AIM                                   | 7         |
| <b>2. MATERIAL &amp; METHODS</b>          | <b>8</b>  |
| 2.1 TRADITIONAL MORPHOMETRICS & MERISTICS | 8         |
| 2.2 MICRO CT                              | 14        |
| 2.3 STATISTICS                            | 14        |
| 2.4 GENETIC ANALYSIS                      | 14        |
| <b>3. RESULTS</b>                         | <b>15</b> |
| 3.1 TRADITIONAL MORPHOMETRICS             | 15        |
| 3.2 STATISTICS                            | 21        |
| 3.3 MERISTICS                             | 26        |
| 3.4 MICRO CT                              | 28        |
| 3.5 GENETIC ANALYSIS                      | 34        |
| <b>4. DISCUSSION</b>                      | <b>37</b> |
| 4.1 CONCLUSION                            | 42        |
| <b>5. ACKNOWLEDGMENTS</b>                 | <b>42</b> |
| <b>6. LITERATURE</b>                      | <b>43</b> |
| <b>7. SUPPLEMENT</b>                      | <b>47</b> |
| <b>8. ABSTRACT</b>                        | <b>58</b> |
| <b>9. ZUSAMMENFASSUNG</b>                 | <b>59</b> |

# 1. Introduction

*Garra barreimiae* is one of nine described species belonging to the genus *Garra* Hamilton 1822 occurring in the Arabian Peninsula. This species lives in fast running rivers as well as in slow moving waters or small pools throughout the Hajar Mountains of Oman and the United Arab Emirates (Freyhof et al., 2015), also a troglomorphic cave population is known (Banister, 1984).

## 1.1 Taxonomy

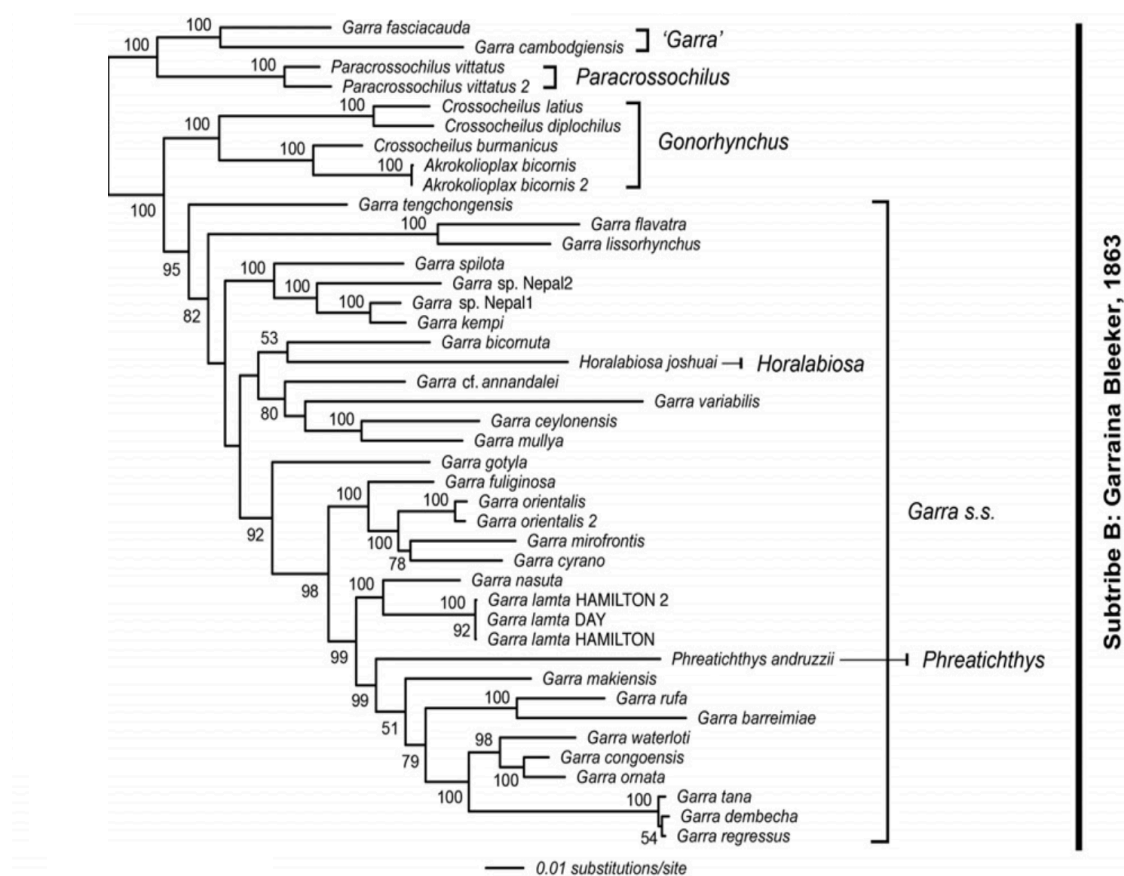
Within Cyprinidae the genus *Garra* belongs to the tribe Labeonini (Yang & Mayden, 2010). Rainboth (1991, 1996) merged Labeine (Reid, 1982) species and Labeoninae (Chen et al., 1984) into the tribe Labeonini. This nomenclature was adopted by Yang & Mayden (2010). Labeonini have been divided into four subtribes on the basis of multiple mitochondrial and nuclear genes: Labeoina Bleeker 1859, Garraina Bleeker 1863, Osteochilina and Semilabeoina (Yang et al., 2012). While the monophyly of Labeonini has been confirmed, many relationships between taxa within Labeonini are still unclear. The Labeonini subtribe Garraina contains most *Garra* species [Fig. 1], including *G. barreimiae* (Yang et al., 2012), but the phylogenetic relationships within the genus are not clearly resolved yet. An example is *Phreatichthys andruzzii* Vinciguerra, 1924, an endemic cave fish from Somalia, which was found to be closely related to *G. barreimiae* Fowler & Steinitz, 1956 and *Garra rufa* (Heckel, 1843) by Colli et al. (2009) and Yang et al. (2012), further on it might be included into the genus *Garra* as it was suggested for *Iranocypris typhlops* Bruun & Kaiser, 1944 which is now valid as *Garra typhlops* Bruun & Kaiser, 1944 (Farashi et al., 2014; Hamidan et al., 2014; Eschmeyer et al., 2016). *Garra* is still considered to be a very complex genus and more generic lineages might be hidden within this taxon (Yang et al., 2012; Zhang, 2005). Only recently, several new species have been described including *Garra jordanica* Hamidan, Geiger & Freyhof, 2014, *Garra mondica* Sayyadzadeh, Esmaeili & Freyhof, 2015 and *G. lorestanensi* Mousavi-Sabet & Eagderi, 2016.

## 1.2 Origin and Dispersion

According to a Bayesian relaxed molecular clock analysis based on the mitochondrial *cytochrome b* gene with *Danio rerio* as root it was proposed that Labeoninae originated between 25 and 17 million years ago in the Early Miocene, by the time of the second uplift of the Tibetan plateau (Tang et al., 2009). They then should have diversified and dispersed into Asia and Africa

and arrived at the Arabian Peninsula about 20.5 to 14.8 million years ago when Eurasia and the Afro-Arabian plate became connected after the gradual closing of the Tethys. 10 to 8 million years ago, when sea levels dropped 60 m below modern levels, Africa and the Arabian Peninsula became connected so that *Garra*-like Labeonins might have entered Eastern Africa via Yemen and dispersed further into the continent (Tang et al., 2009). A Labeo-like pharyngeal tooth, the oldest known African fossil of *Labeo*, was discovered in Kenya and dated to the Early Miocene about 17 million years ago (Van Couvering, 1977; Stewart, 2001; Tang et al., 2009).

The genus *Garra* is widespread in Asia and Africa (Menon, 1964). Yunnan in Southern China is considered to be its geographic origin. *Garra* is supposed to have originated in the Middle or Late Miocene (Menon, 1964; Tang et al., 2009). This period marks the time of the most intensive phase of the Himalayan orogeny. Frequent tectonic movements, the uplift of the Qinghai-Tibetan plateau, constantly rejuvenated streams and climate change in East Asia triggered a rapid evolution of adaptations for fast flowing streams (Tang et al., 2009).



**Fig. 1:** Maximum likelihood tree of the Labeonini subtribe Garraina on the basis of combined nuclear and mitochondrial data. Modified after Yang et al., 2012. [*Garra s.s.*= *Garra sensu stricto*]



Most species of *Garra* are adapted to running waters. They are able to attach to substrate with their adhesive disc and, to a lesser extent, with their fins (Menon, 1964). According to Hennig's progression rule, the phylogenetic relationships of *Garra* support the theory of an Asian origin (Tang et al., 2009). Dispersion might have happened in waves depending on the upheaval of the Himalayas and other mountain ranges in Southeast Asia. *Garra* expanded westward along the southern side of the Himalayas during the Pliocene according to Menon (1964). Yet, there is almost no fossil evidence of *Garra*. The first known fossils have been discovered in Jradzor, Armenia and were dated to the Pliocene. There are no extant species of *Garra* in Armenia, but during the Pliocene the Araks-Kura river drainage could have been connected with the Euphrates and Tigris drainage, in which species of the genus *Garra* are still present (Vasilyan & Carnevale, 2013). Until more fossils are discovered, the point of time of the first migration of *Garra* into Arabia is still very uncertain.

During the Pleistocene glaciations the sea-levels dropped and the Arabo-Persian Gulf basin was disconnected from the deeper Indian Ocean. Rivers from the Mesopotamian region could flow to the Gulf of Oman (Krupp, 1983). With dropped sea levels in the Late Pleistocene and Early Holocene, 74.000 to 8.000 years ago, the Gulf formed an Oasis that was part of the "Fertile Crescent" (sensu Breasted 1916; Rose, 2010). This today inundated area formed a floodplain across northern Arabia created by the rivers Karun, Euphrates, Tigris and Wadi Batin. These streams formed the Ur-Schatt River Valley, a catchment zone over 100.000 km<sup>2</sup>, which was additionally fed by runoff from the Iranian Zagros Mountains and the Rub'Al Khali aquifers of Eastern Arabia. Thus the majority of freshwater of South West Asia drained into the Persian Gulf (Alsharhan et al., 2001; Rose, 2010; Shiraz & Münster, 1992; Sultan et al., 2008). Remains of crocodiles, mastodons and rhinoceroses indicate a former subtropical climate in Central Arabia. Hyperarid conditions probably began about 17.000 years before present (Krupp, 1983). According to Lambeck (1996) the Strait of Hormuz opened up about 14.000 years ago, marine incursion into the Central Gulf Basin started about 12.500 years ago and into the Western Gulf Basin about a thousand years later. Today's shoreline has been existing for about 6.000 years. Since the Persian Gulf is one of the shallowest seas of the world with average water depths of 35 m, it is widely recognised that it has been above sea level in glacial periods (Lambeck, 1996). The flooding of the Persian Gulf Basin probably marks the latest possible time of migration of *Garra* from Asia into the Oman.

Today 21 extant freshwater fish species are known from the Arabian Peninsula with nine species belonging to the genus *Garra*: *G. buettikeri* Krupp, 1983 (VU), *G. dunsirei* Banister, 1987 (EN), *G. lautior* Banister, 1987 (EN), *G. longipinnis* Banister & Clarke, 1977 (DD), *G.*

*mamshuqa* Krupp, 1983 (EN), *G. sahilia* Krupp, 1983 (LC), *G. smarti* Krupp & Budd, 2009 (VU), *G. tibanica* Trewavas, 1941 (LC) and *G. barreimiae* Fowler & Steinitz, 1956 (LC)<sup>1</sup> (Freyhof et al., 2015). These species are adapted to freshwater ecosystems in desert areas, which are 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).

### 1.3 *Garra barreimiae*

*Garra barreimiae* lives in fast running rivers as well as in slowly moving waters or small pools throughout the mountains of Oman and the United Arab Emirates (Freyhof et al., 2015). It was first described by Fowler & Steinitz (1956) from Buraimi (also Al Buraimi, Baraimi, Barreimi), Oman and later divided into the subspecies *Garra barreimiae barreimiae*, *Garra barreimiae shawkahensis* Banister & Clarke, 1977 and *Garra barreimiae gallagheri* Krupp, 1988. A subterranean population of *G. barreimiae*, the Omani blind cave fish, was described by Banister (1984). It is known from one location in the Al Hoota Cave close to Nizwa. It was not considered to be a valid species since, except for the troglomorphic characters, it is very similar to the surface populations. Moreover, although the cave population is genetically isolated, there might be some gene flow from the cave to the adjacent surface populations. This troglomorphic population seems to be of recent origin less than one million years ago (Kruckenhauser et al., 2011). The long-finned *G. longipinnis* Banister & Clarke, 1977 has been described from one wadi near Saiq, Jabal al Akhdar, but since then, only fish identified as *G. barreimiae* have been found at this location. It is still unclear if *G. longipinnis* has become extinct or if atypical specimens of *G. barreimiae* were described as a new species (Freyhof et al., 2015). Many populations of *G. barreimiae* are very isolated from each other and inhabit different drainage systems around the Hajar Mountains (Freyhof et al., 2015). In former times during intermittent pluvial intervals the river network was much denser, so populations might have been more connected. *G. barreimiae* has previously been categorized as threatened, but it is now categorized as least concern. The Omani blind cave fish however is considered to be a unique population that should be protected and managed separately (Freyhof et al., 2015). Kruckenhauser et al. (2011) were the first to use molecular genetic methods to describe genetic

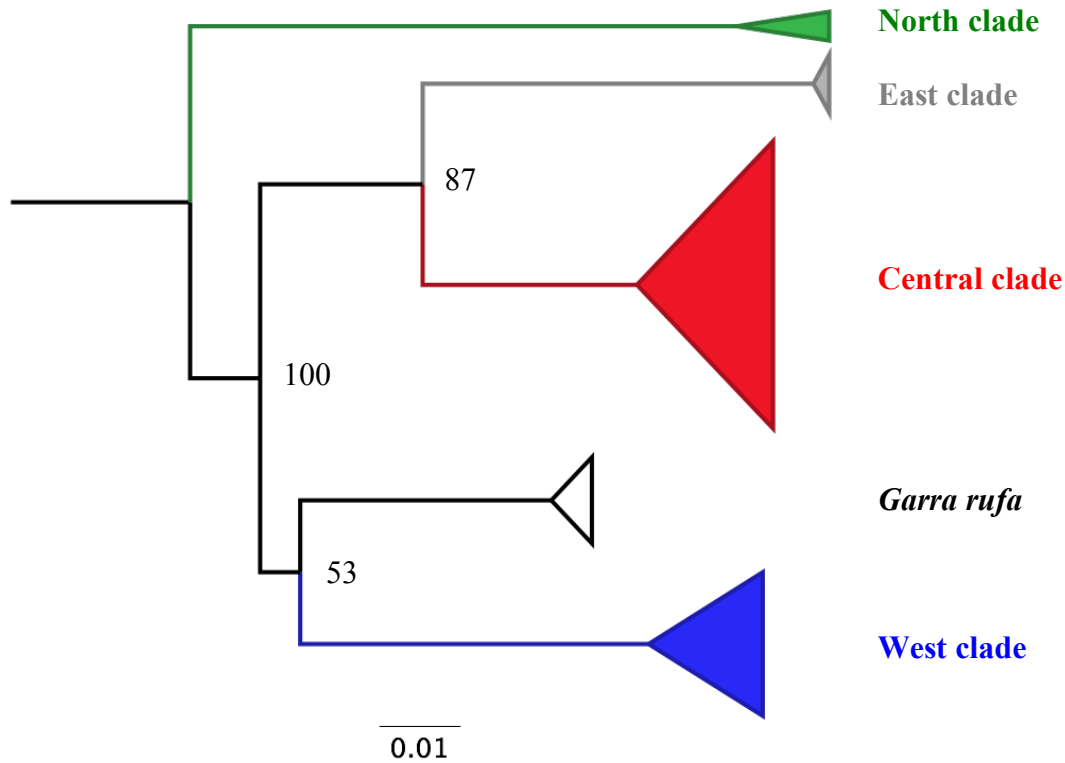
---

<sup>1</sup> IUCN Red List Categories: Extinct (EX), Extinct in the wild (EW), Critically endangered (CR), Endangered (EN), Vulnerable (VU), Near threatened (NT), Least concern (LC), Data deficient (DD), Not evaluated (NE)

differentiations within *G. barreimiae*. On the basis of genetic data (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, the type locality of *G. b. gallagheri*, and one in the northeast of the Hajar Mountains. Subsequent genetic analysis of the surface populations distinguished between at least four different clades with distances between 6% and 10%, as measured in a section of the mt cytochrome c oxidase subunit 1 gene (*COI*) [Fig. 2]. The geographic distribution of these clades does not reflect the differentiation of the known subspecies. Genetic comparison of surface populations with *G. rufa* did not confirm the monophyly of *G. barreimiae* (Kirchner et al., unpublished). Due to the conspicuously individual variation among species, even concerning specimens from the same locality, the taxonomy of *Garra* has been troublesome (Menon, 1964). However, with the foundation of the newly discovered and striking differences in mt sequences, which can 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 is a challenge for the people of Oman but also poses a threat to isolated fish populations. *G. barreimiae* is widely distributed throughout the Oman, but since many populations are isolated (Freyhof et al., 2015) and the type-locality in Buraimi already seems to be lost due to water extraction (Kruckenhauser pers. comm.) it is a necessity to study potentially cryptic species within *G. barreimiae*.

#### 1.4 Aim

This study is part of a broader phylogenetic analysis of *G. barreimiae* and aims to reveal whether morphological differences can be found between previously determined genetic clades using traditional morphometrics and meristics as well as micro CT imaging, which is used for the first time to compare the osteology within *G. barreimiae*. Additionally, the nuclear (nc) *RAG-1* gene was tested for its suitability to analyse the phylogeny of the species. In addition to mt genes the nc marker sequence should give further insight into hitherto possibly overlooked cryptic species.



**Fig. 2:** Maximum Likelihood tree based on combined mitochondrial *COI* and *CR* sequences (Kruckenhauser et al., 2011; Kirchner et al., unpublished) showing the four clades of *Garra barreimiae* in Oman and *Garra rufa*.

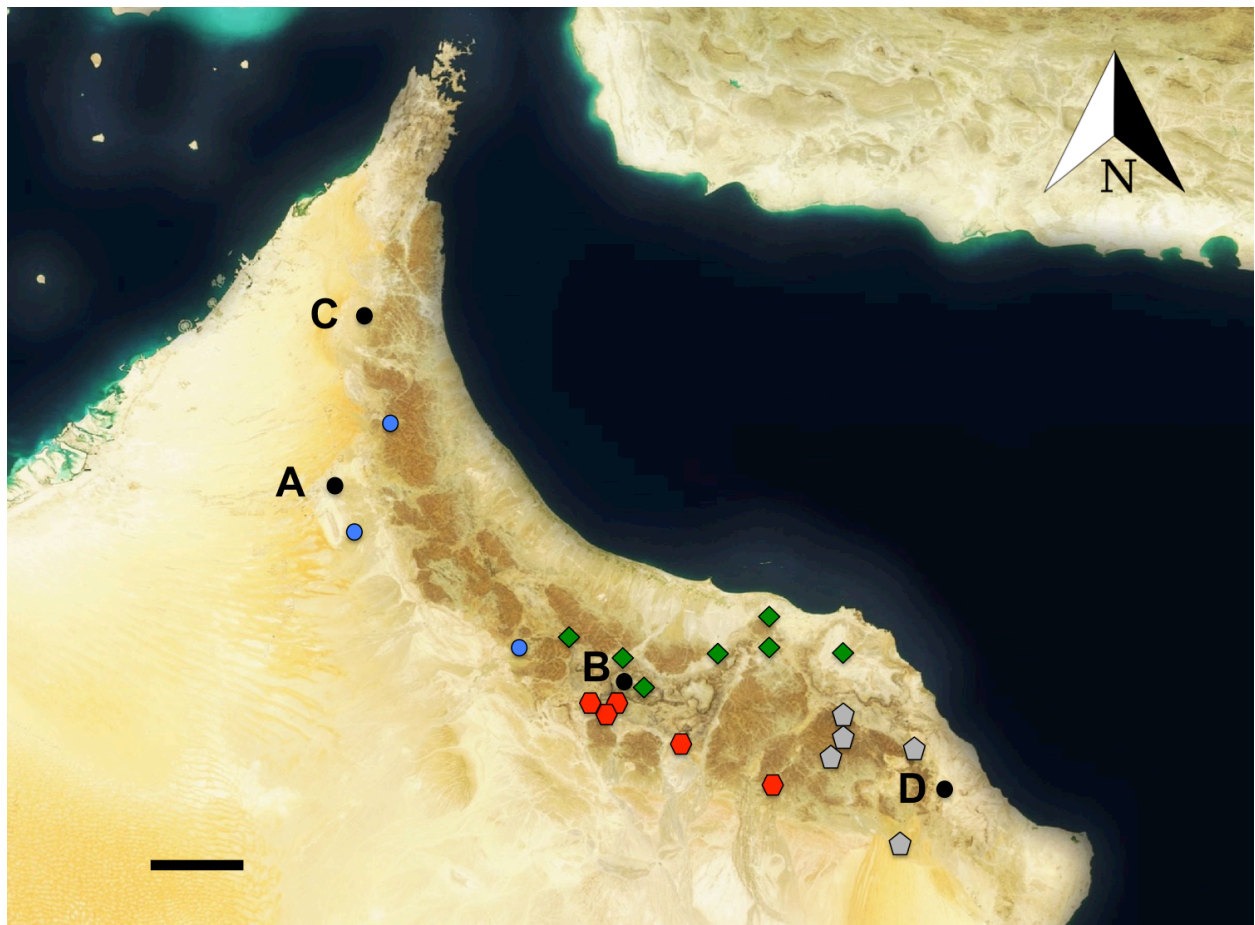
## 2. Material & Methods

### 2.1 Traditional Morphometrics & Meristics

Eighty-seven specimens of *Garra barreimiae*, collected at 20 localities, were included in the morphological analysis (the dubious taxon *G. longipinnis* is considered in the discussion). The individuals, which are part of the collection of the Natural History Museum Vienna, represent four mitochondrial (mt) clades (Kruckenhauser et al., 2011; Kirchner et al., unpublished) [Fig. 2]: West clade (n=19) [Fig. 4], Central clade (n=25) [Fig. 5], North clade (n=19) [Fig. 6] and East clade (n=24) [Fig. 7] named after their relative distribution around the Al Hajar Mountains in the Sultanate of Oman [Fig. 3]. Coordinates and number of specimens of each sampling site are given in Tab. 1. Specimens were fixed in 80% ethanol.

