Journal of Applied Ichthyology

J. Appl. Ichthyol. 31 (2015), 381–389

ISSN 0175–8659

Received: June 24, 2014
Accepted: July 12, 2014
doi: 10.1111/jai.12674

New insights to discriminate between *Sympterygia acuta* Garman 1877 and *Sympterygia bonapartii* Müller & Henle, 1841 (Rajidae) of the Southwest Atlantic Ocean: on the use of geometric morphometrics and spinulation patterns

By P. Orlando[1], M. González-Castro[2,3] and E. Mabragaña[2,3]

[1]*Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, República Argentina;* [2]*Grupo de Biotaxonomía Morfológica y Molecular de Peces (BIMOPE), IIMyC- CONICET, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Mar del Plata, República Argentina;* [3]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, República Argentina*

Summary

Geometric-morphometric and spinulation pattern approaches were employed to assess the discrimination of two species of *Sympterygia* skates in the Southwest Atlantic: *Sympterygia acuta* Garman 1877 and *Sympterygia bonapartii* Müller & Henle, 1841. Two types of variables were employed: linear morphometrics measurements (LMMs), and interlandmark distances (IlDs). The Principal Component Analysis (PCA) and the discriminant analysis (DA) do not overlap between the species. Morphometrically, *S. bonapartii* is characterized by a wider disc, larger pelvic fins, larger eyes, a greater distance between nostrils and between the first pair of gill slits, a greater interorbital distance between spiracles, and a wider mouth. On the other hand, *S. acuta* has a larger disc due to the snout size. Regarding the allometric coefficient analysis performed on the IlDs, *S. bonapartii* showed a positive allometry for the variables that comprise the Box-Truss 3 (Ventral protocol), which can be related to the mouth width and the first gill slits. This growth type is consistent with those observed in the Box-Truss 3 (Outline protocol), which is related to the disc width. It has been interpreted that both structures could develop together. In opposition, *S. acuta* showed isometric growth for the above-mentioned variables; on the other hand, *S. acuta* showed positive allometry for all variables that defined the snout in the three protocols, indicating that the snout has a distinctive growth relative to the size through ontogeny. *S. bonapartii*, however, showed an isometric snout growth. The remaining variables that defined the morphogeometrical protocols displayed the same type of growth. Regarding spinulation, the thorns and dermal denticles proved to be useful to discriminate both species. Thorns of the caudal region were large and oval with smooth edges in *S. bonapartii*, and oval with lobed edges in *S. acuta*. Dermal denticles in *S. acuta* also presented two elongated ridges with a third small ridge between them, whereas those of *S. bonapartii* presented three elongated ridges of the same size.

Introduction

Skates (Family Rajidae), a group of cartilaginous fishes with benthic habits, are oviparous and present in almost all oceans, from the Arctic to the waters of Antarctica. Like all batoids, members of this family have a compressed disk with pectoral fins fused to the sides of the head, but possess a slender tail and small dorsal and caudal fins. The dorsal body surface is covered with small placoids scales. Some 245 skate species are described worldwide, with about 27 genera (Ebert and Compagno, 2007).

The genus *Sympterygia* is represented by two species in the Southwest Atlantic: the bignose fanskate *Sympterygia acuta* Garman 1877, and the smallnose fanskate *Sympterygia bonapartii* Müller & Henle, 1841. Several taxonomic studies have been conducted on these species (Menni, 1972, 1973; McEachran, 1982; Cousseau et al., 2007), which offer external and internal diagnostic characteristics to differentiate both species. However, the dermal denticles and thorns have never been studied thoroughly. These elements are known to be taxonomically valuable to differentiate species (Gravendeel et al., 2002), and can possibly be very useful in identifying the prey items of top predators.

Morphometric techniques have 'evolved' within the last few decades, with the introduction of promissory methods for archiving the shapes of organisms (Rohlf and Marcus, 1993; González-Castro et al., 2012). With inclusion of the concept of a 'homologous landmark' (true anatomical points identified by some consistent features of the local morphology), Strauss and Bookstein (1982) proposed a geometric protocol for character selection, the 'box-truss network', which largely overcomes the disadvantages of traditional datasets (e.g. characters aligned along one axis; coverage of forms that are highly uneven by region; repeated use of some morphological landmarks, etc.). A box-truss usually constitutes four landmarks and their inter-landmark distances.

Investigations employing morphogeometrical protocols in Elasmobranchii are scarce. Jacob and McEachran (1994) applied the box-truss to compare two species of *Dipturus* from the western North Atlantic; however, they employed landmarks located only on the outline of the body and did not note information related to size. Cavalcanti (2004) set up a box truss composed of eight landmarks in order to discriminate the head shape of *Sphyrna* genus sharks. Torres Palacios (2010) also used morphometry for taxonomic studies on stingrays of the genus *Urotrygon*. To date, there are no morphogeometrical studies on skates in the southwest Atlantic.

The snout length in relation to the total length commonly differentiates *Sympterygia acuta* from *S. bonapartii* as well as their coloration and spinulation patterns (Menni, 1973; Cousseau et al., 2007). Other morphological and skeletal characteristics were also registered by McEachran (1982) to characterize both species; however, they were neither statistically nor morphometrically analyzed.

In morphometrics, size must be considered as a contingent source of variability since it is associated with individual growth; the aim of such studies is usually focused on shape that must be size-free ('size-free shape'). In the general case of allometric growth, there is a shape variation related to a size variation (Lleonart et al., 2000). The influence of size due to allometric growth should be removed by appropriate statistical procedures (Lleonart et al., 2000; González-Castro et al., 2008, 2012). Lleonart et al. (2000) presented a technique to scale the data exhibiting allometric growth; this technique eliminates information related to size and adjusts for the new shape they would have in their new size according to the allometry. In this sense, morphogeometrical studies associated with a normalization technique are suitable to answer these concerns (Heras et al., 2006; González-Castro et al., 2008, 2012; Sabadín et al., 2010; Díaz de Astarloa et al., 2011).

The aims of this study were to assess: (i) the interspecific body-shape differences between the *Sympterygia* species, *S. acuta* and *S. bonapartii,* that inhabit Argentine waters; (ii) the character of the allometric coefficients of the morphometric variables in both species; (iii) the weight-length relationships for both species; and (iv) the taxonomic value of thorns and dermal denticles to differentiate *S. acuta* from *S. bonapartii*.

