Zootaxa 3182: 57–64 (2012)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Description of the clasper of the Southern Thorny Skate *Amblyraja doellojuradoi* (Pozzi, 1935) (Chondrichthyes: Rajidae)

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Abstract

This work constitutes the first description of the clasper of *Amblyraja doellojuradoi*. The characterization was based on both external morphology and the composition of the internal cartilages. A comparison based on these structures with other species of the genus is provided. Considering all of the cartilages, the basal and terminal series of *A. doellojuradoi* are more similar in shape and disposition to the equivalent series in *A. radiata* than to *A. hyperborea*. However, marked differences in clasper cartilages permit *A. doellojuradoi* to be distinguished from these congeners.

Key words: Rajiformes, morphology, male copulatory organ, systematics

Introduction

Studies of the anatomy and systematics of batoids have revealed significant variation among many anatomical systems (Compagno 1977; Miyake 1988; Miyake & McEachran 1991), particularly in the neurocranium, pectoral and pelvic girdles, and especially the male copulatory organs, which provide useful diagnostic characters at the specific level. (Ishiyama & Hubbs 1968; Hulley 1970; Stehmann 1970; Hulley 1972; McEachran 1982, 1983). Concerning these organs, special attention is paid to the distal components, as they are most useful in separating species (Ishiyama 1958). Several studies related to this subject have been carried out; however, for some genera this knowledge is scarce. Such is the case of the genus *Amblyraja*, in which the claspers have been described for only two species: *Amblyraja radiata* (Stehmann 1970; Hulley 1972) and *A. hyperborea* (Stehmann 1970).

The genus *Amblyraja*, with 10 species, has a cosmopolitan distribution (Ebert & Compagno 2007). One of the species, the southern thorny skate (*Amblyraja doellojuradoi*), inhabits the Southwest Atlantic Ocean (Menni & Stehmann 2000; Cousseau *et al*. 2007), and is distributed from 35º to 55º S in the Argentinean continental shelf. Information about the southern thorny skate is rarely encountered, referring almost exclusively to its taxonomy and distribution (Pozzi 1935; Bellisio *et al*. 1979; Menni *et al*. 1984; Menni & Stehmann 2000; Sánchez & Mabragaña 2002; Cousseau *et al*. 2007). This work constitutes the first description of the copulatory organ of *Amblyraja doellojuradoi*.

Material and methods

Specimens of the southern thorny skate were collected from research cruises carried out by the National Institute of Fisheries Research and Development in the Southwest Atlantic Ocean (SWA) from 36º to 47º S. The skates were identified as *A. doellojuradoi* through Cousseau *et al.* (2007). A total of 10 claspers belonging to mature specimens ranging from 519 to 551 mm TL were analyzed and preserved in the Laboratorio de Ictiología de la Universidad Nacional de Mar del Plata.

Accepted by M. R. de Carvalho: 9 Dec. 2011; published: 3 Feb. 2012

The clasper dissection was accomplished using the methodology proposed by Stehmann (1970) that consists in the immersion of the organ in a 10% NaOH solution during 20 minutes, to allow the visualization of the cartilages. Nomenclature was based on Stehmann (1970) and McEachran & Compagno (1979).

Results

Since the external structures are closely related to skeletal structures, the description of the clasper cartilages precedes that of the external morphological characteristics.

Skeletal structures. The axial, ventral marginal and dorsal marginal cartilages are located in parallel along the clasper, although only the axial cartilage reached the distal end (Figure 1A). The **axial** is expanded and spatulate with rounded edges, and has its distal extreme less calcified than the proximal region. The **dorsal** and **ventral marginal** cartilages occur together and are positioned in parallel, diverging at the proximal end where the accessory cartilages are located. The distal end of the dorsal marginal cartilage is truncated. The ventral marginal cartilage appears at a short distance from the union of the dorsal and axial cartilages and is tongue-shaped.

