# Morphological and Osteological Variation in Cichlids (Perciformes: Cichlidae)

by

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# Abstract

The family Cichlidae is a species-rich family in the order Perciformes, with estimates of about 1,703 validly described species, which inhabit freshwater and marginal marine environments in Central and South America, Africa, Madagascar, the West Indies, Sri Lanka, and coastal areas of India and Iran. Cichlids are the classical examples of adaptive radiation which has led to an outstanding diversity of body shapes, color patterns, behavior, and an enormous variety of trophic and ecological specializations. Most of the recent work to determine phylogenetic relationships within this family are based on molecular data and neglect morphological characters, which are very important specifically for including fossils in phylogenies, and play a crucial role in identifying cichlid species in the field. My project is in part to rectify this situation, and will include morphological (including osteological) studies. An assessment of the variability of skeletons of extant cichlids within a single population and the variation of the skeleton that might occur with growth, will provide a baseline of the amount of variation that might be expected in a single species of extinct cichlid. This baseline would then allow us to indicate whether or not a sample of fossil cichlids is likely to represent more than a single species. I conducted the morphological and osteological studies within a sample (n=22) of a single population of *Mesonauta festivus.* The measurements and meristics for specimens are found to be normally distributed with the standard deviation ranges from 0.01-0.07. In the current study, the osteology of *Mesonauta festivus* has been described for the first time. I cleared and stained five specimens of *Mesonauta festivus* and used CT scans of the rest of the specimens for the analyses. All the bones examined were found to be morphological similar within the specimens. I reported the morphological analyses and osteological descriptions for a size series of *Iranocichla hormuzensis* (n=107). The standard deviation ranges from 0.01-0.09 among the measurements of the specimens. I documented isometric growth among the *Iranocichla hormuzensis* specimens.

Among the variable sized specimens of *Iranocichla hormuzensis,* the bones were found to be similar with the exception of cartilage being more in the juvenile samples. The osteology of the cichlid species in this thesis is found to be conserved. Consequently, if a sample of fossils shows any osteological variation, it is likely that the sample includes more than one different species. For future research, studies in my thesis can be combined with genetic data to determine if there is any variation among the DNA sequences in the individuals of the same species. Further, these morphological and osteological analysis can be included with the molecular studies along with the fossils to better understand the phylogenetic relationships of cichlids.

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# List of Abbreviations

| | |
|---|---|
| aa | anguloarticular |
| ach | anterior ceratohyal |
| ant | anteroventral process |
| bpt | basipterygium |
| bh | basihyal |
| bb | basibranchial |
| boc | basiooccipital |
| brst | branchiostegal rays |
| cb | ceratobranchials |
| cl | cleithrum |
| cor | coracoid |
| dent | dentary |
| dp | distal process |
| dhh | dorsal hypohyal |
| eb | epibranchials |
| epo | epioccipital |
| exo | exoccipital |
| ect | ectopterygoid |
| end | endopterygoid |
| ep | epurals |
| fr | frontal |
| hyo | hyomandibula |
| hb | hypobranchials |
| HL | head length |
| hy | hypurals |
| io | infraorbitals |
| iop | interopercle |
| lac | lacrimal |
| leth | lateral ethmoid |

| | |
|---|---|
| l phyj | lower phyaryngeal jaw |
| met | metapterygoid |
| meth | mesethmoid |
| max | maxilla |
| na | nasal |
| osph | orbitosphenoid |
| op | opercle |
| psph | parasphenoid |
| pa | parietal |
| pro | prootic |
| pto | pterotic |
| pal | palatine |
| pmx | premaxilla |
| pop | preopercle |
| pch | posterior ceratohyal |
| ptt | posttemporal |
| PCA | principal component analysis |
| phyj | pharyngeal jaw |
| pb | pharyngobranchials |
| pcl | postcleithra |
| pu | preural centra |
| pp | hypurapophysis |
| ph | parhypural |
| popr | posterior process |
| prp | parapophysis |
| qu | quadrate |
| ra | retroarticular |
| soc | supraoccipital crest |
| sph | sphenotic |
| sym | symplectic |
| sop | subopercle |

| | |
|---|---|
| scl | supracleithrum |
| sca | scapula |
| SL | standard length |
| TL | total length |
| u | ural centrum |
| un | uroneural |
| vo | vomer |
| vhh | ventral hypohyal |

# Chapter 1: Introduction

## 1.1 General Introduction

The family Cichlidae (Bonaparte 1835) is one of 469 families of teleost fishes (Nelson 2016). Etymologically, the family name is derived from the Greek word κίχλη (‘*kichle’*) for thrushes and some marine wrasses (Barlow 2000, Froese and Pauly 2017). The oldest known written record of a cichlid is a hieroglyph in ancient Egypt specifically assigned to the Nile tilapia (*Oreochromis niloticus*) (Gardiner 1957). The first cichlid artefacts also appeared in ancient Egypt, displaying the mouthbrooding tilapia as a symbol of rebirth. Another tilapia, *Sarotherodon galilaeus*, embodies the biblical St. Peter’s fish in the Sea of Galilee (Robins 1993).

The first monograph on the family Cichlidae was written by Heckel (1840), based on the Natterer collection from Brazil (illustrations in Riedl-Dorn, 2000). Another early major treatise is by Jardine (1843), from Guyana, Brazil and Venezuela (Kullander & Stawikowski 1997a-b, for identifications). Günther (1868) described and illustrated a large part of the Central American cichlid fauna, followed by Regan (1905-1908a). Pellegrin (1904) revised the family with diagnoses of all genera and species known to him, which remained the basis for all Neotropical cichlid taxonomy until the 1980s (Kullander 1998). The first phylogenetic revision of the Neotropical cichlids was presented by Cichocki (1976), and most recently Kullander (1998) and Farias et al. (1999) have provided phylogenetic hypotheses based on morphology and molecular data respectively. A formal classification down to tribe is provided by Kullander (1998).

Despite this long history of study, there is much that we do not know about cichlid fishes, and the family is still of great interest and the subject of numerous new studies. Of the about 1703 validly described species, 195 have been described only in the last ten years. With at least 2200 species known (Eschmeyer & Fong 2017), the family Cichlidae is the most species-rich non-ostariophysan fish family and the second most species-rich family of freshwater fish worldwide after the family Cyprinidae (2,700 described species) (Kullander 1998). In total, cichlids represent around 13% of extant teleost fishes (Koblmüller et al. 2015). Cichlids have a geographically widespread distribution in tropical freshwater environments, with species found across most of Africa (estimated more than 1300 species), in the Middle East (4 species), Iran (2 species), the Indian sub-continent (3 species), Madagascar (29 species), and from southern North America to southern South America (570 species) (Kullander 1998; Eschmeyer & Fong 2017).

Cichlid fishes are one of the classical examples of adaptive radiation. The group has given rise to an extraordinary variation of body shapes, color patterns, behaviour and eco-morphological specializations (e.g., Schliewen et al. 1994; Danley & Kocher 2001; Joyce et al. 2005; Barluenga et al. 2006; Muschick et al. 2012; López-Fernández et al. 2013; Ronco et al. 2021). In evolutionary biology, adaptive radiation is the rapid diversification of organisms from a single lineage of ancestral species into a multitude of new species in response to a change in the environment that makes new resources available (Simpson 1953). Simultaneously with the expansion of diversity in ecology within a lineage, this response also results in morphological and physiological adaptations (Simpson 1953; Schluter 2000; Givnish 2015). This outstanding diversity and explosive speciation in cichlid fishes make them a powerful vertebrate model and natural laboratory for studying evolutionary biology, along with Darwin finches, *Anolis* lizards and next to experimental microbial evolution (Kocher 2004; Seehausen 2006).

As an outcome of adaptive radiation, cichlids tend to form species flocks. A species flock is a monophyletic group of closely related organisms with a shared common ancestor that are endemic to the same geographically isolated ecosystem (Ribbink 1984). The species flocks of cichlid fishes in lakes Tanganyika, Malawi and Victoria are characterized by their fast divergence rates and represent an enormous species richness among the vertebrates (Turner et al. 2001; Kocher 2004). To put the divergence rates of cichlids into a temporal context, approximately 2,000 cichlid species evolved during the evolutionary time span of our own species, starting from the split of chimpanzees and humans about 5-7 million years ago (Salzburger 2018).

Across various levels of biological organization, cichlids show considerable diversity:
1. Taxonomic diversity: There are 1700 validly described species in more than 200 genera in the family Cichlidae known to date, but many more of them are yet to be formally described (Nelson 2016; Froese and Pauly, 2017). It has been estimated that the total number of cichlid species is in the range of 3,000–4,000 (Kocher 2004); more than half of this taxonomic diversity is found in the faunas of the East African Great lakes: Tanganyika (250 species), Malawi (800–1,000 species) and Victoria (700 species) (Snoeks 2000; Salzburger 2004; Turner et al. 2001). Due to the large number of species and the obscurity of species boundaries, cataloging the diversity of cichlid species is difficult (Sturmbauer 1998; Goldschmidt 1996).

2. Morphological diversity*:* Morphologically, cichlids have a single nostril on each side of their snout rather than two as in most other fish; their lateral line is interrupted; and they possess a characteristic pharyngeal jaw apparatus, which is a second set of jaws for processing food located in the pharynx that is functionally decoupled from the oral jaw apparatus (Nelson 2016; Froese and Pauly 2017). Although none of these characteristics is unique to cichlids, the combination of all these characters allows us to recognize cichlids as a monophyletic group. Within the family, cichlids vary widely in body shape — from rounded, as exemplified by the freshwater angelfish (*Pterophyllum scalare*) or the discus (*Symphysodon discus*), to elongated, as in *Crenicichla percna* — as well as in body size — from less than 3 cm total length and a mass of 0.1 g in, for example, *Telmatochromis bifrenatus*, up to >80 cm total length and >3 kg mass in *Boulengerochromis microlepis*, both from Lake Tanganyika. Above all, cichlids differ in ecologically relevant traits, such as the overall shape of the head and the form of the mouth apparatus (Fryer and Iles 1972; Muschick et al. 2012).
3. Ecological diversity: Cichlids occupy a wide range of feeding niches, ranging from eating algae, sponges, invertebrates or other fish, to mollusc crushing or scale eating, and habitat types, ranging from small streams or rivers to lakes and, within lakes, from the shallow to the deep benthic and limnetic zones. Cichlids occur on rocky, sandy, weedy and muddy substrates, yet others are found in caves or use empty snail shells as shelter (Fryer and Iles 1972; Salzburger 2009). As in other cases of adaptive radiation, there is a strong correlation between phenotype and environment in cichlids (Fryer and Iles 1972; Muschick et al. 2012).
4. Colour diversity*:* Cichlids are highly diverse with respect to body coloration and pigmentation, hence their German name '*Buntbarsche*' (colourful perches) (Seehausen and van Alphen, 1999). Coloration in cichlids has been implicated in sexual selection via female choice with respect to male nuptial coloration but also mimicry and camouflage (Fryer and Iles 1972; Seehausen and van Alphen 1999; Boileau 2015). Not least because of their conspicuous coloration, cichlids are popular with aquarists and biologists for their enormous colour diversity (Fryer and Iles 1972).

Taxonomically, cichlids are divided into several tribes, among which, for example, the African Haplochromini are renowned as particularly species rich and colourful. Closely related species often differ in little else but the colour of body and fins (Maan and Sefc 2013). In many species, body colours are overlaid with dark vertical bars or horizontal stripes. Frequently, the differently coloured body regions are not defined by sharp boundaries but rather shade into one another—in contrast to the sharp-edged patterns of many well-known coral reef fishes (Maan and Sefc 2013).

Colour patterns vary not only between cichlid species, but also within and among populations of a species (e.g., sexual dichromatism, polychromatism and geographic variation), as well as within individuals, depending on their age and social status (Andersson 1994; Barlow 2000; Duftner 2007; Sefc 2011).

5.Behavioural diversity: Cichlids show a very broad behavioural range. Many cichlid species are territorial, while others form large schools. All cichlids show some kind of brood-care behaviour, ranging from substrate spawning to maternal, paternal or biparental mouthbrooding (Fryer and Iles 1972, Salzburger 2009, Barlow 2000). Most members of the Cichlidae are moderately to strongly sexually dimorphic, and have pairwise breeding systems. Eggs are typically deposited on a substrate and both parents guard offspring or several weeks, even for some time after the young are free-swimming. Oral incubation, or mouth-brooding, is fairly common in African cichlids and also has been recorded for many species of the South American genera (Kullander and Ferarris 2003).

Cichlid diversity has been explained both by the versatile design of the pharyngeal jaw complex used for food mastication and by their advanced brood care. The unpaired lower pharyngeal toothplate and the opposed upper pharyngeal tooth plates are contained in a muscular sling characterizing labroid fishes (Kullander and Ferraris 2003). There is considerable variation in the shape of the toothplates and associated dentition, reflecting diet specializations. The oral jaws are generally highly mobile and protrusible, and tooth shape varies greatly, although most Neotropical cichlids have simple, subconical, unicuspid teeth, whereas African cichlids commonly have laterally bicuspid or tricuspid oral teeth (Kullander and Ferarris 2003).

Over the last few decades, a multitude of studies have employed various approaches to shed light on the factors responsible for the stunning diversity within this family. The elaborate reproductive behaviour and a highly developed pharyngeal jaw have long been recognized as important key factors for the evolutionary success of cichlids (Liem 1973; Keenleyside 1991). However, the picture is not simple as additional intrinsic factors like mouth morphology, body shape and size, coloration, color perception, sound and smell, and phenotypic plasticity, combined with ecological opportunity, appear equally important for driving diversification of cichlids (Salzburger 2009; Takahashi & Koblmüller 2011; Wagner et al. 2012; Maan & Sefc 2013; Salzburger et al. 2014).

The center of biodiversity for the family is located in the East African Great Lakes (Victoria, Malawi, and Tanganyika) that harbor more than two- thirds of the known 2,200 species in the family (Sturmbauer 1998; Stiassny and Meyer 1999). The massive monophyletic group of the East African cichlid radiations (EAR) includes thousands of species inhabiting East African lakes and rivers (Schwarzer et al. 2009; Dunz and Schliewen 2013). In Lake Tanganyika (Salzburger et al. 2002, Genner et al. 2007; Schwarzer et al. 2009), Lake Malawi (Sturmbauer et al. 2001), Lake Victoria (Verheyen et al. 2003) and the extinct Lake Makgadikgadi (Joyce et al. 2005), large-scale adaptive radiations have taken place. Lake Tanganyika, which is likely the oldest lake in Africa (9–12 million years, Cohen et al. 1993), is an evolutionary reservoir of old lineages of EAR (Nishida 1991); indeed, many EAR lineages are composed entirely of species endemic to this lake. Therefore, Lake Tanganyikan cichlid fishes are important for resolving the phylogeny of the African cichlid fishes. Large haplochromine species flocks of Lake Malawi and the Lake Victoria basin, which are well-known examples of rapid adaptive radiation, originated in the Lake Tanganyika radiation (Salzburger et al. 2005; Koblmüller et al. 2008a).

The explosive speciation of cichlid fish in the lakes of East Africa has long been a focus for controversy among evolutionary theorists (Mayr 1963; Fryer and Iles 1972). Lake Malawi, with over 400 species of endemic cichlids, has more species of fish than any other lake in the world. Lakes Victoria (>200 species) and Tanganyika (>170 species) run a close second and third. These species probably arose in a relatively short period of time. Recent geological studies suggest that Lake Tanganyika is no more than 12 my old (Cohen et al. 1993), while Lake Malawi is thought to be considerably younger, probably less than 2 my old (Banister and Clarke, 1980). Several authors have suggested an extremely recent (<200,000 years) common ancestor for the Lake Victoria cichlid flock (Sage et al. 1984; Meyer et al. 1990). The mechanisms by which so many species have arisen in such a short time, within closed lake basins are a fascinating subject for research. Both morphological and molecular data have been examined to determine the relationships among cichlids and to reconstruct their biogeography.

Numerous studies have attempted to reconstruct the evolutionary time scale and biogeographic history of cichlids distributed in the Americas, Africa, the Middle East, Madagascar and the Indian subcontinent (Murray 2000; Murray 2001; Genner et al. 2007; Azuma et al. 2008; Friedman et al. 2013; McMahan et al. 2013; Matschiner 2019). The first classification of the Lake Tanganyika cichlid fishes into 12 tribes based on morphological features was done by Poll (1986). Takahashi (2003a) revised Poll's (1986) classification and recognised 16 tribes based on cladistic analysis of anatomical data, of which 14 tribes composed the EAR. Subsequently, further revisions were made for the framework of the EAR tribes. Phylogenetic relationships among the African cichlids have repeatedly been assessed based on different molecular markers, although statistical support for these topologies is often weak (Takahashi and Sota 2016).

Both molecular and morphological studies with a focus on recovering early divergences within Cichlidae have met with limited success. Almost all the molecular studies have relied on one or two molecular markers, which have proven to be insufficient for recovering robust higher-level intrafamilial relationships (e.g., Zardoya et al. 1996; Streelman et al.1998; Farias et al. 1999, 2000, 2001; Sparks 2004a). The combined molecular and total evidence phylogenetic studies done by Farias et al. (2000) do not include all the cichlids from Madagascar. No family level hypothesis of cichlid intra-relationships to date, based on equitable family-wide taxonomic sampling, has shown strong support for a transoceanic sister-group relationship between major geographic assemblages of cichlid fishes (e.g., African- Neotropical), excluding the well-supported sister-group relationship that is consistently recovered between the Malagasy (*Paretroplus*) and South Asian (*Etroplus*) etropline lineages (Sparks 2004a).

The timescale of cichlid evolution has proven difficult to determine, as molecular phylogenies calibrated by biogeographic and fossil calibrations have often generated strongly contrasting results. Did American and African cichlid fishes diverge when the South American and African continents separated 100 million years ago, or did cichlid fishes disperse across the Atlantic at a later date? Matschiner (2019) reviewed the datasets and phylogenetic approaches that have been used to investigate this issue, and re-analysed the data using more contemporary approaches. Matschiner (2019) concluded that the most likely timescale of divergence between American and African cichlids would be 75–60 million years ago and therefore that one or more marine dispersal events must have taken place.

Generally, one major problem that hampers robust divergence time inference in cichlids is the paucity and identity of fossils that can be used to calibrate time-trees. Fossils that are reliably identified and reliably dated are necessary for cichlid molecular researchers to use in their struggle to put cichlid diversification in a temporal context. One of the major issues is the lack of unambiguous and important calibration points for molecular clock estimates, e.g., a consolidated root age of the family Cichlidae, or a lack of cichlid fossils within EAR with the phylogenetically clear position (Schedel et al. 2019).

Because an understanding of cichlid fossils is key to understanding the diversity of this family, it is of interest to determine how much data we might expect to be lost from the fossil record. Fossils, by their nature, preserve mainly hard parts (bone in vertebrates) with the rare preservation of soft tissues, but little else. For example, colour, which is at least partly responsible for the great diversity of extant cichlid species, and possibly one of the key features that has allowed speciation through sexual selection, is not preserved in fossil cichlids. It is likely that the lack of colour in fossil cichlids, as well as other features that are not preserved, would cause us to underestimate the true diversity that is present in the fossil record, because the reported diversity is based only on the skeletons. An assessment of the variability of skeletons of extant cichlids within a single population and the variation of the skeleton that might occur with growth, will provide a baseline of the amount of variation that might be expected in a single species of extinct cichlid. This baseline would then allow us to indicate whether or not a sample of fossil cichlids is likely to represent more than a single species.