All measurements [Tab. 2] and counts were taken by the author. Twenty-five measurements 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 follow Armbruster (2012). Not all measurements and counts could be taken of every specimen due to their preservation and handling in

examinations prior to this study. Measurements are described in percent of standard length unless stated otherwise. 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 numbers of scale rows were taken. Vertebrae were counted as precaudal, intermediate and caudal vertebrae without including the hypural. Five precaudal vertebrae were included for the Weberian Complex. Precaudal vertebrae have no closed haemal arches and caudal vertebrae have a joined parapophyses forming an arch (Birdsong et al., 1988; Rojo, 1991). 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 & 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. It has to be kept in mind that these rays often are counted as 1, thus misconceptions may arise. Unbranched dorsal fin rays and unbranched anal fin rays could only be counted via micro CT images.



**Fig. 3:** 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 & Steinitz, 1956; **B:** Wadi Sahtan; assigned to *Garra barreimiae barreimiae* by Banister & Clarke (1977); **C:** Wadi Shawkah, type locality of *Garra barreimiae shawkahensis* Banister & Clarke, 1977; **D:** Wadi Bani Khalid; type locality of *Garra barreimiae gallagheri* Krupp, 1988, not included in this study. Scale bar: 50 km.

**Tab. 1:** Sampling sites with corresponding coordinates as decimal degrees [longitude, latitude] and number of morphologically examined specimens.

| Clade                | Sampling Site       | Coordinates    | n  |
|----------------------|---------------------|----------------|----|
| <b>West clade</b>    | Al Hayyal           | [23.43, 56.74] | 7  |
|                      | Al Juwayf           | [24.54, 56.10] | 7  |
|                      | Hafeet              | [23.99, 55.84] | 5  |
| <b>Central clade</b> | Al Hamra            | [23.11, 57.28] | 1  |
|                      | Birkat al Mawz      | [22.92, 57.66] | 2  |
|                      | Lizq                | [22.71, 58.17] | 4  |
|                      | Misfat al Arbriyyin | [23.14, 57.31] | 10 |
|                      | Wadi an Nakhar      | [23.17, 57.20] | 8  |
| <b>North clade</b>   | 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  |
| <b>East clade</b>    | 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  |



**Fig. 4:** West clade specimen [Gbar294 32.3 mm SL] with prominent squamation ventrally between pectoral and pelvic fins.



**Fig. 5:** Central clade specimen [Gbar285 30.6 mm SL].





**Fig. 6:** North clade specimen [Gbar231 37.0 mm SL].



**Fig. 7:** East clade specimen [Gbar317 34.3 mm SL].



**Tab. 2:** Length measurements used in this study largely follow Armbruster (2012).

| Abbreviation | Measurement                        | Description                                                                                 |
|--------------|------------------------------------|---------------------------------------------------------------------------------------------|
| AFA          | Distance Anal fin - Anus           | From the origin of the anal fin to the center of the anus                                   |
| AFCDF        | Distance Anal fin - Caudal fin     | From the origin of the anal fin to the end of the vertebral column                          |
| AFL          | Anal fin height                    | From the origin of the anal fin to the tip of the longest fin ray                           |
| BD           | Body depth                         | Vertically measured at origin of the dorsal fin                                             |
| CPD          | Caudal peduncle depth              | Least caudal peduncle depth                                                                 |
| CPL          | Caudal peduncle length             | From the base of the last anal fin ray to the end of the vertebral column                   |
| DFL          | Dorsal fin height                  | From the origin of the dorsal fin to the tip of the longest fin ray                         |
| HD           | Head depth                         | From the end of supraoccipital to ventromedial point vertically                             |
| HL           | Head length                        | From the tip of the snout to the edge of the operculum (excluding gill membrane)            |
| HW           | Head width                         | Largest head width at the edge of the operculum                                             |
| INTN         | Internostril distance              | Smallest distance between the nostrils                                                      |
| IOW          | Interorbital width                 | Smallest distance between the eyes                                                          |
| OD           | Orbit diameter                     | Diameter of the eye                                                                         |
| PCTFL        | Pectoral fin length                | From the origin of the pectoral fin to the tip of the longest fin ray                       |
| PCTPV        | Distance Pectoral fin - Pelvic fin | From the origin of the pectoral fin to the origin of the pelvic fin                         |
| PO           | Postorbital distance               | From the posterior edge of the orbit to the edge of the operculum (excluding gill membrane) |
| POL          | Postdorsal length                  | From the base of the last dorsal fin ray to the end of the vertebral column                 |
| PRAL         | Preanal length                     | From the tip of the snout to the center of the anus                                         |
| PRL          | Predorsal length                   | From the tip of the snout to the origin of the dorsal fin                                   |
| PRPV         | Prepelvic length                   | From the tip of the snout to the origin of the pelvic fin                                   |
| PVFAF        | Distance Pelvic fin - Anal fin     | From the origin of the pelvic fin to the origin of the anal fin                             |
| PVFCDF       | Distance Pelvic fin - Caudal fin   | From the origin of the pelvic fin to the end of the vertebral column                        |
| PVFL         | Pelvic fin length                  | From the origin of the pelvic fin to the tip of the longest fin ray                         |
| SL           | Standard length                    | From the tip of the snout to the end of the vertebral column                                |
| SNL          | Snout length                       | From the tip of the snout to the orbit                                                      |

## 2.2 Micro CT

For micro CT scanning 30 fish 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  $\mu$ CT35 (SCANCO Medical AG, Brüttisellen, Switzerland) at 70 keV source voltage and 114  $\mu$ A intensity. Projections were recorded with 420 ms integration time (camera binning = 2) and an angular increment of 0.36°. Reconstructed slices measured 1024x1024 pixel, and isotropic voxel resolution of reconstructed volumes was 12  $\mu$ m. Imaging was done using Fiji (Schindelin et al., 2012) and Amira 6 (Stalling et al., 2005).

## 2.3 Statistics

Measurements were tested for normality (Shapiro Wilk's test) and equality of variance (Levene's Test) before conducting ANOVA, Kruskal-Wallis test and Tukey's honest significant difference test using R (R Core Team, 2014), SPSS (IBM Corp., 2012) and Past3 (Hammer et al., 2001). Principal component analysis (PCA) and linear discriminant analysis (LDA) were conducted using the following log-transformed characters, which were chosen because of their parametric nature and significant differences between the clades: HL, HW, HW (in % of HL), SNL (in % of HL), OD (in % of HL), IOW, PVFAF, INTN, INTN (in % of HL), PO, PRAL, PCTPV, PRPV. In PCA groups were disregarded.

## 2.4 Genetic analysis

Total genomic DNA was extracted from ethanol preserved specimens using Dneasy blood & tissue kit (Qiagen, USA). An about 1520 bp long section of the *nuclear recombination activating gene 1* (*RAG-1*) was amplified with the forward primer R1 2533F (5' to 3': CTGAGCTGCAGTCAGTACCATAAGATGT) and R1 4090R (5' to 3': CTGAGTCCTTGTGAGCTTCCATRAAYTT) (López et al., 2004).

Amplifications were carried out in 25  $\mu$ l reactions with 2.5  $\mu$ l reaction buffer [10x], 0.2 mM of each dNTP, 0.5  $\mu$ M of each primer, 1 unit TopTaq; 1 $\mu$ l template DNA with following conditions: 94°C pre-denaturing (4 min), 95°C denaturing (40 s), 58°C annealing (40 s), 72°C extension (90 s) for 35 cycles and 72°C final extension (7 min). PCR fragments were sequenced at LGC genomics (Berlin, Germany) using the primers R1 2533F and R1\_int-f (5' to 3':

CATGGTGCTACATTCCATCACG), which was designed for this study. Sequences were edited and aligned with BioEdit version 7.2. (Hall, 1999). *Garra lamta* was used as outgroup to root the tree (GeneBank accession number: JX074459.1). A Maximum Likelihood Tree was constructed using *MEGA* version 6 (Tamura et al., 2013) with 1000 bootstrap replicates. A Median Joining Network was constructed using PopART 1.7 (<http://popart.otago.ac.nz>).

### 3. Results

There is no measurement without overlaps between all of the mt clades of *G. barreimiae*, although many measurements show significant differences between the clades [Tab. 7-8]. Coloration is very variable and ranges from light brown to a very dark grey. Tubercles on the snout are often present. Meristics of gill rakers, intermediate vertebrae and branched pelvic fin rays can be used to distinguish the West clade from the other clades [Tab. 10]. The analysis of the nuclear gene *RAG-1* yielded three distinct but closely related clades [Fig. 19].

#### 3.1 Traditional Morphometrics

Traditional morphometric measurements of the preserved specimens of *G. barreimiae* are given for each clade in Tab. 3-6. Box plots in Fig. 8 illustrate the most noticeable differences between the mt clades. The box plots of the remaining measurements are attached in the supplement. Mean standard lengths of the Central clade (35.5 mm) and the East clade (35.4 mm) are significantly lower than those of the North clade (41.5 mm) and the West clade (40.1 mm) [Fig. 8 A]. The mean values of the distance from the origin of the anal fin to the center of the anus is the lowest in the Central clade (4.2%) compared to the other clades (East clade: 5.6%, North clade: 5.2%, West clade: 4.8%) [Fig. 8 B]. Mean body depth is lower in fishes from the East clade (22.6%) and the West clade (23.0%) [Fig. 8 C] and higher in the Central clade (24.8%) and the North clade (25.2%). The head width of the Central clade has the highest mean value (19.5%) compared to the other clades (East clade: 18.0 %, North clade: 18.7%, West clade: 18.8%) [Fig. 8 D]. The interorbital width [Fig. 8 E] has the highest mean in the Central clade (12.7%) and the lowest in the West clade (11%). The postorbital distance [Fig. 8 F] has the highest mean in the Central clade (40.4% of HL) and the lowest in the East clade (36.6% of HL). Prepelvic length [Fig. 8 G] and snout length [Fig. 8 H] have the highest mean in the West clade. Pectoral fin length [Fig. 8 I] has the highest mean in the Central clade. The mean predorsal

length [Fig. 8 J] of the East clade is similar to the North clade, but a lower minimum is given. Caudal peduncle depth [Fig. 8 K] has the highest mean in the North clade (13.1%) and the lowest in the West clade (12.1%). Caudal peduncle length [Fig. 8 L] has the highest mean in the West clade (15.1%) and the lowest mean in the Central clade (13.8%).

**Tab. 3:** Morphometric measurements of the West clade. For abbreviations of measurements see Tab. 2.

| <b>West clade</b>                    |             |              |           |          |
|--------------------------------------|-------------|--------------|-----------|----------|
|                                      | <b>mean</b> | <b>range</b> | <b>SD</b> | <b>n</b> |
| SL (mm)                              | 40.1        | 28.3-54.7    |           | 19       |
| <b>In percent of standard length</b> |             |              |           |          |
| AFA                                  | 4.8         | 3.4-6.4      | 0.9       | 18       |
| AFCDF                                | 21.6        | 19.0-25.0    | 1.7       | 19       |
| AFL                                  | 15.9        | 14.4-17.9    | 0.9       | 19       |
| BD                                   | 23.0        | 20.9-25.7    | 1.7       | 18       |
| CPD                                  | 12.1        | 11.1-13.1    | 0.7       | 19       |
| CPL                                  | 15.1        | 12.9-17.5    | 1.4       | 19       |
| DFL                                  | 21.3        | 19.9-23.7    | 1.2       | 18       |
| HD                                   | 16.6        | 14.8-19.0    | 1.0       | 19       |
| HL                                   | 23.9        | 21.2-27.2    | 1.4       | 19       |
| HW                                   | 18.8        | 16.6-21.4    | 1.2       | 19       |
| INTN                                 | 7.2         | 5.8-8.4      | 0.6       | 19       |
| IOW                                  | 11.0        | 9.9-11.9     | 0.7       | 19       |
| PCTFL                                | 20.2        | 18.7-23.7    | 1.2       | 19       |
| PCTPV                                | 33.0        | 30.2-35.1    | 1.3       | 18       |
| PO                                   | 9.23        | 7.8-10.6     | 0.8       | 19       |
| POL                                  | 35.8        | 32.0-38.4    | 1.8       | 19       |
| PRAL                                 | 78.6        | 76.0-81.1    | 1.5       | 19       |
| PRL                                  | 51.6        | 49.5-54.5    | 1.4       | 19       |
| PRPV                                 | 56.0        | 52.2-59.2    | 2.0       | 19       |
| PVFAF                                | 22.5        | 20.3-25.1    | 1.6       | 19       |
| PVFCDF                               | 44.2        | 39.9-48.5    | 1.9       | 19       |
| PVFL                                 | 16.6        | 15.2-18.8    | 1.0       | 19       |
| SNL                                  | 10.5        | 8.2-12.2     | 1.0       | 19       |
| <b>In percent of head length</b>     |             |              |           |          |
| HD                                   | 69.4        | 62.4-74.8    | 3.1       | 19       |
| HW                                   | 78.7        | 70.0-90.2    | 5.4       | 19       |
| INTN                                 | 30.2        | 27.3-33.7    | 1.6       | 19       |
| IOW                                  | 46.4        | 41.7-50.2    | 2.9       | 19       |
| OD                                   | 20.8        | 19.5-23.5    | 1.1       | 19       |
| PO                                   | 38.7        | 33.6-43.9    | 2.8       | 19       |
| SNL                                  | 44.1        | 38.1-49.0    | 3.2       | 19       |

**Tab. 4:** Morphometric measurements of the Central clade. For abbreviations of measurements see Tab. 2.

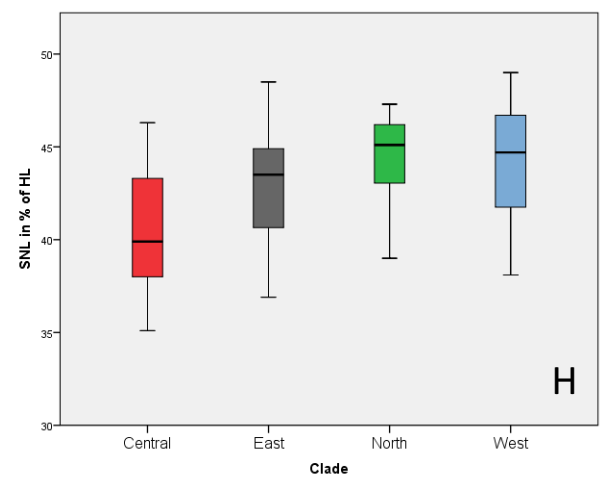
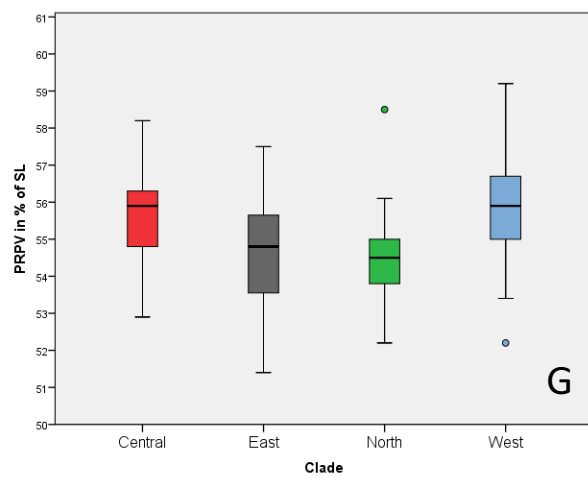
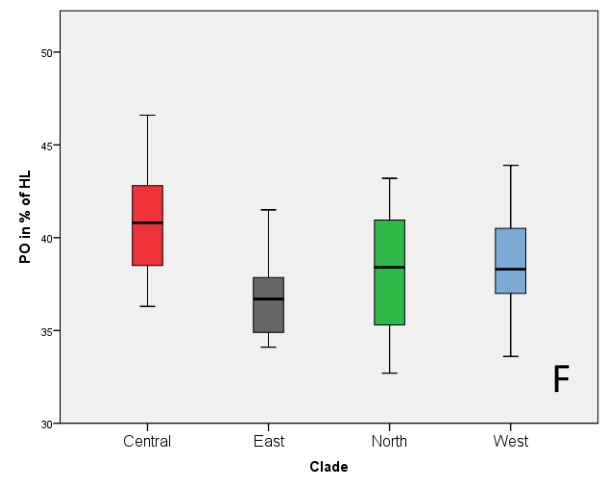
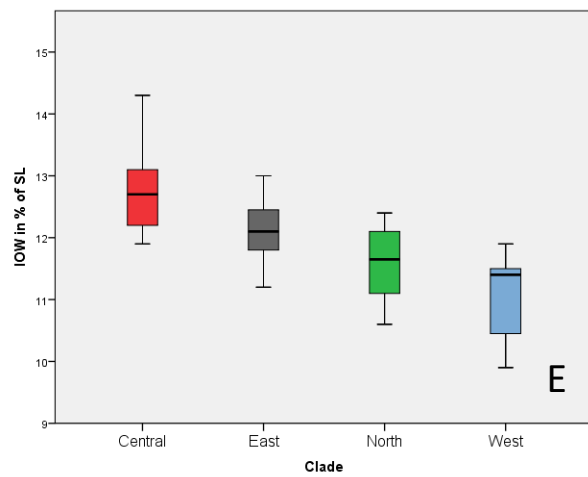
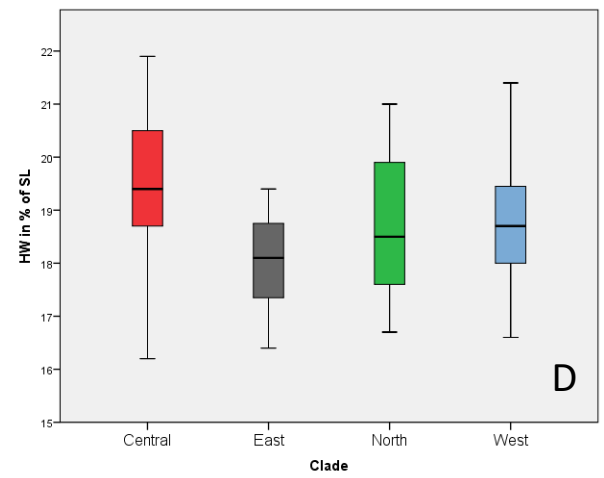
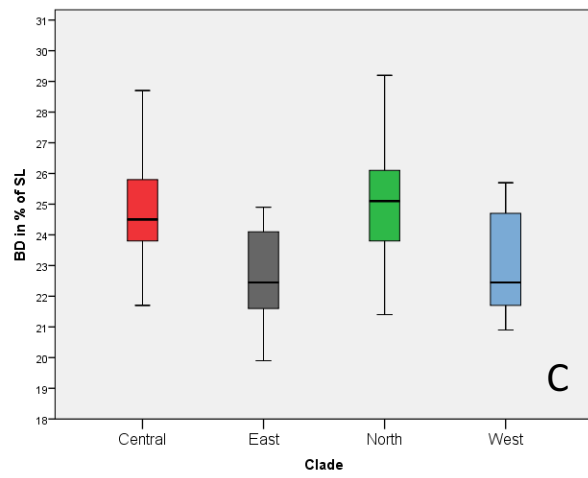
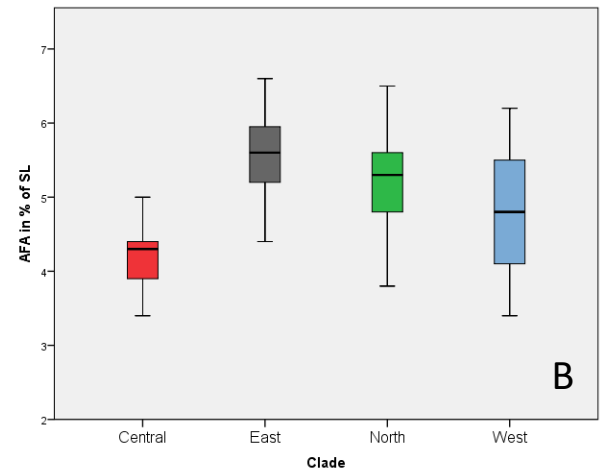
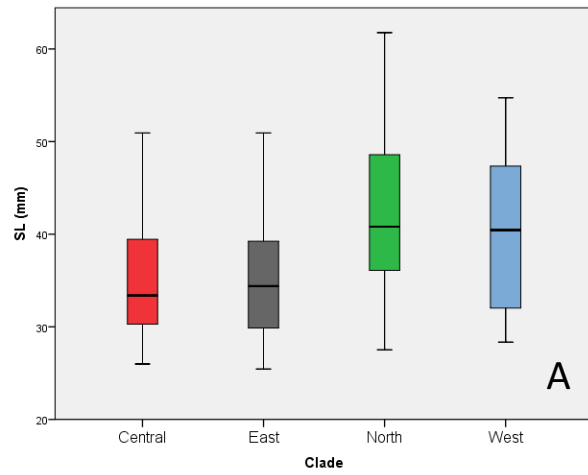
| <b>Central clade</b>                 |             |              |           |          |
|--------------------------------------|-------------|--------------|-----------|----------|
|                                      | <b>mean</b> | <b>range</b> | <b>SD</b> | <b>n</b> |
| SL (mm)                              | 35.5        | 26.0-50.9    |           | 25       |
| <b>In percent of standard length</b> |             |              |           |          |
| AFA                                  | 4.2         | 3.4-5.0      | 0.5       | 25       |
| AFCDF                                | 20.2        | 17.6-22.3    | 1.1       | 25       |
| AFL                                  | 16.2        | 14.3-17.7    | 0.8       | 25       |
| BD                                   | 24.8        | 21.7-28.7    | 1.6       | 25       |
| CPD                                  | 12.4        | 11.4-13.2    | 0.5       | 25       |
| CPL                                  | 13.8        | 11.9-15.6    | 0.8       | 25       |
| DFL                                  | 20.6        | 19.0-22.9    | 1.1       | 25       |
| HD                                   | 17.8        | 16.0-19.5    | 0.9       | 25       |
| HL                                   | 25.3        | 22.0-27.1    | 1.2       | 25       |
| HW                                   | 19.5        | 16.2-21.9    | 1.4       | 25       |
| INTN                                 | 7.8         | 6.9-9.0      | 0.5       | 25       |
| IOW                                  | 12.7        | 11.9-14.3    | 0.6       | 25       |
| PCTFL                                | 20.8        | 17.9-24.8    | 1.8       | 25       |
| PCTPV                                | 30.9        | 27.5-33.8    | 1.6       | 25       |
| PO                                   | 10.2        | 8.6-12.0     | 0.8       | 25       |
| POL                                  | 34.8        | 31.6-37.8    | 1.4       | 25       |
| PRAL                                 | 79.3        | 77.4-81.3    | 1.0       | 25       |
| PRL                                  | 51.9        | 50.5-54.2    | 1.1       | 25       |
| PRPV                                 | 55.6        | 52.9-58.2    | 1.4       | 25       |
| PVFAF                                | 23.3        | 20.5-25.1    | 1.2       | 25       |
| PVFCDF                               | 43.9        | 40.7-46.4    | 1.3       | 25       |
| PVFL                                 | 16.8        | 15.2-19.2    | 1.0       | 25       |
| SNL                                  | 10.3        | 9.0-12.1     | 1.0       | 25       |
| <b>In percent of head length</b>     |             |              |           |          |
| HD                                   | 70.3        | 62.4-74.7    | 2.9       | 25       |
| HW                                   | 77.2        | 64.8-89.9    | 5.2       | 25       |
| INTN                                 | 30.8        | 27.0-34.8    | 1.7       | 25       |
| IOW                                  | 50.1        | 46.3-54.9    | 2.3       | 25       |
| OD                                   | 21.4        | 18.9-25.0    | 1.6       | 25       |
| PO                                   | 40.4        | 36.3-46.6    | 2.6       | 25       |
| SNL                                  | 40.6        | 35.1-46.3    | 3.4       | 25       |