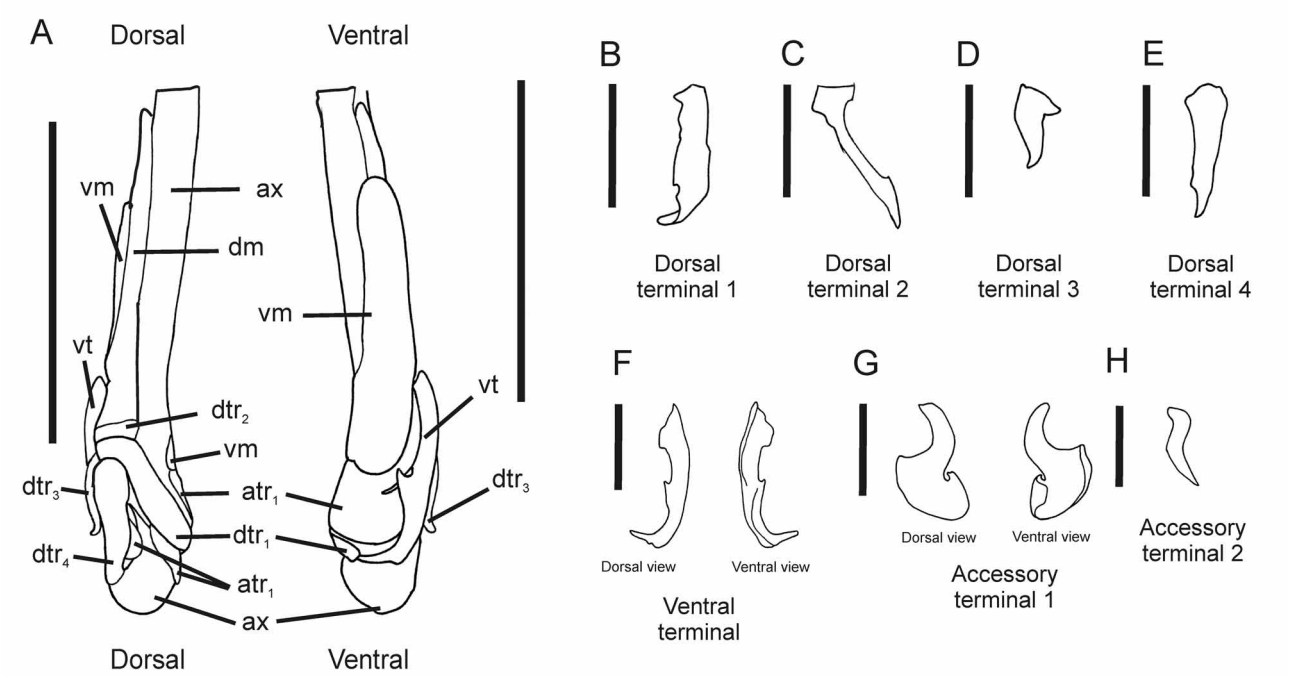

FIGURE 1. A) Clasper cartilages in *Amblyraja doellojuradoi* in dorsal and ventral view. Scale 10 cm. **B**) Dorsal terminal 1 cartilage. **C**) Dorsal terminal 2 cartilage. **D**) Dorsal terminal 3 cartilage. **E**) Dorsal terminal 4 cartilage. **F**) Ventral terminal cartilage. **G**) Accessory terminal 1 cartilage. **H**) Accessory terminal 2 cartilage. Scale 2 cm. All cartilages are in dorsal view, except those noted otherwise.

The **dorsal terminal 1** cartilage crosses obliquely the dorsal face of the clasper, from the union of the dorsal terminal 2 and 3 cartilages until nearly coming into contact with the distal end of the ventral terminal cartilage. It is ribbon-shaped, diminishing its width toward the ends (Figure 1B). The **dorsal terminal 2** cartilage is attached to the distal region of the dorsal marginal cartilage and to the axial cartilage at its distal end. The proximal end is square, becoming sharp toward the distal end, where it reaches the axial cartilage. Proximal end has straight edges and extends rod-shaped to the distal end oriented diagonally to the right (Figure 1C). The **dorsal terminal 3** cartilage is question mark-shaped, and its proximal end is wide and attached to the dorsal terminal 2 cartilage, but its free distal end (spur) is sharp and curved distally (Figure 1D). The **dorsal terminal 4** cartilage is bound externally to the dorsal terminal 3, at the back of the dorsal junction of terminal 2 to the dorsal terminal 3. Its proximal end is wide and reduced toward the distal end, and the edges are straight (Figure 1E). The **ventral terminal** cartilage extends from the joint of the dorsal marginal and the dorsal terminal 2 cartilages to the distal end of the clasper,

externally surrounding the terminal accessory 1. It is J-shaped, with a bulge in the middle of the cartilage that is joined to the terminal accessory 1, with a groove along the opposite lateral margin (Figure 1F).