## 1.2 Thesis Objectives

There are two goals of research for this thesis:

1. To evaluate individual variation in a *Mesonauta festivus*. The morphological and osteological examination of the South American cichlid *Mesonauta festivus* from a single population will allow me to assess how much variation exists in a single population of extant cichlids. This study will provide a baseline of the amount of variation that may be expected in a fossil species, which can then be used to assess the probable number of taxa present in fossil samples.
2. To describe the osteology of the *Iranocichla hormuzensis* and document how it changes over a developmental series. This study will allow me to assess the amount of variation that occurs with growth in a single species, and provide a baseline that can be used to assess whether a sample of fossil specimens of varying size might belong to one or more species.

The significance of this project will be a better understanding of the concept of speciation in evolution as it relates to morphology. The studies based on the morphological and osteological studies will help to improve our understanding of whether variation identified in cichlid fossils might represent individual variation or variation that represents more than one species. This study will help systematists and taxonomists to evaluate cichlid fossils for phylogenetic studies.

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# Chapter 2: Individual variation in a single population of *Mesonauta festivus* (Perciformes: Cichlidae)

## 2.1 Introduction

The genus *Mesonauta* was first established by Günther (1862), and later assessed by Kullander (1983, 1986) who provided a revised diagnosis for the genus. The species *Heros insignis* Heckel, 1840, *Heros festivus* Heckel, 1840 and *Chromys acora* Castelnau, 1855 were included in the genus *Mesonauta* by most authors following Günther (1862) and Steindachner (1875). Gunther (1862) synonymized all these species under the name of *Mesonauta insignis*. A preliminary survey of *Mesonauta* material by Kullander (1983, 1986) from the vast range of the genus, including the Amazonas, Paraguay and Orinoco drainages as well as Guianan rivers, suggests the presence of more than one species. Hence, Kullander (1986) resurrected the name *Mesonauta festivus* and justified the rehabilation of *Mesonauta insignis* as distinct from *Mesonauta festivus.* Both Kullander (1986) and Kullander and Nijssen (1989) considered *Chromys acora* as a valid species of *Mesonauta* as *M. acora,* but none of these papers provided any reasons for the revalidation nor gave diagnostic characters.

*Mesonauta* species are all very similar in shape and colour pattern (Kullander and Silfvergrip 1991). *Mesonauta* species have been found in the Orinoco, the middle and upper Paraguay basin, most of the Brazilian and Peruvian Amazon basin (below lowermost falls except in the Xingu, the Jamari and perhaps the Araguaia, the Bolivian Amazon, Guyana and Amapá) (Kullander and Silfvergrip 1991). The species *Mesonauta festivus* is common and abundant among macrophyte stands along a large geographical range of the Amazonas and Paraná- Paraguay basins, in South America (Pires et al. 2015).

Kullander and Silfvergrip (1991) were able to collect *Mesonauta* from the type locality of *M. festivus*, enabling comparison with material from the Rio Madre de Dios drainage previously identified as *M. festivus* by Kullander (1986). Kullander and Silfvergrip (1991) also studied a series of *Mesonauta* from the Rio Tapajós, Rio Tocantis, Rio Xingu, Rio Paraguay, Rio Orinoco and upper Rio Negro drainages. In comparing those collections, it became evident also that Kullander's (1986) Peruvian *M. insignis* were misidentified. Kullander and Silfvergrip (1986) revised the diagnoses for the three named species of *Mesonauta: M. insignis, M. festivus and M. acora*.

*Mesonauta* has been identified as a member of the Cichlidae (Kullander 1986), based on its having four lateralis canal foramina on the dentary bone. Kullander (1986) also suggested that it has a close relationship with *Pterophyllum* Heckel 1840, with which it shares some morphological characteristics such as an extremely elongated, thickened first pelvic fin ray and ribs on the anteriormost four caudal vertebrae. *Mesonauta* is distinguished from *Pterophyllum* by the former having a more slender shape, with a body depth not over 61% SL compared to more than 65% SL in the latter, larger horizontal scales than the latter, dorsal rays fewer than 13 instead of more than 18, anal rays fewer than 15 instead of more than 18 and more dorsal spines (14-16 instead of 11-13) (Kullander and Silfvergrip 1991). *Mesonauta* species have an oblique lateral band extending from the mouth to the dorsal fin, and often have irregular vertical bars below the band, and a prominent ocellated spot on the caudal fin base, whereas *Pterophyllum* species have a noticeable pattern of dark vertical bars (Kullander and Silfvergrip 1991).

*Mesonauta, Pterophyllum, Symphysodon* Heckel, 1840, *Uaru* Heckel, 1840, and *Heros* Heckel, 1840 probably form a monophyletic assemblage among the cichlasomines (Kullander and Silfvergrip 1991). They all have long projections of the swimbladder extending well back into the caudal region (Kullander and Silfvergrip 1991). The lower pharyngeal tooth plate is stout, with a heart shaped dentigerous area and bears long, strongly compressed teeth (Kullander and Silfvergrip 1991). The anal fin is long, with 5-9, usually 7-8 spines, and 9-31 rays (either about 10-14, or nearly 30) (Kullander and Silfvergrip 1991). Usually, they have more caudal than abdominal vertebrae (Kullander and Silfvergrip 1991). Except in *Symphysodon*, the anterior jaw teeth bear a ledge on the lingual face, topped by one or more small cusps (Kullander and Silfvergrip 1991). In *Symphysodon*, the teeth are simple and reduced in number (Kullander and Silfvergrip 1991).

Currently, there are six species of *Mesonauta* that are accepted as valid: *M. acrora, M. egregious, M. festivus, M. guyanae, M. insignis* and *M. mirificus* (Froese and Pauly, 2021). *Mesonauta mirificus, M. festivus* and *M. acora*, are sympatric species (Kullander and Silfvergrip 1991). However, on the whole, *Mesonauta* species seem to have diversified by allopatric speciation (Kullander and Silfvergrip 1991); notably the sympatric occurrences are in the lowland Amazon, which has undergone the most dramatic hydrographic changes since the reversal of the Amazon outlet in the late Tertiary, and is an area that is most likely to have a fauna of mixed origin (Kullander and Silfvergrip 1991).

*Mesonauta festivus* (Heckel 1840), found in the Paraguay and Bolivian Amazon basins, Rio Jamari and lower Rio Tapajós, is the deepest bodied *Mesonauta* species (Kullander and Silfvergrip 1991) and differs from all others except *M. acora* by having microbranchiospines on all four gill arches (Kullander and Silfvergrip 1991). The Rio Tapajós population can be distinguished by being more slender and by averaging higher anal spine counts and lower abdominal vertebral counts than the more southern populations of *M. festivus* (Kullander and

Silfvergrip 1991). There are no known sexual differences in body shape, fin shape or colour pattern (Kullander and Silfvergrip 1991). The preopercle is rarely scaled, unlike in *M. acora* in which it is invariably scaled (Kullander and Silfvergrip 1991), and the fifth vertical bar on the body is not interrupted as in that species (Kullander and Silfvergrip 1991).

### 2.1.1 Objective

Variation in osteology among individuals may be the result of individual variation of members of the same species, or it may indicate that the individuals belong to different species. In the fossil record, it is not possible to definitively determine the cause of such variation. The main purpose of this chapter is to determine how much variation can be expected in a single population of a species. Previously, the variation in two different populations of *Mesonauta festivus* has been found by Kullander and Silfvergrip (1991). They distinguished the northern Rio Tapajós population from the *M.festivus* specimens collected from southern Rio Tapajós by the former having a more slender body shape, higher anal fin spine count and lower abdominal vertebral count (Kullander and Silfvergrip 1991). The osteology of a population of *M. festivus* is analysed here to determine the amount of variation that might be expected among closely related individuals. This information can then be used to assess whether the amount of variation among fossil specimens would indicate that the fossils are likely from a single population.

## 2.2 Materials and Methods

### 2.2.1 Materials

The specimens of *Mesonauta festivus* are from the collections of the University of Alberta Museum of Zoology, and are ethanol preserved specimens. These fish all come from a single population of wild-caught individuals from South America. Specific locality data was not available. Five specimens were double stained with alcian blue for cartilage, and alizarin red S for bone, and then cleared with trypsin following the procedures of Taylor and van Dyke (1985). I examined an additional 17 specimens, for a total of 22 individuals. I confirmed the species identification of each specimen for this project based on the incomplete lateral line, terminal mouth, oblique stripe on the body and ocellated spot on the caudal fin base.

### 2.2.2 Measurements:

Measurements and counts are taken as described by Daget (1954) and Paugy (1986). I used nine meristic counts and 19 measurements in order to capture morphological variability. The data are summarized in Tables 2.1 and 2.2. Vertebral counts were made for all specimens with the aid of CT scans using a Bruker micro CT Skyscan 1172 (no filter, 50 kV, and 83µA).

Comparative photographs for osteological drawings were taken with a Nikon DXM 1200C digital camera mounted on a Zeiss Stereo Discovery.V8 stereomicroscope with a Carl Zeiss 44403 6-9000 eyepiece (8x) and a variety of lenses (Zeiss Achromat S 0,3x FWD 236 mm, Zeiss Plan Apo S 0.63x FWD 81 mm, and Zeiss Plan Apo S 1.0x FWD 60 mm), using the NIS-Elements F package 2.20, version 5.03 software. For morphological observations, a Nikon SMZ1000 stereomicroscope with a Nikon C-W 10xB/22 using lens Plan Apo 0.5x WD 123 Nikon was used. The colour photographs of the specimens were taken with an OM-D Olympus E-M10 II digital camera with 60mm lens and has been processed using Adobe Photoshop version 23.1.1).

## 2.3 Results

### 2.3.1 General body form and external characteristics:

Size: The longest specimen included in this study is, 39.44 mm SL (Table 2.1), but according to Stawikowski & Werner (1989), *Mesonauta festivus* can reach more than 200 mm total length. Therefore, all the specimens included here are probably juvenile or sub-adult.

Body shape and fins: *Mesonauta festivus* is a deep-bodied (44%-54% SL, mean=49% SL, Table 2.1, Figure 2.1), laterally compressed fish with a terminal mouth, with wide nape and thin posterior trunk. The eyes occupy a large portion of the head (33%-39% HL, mean 36% HL). The predorsal body contour is slightly convex and the prepelvic body contour is concave. The dorsal fin insertion is slightly posterior (43%-52% SL, mean= 48% SL) to the level of the pelvic fin origin and anterior to the level of the anal fin insertion. The pelvic fin insertion is just posterior to the pectoral fin insertion and posteroventral to the operculum. Gunther (1862) found the pelvic fin origin to be anterior to the origin of the dorsal fin insertion in *Mesonauta* whereas it is posterior to the dorsal fin origin in other cichlids. The dorsal fin base (48%-60% SL, mean=55% SL) is larger than the anal fin base (32%-44% SL, mean=37% SL). The caudal peduncle is notably small with equal length margins or dorsal margin slightly longer than the ventral margin as the dorsal fin base is larger compared to the anal fin base. The caudal fin is rounded and has 16 principal rays.

Scales: Ctenoid scales cover the body (Figure 2.1). The interrupted lateral line begins on the first scale next to the operculum with a variable number of scales included in the upper row (13-16 in 22 specimens) and then drops ventrally with 2 intermediate scale rows between the two lateral line rows. The number of scales varies between 9-10 in the lower row of the lateral line (Table 2.2).

Colour pattern: Alcohol-preserved specimens are light yellow with a dark brown oblique stripe above the lateral line starting from the tip of the snout running through the orbit and continuing to the distal end of the dorsal fin rays (Figure 2.1). This oblique stripe may appear as a row of confluent blotches. The top of the head and the back above this stripe are darker than the ventral side below it. There are no dark spots on the prepelvic contour. There are a total of eight light brown vertical bars including the caudal spot on the caudal peduncle. All fins except the pectoral fin are spotted and dark brown in colour. The pectoral fin is a translucent yellow.

### 2.3.2 Skull

The posterior part of the skull is wider than the anterior part of the skull (Figure 2.2a). In dorsal view, the lateral ethmoid is rectangular in shape with curved margins and is connected to the frontal. The nasal is small, tubular in shape and bears a sensory canal (Figure 2.2a). The vomer has a triangular head with an elongate posterior end that is attached to the parasphenoid (Figure 2.2b). The anterior part of the vomer is visible in dorsal view and attached to the mesethmoid which is narrow anteriorly as compared to the posterior end and makes almost triangular in shape.

A large portion of the dorsal roof consists of the frontals with an elongated posterior edge which sutures with the sphenotic and parietal. The supraorbital sensory canal extends from the anterior to posterior of the frontals. The parietals narrow posteriorly where the supraorbital canal is positioned. The sphenotic has a small process anterodorsally. The pterotic is elongated posteriorly and connected ventrally to the parietal, epiotic and sphenotic, and laterally to the prootic. The prootic roughly forms a rectangle with a rounded corners, bears foramen and ventrally contacts the orbitosphenoid and frontal, and posteriorly the basioccipital and pterotic (Figure 2.2a & b). The epiotic is square with sharp edges. The exoccipitals are attached to the basioccipital laterally and have curved posterior edges. The supraoccipital is wide in the middle and linked to the parietal by its anterior process and posterolaterally to the exoccipitals and epiotic and has a sharp crest posteriorly (Figure 2.2a). The posterior part of the basioccipital is almost rounded, similar to the shape of the centrum and is connected to the first centrum. In ventral view, the parasphenoid connects to the prootic by its posteriolateral edges. The two orbitosphenoids are attached to the frontal laterally and to the parasphenoid dorsally (Figure 2.2b).

### 2.3.3 Jaws and Teeth

The premaxilla has a pointed sharp anterior ascending process which is longer than the premaxillary length. The maxilla has a mid-lateral ascending process and a descending process posteriorly (Figure 2.3b). It has a foramen in the middle of the ascending process which articulates with the ascending process of the premaxilla. The dentary has two limbs joining to form an approximately 45 degree angle to accommodate the anterior shaft of the anguloarticular bone. The retroarticular is a small bone attached to the anguloarticular posteroventrally.

Cylindrical, laterally compressed teeth are present on the premaxillae and dentaries. The teeth decrease substantially in size posteriorly and increase in size toward the symphysis. The teeth of the outer rows are larger than the inner rows. Each dentary has five teeth which are distinctly enlarged compared to the rest of the teeth.

The pharyngeal toothplate is heartshaped (Figure 2.5d). According to Kullander and Silfvergrip, 1991, there are 26-27 teeth along the posterior margin and 8-9 teeth along the median. I find 21-23 teeth along the posterior margin and 6-8 along the median. The teeth are unicuspid (Figure 2.5e), curved or subconical in shape which is a characteristic of the neotropical cichlids (Kullander and Ferarris 2003).

### 2.3.4 Opercular series

The preopercle is L-shaped and the horizontal limb is broader than the vertical limb (Figure 2.4). Both limbs of the preopercle form a right angle with the sensory canal running along both the limbs. The sensory canal opens to the surface ventrally via pores on the horizontal and vertical arms of the preopercle. The interopercle is thinner anteriorly compared to the posterior side. The opercle is the largest bone of the opercular series. The opercle is pointed anteroventrally with an almost straight anterior edge and convex posterior edge; it is nearly triangular or semi-circular in shape. The subopercle has a small, sharp ascending process which is positioned between the posteroventral part of the preopercle and anteroventral part of the opercle bone.

### 2.3.5 Suspensorium

The palatine is small, narrow and rod-like anteriorly with a round posterior end (Figure

2.3c). The ectopterygoid is a narrow bone, slightly curved anteriorly. It is attached to the palatine ventrally and with quadrate laterally. The endopterygoid is small and borders the anterior part of the metapterygoid. The quadrate's upper limb looks like a fan shaped with narrow lower limb. The symplectic is an elongate slim bone, inserting into a groove of the quadrate. The hyomandibular is broad dorsally, narrows ventrally and articulates with the anterior edge of the dorsal part of the preopercle.

### 2.3.6 Infraorbital bones

No cichlids retain an antorbital bone (Oliver 1984). The anterior bone in the series, the lacrimal, is the largest element and is followed by the six other bones (infraorbitals 2-7, the 7th dorsal element is the dermosphenotic) (Figure 2.3a). The lacrimal is approximately square and bears three neuromasts. There is no supraorbital bone, therefore the lateral edge of the frontal completes the dorsal margin of the orbit. The circumorbital sensory canal passes through all the infraorbital bones.

### 2.3.7 Branchial arches and pharyngeal region

The first basibranchial is largest in size and rod-like in shape with slightly concave sides (Figure 2.5a & b). The second is similar to the first one but smaller in size. The third basibranchial is smaller in size than the first one but larger than the second one, rod-like in shape but narrower posteriorly. The fourth basibrachial is the smallest and is entirely cartilaginous in the examined specimens. There are three pairs of hypobranchials; these are rectangular with cartilaginous tips. There are four pairs of epibrachials and four pairs of ceratobranchials which are long slender rods that become progressively shorter posteriorly through the series. The fifth ceratobranchial is modified and bears the dermal toothplate, called the lower pharyngeal jaw.

The dorsal and ventral hypohyals articulate with the anterior tip of the ceratohyal. The ceratohyal is broad and thick anteriorly, with a flat posterior end that is connected with the epihyal. The epihyal is almost triangular in shape. There are five branchiostegal rays on each side (Figure 2.5c), three attached to the ceratohyal and two articulate with the epihyal. They are narrow, long and curved with the first branchiostegal ray shorter than the following rays.

### 2.3.8 Paired fins and Girdles

The posttemporal is curved and the supracleithrum is elongate (Figure 2.6a). The largest element of the pectoral girdle is the cleithrum. The posterior edge of this bone articulates with the coracoid and scapula. The coracoid is curved with a sharp projection forming a large foramen between itself and the cleithrum. The scapula is almost square with rounded edges and has a foramen in the centre. The two postcleithra meet each other and are positioned posterior to the scapula (Figure 2.6b). The postcleithra are sharp, elongated and thin. The pectoral fin is short and rounded with 9-11 rays.

The pelvic girdle is situated horizontally in the thoracic area and attached to the pectoral girdle by muscles and ligaments (Figure 2.6c). The pelvic girdle consists of distal processes, basipterygia, posterior processes and anteroventral processes. The pelvic fins articulate on the posterior processes. The pelvic fin has one spine and five fin rays including the first ray producing a filament that may exceed the standard length in length.

### 2.3.9 Vertebral Column and predorsal bones

The vertebral column consists of 25-27 centra, of which 11-12 are abdominal and 13-15 are caudal. There are two predorsal bones in front of the first dorsal pterygiophore. The first neural spine is located between the first and second predorsal. The first ptergiophore is located before the second neural spine.

### 2.3.10 Dorsal and Anal fins and supports

The dorsal fin has XIII-XV spines and 9-12 fin rays. The spines increase in length from anterior to posterior, and the rays decrease in the size from anterior to posterior. The longest ray can reach posteriorly to almost three-quarters of the length of the caudal fin. The dorsal fin has 23-25 pterygioophores to support the fin.

The anal fin has VII-IX spines and 9-12 fin rays with 17-19 anal pterygiophores. Similar to the dorsal fin, spines increase in length and rays decrease in size posteriorly.

### 2.3.11 Caudal fin and skeleton

The caudal fine is small and rounded with 16 principal caudal fin rays (Figure 2.7). In the caudal skeleton, preural centrum 1 is fused to the first and second ural centra. A urostyle is present, and is conical in shape and pointed distally. Preural centrum 2 has a reduced neural spine and fully developed haemal spine with a thick and curved anterior region, whereas preural centrum 3 has a fully developed neural spine and a long, thin haemal spine.

