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RESEARCH NOTE

Description of the dermal denticles on pre-pelvic claspers of the Cockfish, *Callorhinchus callorynchus* (Holocephali: Callorhinchidae), from Coquimbo, Chile

Descripción de los dentículos dérmicos en claspers pre-pélvicos del pejegallo *Callorhinchus callorynchus* (Holocephali: Callorhinchidae), en Coquimbo, Chile

Álvaro López-Jofré[1], Sebastián Hernández[1,2] and Héctor Flores[3,4]

[1]Sala de Colecciones Biológicas, Facultad de Ciencias del Mar, Universidad Católica del Norte, Casilla 117, Coquimbo, Chile
[2]Biomolecular Laboratory, Center for International Programs and Sustainability Studies, Universidad Veritas, San José 10105, Costa Rica
[3]Departamento de Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte, Casilla 117, Coquimbo, Chile
[4]Centro de Innovación Acuícola (AquaPacífico), Coquimbo, Chile
*Corresponding author: alvaro.lopez@alumnos.ucn.cl

Abstract.- Chimaeras or Ghost sharks (Subclass Holocepahli) have sexual structures called pre-pelvic claspers, and these structures are covered by dermal denticles. In this study, dermal denticles present in pre-pelvic claspers of the Cockfish, *Callorhinchus callorynchus*, were described in order to compare them with those of other known species of this subclass. Pre-pelvic claspers of 22 specimens were removed for further examination. Dermal denticles on the flat surface of pre-pelvic claspers were different when examining their size, shape, and number of cusps. Dermal denticles were classified as type A (three cusps), B (four cusps), C (five cusps), D (six cusps) and E (eight cusps). Dermal denticles with fewer cusps were located near the center, whereas those with more cusps were located towards the periphery. All types of dermal denticles were found in the largest individuals (> 40 cm length). Possible functions of the dermal denticles and the pre-pelvic clasper in the reproductive activity of this species are suggested.

Key words: Cartilaginous fish, morphology, reproduction, sexual character, chimaera

INTRODUCTION

Placoid scales or dermal denticles cover the skin of cartilaginous fishes (Raschi & Tabit 1992, Hamlett *et al.* 2005). The function of these structures is to provide protection against abrasion, predators, and parasites, reduction of hydrodynamic drag, and bioluminescence (Raschi & Tabit 1992, Sullivan & Regan 2001, Southall & Sims 2003, Dean & Bhushan 2010, Lang *et al*. 2012). Moreover, dermal denticles can be useful for species identification (MacLeod 1982, Sire *et al*. 1998, Miyake *et al*. 1999) or age and growth estimates (Serra-Pereira *et al*. 2008). According to these functions, there are several morphological traits of dermal denticles that may differ among species as well as within species, showing ontogenic differences and/or varying in their body location (Gravendeel *et al.* 2002, Lang *et al.* 2012). The length of the scales and the number of keels or riblets on the crown surface varies among mobile swimming sharks. For instance, the fast-swimming shortfin mako, *Isurus oxyrinchus* has small crown length (0.18 mm) and has only three keels, whereas the crown of the slower swimming blacktip shark *Carcharhinus limbatus* is almost twice as long (0.32 mm) and has five keels (Lang *et al.* 2012).

During the reproduction of cartilaginous fishes, internal fertilization is carried out by the paired claspers of the male (Hamlett *et al*. 2005, Ahlberg *et al*. 2009). Claspers represent sexual structures whose interspecific variation is used in alpha taxonomy (Leigh-Sharpe 1924, Taniuchi & Ishihara 1990). Males of the family Callorhinchidae have simple rod-like intromittent claspers without broaden tips, and additional appendages on the head and the pre-pelvic area of mature males (Last & Stevens 2009). In the pre-pelvic area, there is a slit that has a small and retractable structure anterior to each side of the pelvic fins. This structure is known as pre-pelvic clasper and it has been suggested males use the dermal denticles of this structure to hold females during copulation (Last & Stevens 2009). The shape, size, distribution, and number of dermal denticles of the pre-pelvic claspers vary across families Callorhinchidae, Chimaeridae, and Rhinochimaeridae; and they are described for several species such as *Hydrolagus alphus* (Quaranta *et al*. 2006); *Callorhinchus milii*, *Chimaera fulva*, *Hydrolagus homonycteris*, *Harriotta raleighana*, and *Rhinochimaera pacifica* (Last & Stevens 2009); *Hydrolagus macrophthalmus* (González-Acosta *et al*. 2010); *Hydrolagus melanophasma* (Bustamante *et al.* 2012); *Hydrolagus lusitanicus* (Moura *et al.* 2005), and *Chimaera opalescens* (Luchetti *et al*. 2011).