The **accessory terminal 1**, located in between the ventral terminal and ventral marginal cartilages, surrounds the accessory terminal 2. It is somewhat L-shaped with a thin proximal end becoming wider at the distal end. These cartilages fold ventrally forming a cavity (Figure 1G). Finally, the **accessory terminal 2** is surrounded by the accessory terminal 1 and displays an S-shaped curve, thus fitting perfectly into the cavity formed by the accessory terminal 1 cartilage (Figures 1H).

External morphological features. The studied claspers ranged from 110 mm to 150 mm in outer length, with an average length of 135.1 mm, and are devoid of dermal denticles. In the external lateral face of the dorsal lobe two blind cavities occur: the **apopyle** (near the proximal end) and the **pseudosiphon** (near the distal end) (Figure 2A). The latter opening is bounded below by the dorsal marginal and distally by the dorsal terminal 1 cartilage. Its length is 10.52% of clasper total length. Also, internally in the dorsal lobe, two blind cavities (**slit** and **cleft**) are present, as well as the **spur**. The spur is a cartilaginous structure formed by the distal tip of the dorsal terminal 3 cartilage and is visible externally. The slit and cleft are both blind cavities situated on the interior part of the dorsal lobe. The cleft is bounded by the axial and dorsal terminal 4 cartilages. The slit is bordered by the axial and the dorsal terminal 2 cartilages (Figure 2B).

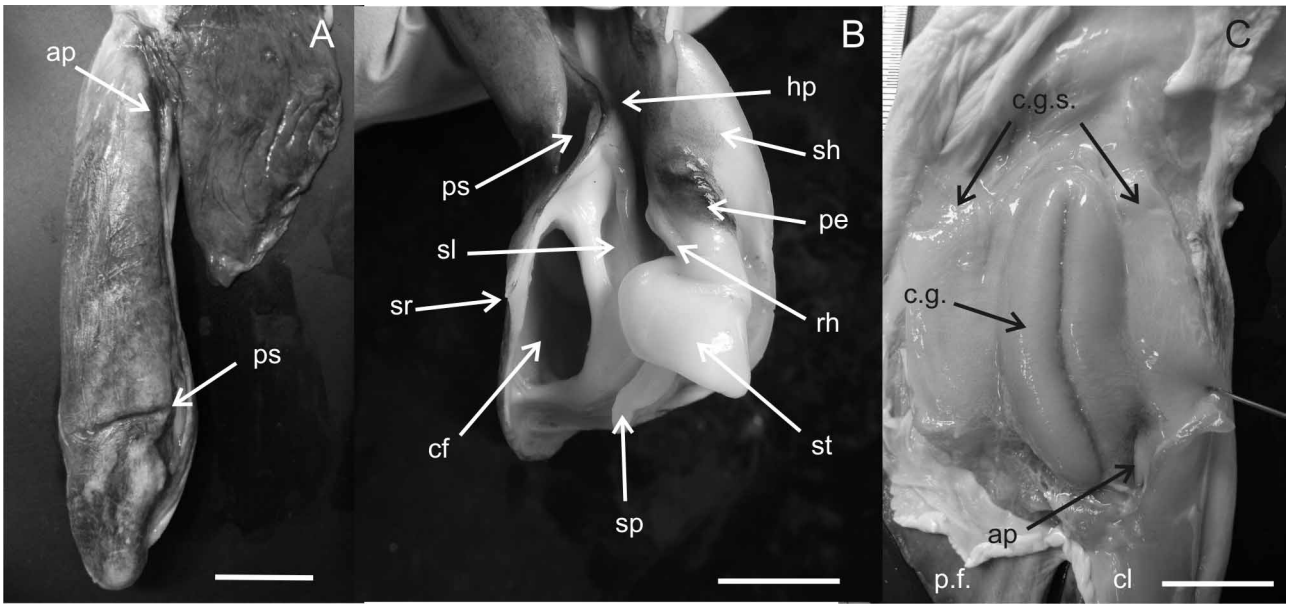

FIGURE 2. External features of right clasper of *Amblyraja doellojuradoi*. Scale 2 cm. **A**) Dorsal view of the clasper of *A. doellojuradoi*. **B**) Open distal end of the clasper of *A. doellojuradoi*. **C**) Clasper gland of *A. doellojuradoi*. Abbreviations: *ap* apopyle, *cf* cleft, *cg* clasper gland, *cgs* clasper gland sac, *cl* clasper, *hp* hypopyle, *pe* pent, *pf* pelvic fin, *ps* pseudosiphon, *rh* rhipidion, *sh* shield, *sl* slit, *sp* spike, *sr* spur, *st* sentinel.