**Tab. 5:** Morphometric measurements of the North clade. For abbreviations of measurements see Tab. 2.

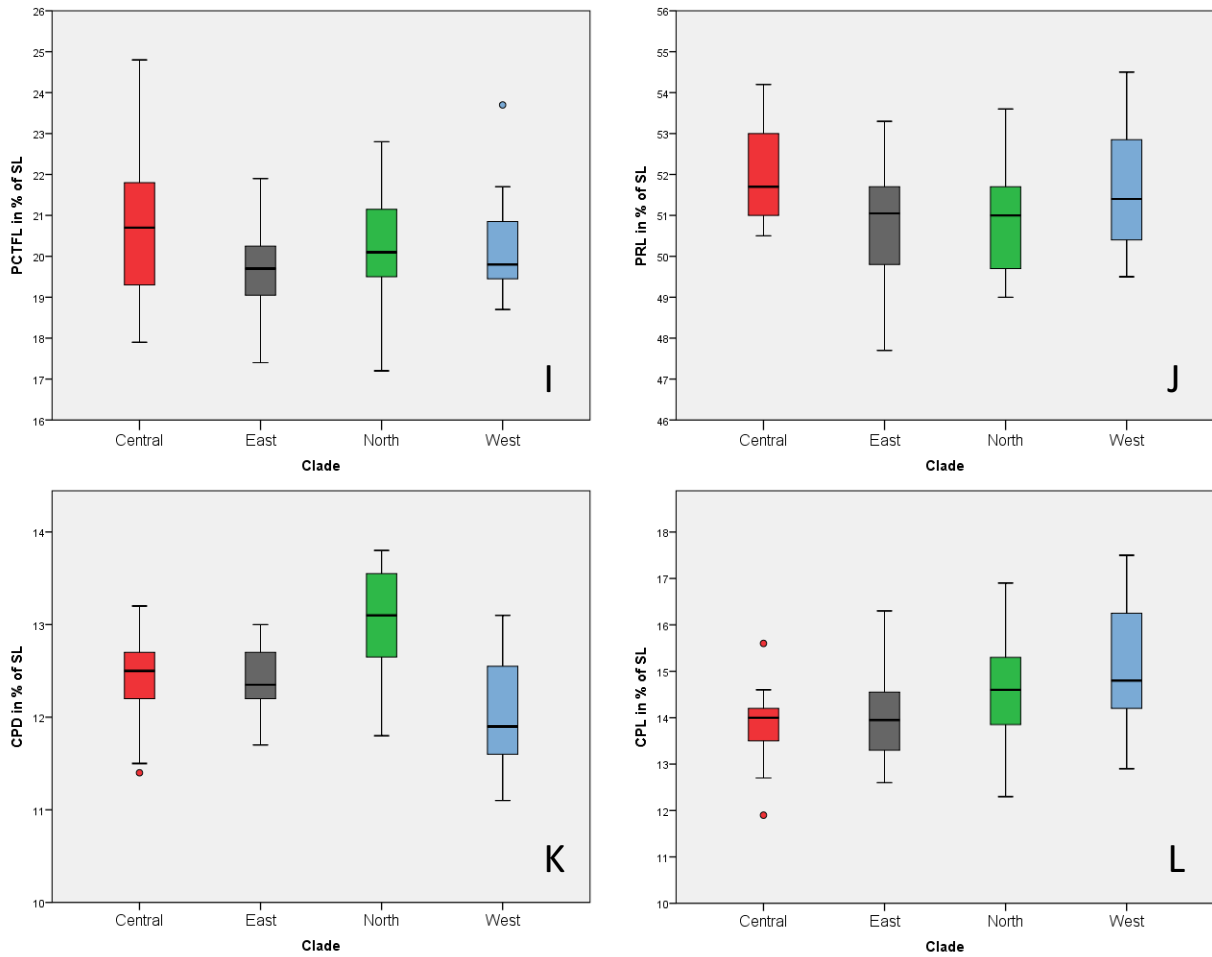
| <b>North clade</b>                   |             |              |           |          |
|--------------------------------------|-------------|--------------|-----------|----------|
|                                      | <b>mean</b> | <b>range</b> | <b>SD</b> | <b>n</b> |
| SL (mm)                              | 41.5        | 27.5-61.8    |           | 19       |
| <b>In percent of standard length</b> |             |              |           |          |
| AFA                                  | 5.2         | 3.8-6.5      | 0.7       | 19       |
| AFCDF                                | 20.9        | 18.8-24.4    | 1.3       | 19       |
| AFL                                  | 16.7        | 14.5-19.4    | 1.2       | 19       |
| BD                                   | 25.2        | 21.4-29.2    | 2.1       | 19       |
| CPD                                  | 13.1        | 11.8-13.8    | 0.6       | 19       |
| CPL                                  | 14.6        | 12.3-16.9    | 1.1       | 19       |
| DFL                                  | 22.2        | 20.3-28.0    | 1.6       | 19       |
| HD                                   | 17.4        | 16.1-18.8    | 0.9       | 19       |
| HL                                   | 24.2        | 21.9-27.5    | 1.5       | 19       |
| HW                                   | 18.7        | 16.7-21.0    | 1.3       | 19       |
| INTN                                 | 7.7         | 6.4-8.6      | 0.5       | 19       |
| IOW                                  | 11.5        | 10.6-12.4    | 0.6       | 18       |
| PCTFL                                | 20.2        | 17.2-22.8    | 1.4       | 19       |
| PCTPV                                | 31.7        | 28.6-33.8    | 1.4       | 17       |
| PO                                   | 9.1         | 8.0-10.5     | 0.6       | 19       |
| POL                                  | 35.9        | 33.4-38.5    | 1.7       | 19       |
| PRAL                                 | 78.6        | 75.3-81.2    | 1.4       | 19       |
| PRL                                  | 50.9        | 49.0-53.6    | 1.2       | 19       |
| PRPV                                 | 54.6        | 52.2-58.5    | 1.4       | 17       |
| PVFAF                                | 23.6        | 22.1-26.4    | 1.2       | 17       |
| PVFCDF                               | 45.0        | 39.9-47.5    | 1.8       | 17       |
| PVFL                                 | 16.9        | 15.0-19.8    | 1.2       | 19       |
| SNL                                  | 10.8        | 9.7-12.5     | 0.8       | 19       |
| <b>In percent of head length</b>     |             |              |           |          |
| HD                                   | 72.4        | 63.2-78.2    | 4.0       | 19       |
| HW                                   | 77.6        | 70.3-84.2    | 4.0       | 19       |
| INTN                                 | 31.7        | 27.2-34.7    | 1.9       | 19       |
| IOW                                  | 48.03       | 38.5-55.7    | 4.4       | 18       |
| OD                                   | 22.1        | 19.4-25.0    | 1.6       | 19       |
| PO                                   | 38.0        | 32.7-43.2    | 3.4       | 19       |
| SNL                                  | 44.7        | 36.9-48.5    | 2.2       | 19       |

**Tab. 6:** Morphometric measurements of the East clade. For abbreviations of measurements see Tab. 2.

| <b>East clade</b>                    |             |              |           |          |
|--------------------------------------|-------------|--------------|-----------|----------|
|                                      | <b>mean</b> | <b>range</b> | <b>SD</b> | <b>n</b> |
| SL (mm)                              | 35.4        | 25.4-50.9    |           | 24       |
| <b>In percent of standard length</b> |             |              |           |          |
| AFA                                  | 5.6         | 4.4-6.6      | 0.6       | 24       |
| AFCDF                                | 20.9        | 19.0-23.6    | 1.3       | 24       |
| AFL                                  | 16.0        | 13.9-18.7    | 1.3       | 24       |
| BD                                   | 22.6        | 19.9-24.9    | 1.4       | 24       |
| CPD                                  | 12.4        | 11.7-13.0    | 0.3       | 24       |
| CPL                                  | 14.1        | 12.6-16.3    | 1.0       | 24       |
| DFL                                  | 21.9        | 20.2-25.6    | 1.3       | 24       |
| HD                                   | 16.9        | 15.7-18.4    | 0.8       | 24       |
| HL                                   | 24.8        | 21.7-27.3    | 1.3       | 24       |
| HW                                   | 18.0        | 16.4-19.4    | 0.9       | 24       |
| INTN                                 | 7.8         | 6.9-8.5      | 0.4       | 24       |
| IOW                                  | 12.1        | 11.2-13.0    | 0.4       | 24       |
| PCTFL                                | 19.7        | 17.4-21.9    | 1.0       | 24       |
| PCTPV                                | 31.3        | 29.4-34.1    | 1.4       | 24       |
| PO                                   | 9.1         | 7.8-11.1     | 0.8       | 24       |
| POL                                  | 35.1        | 32.2-39.0    | 1.6       | 24       |
| PRAL                                 | 79.0        | 76.0-81.0    | 1.2       | 24       |
| PRL                                  | 50.8        | 47.7-53.3    | 1.4       | 24       |
| PRPV                                 | 54.7        | 51.4-57.5    | 1.4       | 24       |
| PVFAF                                | 24.0        | 19.7-26.8    | 1.6       | 24       |
| PVFCDF                               | 45.0        | 41.3-49.0    | 1.6       | 24       |
| PVFL                                 | 16.8        | 14.5-18.4    | 1.0       | 24       |
| SNL                                  | 10.6        | 9.2-12.7     | 1.0       | 24       |
| <b>In percent of head length</b>     |             |              |           |          |
| HD                                   | 68.1        | 63.6-78.6    | 3.4       | 24       |
| HW                                   | 72.8        | 65.4-79.6    | 3.4       | 24       |
| INTN                                 | 31.4        | 26.9-34.1    | 1.7       | 24       |
| IOW                                  | 49.0        | 44.0-54.0    | 2.6       | 24       |
| OD                                   | 22.7        | 20.4-24.7    | 1.3       | 24       |
| PO                                   | 36.6        | 34.1-41.5    | 1.9       | 24       |
| SNL                                  | 42.9        | 36.9-48.5    | 3.0       | 24       |







**Fig. 8:** A-L: Selected length measurements of the four mt clades [West clade: blue, Central clade: red, North clade: green, East clade: grey]. Outliers are marked by coloured dots. For abbreviations of measurements see Tab. 2.

### 3.2 Statistics

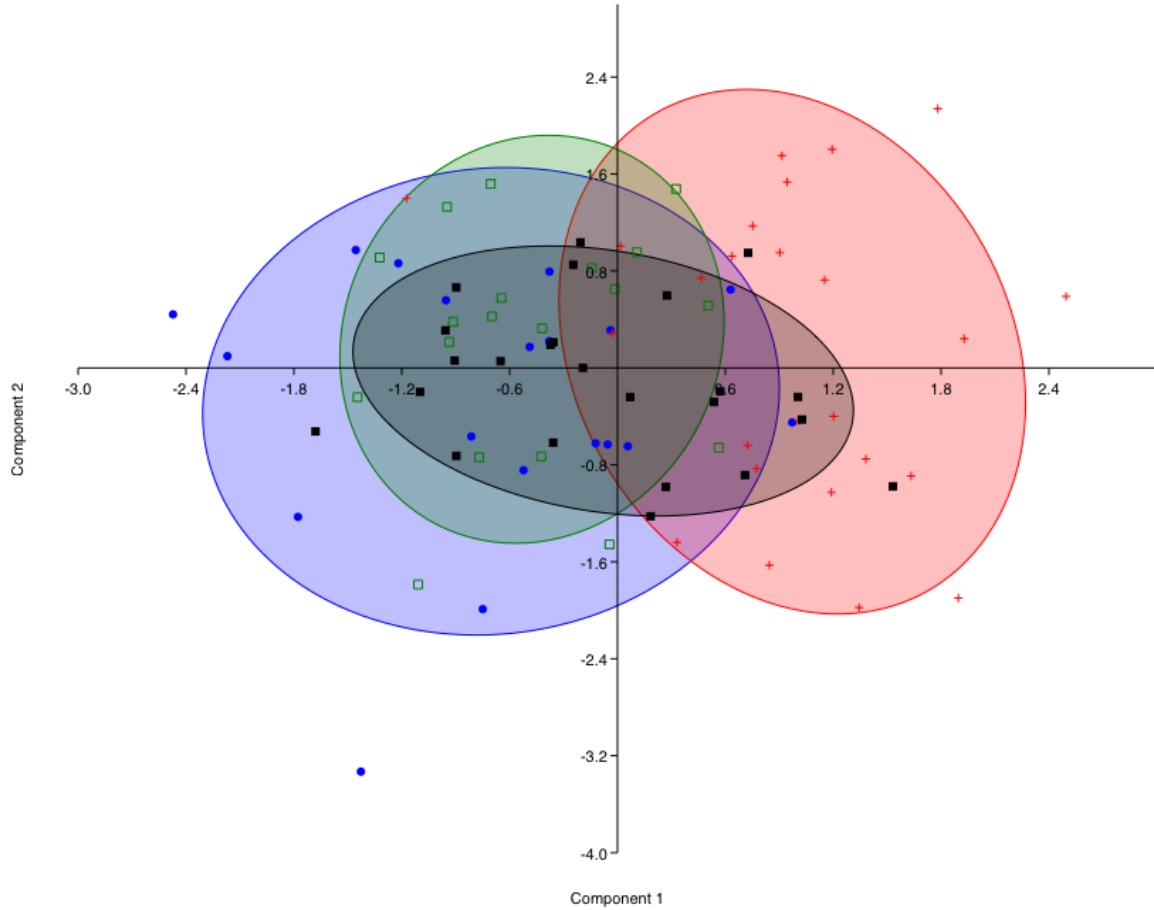
The following measurements are not normally distributed and are thus not suitable for ANOVA, Tukey's HSD and PCA: BD [West clade], DFL [North clade], HD and HD in % of HL [East clade], PRL [Central clade], PCTFL [West clade]. The following measurements have no equality of variance and are thus not suitable for ANOVA, Tukey's HSD and PCA [Tab. 7]: PCTFL, CPL, IOW (in % of HL), AFA, PO (in % of HL), CPD. In the case of non-parametric data the Kruskal-Wallis test is a suitable alternative to ANOVA. Results for Levene's test, ANOVA and Kruskal-Wallis test are given in Tab. 7. Results for Tukeys HSD test are shown in Tab. 8 on the basis of the results of ANOVA.

**Tab. 7:** 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 is preferable over ANOVA. In this case results of the Kruskal-Wallis test are written in bold letters. Signif. codes: 0 "\*\*\*\*", 0.001 "\*\*\*", 0.01 "\*\*". For abbreviations of measurements see Tab. 2.

|                | Levene's test | ANOVA         | Kruskal-Wallis test  |
|----------------|---------------|---------------|----------------------|
| AFA            | 0.0012**      | 2.460E-10***  | <b>3.652E-08***</b>  |
| AFCDF          | 0.2325        | 0.0125*       | 0.0430*              |
| AFL            | 0.0881        | 0.0808        | 0.1639               |
| BD             | 0.6192        | 1.263E-06 *** | <b>1.445E-05 ***</b> |
| CPD            | 0.0035**      | 1.649E-06***  | <b>9.943E-05***</b>  |
| CPL            | 0.0170*       | 0.0006***     | <b>0.0033**</b>      |
| DFL            | 0.9175        | 0.0003***     | <b>0.0002***</b>     |
| HD (% of HL)   | 0.4949        | 0.0007***     | <b>0.0005***</b>     |
| HL             | 0.8436        | 0.0023**      | 0.0013**             |
| HW (% of HL)   | 0.1002        | 0.0002***     | 0.0002***            |
| INTN (% of HL) | 0.8597        | 0.0196*       | 0.0114*              |
| IOW (% of HL)  | 0.0367*       | 0.0012**      | <b>0.0032**</b>      |
| OD (% of HL)   | 0.2250        | 0.0001***     | 0.0002***            |
| PCTFL          | 0.0100*       | 0.0625        | <b>0.1778</b>        |
| PCTPV          | 0.7127        | 0.0001***     | 0.0030**             |
| PO (% of HL)   | 0.0040**      | 4.836E-05***  | <b>0.0001***</b>     |
| POL            | 0.3254        | 0.0539        | 0.0777               |
| PRAL           | 0.1987        | 0.1715        | 0.1955               |
| PRL            | 0.7126        | 0.0079 **     | <b>0.0335*</b>       |
| PRPV           | 0.3664        | 0.0105*       | 0.0036**             |
| PVFAF          | 0.4907        | 0.0063**      | 0.0180*              |
| PVFCDF         | 0.6056        | 0.0895        | 0.1220               |
| PVFL           | 0.8987        | 0.8523        | 0.7766               |
| SNL (% of HL)  | 0.1126        | 9.016E-05***  | 0.0003***            |

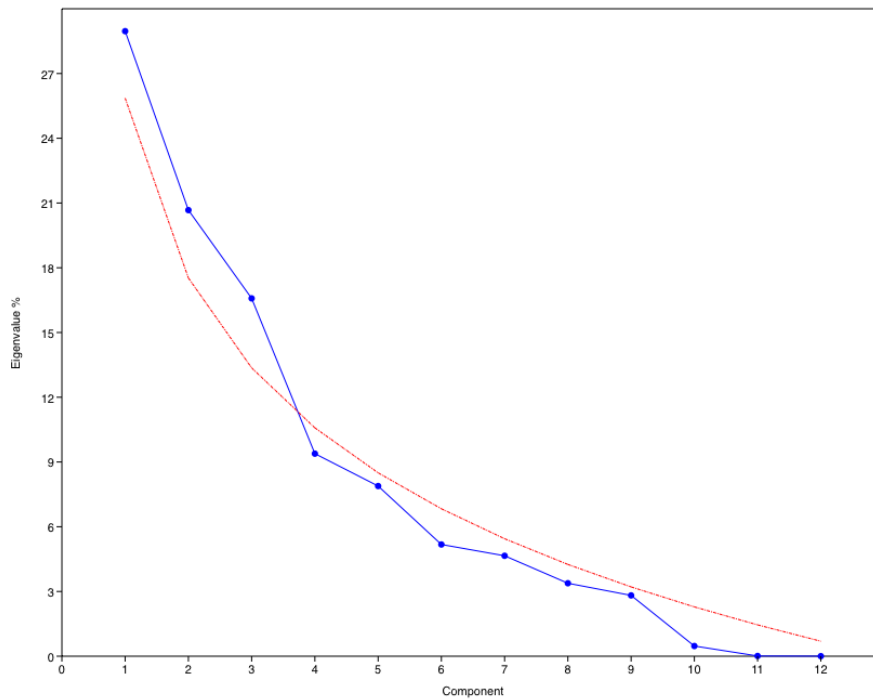
**Tab. 8:** Tukey's honest significant difference test of parametric morphometric measurements of the four clades. Signif. codes: 0 "\*\*\*\*", 0.001 "\*\*\*", 0.01 "\*\*". For abbreviations of measurements see Tab. 2.

| <b>West<br/>Central</b>      | <b>West<br/>North</b>        | <b>West<br/>East</b>          | <b>Central<br/>East</b>       | <b>Central<br/>North</b>       | <b>North<br/>East</b>       |
|------------------------------|------------------------------|-------------------------------|-------------------------------|--------------------------------|-----------------------------|
| AFCDF<br>0.0069**            | HD<br>0.0113*                | HW<br>(% of HL)<br>0.0005**** | BD<br>0.0006****              | HL<br>0.0266*                  | BD<br>0.0002****            |
| HD<br>0.0003****             | HD<br>(% of HL)<br>0.0234*   | INTN<br>0.001**               | DFL<br>0.0071**               | IOW<br>0.0001****              | HW<br>(% of HL)<br>0.0049** |
| HL 0.0036**                  | INTN<br>0.0125*              | IOW<br>0.0001****             | HW<br>0.0009****              | PO<br>0.0002****               | IOW<br>0.0053**             |
| INTN<br>0.0013**             | INTN<br>(% of HL)<br>0.0152* | OD<br>(% of HL)<br>0.0003**** | HW<br>(% of HL)<br>0.0125**** | SNL<br>(% of HL)<br>0.0003**** |                             |
| IOW<br>0.0001****            | OD<br>(% of HL)<br>0.0159*   | PCTPV<br>0.002**              | IOW<br>0.0135*                |                                |                             |
| PCTPV<br>0.0002****          | PCTPV<br>0.0217*             | PRPV<br>0.038*                | OD<br>(% of HL)<br>0.0158*    |                                |                             |
| PO<br>0.0003****             | PRPV<br>0.019*               | PVFAF<br>0.0045**             | PO<br>0.0002****              |                                |                             |
| SNL<br>(% of HL)<br>0.0016** | PVFAF<br>0.0464*             |                               |                               |                                |                             |

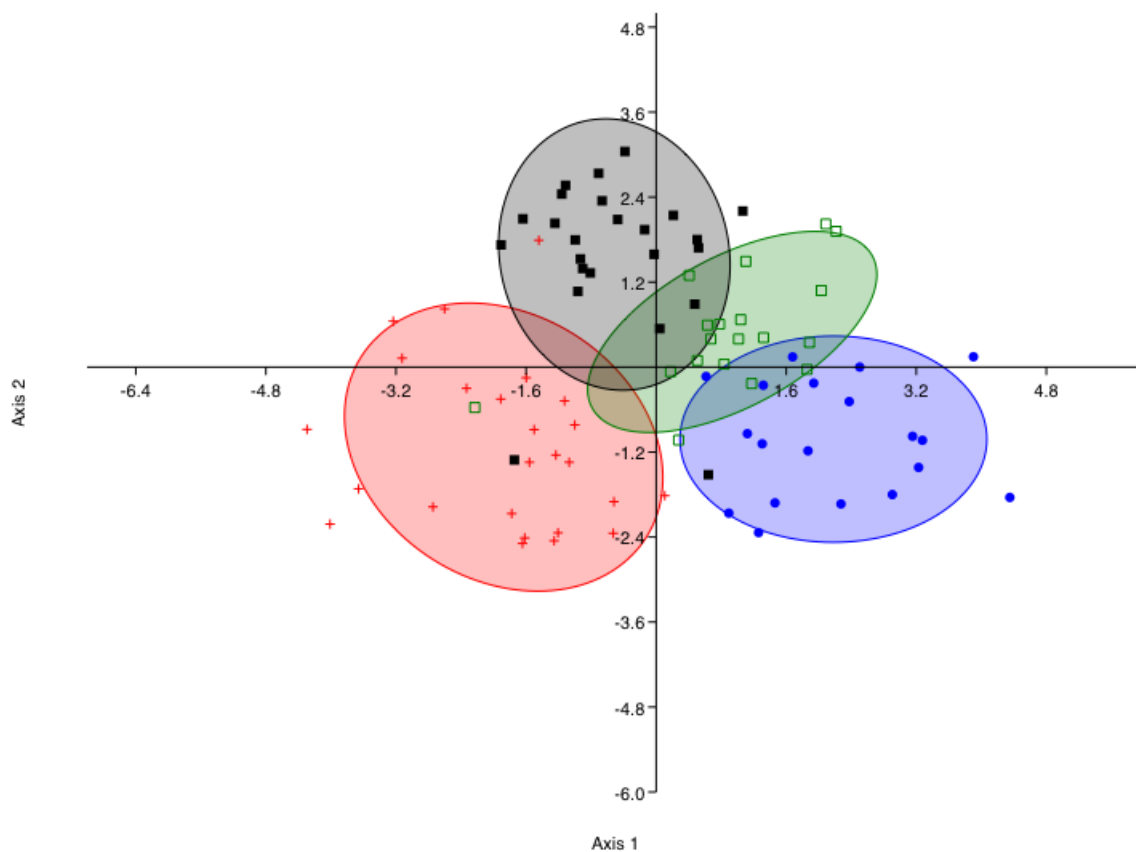


**Fig 9:** PCA of log-transformations of significant parametric measurements with ellipses indicating 75% tolerance. [PC1: eigenvalue 0.00291, 28.96% variance; PC2: eigenvalue 0.00208, 20.67% variance; West clade: blue, Central clade: red, North clade: green, East clade: black]

The PCA [Fig. 9] was carried out with log-transformations of parametric and significant morphometric measurements. PC1 has an eigenvalue of 0.00291 and explains 28.96% of variance and PC2 with an eigenvalue of 0.00208 explains 20.67% of variance. Ellipses with 75% tolerance are used in the plot. PC1, PC2 and PC3 were higher than the random broken stick model, which functions as a stopping rule in PCA, in which components should only be retained as long as their eigenvalues are higher than corresponding random broken stick components [Fig. 10]. Since PC4 to PC12 are below the broken stick model they had to be rejected. LDA [Fig. 11] was carried out with log-transformations of parametric and significant morphometric measurements. Axis 1 has an eigenvalue of 2.42 and explains 60.03% of variance. Axis 2 has an eigenvalue of 1.41 and explains 34.87% of variance. Axis 3, which was not used for the plot, has an eigenvalue of 0.206 and explains 5.1% of variance. 86.21% of specimens are correctly classified in the confusion matrix [Tab. 9].