Materials and methods

Sampling

Specimens of *Sympterygia acuta* (n = 71) and *S. bonapartii* (n = 130) were collected with a 6 m long shrimp net (mesh size 50 mm in the wings, 20 mm in the cod end) by trawl fishing at the coast near Mar Chiquita coastal lagoon (37°46′S, 57°27′W), and Mar del Plata (37°59′S, 57°24′58W), Argentina, in 2011 and 2012. Fish were frozen and transported to the laboratory where they were identified, measured, sexed and weighed.

Morphometry

Two types of variables were employed: linear morphometric measurements (LMMs), and inter-landmark distances (ILDs).

Morphometric analysis based on LMMs

Using dial calipers to the nearest 0.1 mm, eight LMMs were measured on the dorsal side and seven LMMs on the ventral side of specimens, following Last et al., 2008. Dorsal variables were: total length (Tl); disc width (Dw); disc length (Dl); snout to maximum width (S-m); pre-orbital distance (Pd); head length (Hl); eye diameter (El); interorbital distance (Il). Ventral variables were: distance snout-cloaca (S-N); distance cloaca-caudal fin (C-C); preoral length (Po); prenasal length (Pn); pelvic fin length (Pel); Clasper length (Cl); Distance cloaca-clasper (Dcc). Morphometric characters were organized by species. A normalization technique to scale the data that exhibited allometric growth was employed following Lleonart et al. (2000), a method derived from theoretical equations of allometric growth and completely removing all information related to size, not only scaling all individuals to the same size, but also adjusting their shape to a standard form according to allometry. Total length was used as the independent variable, while the remaining fourteen variables were considered as dependent. X_o represents a reference value of size (X_o = 380 mm) to which all individuals are either reduced or amplified (Lombarte and Lleonart, 1993; Ibáñez-Aguirre and Lleonart, 1996; González-Castro et al., 2008, 2012). After transformation, a Principal Component Analysis (PCA) was performed using the R software (R Development Core Team, 2012). The principal component scores (PCs) were then submitted to cross-validate discriminant analysis (DA) using PASW Statistic version 18.0, in order to build a predictive model of group membership based on the observed characteristics of each case.

Morphometric analysis based on IlDs

Three different morpho-geometrical protocols were performed, employing different Box Truss network sites (Strauss and Bookstein, 1982; González-Castro, 2007; González-Castro et al., 2012):

Ventral protocol (VP), based on nine landmarks, with eighteen IlDs defined: (1–2, 1–3, 2–3, 2–4, 2–5, 3–4, 3–5, 4–5, 4–6, 4–7, 5–6, 5–7, 6–7, 6–8, 6–9, 7–8, 7–9, and 8–9) (Fig. 1a, Table 1). Four box trusses constituted this protocol.

Dorsal protocol (DP), with seven landmarks and thirteen inter-landmarks distances taken: 1–2, 1–3, 2–3, 2–4, 2–5, 3–4, 3–5, 4–5, 4–6, 4–7, 5–6, 5–7, and 6–7, with a total of three box trusses (Fig. 1b, Table 1).

Outline protocol (OP), defined for thirteen landmarks, covered the outline of the specimens (Table 1). Twenty-eight inter-landmarks distances were registered: 1–2, 1–3, 2–3, 2–4, 2–5, 3–4, 3–5, 4–5, 4–6, 4–7, 5–6, 5–7, 6–7, 6–8, 6–9, 7–8, 7–9, 8–9, 8–10, 8–11, 9–10, 9–11, 10–11, 10–12, 10–13, 11–12, 11–13, and 12–13. Six box trusses formed this protocol (Fig. 1c). The inter-landmark distances were measured with a digital caliper.

As in the LMM analysis, the normalization technique of Lleonart et al. (2000) was employed. Three different L_{to} or X_o were used. For the ventral protocol, the length from snout to cloaca (X_o = 200 mm) was chosen as the independent variable (Fig. 1a). For the dorsal protocol, the independent variable was the head length (X_o = 80 mm) (Fig. 1b); for the other outline protocol (Fig. 1c) the total length was considered to be the independent variable (X_o = 380 mm). After this transformation, a new matrix with the normalized results was made, and a principal component analysis (PCA) was performed. Finally, principal component scores (PCs) were used to cross-validate discriminant analysis (DA).

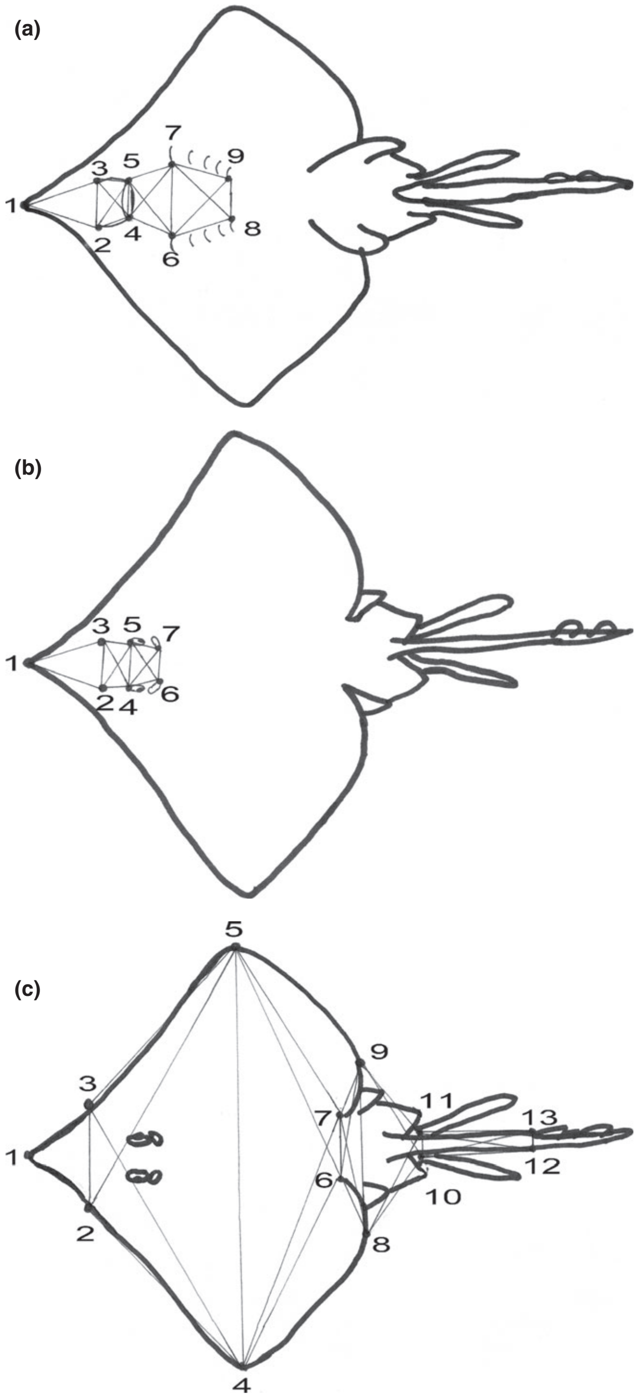

Fig. 1. Truss network landmarks for morphometric analyses in *Sympterygia bonapartii* and *S. acuta*. (a) Ventral protocol; (b) Dorsal protocol; (c) Outline protocol

Weight-length relationships

The equation $W = a*L^b$ expresses the relationship between total length (Tl) and total weight (W) for almost all fish species. Growth type of the two *Sympterygia* species was identified using Fisher's test to ascertain if parameter *b* with its confidence ($\alpha = 0.05$) covers three, or is significantly different from three.