The Cockfish *Callorhinchus callorynchus* (Linnaeus, 1758) (Subclass Holocephali: Family Callorhinchidae), is related to sharks, skates and rays (Subclass Elasmobranchii) (Last & Stevens 2009). Distribution of this species is restricted to South America including Peru, Chile, Argentina, Uruguay and southern Brazil (Menni & López 1984, Di Giacomo 1992, López *et al*. 2000, Gowert & Oddone 2019, Siciliano *et al*. 2020). The Cockfish is an important commercial species that is commonly captured as bycatch of both artisanal fisheries targeting on flounders and industrial trawlers targeting on crustaceans off Coquimbo Bay and it has been suggested that reproductive aggregations of this species are found from early spring and summer (Acuña *et al.* 2007, Hernández *et al*. 2010). However, despite the occurrence of the Cockfish in the country's fisheries, seasonality of its reproductive peaks at some regions has been putted into question (Alarcón *et al*. 2011) and its reproductive behavior remains poorly understood. The description of sexual characters such as pre-pelvic claspers may indicate some reproductive aspects associated to behavior during copulation of the Cockfish (Colonello *et al.* 2011*)*. Therefore, the objective of this study was to describe the dermal denticles of pre-pelvic claspers of the Cockfish and compare them with those of other chimaeras.

Materials and methods

A total of 22 fresh male individuals of *Callorhinchus callorynchus* were collected from landings at the Coquimbo fishing village (29°57'S; 71°20'W). The pre-pelvic claspers were dissected from each fish and stored in 95% ethanol (approximately one hour after capture). Tissue samples of 1 cm^2 were dissected from the inner face of the blade where the dermal denticles are anchored to the skin and lysed at 56 °C for 4 h using a lysis buffer (10 mM Tris-Hcl, 10 mM NaCl, 2 mM EDTA, 1% SDS) containing 40 µL of proteinase K (Ambion 20 mg mL^{-1}). Samples were vortexed to release the dermal denticles from the skin which were subsequently stored in vials containing 95% ethanol. Then, samples were observed and analyzed using an electronic magnifying glass (x3.2 - x4.0, Stemi 2000-C ZEISS®). Line drawings were made using digital images (Appendix, Fig. S1). Samples were finally deposited in the Sala de Colecciones Biológicas, Facultad de Ciencias del Mar, Universidad Católica del Norte of Coquimbo, Chile (catalog 7137: CBUCN 5500-5521) for further registration and analysis.

Results and discussion

This study provided the first morphological description of the skin-anchored dermal denticles of pre-pelvic claspers of *Callorhinchus callorynchus*. The examined specimens ranged from 35.5 cm to 60.3 cm in total length, with an average size of 49.9 cm, whereas ranging in weight from 0.70 kg to 1.34 kg, with an average weight of 1.03 kg. (Appendix, Table S1).

The pre-pelvic claspers of *C. callorynchus* are located inside the pre-pelvic cavity, anterior to the pelvic fins (Fig. 1A). The extracted pre-pelvic claspers are twisted-shaped and are composed of cartilaginous appendages ending in a funnel-shaped lobe (Fig. 1B). These appendages protrude beyond the pre-pelvic cavity (Fig. 1B). There are several dermal denticles with multiple cusps along the inner side of the horizontally flattened surface in which the denticles with fewer cusps are located in the center and those with more cusps are located towards the periphery of this side (Fig. 1C).

