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Assessment of the morphometry of saccular otoliths as a tool to identify triplefin species (Tripterygiidae)

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In the present work we describe nine saccular otolith morphometric indices (circularity, rectangularity, aspect ratio, percentage of the otolith surface occupied by the sulcus, percentage of the sulcus length occupied by the cauda length and ostium length, otolith length relative to the length of the fish, rostrum aspect ratio and percentage of the rostrum length occupied by the otolith length) of 41 species of the Tripterygiidae family collected mainly from New Zealand, Australia, Chile, South Africa, Mediterranean Sea and North America. The principal component of analysis showed that the indices that best explain the variability between species were related to sulcus and rostrum morphometry. According to cluster analysis, otolith morphometry could reflect the diversity of microenvironments for some genera such as Notoclinops *and* Forsterygion*, while this does not happen to genera like* Enneapterygius *and* Ruanoho*. The discriminant analysis showed that the species* Helcogrammoides cunninghami, Karalepis stewarti, Lepidoblennius haplodactylus, Notoclinus compressus, Ucla xenogrammus *can be discriminated by using the morphometric indices. Two new indices related to the sulcus that were of great value for the discrimination of these species are described for the first time. This information will be a useful tool for palaeontological, taxonomic and trophic ecology studies.*

Keywords: Tripterygiidae, otolith morphometry, ecomorphology, phylogenetic variability, electron microscopy

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INTRODUCTION

The Tripterygiidae family with 29 genera and around 171 species (Eschmeyer & Fong, 2014) is distributed in temperate and tropical regions of the Atlantic, Indian and Pacific Oceans. Several species of triplefins are used in the aquarium fish trade. Furthermore, they are strongly site-associated and guard benthic eggs, characteristics that may make populations vulnerable to habitat impacts (Baker, 2009). Their taxonomic identification is difficult due to their similarities in gross morphological and meristic characters (Gon, 1990; Cancino *et al.*, 2010). Therefore, the use of otoliths could provide a proper tool for the identification of the species of this family. Moreover, otoliths are often found in the stomach content of various organisms as well as in fossil sediments, thus being a very useful tool for taxonomic, ecological and paleontological studies (Wirtz, 1976; Schwarzhans, 1980; Schwarzhans & Grenfell, 2002; Reichenbacher *et al.*, 2007). Otoliths are complex polycrystalline structures composed of calcium carbonate (approximately 96%) and trace elements immersed in a protein matrix (Campana *et al.*, 1997). These structures are located in the inner ear of fishes and have a role in hearing and maintenance of equilibrium (Popper & Zhongmin, 2000). They are enclosed in three end-organs of the inner ear in teleost fishes (Popper *et al.*, 1988). The saccular otolith (sagitta) is the largest, at least in most teleost families (Schulz-Mirbach & Reichenbacher, 2006).

The morphometry and morphology of the otoliths have been widely used to identify species of other families. For example, Callicó Fortunato *et al.* (2014) have used the morphometry to identify species of mullets (Mugilidae) from the North-eastern Atlantic and Mediterranean Sea, while Tuset *et al.* (2008) performed the characterization of 348 fish species using otolith morphology and morphometry. Reichenbacher *et al.* (2007) used otolith morphology and morphometry for assessing taxonomy and diversity in fossil and extant killifish (*Aphanius*). This paper is one of the first studies dedicated to the combined use of otolith morphology and morphometry. Otolith morphometry has also been used for the identification of fish stocks (e.g. Avigliano *et al.*, 2014; Avigliano *et al.*, 2015a, c), and the simultaneous use of morphometry and morphology has been employed for the study of ecological patterns in fish (Volpedo & Echeverría, 2003; Volpedo & Fuchs, 2010; Curcio *et al.*, 2014). However, the studies related to the identification of species of Tripterygiidae family using otoliths are few. Chaine (1956) described the saccular otolith of *Tripterygion tripteronotus* (Risso, 1810). Wirtz (1976) briefly described the otolith morphology of three existing members of the genus *Tripterygion* in the Mediterranean Sea, while Smale *et al.* (1995) described the morphology of the saccular otolith of two triplefin fishes (*Cremnochorites capensis* (Gilchrist & Thompson, 1908) and *Helgogramma obtusirostris* (Klunzinger, 1871)). Furthermore, Schwarzhans & Grenfell (2002) reported on the presence of otoliths of four triplefin

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fishes from New Zealand. Later, Jawad (2007) described the morphology of the otoliths of several tripterygiids species in order to contribute to the taxonomy of the species of this family.

In the present work, we describe for the first time nine morphometric otolith indices of 41 species of the Tripterygiidae family collected mainly from New Zealand, Australia, Chile, South Africa, Mediterranean Sea and North America. This work is considered a potentially important tool for the identification of species of triplefins using saccular otoliths. The results of this study may also be important for the fossil record, taxonomic and diet studies (stomach contents of predators).

MATERIALS AND METHODS

Ichthyological material

Fish specimens are from New Zealand (several localities) (number of species = 24); Australia (Tasmania, Lizard Island, Avalon, Port Phillip Bay) (N = 9); South Africa (False Bay, Sodwana Bay) (N = 5); Chile (Quintero) (N = 1); USA (California) (N = 1); Spain (Ibiza) (N = 1). Examined material (Table 1) comes from the Museum of New Zealand (Wellington, New Zealand), Australian Museum (Sydney, Australia) and School of Biological Sciences (University of Auckland, Auckland, New Zealand). Those specimens belonging to the School of Biological Sciences were made available by Kendall Clements (University of Auckland) by means of scuba diving using slurp guns. Specimens without registration number are non-museum specimens and they are being kept in the School of Biological Sciences (University of Auckland).

Specimens of all species were identified using the traditional taxonomic identification methods and no genetic study has been applied. The museum specimens used in this study were already identified when they have been borrowed. The non-museum specimens were identified using the following references: for Australian and New Zealand specimens, Fricke (1994); for Chile, Fricke (1997), for South Africa, Holleman (1986) and Fricke (1997), for Spain, Carreras-Carbonell *et al.* (2007) and for USA, Allen & Robertson (1994).

The animals were measured (SL; most anterior point to the posterior tip of the vertebral column) using a digital caliper (model IP54, 150 mm moisture-proof electronic digital caliper, Shenzhen Pride Instruments, Inc., China) to the nearest 1 mm. The saccular otoliths were removed by turning the ventral side of the fish upward to allow removal of the lower jaw, the gills and the hypobranchial apparatus, and to expose the base of the skull. Later, the otoliths were cleaned with 70% ethanol and stored dry in a small plastic tube.

Otolith morphometry

Scanning electron microscopy (SEM) was used to investigate right saccular otolith ultrastructure. Otoliths examined by SEM were air dried and mounted on aluminium stubs using double-sided sticky tape. When dry, the otoliths and stubs were sputter coated with gold to a thickness of 28–30 nm in a vacuum of about 40×10^{-3} Torr. Otoliths were viewed using the secondary electron image of Philips XL45 FEG at an accelerating voltage of 5.0 KV.

According to the terminology used by Avigliano *et al.* (2014) the following morphometric variables have been determined based on the images (Figure 1): otolith length (OL, mm), otolith width (OW, mm), otolith perimeter (PO, mm), otolith surface (OS, mm^2), sulcus perimeter (SP, mm), sulcus surface (SS, mm^2), sulcus length (SuL, mm), cauda length (CL, mm), ostium length (OSL, mm), rostrum width (RW, mm) and rostrum length (RL, mm). These parameters were measured in all the otoliths using image processing systems (Image-Pro Plus 4.5®). Subsequently, otolith shape indices were calculated: circularity (PO^2/OS), rectangularity ($OS/(OL \times OH)$), aspect ratio (OW/OL, %), percentage of the otolith surface occupied by the sulcus (SS/OS, %), percentage of the sulcus length occupied by the cauda length (CL/SuL, %), percentage of the sulcus length occupied by the ostium length (OSL/SuL, %), rostrum aspect ratio (RW/RL, %) and percentage of the rostrum length occupied by the otolith length (RL/OL, %). CL/SuL and OSL/SuL indices were used for the first time in this work.

Data analysis

Analysis of covariance (ANCOVA) with fish size (standard length) as a covariate was carried out for each index to test the effect of size on indices (Campana *et al.*, 2000; Kerr & Campana, 2014). ANCOVA is robust to violations of the assumption of homogeneity of variance (Olejnik & Algina, 1984). All indices varied significantly with fish size (ANCOVA, $P < 0.05$) and they were corrected using the common within-group slope (b) for each variable on fish standard length (e.g. Longmore *et al.*, 2010; Kerr & Campana, 2013; Avigliano *et al.*, 2015a, b, c).