Analysis of allometric coefficients

For the analysis of the allometric coefficients of all variables (LMM and IlDs), a different matrix was constructed, where the columns represent the values of each ln transformed-variable, and the rows represent the individuals. In all cases the total length was taken as the independent variable, while the remainder were considered dependent variables.

Hypothesis test for isometry:

With the equation $Y = a\ X^b$ that linearized $\ln(Y) = \ln(a) + b\ \ln(X)$, the null hypothesis Ho: $b = 1$ with the alternative H1: $b \neq 1$ was tested. For this, the statistic $t = (b-1)/s(b)$, where s(b) is the standard deviation of *b* was calculated. The absolute value of $|t|$ is compared with the critical value t (n-2, 1-α/2) of a t-distribution with n-2 degrees of freedom.

Identification of dermal denticles and thorns

Five adult individuals of each species were selected. A 4 cm^2 piece of skin from different body regions was extracted from each specimen (Gravendeel et al., 2002). After maceration of the skin, the dermal denticles and the thorns were removed. These structures were recovered by decantation and then dried. Denticles were observed with an Arcano (40×) microscope and digital photographs were taken with an Arcano 9 mega pixel digital camera (Wuxi, China) attached to the binoculars with a 40× video-zoom connector. Thorns were photographed with a Nikon CoolPix P120 (Indonesia).

For identification of dermal denticles and thorns, the methodology and areas proposed by Gravendeel et al. (2002) were followed. The midline thorns were separated into two different sub-zones: the disc region and the caudal region.

Results

Morphometry

The mean, range and standard deviation of the different morphometric variables employed for both species of *Sympterygia* are summarized in Table 2.

Morphometric analysis based on LMMs

The PCA of the correlation matrix generated by the normalization procedure produced two eigenvalues <1 (Table 3). Correlations between variables and components <0.6 were considered significant (data not shown). The first two PCs explain more than 70% of the total variance in the data (Table 3). The PCA based on LLMs allowed the differentiation between *Sympterygia acuta* and *S. bonapartii*, as well as the characterization of their particular shapes. In this respect, *S. acuta* showed higher loadings for the variables Pn, Pd, Hl, but also in a minor way DL, S-m and S-N. *S. bonapartii*, however, showed higher loadings for the variables DW, Id, E and Pel. *S. bonapartii* also showed low values for the variables that characterized *S. acuta* (Fig. 2a). The DA correctly classified 100% of the fish according to body shape.

Table 1
Anatomical location of landmarks selected for the three protocols employed

Landmarks	Ventral protocol (VP)	Dorsal protocol (DP)	Outline protocol (OP)
1	Tip of snout.	Tip of snout.	Tip of snout.
2	Lateral posterior edge of the left nostril.	Anterior end of the left side of the braincase.	Point that indicates the beginning of the braincase in the left outline of the fish.
3	Lateral posterior edge of the right nostril.	Anterior end of the right side of the braincase.	Point that indicates the beginning of the braincase in the right outline of the fish.
4	Left corner of the mouth.	Upper edge of the left eye.	Point of greater convexity on the left side of the fish (maximum width of the disc).
5	Right corner of the mouth.	Upper edge of the right eye.	Point of greater convexity on the right side of the fish (maximum width of the disc).
6	Inner edge of the first left gill slits.	Posterior corner of the left spiracle.	Left internal notch of the disc.
7	Inner edge of the first right gill slits.	Posterior corner of the right spiracle.	Right internal notch of the disc
8	Inner edge of the fifth left gill slits.		Point of maximum convexity on the caudal portion of the disc of the left side of the fish.
9	Inner edge of the fifth right gill slits.		Point of maximum convexity on the caudal portion of the disc of the right side of the fish.
10			Left side of the tail at the end of the pelvic fin.
11			Right side of the tail at the end of the pelvic fin.
12			Point that mark the beginning of the first dorsal fin to the left side of the tail.
13			Point that mark the beginning of the first dorsal fin to the right side of the tail.

Morphometric analysis based on IlDs

Ventral protocol. PCA of the correlation matrix, generated by normalization procedure, produced two eigenvalues <1 (Table 3). The first two PCs explained more than 74% of the variance in the data (Table 3). Correlations between variables and components <0.6 were considered as significant (data not shown). The PCA based on inter-landmarks distances (ventral protocol) allowed a clear differentiation of *Sympterygia acuta* and *S. bonapartii* (Fig. 2b). *S. bonapartii* has a wider mouth, a greater distance between nostrils and between the first pair of gill slits (box truss 2, 3 and 4). On the other hand, *S. acuta* has a longer snout (box truss 1). The DA correctly classified 100% of the fish according to body shape.

Dorsal protocol. PCA of the correlation matrix generated by normalization procedure, produced one PC with eigenvalue <1 (Table 3). The first two PCs explained more than 90% of the variance in the data (Table 3). Correlations between variables and components <0.6 were considered significant (data not shown). The PCA based on inter-landmark distances (dorsal protocol) allowed a clear differentiation of *Sympterygia acuta* and *S. bonapartii* (Fig. 2c). *S. bonapartii* has greater interorbital and interspiracular distances (box truss 2 and 3), whereas *S. acuta* has a longer snout (box truss 1) and lower loadings for the interorbital and interspiracular distances. The DA correctly classified 100% of the fish according to body shape.