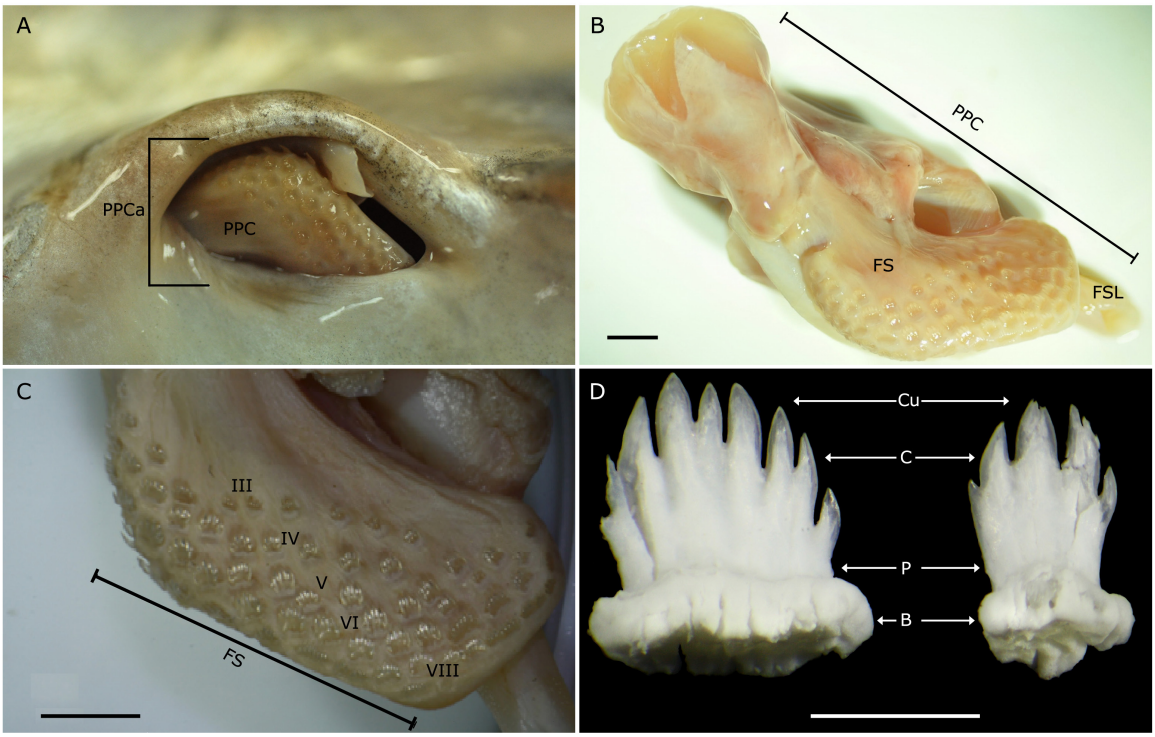

Figure 1. Pre-pelvic clasper of an adult specimen of *C. callorynchus*, and its dermal denticles. A) pre-pelvic clasper (PPC) inside the pre-pelvic cavity (PPCa) anterior to the pelvic clasper. B) pre-pelvic clasper (PPC) out of the pre-pelvic cavity (FS= flat surface; FSL= funnel-shaped lobule). Bar scale= 0.5 cm. C) Dermal denticles on the flat surface (FS) of the pre-pelvic claspers (III= denticles of three cusps, IV= denticles of four cusps, V= denticles of five cusps, VI= denticles of six cusps, VIII= denticles of eight cusps). Bar scale= 0.5 cm. D) typical shape of dermal denticles found in the pre-pelvic clasper, showing the main parts of the structure (Cu= cusp, C= crown, P= peduncle, B= base). Bar scale= 1 mm / Clasper pre-pélvico de especímen adulto de *C. callorynchus*, y sus dentículos dérmicos. A) clasper pre-pélvico (PPC) dentro de la cavidad pre-pélvica anterior al clasper pélvico (PPCa); B) clasper pre-pélvico (PPC) extraído de su cavidad (FS= superficie plana; FSL= lóbulo en forma de embudo). Escala de barra= 0,5 cm; C) dentículos dérmicos en la superficie plana (FS) del clasper pre-pélvico (III= dentículos de tres cúspides, IV= dentículos de cuatro cúspides, V= dentículos de cinco cúspides, VI= dentículos de seis cúspides, VIII= dentículos de ocho cúspides). Escala de barra= 0,5 cm; D) forma típica de los dentículos hallados en el clasper pre-pélvico, mostrando las principales partes de la estructura (Cu= cúspides, C= corona, P= pedúnculo, B= base). Escala de barra= 1 mm