There are five hypurals. The first and fourth are the largest, of similar proportions and triangular in shape. The second hypural rests dorsally on the first hypural. There is a space (= diastema) between the second and third hypural. The fourth hypural fits firmly into the urostyle and rests on the third hypural dorsally and the fifth hypural is the smallest of all. The uroneural is attached to the urostyle on the dorsal side and rests dorsally on the fifth hypural. The parahypural has an anterior flange at the base which projects posteriorly over the hypural called a hypuraphophysis. In the space between the neural spine of the third preural centrum and the uroneural, there are two epurals of different sizes. The first one is larger than the second one.

### 2.4 Variation among specimens

I assessed the intraspecific variation within a sample (N=22) of *Mesonauta festivus.* Most measurements and meristics are normally distributed for this sample (Table 2.1,2.2). I recorded a range of 33-39.4 mm SL for specimens of *M. festivus*, whereas Kullander and Silfvergrip (1991) recorded a range of 40.3-76.0 mm SL (N=16) for the specimens of *M. festivus* from Paraguay, 35.3-80.3 mm SL (N=13) for the specimens of *M. festivus* from Guaporé, 41.7-75.8mm SL (N=12) for the specimens of *M. festivus* from Tapajos, 40.8-78.3mm SL (N=25) for the specimens of *M. festivus* from Peru.

I recorded unicuspid subconical teeth in the specimens which is similar to work done by Kullander and Silfvergrip (1991). The gill rakers range from 6-7 in the present study as compared to Kullander and Silfvergrip (1991) who found ranges between 5-7. I found the number of scales in the upper row of lateral line ranges between 13-16 and in the lower row ranges between 9-10 and 25-27 number of total vertebraes. All the ranges found in the number of fin spines and rays, pterygiophores are reported in Table 2.2. The comparison of meristics of the current study with Kullander and Silfvergrip (1991) can be found in the Table 2.3.

The variation found within the specimens of the present study falls in the normal range that is usually expected in specimens of the same population (Kullander and Silfvergrip 1991). In terms of osteology, I examined five cleared and stained specimens of *Mesonauta festivus* along with the CT scans of the rest of the specimens and I did not find any significant variation in the bones.

### 2.5 Discussion

The osteology of the *Mesonauta festivus* is described for the first time. The bony elements did not show any significant differences among the individuals along with the normal distribution of most measurements and meristics. This suggests that if much variation is found in fossil specimens it may indicate that there are more than one species present.

*Mesonauta festivus* is a generalist species (Pires et al. 2015). *Mesonauta festivus* is capable of living in strikingly different physical-chemical water conditions (Pires et al. 2015), and has a high tolerance to very high variation in environmental conditions observed throughout the hydrological cycles along the entire year (de Almeida and Melo 2009). The low values of dissolved oxygen recorded for some samples suggest that *Mesonauta festivus* is capable of living in hypoxic conditions, similar to many other cichlid species in the Amazon (Almeida-Val et al. 1995; Chippari-Gomes et al. 2005). *Mesonauta festivus* has a capability of reproducing throughout the entire year, which demonstrates its lack of dependence on specific environmental conditions (Pires et al. 2015). There is no known sexual differences in body shape, fin shape or colour pattern in *Mesonauta festivus* (Kullander and Silfvergrip 1991). These traits here mentioned contribute to the species broad geographical range by buffering selective pressures posed by the different environments that the species inhabits (Schlichting 2004). Therefore, these traits may allow the evolutionary lineage to remain similar even when populations experience distinctly different biotic and abiotic conditions. If one population experiencing local selective pressures reached the species status, the geographical range of *M. festivus* lineage would be smaller by consequence. This may aid in explaining the low phenotypic variation of this evolutionary lineage, as seen from the remarkable morphological similarity among the species of *Mesonauta* (Kullander and Silfvergrip 1991) or even the low diversity in morphology of its tribe (López-Fernández et al. 2013). By the same token, the remarkably active dispersion ability of this species may add an important contribution by increasing the gene flow between populations (Malcom 2011).

### 2.5.1 Conclusion

This assessment of individual variation within a single population of a cichlid fish provides a baseline for assessing the likelihood of multiple species being represented among a collection of fossil specimens from a single locality. I found there to be a lack of variation in *Mesonauta festivus* from a single population. In contrast, Kullander and Silfvergrip (1991) found variation to be present in different populations of *M. festivus*. The lack of variation within a population combined with presence of variation between populations suggests that a fossil sample of cichlids containing specimens with some variation are unlikely to belong to a single population. Whether or not the fossil sample should be interpreted as coming from more than one population, or more than one species will need further research to determine.

## 2.6 Tables

Table 2.1. Measurements and ratios for *Mesonauta festivus* (n=22).

| | **Range** | **Mean** | **SD** |
|---|---|---|---|
| Standard lengh (SL mm) | 33-39.44 | 35.38 | |
| Total length (TL mm) | 34.7-51.45 | 46.89 | |
| SL:TL | 0.67-0.99 | 0.75 | 0.07 |
| Body depth $SL^{-1}$ | 0.44-0.54 | 0.49 | 0.02 |
| Head length $SL^{-1}$ | 0.33-0.38 | 0.37 | 0.02 |
| Interorbital distance $HL^{-1}$ | 0.33-0.44 | 0.40 | 0.02 |
| Eye socket diameter $HL^{-1}$ | 0.33-0.39 | 0.37 | 0.02 |
| Snout length $HL^{-1}$ | 0.33-0.43 | 0.37 | 0.03 |
| Caudal peduncle depth:Caudal peduncle length | 1.56-2.45 | 1.97 | 0.22 |
| Length of upper jaw $HL^{-1}$ | 0.21-0.27 | 0.24 | 0.01 |
| Predorsal length $SL^{-1}$ | 0.43-0.52 | 0.48 | 0.02 |
| Preanal length $SL^{-1}$ | 0.52-0.65 | 0.61 | 0.03 |
| Prepelvic length $SL^{-1}$ | 0.41-0.50 | 0.45 | 0.02 |
| Prepectoral length $SL^{-1}$ | 0.35-0.42 | 0.39 | 0.01 |
| Dorsal fin base $SL^{-1}$ | 0.48-0.60 | 0.55 | 0.03 |
| Anal fin base $SL^{-1}$ | 0.32-0.44 | 0.39 | 0.03 |

Table 2.2. Meristics for *Mesonauta festivus*. The number of specimens that showed each count is in brackets.

| **Number of** | **Sample Size** | | | | |
|---|---|---|---|---|---|
| Dorsal fin spines | 22 | XIII (1) | XIV (7) | XV (14) | |
| Dorsal fin rays | 22 | 9 (1) | 10 (8) | 11(9) | 12 (4) |
| Anal fin spines | 22 | VII (2) | VIII (14) | IX (6) | |
| Anal fin rays | 22 | 9 (1) | 10 (6) | 11 (10) | 12 (5) |
| Pectoral fin rays | 22 | 9 (3) | 10 (12) | 11 (6) | |
| Abdominal vertebrae | 17 | 11 (1) | 12 (16) | | |
| Caudal vertebrae | 17 | 13 (4) | 14 (9) | 15 (3) | |
| Total vertebrae | 17 | 25 (5) | 26 (10) | 27 (2) | |
| Lateral line: | | | | | |
| -Scales in upper row | 22 | 13 (1) | 14 (1) | 15 (16) | 16 (4) |
| -Scales in lower row | 22 | 9 (16) | 10 (6) | | |
| - Scales overlapped in upper row and lower row | 22 | 0 (2) | 1 (1) | 2 (19) | |
| -Intermediate scale rows between upper row and lower row | 22 | 2 (22) | | | |
| Gill rakers | 22 | 6 (9) | 7 (13) | | |
| Dorsal pterygiophores | 17 | 23(1) | 24(13) | 25(3) | |
| Anal pterygiophores | 17 | 17(14) | 18(1) | 19(2) | |

Table 2.3. Comparison of meristics from this study to Kullander and Silfvergrip (1991) study.

| **Number of** | **Current Study** | **Kullander and Silfvergrip (1991)** |
|---|---|---|
| Gill Rakers | 6-7 | 5-7 |
| Lateral line scales | | |
| -Upper row | 13-16 | 14-19 |
| -Lower row | 9-10 | 6-10 |
| Dorsal fin spines | XIII-XV | XIV-XVI |
| Dorsal fin rays | 9-12 | 9-12 |
| Anal fin spines | VII-IX | VII-IX |
| Anal fin rays | 9-12 | 10-14 |
| Total Vertebrae | 25-27 | 25-27 |
| Abdominal Vertebrae | 11-12 | 11-13 |
| Caudal Vertebrae | 13-15 | 13-15 |

## 2.7 Figures

Figure 2.1. Photograph of a preserved specimen of *Mesonauta festivus*, specimen number 5, SL = 34.2 mm. Scale bar measurements = mm.

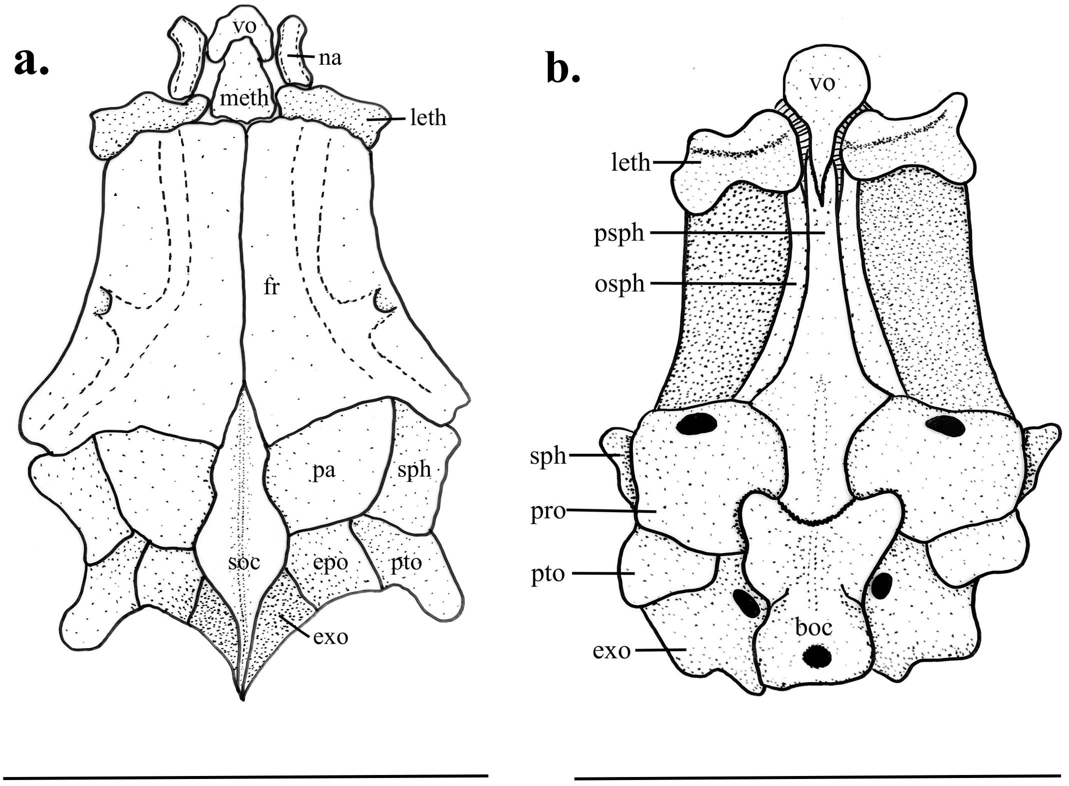

Figure 2.2. Drawing of the skull of *Mesonauta festivus*, specimen number 2, SL = 38.18mm, a. dorsal, b. ventral. Hatching represents cartilage. Scale bar = 5 mm.

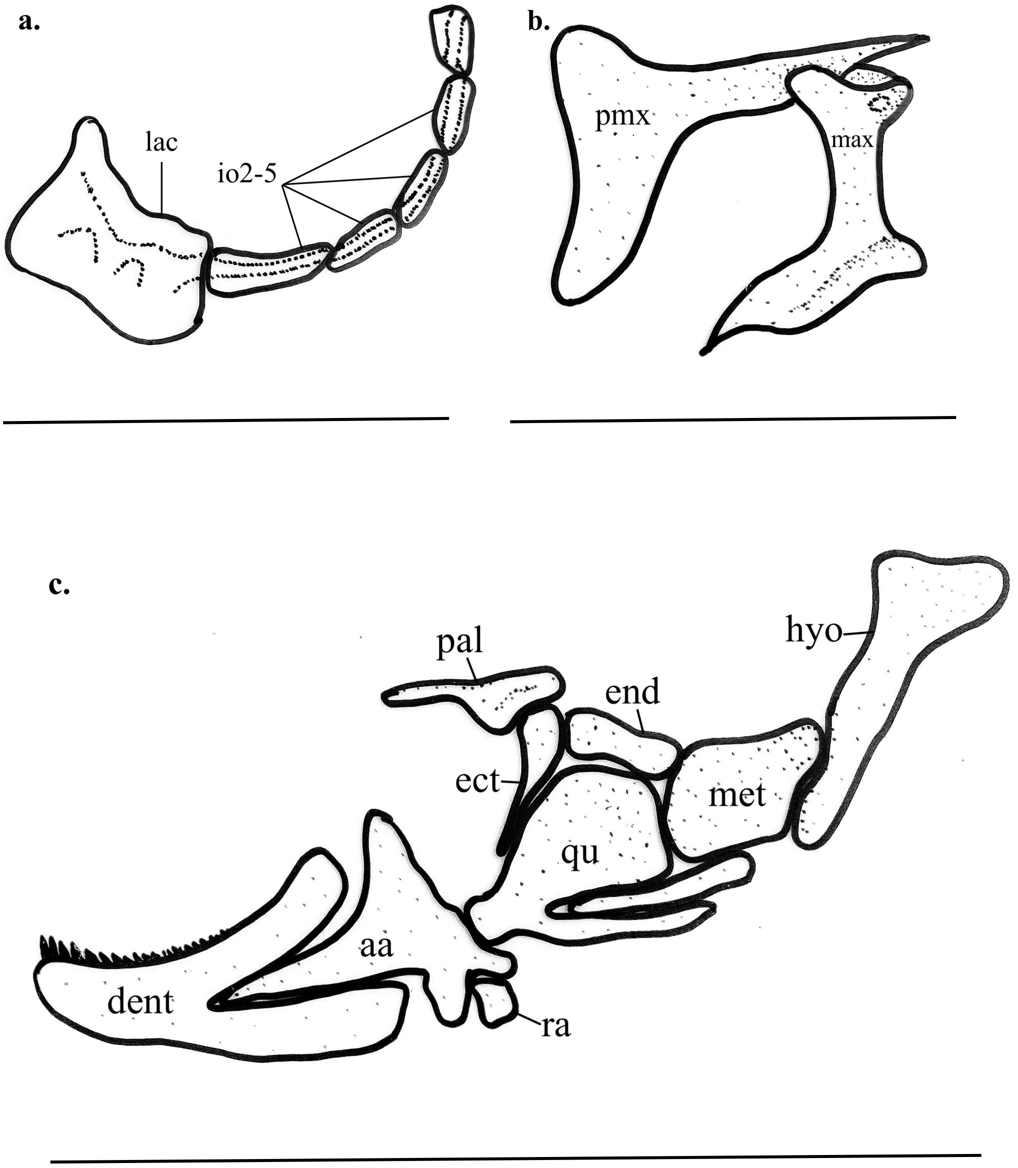

Figure 2.3. a. Drawing of the infraorbital series (scale bar = 2 mm) b. Drawing of the premaxilla and maxilla of the left jaw in lateral view (scale bar = 2 mm) c. Drawing of the dentary and suspensorium (scale bar = 5 mm) of *Mesonauta festivus,* Specimen number 2 , SL = 38.18 mm.

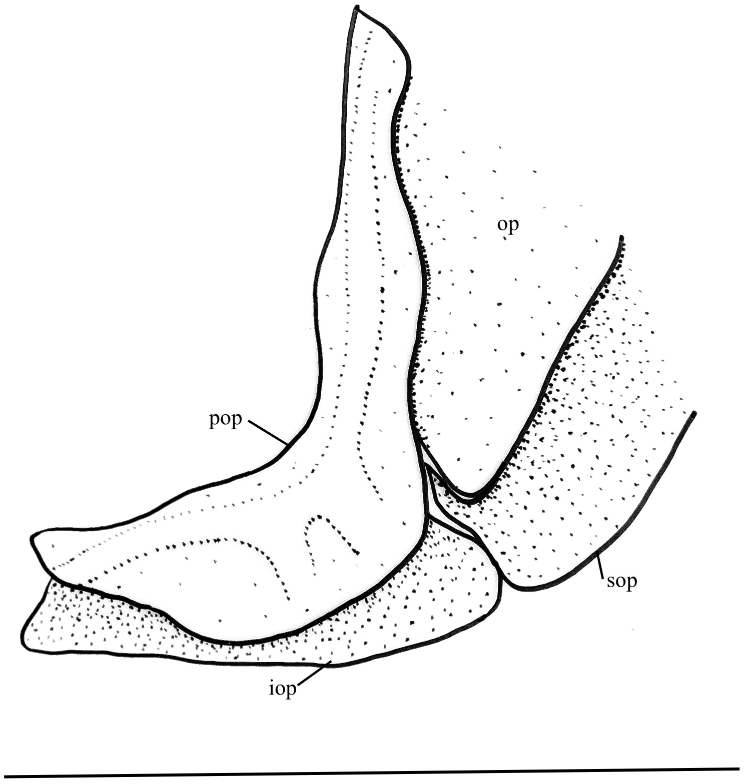

Figure 2.4. Drawing of the opercular series of *Mesonauta festivus*, specimen number 2, SL = 38.18 mm. Scale bar = 5 mm.

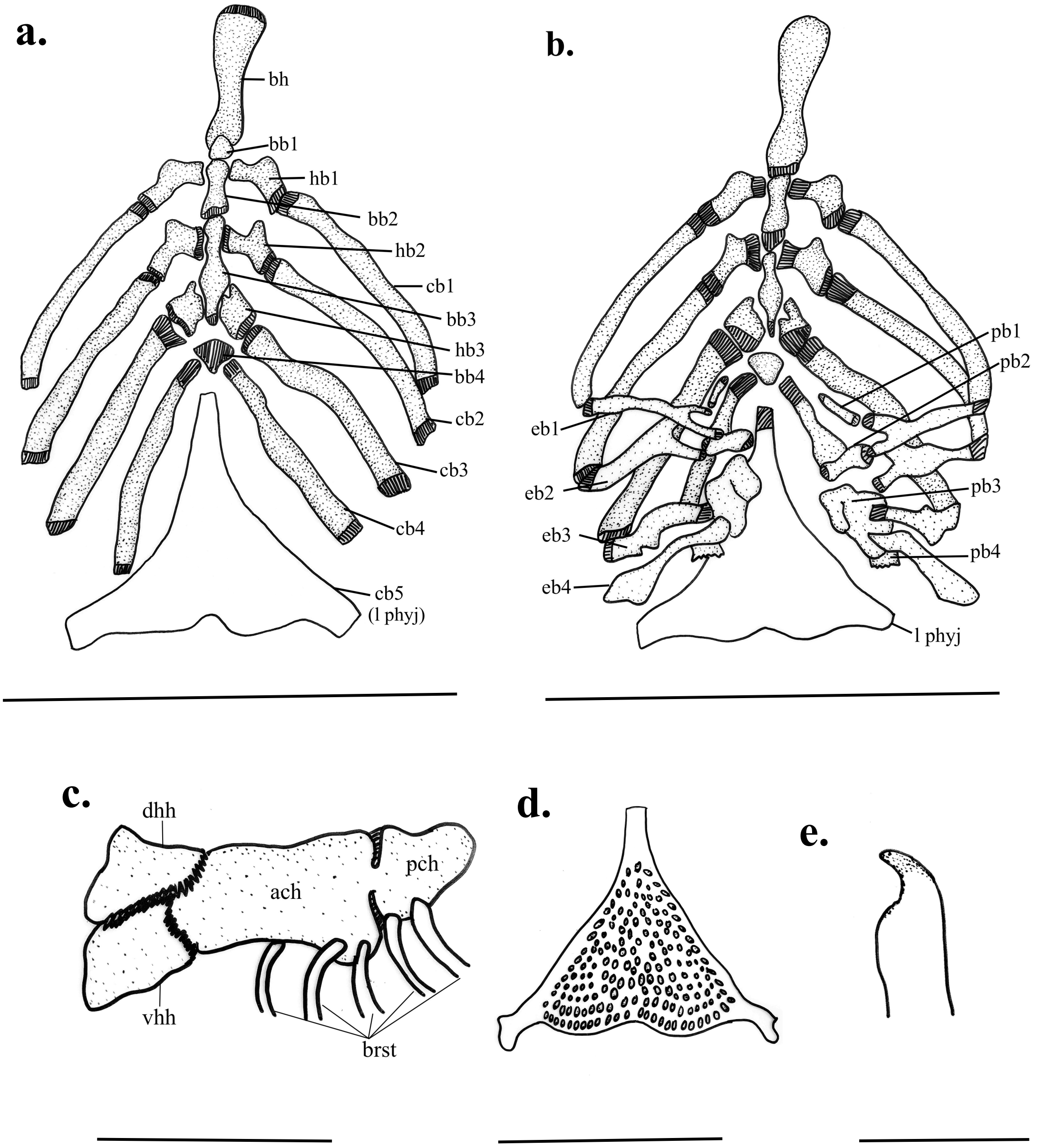

Figure 2.5. Drawings of the branchial arches and pharyngeal region of *Mesonauta festivus,* specimen number 2, SL = 38.18mm, (a) Branchial apparatus in ventral view (b) Branchial apparatus in dorsal view (c) Hyoid arch in lateral view (d) Lower pharyngeal toothplate (e) teeth in lateral view. Hatching represents cartilage. Scale bar for a = 5 mm, for b = 5 mm, for c,d,e = 2 mm.

