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Morphology and DNA Barcoding Reveal a New Species of Eagle Ray from the Southwestern Atlantic: *Myliobatis ridens* sp. nov. (Chondrichthyes: Myliobatiformes: Myliobatidae)

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Natalia L. Ruocco, Luis O. Lucifora, Juan M. Díaz de Astarloa, Ezequiel Mabragaña, and Sergio M. Delpiani (2012) Morphology and DNA barcoding reveal a new species of eagle ray from the southwestern Atlantic: *Myliobatis ridens* sp. nov. (Chondrichthyes: Myliobatiformes: Myliobatidae). *Zoological Studies* **51**(6): 862-873. Two species of *Myliobatis*, the southern eagle ray *M. goodei* Garman and the bullnose eagle ray *M. freminvillii* Le Sueur, have long been recognized to occur in coastal Argentinean waters. Several unusual specimens belonging to the family Myliobatidae were recently collected off Buenos Aires Province, Argentina. These specimens clearly belong to the genus *Myliobatis*, since they have a broad disk with long sharply pointed pectoral fins, a projecting snout, a very long and thin tail, and a smaller dorsal fin set farther back on the tail, well beyond the pelvic fins. However, the specimens were distinct from all sympatric congeners in several characters, and they are described here as a new species. *Myliobatis ridens* sp. nov. is distinguished from *M. goodei* in having a relatively shorter snout, a wider interorbital space, a wider mouth, and different shapes of the ventral and dorsal marginal cartilages of the claspers than the latter; and from *M. freminvillii* by having smaller eyes, a smaller dorsal fin, a plain dorsal coloration, and a different shape of the dorsal marginal cartilage of the claspers. In order to test this morphological differentiation, cytochrome *c* oxidase subunit I (COI) sequence data were obtained from the new species and compared to those of its congeners. Analysis of COI sequences showed a congeneric sequence divergence of > 6%, supporting species differentiation. Therefore combining both traditional taxonomy and DNA barcoding, a new eagle ray species, *M. ridens* sp. nov., from the Southwest Atlantic Ocean was discovered. http://zoolstud.sinica.edu.tw/Journals/51.6/862.pdf

Key words: Elasmobranchii, *Myliobatis*, Cytochrome *c* oxidase subunit I gene, South America, Argentina.

The order Myliobatiformes is distributed worldwide from tropical to temperate waters (Bigelow and Schroeder 1953, McEachran and Aschliman 2004). These organisms tolerate a wide range of depth and salinity, from fresh water to estuarine and marine waters (Cousseau and Perrotta 2004, McEachran and Aschliman 2004).

In the Bonaerensean District of the Argentine Biogeographic province (off Uruguay and northern Argentina at 33°- 40°S, Menni and

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Gosztonyi 1982, Menni and López 1984, Menni and Stehmann 2000, Menni et al. 2010), the order is represented by the families Dasyatidae, Gymnuridae, Mobulidae, and Myliobatidae (Menni and Stehmann 2000, Cousseau et al. 2007), and 10 different species of the Myliobatiformes have been recorded, although only 3 are part of the permanent fauna of the region, while the rest are tropical species that only occasionally reach the district (Menni and Stehmann 2000, Cousseau et al. 2007).

The genus *Myliobatis* can be recognized, among other features, by having a rhombic disc broader than long, a head distinct from the disc with a simple cephalic lobe projected forward, and the presence of 1 venomous spine located near the base of the tail behind the dorsal fin (Bigelow and Schroeder 1953). According to Compagno (2005), the genus is composed of 11 valid species, plus 1 dubious species. In the western Atlantic, only 2 species of *Myliobatis* have been recorded: *M. goodei* Garman and *M. freminvillii* Le Sueur. *Myliobatis goodei* is distributed from North Carolina, USA, to Central America, and from Rio de Janeiro to Buenos Aires Province, Argentina (Castello 1974), while *M. freminvillii* occurs from Cape Cod, USA, to the northern Gulf of Mexico, off the northern coast of South America, and from southern Brazil to northern Argentina (McEachran and de Carvalho 2002). Until the 1990s, *M. goodei* Garman was the only species of the genus recognized as permanently present off Argentina, while *M. freminvillii* Le Sueur appeared only occasionally (Refi 1975). Since the 1990s, evidence has accumulated that indicates the presence of 2 species of *Myliobatis* which are permanent residents of the Bonaerensean District: *M. goodei* and an undescribed species (Cousseau and Perrotta 2004, Christiansen and Cousseau 2005, Cousseau et al. 2007), which was also reported from southern Brazil (Levy and Conceição 1989, Vooren 1997). In this paper, we combined both morphological and molecular identification approaches to describe the new species of *Myliobatis* from Argentina. Comparisons of this species with the sympatric *M. goodei* and *M. freminvillii* are also made.

MATERIALS AND METHODS

Specimens examined were collected by vessels of Fundación Mundo Marino, San Clemente del Tuyú, from Bahía Samborombón, and by artisanal fishing boats off the Mar Chiquita coastal lagoon (Argentina); these specimens were deposited in the collection of the Laboratorio de Biotaxonomía Morfológica y Molecular de Peces of the Univ. Nacional de Mar del Plata (UNMDP), Mar del Plata, Argentina.

Morphometric measurements followed Bigelow and Schroeder (1953) and Refi (1975). The left clasper of the holotype and two paratypes of *M. ridens* sp. nov. and 1 mature individual of *M. goodei* were dissected to reveal its internal structure for comparison. The terminology of clasper elements followed Hulley (1972) and Refi (1975). In total, 23 measurements, expressed as proportions of the disc width (DW), were taken from 22 specimens: 6 individuals of *M. goodei*, 1 of *M. freminvillii*, and 15 of *M. ridens* sp. nov., including the holotype and 14 paratypes (Table 1).