These dermal denticles are anchored to the skin and oriented to the inner side of the pre-pelvic clasper, where the crown is flexible and mobile. In general, all types of denticles have a regular base with elongated and elliptical shape. The peduncle is relatively short and the crown is flat, with cusps inwardly oriented (Fig. 1D). Dermal denticles varied in size and number of cusps (Fig. 2). According to the number of cusps, they were classified as type A (three cusps), type B (four cusps), type C (five cusps), type D (six cusps) and type E (eight cusps). In specimens exceeding 40 cm in total length, the observed pre-pelvic claspers have all mentioned types of dermal denticles, whereas the two smaller specimens (< 40 cm length) lacked these structures (Table 1). In general, denticles with fewer cusps have a shorter base, more elongated peduncles, and are located in more central areas of the pre-pelvic clasper, while those with more cusps have a more elongated base, narrower peduncles and are located in greater proportion towards the periphery of the pre-pelvic clasper (Appendix, Table S2).

Dermal denticles associated with claspers have been described for other chimaerid species (Moura *et al.* 2005, Quaranta *et al.* 2006, González-Acosta *et al.* 2010, Luchetti *et al.* 2011, Bustamante *et al.* 2012). However, variations in number, shape and orientation of dermal denticles have been described for different species. For example, *Hydrolagus alphus* have at least four pre-pelvic claspers in the form of hooks located on the external margin of the structure (Quaranta *et al.* 2006). This species has few dermal denticles on its pre-pelvic claspers compared to *C. callorhynchus* which has 60-70 denticles on the posterior area of the clasper (Fig. 1C). The species *Hydrolagus melanophasma* has four to five dermal denticles along the medial edge of the pre-pelvic clasper (Bustamante *et al.* 2012) and *H. macrophthalmus* has only three dermal denticles on the lateral margin (González-Acosta *et al.* 2010). Other species of *Hydrolagus* have pre-pelvic claspers with denticles on their external margin; about three denticles in *H. homonycteris* (Last & Stevens 2009),

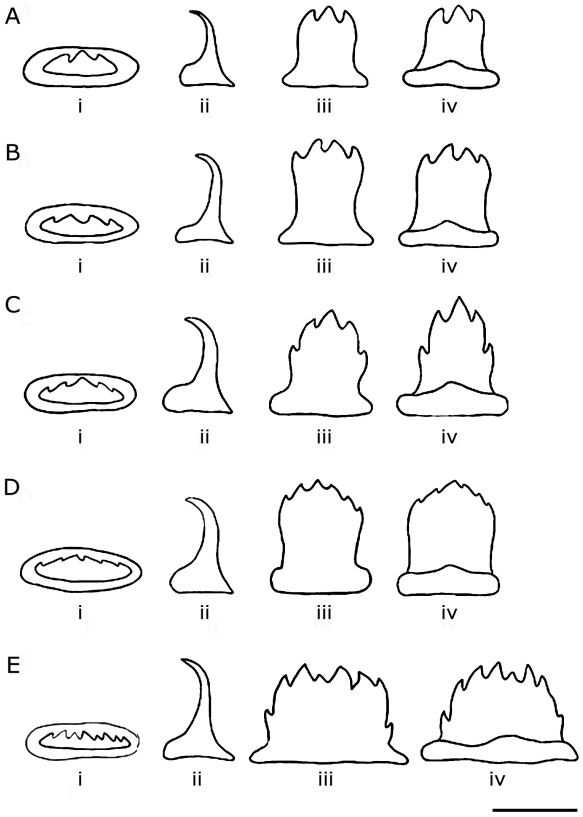

Figure 2. Shape of the dermal denticles of the pre-pelvic clasper of *C. callorynchus* from different angles. A) denticles of three cusps; B) denticles of four cusps; C) denticles of five cusps; D) denticles of six cusps; E) denticles of eight cusps. i= top view, ii= lateral view, iii= back view, iv= frontal view. Bar scale= 1 mm / Forma de los dentículos dérmicos del clasper pre-pélvico de *C. callorynchus*, vistos desde distintos ángulos. A) dentículos de tres cúspides; B) dentículos de cuatro cúspides; C) dentículos de cinco cúspides; D) dentículos de seis cúspides; E) dentículos de ocho cúspides. i= vista superior, ii= vista lateral, iii= vista trasera, iv= vista frontal. Escala de barra= 1 mm