A Principal Component Analysis (PCA) was applied to identify the variables that explain the highest proportion of variability and to investigate the morphometric patterns shown between species. The selection of axes for interpretation was performed using a screen plot (Hubert *et al.*, 2009).

A cluster analysis was performed using the unweighted pair group method with arithmetic average (UPGMA) on an Euclidean distance matrix to assess morphological dissimilarity among species. In order to estimate the good fit between similarity matrix and the dendrogram, the coefficient of cophenetic correlation was calculated. A high cophenetic correlation suggests a good fit among the similarity matrix and dendrogram. Prior to Euclidean distance calculation the data were standardized to have a mean of zero and a variance of one.

Finally a Canonical Discriminant Analysis (CDA) was performed to test the accuracy of using those indices for the identification of fish species. To determine the discriminatory importance of each index (i.e. the value of each index that contributed most to the separation of the species) across all discriminant functions, the mean discriminant coefficient was calculated using the following equation (Backhaus *et al.*, 2006): Mean discriminant coefficient $bj = \Sigma|bjk|^*EAk$ ($k = 1, k = \ldots.$) where bjk is the standardized discriminant function coefficient for the variable j with respect to the discriminant function k, and EAk is the proportion of the eigenvalue of the discriminant function k in relation to the sum of all eigenvalues.

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Table 1. Examined materials of studied triplefin. AM, Australian Museum, Sydney; NMNZ, Museum of New Zealand.

Species	Specimen number	Standard length (mm)	Locality	Date of collection	Catalogue number
Australia					
Ceratobregma acanthops (Whitley, 1964)	4	20–30	Lizard Island	24 December 1997	–
Enneapterygius atrogulare (Günther, 1873)	4	18–30	Lizard Island	19 December 1997	–
Enneapterygius gracilis Fricke, 1994	3	21–28	Lizard Island	19 December 1997	–
Enneapterygius pausifasciatus Fricke, 1994	4	20–30	Lizard Island	23 December 1997	–
Enneapterygius rufopileus (Waite, 1904)	4	30–48	–	18 April 1997	AM.7710–014
Helcogramma springeri Hansen, 1986	4	18–32	Lizard Island	24 December 1997	–
Lepidoblennius haplodactylus Steindachner, 1867	4	36–76	Avalon, Sydney	23 April 1997	–
Trinorfolkia clarkei (Morton, 1888)	4	29–57	Port Phillip Bay, Victoria	9 April 1997	–
Ucla xenogrammus Holleman, 1993	4	18–44	Lizard Island	14 December 1997	–
Chile					
Helcogrammoides cunninghami (Smitt, 1898)	4	15–24	Playa El Durazno, Quintero	28 November 1999	–
New Zealand					
Apopterygion oculus Fricke & Robertsin, 1994	4	20–50	Mernoo Bank, Chatham Rise, Tangaroa	12 January 1979	NMNZ P. 25176
Bellapiscis lesleyae Hardy, 1987	4	28–52	Mathesons Bay, Hauraki Gulf	27 January 1997	–
Bellapiscis medius Hardy, 1987	4	28–82	Horseshoe Bay, Stewart Island	3 January 1998	–
Blennodon dorsal (Clarke, 1879)	4	25–135	Muriwai	9 October 1999	–
Cryptichthys jojettae Hardy, 1987	4	20–44	Breaker Bay, Wellington	9 February 1998	–
Forsterygion capito (Jenyns, 1841)	4	25–82	Island Bay, Wellington	7 January 2000	–
Forsterygion flavonigrum Fricke & Roberts in Fricke, 1994	4	26–43	Ulva Islands, Stewart Island	30 January 1998	–
Forsterygion gymnota Scott, 1977	4	36–85	Queens Wharf, Wellington	23 April 2000	–
Forsterygion lapillum Hardy, 1989	5	24–59	Ulva Islands, Stewart Island	February 1998	–
Forsterygion malcomi Hardy, 1987	4	38–105	Mokohinau Islands	18 February 1998	–
Forsterygion maryannae (Hardy, 1987)	4	27–52	Three Kings Islands	1 March 1999	–
Forsterygion nigripenne Valenciennes 1836	4	33–36	Whangateau Wharf, Whangateau Estuary	13 December 1999	–
Forsterygion varium Schneider (1801)	4	93–110	Island Bay, Wellington	7 February 1998	–
Gilloblennius abditus Hardy, 1986	4	27–63	Kapiti Island	6 March 1996	NMNZ P. 33278
Gilloblennius tripennis (Forster, 1801)	4	73–113	Ringaringa Bay, Oban, Stewart Island	7 March 1992	–
Karalepis stewarti Hardy, 1984	5	36–120	Three Kings Islands	1 March 1999	–
Matanui bathytaton (Hardy, 1989)	4	38–90	Mernoo Bank	12 Jan 1979	NMNZ P. 25319
Matanui profundum (Fricke & Roberts, 1994)	4	27–68	Omaha Bay	19 Nov 1999	–
Notoclinops caerulepunctus Hardy, 1989	4	18–36	Cathedral Rock	20 January 1998	–
Notoclinops segmentatus (McCulloch & Phillips, 1923)	4	15–47	Hen and Chicken Islands, Hauraki Gulf	6 February 1997	–
Notoclinops yaldwyni Hardy, 1987	4	20–51	Mokohinau Islands	20 January 1998	–
Notoclinus compressus (Hutton, 1872)	4	25–80	Manukau Bay, Owenga, Chatham Island	4 February 1991	–
Ruanoho decemdigitatus (Clarke, 1879)	4	38–102	Breaker Bay	9 February 1998	–
Ruanoho whero Hardy, 1986	4	30–77	Ulva Islands, Stewart Island	30 January 1998	–
South Africa					
Acanthanectes rufus Holleman & Buxton, 1993	4	15–28	Sodwana Bay	1999	–
Enneapterygius abeli (Klausewitz, 1960)	4	15–23	Sodwana Bay	29 May 2001	–
Enneapterygius ventermaculatus Holleman, 1982	4	12–20	Sodwana Bay	28 May 2001	–
Helcogramma obtusirostris (Klunzinger, 1871) Spain	4	18–28	Sodwana Bay	27 May 2001	–
Tripterygion tartessicum Carreras-Carbonell *et al.*, 2007	4	30–48	Portinax, Ibiza	July 2001	–
United States of America					
Crocodilichthys gracilis Allen & Robertson, 1991	4	20–31	West Ventura, California	1997	–

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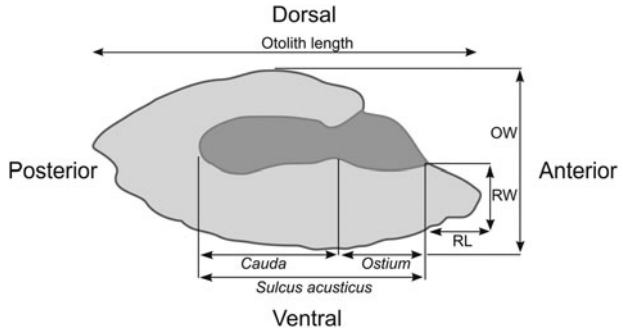

Fig. 1. Generalized scheme of the inner surface of saccular otoliths of triplefins illustrating the most relevant features. OW, otolith width; RW, rostrum width; RL, rostrum length.

Data processing was performed using SPSS 19 and INFOSTAT statistical programs.

RESULTS

The mean and range of the indices calculated are shown in Table 2 and the otolith images of all species studied are shown in Figure 2.

Four principal components were extracted from the PCA which accounted for 77% of the total variance of the original nine morphological variables (Figure 3A, B). The first axis (PC1) explained 30% of the total variability (Figure 3A). The morphometric variables that contributed most to the formation of the spatial gradient of the PC1 scores were CL/SuL (eigenvector = 0.50), OW/OL (eigenvector = 0.46) and RL/OL (eigenvector = 0.48) (Figure 3A). The second axis (PC2) explained 19% of the total variability with the most important variables being RW/RL (eigenvector = 0.59), CI (eigenvector = −0.43) and OSL/SuL (eigenvector = −0.40) (Figure 3A). In the plot (Figure 3A), an association between CL/SuL and RL/OL indices and the species *F. flavonigrum, R. whero* and *R. decemdigitatus* were observed. The OW/OL index was associated with *B. medius, F. malcolmi* and *N. yaldwyni* (Figure 3A). In addition, RW/RL was associated with *F. lapillum, F. varium, H. cunninghami* and *K*, while CI with *F. nigripenne*, among other species (Figure 3A).

