

# LAEVIPILINA CACHUCHENSIS, A NEW NEOPILINID (MOLLUSCA: TRYBLIDIA) FROM OFF NORTH SPAIN

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## ABSTRACT

A new tryblidian species, *Laevipilina cachuchensis*, is described from a depth of 580–600 m on ‘El Cachucho’ Bank (Asturias, north Spain). This resembles the three described species of *Laevipilina* McLean, 1979, differing mainly in shell characters (height/length ratio, apex position, size of prisms forming prismatic layer, relative size and arrangement of insertion areas of dorsoventral muscles), and details of radular morphology. Two living specimens (1.9 and 1.6 mm in length) were found on ferromanganese laminar nodules. The digestive content is of organic particles, sediment and fragments of foraminiferans and radiolarians.

## INTRODUCTION

Twenty-five recent species of tryblidians are currently known (Warén & Gofas, 1996; Marshall, 1998; Warén & Bouchet, 2001). Eight of them were described from the Atlantic Ocean: *Neopilina rebainsi* Moskaliev, Starobogatov & Filatova, 1983, from the Scotland Sea, SE Malvinas Islands; *Micropilina minuta* Warén, 1989, from Iceland; *Rokopella euglypta* (Dautzenberg & Fischer, 1897) from the Azores; *R. brummeri* Goud & Gittenberger, 1993, and *R. segonzaci* Warén & Bouchet, 2001, from the mid-Atlantic Ridge; *Veleropilina goesi* Warén, 1988, from the Virgin Islands; *V. zografii* (Dautzenberg & Fischer, 1896) from the Azores; and *Laevipilina rolani* Warén & Bouchet, 1990, from NW Spain (Goud & Gittenberger, 1993; Moskaliev *et al.*, 1983; Urgorri & Troncoso, 1994; Warén, 1988, 1989; Warén & Bouchet, 1990, 2001; Warén & Gofas, 1996).

Tryblidian taxonomy is based mainly on shell morphology and structure, and radular features and soft parts, when the latter are known. Even on the few occasions in which a number of complete specimens of the same species have been studied, little is known about the intraspecific variation of the taxonomic characters used. This makes it difficult to describe a new species, even in a relatively well known genus such as *Laevipilina* McLean, 1979, which comprises three similar species: *L. hyalina* McLean, 1979, off California, the above-mentioned *L. rolani*, and *L. antarctica* Warén & Hain, 1992, from the Lazarev and eastern Weddell Sea (Antarctica) (McLean, 1979; Warén & Hain, 1992).

Two specimens of *Laevipilina* were collected alive from off north Spain during the ‘Fauna Ibérica II’ expedition (1991). The study of shell, soft parts and radula of these specimens and the comparison with the three known species of *Laevipilina* revealed enough differences to be recognized as a different species, and it is herein described.

## MATERIAL AND METHODS

The two specimens studied were preserved in 70% ethanol after being photographed alive. Soft parts of the holotype were dehydrated in an ethanol series, embedded in Spurr’s resin, serially cross-sectioned (2 µm), and stained with methylene blue and 1.5% borax. Unfortunately, several of the serial sections were lost and the staining was not good. Soft parts of the paratype were

critical-point dried in their shell, SEM photographed, then removed and soaked in 5% potassium hydroxide to prepare the radula. Finally, shells of both specimens and the radula of the paratype were photographed with SEM.

## SYSTEMATIC DESCRIPTION

### Family Neopilinidae Knight & Yochelson, 1958

*Diagnosis:* See McLean (1979) and Haszprunar & Schaefer (1996).

### Genus *Laevipilina* McLean, 1979

*Type species:* *Vema (Laevipilina) hyalina* McLean, 1979, by original designation.

*Diagnosis:* See McLean (1979) and Warén & Bouchet (1990).