The following structures are observed in the ventral lobe, from the proximal to the distal end: **shield**, **rhipidion**, **pent**, **sentinel** and **spike**. The well-developed shield is almost entirely covered with a thin epithelium. The rhipidion is a pinkish, porous and erectile tissue located posterior to the distal end of the dorsal marginal cartilage, near the proximal end of the sentinel. The pent is a dark adipose tissue associated with the inner edge of the ventral terminal cartilage, and is vertically elongated, extending from the distal end of the rhipidion to near the sentinel. The well-developed sentinel is also covered with a thin epithelium. The spike, a cartilaginous structure covered by integument, is formed by the distal end of the accessory terminal 2 emerging from the central part of the accessory terminal 1 cartilage (Figure 2B).

The clasper gland in *A. doellojuradoi* is a solid cylindrical structure that is oriented towards the anterior end and was folded toward the posterior end, culminating in the apopyle. As Friedman (1935) described for other batoids, the clasper gland of *A. doellojuradoi* is located in the lumen of each “clasper gland sac” which is situated subdermally on the ventral surface of the pelvic fin (Figure 2C).

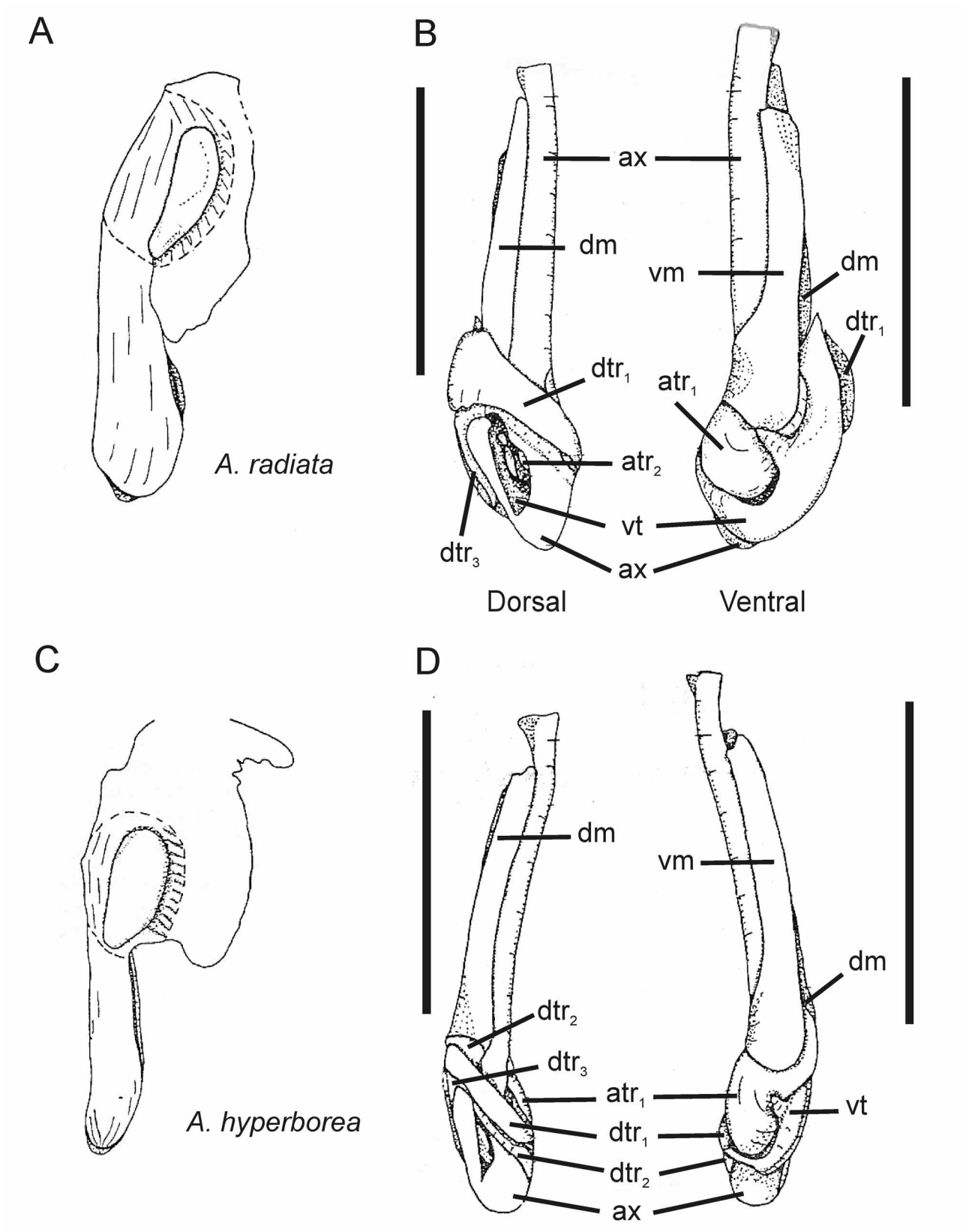

FIGURE 3. A) Right clasper of *Amblyraja radiata*. **B**) Clasper cartilages of *A. radiata* in dorsal and ventral view. **C**) Right clasper of *A. hyperborea*. **D**) Clasper cartilages in *A. hyperborea* in dorsal and ventral view. Scale 10cm. Abbreviations: atr_1 accessory terminal 1 cartilage, atr_2 accessory terminal 2 cartilage, *ax* axial cartilage, *dm* dorsal marginal cartilage, dtr_1 dorsal terminal 1 cartilage, dtr_2 dorsal terminal 2 cartilage, dtr_3 dorsal terminal 3 cartilage, dtr_4 dorsal terminal 4 cartilage, *vm* ventral marginal cartilage, *vtr* ventral terminal cartilage. Figures modified from Stehmann (1970).