In order to obtain an independent test of any observed morphological divergence, we compared the sequence of the cytochrome *c* oxidase subunit I (COI) gene of the new species and of all other publicly available species of *Myliobatis*. The COI gene is an effective molecular marker for identifying species of many taxa (Chen et al. 2010, Chullasorn et al. 2011, Iamsuwansuk et al. 2012), including chondrichthyans (Ward et al. 2008). Muscle tissue samples were taken from 24 specimens, including 16 *M. ridens* sp. nov, (holotype, paratypes, and comparative material), 7 *M. goodei*, and 1 *Dasyatis hypostigma* Santos and de Carvalho (Table 2), and samples were preserved in 99.5% ethanol at -20°C for the genetic analysis. Genomic DNA was extracted according to the protocol of Ivanova et al. (2006). Amplification of the 5' barcode region of COI was attempted using C_FishF1t1/C_FishR1t1 primer fish cocktails (Ivanova et al. 2007). All primers were appended with M13 tails to facilitate sequencing. Polymerase chain reactions (PCRs) were performed in 96-well plates containing tissues of other marine fishes from Argentina. The reaction master mix consisted of 825 μl water, 125 μl 10× buffer, 62.5 μl 25 mM $MgCl_2$, 6.25 μl 10 mM dNTP, 12.5 μl of each primer (0.01 mM), and 6.25 μl 5 U/μl *Taq* DNA polymerase and was prepared for each plate; each well contained 10.5 μl of the mixture and 2 μl genomic DNA. The PCR profile consisted of an initial denaturation of 1 min at 94°C, 5 cycles of 94°C for 30 s, annealing at 50°C for 40 s, and extension at 72°C for 1 min, followed by 30 cycles of 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min and held at 4°C. Amplicons were visualized on a 2% agarose E-Gel® 96-well system

Table 1. Body proportions expressed as a percent (%) of the disc width (DW) (in mm) for the holotype and paratypes of *Myliobatis ridens* sp. nov., *M. goodei*, and *M. freminvillii*. *n*, number of specimens from which measurements were taken; SD, standard deviation

Measurements	Holotype	*M. ridens* sp. nov.				
		Paratypes				
		Min	Max	*n*	Mean	S.D.
Total length	tail cut	157.1	167.2	3	164	5.8
Disc width	531	290	690	14	478	128.8
Predorsal distance	77.6	72.1	81.1	14	76.8	2.6
Preorbital length	7.7	4.9	8.0	14	6.4	0.9
Interorbital distance	13.6	13.6	16.5	14	14.8	0.8
Disc length	61.6	57.8	63.6	14	59.7	1.7
Precloacal distance	57.4	53.4	57.5	14	55.6	1.5
Preoral length	9.6	7.9	10.5	14	9.4	0.7
Internarial distance	6.6	5.3	6.8	14	6.0	0.5
First interbranchial distance	15.6	14.4	17.4	14	16.0	0.9
Fifth interbranchial distance	9.2	9.7	11.0	14	10.3	0.4
Mouth width	8.9	7.9	10.7	14	9.2	0.8
Distance between the edge of the disc to the first gill opening	7.0	5.9	7.2	14	6.7	0.4
Dorsal fin height	1.9	1.5	2.8	14	2.2	0.4
Dorsal fin base length	4.3	3.5	5.2	14	4.4	0.5
Distance between pelvic fin tip and origin of dorsal fin	6.4	4.8	9.1	14	7.2	1.2
Spine length	-	11.7	14.9	12	13.2	1.1
Horizontal diameter of eye ball	1.7	1.2	2.3	14	1.8	0.3
Spiracle length	6.0	4.9	6.8	14	5.8	0.6
Interspiracular distance	13.0	12.6	14.5	14	13.4	0.5
Distance between dorsal fin tip and origin of spine	0.6	0.3	1.4	14	0.9	0.3
Clasper, postcloacal length	15.9	15.0	15.5	2	15.3	0.4
Clasper, length from pelvic axil	5.9	3.4	5.8	2	4.6	1.7

Measurements	*M. goodei*					*M. freminvilli*
	Min	Max	*n*	Mean	S.D.	value
Total length	170.7	197.4	5	181	10.4	tail cut
Disc width	385	575	6	470	76.7	608
Predorsal distance	72.4	77.3	6	74.7	1.9	82.7
Preorbital length	6.4	9.0	6	7.7	1.1	13.4
Interorbital distance	10.7	13.5	6	12.5	1.0	15.6
Disc length	58.8	64.6	6	61.0	2.1	72.4
Precloacal distance	54.5	58.5	6	55.9	1.9	67.3
Preoral length	9.6	12.6	6	11.0	1.1	15.6
Internarial distance	5.3	6.0	6	5.8	0.3	8.9
First interbranchial distance	13.9	15.0	6	14.4	0.4	16.6
Fifth interbranchial distance	9.1	10.0	6	9.7	0.3	7.7
Mouth width	6.5	7.4	6	6.9	0.3	10.7
Distance between the edge of the disc to the first gill opening	7.2	8.5	6	8.1	0.5	8.9
Dorsal fin height	1.2	1.7	6	1.4	0.2	2.9
Dorsal fin base length	3.6	4.9	6	4.1	0.5	5.7
Distance between pelvic fin tip and origin of dorsal fin	4.0	6.2	6	5.5	0.8	4.4
Spine length	10.7	13.2	5	9.7	5.5	-
Horizontal diameter of eye ball	1.4	1.9	6	1.6	0.2	3.3
Spiracle length	4.8	6.5	6	5.8	0.6	6.2
Interspiracular distance	12.2	13.8	6	12.9	0.5	15.6
Distance between dorsal fin tip and origin of spine	0.3	0.8	6	0.6	0.2	-
Clasper, postcloacal length	15.6	1	-	-		13.8
Clasper, length from pelvic axil	5.3	1	-	-		3.9