### *Laevipilina cachuchensis* new species

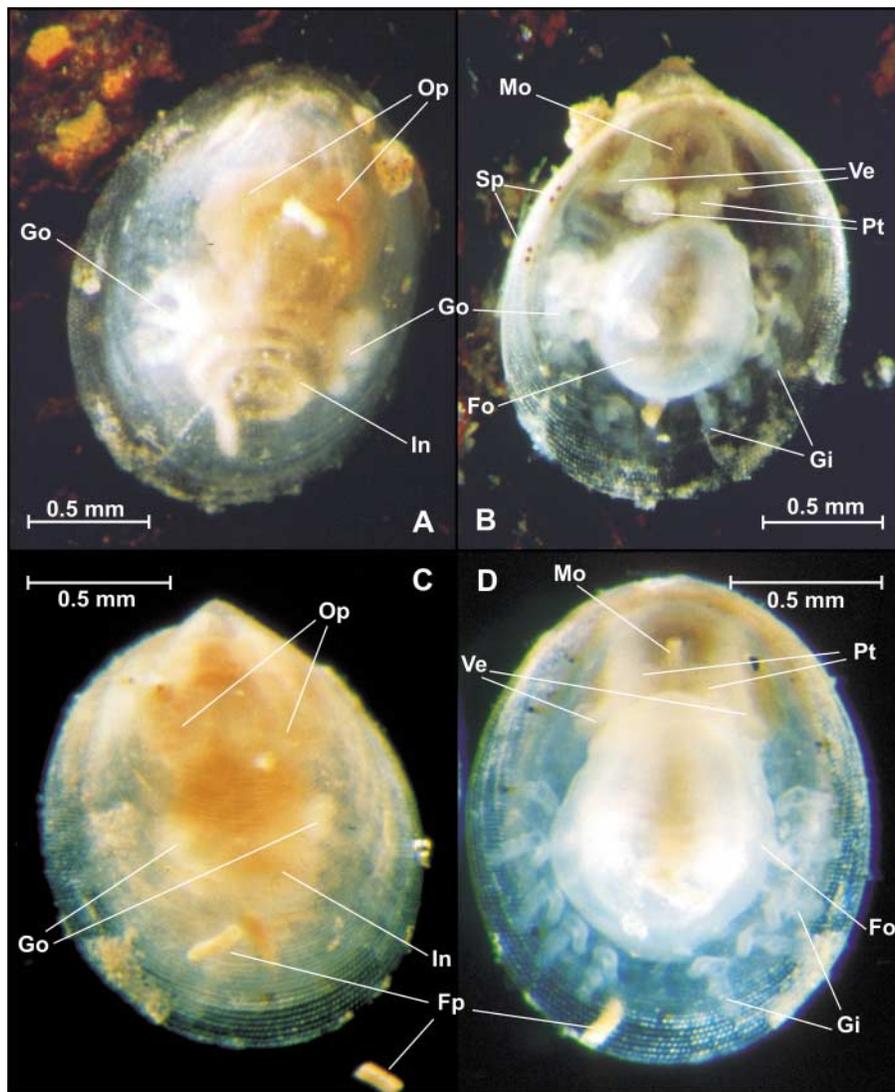
*Type material:* Holotype (Figs 1A, B, 2, 3A, B, 6) (Museo Nacional de Ciencias Naturales, Madrid, MNCN 15.04/1), 1.9 mm length, 1.5 mm width, 0.6 mm height (soft parts sectioned, shell SEM mounted). Paratype (Figs 1C, D, 3C, D, 4, 5) (MNCN 15.04/1), 1.6 mm length, 1.3 mm width, and 0.5 mm height (critical-point dried, radula extracted, shell SEM mounted).

*Type locality:* Fauna Ibérica II Expedition, station 162A (44° 02' 13" / 19" N, 04° 50' 28" / 51' 05" W) ‘El Cachucho’ Bank, north of Asturias (north Spain), 580–600 m depth, 26 June 1991. This station is a deep depression with laminar ferromanganese nodules; on the horizontal bottom there is a dense population of the gorgonian *Callogorgia verticillata* (Pallas, 1766). Both specimens were found in crevices of nodules. The accompanying fauna in the sample was scarce, with numerous specimens of the holothurian *Psolidium* sp., and some specimens of the gastropod *Anatoma* sp. and the bivalve *Cuspidaria* sp.

*Etymology:* From the type locality (‘El Cachucho’).

*Shell.* The shell is small (to 1.9 mm length, 1.5 mm width; 0.6 mm height), oval, thin, fragile, transparent and iridescent (Figs 1, 2), with a height/length ratio of 0.31. The posterior surface is convex and the anterior surface (under the apex) concave, forming an angle of almost 90° with the base (Fig. 2A, B). The shell margin is regular and smooth (Fig. 1B, D). The apex is pointed