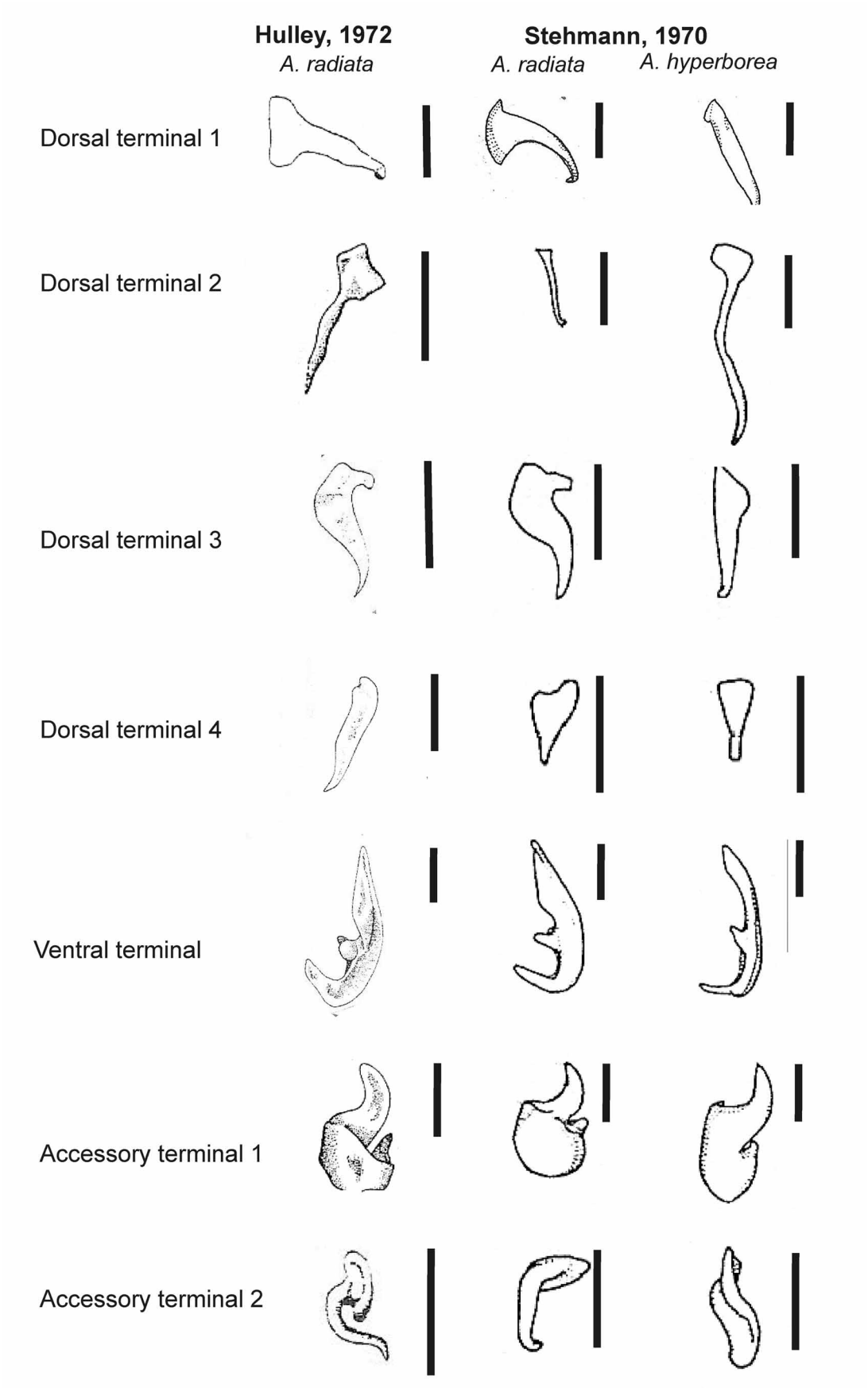

FIGURE 4. Comparison of the terminal series cartilages of the species belonging to the genus *Amblyraja*, described by Stehmann (1970) and Hulley (1972). Scale 2 cm.

TABLE 1. Comparison of the cartilages that constitute the clasper of the species *Amblyraja doellojuradoi* (present study) with those of *A. hyperborea* (Stehmann 1970) and *A. radiata* (Stehmann 1970; Hulley 1972).

		A. doellojuradoi	*A. hyperborea*	*A. radiata*
Cartilages of the basal series	**Axial cartilage (ax)**	Robust	Thin	Robust
	Dorsal marginal cartilage (dm)	Rod-shaped; with blunt ends but the proximal one wider than the distal end	Rod-shaped with blunt ends; width almost uniform	Rod-shaped; with blunt ends but the proximal one wider than the distal end
	Ventral marginal cartilage (vm)	Proximal end beginning distally to the origin of the dorsal marginal cartilage	Proximal end starting at the level of dorsal marginal cartilage	Proximal end beginning distally to the origin of the dorsal marginal cartilage
Cartilages of the terminal series	**Dorsal terminal 1 cartilage (dtr_1)**	Thin Both ends similar Proximal end not covering the union of the dorsal marginal and dorsal terminal 2 cartilages	Thin Both ends similar Proximal end not covering the union of the dorsal marginal and dorsal terminal 2 cartilages	Robust Proximal end more robust than distal end Proximal end covering the union of the dorsal marginal and dorsal terminal 2 cartilages