and up to six denticles in *H. lusitanicus* (Moura *et al.* 2005). Chimaera species such as *Chimaera fulva* and *C. opalescens* also have pre-pelvic claspers with rows of five to eight dermal denticles on the internal margin, and one or two irregularly shaped denticles in some specimens (Last & Stevens 2009, Luchetti *et al.* 2011). Rhinochimaeridae species, such as *Rinochimaera pacifica* and *Harriotta raleighana*, have four to five well-formed denticles on the internal margin of the pre-pelvic clasper (Last & Stevens 2009). Most of the species mentioned have single-cusp denticles on their reproductive structures and only in *C. mili* is it possible to observe dermal denticles with different numbers of cusps, as well as *C. callorynchus*. The two pre-pelvic dermal denticles of species of *Callorhinchus* have a similar shape, and differ from the pre-pelvic dermal denticles in species within Chimaeridae (*i.e.*, *Hydrolagus* and *Chimaera*), which have a simple discoidal shape (Last & Stevens 2009).

A pattern in the number of dermal denticles was also observed when comparing different genera or families of the species mentioned above. For example, within *Callorhinchus*, the number of denticles is notoriously greater than within *Hydrolagus* or *Chimaera*. Although it can also be found at great depths, *C. callorynchus* is mostly found in coastal waters, not exceeding 200 m depth (Alarcón *et al*. 2011, Chierichetti *et al*. 2017, Siciliano *et al*. 2020), while species of *Hydrolagus* and *Chimaera* are associated with depths greater than 1000 m (Moura *et al.* 2005, Luchetti *et al.* 2011, Bustamante *et al.* 2012). By living at different depths, differences in the environmental conditions in which the species of these groups develop could be inferred. This, in turn, could suggest that morphological differences in reproductive structures between species are due to these differences in environmental conditions. However, it is very likely that the environmental conditions in which these organisms develop determine behaviors and processes (Walker 2007, Elisio *et al.* 2017) rather than the development of reproductive sexual characteristics.

Table 1. Types of dermal denticles found in pre-pelvic claspers of each *C. callorynchus* specimen according to body size (TL= total length). Dermal denticles are classified according to the number of cusps they have; III= three cusps, IV= four cusps, V= five cusps, VI= six cusps, and VIII= eight cusps. The presences (p), absences (a) and the number (N) of types of dermal denticles are shown for each specimen / Tipos de dentículos dérmicos encontrados en clasper pre-pélvicos de cada ejemplar de *C. callorynchus* según su tamaño corporal (LT= longitud total). Los dentículos dérmicos se clasifican según el número de cúspides que tienen; III= tres cúspides, IV= cuatro cúspides, V= cinco cúspides, VI= seis cúspides y VIII= ocho cúspides. Se muestran las presencias (p), ausencias (a) y el número (N) de tipos de dentículos dérmicos para cada ejemplar

LT (cm)	Type of dermal denticle					N	LT (cm)	Type of dermal denticle					N
	III	IV	V	VI	VIII			III	IV	V	VI	VIII	
35.5	a	a	a	a	a	0	49.6	p	p	p	p	p	5
36.9	a	a	a	a	a	0	51.3	p	p	p	p	a	4
40.2	p	p	p	p	p	5	55.6	p	p	p	p	p	5
40.4	p	p	p	p	p	5	56.3	p	p	p	p	p	5
42.3	p	p	p	p	p	5	56.6	p	p	p	p	p	5
44.2	p	p	p	p	p	5	56.8	p	p	p	p	p	5
44.5	p	p	p	p	p	5	57.4	p	p	p	p	p	5
47.9	p	p	p	p	p	5	57.9	p	p	p	p	p	5
48.2	p	p	p	p	p	5	58.4	p	p	p	p	p	5
48.5	p	p	p	p	p	5	60.2	p	p	p	p	p	5
49.3	p	p	p	p	p	5	60.3	p	p	p	p	p	5

Another notable aspect is the apparent relationship between the size of a Cockfish individual and the variety of dermal denticles it may have in its pre-pelvic clasper. This can be observed in the smallest individuals (35-40 cm total length) examined in this study, which completely lacked dermal denticles on their pre-pelvic claspers, while in larger individuals all types of dermal denticles were found. This is important considering that the average size at which males of this species reach sexual maturity has been estimated at 43 cm in total length (Alarcón *et al*. 2011, Bernasconi *et al*. 2015, Chierichetti *et al.* 2017). Therefore, individuals smaller than 40 cm (juveniles) probably do not develop dermal denticles, being useful structures to identify mature males.