Outline protocol. The twenty-eight normalized IlDs analyzed by the PCA of the correlation matrix, produced four eigenvalues <1 (Table 3). The first four PCs explained more than 80% of the data variance (Table 3). Correlations between variables and components <0.6 were considered significant (data not shown). The PCA based on inter-landmark distances (outline protocol) allowed a clear differentiation of *Sympterygia acuta* and *S. bonapartii* (Fig. 2d). *S. bonapartii* has a wider disc (box trusses 2, 3 and 4) and has larger pelvic fins (box truss 5). Furthermore, the dorsal fin was inserted posteriorly in the tail (data not shown), whereas *S. acuta* has a longer snout (box truss 1) and lower loadings for the variables that otherwise characterize *S. bonapartii*. The DA correctly classified 100% of the fish according to body shape.

Weight-length relationships

The weight-length relationships for both species showed the best fit to the potential function ($Pt = a*Lt^b$). High regression coefficients were obtained ($r^2 > 0.98$). The Fisher-test revealed significant differences ($p = 0.05$) between *S. acuta* and *S. bonapartii* (Fig. 3). Estimates of parameter *b* and growth type were statistically different between both species, with b = 3.14 and b = 3.23 for *S. acuta* and *S. bonapartii,* respectively.

Allometric coefficients analysis

Morphometric analysis based on LMMs. All analyzed LMMs showed a positive allometry for both species, with the exception of E and C-C, which displayed negative allometry. Remarkably, DW was isometric in *S. acuta* but positively allometric in *S. bonapartii.*

Morphometric analysis based on IlDs. Ventral protocol: The variables 1–2 and 1–3 showed a positive allometry for both species. Remarkably, 2–3 was negatively allometric in *S. acuta* but isometric in *S. bonapartii* (box truss 1). All variables

Table 2
Mean, range and standard deviation of the different morphometric variables employed for both species of *Sympterygia*

	Sympterygia acuta				*Sympterygia bonapartii*			
Variables	N	Range	Mean	SD	N	Range	Mean	SD
LMMs (%Tl)								
Total length (Tl) (mm)	71	118–578	413.5	97.1	130	0.60–0.94	396.1	108.4
Disc width	71	0.54–0.79	0.585	0.030	130	0.60–0.95	0.683	0.034
Disc length	71	0.45–0.58	0.540	0.025	130	0.46–0.72	0.528	0.030
Snout to maximum width	71	0.23–0.37	0.317	0.025	130	0.21–0.33	0.275	0.023
Pre-orbital distance	71	0.06–0.26	0.211	0.026	130	0.13–0.17	0.147	0.008
Head length (Hl)	71	0.22–0.30	0.269	0.017	130	0.79–0.24	0.209	0.009
Eye diameter	71	0.02–0.2	0.022	0.022	130	0.02–0.04	0.026	0.003
Distance snout-cloaca (S-N)	71	0.44–0.57	0.526	0.025	130	0.42–0.55	0.491	0.023
Distance cloaca-caudal fin	71	0.29–0.56	0.472	0.033	130	0.45–0.58	0.509	0.023
Preoral length	71	0.14–0.23	0.199	0.017	130	0.07–1.36	0.141	0.109
Prenasal length	71	0.12–0.21	0.179	0.015	130	0.1–0.13	0.113	0.006
Pelvic fin length	71	0.15–0.21	0.172	0.012	130	0.12–0.21	0.176	0.015
Clasper length	33	0.01–0.17	0.074	0.066	61	0.004–0.14	0.027	0.034
Distance cloaca-clasper	33	0.09–0.30	0.170	0.075	61	0.07–0.26	0.125	0.034
Interorbital distance	71	0.05–0.06	0.055	0.003	130	0.04–5.4	0.097	0.472
IlDs (Ventral Protocol) (% S-N)								
1–2	71	0.32–0.39	0.359	0.016	130	0.22–0.3	0.26	0.01
1–3	71	0.31–0.39	0.358	0.017	130	0.22–0.3	0.25	0.01
2–3	71	0.12–0.19	0.140	0.012	130	0.15–0.2	0.17	0.01
2–4	71	0.05–0.09	0.066	0.007	130	0.06–0.09	0.07	0.00
3–5	71	0.05–0.08	0.064	0.006	130	0.07–0.09	0.07	0.00
4–5	71	0.1–0.15	0.122	0.009	130	0.12–0.16	0.13	0.01
4–6	71	0.11–0.15	0.127	0.008	130	0.13–0.17	0.15	0.01
4–7	71	0.2–0.24	0.219	0.010	130	0.22–0.28	0.25	0.01
5–6	71	0.2–0.25	0.221	0.010	130	0.09–0.28	0.25	0.02
5–7	71	0.1–0.14	0.125	0.007	130	0.13–0.17	0.15	0.01
6–7	71	0.24–0.37	0.275	0.017	130	0.25–0.33	0.30	0.01
6–8	71	0.12–0.16	0.136	0.007	130	0.13–0.17	0.15	0.01
6–9	71	0.23–0.28	0.256	0.010	130	0.25–0.32	0.27	0.01
7–8	71	0.23–0.28	0.258	0.010	130	0.25–0.31	0.28	0.01
7–9	71	0.12–0.16	0.137	0.008	130	0.13–0.18	0.15	0.01
8–9	71	0.14–0.2	0.175	0.010	130	0.15–0.21	0.18	0.01
IlDs (Dorsal Protocol) (% Hl)								
1–2	71	0.55–0.72	0.665	0.030	130	0.54–0.67	0.599	0.021
1–3	71	0.52–0.72	0.666	0.032	130	0.54–0.65	0.594	0.019
2–3	71	0.27–0.51	0.331	0.036	130	0.35–0.45	0.392	0.020
2–4	71	0.14–0.21	0.161	0.020	130	0.15–0.20	0.176	0.010
2–5	71	0.29–0.46	0.330	0.030	130	0.34–0.43	0.389	0.019
4–5	71	0.22–0.38	0.255	0.024	130	0.26–0.37	0.316	0.022
4–6	71	0.10–0.20	0.129	0.014	130	0.31–0.4	0.352	0.017
4–7	71	0.25–0.41	0.283	0.025	130	0.51–0.65	0.586	0.024
5–6	71	0.25–0.4	0.284	0.024	130	0.22–0.64	0.583	0.040
5–7	71	0.11–0.19	0.127	0.014	130	0.31–0.4	0.352	0.017
6–7	71	0.22–035	0.256	0.023	130	0.59–0.76	0.700	0.030
IlDs (Outline Protocol) (%Tl)								
1–2	71	0.18–0.28	0.226	0.018	130	0.15–0.22	0.191	0.012
1–3	71	0.18–0.27	0.225	0.017	130	0.15–0.22	0.190	0.013
2–3	71	0.24–0.39	0.296	0.024	130	0.25–0.4	0.323	0.024
2–4	71	0.15–0.25	0.203	0.017	130	0.18–0.29	0.244	0.018
2–5	71	0.43–0.51	0.460	0.016	130	0.48–0.6	0.529	0.021
3–4	71	0.43–0.51	0.462	0.016	130	0.47–0.59	0.530	0.020
4–6	71	0.27–0.35	0.297	0.012	130	0.29–0.39	0.345	0.015
4–7	71	0.38–0.42	0.401	0.012	130	0.37–0.52	0.449	0.021
5–6	71	0.38–0.42	0.401	0.011	130	0.39–0.52	0.449	0.020
5–7	71	0.28–0.32	0.298	0.010	130	0.31–0.4	0.346	0.015
6–7	71	0.11–0.15	0.126	0.009	130	0.1–0.15	0.121	0.010
6–8	71	0.05–0.09	0.075	0.009	130	0.08–0.12	0.097	0.008
6–9	71	0.17–0.21	0.189	0.009	130	0.17–0.24	0.203	0.013
7–8	71	0.17–0.21	0.189	0.009	130	0.17–0.24	0.202	0.013
7–9	71	0.05–0.09	0.073	0.008	130	0.08–0.12	0.096	0.008
8–9	71	0.12–0.28	0.237	0.021	130	0.2–0.32	0.260	0.022
8–10	71	0.11–0.17	0.141	0.013	130	0.11–0.18	0.144	0.016