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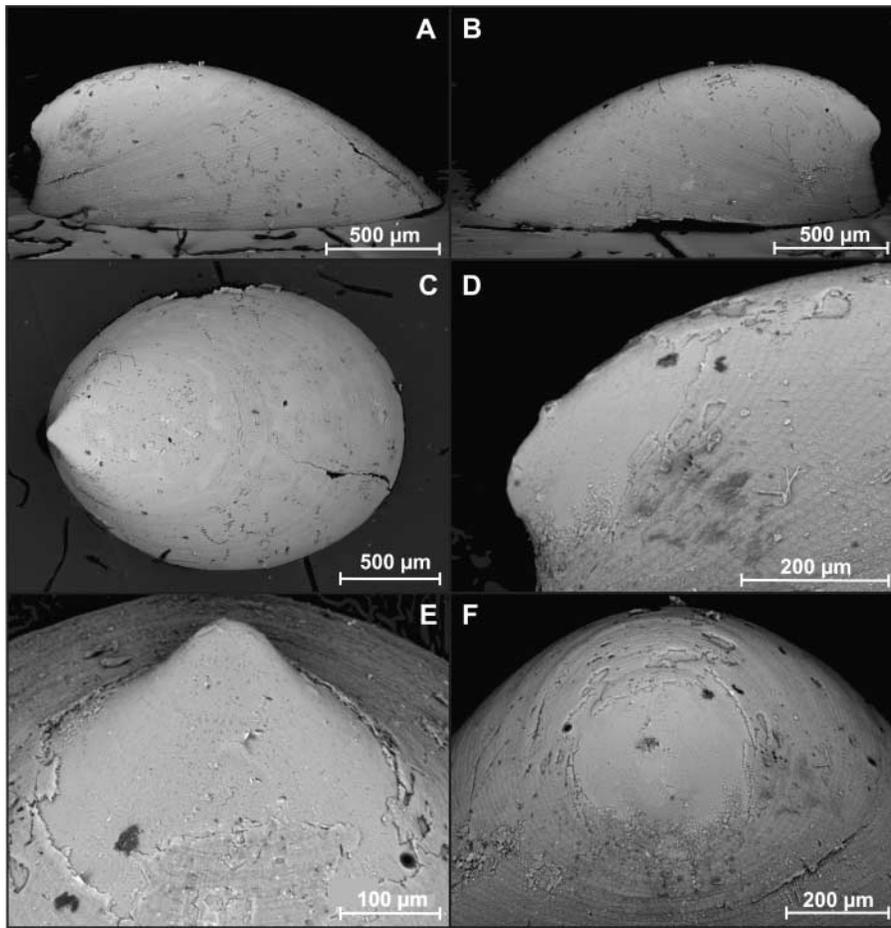


**Figure 1.** *Laevipilina cachuchensis* n. sp. **A, B.** Dorsal and ventral view of the living holotype. **C, D.** Dorsal and ventral view of living paratype. Abbreviations: Fo, foot; Fp, faecal pellets; Gi, gill; Go, gonad; In, intestine; Mo, mouth; Op, oesophageal pouches; Pt, postoral tentacles; Sp, reddish brown spots; Ve, velum.

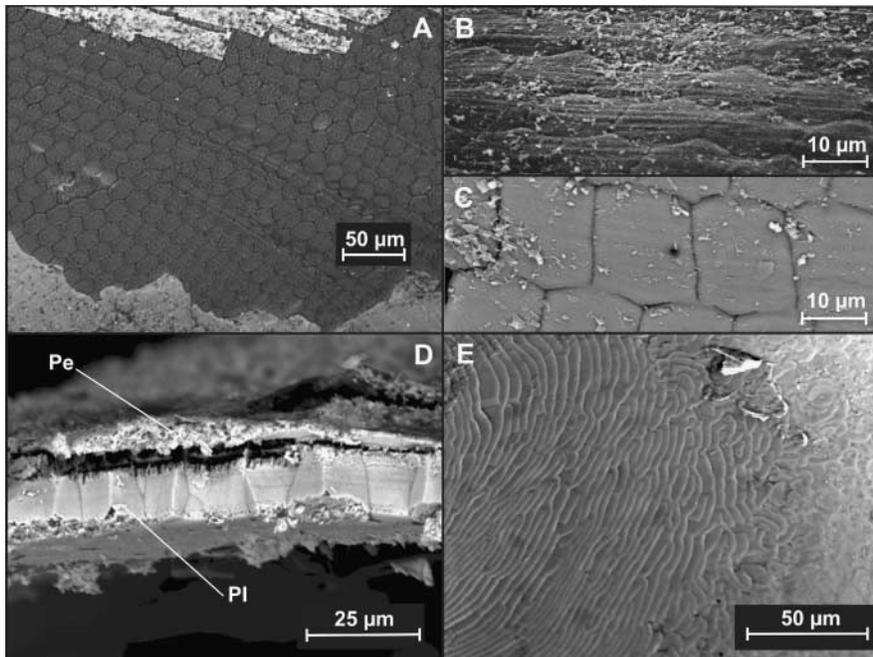
and directed forward, and is situated just above the anterior margin (Fig 2C). The apical area is eroded in both specimens and thus it cannot be observed if there is any distinct sculpture or a clear separation from the rest of the shell (Fig. 2D–F). Shell sculpture comprises hexagonal prism-shaped concentric rows, smaller and more tightly packed under the apex than on the dorsal-rear surface of the shell; SEM revealed extremely faint, almost imperceptible concentric lines on the surface of the prisms (Fig. 3A–C). The shell structure (Fig. 3A–D) consists of a thin periostracum, a prismatic layer and an inner nacreous layer. The prismatic layer (Fig. 3A–C) is formed by concentric lines of hexagonal prisms, 13.5  $\mu\text{m}$  in height and 12–26.5  $\mu\text{m}$  in diameter; the periostracum and prismatic structure is eroded at the apical area. The nacreous layer is thin, with more densely packed sheets at the central part of the shell (Fig. 3D). On the inner surface of the paratype are visible the insertion areas of eight pairs of dorsoventral muscles (A to H, according to the nomenclature of Lemche & Wingstrand, 1959, and Schaefer & Haszprunar, 1996), as well as the bundles forming the different muscles (Fig. 4). The diameter of the muscles decreases progressively from the anterior to the posterior part. The most anterior pairs of muscles are the partly fused A and B (A + B),