**Fig. 10:** Scree plot of the PCA. PC1 to PC3 eigenvalues (blue) are higher than the random broken stick model (red) and therefore may be used for PCA.



**Fig. 11:** LDA of log-transformations of significant parametric measurements with ellipses indicating 75%

tolerance [Axis 1: eigenvalue 2.42, 60.03% of variance, Axis 2: eigenvalue 1.41, 34.87% of variance; West clade: blue, Central clade: red, North clade: green, East clade: black]

**Tab 9:** Confusion matrix of LDA. 86.21% of specimens were correctly classified.

|                | <b>West</b> | <b>Central</b> | <b>North</b> | <b>East</b> | <b>Total</b> |
|----------------|-------------|----------------|--------------|-------------|--------------|
| <b>West</b>    | 15          | 0              | 4            | 0           | 19           |
| <b>Central</b> | 0           | 24             | 0            | 1           | 25           |
| <b>North</b>   | 1           | 1              | 17           | 0           | 19           |
| <b>East</b>    | 1           | 1              | 3            | 19          | 24           |
| <b>Total</b>   | 17          | 26             | 24           | 20          | 87           |

### 3.3 Meristics

Vertebrae 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. Meristic counts are summarized in **Tab. 10**.

Main differences were found in the number of intermediate vertebrae: 1 in the West clade [**Fig. 12**] versus 2 in the other clades [**Fig. 13-15**], i,7 pelvic fin rays in the West clade and i,8 in the other clades [**Fig. 16**], 17 precaudal vertebrae in the East clade, 18 in the Central clade and North clade and 19 in the West clade [**Tab. 10**], although there are overlaps. There are 15 to 17 gill rakers on the ceratobranchial on the lower limb of the first gill arch in the West clade [mean value: 15.5; 15=64%, 16=27%, 17=9%], clearly separating it from the other clades with 12 to 14 in the Central clade [mean value: 12.5; 12=60%, 13=30%, 14=10%] and 10 to 14 in the North clade [mean value: 12.1; 10=18%; 11=18%; 12=18%; 13=35%; 14=12%] and East clade [mean value: 12.2; 10=9%; 11=27%; 12=23%; 13=18%; 14=23%].

Across all clades the following traits are quite stable: Dorsal fin rays of all clades are iii,7½ and anal fin rays are iii,5½ [**Fig. 12-15**]. Individual cases of 2 unbranched anal fin rays [**Fig. 18 A**] as well as 4½ branched anal fin rays occur. 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 has 10 upper and 9 lower principal fin rays (=9+8 branched caudal fin rays). Individual cases of 11+10 principal caudal fin rays occur [**Fig 18 B**]. Gill rakers are small, sometimes only knob-like, very fragile and present on the ceratobranchial, but not always on the epibranchial. There are 12 or 13 predorsal scales with individual cases of 11 or 14 predorsal scales. There are 4 scales above the lateral line and 3 scales below the lateral line. Individual cases of 5 scales above the lateral line and 4 scales below the lateral line occur. All fish have four barbels, although cases of single missing barbels occur. Pharyngeal teeth in three rows: typically 2.4.5-5.4.2 [**Fig. 17**]. Teeth numbers of 1.2.5, 1.3.5 or 2.3.5 occur.

**Tab. 10:** Meristic counts and associated number of specimens per clade. Gill rakers counted on lower limb of the first gill arch. Dorsal fin pterygophore insertion refers to the precaudal vertebra number anterior to the first major dorsal fin pterygophore. Anal fin pterygophore insertion refers to the caudal vertebra number anterior to the first major anal fin pterygophore. Presence and absence of tubercles and breast scales are given.

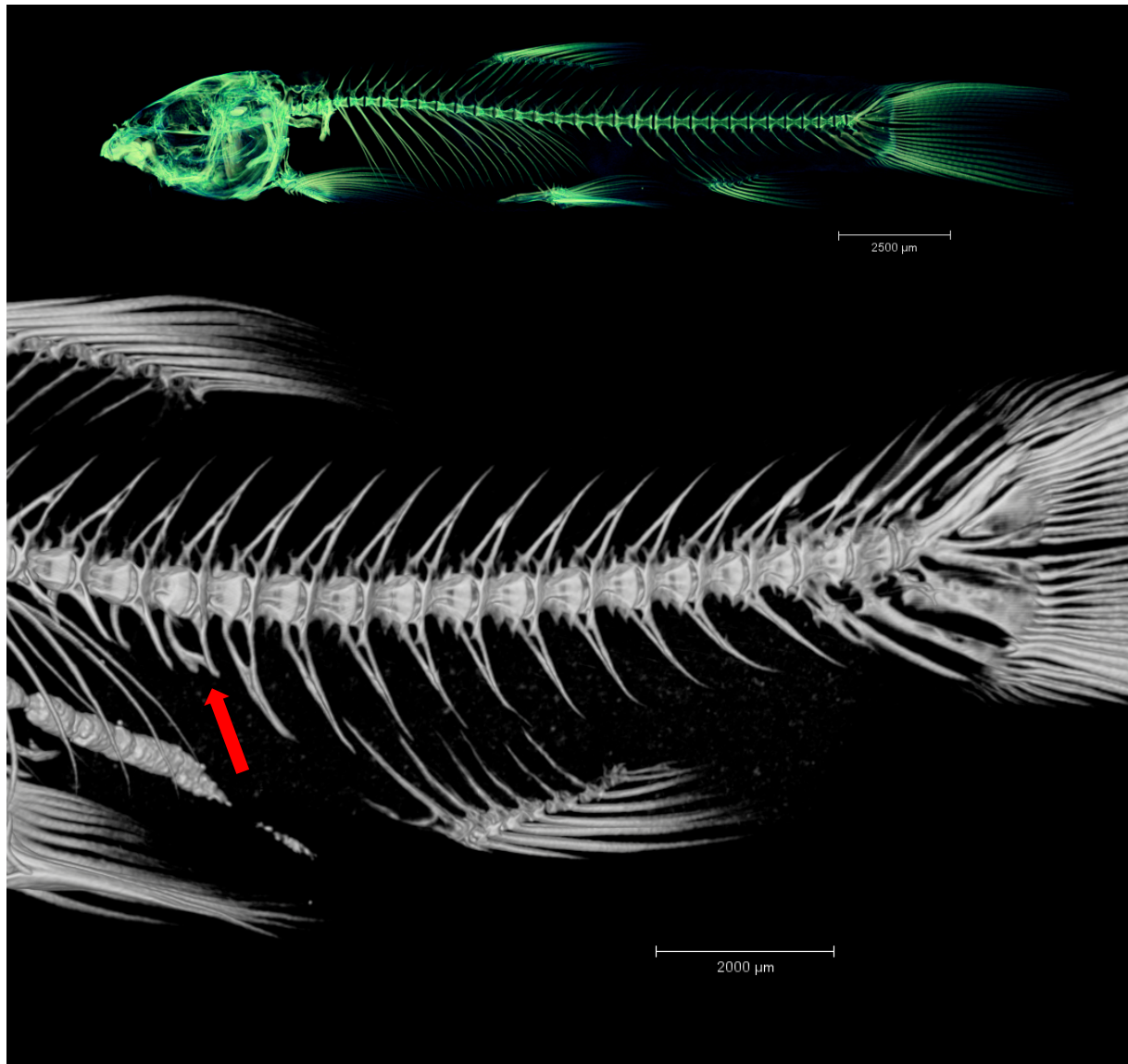
|                                          | count | West | Central | North | East |
|------------------------------------------|-------|------|---------|-------|------|
| precaudal<br>vertebrae                   | 17    | -    | -       | 2     | 7    |
|                                          | 18    | 1    | 6       | 5     | -    |
|                                          | 19    | 6    | 1       | 1     | -    |
| intermediate<br>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<br>pterygophore<br>insertion  | 10    | -    | 3       | 4     | 6    |
|                                          | 11    | 6    | 4       | 4     | 1    |
|                                          | 12    | 1    | -       | -     | -    |
| anal fin<br>pterygophore<br>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<br>caudal fin base     | 1     | -    | 1       | -     | -    |
|                                          | 2     | 11   | 23      | 8     | 17   |
|                                          | 3     | 8    | 1       | 11    | 6    |
| total lateral scale<br>count             | 32    | -    | 6       | -     | 2    |
|                                          | 33    | 6    | 16      | 13    | 7    |
|                                          | 34    | 8    | 3       | 5     | 14   |
|                                          | 35    | 5    | -       | 1     | -    |
| scales between<br>anus and anal fin      | 1     | -    | 2       | 1     | 3    |
|                                          | 2     | 17   | 20      | 10    | 11   |
|                                          | 3     | 2    | 3       | 8     | 7    |
|                                          | 4     | -    | -       | -     | 1    |
| scales between<br>anus and pelvic<br>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<br>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<br>lower limb of the<br>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  | -  | -  | -  |
| branched pelvic<br>fin rays                                | 7   | 18 | 3  | 4  | 4  |
|                                                            | 8   | 1  | 22 | 15 | 19 |
| unbranched<br>pectoral fin rays                            | 1   | 16 | 24 | 15 | 18 |
|                                                            | 2   | 3  | 1  | 4  | 4  |
| branched pectoral<br>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 |

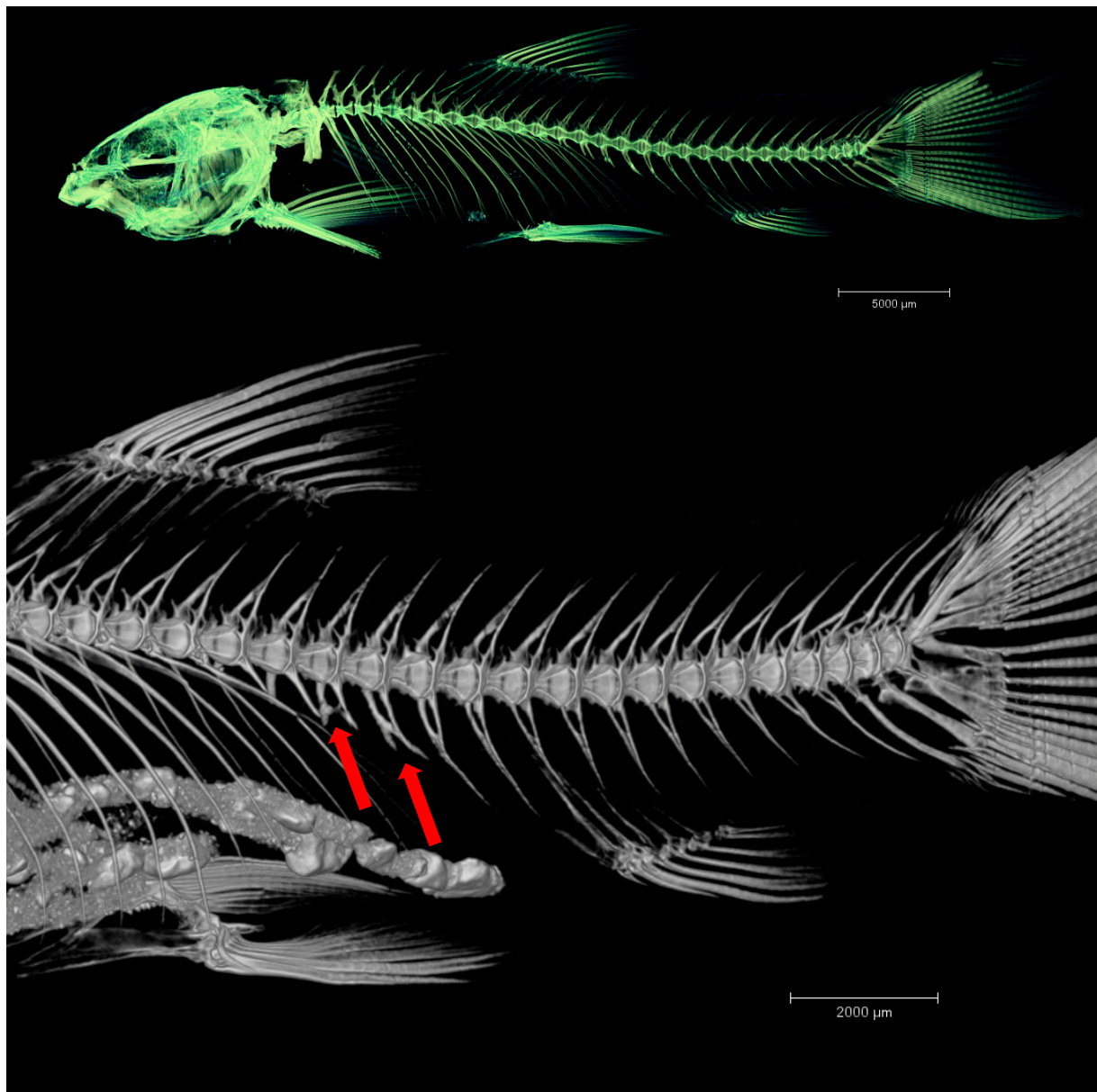
### 3.4 Micro CT

Results of micro CT imaging have been incorporated into the meristic results [Tab. 10]. The number of intermediate vertebrae [Fig. 12-15] and branched pelvic fin rays [Fig. 16] could be used to distinguish the West clade from the other clades. Pharyngeal teeth numbers are variable but typically 2.4.5-5.4.2 [Fig. 17].

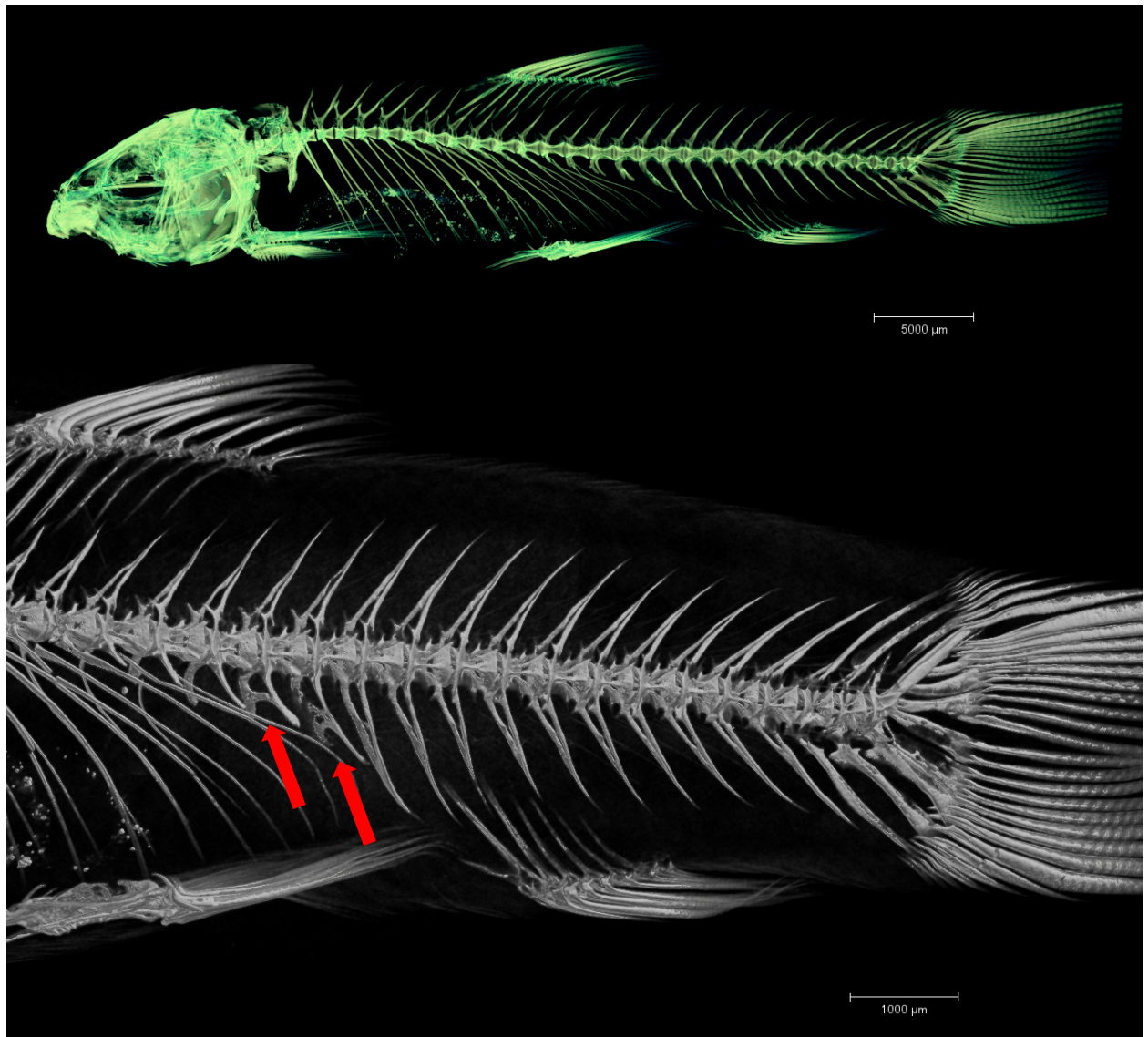




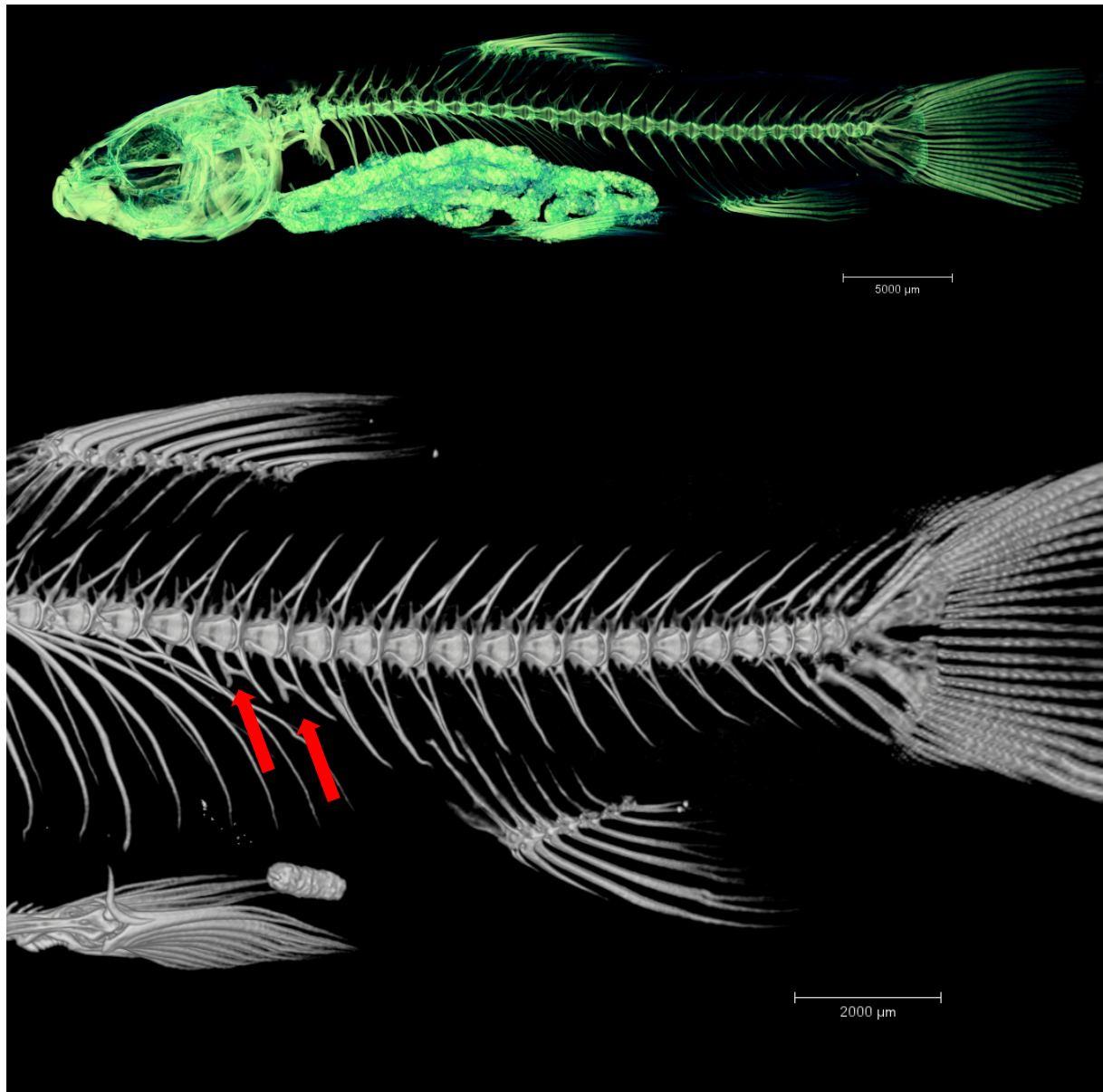
**Fig. 12:** Micro CT images of typical West clade specimens [upper picture: Gbar 402; lower picture: Gbar 294] with one individual intermediate vertebra (arrow), directly followed by two caudal vertebrae before the anal fin pterygophore insertion.