Table 2
(Continued)

	Sympterygia acuta				*Sympterygia bonapartii*			
Variables	N	Range	Mean	SD	N	Range	Mean	SD
8–11	71	0.15–0.20	0.177	0.012	130	0.14–0.22	0.178	0.015
9–10	71	0.16–0.20	0.180	0.011	130	0.14–0.22	0.180	0.016
9–11	71	0.12–0.17	0.143	0.012	130	0.11–0.18	0.145	0.016
10–11	71	0.03–0.06	0.050	0.005	130	0.03–0.06	0.044	0.004
10–12	71	0.19–0.31	0.235	0.025	130	0.19–0.34	0.241	0.024
10–13	71	0.19–0.31	0.238	0.025	130	0.20–0.34	0.244	0.024
11–12	71	0.19–0.31	0.239	0.025	130	0.20–0.34	0.244	0.024
11–13	71	0.19–0.31	0.235	0.025	130	0.19–0.34	0.241	0.024
12–13	71	0.02–0.03	0.022	0.002	130	0.02–0.03	0.027	0.003

Table 3
Eigenvalues (<1) and cumulative variance obtained in the Principal Components Analysis

	Linear morphometrics measurements		Inter-landmark distances							
			Ventral protocol		Dorsal protocol		Outline protocol			
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC3	PC4
Eigenvalues	6.057	2.432	11.862	1.536	11.49	–	12.524	5.607	3.421	2.222
Cumulative variance	50.5	70.7	65.9	74.4	88.4	93.2	44.7	64.7	77	85

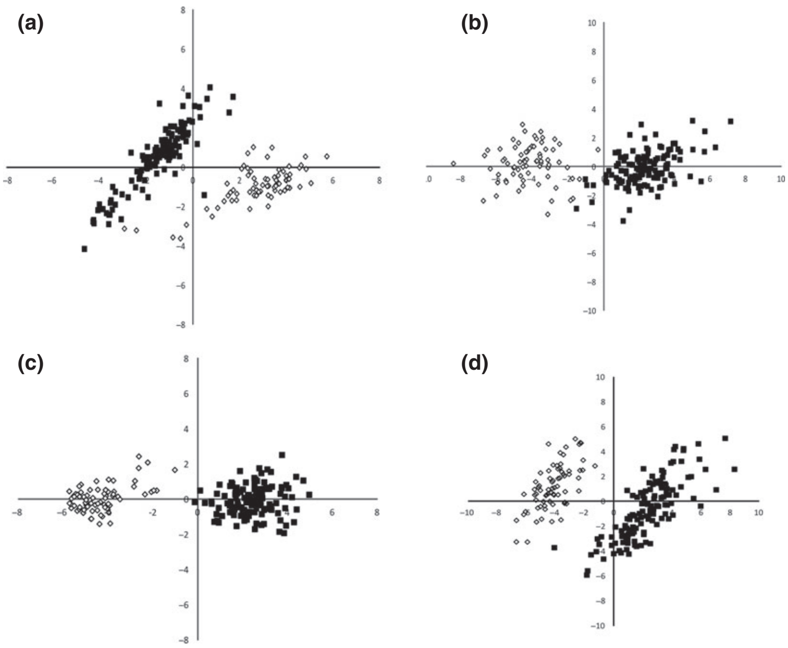

Fig. 2. *Sympterygia bonapartii* (black squares) and *Sympterygia acuta* (white diamonds). (a) Principal component (PC) analysis (PC1 vs PC2) based on linear morphometric measurements. (b) Principal component (PC) analysis (PC1 vs PC2) based on inter-landmarks distances (ventral protocol). (c) Principal component (PC) analysis (PC1 vs PC2) based on inter-landmarks distances (dorsal protocol). (d) Principal component (PC) analysis (PC1 vs PC2) based on inter-landmarks distances (outline protocol)

that constitute box trusses 2, 3 and 4 showed a positive allometry for both species, except for the 4–5 and 6–7 variables, which displayed isometry in *S. acuta*.

Dorsal protocol: All variables that constitute the 2nd and 3rd box truss showed a negative allometry for both species with the exception of 2–3, which displayed positive allometry. Remarkably, 1–2 and 1–3 (which determine the distances between the beginning of the braincase and the snout) were positively allometric in *S. acuta* but isometric in *S. bonapartii.*

Outline protocol: The variables 1–2 and 1–3 (which characterize the snout shape) displayed a positive allometry in *S. acuta*, but were isometric in *S. bonapartii*. Remarkably, the 2–3 variables showed negative allometry in both species (box truss 1). All variables that constitute box truss 3 showed isometry for *S. acuta*, and positive allometry for

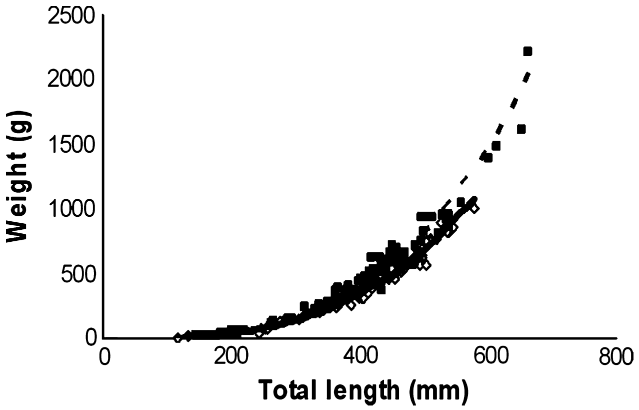

Fig. 3. Weight-length relationships: *S. acuta* (white diamonds, solid line) and *S. bonapartii* (black squares, dotted line)

S. bonapartii. Both species had negative allometry in 6–8 and 7–9 and isometry in 6–9, 7–8 and 8–9 (box truss 4). All variables that constitute box truss 5 (which represent the shape of the pelvic fin) showed a positive allometry for both species, with the exception of 10–11 (which is the width of the tail at the level of the caudal portion of the pelvic fins). The last one showed negative allometry in *S. acuta*, but was isometric in *S. bonapartii*. All variables that constitute box truss 6 (which represents the shape of the tail) showed a negative allometry for both species, with the exception of 12–13, which displayed isometry in *S. bonapartii*.