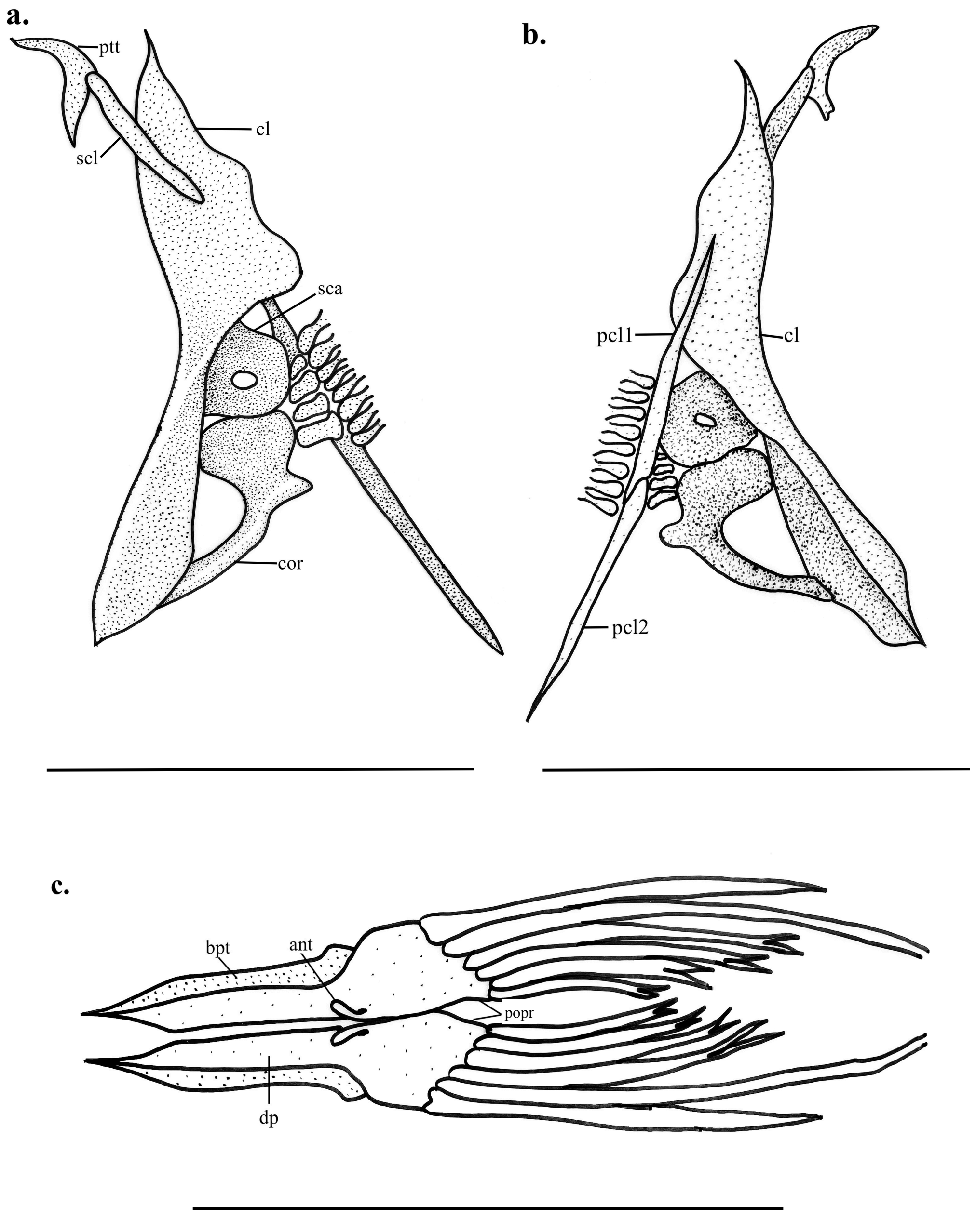

Figure 2.6. Drawing of (a) the left pectoral girdle in lateral view, (b) the left pectoral girdle in medial view, (c) the pelvic girdle in the ventral view of *Mesonauta festivus*, specimen number 7, SL = 37.96mm. Scale bar for a,b,c = 5 mm.

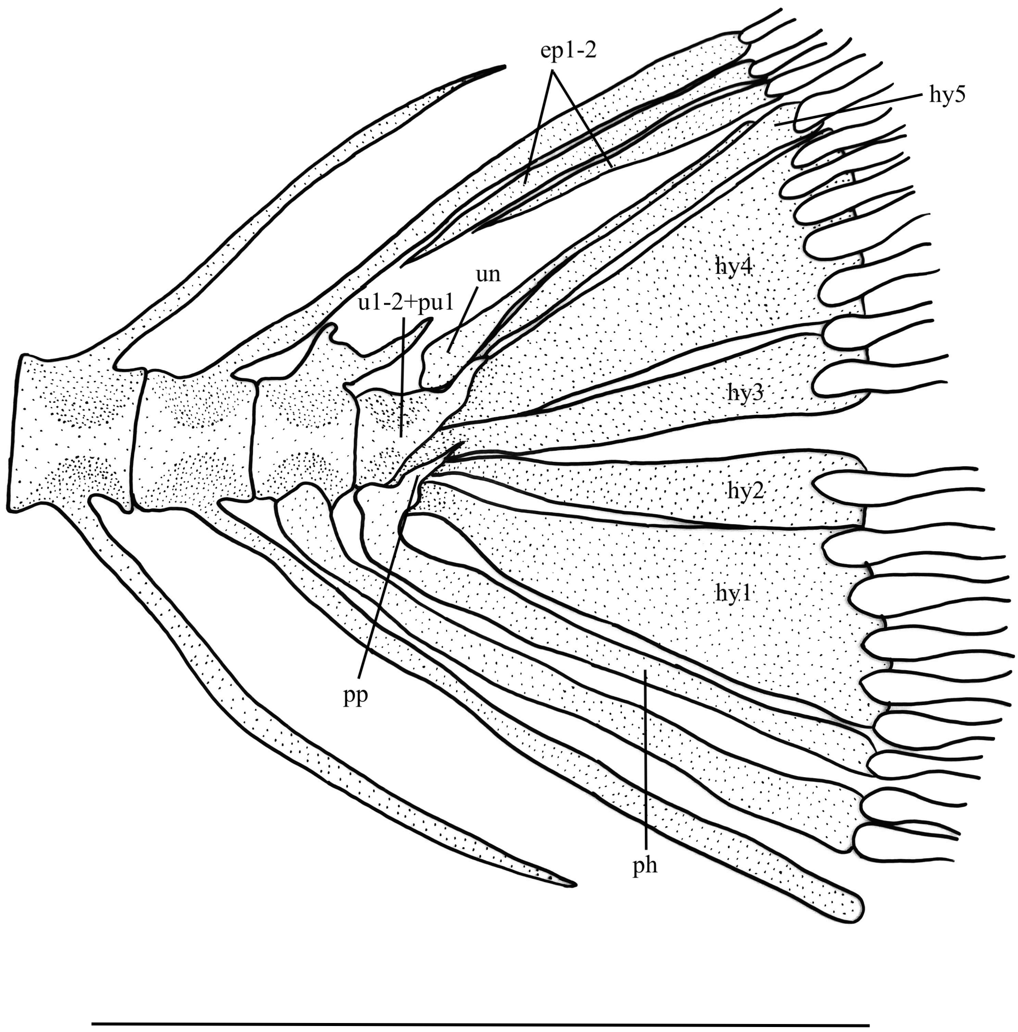

Figure 2.7. Drawing of the caudal fin of *Mesonauta festivus,* specimen number 7, SL = 37.96mm. Scale bar = 5 mm.

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# Chapter 3: Morphological and Osteological variation among a developmental series of the *Iranocichla hormuzensis* (Perciformes: Cichlidae)

## 3.1 Introduction

Behnke (1975) was the first to report the existence of an endemic cichlid species in Southern Iran. A brief description was provided by Saadati (1977) in his MS thesis based on the nine specimens collected by Behnke (1975). These Iranian cichlids were first described as a new genus and species, *Iranocichla hormuzensis* by Coad (1982) based on the 221 specimens that he collected from the Mehran River drainage. This was the only species of known in the genus *Irancichla* until recently when Esmaeili et al. (2016) described a second species as *Iranocichla persa* from Shur, Hasanlangi and Minab river drainages in the eastern part of the Iran, and a third morph that remains unnamed, *Iranoncichla* sp., was reported from the Kol River drainage by Schwarzer et al. (2016). Coad (1982) suggested that *I. hormuzensis* is closely related to the genus *Tristramella* which is endemic to the Levant Rift valley and waters around Damascus based on several characters shared between these genera. These characters include a *Tilapia*-type pharyngeal apophysis; the inferior apophyses for support of the anterior end of the swim bladder centering on the fourth vertebra; and the posterior scale circuli being granular, such that the exposed scale surface has rows of rounded or irregular protuberances (Coad 1982).

### 3.1.1 Objective

The main purpose of this chapter is to describe the osteology of *Iranocichla hormuzensis* focusing on how it changes over a developmental series. This study will allow me to assess the amount of variation that occurs with growth in a single species, and provide a baseline that can be used to assess whether a sample of fossil specimens of varying size might be expected to belong to one or more species.

## 3.2 Materials and Methods

### 3.2.1 Materials

The specimens of *Iranocichla hormuzensis* are from the Icthyology collection of the Canadian Museum of Nature (formerly the National Museum of Natural Science), Ottawa, Canada: catalogue numbers CMN 79-0142, 79-0140, and are specimens that were fixed in formaldehyde and permanently stored in ethanol. Specimens were collected by B.W. Coad from

Baghu River at road bridge (27$^0$17’N, 56$^0$28’E) and Kul River drainage (27$^0$14’N, 55$^0$46’E), Iran in 1976. I examined a total of 107 individuals including 94 specimens used for measurements and meristics and 13 previously stained specimens used for osteological studies. I confirmed the identification of each specimen for this project based on the tilapia mark on the dorsal fin (Coad, 1982), and the caudal skeleton being as described by Dastanpoor et al. (2021).

### 3.2.2 Measurements

Measurements and counts for specimens were collected as described by Daget (1954) and Paugy (1986). I used ten meristic counts and nineteen measurements in order to capture morphological variability. The data are summarized in Tables 3.1 and 3.2. Vertebral counts were made for all specimens with the aid of CT scans using a Bruker micro CT Skyscan 1172 (no filter, 50 kV, and 83µA, 4w). Measurements were made using digital calipers (0-150 mm Electronic Digital Caliper) and fin, gill raker and scale counts were made using a Zeiss SteREO Discovery.V8 stereomicroscope using a Carl Zeiss 44403 6-9000 eyepiece (8x) and a Zeiss Achromat S 0.3x FWD 236 mm lens. Measurements were made using a Nikon SMZ1000 stereomicroscope using a WD 123 Nikon Plan Apo 0.5x lens and a C-W 10xB/22 eyepiece reticle. A summary of all measurements and meristics is available in Appendix A.

I assessed 13 specimens that were previously cleared and double stained for cartilage (alcian blue stain) and bone (alizarin red S stain). Comparative photographs for osteological studies were taken with a Nikon DCM1200C digital camera mounted on a Zeiss Stereo Discovery.V8 stereomicroscope with a Carl Zeiss 44403 6-9000 eyepiece (8x) and a variety of lenses (Zeiss Achromat S 0.3x FWD 236 mm, Zeiss Plan Apo S 0.63x FWD 81 mm, and Zeiss Plan Apo S 1.0x FWD 60 mm), with NIS-Elements F package 2.20, version 5.03. I processed the figures using Adobe Photoshop CS6.

I completed all statistical analyses using R version 4.1.1 (R Core Team, 2017). To test the distribution of the studied specimens, I calculated the skewness, kurtosis and Shapiro-Wilk test value using the package e1071 (Meyer *et al.,* 2019). Histograms and scatterplots were completed for all measurements and meristics (Appendix B). To determine whether *Iranocichla hormuzensis* exhibits allometric or isometric growth, all measurements were changed to the logarithmic form and the slopes were calculated using MS Excel (version 14.0.0).

## 3.3 Results

### 3.3.1 General Body Form and External Characteristics

Size: The largest specimen in this study is, 59.79 mm SL (Table 3.1). According to Esmaeili et al. (2016), the maximum standard length for the *Iranocichla hormuzensis* adults can reach up to 100 mm. The standard length ranges from 20.17-59.79 mm for this study which means it includes both juveniles and adults. Measurements and meristics are summarized in Tables 3.1 and 3.2, respectively.

Body shape and fins: *Iranocichla hormuzensis* has a fusiform body with a terminal mouth, with wide nape and thin posterior trunk (Figure 3.1). The snout profile is convex in shape and the maxilla extends posteriorly to the nostril. The body depth is approximately a third of the standard length with the greatest depth just before the dorsal fin insertion (23%-40%, mean=31%; Table 3.1). The dorsal fin insertion is slightly anterior (22%-61%, mean= 38% SL) to the level of the pelvic fin origin (26%-64%, mean= 40%). The pelvic fin insertion is just posterior to the pectoral fin insertion and posteroventral to the operculum. The anal fin insertion is posterior on the body (49-121%, mean= 73% SL). The dorsal fin base (40-70% SL, mean=53% SL) is larger than the anal fin base (9-16% SL, mean=12% SL). The pectoral and pelvic fins are short. The caudal fin is truncate in shape.

Scales: Cycloid scales or scales with very small ctenus-like structure cover the body (Figure 3.1). In some larger specimens the regular scale rows are interspersed with irregularly distributed smaller scales. Scales are absent from the head, operculum, from the dorsal and anal fin bases, absent between the pectoral and pelvic fin bases, on the belly and isthmus anterior to the pelvic fin. The upper row of the lateral line has scales ranges from 17-21 whereas it ranges from 9-12 in the lower row.

Colour pattern: Alcohol-preserved specimens are pale brown in colour. All the specimens (including juveniles and adults) have a distinct tilapia-mark (black spot) on the soft rays of the dorsal fin along with a second spot on some of the specimens. Juvenile specimens have 7-11 brown vertical bars on the body which are also present in the adults but less distinct. There are irregular brown blotches on the dorsal and anal fins in adults but these are absent in the juvenile specimens. The pectoral and pelvic fin do not have any pigmentation and are lightly coloured.

The measurements that were standardized by SL demonstrated a normal distribution for examined specimens (Appendix B). All length and depth measurements are positively correlated with a slope of approximately 1.0 (Figure 3.3, Appendix B). There were no strong correlations (with strong defined as $r > 0.70$) found in relation to standard length and count data (Appendix B).

### 3.3.2 Skull

The nasals are tubular in shape anteriorly and broaden into a triangle with a flat base towards the posterior end (Figure 3.4a). The nasal bears a sensory canal. The lateral ethmoid has a heart shape in ventral view. The frontals broaden posteriorly. The parietal is semi-circular in shape. The sensory canal passes from the frontals and continues onto the parietals. The sphenoid attaches to the lateral edge of the parietal. It has a lateral projection and is tubular in shape. The pterotic, positioned posterior to the sphenoid, has an almost rhomboid shape. The posttemporal is connected to the pterotic. The epiotic is almost rectangular in shape and sits between the supraoccipital crest and pterotic.

The vomer is conical with an elongated posterior end which joins with the parasphenoid (Figure 3.4b). The prootic is almost square and located at the posterolateral end of the parashenoid. Each prootic bears a foramen anteriorly. The orbitosphenoid has a concave outline in ventral view. From the ventral side, the exoccipital is triangular in shape and laterally attached to the basioccipital.

### 3.3.3 Jaws and Teeth

A pointed, sharp ascending process is present on the premaxilla. A mid-lateral ascending process and a posterior descending process are present on the maxilla (Figure 3.4). The maxilla bears a foramen in the middle of the ascending process; this process attaches to the ascending process of the premaxilla. The dentary contains two limbs joining to make a V-shape (Figure 3.5). The anterior shaft of the anguloarticular bone fits between the two limbs of the dentary. The premaxilla and dentary is quite similar to the south American cichlid *Mesonauta festivus* which was discussed in the previous chapter (Chapter 2).

The premaxilla and dentary have multiple rows of unicuspid, bicuspid and tricuspid laterally compressed teeth. The teeth are arranged irregularly. The teeth decrease substantially in size posteriorly and increase in size toward the symphysis.

The pharyngeal toothplate is in cordiform shape (Figure 3.6b). The teeth are cylindrical unicuspid, or subconical unicuspid in shape.

### 3.3.4 Opercular Series

The vertical limb of the preopercle forms a $90^0$ angle with the horizontal limb of the preopercle (Figure 3.7). There are three sensory canal openings on both of the limbs of the preopercle. The interopercle fits at the bottom of the preopercle and curves along the posteroventral edge of the preopercle. The opercle is the largest element of the opercular series. It is conical in shape with sharp edges. The subopercle has a triangular, sharp ascending process which is situated between the posteroventral part of the preopercle and the anteroventral part of the opercle bone. The subopercle also contacts the posterior base of the interopercle.

### 3.3.5 Suspensorium

The palatine is small, elongated anteriorly, with a broad posterior end (Figure 3.5). The ectopterygoid is a small, slightly curved, bony element with a broad anterior edge. It is attached to the palatine ventrally and the quadrate laterally. The endopterygoid is rectangular in shape and a relatively small bone. The quadrate has two limbs. The anterodorsal limb is fan-shaped and fused with the narrower posteroventral to form the space for the elongated sympletic. The hyomandibula is broad dorsally, elongated ventrally and articulates along the posterior edge of the dorsal part of the preopercle.