**Fig. 13:** Micro CT images of a typical specimen of the Central clade [Gbar283] with two intermediate vertebrae (arrows). Substrate within the intestine is clearly visible.

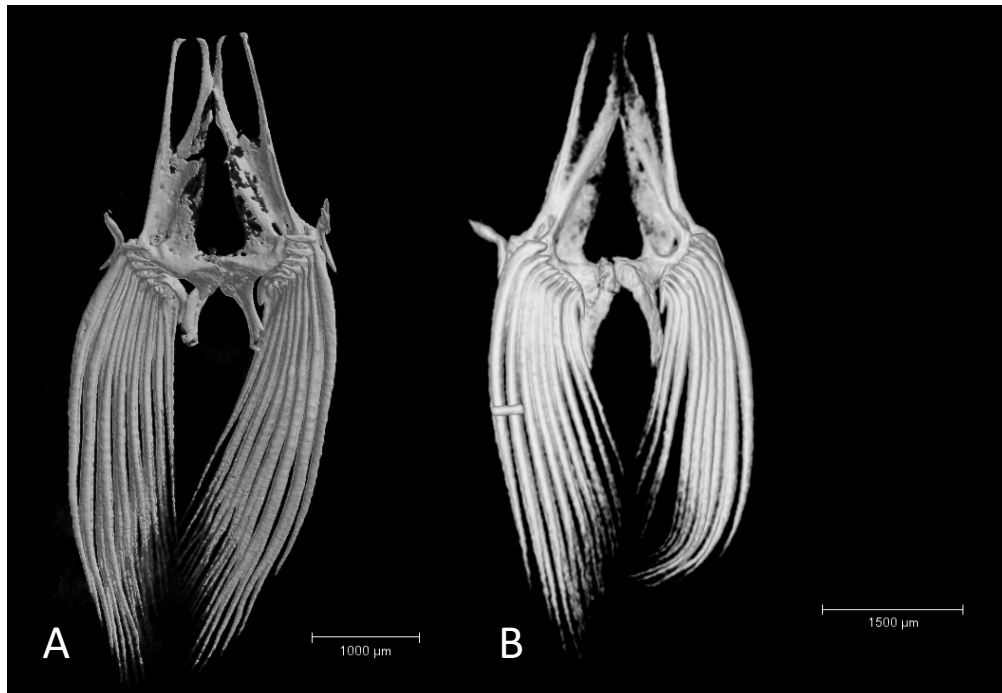


**Fig. 14:** Micro CT images of a typical specimen of the North clade [Gbar242] with two intermediate vertebrae (arrows).

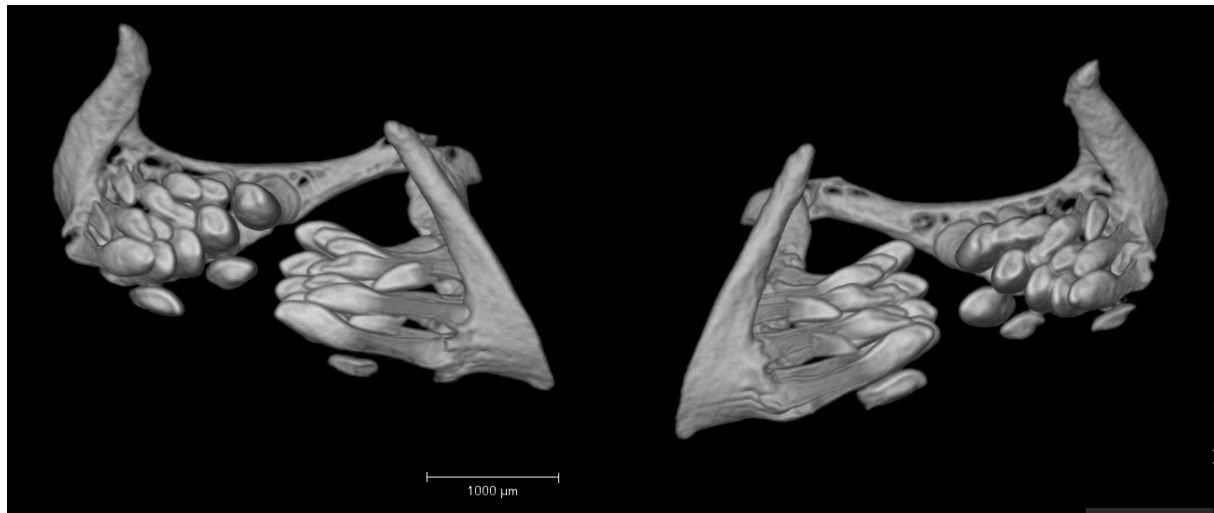


**Fig. 15:** Micro CT images of typical specimens of the East clade [upper picture: Gbar260; lower picture: Gbar315] with two intermediate vertebrae (arrows). The intestine is filled with substrate and thus clearly visible.

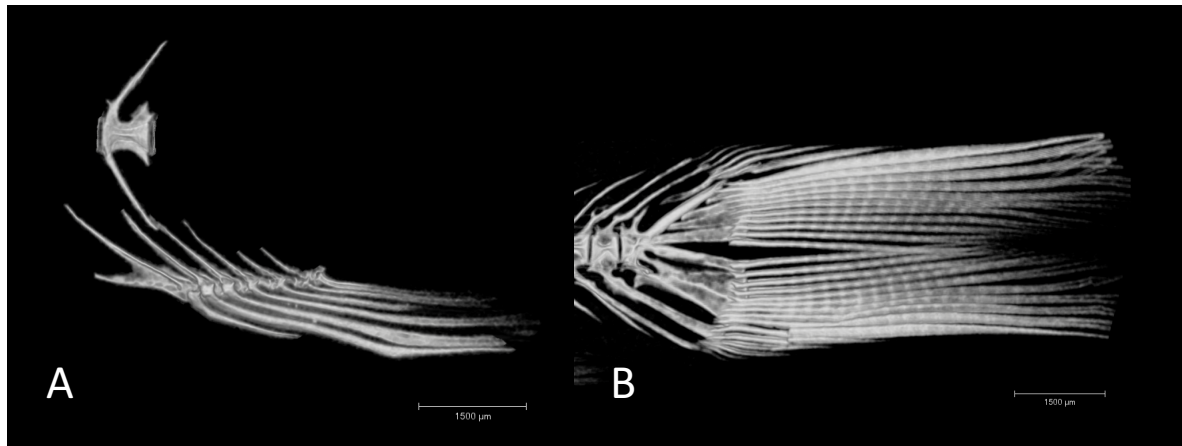




**Fig. 16:** Variations of pelvic fin ray numbers in specimens belonging to the West clade [A] and the Central clade [B]. A (i,7) is more common in West clade specimens, while B (i,8) is more common in the other three clades. See also Tab. 10.



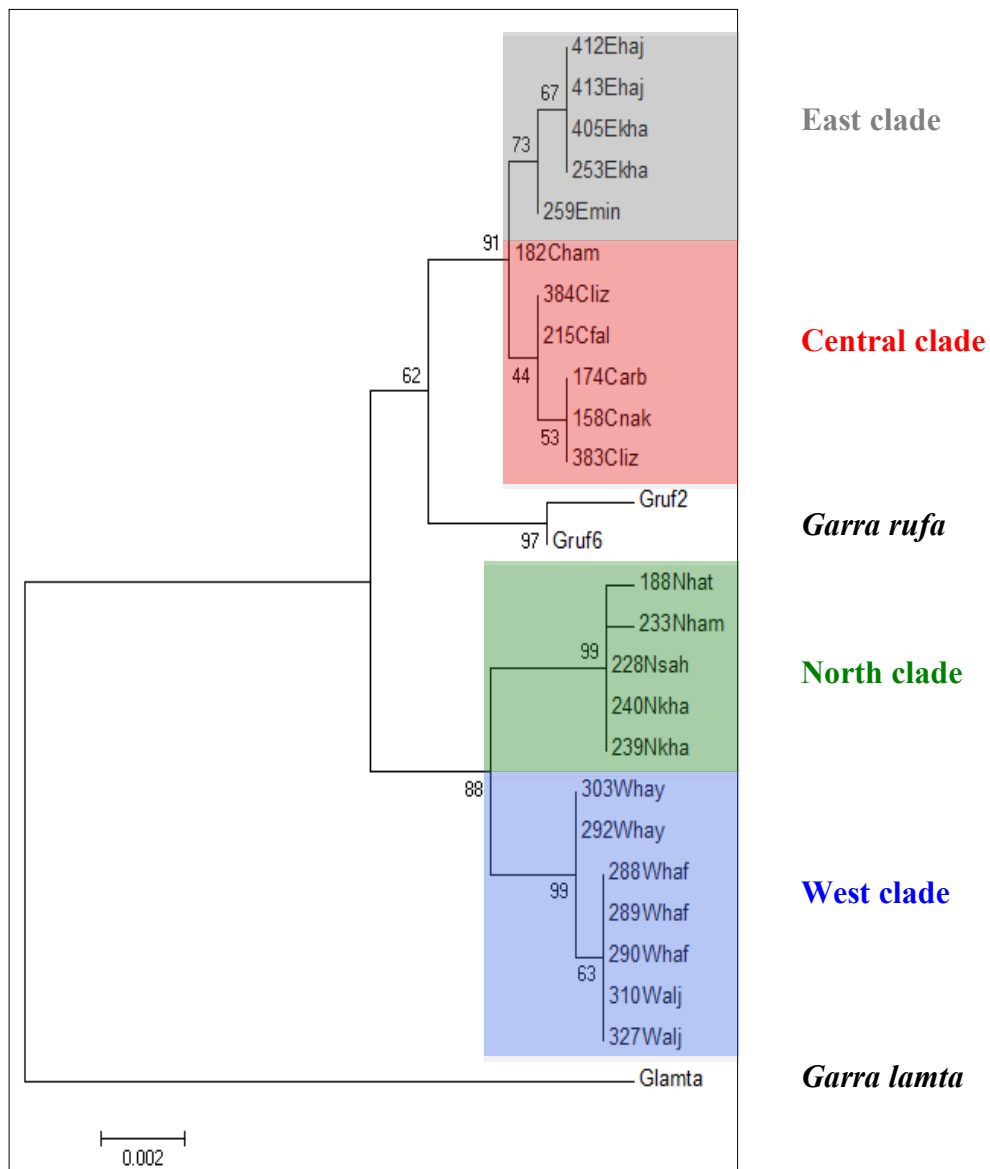
**Fig. 17:** Pharyngeal teeth numbers 2.4.5-5.4.2 are typical for the genus *Garra*. Pictured left and right teeth belong to a North clade specimen [Gbar231 37.0 mm SL].



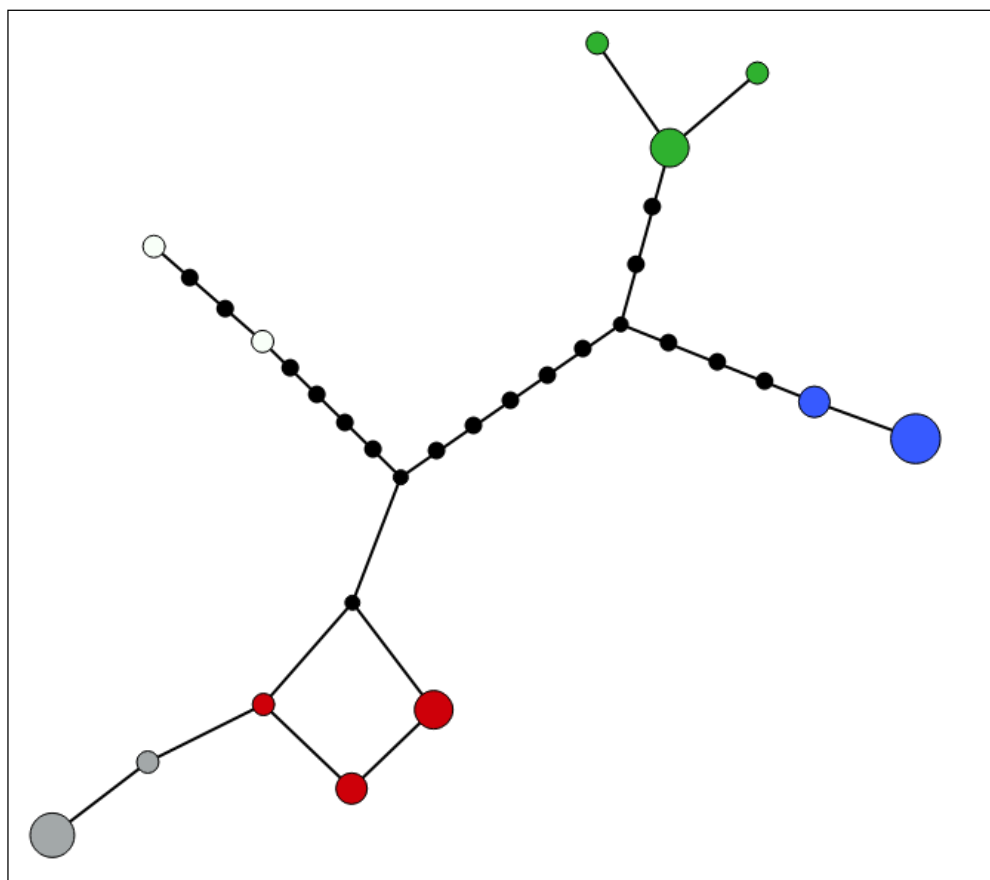
**Fig. 18:** Atypical fin ray numbers. A: Anal fin with ii,5½ fin rays of a North clade specimen; B: Caudal fin with 11+10 principal fin rays of a West clade specimen.

### 3.5 Genetic analysis

From 25 specimens a section of the *RAG-1* gene was amplified, none of the sequences had an insertion/deletion, hence the alignment was straightforward and had a length of 1459 bp. The Maximum Likelihood tree is given in **Fig. 19**. Three of the four mt clades of *G. barreimiae* can also be found in the tree from the *RAG-1* sequences: The sequences of the East clade and those of the Central clade are closely related and form one clade together. The North clade and the West clade are clearly distinguishable. *Garra barreimiae* does not form a monophylum as *G. rufa* is the sister group of the clade comprising the Central clade and the East clade. However, the support value for this node is not very high. In the Median Joining Network [**Fig. 20**] a single substitution separates the East clade from the Central clade and 7 substitutions separate the North clade from the West clade. The Central clade and the East clade have less substitutions to *G. rufa* than to the North clade and West clade. Mean p distances [**Tab. 11**] between the three clades are very low.



**Fig. 19:** Maximum Likelihood Tree of a 1459 bp long fragment of the nuclear gene *RAG-1*. *Garra lamta* was used as outgroup. The mt Central clade and East clade together form the nc Central-East clade. Numbers of the individuals refer to collection specimen numbers (Gbar) of the NHM Vienna.



**Fig. 20:** Median Joining Network of *RAG-I* [West clade: blue; Central clade: red; North clade: green; East clade: grey; *Garra rufa*: white]. The size of the colored circles is proportional to the number of individuals sharing the same haplotype.

**Tab. 11:** Mean p distances between three nuclear clades of *G. barreimiae* and *G. rufa*.

| Clade              |                    | p distance | Std. Err |
|--------------------|--------------------|------------|----------|
| West clade         | North clade        | 0.0056     | 0.0019   |
| West clade         | Central-East clade | 0.0094     | 0.0024   |
| North clade        | Central-East clade | 0.0085     | 0.0023   |
| West clade         | <i>G. rufa</i>     | 0.0104     | 0.0025   |
| North clade        | <i>G. rufa</i>     | 0.0109     | 0.0028   |
| Central-East clade | <i>G. rufa</i>     | 0.0062     | 0.0019   |



## 4. Discussion

In the past, the taxonomy and species delimitation of several species of the genus *Garra* has been problematic because of the high variability of morphological characters even concerning specimens from the same species and the same locality (Menon, 1964). According to Menon, the number of barbels, the absence or presence of the proboscis, the position of the anus, the number of lateral line scales and gill rakers, as well as the coloration are the most important taxonomic characters in the genus. He also stated that many characters like body depth, the length of the head, the position of dorsal fin or anal fin, the presence of scales on the chest, belly and back as well as the interorbital width vary depending on environment or growth and thus are not suitable for taxonomy. Also fin rays, pharyngeal teeth and plastic characters like tubercles on the snout, the size of the adhesive disc, the snout or the eyes, among others, are not helpful in separating species (Menon, 1964), however in more recent publications morphological traits like the number of fin rays could be used in the description of new species (Sayyadzadeh et al., 2015).

With the introduction of molecular genetic methods however, distant lineages could be separated into clades, which eventually present diverging evolutionary units with hidden morphological differences. All specimens of this study have originally been caught for genetic analyses only. Therefore, the samples did sometimes not represent the full size spectrum of a population and contained many subadult individuals. However, this study is based on the largest number of morphologically examined specimens of *Garra barreimiae* to this point. For an evaluation of the morphological differences of the four mt clades that are found in the current study, it is necessary to recapitulate the taxonomic work that has been performed until now [Tab. 12].

*G. barreimiae* was originally described by Fowler & Steinitz (1956) from Buraimi, Oman, on the basis of 6 specimens. The dorsal fin had iii,7 rays, the anal fin iii,5, the pectoral fin i,15 and the ventral fin i,7. There were 32+2 lateral line scales, 12 predorsal scales and 5.3.2 right pharyngeal teeth of one pharyngeal bone examined. The paratypes had the same characteristics like the holotype.

Menon (1964) later received 4 specimens of *G. barreimiae* from Henry Fowler. Their origin was simply stated as "from Oman". He compared *G. barreimiae* to *G. rufa* from Aleppo (Syria) and concluded that *G. barreimiae* was more elongated and had a shorter head. He counted 12 to 14 outer gill rakers on the lower limb of the anterior arch, 33-34 lateral line scales and iii,6 dorsal fin rays for *G. barreimiae*. Menon put *G. rufa*, *G. rufa obtusa* and *G. barreimiae* into the 'Rufa'-complex because of their larger number of gill rakers (12-20) on the lower limb of the anterior arch and their intensely darker pigmentation along the mid-lateral band compared to the closely

related 'Tibanica'-complex.

Banister & Clarke (1977) examined 8 specimens from Wadi Sahtan [23°14' N, 57°19' E], which is at the southern border of our North clade distribution, 14 specimens from Wadi Shawkah and 3 fishes from a collection from Muscat. Two fishes from Buraimi were "examined but not measured because of their fragile condition". The authors described the pharyngeal tooth formula of *G. barreimiae* as 2.4.5-5.4.2. The dorsal fin was characterized by four unbranched rays and six or eight branched rays, which differs strikingly from this study's counts. Krupp (1988) later declared this count to be "obviously an error". The 25 specimens radiographed by Banister & Clarke had 30-33 vertebrae. Only one specimen had 18 precaudal vertebrae, all the others had 16 or 17 precaudal vertebrae. 13 to 16 caudal vertebrae were counted without mentioning intermediate vertebrae. In this study only East clade and North clade specimens had 17 precaudal vertebrae and none had 16 [Tab. 10]. Banister & Clarke further agreed with Menon in aligning *G. rufa* with *G. barreimiae* because of the similar colour pattern and body shape, but the anus of *G. rufa* is further away from the anal fin. A smaller distance between anus and anal fin is considered to be a derived condition. 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*: 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 *Garra longipinnis* from the Saiq region [23°02' N, 57° 28' E] based on 8 specimens with strikingly long paired fins (Banister & Clarke, 1977). The locality is within the region of our Central clade. Since fish with the described morphology of *G. longipinnis* have not been found again, it is as of yet unclear if this species has become extinct or if atypical specimens of *G. barreimiae* were described as a new species (Freyhof et al., 2015).