	Dorsal terminal 2 cartilage (dtr_2)	Square-shaped proximal end, shrinking into a thin cartilage at distal end.	Square-shaped proximal end, shrinking into a thin cartilage at distal end. *A. hyperborea* has the thinnest part twice as long as *A.doellojuradoi*.	Square-shaped proximal end, shrinking into a thin cartilage at distal end (Hulley, 1972). Rod-shaped with a relatively uniform width (Stehmann, 1970).
	Dorsal terminal 3 cartilage (dtr_3)	Question mark- shaped, with one side concave and the other convex; slightly curved distal end.	Triangular; straight distal end.	Question mark- shaped, with one side concave and the other convex; slightly curved distal end.
	Dorsal terminal 4 cartilage (dtr_4)	Elongated cartilage, with pointed distal end.	Triangular cartilage; becoming sharpened in a funnel-shaped distal end	Elongated cartilage, with pointed distal end (Hulley, 1970). Triangular cartilage, somewhat wide and short, sharpened distal end (Stehmann, 1970).
	Ventral terminal cartilage (vt)	J-shaped, with a bulge in the middle. Proximal end exposed. The distal end does not reach the axial cartilage tip.	J-shaped, with a bulge in the middle. Proximal end hidden beneath the terminal accessory 1 The distal end does not reach the axial cartilage tip.	J-shaped, with a bulge in the middle Proximal end located above the terminal accessory 1 The distal end extends to the level of axial cartilage.
	Accessory terminal 1 cartilage (atr_1)	L-shaped	L-shaped	L-shaped
	Accessory terminal 2 cartilage (atr_2)	S-shaped curve with blunt ends	S-shaped curve with blunt ends	S-shaped curve with widened proximal end