The third axis (PC3) explained 16% of the variability with the most important indices being SS/OS (eigenvector = 0.59) and OSL/SuL (eigenvector = −0.49) (Figure 3B). Finally, the fourth component explained 12% of the variability with the most significant variables OL/SuL (eigenvector = 0.57) and CI (eigenvector = 0.47) (Figure 3B). According to the third and fifth component, an association between SS/OS and the species *B. medius, H. obtusirostris* and *L. haplodactylus* were observed (Figure 3B). The OSL/SuL index was associated with *C. gracilis* and *E. rufopileus* (Figure 3B). In addition, OL/SuL and CI were associated with *B. dorsalis* and *F. flavonigrum* (Figure 3B).

The cophenetic correlation coefficient (UPGMA dendrogram) was 0.85, suggesting a good fit between the similarity matrix and the matrix derived from the dendrogram. The similarity analysis showed the existence of at least five main different groups (Figure 4). Group 1 is grouped by *B. medius, N. caerulpunctus* and the genera *Cryptichthys, Gilloblennius* and *Ceratobregma*. This subgroup is associated with higher values of the index RL/OL (>22).

Group 2 is characterized by *L. haplodactylus* while group 3 by the studied species of the genera *Apopterygion* and *Blennodon*.

Group 4 can be divided into several subgroups and contains all species of the genera *Enneapterygius, Forsterygion, Notoclinus, Ruanoho, Matanui, Trinorfolkia, Karalepis* and *Ucla*. It shares species of the genera *Bellapiscis* and *Notoclinops* with group 1. Subgroup 4a contains *E. paucifaciatus, F. nigripenne, N. segmentatus* and *E. gracilis*, while subgroup 4b contains the other species and genera mentioned previously for group 4. Overall, the fish in group 4 (Figure 4) had otoliths with developed rostrum lengths (RL/OL > 7) except in the genera *Karalepis* and *Ucla* (RL/OL: 0–4.8). On the other hand the species of subgroup 4a showed low rectangularity (RE < 0.9) and very variable relative size of rostrum. The rostrum is absent in *E. paucifaciatus* (Table 2). The species of subgroup 4bii showed the lowest values of RW/RL index (<152) and the SS/OS and OSL/SuL indexes were similar for all species of this subgroup (Figure 4).

Finally high similarity was also observed for species of the genera *Crocodilichthys, Tripterygion* and *Acanthanectes* and the species *H. springeri* (group 5). These species showed high rectangularity (RE > 0.1) and low relative size of rostrum (RL/OL < 6.5) (Table 2) (Figure 4).

The species *H. obtusirostris* and *Cremnochorites* and *Helcogrammoides* genera showed low similarity in relation to the mentioned groups (Figure 4). Particularly *Cremnochorites* has the lowest CL/SuL index.

The Canonical Discriminant Analysis showed a separation between some species (Table 3). The CDA proved to have greater accuracy in classifying the species *H. cunninghami, K. stewarti, L. haplodactylus, N. compressus, U. xenogrammus* (66–100%) (Table 3). However, the percentage of correctly classified individuals was low (50%) for *A. rufus, B. dorsalis, C. jojettae, F. flavonigrum, F. varium, F. gymnotum, M. bathytaton, N. caerulpunctus* and bad (<50%) for the other species.

Based on the mean discriminant coefficients the CL/SL was identified as the most important index followed by the OSL/SuL, OL/SuL and RW/RL indices ($bj = -1.01$, $bj = 0.59$, $bj = 0.33$, -0.32 respectively).

DISCUSSION

Environmental factors such as salinity, water temperature and depth have been suggested to be responsible for some inter- and intra-specific differences in, for example, sulcus area and otolith length (e.g. Lombarte, 1992; Lombarte *et al.*, 2010, Avigliano *et al.*, 2014; Reichenbacher & Reichard, 2014). However several variables such as otolith size, rostrum and sulcus morphology are principally under genetic control in the same groups of fishes. Therefore, the taxonomic value of otoliths is well established (e.g. Gierl *et al.*, 2013, Reichenbacher & Reichard, 2014). Because of these characteristics, otolith morphometry has been widely used to identify fish stocks (e.g. Campana & Casselman, 1993; Burke *et al.*, 2008; Cañas *et al.*, 2012; Avigliano *et al.*, 2014), to differentiate species (e.g. Nolf, 1985; Smale *et al.*, 1995; Tuset *et al.*, 2011; Tuset *et al.*, 2013; Zhuang *et al.*, 2014), and to describe ecomorphological patterns of species (e.g. Platt & Popper, 1981; Gauldie, 1988; Lombarte *et al.*, 2003; Volpedo & Echeverría, 2003;

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Table 2. Mean and standard deviation and range (minimum–maximum) of the morphological indices of 41 species of Tripterygiidae. SL, Fish standard length; OL, otolith length; OW, otolith width; PO, otolith perimeter; OS, otolith surface; SP, sulcus perimeter; SS, sulcus surface, SuL, sulcus length; CL, cauda length; OSL, ostium length; RW, rostrum width; RL, rostrum length; CI, circularity and RE, rectangularity.