(Invitrogen, Grand I., NY, USA). Extraction and amplification were performed at the International Barcode of Life Argentinean reference Barcode Laboratory of CONICET at the Museo Argentino de Ciencias Naturales (Buenos Aires, Argentina). Sequencing was performed in the Canadian Centre for DNA Barcoding (Ontario, Canada). Sequencing reactions used M13 forward and reverse primers with the BigDye® Terminator vers. 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.), and the reaction profile was comprised of an initial step of 2 min at 96°C and 35 cycles of 30 s at 96°C, 15 s at 55°C, and 4 min at 60°C. Products were directly sequenced using an ABI 3730 capillary sequencer according to the manufacturer's instructions. DNA sequences were aligned with SeqScape vers. 2.1.1 software (Applied Biosystems). Sequence divergences were calculated using the Kimura two-parameter (K2P) distance model (Kimura 1980), and unrooted Neighbor-joining (NJ) phenograms based on K2P distances were created using MEGA 5 (Tamura et al. 2011) and were bootstrapped 500 times to provide percentage bootstrap values for branch points. Sequence data were available from both the Barcode of Life Data System (BOLD) and GenBank (Table 2).

In order to compare the DNA barcode of the new species with its congenerics, available public sequences of *Myliobatis* were obtained from BOLD, and unrooted NJ phenograms based on K2P distances were created using MEGA 5 and were bootstrapped 500 times to provide percentage bootstrap values for branch points.

Finally, using one of the tools available on BOLD, called "identify specimen", the closest matches of our sequences were obtained. For this purpose, we used one of the available databases to identify COI sequences: "Species Level Barcode Database" (which includes every COI barcode record, public and unpublished, with a species level identification and a minimum sequence length of 500 bp). In this case, the unique information obtained was a percentage similarity of our sequence to existing Barcode records.

Table 2. Voucher specimens of *Myliobatis* spp. sequenced for the barcode region of the mitochondrial DNA cytochrome *c* oxidase subunit 1

Species	Sample ID	GenBank N°	Voucher no.	Collection site
M. ridens sp. nov.	UNMDP-T 0459	JQ305818	UNMDP 459	Off Mar Chiquita lagoon
M. ridens sp. nov.	UNMDP-T 0461	JQ305815	UNMDP 461	Off Mar Chiquita lagoon
M. ridens sp. nov.	UNMDP-T 0462	JQ305816	UNMDP 462	Off Mar Chiquita lagoon
M. ridens sp. nov.	UNMDP-T 0507	JQ305813	UNMDP 507	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0508	JQ305812	UNMDP 508	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0509	JQ305817	UNMDP 509	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0511	JQ305811	UNMDP 511	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0512	JQ305810	UNMDP 512	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0513	JQ305825	UNMDP 513	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0514	JQ305824	UNMDP 514	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0515	JQ305823	UNMDP 515	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0516	JQ305822	UNMDP 516	San Clemente del Tuyú
M. ridens sp. nov.	UNMDP-T 0528	JQ305821	UNMDP 528	Off Mar Chiquita lagoon
M. ridens sp. nov.	UNMDP-T 0529	JQ305820	MNHN 2011-0870	Off Mar Chiquita lagoon
M. ridens sp. nov.	UNMDP-T 0530	JQ305819	UNMDP 530	Off Mar Chiquita lagoon
M. ridens sp. nov.	UNMDP-T 0531	JQ305814	UNMDP 531	Off Mar Chiquita lagoon
M. goodei	UNMDP-T 0080	JQ305805	UNMDP 80	Off Mar Chiquita lagoon
M. goodei	UNMDP-T 0493	JQ305808	UNMDP 493	San Clemente del Tuyú
M. goodei	UNMDP-T 0494	JQ305803	UNMDP 494	San Clemente del Tuyú
M. goodei	UNMDP-T 0495	JQ305804	UNMDP 495	San Clemente del Tuyú
M. goodei	UNMDP-T 0496	JQ305809	UNMDP 496	San Clemente del Tuyú
M. goodei	UNMDP-T 0497	JQ305807	UNMDP 497	San Clemente del Tuyú
M. goodei	UNMDP-T 0498	JQ305806	UNMDP 498	San Clemente del Tuyú
Dasyatis hypostigma	UNMDP-T 0460	JQ305802	UNMDP 460	Off Mar Chiquita lagoon

RESULTS

Myliobatis ridens sp. nov.
(Fig. 1)

Synonyms: *Myliobatis* "DL" (Levy and Conceição 1989), *Myliobatis* sp. (Vooren 1997, Cousseau and Perrotta 2004, Christiansen and Cousseau 2005, Vooren et al. 2005, Cousseau et al. 2007, Ruocco et al. 2008), *Myliobatis goodei* tipo 2 (Cruz 1983), *Myliobatis* sp. DL (dentes largos) (Rezende et al. 2006).

Material examined

Holotype: UNMDP 511, 531 mm DW, mature male, tip of the tail missing, from Bahía Samborombón, San Clemente del Tuyú, 4 Jan. 2011.

Paratypes: 14 specimens: UNMDP 507, 465 mm DW, mature male; UNMDP 508, 542 mm DW, mature female; UNMDP 509, 491 mm DW, mature male; UNMDP 510, 540 mm DW, mature male; UNMDP 512, 574 mm DW, mature male; UNMDP 513, 490 mm DW, immature female; UNMDP 514, 582 mm DW, mature female; UNMDP 515, 690 mm DW, mature female, UNMDP 516, 654 mm DW, mature female, tail missing, collected in Bahía Samborombón, San Clemente del Tuyú, 4 Jan. 2011; UNMDP 527, 290 mm DW, tail missing, immature female; UNMDP 528, 302 mm DW, tail missing, immature female; MNHN 2011-0870, 340 mm DW, 534 mm total length (TL), immature female; UNMDP 530, 360 mm DW, 602 mm TL, immature male; UNMDP 531, 368 mm DW, 615 mm TL, immature female. These last 5 individuals were caught off Mar Chiquita coastal lagoon, 28 Dec. 2010.