Krupp (1983) published a species key for Arabian *Garra* in which *G. barreimiae* was described as having 12 scales around the least circumference of the caudal peduncle and 11 to 18 gill rakers on the lower limb of the first gill arch. *G. b. barreimiae* should have less than 15 gill rakers on the lower limb of the first gill arch and *G. b. shawkahensis* should have more than 14 gill rakers, which was the substantial morphological difference between the two subspecies. Further *G. b. barreimiae* was characterized as having 4 unbranched dorsal fin rays and 7 to 8 branched rays. The only traditional morphometric measurement to distinguish between *G. b. barreimiae* and *G. b. shawkahensis* mentioned in previous studies was the prepelvic distance, which is larger in *G. b. shawkahensis*. In this study, the mean prepelvic distance is the largest in

**Tab. 12:** Meristic counts of *G. barreimiae* of previous studies. For this study's clades see Tab. 10 [*G. b.* = *Garra barreimiae*, *G. b. b.* = *Garra barreimiae barreimiae*; *G. b. s.* = *Garra barreimiae shawkahensis*].

| (Sub-)Species | Fowler & Steinitz (1956) | Menon (1964)   | Banister & Clarke (1977)                  |                 | Krupp (1983)                 |                 |
|---------------|--------------------------|----------------|-------------------------------------------|-----------------|------------------------------|-----------------|
|               |                          |                | <i>G. b. b.</i>                           | <i>G. b. s.</i> | <i>G. b. b.</i>              | <i>G. b. s.</i> |
| Location      | Buraiimi                 | Oman (unspec.) | Wadi Sahtan & Muscat collection (unspec.) | Wadi Shawkah    | Wadi Sahtan & Oman (unspec.) | Wadi Shawkah    |
| Dorsal fin    | iii,7                    | iii, 6         | iv, 6 or 8                                | iv, 6 or 8      | iv, 7-8                      | iv, 6-7         |
| Ventral fin   | i,7                      | /              | /                                         | /               | /                            | /               |
| Pectoral fin  | i,15                     | /              | /                                         | /               | /                            | /               |
| LLSC          | 32+2                     | 33-34          | 29-32                                     | 30, 31 or 33    | 31-34                        | 32-35           |
| Gill rakers   | /                        | 12-14          | 11-14                                     | 15-18           | 11-14                        | 16-18           |
| Pharyngeal    | -5.3.2 (only 1 examined) | /              | 2.4.5-5.4.2                               |                 | 2.4.5-5.4.2                  |                 |
| Vertebrae     | /                        | /              | 16+15-16; 17+13-16; 18+15                 |                 | /                            | /               |

the West clade with 56.0% [Fig. 8; Tab. 3] and thus in fact very close to the measurement of Banister & Clarke (56.2%) for *G. b. shawkahensis*. In addition, all of the 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 & 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 & Steinitz did not measure the prepelvic distance and did not count gill rakers in their original description of *Garra barreimiae*. Banister & Clarke did not take measurements of specimens from Buraimi, but put them together with specimens from Wadi Sahtan, which clearly belongs to another genetic clade (North clade), as well as fishes from undisclosed regions of Oman. Fishes from Wadi Shawkah did cluster together with specimens from Hafeet, Hayyal and Al Juwayf in an analysis of mt sequences (Kirchner et al, unpublished). Further 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. 3]. In the Catalog of Fishes *G. b. shawkahensis* is considered to be a synonym of *G. barreimiae* (Eschmeyer et al., 2016). During the collection trip of the Natural History Museum Vienna, the original description site of *G. barreimiae*, Buraimi, was visited, but no water carrying falaj (artificial irrigation channel) could be found. Native people commented that due to the building of standpipes the aflaj are no longer maintained and do not carry water anymore. One has to conclude that the locality of the original description is lost (Kruckenhauser pers. comm.). 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 genetic clade. 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*.

All of the traditional morphometric measurements between the clades have huge overlaps and are very variable within the clades [Fig. 8]. Specimens from the Central clade and the East clade are generally smaller than those of the West clade and the North clade, but this might be due to stochastic sampling. The West clade and East clade specimens are more slender than the other two clades. AFA, IOW and PO (in % of HL) differentiate the Central clade from the others. The pectoral fin length is very variable in the Central clade but its maximum does not come close to the length of *G. longipinnis* measured by Banister & Clarke (1977), thus if *G. longipinnis* exists it was obviously not part of the material analyzed. The clades are not separated in the PCA [Fig.

9], although in the LDA [Fig. 11] they are discriminable and 86% of specimens can be correctly classified based on the same measurements in the confusion matrix [Tab. 9]. A noteworthy difference is the pelvic fin ray count with 7 branched rays in most of the West clade specimens and 8 branched rays in most specimens of the other clades [Tab. 10; Fig. 16]. The West clade also has generally 19 precaudal and 1 intermediate vertebrae, both of which are very uncommon in the other clades. All of the East clade specimens have 17 precaudal vertebrae, a feature apart from that only found in two of the North clade specimens. The first two pectoral fin rays of *Garra* are usually unbranched (Lundberg & Marsh, 1976; Stiassny & 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 variable. Unlike in previous studies (Banister & Clarke, 1977; Krupp, 1983) the dorsal fin ray numbers were quite stable with iii,7½. While the variability of this characteristic could be higher than shown in this study, the possibility of differing counting methods is given, since it is often not clearly stated how fin rays have been counted and no visual proof is provided. Additionally, it has to be noted that the first unbranched dorsal fin ray as well as the final branched pectoral fin rays are very small and thus hard to see both using light microscopy and the chosen resolution of the micro CT.

Although there are only slight differences in the sequences of *RAG-1* between the clades, the topology of the mt clades is well supported [Fig. 19]. The Central clade and the East clade form one nuclear clade which is well supported. The nuclear West clade and North clade are also rather well supported, just as the node connecting West clade and North clade. Again, like in the previous mitochondrial study, *G. rufa* prevents the monophyly of *G. barreimiae*. The node connecting the Central clade and East clade with *G. rufa* however is poorly supported. Mean p distances [Tab. 11] are between 0.6% and 1% and thus very low, further indicating a very recent diversification, as mentioned by Kruckenhauser et al. (2011). *RAG-1* is a popular and widely used nuclear marker in phylogenetic studies of fish and other vertebrates (López et al., 2004), however it is suggested to investigate if different nuclear markers (e.g. *ITS*) will come to the same conclusions, to clarify if the four mt clades are indeed monophyletic. Further, the relationships to *G. rufa* and other closely related species need to be investigated.

There is no generally accepted classification in ichthyology and fish taxonomy is still very much disputed, which may be due to rapid speciation by adaptive radiation or geographic isolation in many groups of teleosts including cyprinids (Nellen & Dulčić, 2008). Just as it is the case with *G. barreimiae*, which probably had a larger distribution in the Arabian Peninsula in former times and now consists of many isolated populations (Freyhof et al., 2015). 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).

#### **4.1 Conclusion**

Three well supported nuclear *RAG-1* clades of *G. barreimiae* have been identified (West clade and North clade, which are congruent with the mt clades, and Central-East clade, which consists of the mt Central clade and East clade). Besides the number of gill rakers, which separate the West clade from the other clades, no clearly well-defined morphological difference could be identified with the methods applied. Several morphometric measurements however, display significant differences between the clades and besides a few overlaps, the numbers of intermediate vertebrae and branched pelvic fin rays indicate that the West clade is distinct from the other clades not only at the DNA sequence level, but also morphologically. The original descriptions of *G. barreimiae* and *G. b. shawkahensis* correspond to the West clade both geographically and morphologically. The subspecies *G. b. barreimiae* and *G. b. shawkahensis* probably belong to the same taxon. The North clade, and Central-East clade are clearly distinct on a genetic level, but have as of yet not been described as any valid species.

### **5. Acknowledgments**

I want to thank Elisabeth Haring and Helmut Sattmann for giving me the opportunity to write my thesis at the Natural History Museum Vienna, which has been a place of wonder since my early childhood and certainly influenced me in my choice of studies. Special thanks to Luise Kruckenhauser for investing time in supervision, scientific discussion and troubleshooting. Many thanks to Harald Ahnelt and Jörg Freyhof for giving insights into fish morphology and taxonomy. I am grateful to Gerhard Forstenpointner and Stephan Handschuh for providing the possibility to use a micro CT scanner for this study. Thank you Sandra Kirchner, Oliver Macek, Julia Schindelar, Barbara Däubel and Lorena Toscano for your continued support in the lab and for having gallons of coffee. Lastly I would like to thank my family. Without their support and appreciation of education the expenditure of completing such elaborate and protracted studies would not have been possible. So long, and thanks for all the fish.

## 6. Literature

- Ahnelt H and Duchkowitsch M. 2003. The postcranial skeleton of *Proterorhinus marmoratus* with remarks on the relationships of the genus *Proterorhinus* (Teleostei: Gobiidae). *Journal of Natural History* 38 (7): 913-924
- Alsharhan AS, Rizk ZA, Nairn AEM, Bakhit DW and Alhajari SA. 2001. Hydrogeology of an arid region: the Arabian Gulf and adjoining areas. New York: Elsevier.
- Al-Rawahi MN, Brinkmann K, Schlecht E and Buerkert A. 2014. Effects of changing water availability on land use in irrigated mountain oases of Al Jabal Al Akhdar, northern Oman. *Erde* 145 (4): 197–211.
- Armbruster JW. 2012. Standardized measurements, landmarks, and meristic counts for cypriniform fishes. *Zootaxa* 3586: 8–16.
- Banister KE and Clarke MA. 1977. The freshwater fishes of the Arabian Peninsula. *Journal of Oman Studies* 132–144.
- Banister KE. 1984. A subterranean population of *Garra barreimiae* (Teleostei: Cyprinidae) from Oman, with comments on the concept of regressive evolution. *Journal of Natural History* 18: 927-938.
- Birdsong RS, Murdy EO and Pezold FL. 1988. A study of the vertebral column and median fin osteology in gobiid fishes with comments on gobioid relationships. *Bulletin of Marine Science* 42, 174–214.
- Breasted JH. 1916. *Ancient times: a history of the early world*. Boston: Ginn.
- Chen XL, Yue PQ and Lin RD. 1984. Major groups within the family Cyprinidae and their phylogenetic relationships. *Acta Zootaxonomica Sinica* 9: 424–440 (in Chinese with English summary).
- Colli L, Paglianti A, Berti R, Gandolfi G and Tagliavini J. 2009. Molecular phylogeny of the blind cavefish *Phreatichthys andruzzii* and *Garra barreimiae* within the family Cyprinidae. *Environmental Biology of Fishes* 84 (1): 95-107.
- Eschmeyer WN, Fricke R and van der Laan R (eds). CATALOG OF FISHES: GENERA, SPECIES, REFERENCES. Electronic version accessed 01 07 2016.
- Farashi A, Kaboli M, Rezaei H, Naghavi M, Coad B. 2014. Reassessment of the taxonomic position of *Iranocypris typhlops* Bruun & Kaiser, 1944 (Actinopterygii, Cyprinidae). *ZooKeys* 374: 69-77.
- Fowler HW and Steinitz H. 1956. Fishes from Cyprus, Iran, Iraq, Israel and Oman. *Bulletin of the Research Council of Israel* 5B: 262–289.

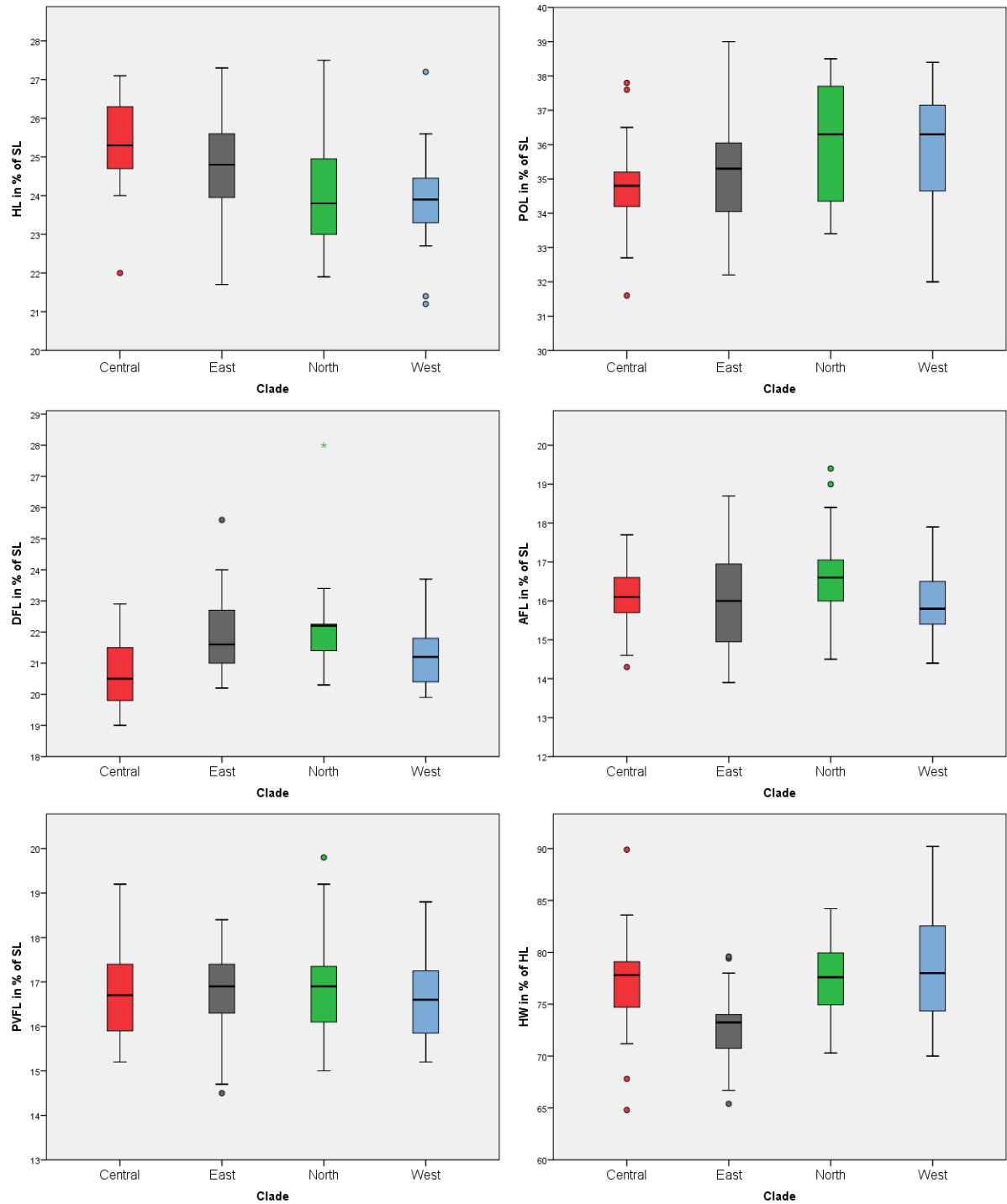
- Freyhof J, Hamidan NA, Feulner GR and Harrison I. 2015. Chapter 3. The Status and Distribution of Freshwater Fishes of the Arabian Peninsula. 16-29. In: García N, Harrison I, Cox N and Tognelli MF (compilers) (2015) The Status and Distribution of Freshwater Biodiversity in the Arabian Peninsula. Gland, Switzerland, Cambridge, UK & Arlington, USA: IUCN.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95–98.
- Hamidan NA, Geiger MF and Freyhof J. 2014. *Garra jordanica*, a new species from the Dead Sea basin with remarks on the relationship of *G. ghorensis*, *G. tibanica* and *G. rufa* (Teleostei: Cyprinidae). Zootaxa 4048: 75–89.
- Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4 (1): 9pp.
- IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.
- Kruckenhauser L, Haring E, Seemann R and Sattmann H. 2011. Genetic differentiation between cave and surface-dwelling populations of *Garra barreimiae* (Cyprinidae) in Oman. BMC Evolutionary Biology 11 (1): 172-186.
- Krupp F. 1983. Freshwater Fishes of Saudi Arabia and Adjacent Regions of the Arabian Peninsula. In: Fauna of Saudi Arabia. Basel & Jeddah. 568–636.
- Krupp F. 1988. Freshwater Fishes of the Wadi Batha Drainage. Journal of Oman Studies (3): 401–404.
- Krupp F and Budd K. 2009. A new species of the genus *Garra* (Teleostei: Cyprinidae) from Oman. Aqua, International Journal of Ichthyology 15 (2): 117–120.
- Lambeck K. 1996. Shoreline reconstructions for the Persian Gulf since the last glacial maximum. Earth and Planetary Science Letters 142 (1-2): 43–57.
- López JA, Chen W and Ortí G. 2004. Esociform Phylogeny. Copeia (3): 449–464.
- Lundberg JG and Marsh E. 1976. Evolution and functional anatomy of the pectoral fin rays in cyprinoid fishes, with emphasis on the suckers (Family Catostomidae). American Midland Naturalist 96: 332–349.
- Menon A. 1964. Monograph of the Cyprinid fishes of the genus *Garra* Hamilton. Memoires of the Indian Museum 14 (4): 173–260.
- Nellen W and Dulčić J. 2008. Evolutionary steps in ichthyology and new challenges. Acta Adriatica 49 (3): 201–232.

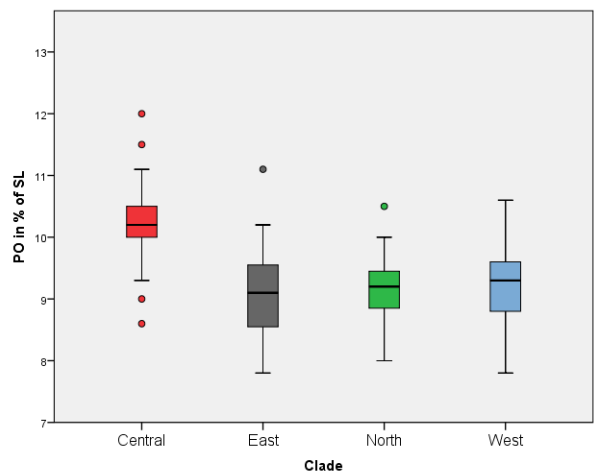
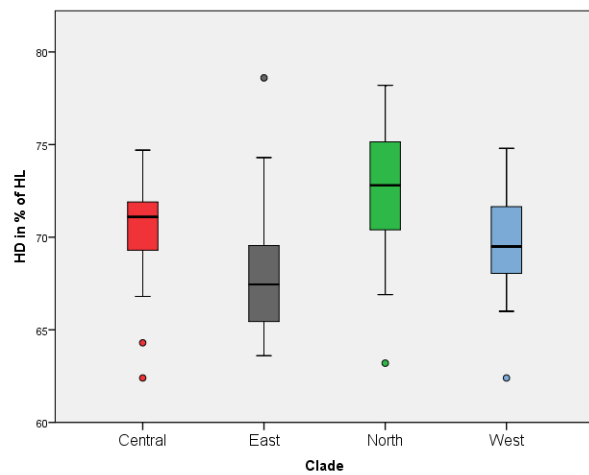
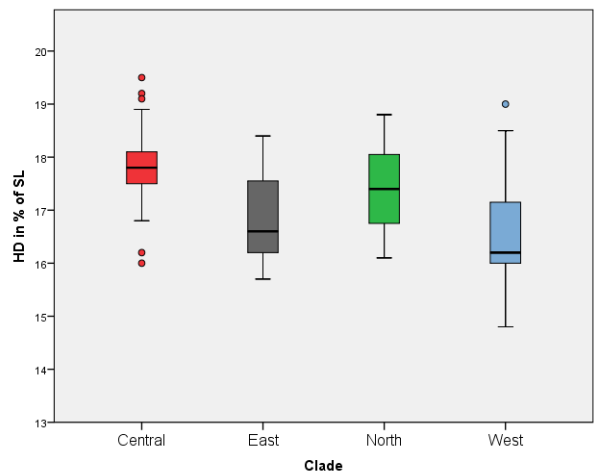
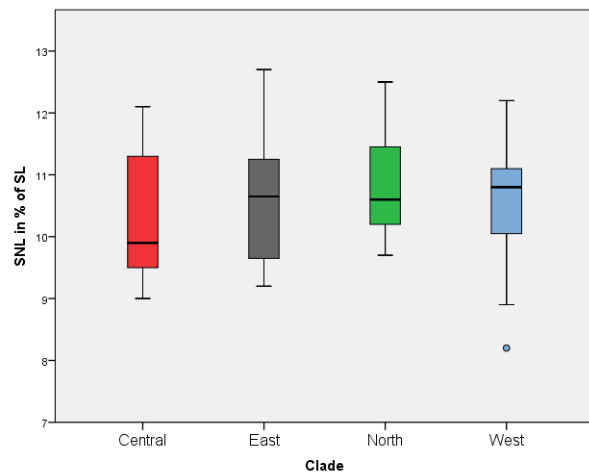
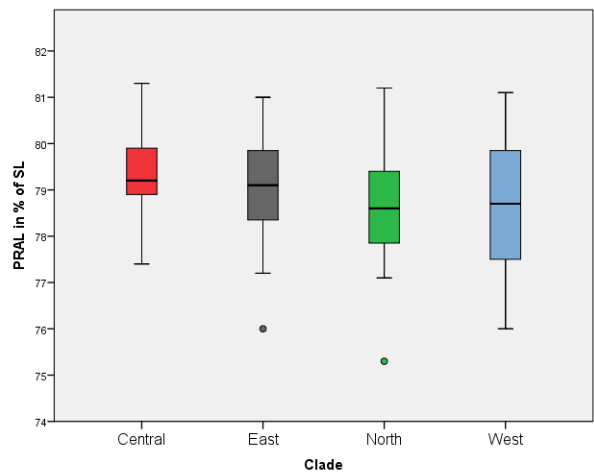
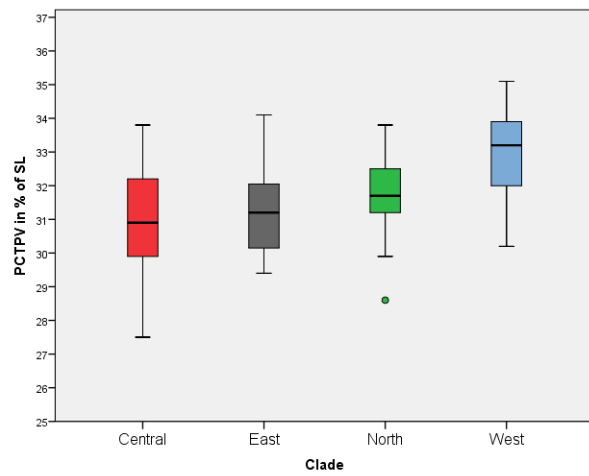