	Species	OW/OL		OL/SuL		CI		RE	
1	*Acanthanectes rufus*	26.3 ± 9.6	(20.0–40.3)	0.05 ± 0.01	(0.03–0.06)	26.1 ± 9.3	(20.8–40.0)	1.3 ± 0.5	(0.7–2.0)
2	*Apopterygion oculus*	48.3 ± 3.1	(45.5–51.7)	0.08 ± 0.02	(0.05–0.11)	18.8 ± 0.8	(18.3–20.1)	0.8 ± 0.1	(0.7–1.0)
3	*Bellapiscis lesleyae*	45.0 ± 7.8	(33.3–50.1)	0.03 ± 0.00	(0.03–0.04)	15.7 ± 11.2	(0.0–26.7)	0.7 ± 0.02	(0.7–0.7)
4	*Bellapiscis medius*	70.0 ± 35.5	(48.5–123.0)	0.03 ± 0.00	(0.03–0.04)	20.8 ± 1.1	(19.1–21.5)	0.7 ± 0.2	(0.5–1.0)
5	*Blennodon dorsalis*	41.3 ± 6.8	(32.4–49.1)	0.05 ± 0.03	(0.04–0.11)	10.2 ± 11.6	(0.2–22.2)	0.8 ± 0.1	(0.7–1.0)
6	*Ceratobregma acanthops*	47.1 ± 8.3	(40.0–58.8)	0.05 ± 0.00	(0.05–0.06)	17.1 ± 6.5	(9.6–24.3)	0.7 ± 0.09	(0.6–0.8)
7	*Cremnochorites capensis*	31.5 ± 9.8	(20.0–40.0)	0.05 ± 0.00	(0.04–0.05)	14.4 ± 9.2	(4.0–23.1)	2.1 ± 2.0	(0.7–5.0)
8	*Crocodilichthys gracilis*	27.4 ± 13.6	(10.0–39.0)	0.05 ± 0.00	(0.05–0.05)	31.4 ± 10.4	(22.0–45.0)	1.1 ± 0.6	(0.7–2.0)
9	*Cryptichthys jojettae*	45.3 ± 7.0	(31.6–54.5)	0.04 ± 0.01	(0.04–0.05)	16.1 ± 4.0	(10.4–19.4)	0.9 ± 0.2	(0.7–1.1)
10	*Enneapterygius gracilis*	38.7 ± 9.7	(25.0–50.0)	0.05 ± 0.01	(0.04–0.06)	24.3 ± 14.5	(11.2–40.0)	0.6 ± 0.05	(0.5–0.6)
11	*Enneapterygius abeli*	45.8 ± 12.6	(43.7–50.0)	0.05 ± 0.00	(0.04–0.05)	19.4 ± 2.0	(17.6–22.1)	0.9 ± 0.2	(0.7–1.1)
12	*Enneapterygius atrogulare*	42.9 ± 2.8	(36.4–47.9)	0.05 ± 0.00	(0.04–0.05)	20.2 ± 5.0	(13.7–26.1)	0.7 ± 0.03	(0.7–0.7)
13	*Enneapterygius paucifaciatus*	37.1 ± 5.9	(30.0–43.8)	0.05 ± 0.00	(0.05–0.05)	32.2 ± 22.1	(16.8–57.6)	0.5 ± 0.18	(0.3–0.7)
14	*Enneapterygius rufopileus*	33.8 ± 6.8	(22.2–42.4)	0.04 ± 0.00	(0.04–0.05)	26.5 ± 4.8	(21.3–31.3)	0.9 ± 0.22	(0.7–1.1)
15	*Enneapterygius ventermaculus*	46.1 ± 9.4	(33.3–60.0)	0.04 ± 0.01	(0.03–0.05)	23.8 ± 2.5	(21.0–26.7)	0.8 ± 0.34	(0.6–1.3)
16	*Forsterygion flavonigrum*	46.8 ± 11.5	(39.3–56.0)	0.04 ± 0.01	(0.04–0.05)	19.9 ± 0.9	(18.6–20.5)	0.7 ± 0.6	(0.6–0.7)
17	*Forsterygion lapillum*	46.3 ± 5.8	(44.2–49.0)	0.04 ± 0.01	(0.03–0.06)	18.8 ± 0.5	(18.2–19.4)	0.7 ± 0.06	(0.7–0.9)
18	*Forsterygion malcolmi*	47.7 ± 7.3	(40.7–54.7)	0.04 ± 0.01	(0.03–0.04)	18.0 ± 4.0	(12.9–21.2)	0.7 ± 0.06	(0.7–0.8)
19	*Forsterygion varium*	40.9 ± 5.8	(38.1–44.1)	0.03 ± 0.01	(0.02–0.04)	21.6 ± 0.3	(21.3–22.0)	0.7 ± 0.02	(0.6–0.7)
20	*Forsterygion gymnota*	43.3 ± 5.8	(38.3–50.0)	0.03 ± 0.00	(0.03–0.03)	20.2 ± 2.9	(17.2–22.8)	0.7 ± 0.02	(0.7–0.7)
21	*Forsterygion capito*	48.9 ± 5.7	(40.9–54.2)	0.04 ± 0.01	(0.03–0.05)	19.8 ± 1.5	(18.2–21.6)	0.7 ± 0.07	(0.7–0.8)
22	*Forsterygion maryannae*	43.8 ± 5.6	(36.9–47.8)	0.04 ± 0.00	(0.04–0.04)	18.4 ± 0.7	(17.4–19.2)	0.9 ± 0.2	(0.7–1.2)
23	*Forsterygion nigripenne*	42.8 ± 0.82	(41.7–43.5)	0.05 ± 0.01	(0.04–0.06)	32.0 ± 26.1	(16.1–71.1)	0.6 ± 0.3	(0.2–0.9)
24	*Gilloblennius abditus*	47.7 ± 4.4	(43.0–52.6)	0.03 ± 0.00	(0.03–0.04)	22.1 ± 1.7	(20.5–24.5)	0.7 ± 0.08	(0.6–0.7)
25	*Gilloblennius tripennis*	44.8 ± 8.4	(39.0–57.1)	0.03 ± 0.00	(0.02–0.03)	21.5 ± 1.9	(20.1–24.3)	0.7 ± 0.1	(0.4–0.8)
26	*Helcogramma obtusirostris*	25.2 ± 13.2	(11.1–42.3)	0.05 ± 0.00	(0.04–0.05)	23.5 ± 6.0	(20.0–32.4)	0.9 ± 0.1	(0.7–1.1)
27	*Helcogramma springeri*	25.6 ± 14.7	(10.0–43.5)	0.05 ± 0.01	(0.05–0.06)	23.7 ± 5.9	(19.1–30.0)	1.2 ± 0.6	(0.7–2.0)
28	*Helcogrammoides cunninghami*	28.0 ± 20.4	(0.0–48.8)	0.04 ± 0.01	(0.03–0.05)	4.5 ± 8.0	(0.0–17.9)	8.4 ± 0.4	(0.7–30.0)
29	*Karalepis stewarti*	44.7 ± 4.2	(40.3–49.7)	0.04 ± 0.01	(0.03–0.05)	17.8 ± 5.7	(8.3–22.4)	0.8 ± 0.1	(0.7–1.1)
30	*Lepidoblennius haplodactylus*	43.1 ± 5.6	(36.4–50.0)	0.03 ± 0.00	(0.03–0.04)	21.3 ± 6.2	(16.0–27.8)	0.6 ± 0.06	(0.5–0.7)
31	*Matanui bathytaton*	51.4 ± 3.8	(46.4–55.2)	0.04 ± 0.01	(0.04–0.06)	14.3 ± 9.3	(0.3–20.2)	0.7 ± 0.04	(0.6–0.7)
32	*Matanui profundum*	57.1 ± 7.5	(48.2–65.0)	0.04 ± 0.01	(0.04–0.04)	16.6 ± 2.3	(13.1–17.9)	0.7 ± 0.1	(0.5–0.8)
33	*Notoclinus compressus*	60.9 ± 4.2	(55.6–70.7)	0.02 ± 0.00	(0.02–0.02)	16.9 ± 2.1	(14.9–19.6)	0.8 ± 0.1	(0.7–0.8)
34	*Notoclinops caerulpunctus*	50.2 ± 6.7	(45.3–55.6)	0.03 ± 0.00	(0.03–0.04)	16.7 ± 0.7	(16.0–17.7)	0.8 ± 0.04	(0.7–1.0)
35	*Notoclinops segmentatus*	39.2 ± 3.7	(34.2–43.1)	0.06 ± 0.02	(0.04–0.07)	19.3 ± 2.3	(15.9–20.9)	0.7 ± 0.1	(0.4–0.9)
36	*Notoclinops yaldwyni*	44.8 ± 4.5	(41.9–51.5)	0.04 ± 0.00	(0.03–0.04)	19.6 ± 1.3	(17.8–20.6)	0.8 ± 0.08	(0.7–0.9)
37	*Ruanoho whero*	42.7 ± 7.0	(35.8–52.6)	0.03 ± 0.01	(0.02–0.05)	20.2 ± 1.4	(19.3–22.2)	0.7 ± 0.1	(0.5–0.7)
38	*Ruanoho decemdigitatus*	42.8 ± 4.0	(37.1–48.3)	0.03 ± 0.00	(0.03–0.03)	18.3 ± 5.2	(10.5–22.5)	0.7 ± 0.04	(0.7–0.8)
39	*Trinorfolkia clarkei*	39.1 ± 5.5	(37.5–40.0)	0.04 ± 0.00	(0.03–0.05)	21.4 ± 1.6	(19.2–23.1)	0.9 ± 0.2	(0.7–1.3)

Continued

Table 2. Continued

	Species	OW/OL		OL/SuL		CI		RE	
40	*Tripterygion tartessicum*	29.9 ± 10.5	(16.7–40.9)	0.04 ± 0.00	(0.04–0.04)	24.2 ± 2.0	(21.7–26.7)	1.1 ± 0.06	(0.7–2.1)
41	*Ucla xenogrammus*	37.8 ± 2.3	(33.3–41.0)	0.04 ± 0.00	(0.03–0.04)	21.9 ± 1.1	(20.6–23.1)	0.8 ± 0.07	(0.7–0.8)