formed by more than 20 bundles. The pairs C, D and E are of similar size, each of them formed by 14 bundles; C is very close to A + B, C and D being the most separate, and D and E are at an intermediate distance. The pairs F, G and H have respectively 8, 11 and 5 muscular bundles; H is the smallest muscle and F is slightly more inner than the others. This dorsoventral musculature was also observed in the serial sections of the holotype, arranged obliquely from the external edge of the foot to the dorsal part of the animal (Fig. 6D).

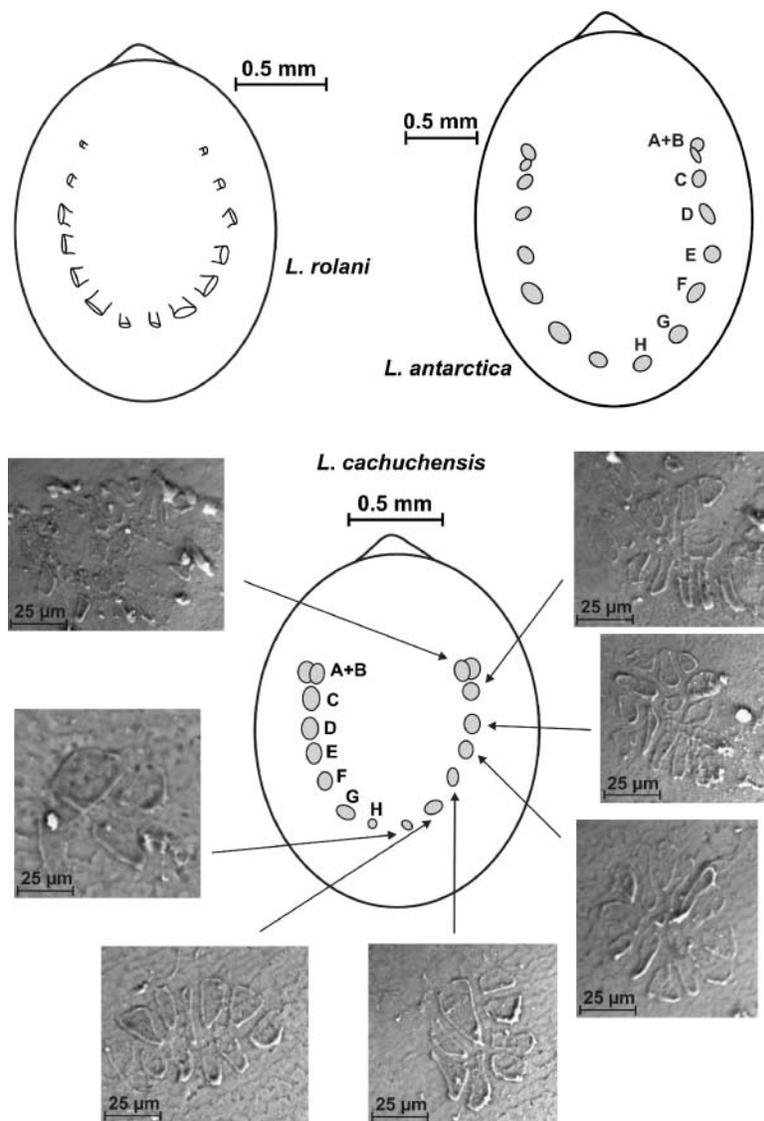
*Soft parts.* The living animal is transparent and lacks any external pigmentation, except for a very pale brown colour in the anterior margin of the mantle and the cephalic area, and eight small rounded reddish brown spots irregularly scattered on the mantle margin (Fig. 1B, D). The velar lobes are well developed, and almost reach back to the foot when both velum and foot are expanded (Fig. 1B, D). The velum is separated from the anterior lip of the mouth by a deep, narrow groove that is clearly visible and somewhat cuticularized. The post-oral tentacles are short and claviform and are arranged in two clusters, each one with more than 18 tentacles (exact number could not be counted). The foot is translucent, sucker-shaped, almost circular when



**Figure 2.** *Laevipilina cachuchensis* n. sp. Shell of the holotype. **A, B.** Lateral view. **C.** Dorsal view. **D, E, F.** Detail of apex and apical area.