Diagnosis: A species of *Myliobatis* distinguished by the following combination of characters: disc rhombic, wider than long, width about 2.2-times TL. Disc length 58%-64% of DW. Anterior margin of disc joining head behind orbits. Head clearly protruding from disc, eyes in lateral position and of moderate size. Snout width equal to interorbital distance (13.6%-16.5% DW), mouth relatively wide, as broad as distance between 5th gill slits (mouth width 0.8-1-times distance between 5th gill slits); mouth width greater than distance between inner ends of nostrils (1.5-1.6-times). Distance between 5th gill slits greater than distance between inner ends of nostrils (1.8-1.6-times).

Description: Measurements and counts given in table 1. Range values of paratypes given in parentheses, following those for holotype. Disc rhombic, broader than long, about twice as wide as long (DW 2.2-times disc length). Head distinctly elevated from disc; snout short, its width equal to distance between orbits which is 13.6% (13.6%-16.5%) DW. Preoral snout length 1.4 (1.5)-times internarial distance and 0.6 (0.5-0.6)-times distance between 1st gill slits; preorbital snout length 0.6 (0.4-0.5)-times interorbital distance; eyes relatively small, lateral, greatly elevated, strongly protruding,

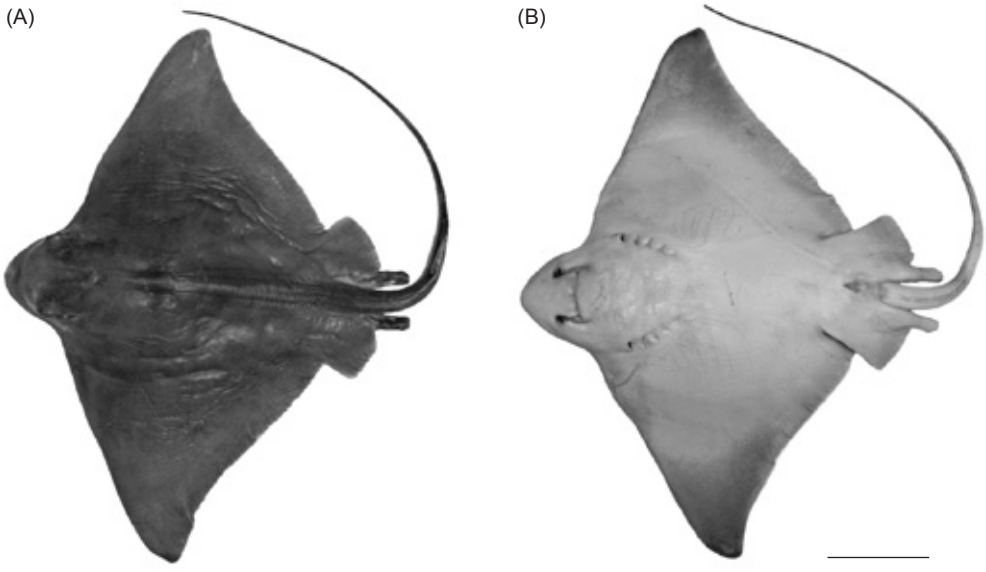

Fig. 1. *Myliobatis ridens* sp. nov., holotype, UNMDP no. 511, mature male, 531 mm disc width. (A) Dorsal view; (B) ventral view. Scale bars = 100 mm.

orbit diameter 0.3 (0.2-0.3)-times spiracle length and 8 (7.2-11.3)-times interorbital length; subrostral lobe located below anterior part of head, rounded, and continuous with pectoral fins. Spiracles tear-shaped and lateral, greatly enlarged, larger than orbits, spiracles length 3.5 (2.9-4.1)-times orbit diameter and 2.2 (2.1-2.5)-times interspiracular distance. Pectoral fins with weakly convex anterior margins, posterior margins moderately concave, outer corners moderately rounded. Mouth relatively wide 8.9% (7.9%-10.7%) DW, its width 1 (0.8-1)-times space between 5th gill slits, and 1.5 (1.5-1.6)-times internarial distance. Teeth flattened, arranged like pavement stones, normally 7 series of teeth in each jaw, teeth of middle series much larger than those located toward corners of mouth. Distance between 5th gill slits 1.4 (1.6-1.8)-times distance between inner ends of nostrils, distance between 5th gill slits about 1/2, 0.6 (0.6-0.7)-times, as great as distance between 1st gill slits. Dorsal fin small, its base length only 0.6 (0.7-0.8)-times as long as distance between nostrils; and height 1.9% (1.5%-2.8%) DW, its origin well behind level of rear edges of pelvic fins, distance between pelvic fin rear tip to dorsal fin origin 6.4% (4.8%-9.1%) DW. Tail elongate and whip-like, markedly differentiated from body, much longer than disc, and without longitudinal folds or ridges. One or 2 serrated tail spines close behind dorsal fin, distance between dorsal fin and origin of spine 0.6% (0.3%-1.4%) DW, spine length 11.7%-14.9% DW. Tail spines serrated with lateral teeth and barbed tip. Skin smooth, lacking denticles or thorns dorsally and ventrally.

Claspers: Claspers in adult males moderately stout, cylindrical, clasper tips not exceeding 1st dorsal-fin origin, tips simple without terminal spines or other rigid projections. They represent 24.8% of disc length in mature individuals (Fig. 2).

In ventral view, dorsal terminal 1 (DT1) cartilage short, broad, and rounded. In dorsal view, ventral marginal (VM) cartilage triangular (scalene), with cartilaginous tip expanded laterally from longitudinal axis. Dorsal marginal (DM) forming triangular cartilage with groove on innermost side; its lower edge parallel to distal edge of DT2. Latter rectangular, with straight distal end. Length of the DM slightly smaller than that of DT2 (Fig. 8).

Color in life: Dorsal surface dark brown to dark olive-green or orange-brown. Ventral surface whitish with darker-orange or black margins of pectoral fins. Lower surface of pelvic fins, claspers, and tail white. Tail paler near base; becoming darker to nearly black towards tip.