	Species	SS/OS		OSL/SuL		CL/SuL		RW/RL		RL/LO	
1	*Acanthanectes rufus*	22.4 ± 7.2	(13.3–30.0)	53.6 ± 7.2	(50.0–64.5)	26.2 ± 6.2	(20.0–34.8)	135.3 ± 25.6	(100.0–157.9)	5.5 ± 2.4	(3.0–8.3)
2	*Apopterygion oculus*	34.5 ± 40.1	(10.9–95.2)	31.8 ± 12.9	(15.4–45.6)	37.5 ± 18.5	(15.4–56.0)	154.8 ± 46.4	(100.0–200.0)	4.7 ± 2.2	(1.8–7.0)
3	*Bellapiscis lesleyae*	23.5 ± 1.4	(21.6–25.0)	45.6 ± 4.1	(40.7–50.9)	59.6 ± 5.8	(54.6–67.9)	272.4 ± 86.3	(209.1–400.0)	7.7 ± 6.4	(1.0–14.5)
4	*Bellapiscis medius*	27.0 ± 3.3	(22.8–30.8)	49.6 ± 3.7	(45.8–53.4)	56.3 ± 4.1	(52.0–61.5)	204.9 ± 212	(25.6–454.1)	33.0 ± 40.2	(2.0–87.6)
5	*Blennodon dorsalis*	16.8 ± 4.4	(11.1–21.9)	56.2 ± 1.4	(54.5–58.0)	43.0 ± 1.6	(40.9–44.7)	173.9 ± 24.6	(146.6–200.0)	7.1 ± 3.2	(2.7–10.5)
6	*Ceratobregma acanthops*	55.2 ± 37.2	(14.3–90.0)	71.6 ± 28.1	(40.0–100.0)	62.2 ± 24.0	(40.0–85.7)	107.3 ± 36.0	(83.3–160.2)	33.9 ± 30.1	(8.2–70.6)
7	*Cremnochorites capensis*	8.6 ± 6.0	(3.0–17.2)	23.1 ± 21.2	(3.8–51.2)	20.5 ± 19.6	(5.0–48.8)	220.2 ± 70.2	(133.3–297.4)	4.0 ± 1.3	(2.7–5.3)
8	*Crocodilichthys gracilis*	16.9 ± 3.2	(13.3–20.0)	88.0 ± 42.4	(57.5–150.0)	59.9 ± 27.1	(39.7–100.0)	167.5 ± 55.4	(133.3–250.0)	5.4 ± 2.6	(3.0–8.3)
9	*Cryptichthys jojettae*	26.7 ± 8.1	(16.7–33.3)	84.1 ± 50.1	(35.8–140.0)	70.4 ± 16.5	(50.5–87.5)	114.5 ± 69.5	(40.0–183.2)	35.3 ± 26.8	(9.4–63.6)
10	*Enneapterygius gracilis*	73.1 ± 46.5	(19.4–100.0)	55.6 ± 32.0	(25.0–88.9)	46.6 ± 20.8	(25.0–66.7)	130.4 ± 26.9	(100.0–151.2)	16.4 ± 13.3	(5.5–31.3)
11	*Enneapterygius abeli*	19.2 ± 6.8	(13.3–28.6)	43.6 ± 7.2	(33.3–50.0)	42.4 ± 10.4	(33.3–52.8)	57.3 ± 35.5	(30.0–109.1)	10.0 ± 2.4	(6.7–12.3)
12	*Enneapterygius atrogulare*	28.6 ± 11.7	(17.2–42.9)	66.2 ± 9.9	(57.1–80.0)	40.4 ± 13.6	(28.6–60.0)	115.4 ± 40.8	(66.7–156.9)	9.8 ± 7.1	(4.3–20.0)
13	*Enneapterygius paucifaciatus*	66.8 ± 41.4	(20.3–100.0)	60.7 ± 20.0	(40.0–80.0)	39.3 ± 20.0	(20.0–60.0)	–		0	
14	*Enneapterygius rufopileus*	16.4 ± 5.7	(10.0–22.4)	43.3 ± 11.8	(33.3–56.6)	52.5 ± 9.8	(43.4–66.5)	120.4 ± 82.2	(40.0–235.6)	11.3 ± 3.2	(8.2–15.9)
15	*Enneapterygius ventermaculus*	4.9 ± 9.7	(0.0–19.4)	59.2 ± 27.5	(40.0–100.0)	45.5 ± 6.4	(40.0–51.8)	152.9 ± 66.3	(100.0–250.0)	11.6 ± 3.11	(8.0–14.3)
16	*Forsterygion flavonigrum*	27.3 ± 12.8	(20.0–46.5)	49.2 ± 7.7	(39.8–56.3)	53.5 ± 8.8	(45.6–61.9)	158.2 ± 19.6	(141.0–180.9)	16.2 ± 0.5	(15.7–16.8)
17	*Forsterygion lapillum*	19.1 ± 1.5	(17.2–21.5)	45.7 ± 2.7	(42.5–49.0)	54.5 ± 2.7	(51.7–59.0)	183.2 ± 32.1	(155.3–231.6)	7.4 ± 1.10	(5.9–8.8)
18	*Forsterygion malcolmi*	23.6 ± 3.7	(21.2–29.0)	38.9 ± 6.8	(29.4–44.5)	59.4 ± 6.6	(52.8–68.2)	139.0 ± 60.8	(87.7–227.3)	17.0 ± 10.9	(9.0–33.2)
19	*Forsterygion varium*	19.8 ± 1.5	(18.0–21.7)	45.5 ± 7.3	(38.7–55.7)	52.2 ± 7.4	(43.2–61.3)	165.4 ± 7.3	(160.0–177.3)	11.1 ± 2.1	(9.5–14.0)
20	*Forsterygion gymnota*	20.8 ± 1.8	(18.5–22.9)	47.2 ± 6.8	(40.0–54.2)	53.6 ± 2.6	(49.7–55.6)	130.4 ± 36.2	(103.3–181.3)	14.2 ± 2.6	(12.0–18.3)
21	*Forsterygion capito*	19.5 ± 4.8	(15.4–23.7)	47.5 ± 1.8	(45.4–50.0)	56.4 ± 8.4	(48.7–68.4)	206.9 ± 30.2	(163.9–234.8)	9.4 ± 3.4	(5.6–13.9)
22	*Forsterygion maryannae*	20.1 ± 2.2	(17.3–22.8)	36.5 ± 11.0	(25.8–46.9)	45.4 ± 10.9	(35.9–56.2)	142.8 ± 26.3	(116.7–177.6)	16.2 ± 5.8	(12.1–24.8)
23	*Forsterygion nigripenne*	36.5 ± 26.6	(20.0–76.3)	56.0 ± 10.1	(44.4–69.1)	49.8 ± 5.3	(44.8–55.8)	155.8 ± 14.8	(137.6–170.6)	9.5 ± 2.6	(6.9–12.6)
24	*Gilloblennius abditus*	45.2 ± 29.2	(19.9–81.8)	69.9 ± 25.6	(44.5–100.0)	62.9 ± 14.3	(46.5–76.9)	118.8 ± 38.9	(85.7–165.0)	35.1 ± 30.1	(9.1–73.7)
25	*Gilloblennius tripennis*	23.0 ± 14.2	(8.3–42.1)	40.3 ± 11.2	(27.3–52.6)	53.2 ± 12.0	(36.4–64.5)	146.9 ±	(100.0–250.0)	12.0 ±	(5.0–21.4)
26	*Helcogramma obtusirostris*	85.7 ± 19.1	(59.5–100.0)	–		–		–		0	
27	*Helcogramma springeri*	24.3 ± 6.6	(18.7–33.3)	59.4 ± 0.00	(53.8–66.7)	40.3 ± 0.00	(33.3–45.0)	103.5 ± 46.1	(50.0–164.0)	6.4 ± 2.4	(4.0–9.1)
28	*Helcogrammoides cunninghami*	25.2 ± 7.6	(17.4–33.3)	48.9 ± 5.4	(45.4–50.0)	58.5 ± 5.0	(50.0–66.7)	160.7 ± 63.8	(100.0–250.0)	12.7 ± 4.2	(8.0–16.7)
29	*Karalepis stewarti*	18.3 ± 5.1	(12.8–24.1)	52.6 ± 2.3	(50.9–54.5)	51.0 ± 7.5	(47.8–53.3)	279.3 ± 69.2	(228.6–400.0)	4.8 ± 1.9	(2.1–6.5)
30	*Lepidoblennius haplodactylus*	69.2 ± 42.7	(29.4–120.0)	20.8 ± 1.3	(17.6–24.1)	21.9 ± 2.3	(17.9–29.2)	238.4 ± 45.1	(187.5–277.8)	17.4 ± 17.4	(3.4–40.0)
31	*Matanui bathytaton*	22.7 ± 2.0	(20.4–25.0)	46.7 ± 3.4	(41.3–51.2)	58.4 ± 5.4	(47.8–72.2)	195.9 ± 66.5	(112.5–253.7)	13.0 ± 10.1	(5.7–27.6)
32	*Matanui profundum*	21.1 ± 7.5	(12.8–31.0)	41.6 ± 4.6	(10.0–64.7)	45.8 ± 10.2	(11.1–64.7)	315.0 ± 344	(114.3–830.0)	13.0 ± 6.5	(8.3–22.6)
33	*Notoclinus compressus*	23.5 ± 2.7	(20.8–26.2)	51.3 ± 5.2	(46.8–58.3)	51.4 ± 3.4	(46.4–53.6)	257.4 ± 52.2	(191.7–318.5)	8.5 ± 3.2	(6.1–13.0)
34	*Notoclinops caerulpunctus*	90.8 ± 152	(5.3–320.0)	43.3 ± 5.9	(37.0–51.1)	60.1 ± 4.6	(54.2–64.4)	122.8 ± 10.0	(109.9–133.3)	22.3 ± 5.1	(18.8–29.6)
35	*Notoclinops segmentatus*	72.2 ± 102	(18.6–226.4)	46.1 ± 6.7	(36.0–50.0)	46.1 ± 5.4	(38.0–49.0)	139.0 ± 19.1	(110.3–150.7)	11.7 ± 2.6	(9.0–15.3)
36	*Notoclinops yaldwyni*	19.1 ± 2.15	(17.1–22.1)	45.2 ± 4.9	(39.1–50.8)	50.0 ± 5.5	(42.0–54.7)	142.6 ± 16.3	(130.6–166.7)	17.5 ± 3.1	(13.2–20.7)
37	*Ruanoho whero*	29.6 ± 17.5	(18.7–55.6)	57.1 ± 16.9	(44.4–81.8)	56.1 ± 11.7	(45.5–72.7)	152.9 ± 34.5	(122.2–201.0)	20.9 ± 17.5	(9.1–47.4)
38	*Ruanoho decemdigitatus*	31.5 ± 20.7	(19.5–62.5)	56.4 ± 18.7	(44.2–84.2)	57.7 ± 10.8	(50.7–73.7)	126.7 ± 34.9	(75.0–152.4)	24.0 ± 19.9	(12.9–53.3)
39	*Trinorfolkia clarkei*	16.7 ± 6.2	(8.0–22.4)	57.0 ± 4.2	(53.8–63.0)	33.7 ± 13.4	(14.3–45.5)	305.9 ± 216	(150.7–625.0)	5.1 ± 2.7	(2.0–8.3)
40	*Tripterygion tartessicum*	17.5 ± 6.9	(8.0–23.7)	42.2 ± 13.4	(28.6–56.9)	36.5 ± 6.9	(28.6–44.1)	169.8 ± 53.9	(133.3–250.0)	4.8 ± 2.6	(2.5–8.0)
41	*Ucla xenogrammus*	25.1 ± 10.3	(16.9–40.0)	49.9 ± 11.7	(33.3–54.1)	38.3 ± 4.0	(33.3–43.1)	–		0	