### 3.3.6 Infraorbital Bones

The lacrimal is the largest element and is approximately square in shape with three rounded edges and a pointed anterodorsal process (Figure 3.5). It bears three neuromasts. It is followed by 2-6 thin, elongated, small infraorbitals. The circumorbital sensory canal passes through all the infraorbital bones.

### 3.3.7 Branchial Arches and Pharyngeal Region

The first basibranchial is smallest in size. The second and third are narrow and rod-like in shape. The third one is the largest of all. There are three pairs of hypobranchials; the third pair is positioned near the third basibranchial. There are four pairs of epibranchials and four pairs of ceratobranchials which are long slender rods. The fifth ceratobranchial is modified into the lower pharyngeal jaw. Toothplates cover the third and fourth pair of pharyngobranchials.

There are two hypohyals (the dorsal and ventral hypohyals) which are attached to the anterior part of the ceratohyal. The ceratohyal is elongated anteriorly with a broad posterior end that is connected with the epihyal. The epihyal is almost conical in shape. Five branchiostegal rays are present on each side, three of them are attached to the ceratohyal and two are positioned with the epihyal. They are narrow, long and curved with increasing size from the first towards the fifth.

### 3.3.8 Paired Fins and Girdles

The cleithrum is the largest bone of the pectoral girdle (Figure 3.9). It has a broad dorsal head with a pointed dorsal tip. The coracoid is curved with two sharp projections forming a large foramen between itself and the cleithrum. The scapula is almost square with pointed edges, has a foramen in the centre, and provides a base for the radials. The two postcleithra are linked to each other and are situated posterior to the scapula. The postcleithra are sharp, elongated and thin. The supracleithrum is elongated and located posterodorsally on the cleithrum, and attaches dorsally to the semicircular base of the posttemporal . The pectoral fin is short and wing shaped with 11-12 rays.

The distal process of the pelvic girdle is narrow anteriorly and widens posteriorly where the fin rays attach. The pelvic girdle is situated horizontally in the thoracic area and attached to the pectoral girdle by muscles and ligaments (Figure 3.10). The anteroventral processes are small, thin and quite bulbous at the anterior edge. The pelvic fins articulate on the posterior processes. The pelvic fin is short and has one spine and five fin rays. The first ray next to the spine is the longest and rays decrease in the size progressively.

### 3.3.9 Vertebral Column and Predorsal Bones

The vertebral column consists of 27-30 centra, of which 13-15 are abdominal and 13-15 are caudal. Parapophyses are located on the posterolateral part of the third and fourth vertebrae (Figure 3.8). There is one predorsal bone in front of the first dorsal pterygiophore; it is positioned just behind the supraoccipital crest and before the first neural spine. The first pterygiophore is located between the first and second neural spines.

### 3.3.10 Dorsal and Anal Fins and Supports

The dorsal fin has XIV-XVI spines and 9-11 fin rays. The spines increase in size progressively, and the rays increase in size until the midpoint then decrease in size posteriorly; therefore, the fin is semi-circular or posteriorly rounded. The dorsal fin has 23-26 pterygiophores to support the fin. The first pterygiophore inserts between the first and second neural spines.

The anal fin has three spines and 5-6 fin rays with 8-9 anal pterygiophores. The first pterygiophore is largest in size. Anal fin spines increase in length progressively and rays vary in size in a pattern similar to the dorsal fin; it has a posteriorly rounded shape.

### 3.3.11 Caudal Fin and Skeleton

The caudal fin is small and truncate (Figure 3.11). A urostyle is present, and has a broad anterior and elongated pointed distal end. The uroneural is thin and bent ventrally along the urostyle. The neural spine on preural centrum 2 is doubled, with the spine varying in length among the specimens and fully developed haemal spine with a thick anterior region, whereas preural centrum 3 has a fully developed neural spine which varies in length among the specimens as compared to the neural spine on preural centrum 2 and a fully developed haemal spine with a broad base, and pointed anterior process.

There are five hypurals. In some specimens, hypural 1 and 2 or 3 and 4 are fused. The first and fourth hypurals are the largest, of similar proportions and triangular in shape. There is a space (= diastema) between the second and third hypural. The fourth hypural fits firmly into the urostyle and the fifth hypural is the smallest of all. The parahypural has an anterior flange at the base which projects posteriorly over the hypural called a hypuraphophysis. There are two long and thin epurals; these vary in size among the specimens.

## 3.4 Discussion

The size series of *Iranocichla hormuzensis* covers a range of 20.17-59.79 mm SL (25.91-72.35mm TL). The measurements recorded in the current study are normally distributed (Table 3.1). All the measurements have a slope of approximately 1.0 which suggests that *I. hormuzensis* has isometric growth. There is variability in the ossification pattern between the juvenile and adult specimens.

Previously, Coad (1982) examined specimens of *I. hormuzensis* ranges from 20.2-97.3 mm SL and Esmaeili et al. (2016) analysed a range of 82.3-100.6 mm SL. The comparisons with these studies can be found in Table 3.3.

### 3.4.1 Osteological Changes in a Growth Series

The most prominent change to the skeleton between juveniles and adults is the amount of ossification. The smallest cleared and stained specimen has a mostly cartilaginous skeleton compared to the adult-sized cleared and stained specimen. Some studies on other fishes have found there is a consistent ossification pattern with the anterior portion of the skull, and bones involved with feeding, ossifying first (e.g., *Seriola dumerili* studied by Liu (2001) and *Hippocampus hippocampus* studied by Novelli *et al.* (2017)). There is a possibility that this is the same in *Iranocichla hormuzensis* but further research is needed on this topic. All the bones retained the same shape in juveniles and adult specimens. Dastanpoor et al. (2021) found that majority of the bony elements in *Iranocichla* species (*Iranocichla hormuzensis, Iranocichla persa*, *Iranocichla* sp.) are similar except for some variability in the caudal skeleton. Esmaeili et al. (2016) described *Iranocichla persa* as a separate species from *Iranocichla hormuzensis* based only on the DNA sequence and the body colouration.

### 3.4.2 Conclusion

The isometric growth and the osteological results in the growth series of *Iranocichla hormuzensis* indicate that the juveniles and adults have the same morphology of bones throughout life. This suggest that cichlid fossils of various sizes found in a single locality would be expected to have the some bone morphology if they represent a single species; there would be no changes in osteology expected with growth. Therefore, if fossil specimens of different body size differ in skeletal morphology, it is likely that they represent different species.

### 3.5 Tables

Table 3.1. Measurements and ratios for *Iranocichla hormuzensis* (n=94), CMN 79-0142 and 79-0140, from Baghu River and Kul River drainage, Iran.

| | **Range** | **Mean** | **SD** |
|---|---|---|---|
| Standard lengh (SL mm) | 20.17-59.79 | 32.28 | |
| Total length (TL mm) | 25.91-72.35 | 39.52 | |
| SL:TL | 0.76-0.88 | 0.81 | 0.02 |
| Body depth $SL^{-1}$ | 0.23-0.40 | 0.31 | 0.03 |
| Head length (HL mm) $SL^{-1}$ | 0.13-0.46 | 0.37 | 0.03 |
| Interorbital distance H $L^{-1}$ | 0.23-1.02 | 0.32 | 0.07 |
| Eye socket diameter $HL^{-1}$ | 0.19-0.91 | 0.27 | 0.07 |
| Snout length $HL^{-1}$ | 0.30-1.20 | 0.38 | 0.09 |
| Caudal peduncle depth:Caudal peduncle length | 0.53-1.05 | 0.77 | 0.10 |
| Length of upper jaw $HL^{-1}$ | 0.13-0.55 | 0.23 | 0.05 |
| Predorsal length $SL^{-1}$ | 0.22-0.61 | 0.38 | 0.04 |
| Preanal length $SL^{-1}$ | 0.45-1.21 | 0.73 | 0.07 |
| Prepelvic length $SL^{-1}$ | 0.26-0.64 | 0.40 | 0.04 |
| Prepectoral length $SL^{-1}$ | 0.25-0.60 | 0.39 | 0.03 |
| Dorsal fin base $SL^{-1}$ | 0.40-0.75 | 0.53 | 0.05 |
| Anal fin base $SL^{-1}$ | 0.09-0.16 | 0.12 | 0.01 |
| Dorsal fin length $SL^{-1}$ | 0.41-0.77 | 0.58 | 0.05 |
| Anal fin length $SL^{-1}$ | 0.12-0.24 | 0.19 | 0.01 |
| Pectoral fin length $SL^{-1}$ | 0.18-0.53 | 0.26 | 0.04 |

Table 3.2. Meristics for *Iranocichla hormuzensis* (n=94), CMN 79-0142 and 79-0140, from Baghu River and Kul River drainage, Iran.The number of specimens that showed each count is in brackets.

| **Number of** | **Sample Size** | | | | | |
|---|---|---|---|---|---|---|
| Dorsal fin rays | 94 | 9 (66) | 10 (13) | 11(15) | | |
| Anal fin rays | 94 | 5 (81) | 6(13) | | | |
| Pectoral fin rays | 94 | 11(16) | 12(78) | | | |
| Abdominal vertebrae | 94 | 13(7) | 14(23) | 15(64) | | |
| Caudal vertebrae | 94 | 13(18) | 14(63) | 15(13) | | |
| Total vertebrae | 94 | 27(8) | 28 (28) | 29(55) | 30(3) | |
| Lateral line: | | | | | | |
| -Scales in upper row | 94 | 17(18) | 18(8) | 19(18) | 20(8) | 21(42) |
| -Scales in lower row | 94 | 9(6) | 10(18) | 11(32) | 12(38) | |
| Gill rakers | 94 | 14(39) | 15(51) | 16(4) | | |
| Dorsal pterygiophores | 94 | 23(5) | 24(85) | 25 (3) | 26(1) | |
| Anal pterygiophores | 94 | 8(80) | 9(14) | | | |

Table 3.3. Comparison of meristics from this study to Coad (1982) and Esmaeili et al. (2016) study.

| **Number of** | **Current Study** | **Coad 1982** | **Esmaeili et al. 2016** |
|---|---|---|---|
| Gill Rakers | 14-16 | 14-19 | 14-17 |
| Lateral line scales | | | |
| -Upper row | 17-21 | 17-29 | 16-21 |
| -Lower row | 9-12 | 9-12 | 9-14 |
| Dorsal fin rays | 9-11 | 9-11 | 9-11 |
| Anal fin rays | 5-6 | 6-9 | 5-7 |
| Pectoral fin rays | 11-12 | 11-12 | 12 |
| Total Vertebrae | 27-30 | 28-30 | - |
| Abdominal Vertebrae | 13-15 | 14-16 | - |
| Caudal Vertebrae | 13-15 | 13-15 | - |

### 3.6 Figures

Figure 3.1. Photographs of *Iranocichla hormuzensis*, CMN 79-0142 and 79-0140, (a) smallest specimen = specimen number 93, SL = 20.17 mm, (b) largest specimen = specimen number 25, SL = 59.79 mm. Scale bar = 10 mm.

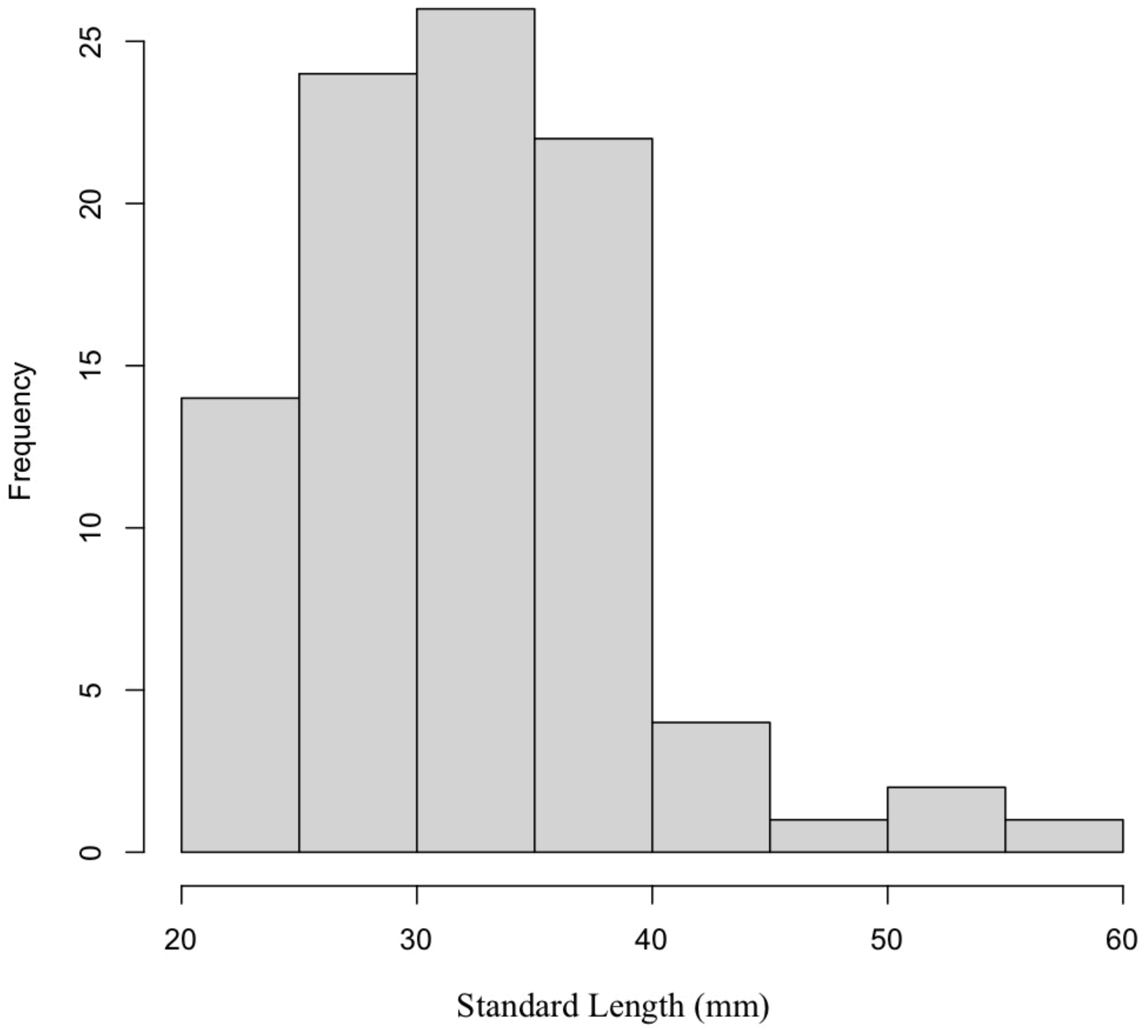

Figure 3.2. Histogram of standard length for *Iranocichla hormuzensis*, CMN 79-0142 and 79-0140. Histograms for remaining measurements are available in Appendix B.

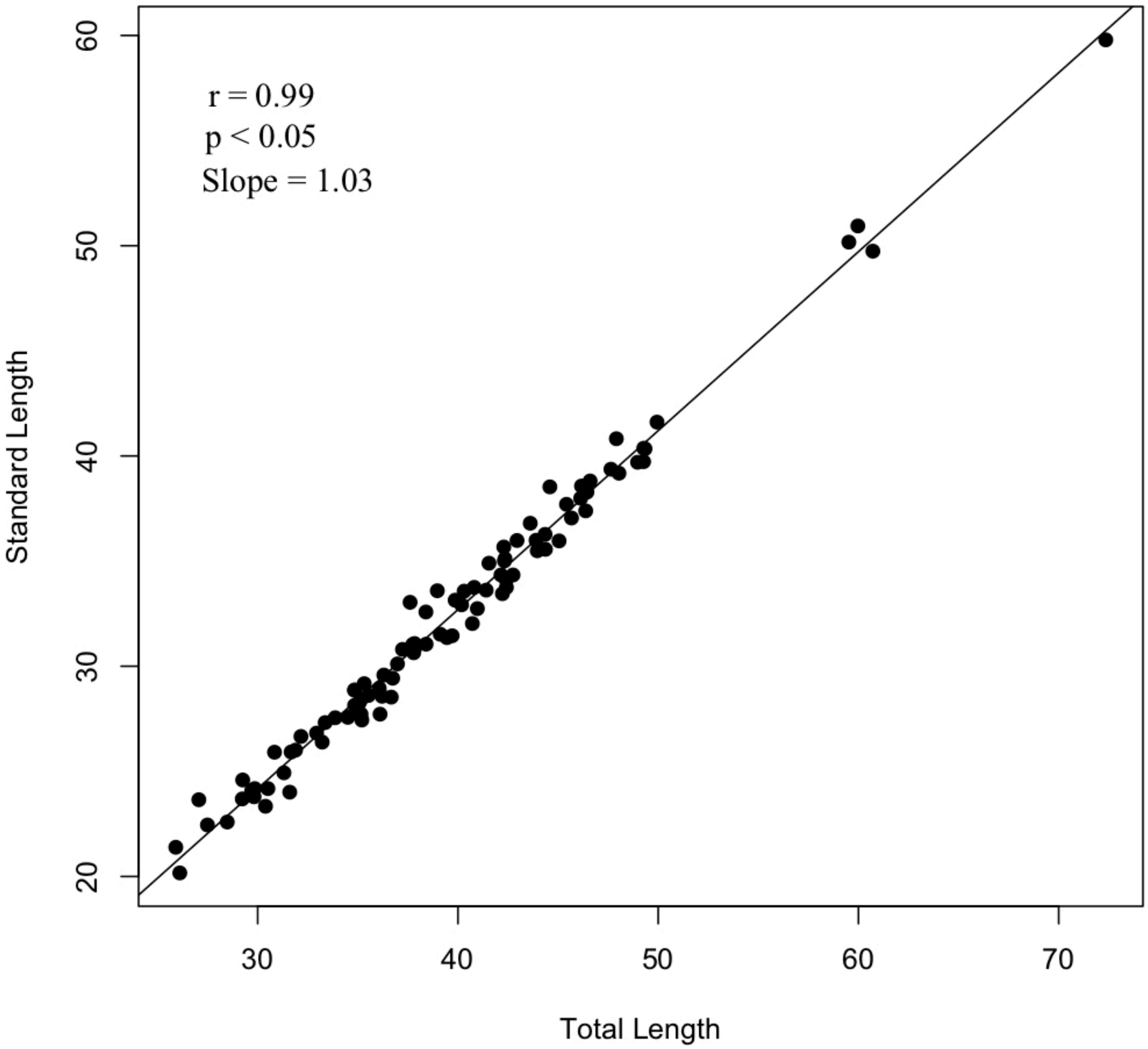

Figure 3.3. Scatterplot of standard length in relation to total length for *Iranocichla hormuzensis*, CMN 79-0142 and 79-0140. Pearson correlation and slope values are in the respective top left corners. Scatterplots for remaining measurements are available in Appendix B.