Size: Maximum size 700 and 630 mm DW for females and males, respectively; both sexes mature at approximately 500-600 mm DW (Ruocco, unpubl. data). Males are typically smaller than females.

Barcode sequence: A 652-bp amplicon from the 5' region of the mitochondrial COI gene was bidirectionally sequenced for the holotype, 12 paratypes, and additional comparative material of *M. ridens* sp. nov. (sequences deposited in GenBank). The holotype and six of the paratype sequences were identical, while the remainder differed by only 1, 2, or 3 nucleotides. The average K2P genetic divergence within species was 0.2%. The mitochondrial (mt) DNA COI barcode profile of the holotype (haplotype) is reported herein as an aspect of the type description:

CCTTTACTTGATCTTTGGTGCATGAGCAGGGAT
AGTGGGTACTGGCCTCAGCCTACTAATTCGAA
CAGAACTAAGTCAACCGGGGGCCTTACTGGGT
GACGACCAAATTTATAATGTAATTGTTACCGCCC
ATGCCTTTGTAATAATCTTCTTCATGGTCATACC
AATCATAATCGGGGGGTTCGGCAATTGATTAGT
TCCCTTAATGATCGGTGCTCCAGACATAGCCTT
CCCACGAATAAATAATATAAGCTTCTGACTTCTC
CCTCCATCTTTTCTTCTACTGCTAGCCTCAGCA
GGAGTAGAAGCCGGGGCTGGTACTGGGTGAA
CAGTTTATCCCCCTCTAGCTGGCAACCTAGCAC
ATGCCGGGGCCTCTGTAGATTTAACTATCTTTT
CCTTACATTTAGCAGGGGTCTCCTCTATTCTGG
CATCAATCAATTTTATCACTACAATTATTAATATA
AAACCACCAGCAATCTCTCAATATCAAACACCA
CTCTTTGTTTGATCTATTCTTATTACAACCATTCT
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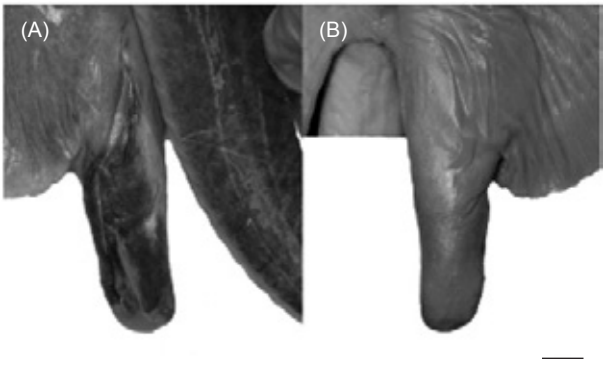

Fig. 2. (A) Dorsal view of left clasper of *M. ridens* sp. nov., paratype, UNMDP no. 510, mature male, 540 mm disc width; (B) ventral view of the same clasper. Scale bar = 10 mm.

The 16 specimens of *M. ridens* sp. nov. examined in this study formed a cohesive cluster which substantially diverged from that of *M. goodei* (Fig. 3), and showed a congeneric sequence divergence of 6.1%, well above the threshold proposed by Hebert et al. (2004) and Lefébure et al. (2006) (i.e., 10-times the mean intraspecific variation for the group under study).

DNA barcoding discriminated specimens of the new species from those of the other 5 *Myliobatis* species from which sequences are publicly available from BOLD. Figure 4 shows the NJ tree obtained from these specimens. Once again, specimens of each species of *Myliobatis* were clustered together and separated from each one's congenerics. Surprisingly, 2 putative specimens of *M. goodei* (see specimens with an asterisk (*) in figure 4) shared the same haplotype with specimens of *M. ridens* sp. nov. These individuals (juvenile females of 277 and 274 mm DW) were captured off Mar Chiquita coastal lagoon in 2006 and 2007, respectively, and were previously misidentified as *M. goodei* (Mabragaña et al. 2011). Re-examination of these 2 specimens from an e-voucher allowed us to identify them as *M. ridens* sp. nov. because of the characteristic head morphology (Fig. 5).

Finally, analysis of the closest matches using "Species Level Barcode Database" showed that specimens of *M. ridens* sp. nov. possessed haplotypes that were substantially divergent from all other *Myliobatis* species represented in the BOLD. Numbers of specimens and percentage of similarity with DNA Barcodes of *M. ridens* sp. nov. are here presented: *M. goodei* (*n* = 6, 94%-94.2%), *M. freminvillii* (*n* = 1, 90.6%-91.2%), *M. californica* (*n* = 7, 93.8%-94.4%), *M. longirostris* (*n* = 2, 90.8%-91.5%), *M. aquila* (*n* = 12, 93.8%-96.2%), *M. tobijei* (*n* = 1, 93.2%-93.7%), and *M. australis* (*n* = 6, 91.3%-92.5%) (www.boldsystem.org).

Etymology: *Myliobatis ridens* sp. nov. from the Latin *ridens*, laughing or smiling, in reference to the peculiar countenance of this species caused by the corners and width of the mouth that resembles a smile.

Common names: Shortnose eagle ray, chucho ñato (in Spanish).