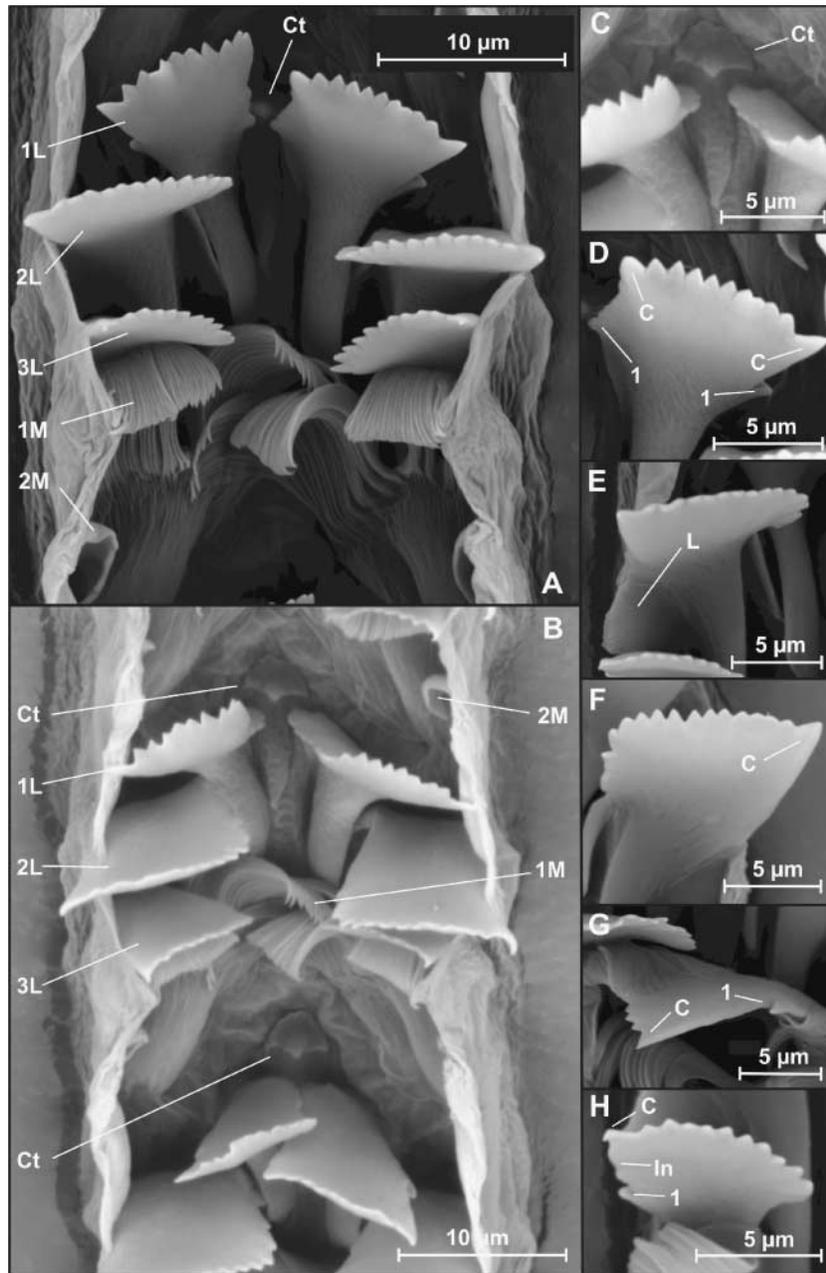
**Figure 3.** *Laevipilina cachuchensis* n. sp. Shell structure. **A.** Periostracum and prismatic layer (holotype). **B, C.** Detail of the prismatic layer (holotype). **D.** Section of the prismatic layer (paratype). **E.** Nacreous layer (paratype). Abbreviations: Pe, periostracum; Pl, prismatic layer.



**Figure 4.** Comparative schematic view of diameter and arrangement of the insertion areas of dorsoventral muscles of *Laevipilina rolani* (according to Urgorri & Troncoso, 1994), *Laevipilina antarctica* (according to Schaefer & Haszprunar, 1996) and *Laevipilina cachuchensis* n. sp., with micrographs of the insertion areas of the latter species in the paratype.

contracted ( $0.90 \times 0.76$  mm) and oval when expanded, with a thin extended margin. The mantle cavity surrounding the foot is wide, becoming narrow towards the cephalic area. There are five pairs of gills placed at the posterior two-thirds of the foot, the last pair near the anal papilla, each one with two to three 'digits' (leaflets). The anus is situated at the end of a small papilla, at the middle of the posterior part of the mantle cavity. With transmitted light, two pairs of light brown voluminous oesophageal pouches were visible dorsally, the anterior ones smaller than the posterior, and the four coils of the intestine (Fig. 1A, C), which shows a diameter in sections of  $50\text{--}100\ \mu\text{m}$  (Fig. 6D, E). The digestive tract contained abundant organic particles and sediment, with some fragments of foraminiferans and radiolarians; some cylindrical faecal pellets were observed during the study of living specimens (Fig. 1C, D). The gonads were also observed through shell as a pair of white and lobate sacs placed ventrally on both sides of the intestine (Fig. 1A, C). The serial sections of the holotype (probably a ripe female) show an ovary containing two to three large and long oocytes (up to  $180 \times 36\ \mu\text{m}$ , Fig. 6B, C).