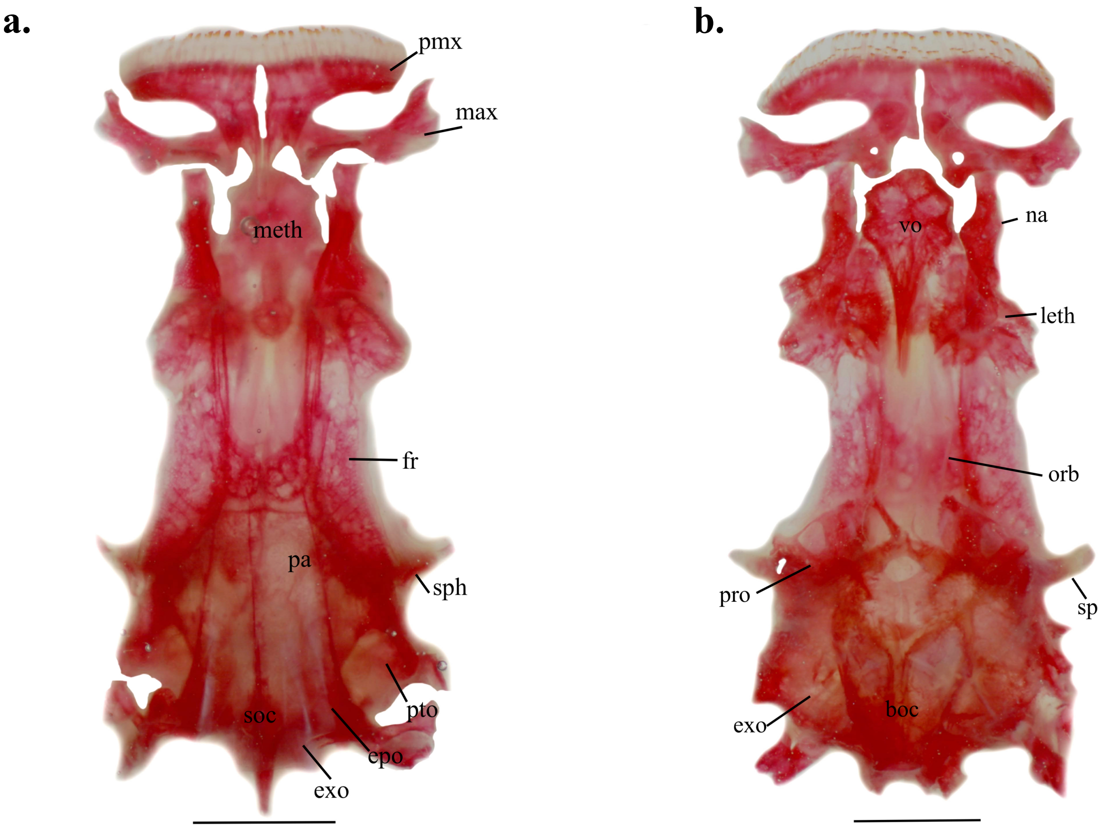

Figure 3.4. Photograph of the skull of *Iranocichla hormuzensis*, a. dorsal, b. ventral (parasphenoid was broken). Scale bar = 5 mm.

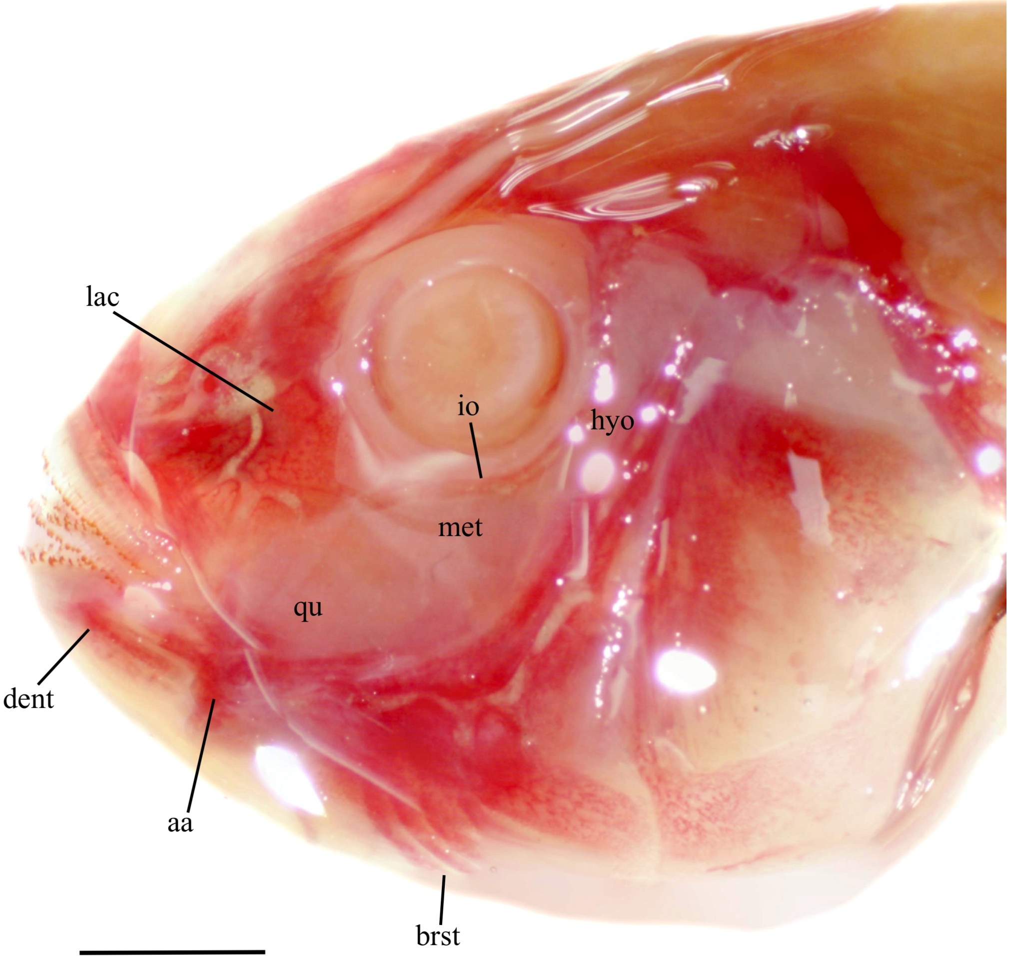

Figure 3.5. Photograph of the dentary, infraorbitals and suspensorium of *Iranocichla hormuzensis*. Scale bar = 5 mm.

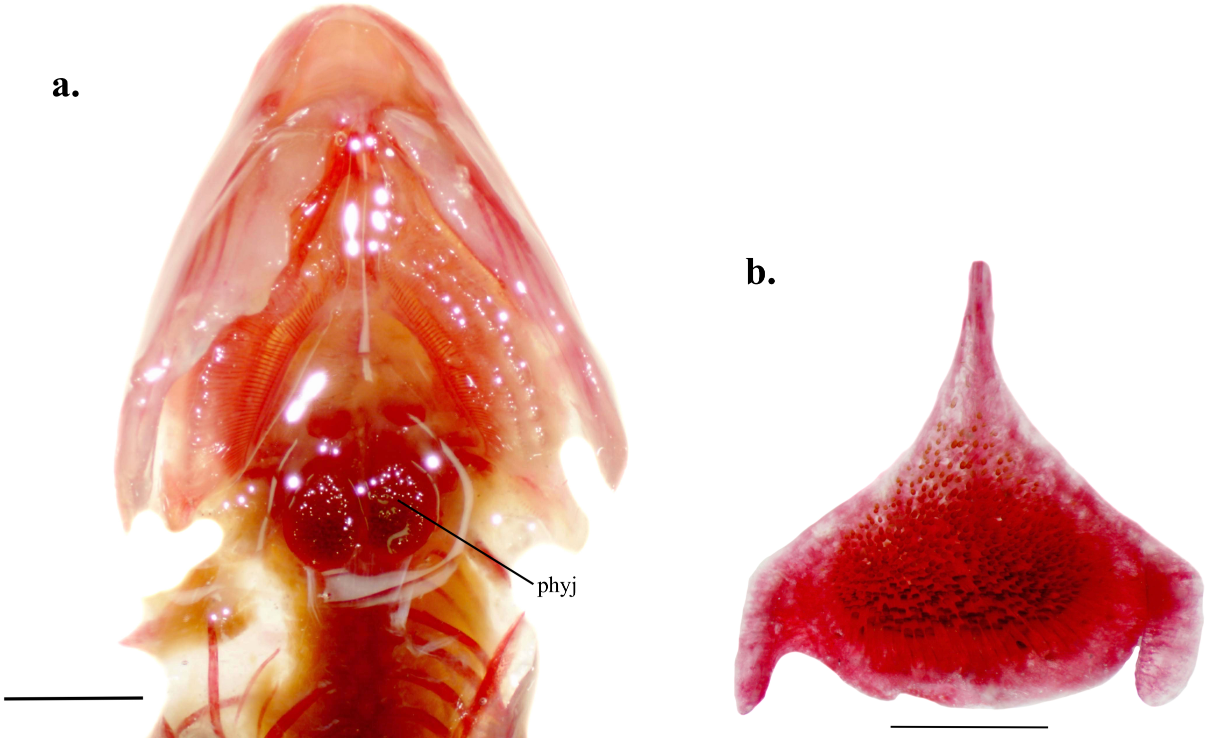

Figure 3.6. (a) Photograph of the branchial arches and pharyngeal region of *Iranocichla hormuzensis*, scale bar= 5mm, (b) Lower pharyngeal toothplate of *Iranocichla hormuzensis*, scale bar= 2 mm.

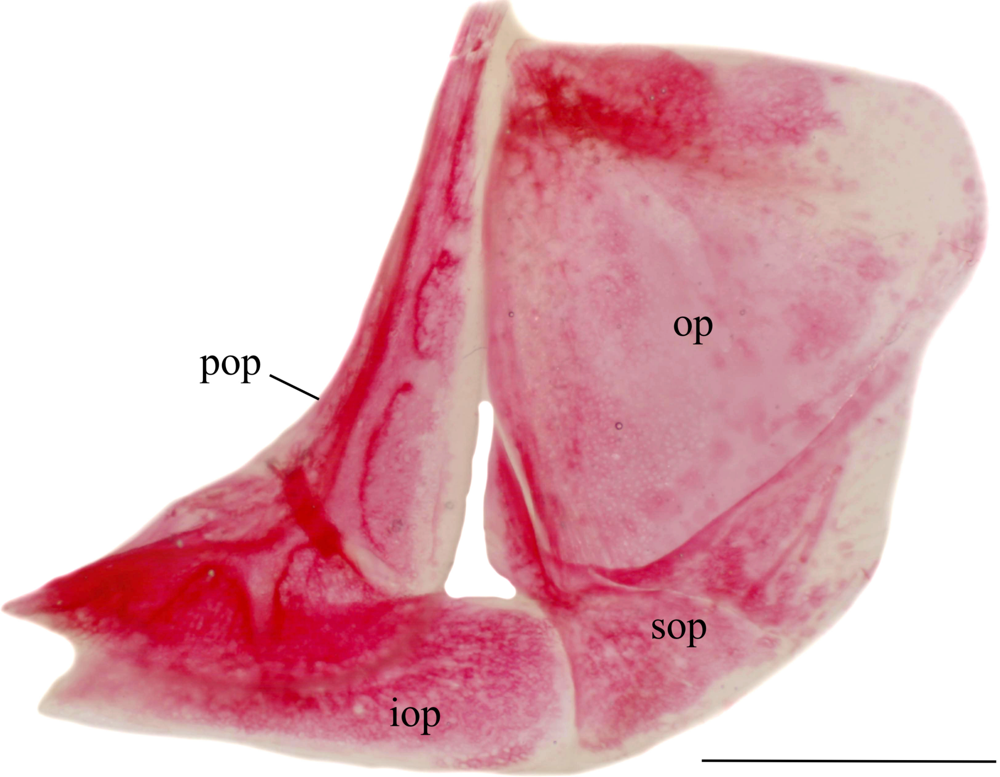

Figure 3.7. Photograph of the opercular series of *Iranocichla hormuzensis*. Scale bar = 5 mm.

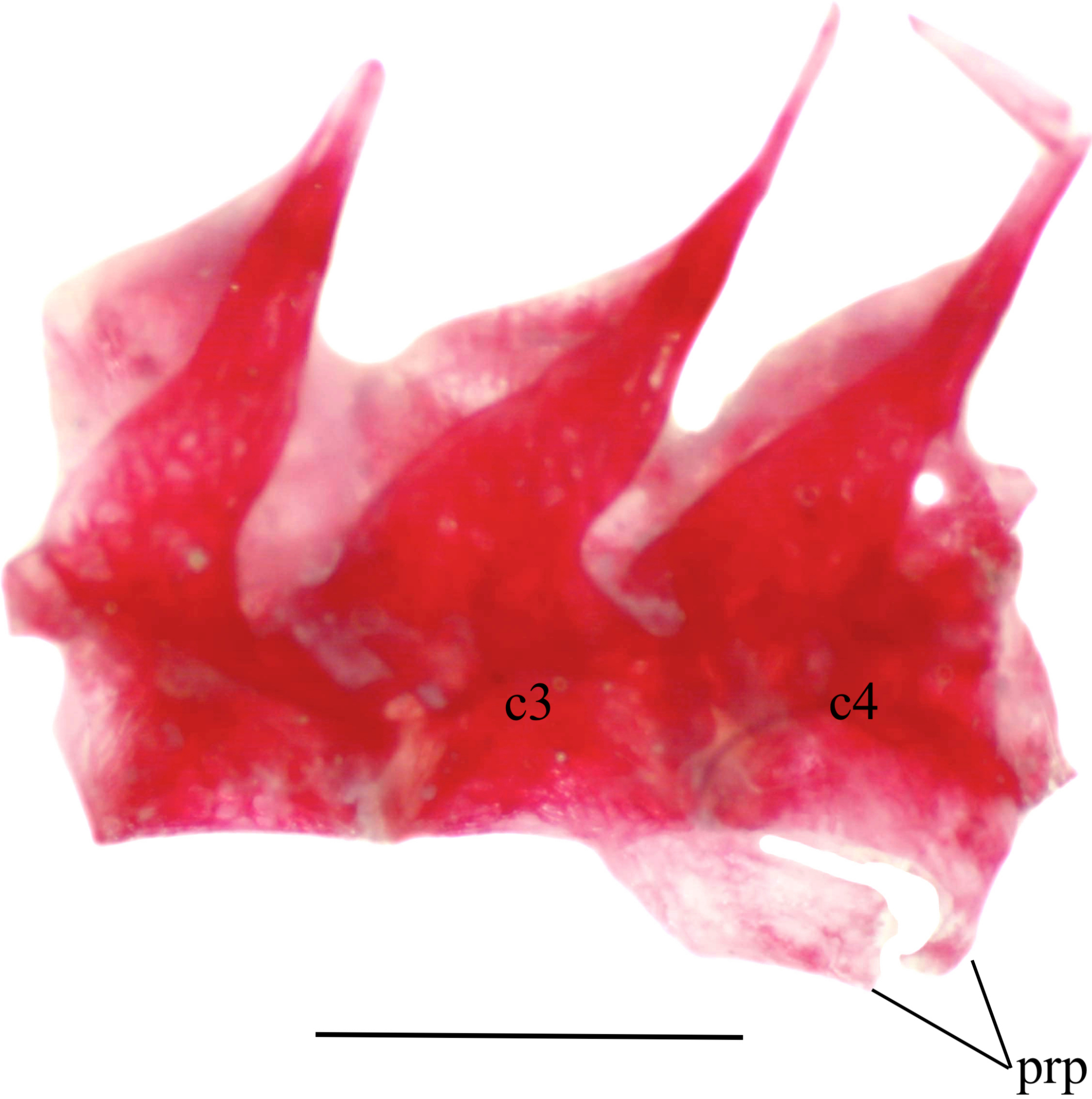

Figure 3.8. Photograph of the lateral view of vertebrae of *Iranocichla hormuzensis*. Scale bar= 5mm.

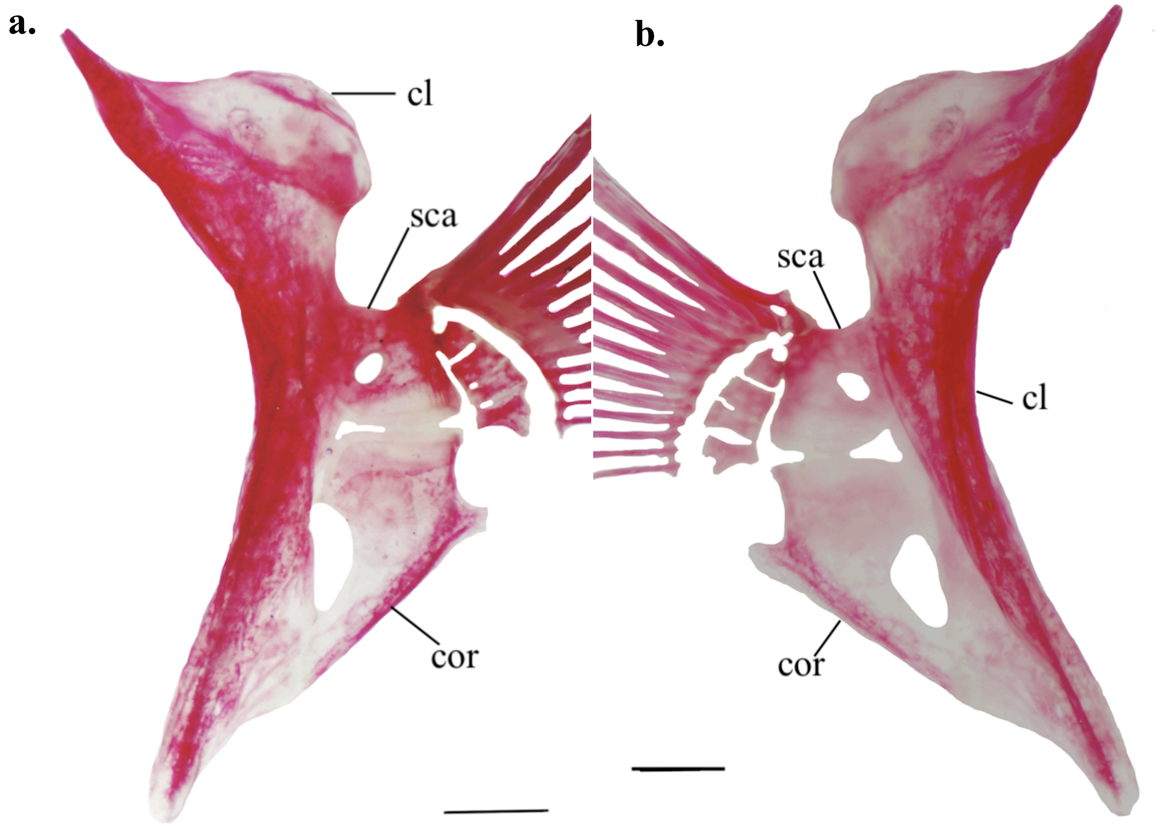

Figure 3.9. Photograph of (a) the left pectoral girdle in lateral view, (b) the left pectoral girdle in medial view of *Iranocichla hormuzensis.* Scale bar= 5mm.