Distribution: The species is endemic to the

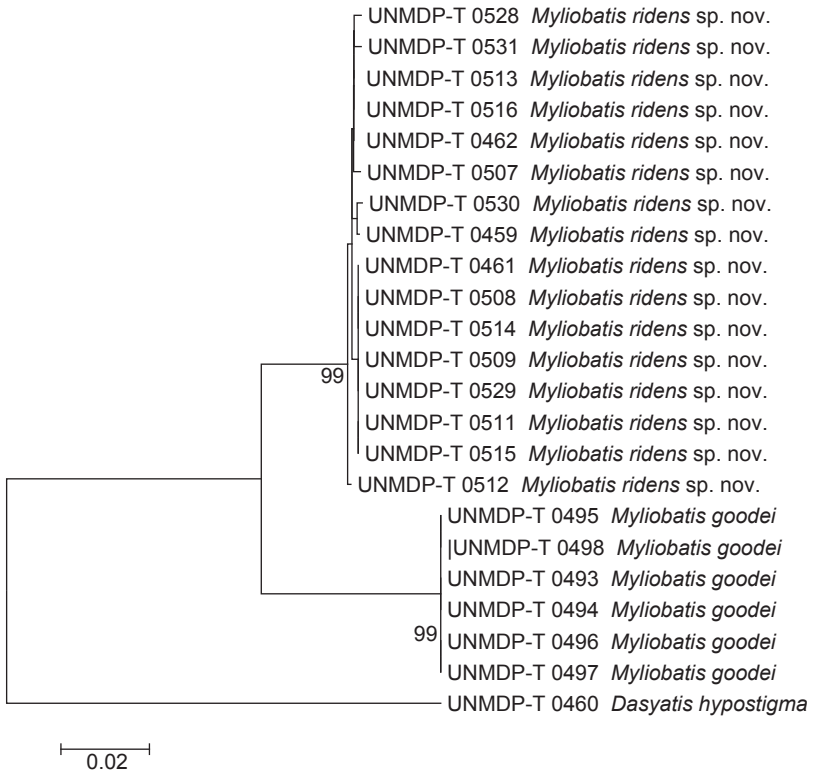

Fig. 3. Neighbor-joining tree of cytochrome *c* oxidase subunit I gene sequences from specimens of *Myliobatis goodei* and *M. ridens* sp. nov. from off Argentina. *Dasyatis hypostigma* was used as an outgroup. Numbers at the nodes are bootstrap values (only values of > 70 are given).

southwestern Atlantic shelf. The occurrence of *M. ridens* sp. nov. has been unequivocally recorded by published descriptions or by our study material from off Cape Santa Marta Grande (28°36'S) (Vooren 1997, Rezende et al. 2006), off Rio Grande do Sul, Brazil (Levy and Conceição 1989, Vooren et al. 2005), and off Uruguay and northern Argentina to as far south as 41°S (Cousseau et al.

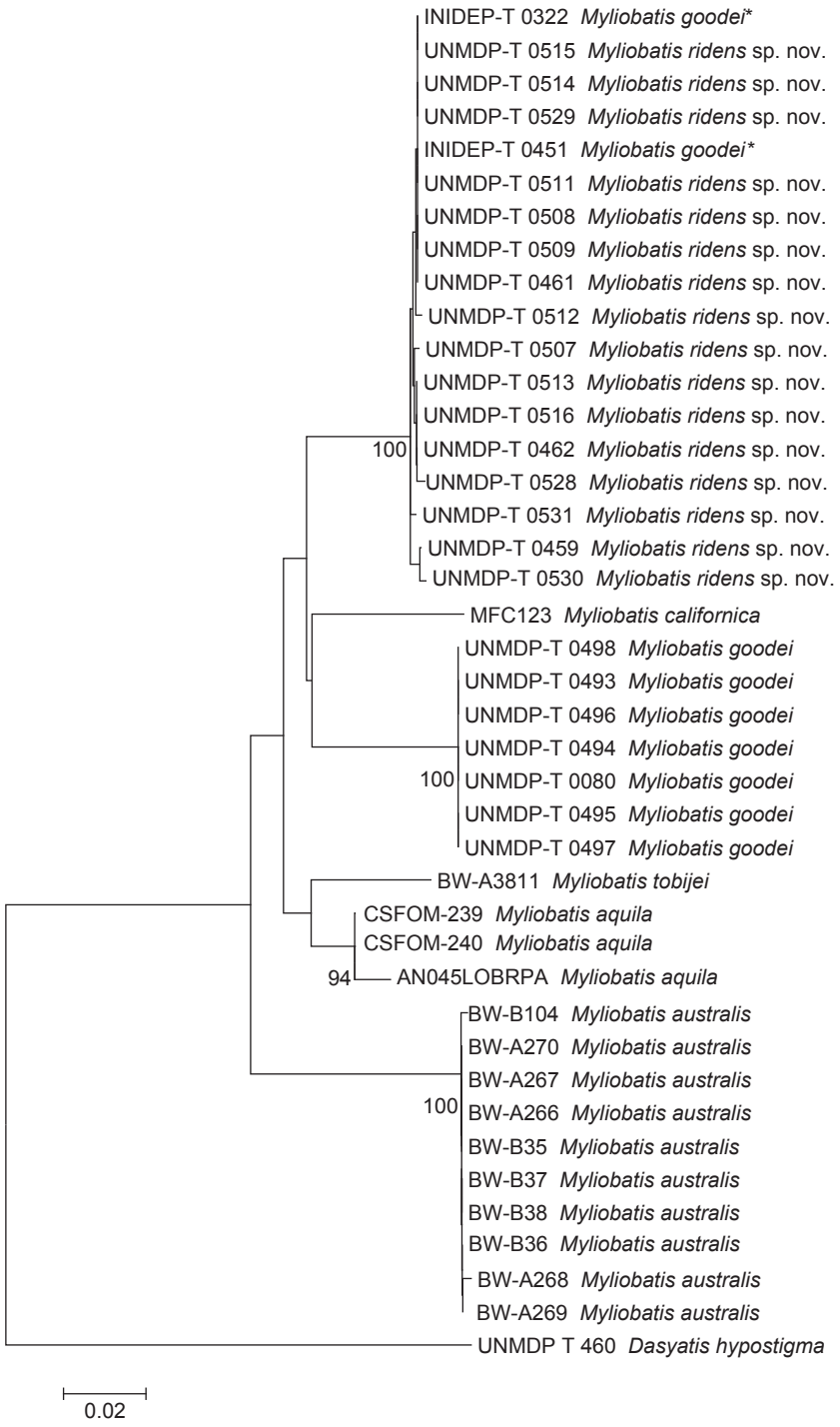

Fig. 4. Neighbor-joining tree of cytochrome *c* oxidase subunit I gene sequences from specimens of *Myliobatis* spp. from Argentina and those (publicly) available on Barcode of Life Data Systems (BOLD). *Dasyatis hypostigma* was used as an outgroup. Numbers at the nodes are bootstrap values (only values of > 70 are given).