Most studies on the reproductive biology of *C. callorhynchus* focus mainly on aspects such as sexual maturity, sexual dimorphism and other sexual characteristics (Di Giacomo & Perier 1994, Chierichetti *et al*. 2017). Studies focusing on more specific aspects such as morphological descriptions of the pre-pelvic clasper and other structures such as the cephalic tenaculum could provide more information on the reproductive biology of this species. Cartilaginous fishes have evolved different mating behaviors according to the reproductive traits possessed by species in different groups (Wourms 1977). For example, in elasmobranch species, males hold the female by softly biting her pectoral fins to perform internal fertilization during copulation (Wourms 1977, Pratt & Carrier 2011). It is suggested that male holocephalans, on the other hand, use the pre-pelvic claspers and cephalic claspers as tools to grasp the female during mating (Wourms 1977). Studies about reproductive biology of *C. callorynchus* made by Di Giacomo & Perier (1994), also suggest the importance of both appendages in the copulation process of this species. Furthermore, the origin and development of the dermal denticles are directly related to the ontogenetic growth of cartilaginous fishes (Miyake *et al.* 1999). Therefore, the dermal denticles found in the pre-pelvic claspers of this species would have their origin in different aspects of its reproductive behavior typical of the group to which it belongs. Further studies on morphology of sexual characters may be useful in understanding reproductive behavior of this and other chimaeras. It is proposed here that *C. callorynchus* has been a good model for the study of sexual characteristics not been addressed in greater detail until present.

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Appendix

Table S1. Body measurements of *Callorhinchus callorynchus* specimens (N= 22); TL= total length of the individuals, PL= peduncle length (length of the head of peduncle), W= weight of the individuals. No weight data for the first three individuals due to loss of samples / Medidas corporales de los especímenes de *Callorhinchus callorynchus* (N= 22); TL= longitud total del individuo, PL= longitud del pedúnculo (longitud de la cabeza del pedúnculo), W= peso del individuo. No hay datos de peso de los tres primeros individuos debido a la pérdida de muestras

N	TL (cm)	PL (cm)	W (g)	N	TL (cm)	PL (cm)	W (g)
1	40.4	20.9	-	12	47.9	32.5	930
2	60.3	40.1	-	13	40.2	29.0	729
3	60.2	40.2	-	14	42.3	30.5	793
4	49.6	40.9	1.134	15	58.4	42.2	1.344
5	55.6	46.2	1.203	16	44.2	31.7	802
6	51.3	42.4	1.195	17	57.9	42.3	1.302
7	56.6	40.3	1.209	18	35.5	26.3	703
8	56.3	41.2	1.198	19	56.8	42.4	1.209
9	49.3	38.0	1.150	20	48.5	32.8	942
10	44.5	32.9	834	21	36.9	26.2	744
11	57.4	41.8	1.210	22	48.2	33.1	955

Table S2. Types of dermal denticles under electronic magnifying glass considering the number of cusps in the crown of the denticule, their shape and their location in the pre-pelvic clasper of *Callorhinchus callorynchus* / Tipos de dentículos dérmicos en lupa electrónica considerando el número de cúspides en la corona del dentículo, su tamaño y su ubicación en el clasper pre-pélvico de *Callorhinchus callorynchus*

N° of cusps	Description	Location of dermal denticles
3	Regular shape with almost same size cusps. The crown (body of the denticle) is approximately twice the size of the base. The crown is projected from the center of the base, and it curve in the upper end. Non-prominent peduncle resembling a small depression at the edge of the denticle just under the crown. The base is wide and it has an irregular discoidal shape higher on one side.	Center
4	Irregular shape and size (no cusp protrude from the rest). The crown is extended and approximately three times the size of the base. The crown is projected from the center of the base in a double sheet (almost imperceptible) and curves at the upper end. Non-prominent peduncle. The base is thin and it has an irregular discoidal shape higher on one side.	Center
5-6	Irregular shape and size. The five cusps follow a pattern of size, with smaller, thinner cusps at the edges, and larger, wider cusps toward the center of the crown. The central cusp is more prominent than the rest. The crown is almost three times the size of the base. The crown is projected from the center of the base in a double sheet (almost imperceptible) and curves at the upper end. The peduncle is not very prominent. The base is big and wide, and it has an irregular discoidal shape higher on one side.	Center and periphery
8	Irregular shape and size. The crown has a saw shape at the top, with finer and more pointed cusps. The crown is almost twice the size of the base, though it is much more flattened than in the rest of the denticules. The crown is projected form the center of the base and curves on the top end. The peduncle is short. The base is thin and it has an irregular discoidal shape higher on one side.	Periphery