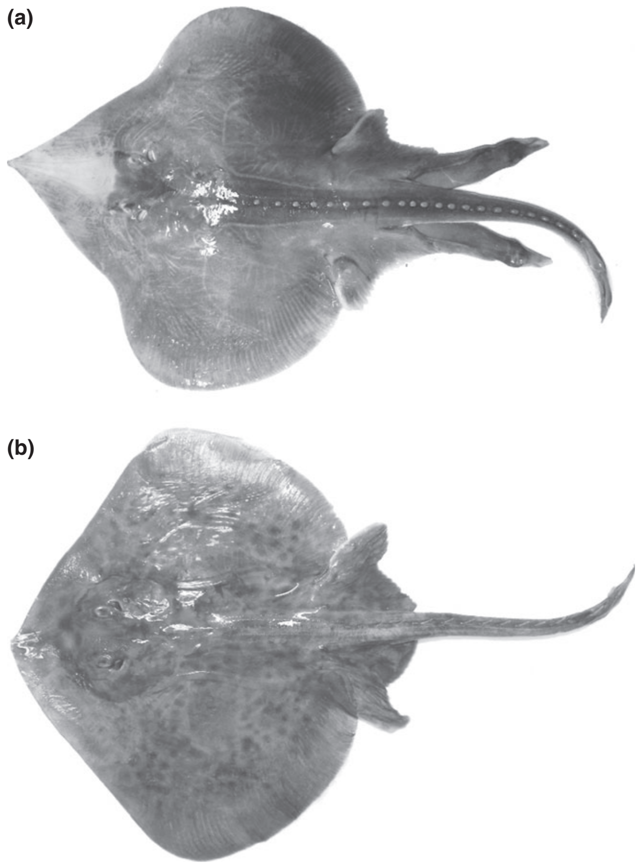

Fig. 4. (a) Male *Sympterygia acuta*, 535 TL. (b) Female *Sympterygia bonapartii*, 417 mm TL

Identification of dermal denticles and thorns

Sympterygia acuta

S. acuta has minute denticles at the anterior edge of the disc. The species has a row of 18–27 thorns from the scapular region to the first dorsal fin, and one thorn between the dorsal fins (Fig. 4a). No orbital thorns are present. In adult males, up to five rows of alar thorns were observed.

Thorns in the midline of the disc region were oval, with lobed edges. The crown was implanted vertically in the posterior part of the basal plate (Fig. 5a). Thorns in the caudal region were similar to those mentioned above, but the crown was implanted centrally in the basal plate. The crown did not exceed the basal plate (Fig. 5a). The anterior spike and the spoon of the alar thorns were clearly visible. The spoon has lobed edges. Both structures have a foramen, which was clearly separated. The crown length measured about ¼ of the total length of the posterior thorn region (Fig. 5a). Denticles of the rostral and malar regions have bilateral symmetry and presented two elongated ridges at an angle of about 90°, with respect to the longitudinal axis. They also presented a third, smaller ridge. The crown was long and vertically implanted (Fig. 5a).

Sympterygia bonapartii

Sympterygia bonapartii has an outer disc edge with densely arranged denticles. There are two thorns on the inner edge of each eye (orbitals), and an incomplete line of 11–18 thorns in the scapular region to the first dorsal fin, and one thorn between the dorsal fins (Fig. 4b).

The orbital thorns have two incisions in the anterior region of the basal plate, which also have a triangular incision in the posterior region (ventral view). The crown is implanted vertically in the posterior part of the basal plate (Fig 5b). Thorns in the midline region are oval, with lobed edges. The crown is implanted vertically in the posterior part of the basal plate (Fig. 5b). In the caudal region, the thorns are oval, much longer than wide, with smooth edges. The crown is implanted vertically and centrally in the basal plate. The crown sometimes can overshoot the basal plate (Fig. 5a). Rostral and malar dermal denticles have a bilateral symmetry with three elongated ridges of the same size. The external ridges form an obtuse angle with the longitudinal axis. The crown is long and implanted vertically (Fig 5b).

Discussion

Morphology has been traditionally employed as a tool to differentiate the Rajidae species. Skeletal structures, coloration patterns, spinulation patterns and lineal morphometric measurements (*sensu* González-Castro et al., 2012) have been used to achieve this (Stehmann, 1970; Menni, 1973; McEachran, 1982; Cousseau et al., 2007; Last et al., 2008). Molecular tools have recently been employed to discriminate SW