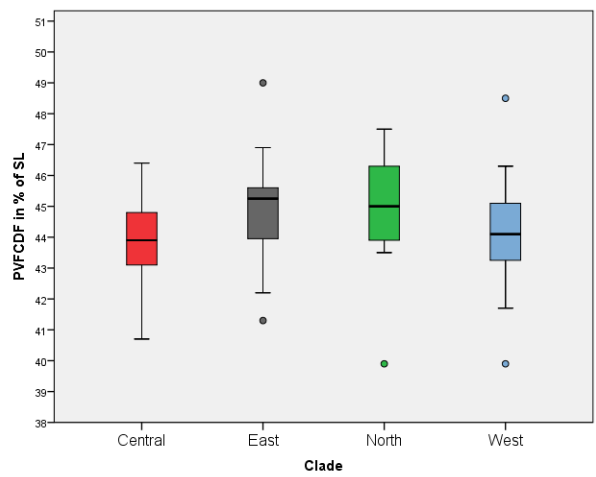
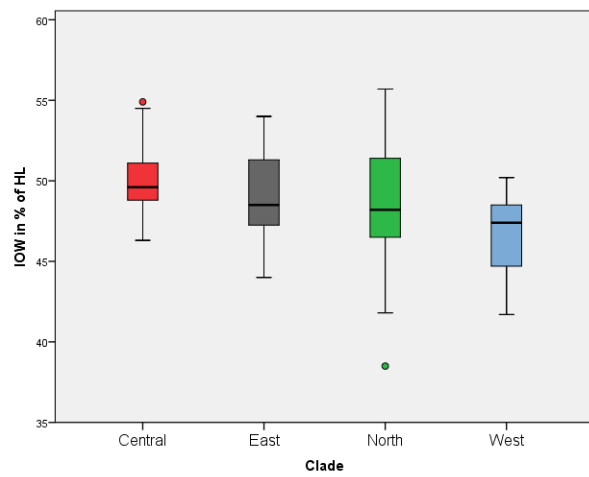
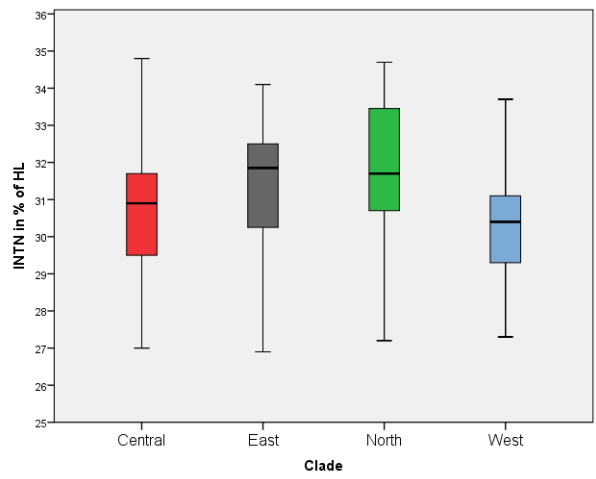
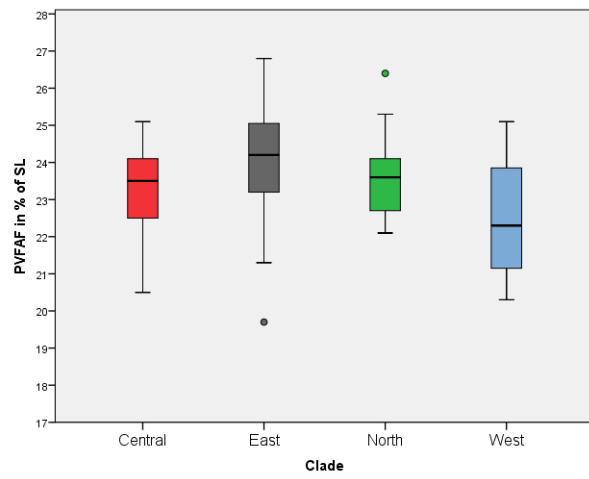
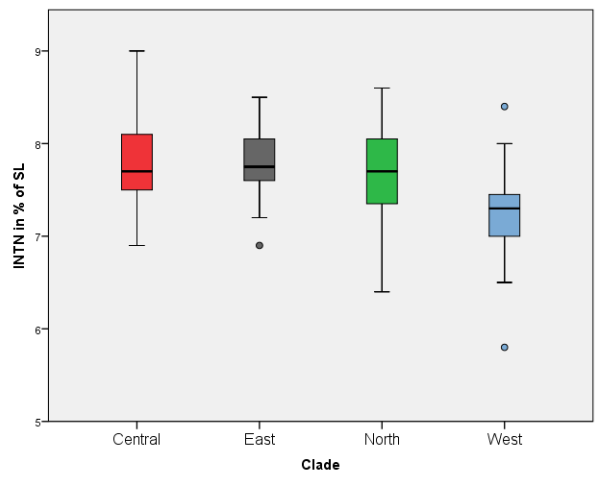
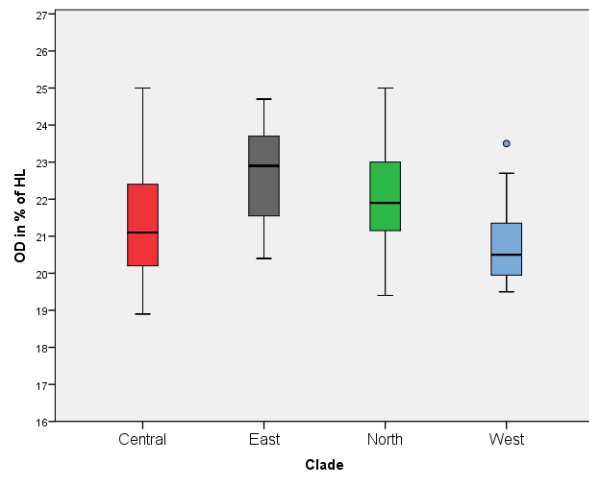
- R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Rainboth WJ. 1991. Cyprinid fishes of Southeast Asia. In: Winfield IJ, Nelson JS (eds.), Cyprinid Fishes: Systematics, Biology and Exploitation. Chapman and Hall, London. 156–210.
- Rainboth WJ. 1996. Fishes of the Cambodian Mekong. FAO species identification field guide for fishery purposes. FAO, Rome.
- Reid GM. 1982. The form, function and phylogenetic significance of the vomero-palatine organ in cyprinid fishes. *Journal of Natural History* 16 (4): 497–510.
- Rojo AL. 1991. Dictionary of Evolutionary Fish Osteology. Boca Raton, FL: CRC Press. 273.
- Rose JJ. 2010. New Light on Human Prehistory in the Arabo-Persian Gulf Oasis. *Current Anthropology* 51 (6): 849-883.
- Sayyadzadeh G, Esmaili HR, Freyhof J. 2015. *Garra mondica*, a new species from the Mond River drainage with remarks on the genus *Garra* from the Persian Gulf basin in Iran (Teleostei: Cyprinidae). *Zootaxa* (1): 75-89.
- Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. 2012. Fiji: an open-source platform for biological-image analysis. *Nature methods* 9 (7): 676-682.
- Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E and Crozier RH. 2010. Integrative Taxonomy: A Multisource Approach to Exploring Biodiversity. *Annual Review of Entomology* 55: 421–38.
- Shiraz GF and Münster KP. 1992. A morphotectonic study of environmental impact on ground water in southern Iran and under the Persian Gulf. *Geologische Rundschau* 81 (2): 581-587.
- Stalling D, Westerhof M and Hege HC. 2005. Amira: a Highly Interactive System for Visual Data Analysis. 749-767. In: Hansen CD and Johnson CR (eds.), *The Visualization Handbook*. Elsevier.
- Stewart KM. 2001. The freshwater fish of Neogene Africa (Miocene-Pleistocene): Systematics and biogeography. *Fish and Fisheries* 2 (3): 177–230.
- Stiassny MLJ and Getahun A. 2007. An overview of labeonin relationships and the phylogenetic placement of the Afro-Asian genus *Garra* Hamilton, 1922 (Teleostei: Cyprinidae), with the description of five new species of *Garra* from Ethiopia and a key to all African species. *Zoological Journal of the Linnean Society* 150: 41–83.

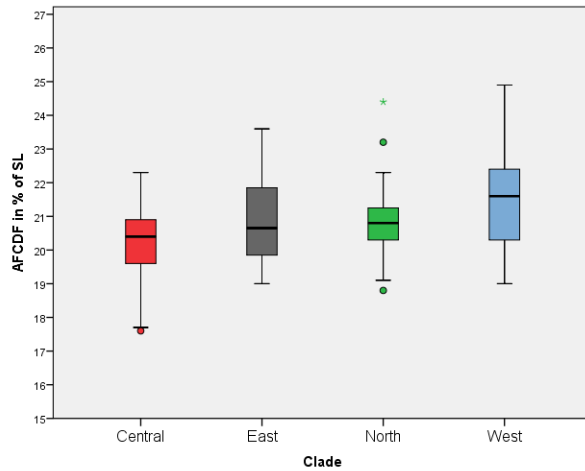
- Sultan M, Sturchio N, Al Sefry S, Milewski A, Becker R, Nasr I and Sagintayev Z. 2008. Geochemical, isotopic, and remote sensing constraints on the origin and evolution of the Rub Al Khali aquifer system, Arabian Peninsula. *Journal of Hydrology* 356: 70- 83.
- Tang Q, Getahun A and Liu H. 2009. Multiple in-to-Africa dispersals of labeonin fishes (Teleostei: Cyprinidae) revealed by molecular phylogenetic analysis. *Hydrobiologia* 632 (1): 261–271.
- Turner GF. 1999. What is a fish species? *Reviews in Fish Biology and Fisheries* 9 (4): 281–297.
- Van Couvering JAH. 1977. Early records of freshwater fishes in Africa. *Copeia* 1977: 163–166.
- Vasilyan D and Carnevale G. 2013. The Afro–Asian labeonine genus *Garra* Hamilton, 1822 (Teleostei, Cyprinidae) in the Pliocene of Central Armenia: Palaeoecological and palaeobiogeographical implications. *Journal of Asian Earth Sciences* 62: 788–796.
- Yang L, Arunachalam M, Sado T, Levin BA, Golubtsov AS, Freyhof J, Friel JP, Chen W, Hirt MV, Manickam R, Agnew MK, Simons AM, Saitoh K, Miya M, Mayden RL and He S. 2012. Molecular phylogeny of the cyprinid tribe Labeonini (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution* 65 (2): 362–379.
- Yang L and Mayden RL. 2010. Phylogenetic relationships, subdivision, and biogeography of the cyprinid tribe Labeonini (sensu Rainboth 1991) (Teleostei: Cypriniformes), with comments on the implications of lips and associated structures in the labeonin classification. *Molecular Phylogenetics and Evolution* 54 (1): 254–65.
- Zhang E. 2005. Phylogenetic relationships of labeonine cyprinids of the disc-bearing group (Pisces: Teleostei). *Zoological Studies* 44 (1): 130-143.

## 7. Supplement





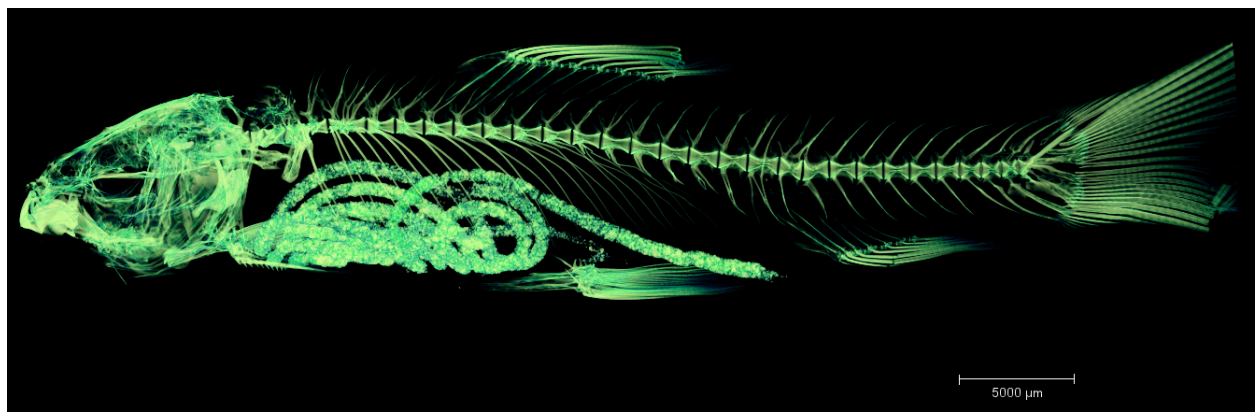




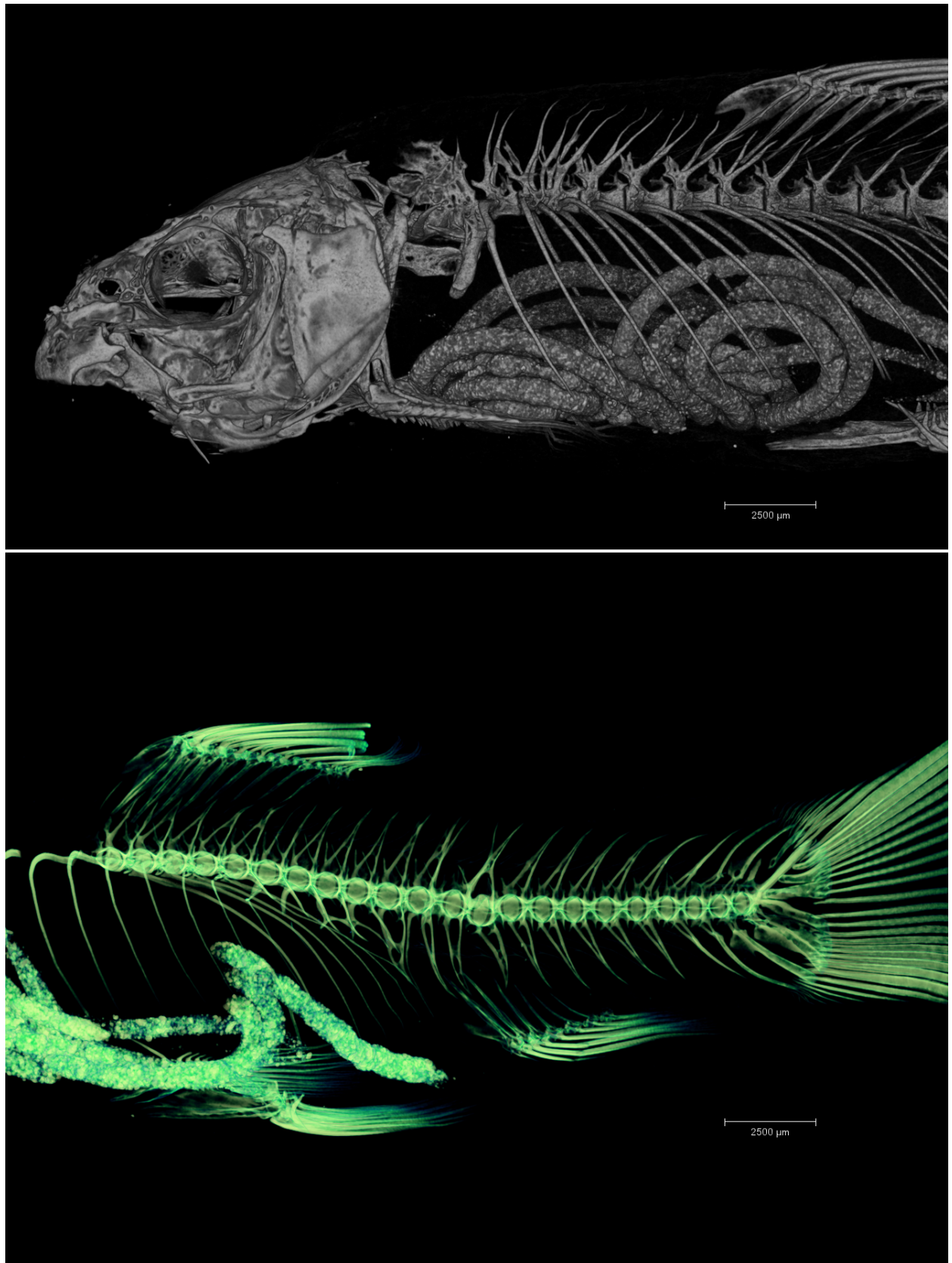
**Supplementary Fig. 1:** Additional box plots of traditional morphometric measurements. Colored dots mark outliers. For abbreviations of measurements see Tab. 2.

**Supplementary Tab. 1:** Additional results of Levene's test for homogeneity of variance, ANOVA and Kruskal-Wallis test for morphometric measurements. In the case of non-parametric data results of the Kruskal-Wallis test is preferable over ANOVA. In this case results of the Kruskal-Wallis test are written in bold letters. Signif. codes: 0 "\*\*\*\*", 0.001 "\*\*\*", 0.01 "\*\*". For abbreviations of measurements see Tab. 2.

|                | Levene's test | ANOVA        | Kruskal-Wallis test |
|----------------|---------------|--------------|---------------------|
| INTN (% of SL) | 0.3457        | 0.0005***    | 0.0010**            |
| HD (% of SL)   | 0.7506        | 5.836E-05*** | <b>0.0001***</b>    |
| HW (% of SL)   | 0.1528        | 0.0008***    | 0.0013**            |
| IOW (% of SL)  | 0.0726        | 1.545E-14*** | 6.940E-11***        |
| PO (% of SL)   | 0.8734        | 3.048E-07*** | 5.027E-06***        |
| SNL (% of SL)  | 0.8455        | 0.3581       | 0.3530              |

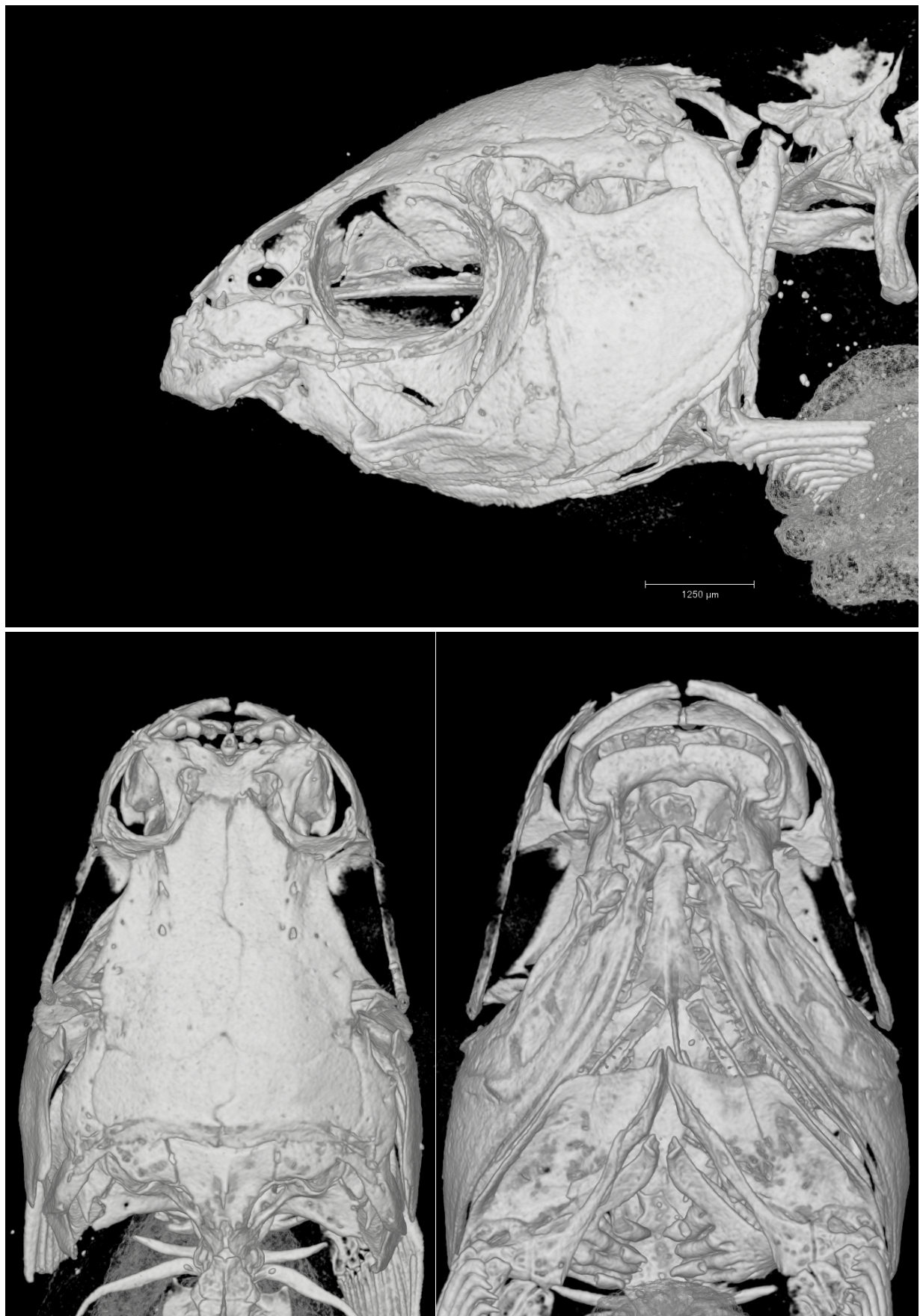


**Supplementary Fig. 2:** Aberrant specimen of the East clade (Gbar254) with coadunated vertebrae which could not be counted.



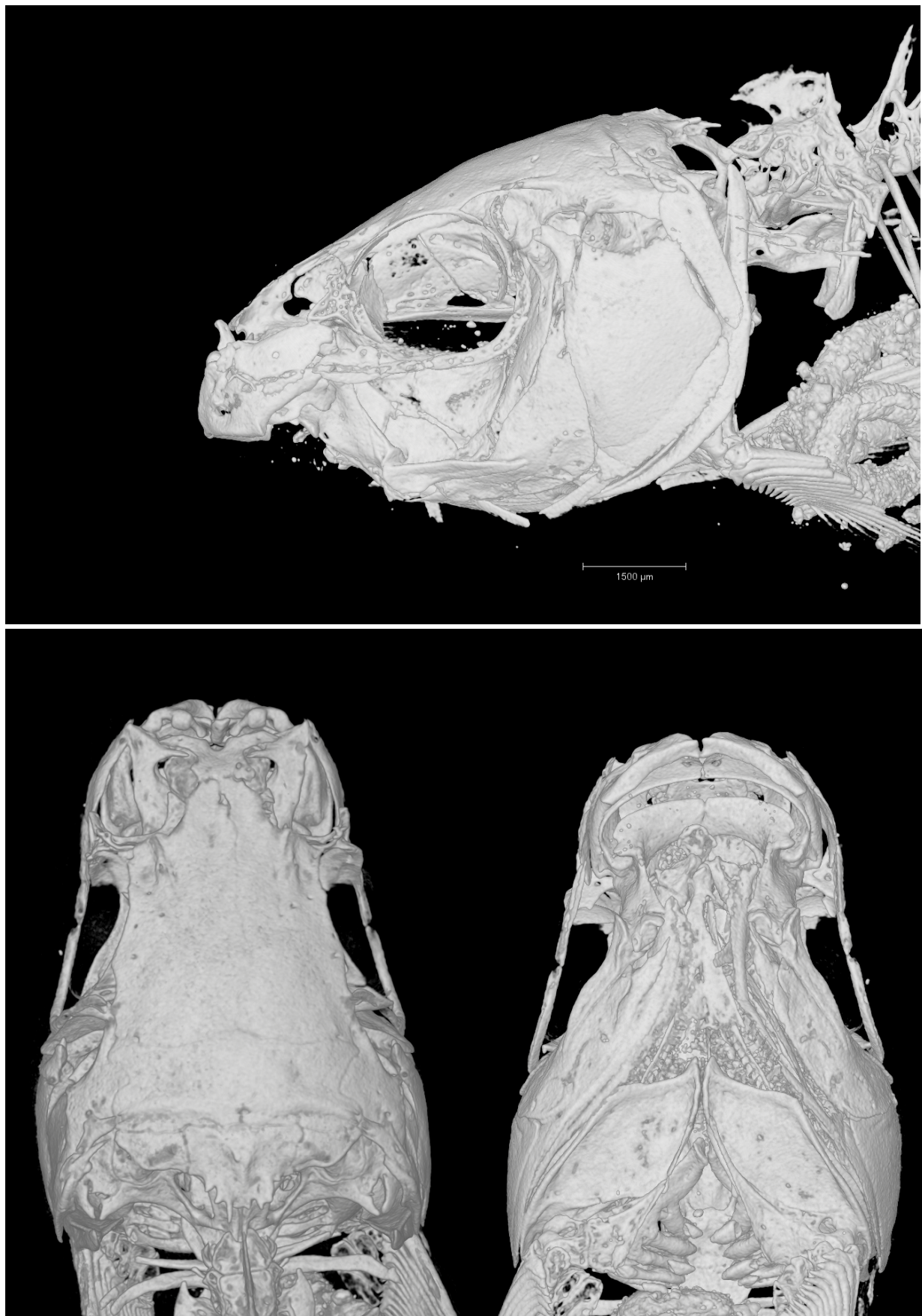
**Supplementary Fig. 3:** Anterior and caudal details of the aberrant East clade specimen (Gbar254).