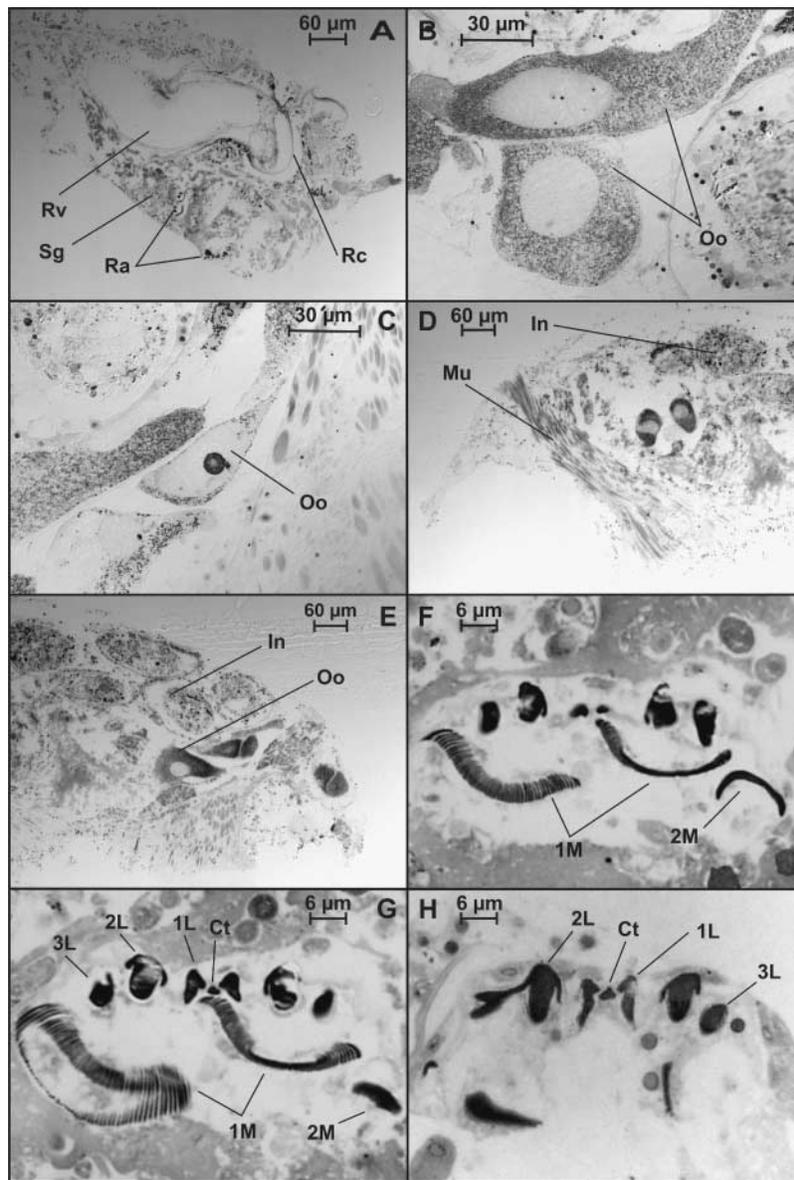
**Radula.** The radula of the paratype (1.6 mm) has a length of  $990\ \mu\text{m}$  and width of  $45\ \mu\text{m}$ , with 44 transverse rows of 11 teeth; the width measured in holotype sections was between 30 and  $60\ \mu\text{m}$ . The radula has two complete whorls and is surrounded by the glandular mass of salivary glands (Fig. 6A). Two large radular cartilages are located dorsally to the radular sac, delimiting a large central radular vesicle (Fig. 6A). The oesophageal pouches are placed on both sides of the radular vesicle. The rows of teeth are arranged in a V, with slight asymmetry (Fig. 5A, B). The central tooth is long and narrow, with a small cusp ending in a main central frontal denticle and one smaller denticle on each side (1-M-1, Fig. 5A–C, see Table 2). In section, the basal part is trilobed, whereas the apical part is rounded (Fig. 6G, H). The lateral teeth have a narrow and elongated base and a frontally truncated and serrate scoop-shaped cusp. The first and third lateral teeth are similar in size, whereas the second tooth is the largest. The first lateral has 13 denticles (1-C-9-C-1, Fig. 5A, B, D; see Table 2); 11 on the frontal margin of the cusp (C-9-C), of which the outermost denticle on each side (C) is stronger than the central ones, and



**Figure 5.** *Laevipilina cachuchensis* n. sp. Radula of paratype. **A.** Postero-frontal view. **B.** Antero-frontal view. **C.** Central tooth. **D.** First lateral tooth. **E, F.** Second lateral tooth. **G, H.** Third lateral tooth. Abbreviations: C, denticle forming corner; Ct, central tooth; In, indistinctly denticulate edge; L, laminar edge; 1, denticle on the cusp margin; 1L, first lateral tooth; 2L, second lateral tooth; 3L, third lateral tooth; 1M, first marginal tooth; 2M, second marginal tooth.