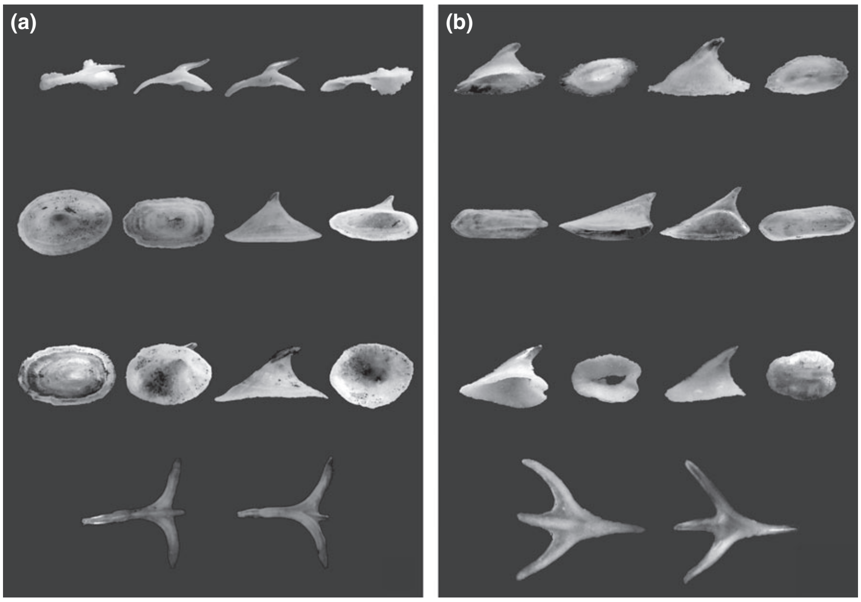

Fig. 5. *Sympterygia acuta*. (a) Top row: alar thorns; Second row: Midline (disc region) thorns; Third row: Midline (caudal region) thorns. Left to right: dorsal, lateral (70°), lateral (90°) and ventral view. Bottom row: dermal denticles, left: dorsal view; right: ventral view. *Sympterygia bonapartii*. (b) Top row: Midline (disc region) thorns; Second row: Midline (caudal region) thorns; Third row: orbital region thorns. Left to right: dorsal, lateral (70°), lateral (90°) and ventral view. Bottom row: dermal denticles, left: dorsal view; right: ventral view

Atlantic skates and are a complementary tool to describe new species (Díaz de Astarloa et al., 2008; Mabragaña et al., 2011). However, there are no previous works related to this methodology (morphogeometrical characterization) for Rajidae in Argentina. The present study represents the first contribution to the morphogeometrical characterization and discrimination at the interespecific level of the *Sympterygia* species for the Southwest Atlantic.

Inter-specific morphological differences evidenced by McEachran (1982) for *Sympterygia* species have been confirmed morphogeometrically and statistically in our study. In this respect, *S. bonapartii* was characterized by a wider disc (variable 4–5, OP), larger pelvic fins (Box-Truss 5, OP), larger eyes, greater distances between nostrils (variable 2–3, VP) and between the first pair of gill slits (variable 6–7, VP), greater interorbital distances (variable 4–5, DP) between spiracles (variable 6–7, DP) and a wider mouth (variable 4–5, VP). However, *S. acuta* has a larger disc due to its snout size.

Regarding inter-specific comparison of allometric coefficient analyses, *S. bonapartii* showed a positive allometry for the variables that comprise the box truss 3 (VP) (related to the mouth width and the first gill slits). This growth type is consistent with those observed in the box truss 3 (OP), which is related to the width disc. It has been interpreted that both structures could develop together. *S. acuta*, however, showed isometric growth in the above-mentioned variables, but showed positive allometry for all variables that defined the snout in the three protocols. This indicates that the snout has distinctive growth relative to the size through ontogeny, whereas *S. bonapartii* showed isometric growth for the snout structure. The remaining variables that defined the morphogeometrical protocols displayed the same type of growth.

Thorns and dermal denticle morphology have been used to discriminate skate species (Gravendeel et al., 2002). Differences in the thorns and dermal denticles of *S. acuta* and *S. bonapartii* are recorded in the present study. Moreover, the form of those structures changed according to the different regions of the analyzed body. Specifically, in both species the thorns in the midline of the disc were ovals, with lobed edges similar to those described by Gravendeel et al. (2002) for the parallel region of *Raja clavata* and *R. undulata*. The thorns of the caudal region were large and oval (much longer than wide) with smooth edges in *S. bonapartii,* and oval with lobed edges in *S. acuta*. The rostral and malar dermal denticles in *S. acuta* have bilateral symmetry with two elongated ridges at an angle of about 90° with respect to the longitudinal axis, as well as a small third ridge. The same elements in *S. bonapartii* also have bilateral symmetry, but with three elongated ridges of the same size. The external ridges form an obtuse angle with the longitudinal axis. These types of denticles were different from those described by Gravendeel et al. (2002) for species of the genus *Raja*, *Leucoraja*, *Dipturus* and *Amblyraja* from the North Sea. *Sympterygia acuta* lacked orbital thorns. In *S. bonapartii* orbital thorns had two notches in the anterior region of the basal plate, which also had a triangular incision in the posterior region (ventral view); this characteristic was not observed in any other aforementioned skates.

In conclusion, the two morphometric analyses (based on LMMs and IlDs) allowed the discrimination of both *Sympterygia* species. The morphogeometric method (IlDs) displayed an adequate coverage of the shape and therefore allowed a detailed description of the shape differences between the two species.

Results of the present work demonstrate the validity of dermal structures to discriminate *Sympterygia* species of the Southwest Atlantic (SWA). Information is lacking on the shape of the thorns and dermal denticles of other Rajidae species in the SWA. Considering its taxonomic value, it would be of importance to characterize these anatomical elements in the remaining species, which could be employed as diagnostic tools for prey identification in the stomachs and fecal contents of top predators, considering that they are one of the few skate structures that remain almost unchanged, especially in the stool of predators (Giardino, 2014).

Acknowledgements

The authors would like to thank: Ariel Martinez, for logistical support; Mar Chiquita coastal lagoon authorities; Matias Delpiani, Daniel Bruno, Damian Castellini, Carlos Jurado and Nicolas Lajud for helping in collecting specimens; Lourdes Corbo by assistance in sampling. This work was supported by CONICET PIP 0942 and 15/E525, EXA 577/2 UNMdP grants.

References

Cavalcanti, M. J., 2004: Geometric morphometric analysis of head shape variation in four species of hammerhead sharks (Carcharhiniformes: Sphyrnidae). In: Morphometrics: Applications in Biology and Paleontology. E. M. T. Ashraf (Ed.). Springer Science and Business Media, Berlin, Germany, pp. 97–113.

Cousseau, M. B.; Figueroa, D. E.; de Díaz Astarloa, J. M.; Mabragaña, E.; Lucífora, L. O., 2007: Rayas, chuchos y otros batoideos del Atlántico Sudoccidental (34°S–55°S). Mar del Plata: Instituto Nacional de Investigación y Desarrollo Pesquero INIDEP, 102 pp. [Skates, rays and other batoids of the Southwest Atlantic (34° S–55° S)] (In Spanish).

Díaz de Astarloa, J. M.; Mabragaña, E.; Hanner, R.; Figueroa, D. E., 2008: Morphological and molecular evidence for a new species of longnose skate (Rajiformes: Rajidae: *Dipturus*) from Argentine waters based on DNA barcoding. Zootaxa **1921**, 35–46.