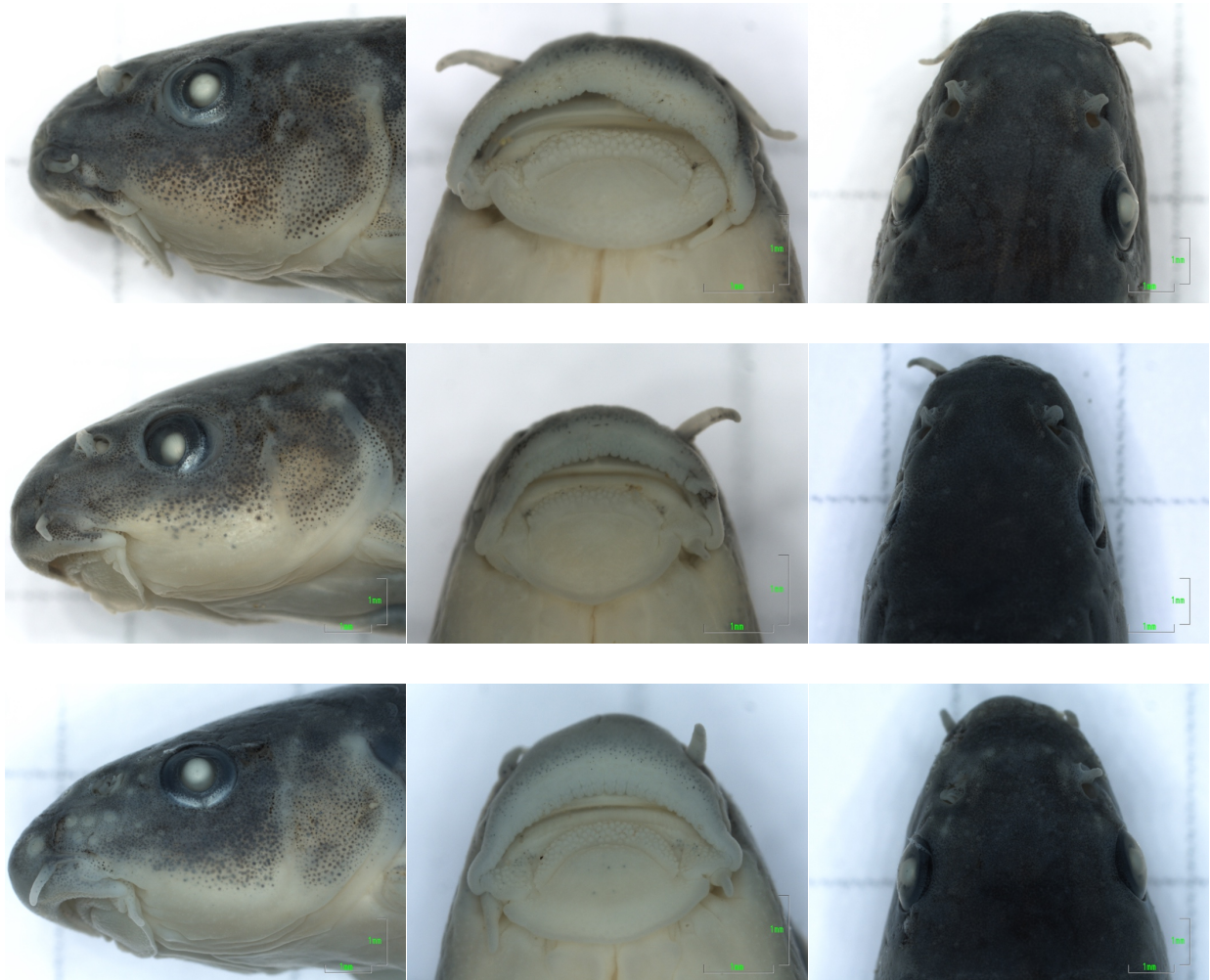


**Supplementary Fig. 4:** Lateral, dorsal and ventral details of the skull of a Central clade specimen (Gbar252 SL 29.6 mm).



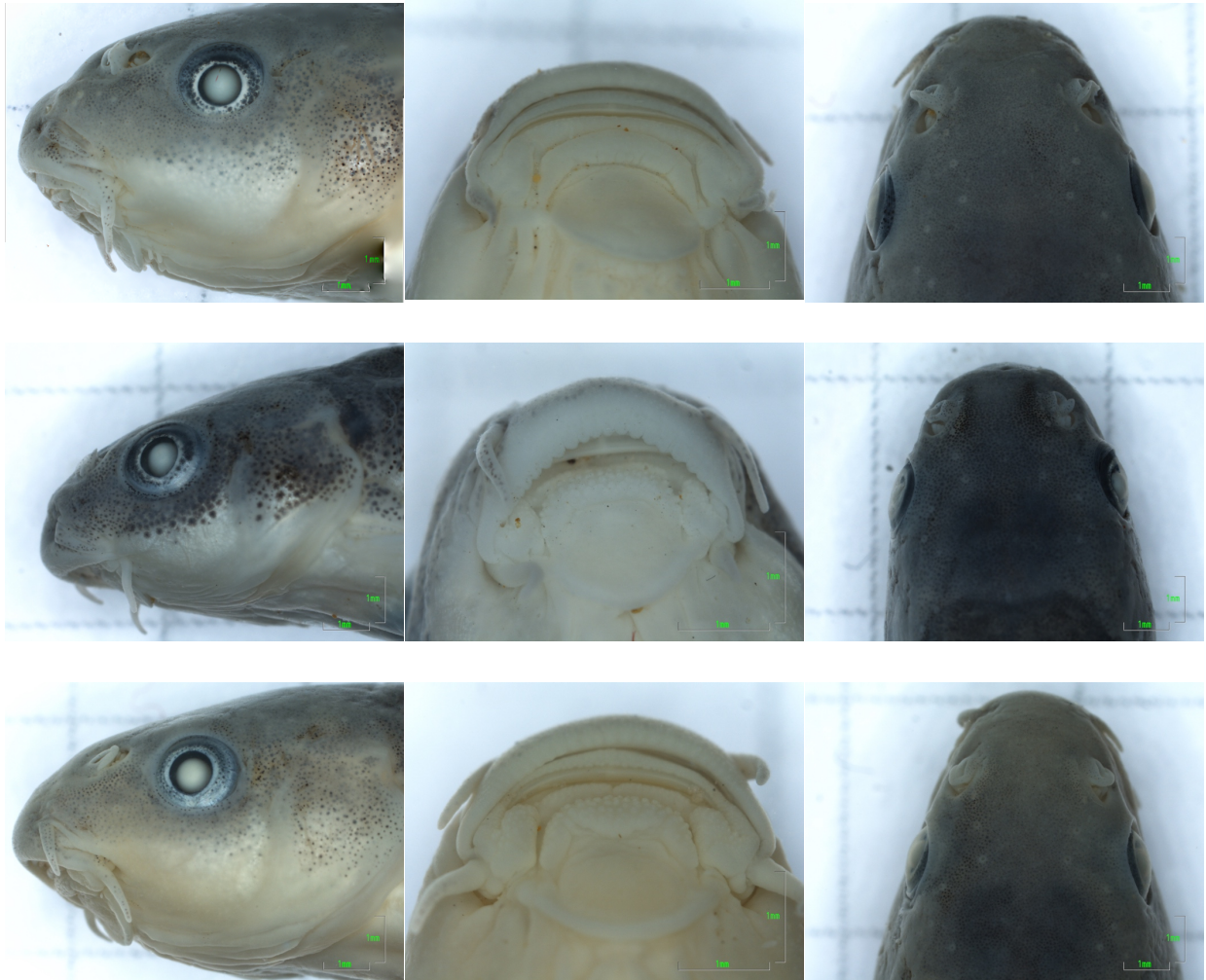


**Supplementary Fig. 5:** Lateral, dorsal and ventral details of the skull of a North clade specimen (Gbar228 SL 32.5 mm).

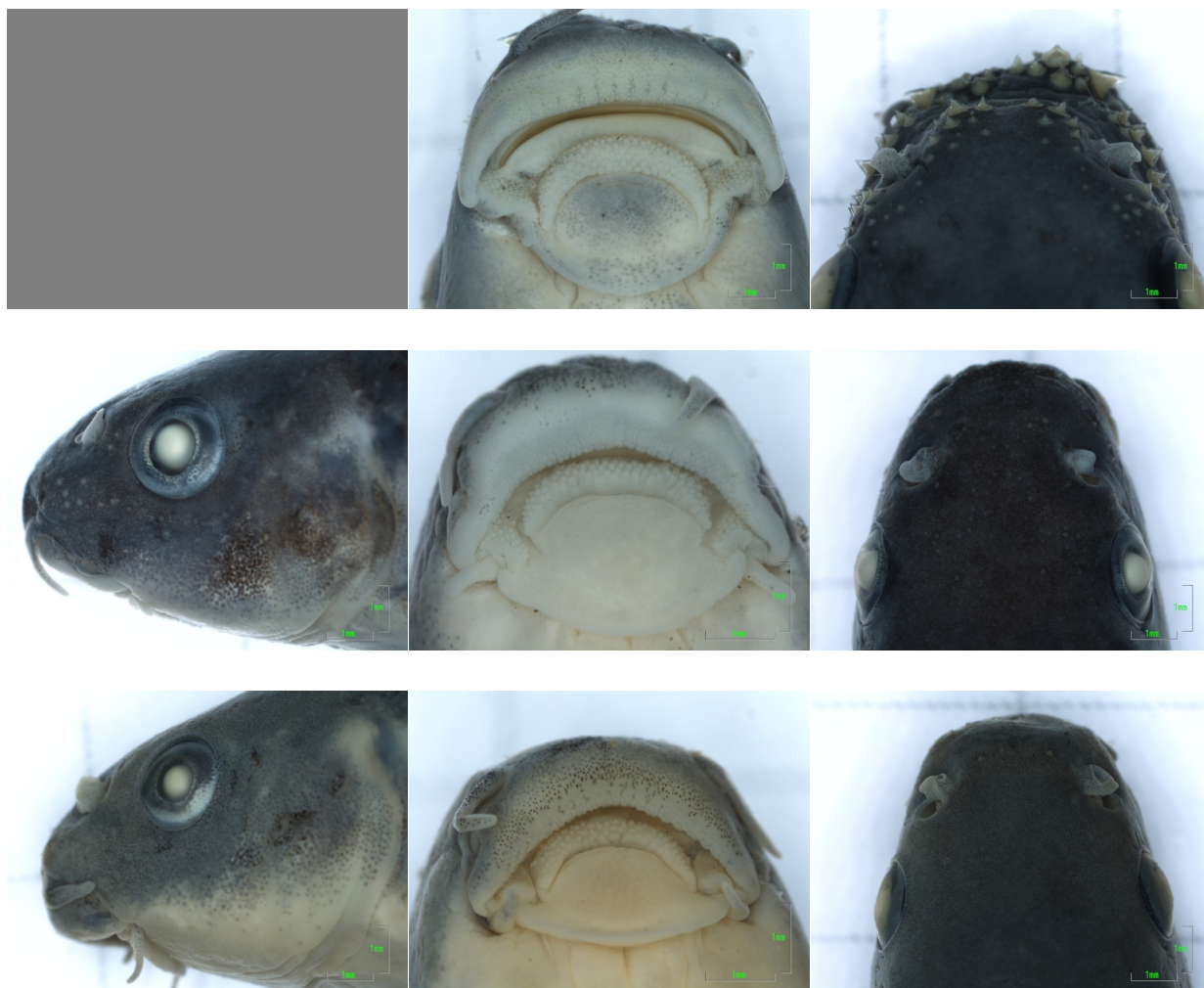


**Supplementary Fig. 6:** Examples of variations of head traits and adhesive discs of specimens of the West clade [first row: Gbar290 SL 29.2 mm; second row: Gbar291 SL 28.7 mm; third row: Gbar294 SL 32.3 mm].

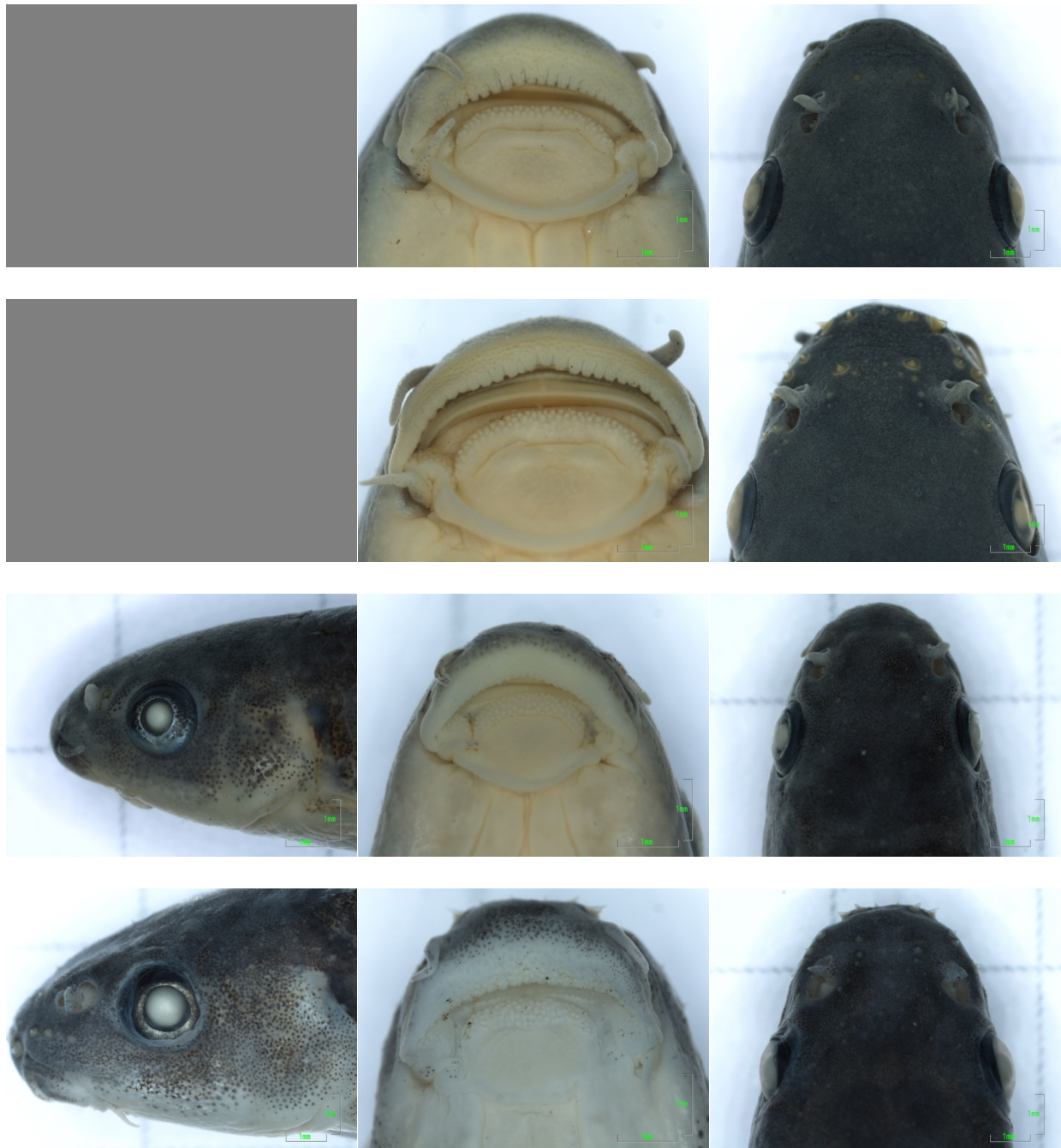




**Supplementary Fig. 7:** Examples of variations of head traits and adhesive discs of specimens of the Central clade [first row: Gbar181; second row: Gbar252 SL 29.6 mm; third row: Gbar157 SL 33.4 mm].



**Supplementary Fig. 8:** Examples of variations of head traits and adhesive discs of specimens of the North clade [first row: Gbar266 SL 48.9 mm; second row: Gbar231 SL 34.0 mm; third row: Gbar242 SL 39.3 mm].



**Supplementary Fig. 9:** Examples of variations of head traits and adhesive discs of specimens of the East clade [first row: Gbar254 SL 40.2 mm; second row: Gbar257 SL 49.6 mm; third row: Gbar253 SL 29.2 mm; fourth row: Gbar260 SL 33.5 mm].



## 8. Abstract

The cyprinid fish *Garra barreimiae* Fowler & Steinitz, 1956 is endemic to the northern Oman and the United Arab Emirates. Three subspecies have been described based on slightly different morphological features: *Garra barreimiae barreimiae* Fowler & Steinitz, 1956, *Garra barreimiae shawkahensis* Banister & Clarke, 1977 and *Garra barreimiae gallagheri* Krupp, 1988 but only *G. barreimiae* and *G. b. shawkahensis* are currently recognized as valid, with *G. b. shawkahensis* considered to be a synonym of *G. barreimiae* (Eschmeyer et al., 2016). Based on DNA sequences of the mitochondrial control region (CR) and the cytochrome c oxydase subunit I (COI) at least four different clades (named West clade, Central clade, North clade and East clade) with genetic distances ranging between 6% and 10% in COI have recently been identified (Kirchner et al., unpublished data) and, moreover, *G. barreimiae* did not appear to be monophyletic in the phylogenetic trees, indicating the possibility of cryptic species. The identification of new species is the first step towards successful species conservation. The goal of the present study was to clarify, if morphological differences between the mitochondrial clades can be found and if nuclear clades of the *RAG-1* gene can be distinguished. Morphological characteristics of *G. barreimiae* are very variable, but the West clade can be clearly distinguished from the other clades by its higher number of gill rakers on the lower limb of the first gill arch with 15-17 versus 10-14 in the other clades. Furthermore, the West clade has one intermediate vertebra, while most specimens of the other clades have two. Another striking difference is found in the number of the branched pelvic fin rays. West clade specimens mostly have seven, while the specimens of the other clades usually have eight. The analysis of the nuclear gene *RAG-1* yielded three distinct but closely related clades: West clade and North clade, which are congruent with the mt clades, and Central-East clade, which consists of the mt Central clade and East clade. *G. barreimiae* does not form a monophylum as *G. rufa* forms a clade with the Central-East clade. It is indicated that the West clade is a distinct species equivalent to *G. barreimiae* and maybe also *G. b. shawkahensis*. The other clades are not clearly distinguishable based on the applied morphological methods, but genetic differences indicate cryptic species.

## 9. Zusammenfassung

Die zu den Cypriniden gehörende Art *Garra barreimiae* Fowler & Steinitz, 1956 ist im nördlichen Oman und den Vereinigten Arabischen Emiraten endemisch. Drei Subspezies sind aufgrund geringfügig unterschiedlicher morphologischer Merkmale beschrieben worden: *Garra barreimiae barreimiae* Fowler & Steinitz, 1956, *Garra barreimiae shawkahensis* Banister & Clarke, 1977 und *Garra barreimiae gallagheri* Krupp, 1988, jedoch gelten momentan alleine *G. barreimiae* und *G. b. shawkahensis* als valide, wobei *G. b. shawkahensis* als Synonym für *G. barreimiae* betrachtet wird (Eschmeyer et al., 2016). Basierend auf DNA Sequenzen der mitochondriellen (mt) Kontrollregion (CR) und der Cytochrome c Oxidase subunit I (COI) wurden mindestens vier unterschiedliche Kladen (West clade, Central clade, North clade und East clade) mit genetischen Distanzen zwischen 6% und 10% in einem Fragment des COI Gens identifiziert (Kirchner et al., unpublished data). Weiters scheint *G. barreimiae* in den phylogenetischen Bäumen nicht monophyletisch zu sein, was auf die Existenz kryptischer Arten hinweist. Die Identifizierung neuer Arten ist notwendig um diese erfolgreich schützen zu können.

Das Ziel dieser Studie war es zu klären, ob morphologische Unterschiede zwischen den mt Kladen gefunden werden und ob nukleäre Kladen des *RAG-I* Genes unterschieden werden können. Die morphologische Merkmale von *G. barreimiae* sind sehr variabel, der West clade jedoch kann aufgrund seiner höheren Anzahl von Kiemenreusendornen auf dem unteren Glied des ersten Kiemenbogens (15-17) deutlich von den anderen Kladen (10-14) unterschieden werden. Im Weiteren besitzt der West clade einen intermediären Wirbel, während die meisten Exemplare der anderen Kladen zwei intermediäre Wirbel aufweisen. Ein weiteres auffälliges Merkmal sind die verzweigten Bauchflossenstrahlen. Exemplare des West clade besitzen meist sieben, während die anderen Kladen für gewöhnlich acht verzweigte Bauchflossenstrahlen haben. Die Analyse des nukleären Gens *RAG-I* ergab drei getrennte aber nahe verwandte Kladen: West clade und North clade, die mit den mt clades übereinstimmen, sowie Central-East clade, der aus dem mt Central clade und East clade besteht. *G. barreimiae* bildet kein Monophylum, da *G. rufa* eine Klade mit dem Central-East clade bildet. Die Daten weisen darauf hin, dass der West clade eine eigenständige Art bildet, die mit *G. barreimiae* und eventuell auch *G. b. shawkahensis* gleichzusetzten ist. Die anderen Kladen sind mittels der angewandten morphologischen Methoden nicht eindeutig zu unterscheiden, jedoch weisen die genetischen Unterschiede auf kryptische Arten hin.