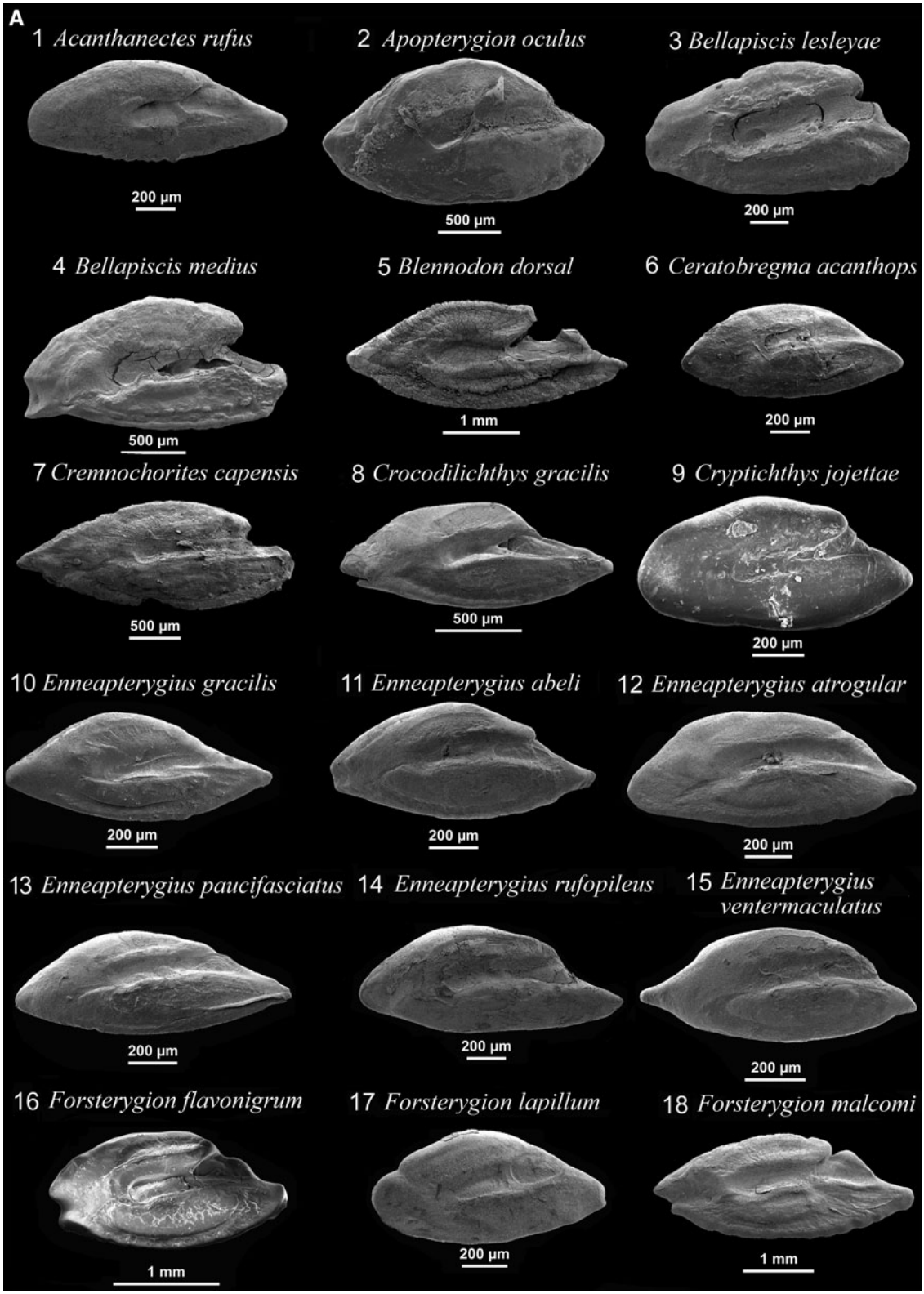

Fig. 2. Left saccular otoliths of different species of triplefins studied.

Volpedo & Fuchs, 2010; Jaramilo *et al.*, 2014; Avigliano *et al.*, 2015c), as an environmental indicator (Nelson *et al.*, 1994; Avigliano *et al.*, 2012, 2015c) and to determine fossilized specimens (e.g. Wirtz, 1976: Schwarzhans, 1980; Reichenbacher *et al.*, 2007). Among the most commonly used indexes are rectangularity, circularity, aspect ratio, OL/SuL (Burke *et al.*, 2008; Tuset *et al.*, 2008; Longmore *et al.*, 2010; Cañas *et al.*, 2012; Jaramilo *et al.*, 2014; Avigliano *et al.*, 2015c, among others), and recently, the RL/OL index has been widely used by various authors (Reichenbacher *et al.*, 2007, 2009; Teimori *et al.*, 2012a, b; Annabi *et al.*, 2013; Reichenbacher & Reichard, 2014,