2007, present study) (Fig. 6). It is also known from estuarine habitats; in Argentina, it was collected in the Ría de Ajó in Bahía Samborombón (a brackish-water environment). The species is most commonly found in depths of 5-15 m, but has been recorded as deep as 47 m.

DISCUSSION

Comparison with other species of *Myliobatis*

There are 2 similar species in the area: *M. goodei* and *M. freminvillii*. In *M. freminvillii*, the distance between the gill slits of the 5th pair are less than or equal to the distance between the internal margin of the nasal openings, while in *M. goodei* and *M. ridens* sp. nov., the distance between the gill slits of the 5th pair are greater than the distance between the internal margin of the nasal openings (Fig. 7).

Myliobatis goodei has eyes located latero-dorsally, the width of the snout is greater than the interorbital distance (distance between orbits 10.7%-13.5% DW); a more pronounced and pointed snout (with a mean preoral length of 11% DW), a small mouth (with a width of 6.5%-7.4% DW), and a larger distance between the edge of the disc to the 1st gill slit (7.2%-8.5% DW). On the other hand, *M. ridens* sp. nov. has eyes in a lateral position, the width of the snout is equal to the interorbital distance (distance between orbits 13.6%-16.5% DW), and it has a blunt short snout (with a mean preoral length of 9.4% DW), a wide mouth (mouth width is 7.9%-10.7% DW), and a smaller distance between the edge of the disc and the 1st gill slit (5.9%-7.2% DW) (Fig. 7).

Myliobatis ridens sp. nov. can be distinguished from *M. freminvillii* because in the latter, the head stands out more in the anterior part of the disc, and adult males have a small horn (cornea lump) in the

Fig. 5. Specimens of *M. ridens* sp. nov. previously identified as *M. goodei* (marked with an asterisk in figure 4 in Mabragaña et al. 2011). (A) INIDEP-T 0451 and (B) INIDEP-T 0322.

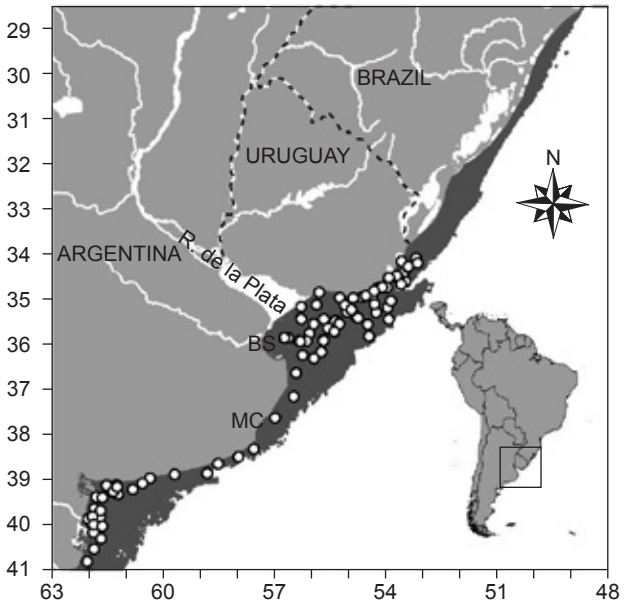

Fig. 6. Known geographic distribution of *M. ridens* sp. nov. (gray area delimited by the 5- and 47-m isobaths). White dots are locations where *M. ridens* sp. nov. was recorded off Uruguay and northern Argentina. BS, Bahía Samborombón; MC, Mar Chiquita coastal lagoon. The inset shows the location of the depicted area in South America.

upper part of each orbit (Fig. 7). The coloration also differs: *M. freminvillii* has a light brown dorsum with white spots, while *M. ridens* sp. nov. has a plain dorsal coloration. In addition, *M. ridens* sp. nov. has smaller eyes (diameter of 1.2%-2.3% DW in *M. ridens* sp. nov., and 3.3% DW in *M. freminvillii*) and a smaller dorsal fin (dorsal fin base of 3.5%-5.2% DW in *M. ridens* sp. nov. and 5.7% DW in *M. freminvillii*) than *M. freminvillii* (Table 1).

Regarding clasper morphology, *M. ridens* sp. nov. can be distinguished from *M. goodei* by several features. In *M. ridens* sp. nov., the ventral marginal is triangular and is expanded laterally from the longitudinal axis; whereas in *M. goodei*, the ventral marginal is rectangular and bluntly expanded laterally. In *M. ridens* sp. nov., the dorsal marginal is triangular (sailing) with a groove on the innermost side, and its lower edge is parallel to the distal edge of the dorsal terminal 2; while in *M. goodei*, the dorsal marginal is oval shaped with a groove located more towards the midline of the element, and its lower edge forms