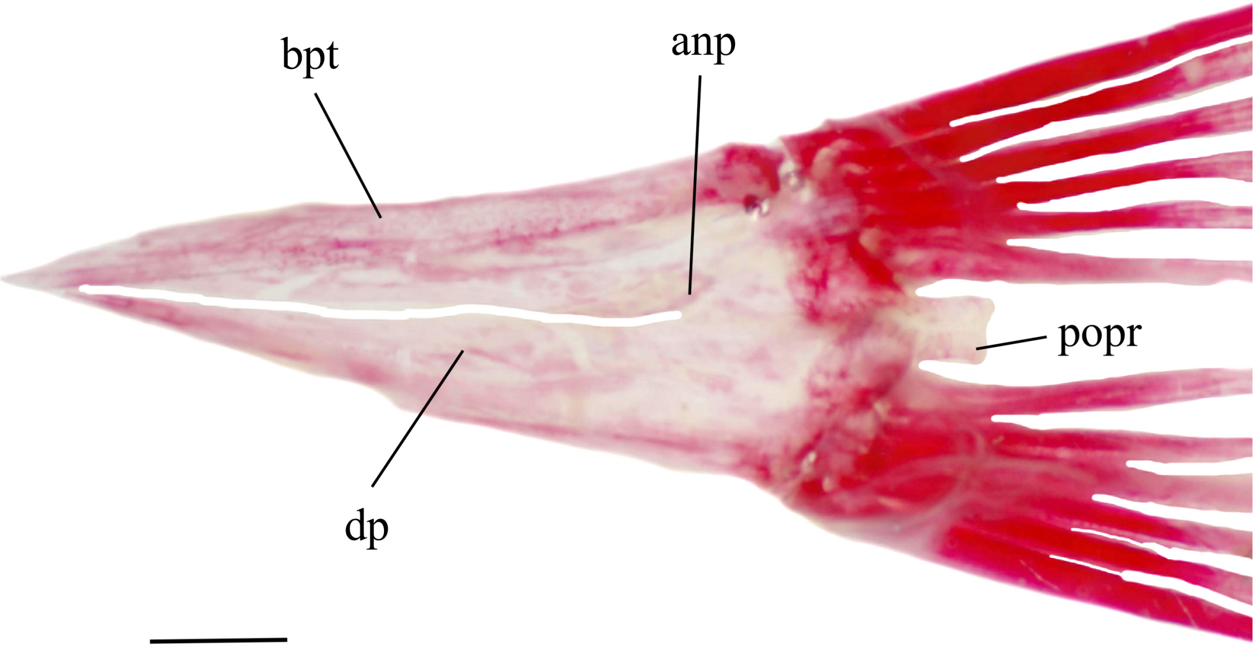

Figure 3.10. Photograph of the pelvic girdle in the ventral view of *Iranocichla hormuzensis*. Scale bar= 5mm.

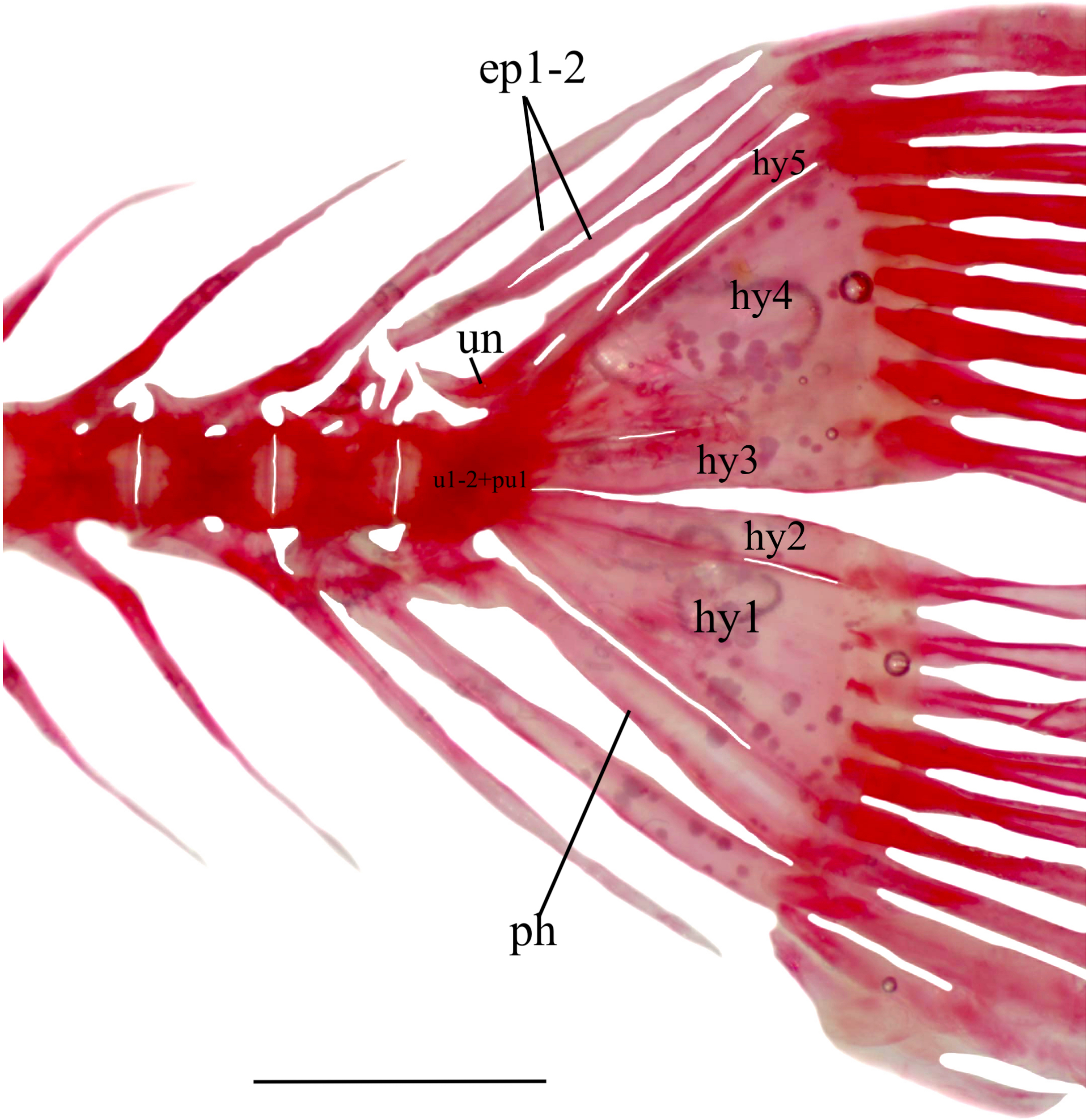

Figure 3.11. Photograph of the caudal fin of *Iranocichla hormuzensis*. Scale bar = 5 mm.

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# Chapter 4: Conclusion

In this thesis I described the osteology of two cichlids: (i) *Mesonauta festivus,* and (ii) *Iranocichla hormuzensis.* There is a lack of detailed osteological descriptions for cichlids in the literature. The comprehensive osteological studies presented here provide  the foundation for future comparisons to other species of cichlids, and to identify fossils.

To put cichlid diversification in a temporal context, molecular researchers need correctly identified and reliably dated fossils. The main problem with building calibrated time trees is the scarcity of confidently, precisely identified cichlid fossils. My first objective was to assess the intraspecific variation in a single population of South American cichlid *Mesonauta fesitvus*. A thorough morphological and osteological study was conducted to determine how much variation can be expected in a single population of a species; this can then be used to assess whether the amount of variation among fossil specimens would indicate that the fossils are likely from a single population or not. The second objective was to describe the osteology of the *Iranocichla hormuzensis* and document how it changes over a developmental series. This study allowed me to assess the amount of variation that occurs with growth in a single species, and provided a baseline that can be used to assess whether a sample of fossil specimens of varying size might belong to one or more species.

## 4.1 Summary of Findings

The second chapter of my thesis assesses the intraspecific variation within a sample (n=22) of a single population of *Mesonauta festivus.* The morphological studies including measurements and meristics for specimens are found to be normally distributed with the standard deviation ranges from 0.01-0.07. The measurements and counts reported in the current study were comparable with a previous study by Kullander and Silfvergrip (1991). The osteology of *Mesonauta festivus* has been described for the first time in the current study. Using five cleared and stained specimens and CT scans of the rest of the specimens, all the bones examined were found to be similar. The ability of *Mesonauta festivus*, being a generalist and having the ability to reproduce throughout the year (Pires et al. 2015), and the dispersion ability of this species (Malcom 2011) may have important contributions to the fact that there is not any significant intraspecific variation among the individuals in this population of *Mesonauta festivus.*

The third chapter of my thesis reported the morphological analyses and osteological descriptions for a size series of *Iranocichla hormuzensis* (n=107). The standard deviation ranges from 0.01-0.09 among the measurements of the specimens. All the depth and length measurements are positively correlated with a slope of approximately 1.0 which explains isometric growth among the *Iranocichla hormuzensis* specimens (Shingelton 2010). The bones were found to be similar among the variable sized specimens with the exception of the amount of cartilage being more in the juvenile samples. Esmaeili et al. (2016) desribed a new species in *Iranocichla* based on DNA sequence analyses and body colouration, and Dastanpoor et al. (2021) reported the osteology to be similar among the three known species, *Iranocichla hormuzensis*, *Iranocichla persa* and *Iranocichla* sp.

The results of both data chapters indicates that there is very little osteological variation in a single species of cichlid, either among a population of similar size, or within a population of variable size. The osteology of the species is conserved. Therefore, if a sample of fossils shows any osteological variation, it is likely that the sample includes more than one different species.

## 4.2 Future Research

There are numerous directions for future research on cichlids that would build on the studies in my thesis. The assessment of a greater range in size of specimens to determine the post-fertilization ossification sequence of *Iranocichla* would allow us to identify the developmental pattern of features with age. Similarly, to better understand the intraspecific variation among a single population of *Mesonauta festivus,* variable sample sizes could be examined. Further, these morphological and osteological analysis can be combined with molecular studies along with the fossils to understand the phylogenetic relationships of cichlids.

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## Appendix A: Guide of Completed Measurements and Counts

Table A.1. Detailed description of collected measurements.

| Measurements | Description |
|---|---|
| Total Length | Distance from tip of snout to the posterior edge of caudal fin |
| Standard Length | Distance from tip of snout to caudal peduncle |
| Head Length | Distance from tip of snout to posterior edge of operculum |
| Length of upper jaw | Distance from the anteriormost point of the premaxilla to the posterior edge of the maxilla |
| Preanal Length | Distance from tip of snout to the level of the first anal-fin spine |
| Predorsal Length | Distance from tip of snout to the level of the first dorsal-fin spine |
| Prepelvic Length | Distance from tip of snout to the level of the pelvic-fin Insertion |
| Prepectoral Length | Distance from tip of snout to the level of the pectoral-fin Insertion |
| Snout Length | Distance from tip of snout to the anterior edge of the orbit |
| Eye Diameter | Distance between anterior and posterior edge of the orbit |
| Interorbital Distance | Distance between orbits |
| Body Depth | Maximum vertical body depth (just anterior to the dorsal fin) |
| Caudal Peduncle Depth | Minimum vertical depth of caudal peduncle |
| Caudal Peduncle Length | Distance between the last anal-fin ray insertion to caudal fin Articulation |
| Pectoral Fin Length | Distance from first pectoral-fin ray to distal end of longest pectoral-fin ray |
| Dorsal Fin Length | Distance from first dorsal-fin ray to distal end of longest dorsal-fin Ray |
| Anal Fin Length | Distance from first anal-fin ray to distal end of longest anal-fin ray |
| Dorsal Fin Base | Distance between first dorsal-fin ray insertion and last dorsal-fin ray insertion |
| Anal Fin Base | Distance between first anal-fin ray insertion and last anal-fin ray Insertion |

Table A.1. Detailed description of collected counts.

| Counts | Description |
|---|---|
| Lateral Line Scales | Number of pored scales on lateral line |
| Gill Rakers | Number of gill rakers on first gill arch |
| Dorsal fin spines | Number of dorsal fin spines |
| Anal fin spines | Number of anal fin spines |
| Anal Fin Rays | Number of anal fin rays |
| Pelvic Fin Rays | Number of pelvic fin rays |
| Dorsal Fin Rays | Number of dorsal fin rays |
| Pectoral Fin Rays | Number of pectoral fin rays |
| Total Vertebrae | Total number of vertebrae |
| Caudal Vertebrae | Number of caudal vertebrae (fully developed haemal arch and spine) |
| Abdominal Vertebrae | Number of abdominal vertebrae (most support pleural ribs) |

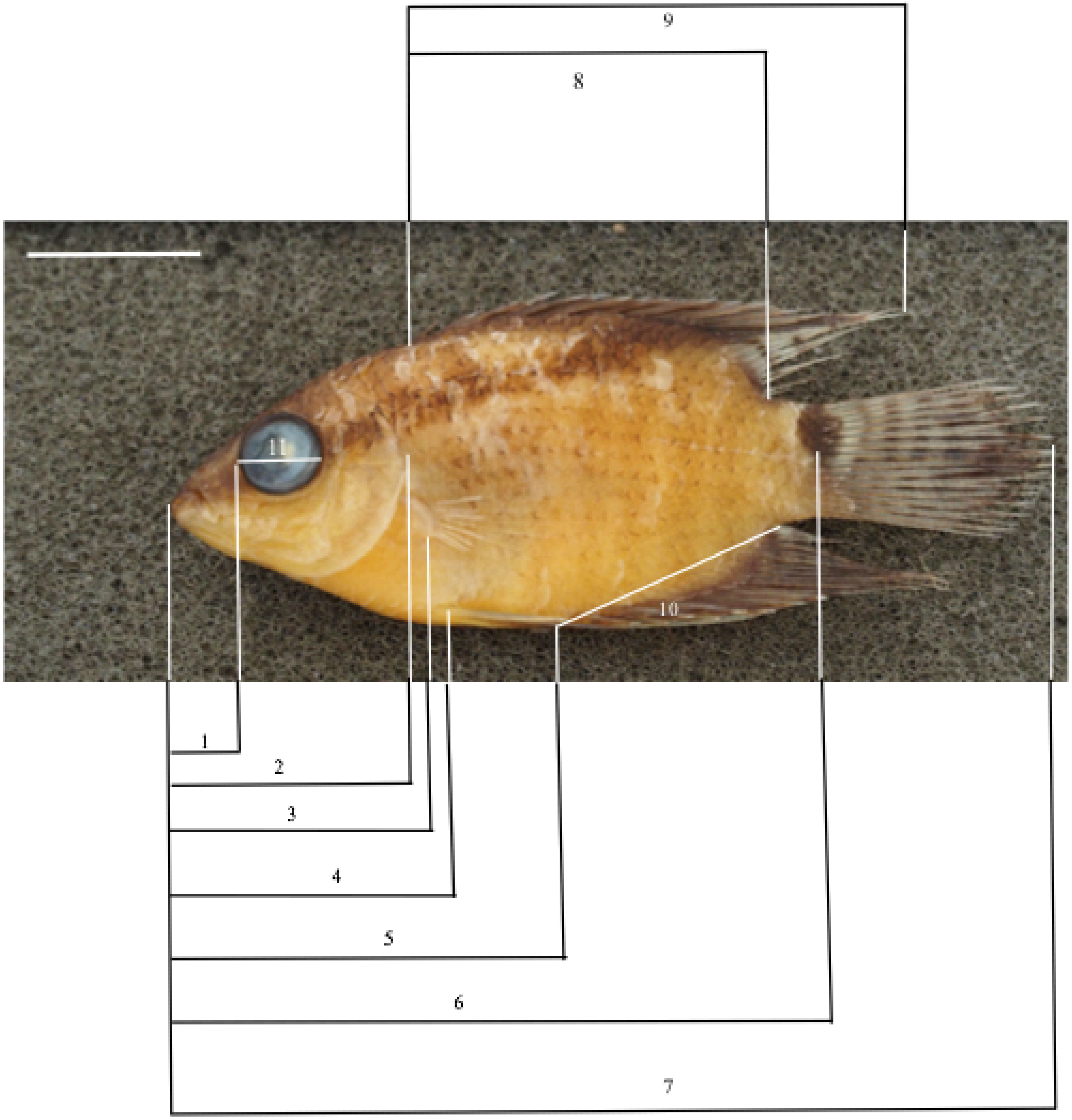


Figure A.1. Schematic of select measurements taken on specimens for this thesis. Photograph of a preserved specimen of *Mesonauta festivus*, specimen number 19, SL = 34.8 mm, scale bar = 10 mm. (1) Snout length, (2) head length, (3) prepectoral length, (4) prepelvic length, (5) preanal length, (6) standard length, (7) total length, (8) dorsal fin base, (9) dorsal fin length, (10) anal fin base, (11) eye diameter.

## Appendix B: Statistical Analyses for Chapter 3

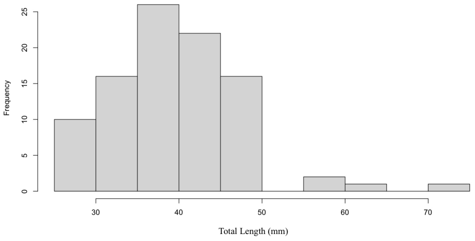
### B.1 Normality Test Results

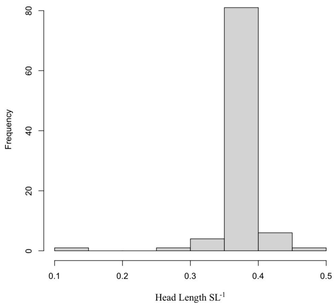
Table B.1.Results of normal distribution tests for completed measurements. The skewness value represents the distortion of a bell-curve (a negative value represents a left-skewed curve, 0 represents a normal distribution, a positive value represents a right-skewed curve). The kurtosis value demonstrates if there are lots of outliers (higher values represent more outliers). The Shapiro-Wilk test was used to test the normal distribution of the data, with a null hypothesis that the data is from a normally distributed population.

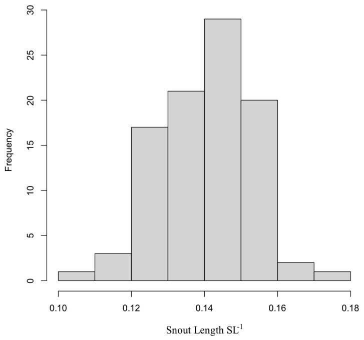
| Measurement | Skewness | Kurtosis | Shapiro-Wilk Test | p-value |
|---|---|---|---|---|
| Total Length | 1.066 | 5.468 | 0.937 | 0.00023 |
| Standard Length | 1.072 | 5.293 | 0.936 | 0.00018 |
| Head Length $SL^{-1}$ | -3.963 | 32.430 | 0.647 | 1.068e-13 |
| Snout Length $SL^{-1}$ | -0.189 | 2.667 | 0.988 | 0.596 |
| Eye Diameter $SL^{-1}$ | -0.139 | 2.976 | 0.989 | 0.681 |
| Interorbital Distance $SL^{-1}$ | 0.223 | 3.399 | 0.984 | 0.334 |
| Body Depth $SL^{-1}$ | 0.294 | 2.383 | 0.973 | 0.057 |
| Preanal Length $SL^{-1}$ | -2.807 | 32.138 | 0.528 | 7.274e-16 |
| Predorsal Length $SL^{-1}$ | -1.909 | 17.440 | 0.780 | 1.592e-10 |
| Prepelvic Length $SL^{-1}$ | -2.837 | 27.126 | 0.643 | 9.105e-14 |
| Prepectoral Length $SL^{-1}$ | -3.590 | 33.665 | 0.554 | 1.998e-15 |
| Caudal Peduncle Depth $SL^{-1}$ | -2.257 | 22.266 | 0.728 | 6.719e-12 |
| Caudal Peduncle Length $SL^{-1}$ | -1.900 | 14.139 | 0.859 | -5.546e-08 |
| Anal Fin Base $SL^{-1}$ | 0.245 | 2.359 | 0.981 | 0.202 |
| Dorsal Fin Base $SL^{-1}$ | 0.466 | 5.560 | 0.959 | 0.0052 |
| Anal Fin Length $SL^{-1}$ | 0.008 | 4.155 | 0.983 | 0.263 |
| Dorsal Fin length $SL^{-1}$ | 0.008 | 4.155 | 0.983 | 0.263 |
| Pectoral Fin Length $SL^{-1}$ | 2.840 | 18.772 | 0.770 | 8.284e-11 |

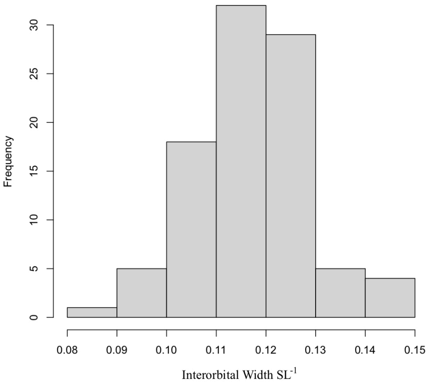
## B.2 Histograms of Measurements and Meristics

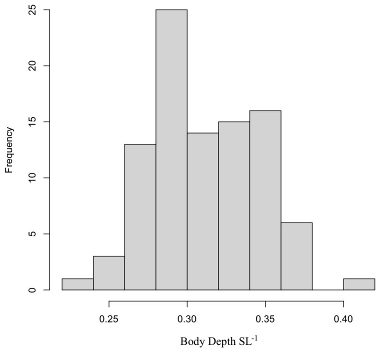
The following histograms represent a normal distribution for the completed measurements and meristics.