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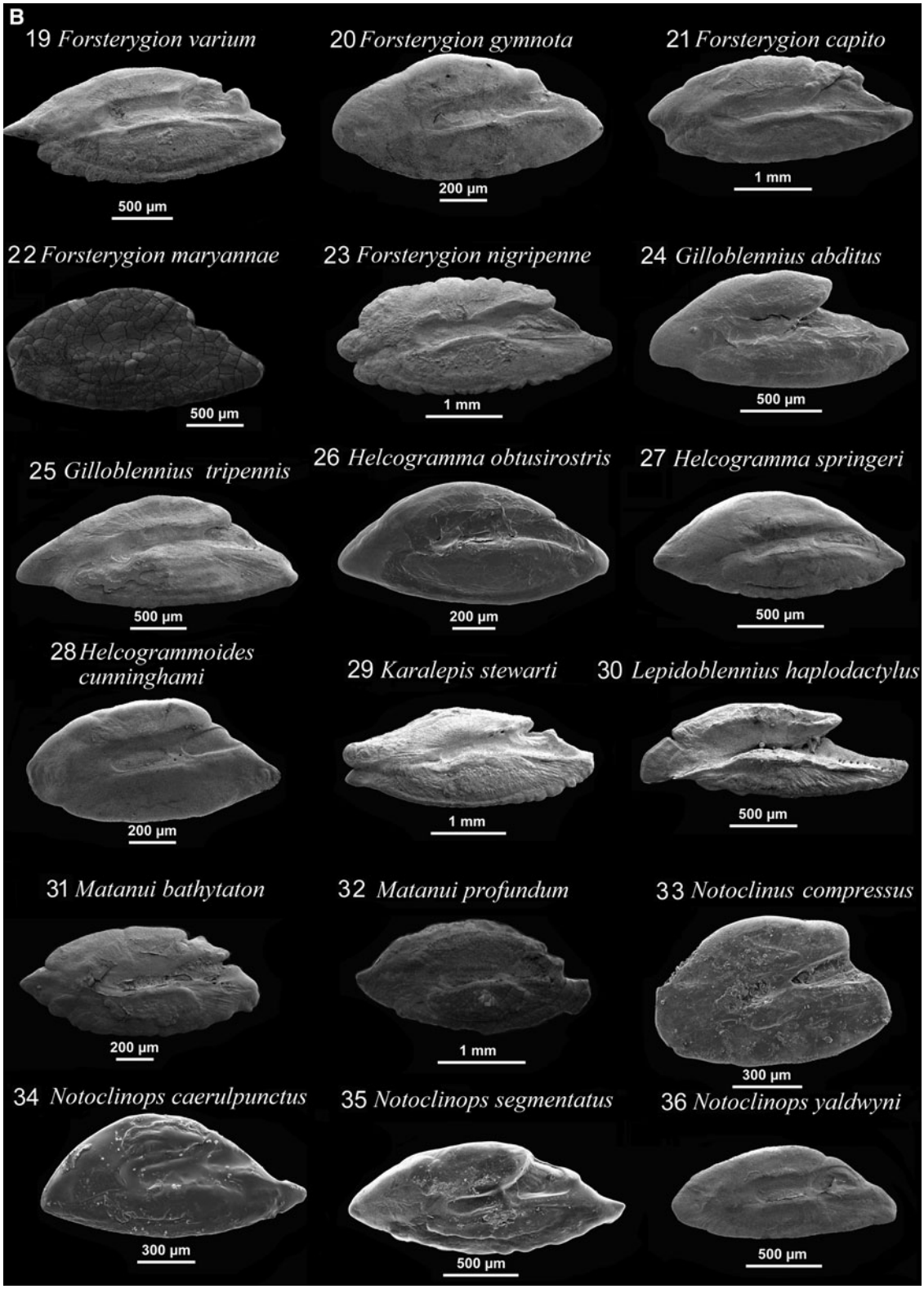

Fig. 2b. Continued

among others). Few studies use relationships based on the sulcus such as SS/OS (Gauldie, 1988, Lombarte, 1992; Avigliano *et al.*, 2014, 2015c; Jaramilo *et al.*, 2014; Zhuang *et al.*, 2014)

Commonly used indexes in this paper such as OL/SuL, circularity and rectangularity were not efficient to characterize the studied species (see PCA, Figure 3). However, the variables that explain the greatest proportion of variability were those associated with rostrum morphometry (RL/OL and RW/RL), OW/OL and the sulcus (CL/SuL, OSL/SuL and SS/OS) (Figure 3), with CL/SuL and OSL/SuL being used for the first time in this paper.

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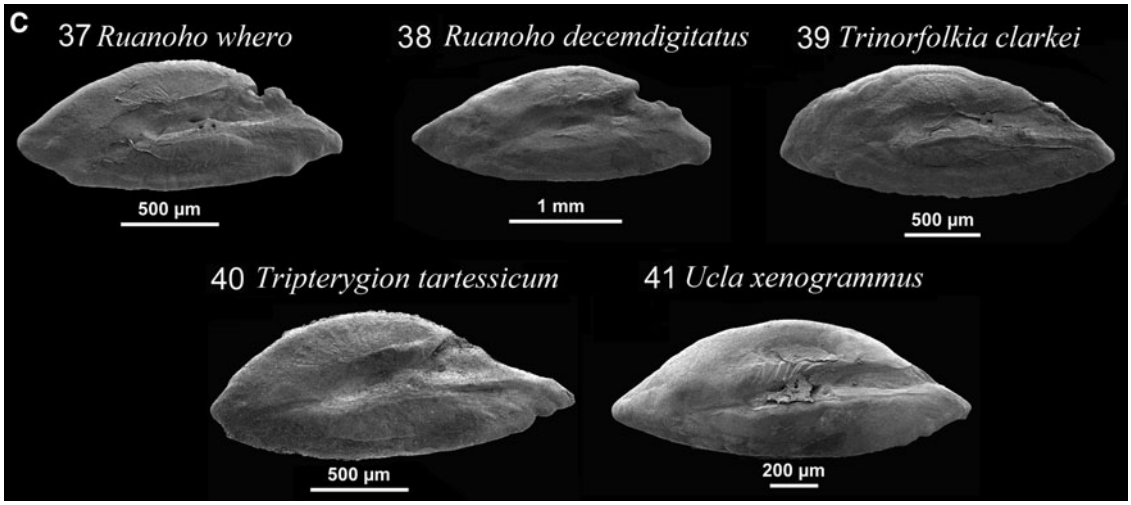

Fig. 2c. Continued

The members of *Enneapterygius* are represented with six species in this work: *E. abeli, E. atrogulare, E. rufopileus, E. ventermaculatus, E. paucifasciatus* and *E. gracilis*. The first four are grouped in the same subclade (4bi) (see Figure 4). *Enneapterygius abeli* is a cryptic and benthic species that can be found on rocky or coral tropical reefs amongst shallow photic waters (Longnecker & Langston, 2005). This species feeds mainly on benthic invertebrates (Longnecker & Langston, 2005). *Enneapterygius atrogulare* is found on intertidal and subtidal areas, specifically on reef surfaces usually in weedy areas, on algal-covered rocks or on rubble (Kuiter, 1993). It prefers silty habitats of upper regions usually on pylons, estuaries and harbours (Kuiter, 1993) and it feeds mainly on tiny invertebrates and algae (Randall *et al*., 1990). *Enneapterygius rufopileus* is a species that prefers cooler water and lives on large green or brown brain corals in shallow water and tidal pools (Fricke, 2002). It is common to find this species in beaches or rockpools with

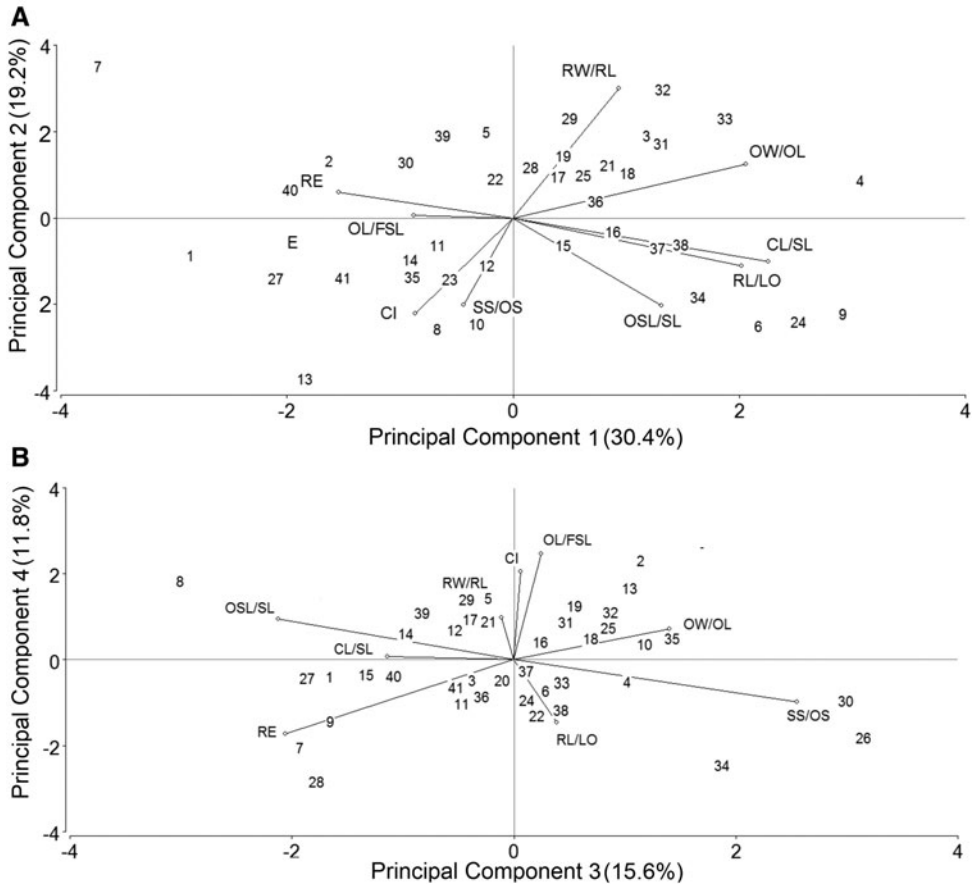

Fig. 3. Biplot on the first four principal components (PC) based on nine morphological indices of 41 species of Tripterygiidae. (A) PC1 *vs* PC2; (B) PC3 *vs* PC4. The species are indicated by numbers (Table 2). RE, rectangularity index; CI, circularity index.