the lateral edges of the cusp also have a prominent denticle (1) on each side. The second lateral tooth has 15 denticles (2–12-C-L, Fig. 5A, B, E, F; see Table 2); 13 denticles are frontal (12-C), and two laterals on the inner edge; among the frontal denticles, the outermost (C) is the strongest. The second lateral also shows a strong laminar expansion (L) on the outer edge of the stem, clearly visible in serial sections (Figs 5E, 6F). The third lateral tooth has 12 denticles (0–10-C-in-1, Fig. 5A, B, G, H; see Table 2), of which 11 are frontal, and the outermost the strongest (C). On the outer lateral edge (in-1) there is a strong basal denticle (1) and the edge is undulated (in) between this denticle and the outermost frontal one (C). Between the prominent denticles on

the extremes (C), the frontal edge of the cusp of the first lateral tooth is almost rectilinear, hardly curved, whereas in the other two lateral teeth only the outer frontal edge is limited by a strong denticle (C), of which the inner frontal part is more curved. The first marginal tooth is formed by 55 falciform teeth with fan-like arrangement (Figs 5A, 6F, G). The second marginal is hardly visible, but seems to have a small non-denticulate, but slightly uneven cusp (Figs 5A, B, 6F, G).



**Figure 6.** *Laevipilina cachuchensis* n. sp. Micrographs of sections of the holotype. **A.** Radular vesicle. **B, C.** Oocytes. **D, E.** Cross-section. **F, G, H.** Radular teeth. Abbreviations: Ct, central radular tooth; In, intestine; Mu, dorsoventral muscle; Oo, oocyte; Ra, radula; Rc, radular cartilage; Rv, radular vesicle; Sg, salivary gland; 1L, first lateral tooth; 2L, second lateral tooth; 3L, third lateral tooth; 1M, first marginal tooth; 2M, second marginal tooth.

## DISCUSSION

The two specimens described above clearly fit in the family Neopilinidae and the genus *Laevipilina*, according to the diagnoses given by McLean (1979), Warén & Bouchet (1990) and Haszprunar & Schaefer (1996).

*Laevipilina cachuchensis* n. sp. shows clear differences from the three previously known species of *Laevipilina* (*L. hyalina*, South California, 174–384 m; *L. rolani*, NW Spain, 985–1000 m; *L. antarctica*, Antarctica, 210–644 m depth, Tables 1 and 2), according to the published descriptions by McLean (1979), Warén & Bouchet (1990), Warén & Hain (1992), Urgorri & Troncoso (1994), Schaefer & Haszprunar (1996), Warén & Gofas (1996) and Haszprunar & Schaefer (1997).

The size of the new species is similar to that of *L. rolani*, but smaller than *L. hyalina* and *L. antarctica*. The height/length shell ratio (0.31) is similar to that of *L. hyalina* (0.32), but differs from the higher *L. rolani* (0.41) and the more depressed *L. antarctica*

(0.25). The apex of *L. cachuchensis* n. sp. is placed just over the anterior shell edge, whereas it does not surpass the anterior edge in *L. hyalina* and *L. antarctica*, and surpasses it in *L. rolani*. The apex is also situated over the half height of the shell in all species, except in *L. hyalina*. The apex of *L. cachuchensis* n. sp. does not have the globular aspect of that of *L. antarctica*, and does not show the clear separation from the shell present in the latter species and *L. rolani*. The prisms of the prismatic layer are hexagonal (sometimes pentagonal and even quadrangular) in all species, but they have a diameter equal to height in *L. hyalina* and *L. rolani* (somewhat higher than height in the latter species, according to Urgorri & Troncoso, 1994), twice the height in *L. antarctica*, and are variable from almost equal to twice in *L. cachuchensis* n. sp.

Regarding soft parts, all species have five pairs of gills, except *L. hyalina* which has six pairs. The living animal of *L. cachuchensis* n. sp. lacks any external pigmentation, except for the pale brown anterior margin of mantle and cephalic area, and the eight

**Table 1.** Comparative shell and soft part traits of *Laevipilina* species.

Species	Shell				Soft parts		
	Max size (mm)	Apex position	Prisms diameter × height (µm)	Ratio H/L	Gills number	Postoral tentacles	Intest. coils
<i>L. hyalina</i> McLean, 1979	2.3	ins	diameter = height <sup>1</sup>	0.27–0.32	6 <sup>1</sup>	Row 6 each side <sup>1</sup>	4 <sup>1</sup>
<i>L. antarctica</i> Warén & Hain, 1992	3	ins	30 × 15 <sup>2</sup>	0.25 <sup>2</sup>	5	Row 7 each side <sup>2</sup>	4 <sup>2</sup>
<i>L. rolani</i> Waren & Bouchet, 1990	1.9	out	25–36 × 20–30 <sup>4</sup>	0.40–0.42 <sup>3,4</sup>	5	Cluster 15–20 each side <sup>3</sup>	4.5 <sup>4</sup> –5 <sup>3</sup>
<i>L. cachuchensis</i> n. sp.	1.9	at	12–26.5 × 13.5	0.31	5	Cluster 18 each side	4

Abbreviations: at, apex above shell periphery; ins, apex inside shell periphery; out, apex outside shell periphery.