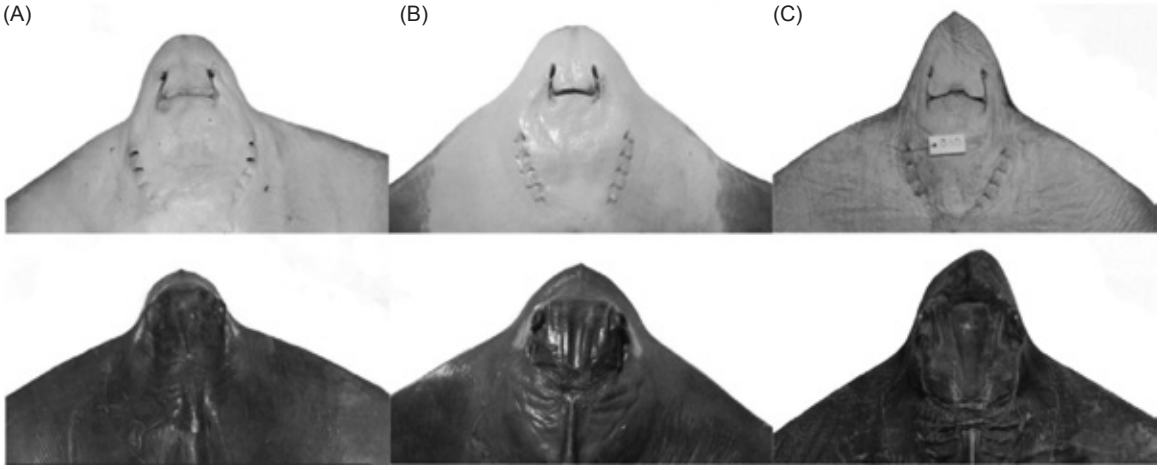

Fig. 7. Ventral (upper row) and dorsal (lower row) views of the head of (A) *M. ridens* sp. nov., UNMDP 510, 540 mm disc width (DW), mature male; (B) *M. goodei*, UNMDP 493, 532 mm DW, mature male; and (C) *M. freminvillii*, INIDEP 365, 680 mm DW, mature male.

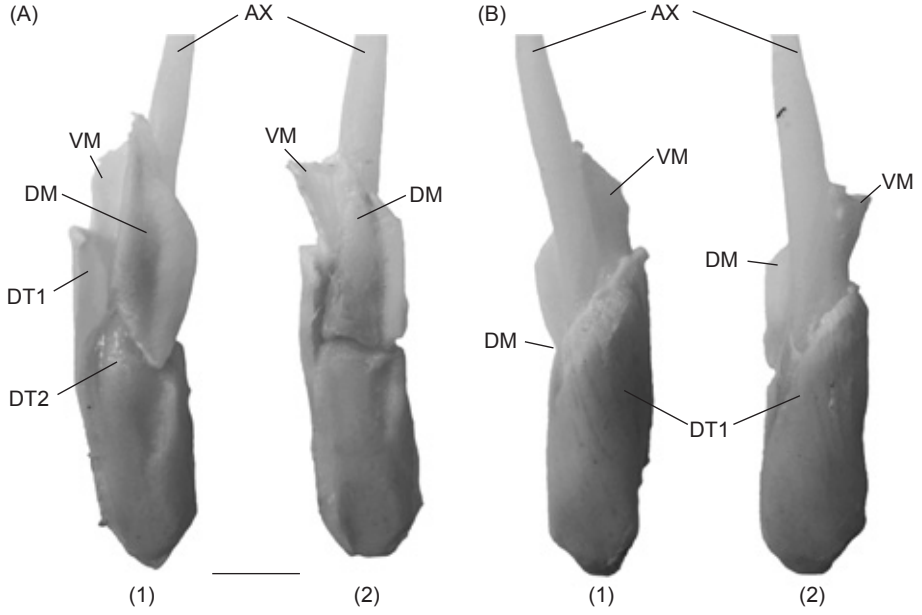

Fig. 8. (A) Dorsal and (B) ventral views of the left clasper cartilages of (1) *M. goodei* and (2) *M. ridens* sp. nov. AX, axial; DM, dorsal marginal; DT1, dorsal terminal 1; DT2, dorsal terminal 2; VM, ventral marginal; VT, ventral terminal. Scale bar = 10 mm.

an acute angle with the distal edge of the dorsal terminal 2 and is parallel to the left edge of dorsal terminal 2 (Fig. 8). In *M. freminvillii*, unlike the other 2 species of *Myliobatis*, the dorsal marginal is rectangular, and reaches only 2/3 of the length of the dorsal terminal 2 cartilage (see figure 3b in Refi 1975), whereas in the other 2 species, the dorsal marginal is more than 80% of the dorsal terminal 2 cartilage.

Comparative material examined: *M. ridens* sp. nov. UNMDP 459, 305 mm DW, immature male; UNMDP 461, 319 mm DW, immature female; UNMDP 462, 297 mm DW immature male; *M. goodei* UNMDP 80, 451 mm DW, immature male; UNMDP 493, 532 mm DW, mature male; UNMDP 494, 500 mm DW, immature female; UNMDP 495, 417 mm DW, immature female; UNMDP 496, 385 mm DW, immature male; UNMDP 497, 575 mm DW, immature female; UNMDP 498, 410 mm DW, immature male; *M. freminvillii* INIDEP 365, 680 mm DW, mature male; *D. hypostigma* UNMDP 460, 284 mm DW, immature female.

Key to the species of the Myliobatidae that occur off Uruguay and Argentina

1a. Distance between gill slits of 5th pair less than or equal to distance between internal margin of nasal openings; base of dorsal fin about equal to distance between exposed nostrils; origin of dorsal fin near posterior margin of pelvic fins. Dorsal surface light brown with white spots .... *M. freminvillii*

1b. Distance between gill slits of 5th pair greater than distance between internal margin of nasal openings, base of dorsal fin about 66%-81% of distance between exposed nostrils; origin of dorsal fin well posterior to posterior margin of pelvic fin. Dorsal surface dark brown, dark olive-green, orange, or reddish-brown 2

2a. Eyes located dorsolaterally, width of snout greater than interorbital distance (interorbital distance 10.7%-13.5% of DW); pronounced and pointed snout, and relatively narrow mouth (its width 6.5%-7.4% of DW) ..... *M. goodei*

2b. Eyes in lateral position, width of snout equal to interorbital distance (interorbital distance 13.6%-16.5% of DW); short and blunt snout, and relatively wide mouth (its width 7.9%-10.7% of DW) ..... *M. ridens* sp. nov.

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