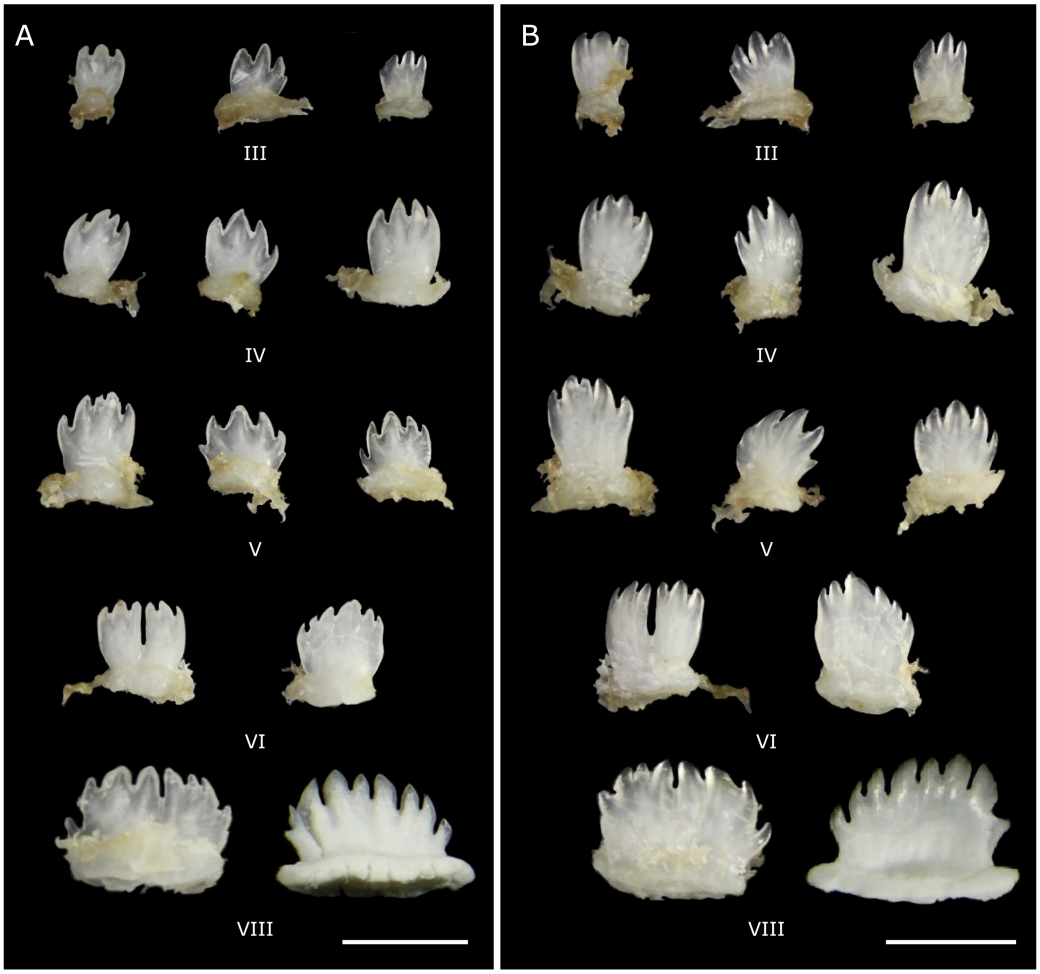

Figure S1. Shape of the dermal denticles in pre-pelvic claspers of *C. callorynchus*. A) Frontal view; B) Back view. Pictures of denticles of three, four, five, six and eight cusps obtained with an electronic magnifying glass (III, IV, V, VI and VIII, respectively). Bar scale= 5 mm / Forma de dentículos dérmicos observados en claspers pre-pélvicos de *C. callorynchus*. A) Vista frontal; B) Vista trasera. Fotografías de dentículos de tres, cuatro, cinco, seis y ocho cúspides obtenidas con lupa electrónica (III, IV, V, VI y VIII, respectivamente). Escala de barra= 5 mm