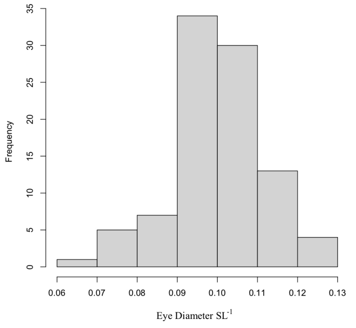


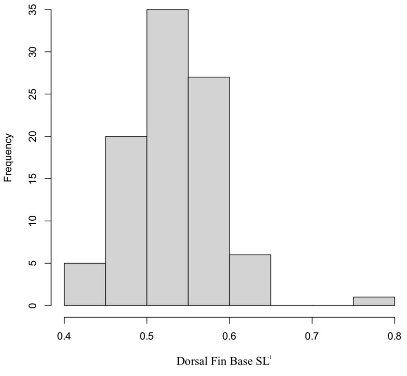


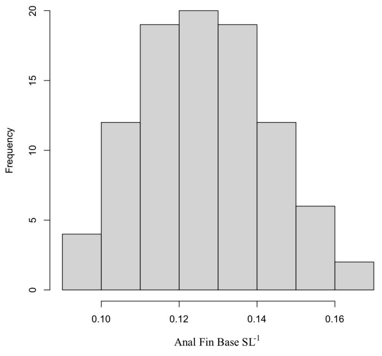


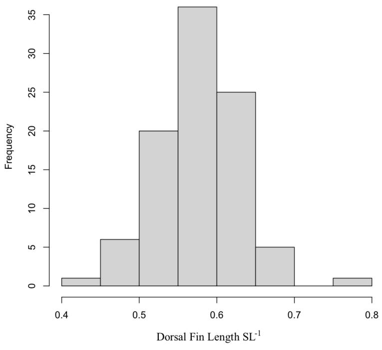


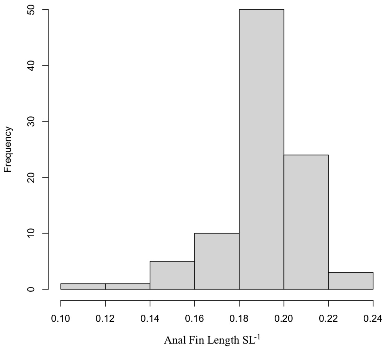


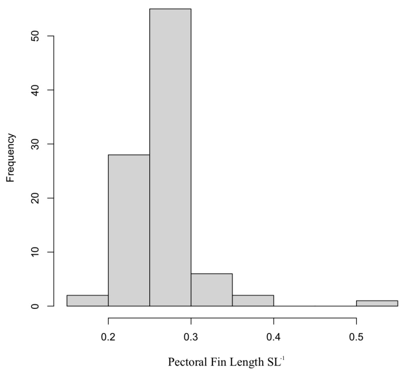


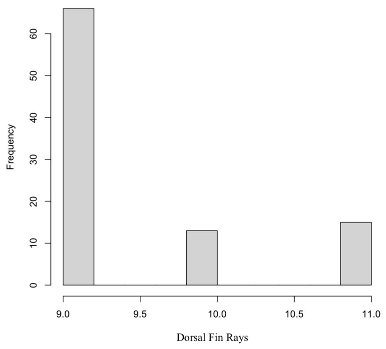


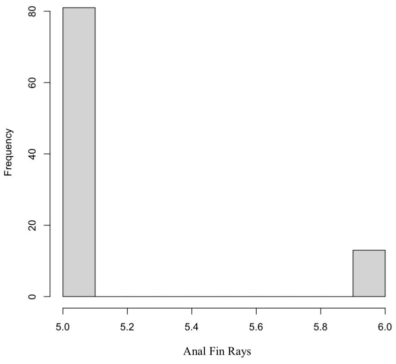


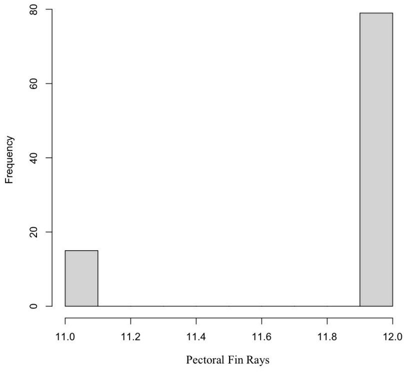


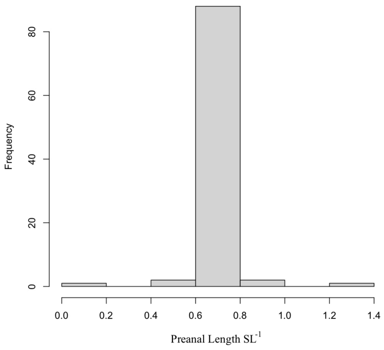

Frequency
0
10
20
30
40
50
0.10
0.12
0.14
0.16
0.18
0.20
0.22
0.24
Anal Fin Length SL$^{-1}$

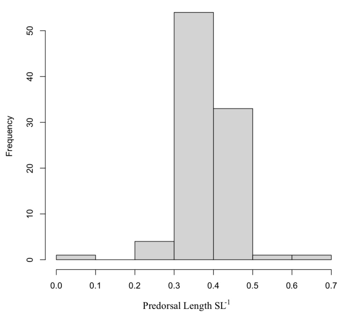

Frequency
0
10
20
30
40
50
0.2
0.3
0.4
0.5
Pectoral Fin Length SL$^{-1}$

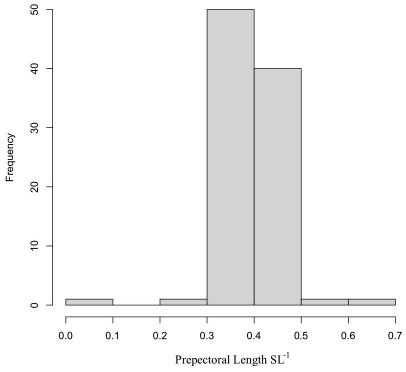

Frequency
0
10
20
30
40
50
60
9.0
9.5
10.0
10.5
11.0
Dorsal Fin Rays

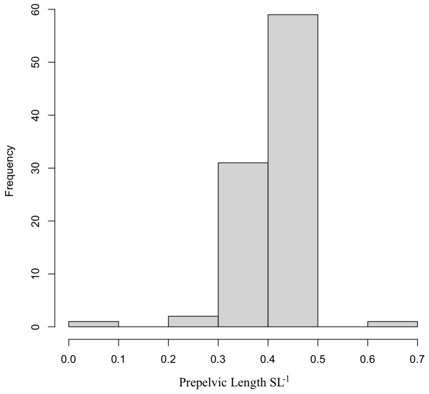

Frequency
0
20
40
60
80
5.0
5.2
5.4
5.6
5.8
6.0
Anal Fin Rays

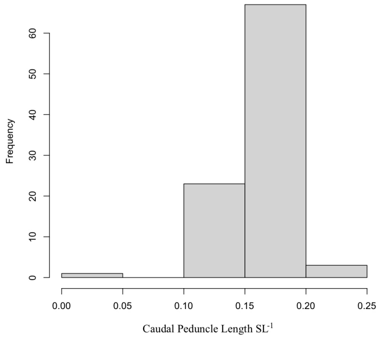

Frequency
0
20
40
60
80
11.0
11.2
11.4
11.6
11.8
12.0
Pectoral Fin Rays

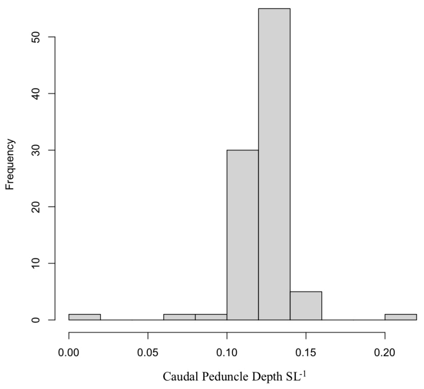

Frequency
0
20
40
60
80
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Preanal Length SL$^{-1}$

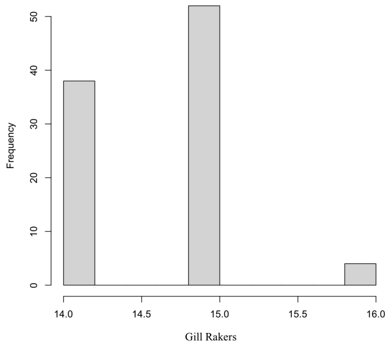

Frequency
0
10
20
30
40
50
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Predorsal Length SL$^{-1}$

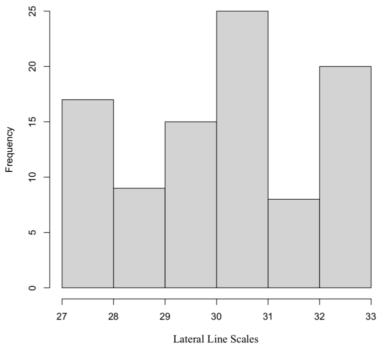

Frequency
0
10
20
30
40
50
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Prepectoral Length SL$^{-1}$

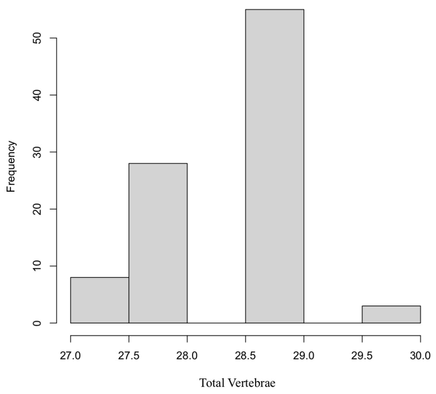

Frequency
0
10
20
30
40
50
60
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Prepelvic Length SL$^{-1}$

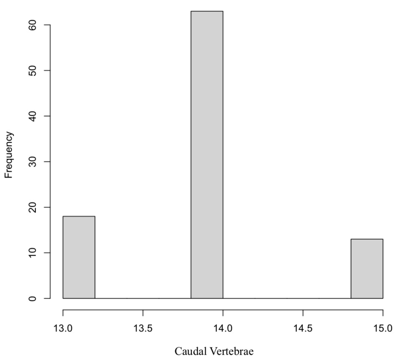


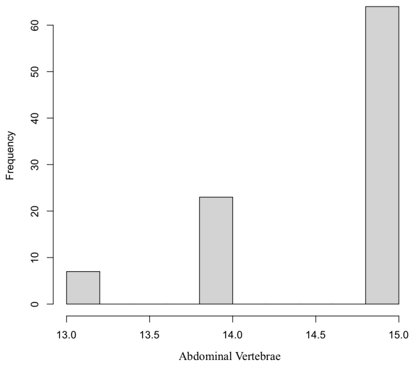








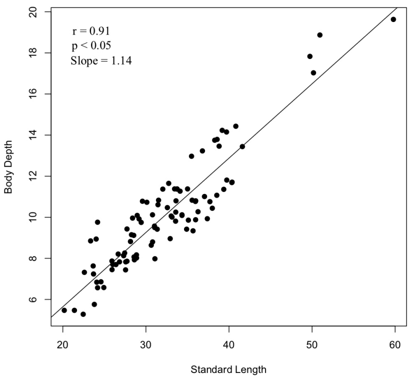


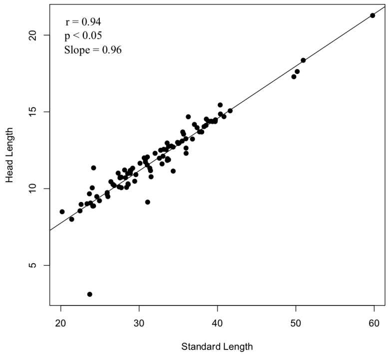


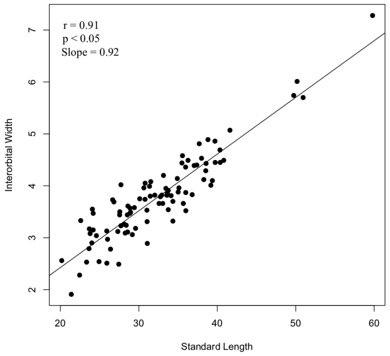
## B.3 Scatterplots of Measurements and Meristics

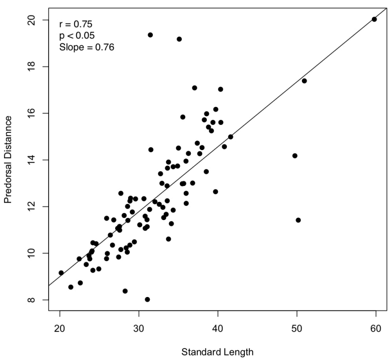
### B.3.1 Scatterplots of Measurements

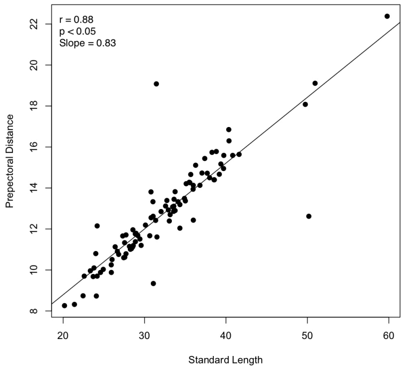
Scatterplots of all measurements were completed using logarithmic data. The Pearson's correlation coefficient (r), p-value, and slope are represented in the top left corner. Scatterplots of measurements represent isometric growth (with a slope ≈ 1.0).

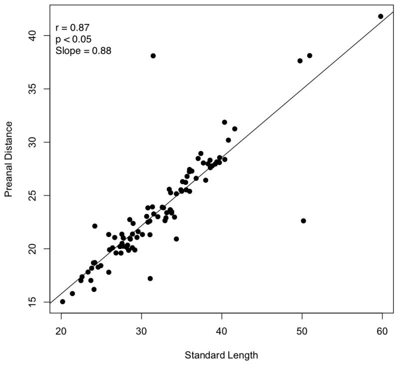

r = 0.91
p < 0.05
Slope = 1.14
Body Depth
Standard Length
6
8
10
12
14
16
18
20
20
30
40
50
60

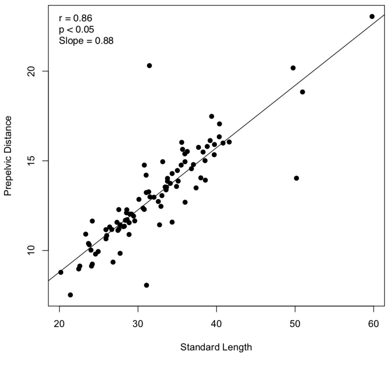

r = 0.94
p < 0.05
Slope = 0.96
Head Length
Standard Length
5
10
15
20
20
30
40
50
60

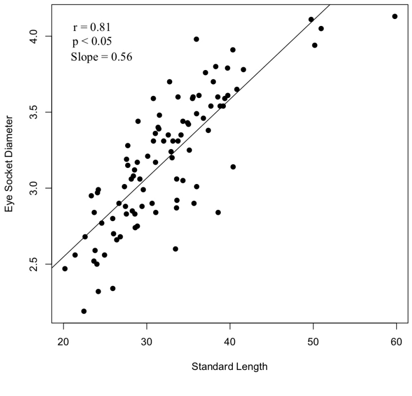

r = 0.91
p < 0.05
Slope = 0.92
Interorbital Width
Standard Length
2
3
4
5
6
7
20
30
40
50
60

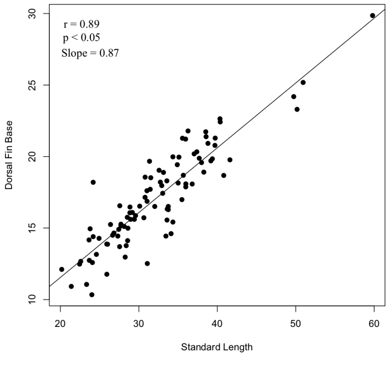

r = 0.75
p < 0.05
Slope = 0.76
Predorsal Distannce
Standard Length
8
10
12
14
16
18
20
20
30
40
50
60

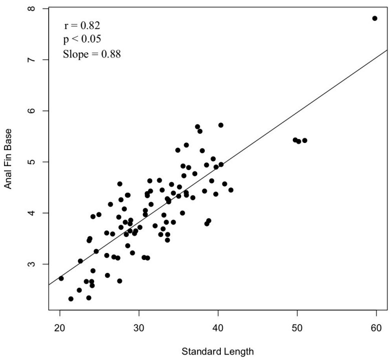

r = 0.88
p < 0.05
Slope = 0.83
Prepectoral Distance
Standard Length
8
10
12
14
16
18
20
22
20
30
40
50
60

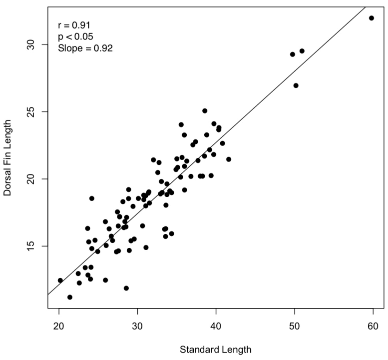

r = 0.87
p < 0.05
Slope = 0.88
Preanal Distance
Standard Length
15
20
25
30
35
40
20
30
40
50
60

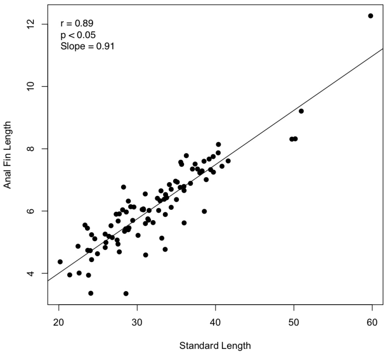

r = 0.86
p < 0.05
Slope = 0.88
Prepelvic Distance
Standard Length
10
15
20
20
30
40
50
60

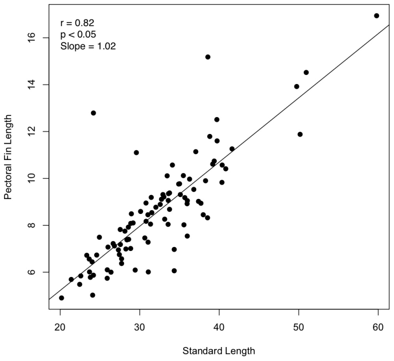

r = 0.81
p < 0.05
Slope = 0.56
Eye Socket Diameter
Standard Length
2.5
3.0
3.5
4.0
20
30
40
50
60

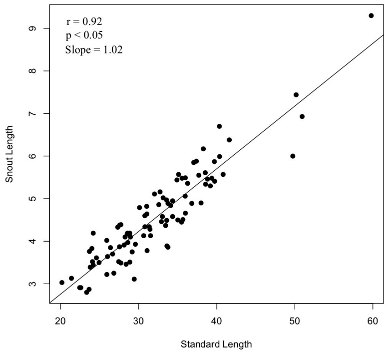

r = 0.89
p < 0.05
Slope = 0.87
Dorsal Fin Base
Standard Length
10
15
20
25
30
20
30
40
50
60











## B.3.2 Scatterplots of Meristics

Scatterplots of counts show no correlation with size.