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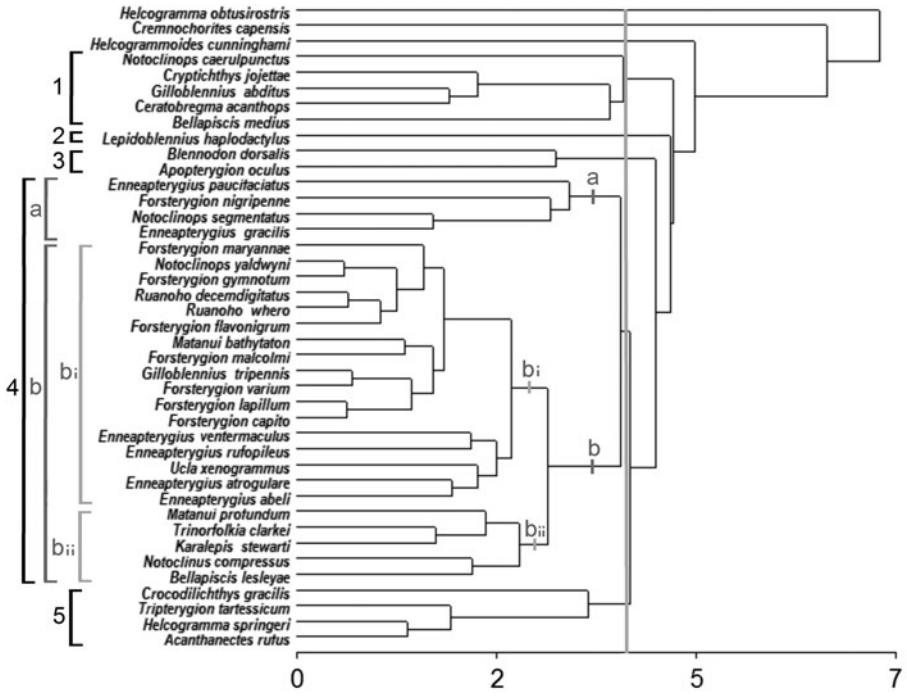

Fig. 4. UPGMA cluster based on morphological indices in 41 species of Tripterygiidae.

overhangs and the algae *Zonaria* sp. and *H. banksii*. *Enneapterygius ventermaculus* is a demersal fish of which little is known and usually inhabits depths below 1 m (Randall, 1995). Considering that *E. abeli*, *E. atrogulare, E. rufopileus* and *E. ventermaculatus* presented high similarity within the subgroup 4bi (Figure 4) and that they inhabit different microenvironments, the otolith morphometry is not reflecting differential use of environments.

Enneapterygius gracilis and *E. paucifasciatus* were the only studied species of the genus *Enneapterygius* that are not in subgroup 4bi (Figure 4) however, all studied indexes except those related to rostrum morphology were similar between these species. This similarity is reflected in the dendrogram (subgroup 4a) (Figure 4). Very little is known about the habitat of these two species. *Enneapterygius paucifasciatus* inhabits coral reefs in depths of 2–4 m (Fricke, 2002), while *E. gracilis* occurs in shallow tidal pools (depth range 0–15 m) and seems to be associated with coralline rocks and seagrass (Fricke, 1994). Once again, the otolith morphometry is not reflecting differential use of environments as happens in other species (Volpedo & Echeverría, 2003; Volpedo & Fuchs, 2010; Curcio *et al.*, 2014). On the other hand, another cause could be related to a great genetic influence. The previously mentioned group was characterized in this work specially by the low size of the rostrum (RL/OL) and there is solid evidence of genetic influence in relation to the size of the rostrum. For example, Reichenbacher *et al.* (2009), Teimori *et al.* (2012a, b) and Reichenbacher & Reichard (2014) have observed a strong correlation between their RL index and genetic factors in species of killifishes. Moreover, the study of Vignon & Morat (2010) performed with *Lutjanus kasmira* (Lutjanidae) also supports this hypothesis. The results on the species of *Ruanoho* also confirm that genetics appears to be more prominent than adaptation to environments in the otoliths of the studied family. According to genetic studies, *R. whero* and *R. decemdigitatus* are sister species (Wellenreuther *et al.*, 2007) however they make a differential use of the habitat and have otoliths with similar morphometric characteristics (Figure 4).

Forsterygion is represented in our study by eight species of which seven are grouped in the same clade (Figure 4, subgroup 4b) and have common ecological features such as dwelling on the top and sides of rocks at low to medium depth (Feary & Clements, 2006; Wellenreuther *et al.*, 2007). The morphometric differences found for *F. nigripenne*, a member of the other subgroup (Figure 4, subgroup 4a), would seem not to respond to genetic factors because studies made with three mitochondrial genes (12S, 16S and region control) and the nuclear gene (ETS2) show high similarity within the genus (Wellenreuther *et al.*, 2010).

Forsterygion nigripenne inhabits shallow estuarine habitats. Besides, it has peculiarities on its lateral line, implying that ecologically divergent species can be caused by a process of functional adaptation with the main selective pressure being the level of background hydrodynamic activity (Feary & Clements, 2006; Berger & Mayr, 1992; Wellenreuther *et al.*, 2010). These features suggest that in the case of *Forsterygion nigripenne* otoliths may reflect bioecological and not genetic differences.

It is interesting to consider that for example *Notoclinops* represented in this work with the species *N. caerulpunctus, N. segmentatus* and *N. yaldwyni* is fragmented into different groups and subgroups (Figure 4, group 1 and subgroup 4a,b). *Notoclinops caerulpunctus* lives at 10 m depth (Feary & Clements, 2006) while *N. segmentatus* and *N. yaldwyni* although sister species make a differential use of the habitat (Wellenreuther *et al.*, 2007). As was observed for *Forsterygion*, otolith morphometry seems to reflect the diversity of microenvironments used by the members of the genus *Notoaclinops*.

Furthermore the genera *Apopterygion, Cremnochorites, Crocodilichthys* and *Lepidoblennius* are isolated in the

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Table 3. Classification matrix of the CDA. The percentages in the last column represent the classification of each species. The species are indicated by numbers, as listed in Table 2. The current classifications for the individual species are marked in bold.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	%
1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	50
2	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	25
3	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	1	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
6	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	25
7	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	25
8	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	50
9	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	25
10	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
12	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	25
15	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
20	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	50
21	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0
25	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**
27	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	**2**	0	0	0	0	0	0	0	0	0	0	0	0	**66**
29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	**4**	0	0	0	0	0	0	0	0	0	0	0	**80**
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**4**	0	0	0	0	0	0	0	0	0	0	**100**
31	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	50
32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**4**	0	0	0	0	0	0	0	**100**
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	50
35	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
37	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	25
39	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
40	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	25
41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**4**	**100**
Total	7	3	2	4	6	5	1	3	5	4	6	1	2	3	1	3	5	4	5	8	6	4	3	4	5	4	4	4	7	5	4	1	2	5	4	0	3	4	3	5	5	

dendrogram (Figure 4) and could not be associated with any pattern in relation to morphometric indexes, these being very variable between them (Table 2).

This work makes it evident that the relationship between otolith morphometry and environmental or genetic factors is extremely complex and can vary between different genera and species.

The CDA allowed identification of only six of the 41 studied species. However, the power of discrimination of used indices may be underestimated due to the relatively small sizes of the collected sample.

The high number of species in the family Tripterygiidae and their wide geographic distribution make it difficult to obtain a larger number of samples. It is expected that a greater number of samples would allow more effective discrimination. However, the results presented are of great value because they make it possible to associate different morphometric indices with several species. Furthermore, the new CL/SuL and OSL/SuL indices were among the most important to discriminate species and could be evaluated for use in other groups of fishes.

In summary, this study shows for the first time a series of nine morphometric indices and high quality images. These data together with the previous explanation related to the morphological descriptions about some of the species studied in this paper (Jawad, 2007) result in an interesting tool for identifying some species of triplefin. This is of great value especially for palaeontological and taxonomic studies. Two new morphometric indices were also described and tested. It is highly useful to discriminate six species of triplefin. In addition the paper is a baseline for further research that needs to intensify studies aimed at separate groups of triplefin. For example, related methodologies such as the analysis of otolith edges (Parisi-Baradad *et al.*, 2005) could provide tools to separate different groups of triplefin that could not be individualized in this work.

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