Discussion

A comparison of the external morphology of the claspers of the three species of the genus *Amblyraja* in which the clasper has been described shows that *A. doellojuradoi* resembles more *A. hyperborea* because of its less robust

aspect (Stehmann 1970). However, it shares other characteristics with *A. radiata*, such as the presence of a slit and the position of the pelvic fin distal tip in relation to the pseudosiphon (Stehmann 1970; Hulley 1972 and results herein) (Figures 2 and 3).

As regards the cartilages composing the clasper, *A. doellojuradoi* presents the same elements as the other two species, amounting to three basal and seven terminal cartilages. The three basal cartilages are universally present in skates and generally exhibit no characteristics of taxonomic value. By contrast, the cartilages of the terminal series are of great value among different species due to their variability in shape and sometimes in number (some species lack some dorsal and accessory terminal cartilages, while others have up to four terminal accessory cartilages) (Stehmann 1970; Hulley 1972; Menni 1972; McEachran 1982, 1983; Last & Gledhill 2007; Jeong & Nakabo 2009).

Table I presents a comparison of the most distinctive features in the clasper cartilages among the three species of *Amblyraja*. To facilitate comparisons, Figures 3 and 4 depict the different claspers. It should be emphasized that the descriptions of the claspers of *A. radiata* made by Stehmann (1970) and Hulley (1972) differ, especially in relation to the dorsal terminal 2 and dorsal terminal 4 cartilages (Figure 4). This may be attributed to different preparation techniques, because an overexposure to NaOH could damage the cartilages changing their original shape. The dorsal terminal 2 and dorsal terminal 4 cartilages of our material of *A. doellojuradoi* are more in agreement with the description of *A. radiata* made by Hulley (1972) than the description of Stehmann (1970).

Considering all the cartilages, the basal and terminal series of *A. doellojuradoi* are more similar in shape and disposition to the equivalent series in *A. radiata* than to *A. hyperborea*. The three cartilages of the basal series share the same characteristics in *A. doellojuradoi* and *A. radiata*. With regard to the terminal series, five cartilages (dtr_2, dtr_3, dtr_4, vt, atr_1) are similar between these species, whereas four cartilages (dtr_1, vt, atr_1, atr_2) are more similar in *A. doellojuradoi* and *A. hyperborea* (Table I).

The presence of four dorsal terminal cartilages is a characteristic observed in all species of the genus *Amblyraja* in which claspers have been described, and is a condition shared at least with the genus *Rajella* (Stehmann 1970; Hulley 1972). McEachran & Dunn (1998) state that the genera *Rajella*, *Leucoraja*, *Breviraja* and *Dactylobatus* have four dorsal terminal cartilages, being this one of the many features used to group these genera in the same clade. However, none of the species of the genera *Breviraja* and *Leucoraja* described by McEachran & Compagno (1982) and Stehmann (1970) have the dorsal terminal 4 cartilage.

In conclusion, characteristics of the clasper are exceptionally important in distinguishing rajid species and in their classification (Stehmann 1970; Hulley 1972; Menni 1972; McEachran 1982, 1983; McEachran & Dunn 1998; Last & Gledhill 2007; Jeong & Nakabo 2009). This statement also applies to *Amblyraja*, as even though *Amblyraja doellojuradoi* has the same elements as the other two species of the genera in which claspers have been described, the shape and disposition of the cartilages, especially those of the terminal series, are not identical and allow them to be distinguished.

Acknowledgments

We would like to thank the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) and the engineer Rocío Gonzalez for providing specimens for this work. We are also grateful to the reviewers that made valuable comments which improved greatly the manuscript and the National University of Mar del Plata for the workplace. E. Mabragaña and G. Delpiani were supported by scholarships from CONICET.

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