Díaz de Astarloa, J. M.; Bezzi, S.; González-Castro, M.; Mabragaña, E.; Hernández, D.; Delpiani, S. M.; Figueroa, D. E.; Cousseau, M. B.; Deli Antoni, M. Y.; Tringali, L., 2011: Morphological, morphometric, meristic and osteological evidence for two species of hake (Actinopterygii: Gadiformes: *Merluccius*) in Argentinean waters. J. Fish Biol. **78**, 1336–1358.

Ebert, D. A.; Compagno, L. J. V., 2007: Biodiversity and systematics of skates (Chondrichthyes: Rajiformes: Rajoidei). Environ. Biol. Fish. **80**, 111–124.

Giardino, G., 2014: Estructura y dinámica de las colonias de Lobos Marinos de un pelo de Provincia de Buenos Aires y su relación con pesquerías de la región, 2008–2011. Tesis doctoral. Facultad de Ciencias Exactas y Naturales. Universidad Nacional de Mar del Plata, 250 pp. [Structure and dynamics of South American sea lions colonies of Buenos Aires Province and its relation to fisheries in the region, 2008–2011. Phd Thesis, University of Mar del Plata]. (In Spanish).

González-Castro, M., 2007: Los peces representantes de la Familia Mugilidae en Argentina. Tesis Doctoral, Universidad Nacional de Mar del Plata, 182 pp. [Mugilidae Fishes in Argentina, PhD Thesis, University of Mar del Plata]. (In Spanish).

González-Castro, M.; Heras, S.; Roldán, M. I.; Cousseau, M. B., 2008: Assessing species validity of *Mugil platanus* Günther, 1880 in relation to *Mugil cephalus* Linnaeus, 1758 (Actinopterygii). Ital. J. Zool. **75**, 319–325.

González-Castro, M.; Ibáñez, A. L.; Heras, S.; Roldán, M. I.; Cousseau, M. B., 2012: Assessment of lineal versus landmark-based morphometry for discriminating species of Mugilidae (Actinopterygii). Zool. Stud. **51**, 1515–1528.

Gravendeel, R.; Van Neer, W.; Brinkhuizen, D., 2002: An identification key for dermal denticles of Rajidae from the North Sea. Int. J. Osteoarchaeol. **12**, 420–441.

Heras, S.; González-Castro, M.; Roldán, M. I., 2006: *Mugil curema* in Argentinean waters: combined morphological and molecular approach. Aquaculture **261**, 473–478.

Ibáñez-Aguirre, A. L.; Lleonart, J., 1996: Relative growth comparative morphometric of *Mugil cephalus* L. and *Mugil curema* V. in the Gulf of Mexico. Sci. Mar. **60**, 361–368.

Jacob, B. A.; McEachran, J. D., 1994: Status of two species of skates, *Raja* (*Dipturus*) *teevani* and *R.* (D) *floridana* (Chondrichthyes: Rajoidei), from the Western North Atlantic. Copeia **2**, 433–445.

Last, P. R.; White, W. T.; Pogonosky, J. J.; Gledhill, D. C., 2008: New Australian skates (Batoidea: Rajoidei) – background and methodology. In: Descriptions of New Australian Skates (Batoidea: Rajoidei). P. R. Last, W. T. White, J. J. Pogonosky, D. C. Gledhill (Eds). CSIRO Marine & Atmospheric Research Paper 021, Hobart, Australia, pp. 1–8.

Lleonart, J.; Salat, J.; Torres, G. J., 2000: Removing allometric effects of body size in morphological analysis. J. Theor. Biol. **205**, 85–93.

Lombarte, A.; Lleonart, J., 1993: Otolith size changes related with body growth, habitat depth and temperature. Environ. Biol. Fish. **37**, 297–306.

Mabragaña, E.; Díaz de Astarloa, J. M.; Hanner, R.; Zhang, J.; González-Castro, M., 2011: DNA barcoding identifies Argentine fishes from marine and brackish waters. PLoS One **6**, e28655.

McEachran, J. D., 1982: Revision of the South American skate genus *Sympterygia* (Elasmobranchii: Rajiformes). Copeia **4**, 867–890.

Menni, R. C., 1972: Anatomía del mixopterigio y diferencias específicas en los géneros *Psammobatis* y *Sympterygia* (Chondrichthyes, Rajidae) [Clasper anatomy and specific differences in the genus *Psammobatis* and *Sympterygia* (Chondrichthyes, Rajidae)]. Neotropica **18**(56), 73–80. (In Spanish).

Menni, R. C., 1973: Rajidae del litoral bonaerense. I. Especies de los Géneros *Raja*, *Bathyraja* y *Sympterygia* (Chondrichthyes) [Rajidae of Buenos Aires coast. I. Species of the genus *Raja*, *Bathyraja* and *Sympterygia* (Chondrichthyes)]. Physis (A) **32**, 413–439. (In Spanish).

R Development Core Team, 2012: R: A Language and Environmental for Statistical Computing. R foundation for Statistical Computing (Version 2.14.2). Available at http://www.R-project.org/.

Rohlf, F. J.; Marcus, L. F., 1993: A revolution in morphometrics. Reprinted from trends. Ecol. Evol. **8**(4), 129–132.

Sabadín, D.; González-Castro, M.; Iudica, C.; Díaz de Astarloa, J. M.; Fernández Iriarte, P. J., 2010: Morphometric and genetic assessment of the *Cynoscion guatucupa* population structure from Buenos Aires coast, Argentine sea. Rev. Biol. Mar. Oceanogr. **45**, 513–517.

Stehmann, M., 1970: Vergleichend morphologische und anatomische Untersuchungen zur Neuordnung der Systematik der nordostatlantischen Rajidae (Chondrichthyes, Batoidei). Arch. Fischereiwiss. **21**(2), 73–164.

Strauss, R. E.; Bookstein, F. L., 1982: The truss: body form reconstructions in morphometrics. Syst. Zool. **131**(2), 113–135.

Torres Palacios, K., 2010: Técnicas morfométricas aplicadas al estudio taxonómico de rayas espinosas americanas del Género *Urotrygon* (Batoidea: Urotrygonidae), del Pacífico Centro-Sur Colombiano. Tesis de Licenciatura. Universidad del Valle, Santiago de Cali. [Morphometric techniques applied to taxonomic study of American spiny stripes genus Urotrygon (Batoidea: Urotrygonidae), from south-central Pacific Colombian. Msc Thesis. Del Valle University, Santiago de Cali.], 78 pp. (In Spanish).

Author's address: M. González-Castro, Grupo de Biotaxonomía Morfológica y Molecular de Peces (BIMOPE), IIMyC- CONICET, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3350, B7602AYL Mar del Plata, República Argentina.
E-mail: gocastro@mdp.edu.ar