After Warén & Gofas (1996) modified after: <sup>1</sup>McLean (1979); <sup>2</sup>Warén & Hain (1992); <sup>3</sup>Waren & Bouchet, 1990; <sup>4</sup>Urgorri & Troncoso, 1990.

**Table 2.** Comparative radular characters of *Laevipilina* species.

Species	Radular tooth shape and denticles					
	Central	1st lateral	2nd lateral	3rd lateral	1st marginal	2nd marginal
<i>L. hyalina</i> McLean, 1979	1-M-1	Truncate 0-C-4-C-0	Truncate 0-C-8-C-0	Truncate ?	Fan 42 <sup>1</sup>	0 <sup>1</sup>
<i>L. antarctica</i> Warén & Hain, 1992	2-M-2	Truncate in-5-C-1 <sup>2</sup>	Truncate in-6-C-in-L <sup>2</sup>	Truncate 0-7-C-0 <sup>2</sup>	Fan 50	0
<i>L. rolani</i> Waren & Bouchet, 1990	1-M-1 <sup>3</sup>	Truncate 1-in-8-C-1 <sup>4</sup>	Truncate 0–11-C-L <sup>4</sup>	Truncate 0–10-C-1 <sup>4</sup>	Fan 45–48 <sup>5</sup> 50–55 <sup>6</sup>	C-in <sup>4,5</sup>
<i>L. cachuchensis</i> n. sp.	1-M-1	Truncate 1-C-9-C-1	Truncate 2–12-C-L	Truncate 0–10-C-in-1	Fan 55	0

The shape and number of denticles is given for each species; the number of denticles is counted from the inner side of the tooth.

Abbreviations: C, prominent denticle forming corner; in, indistinctly denticulate edge; L, laminar edge; M, main denticle; 0, absent.

After Warén & Gofas (1996), modified after: <sup>1</sup> McLean (1979: figure 22); <sup>2</sup> Warén & Hain (1992: figs 10–14); <sup>3</sup> own unpublished data; <sup>4</sup> Warén & Bouchet (1990: figs 7–8); <sup>5</sup> Urgorri & Troncoso (1994: figure 6); Haszprunar & Schaefer (1997: figure 59).

reddish brown spots on the mantle border. No reference is given to any pigmentation in living *L. rolani*, except for 13 black spots scattered irregularly around the mantle edge (Urgorri & Troncoso, 1994), and living *L. hyalina* and *L. antarctica* have a more conspicuous external pigmentation (Lowenstam, 1978; Warén & Hain, 1992, figure 19). *Laevipilina hyalina* and *L. antarctica* have six and seven short and claviform postoral tentacles, respectively, whereas *L. rolani* has 15–20 digitiform tentacles, and *L. cachuchensis* n. sp. has short and claviform tentacles like the two former species, but more numerous (more than 18) as in *L. rolani*. The intestine shows 4 whorls in *L. cachuchensis* n. sp., *L. hyalina* and *L. antarctica* and 4.5–5 whorls in *L. rolani*. The diameter of dorsoventral muscles increases backwards in *L. rolani* (except for the posterior pair). In *L. antarctica* they are similar size, whereas they decrease backwards in *L. cachuchensis* n. sp. judging from the diameter of the insertion areas.

Detailed study of radular morphology also shows several differences in the arrangement of the denticles of each tooth, as summarized in Table 2. *Laevipilina hyalina*, *L. rolani* and *L. cachuchensis* n. sp. have a denticle on each side of the cusp of the central tooth, which is smaller than the mid-front tooth, whereas in *L. antarctica* there are two denticles. The first lateral tooth in *L. cachuchensis* n. sp. and in *L. hyalina* has the cusp limited by two prominent denticles. Conversely *L. rolani* and *L. antarctica* have a single prominent denticle in the external part. The second and third lateral teeth in *L. cachuchensis* n. sp. are very similar to those in *L. rolani*, although the latter lacks the two internal lateral denticles of the second tooth and the undifferentiated denticula-

tion on the external edge of the third. The number of hooks or falciform teeth forming the first marginal row is different in the four species (Table 2). The edge of the cusp of the second marginal tooth is smooth in *L. cachuchensis* n. sp., *L. hyalina* and *L. antarctica*, whereas *L. rolani* presents a prominent denticle on the internal edge (McLean, 1979; Warén & Bouchet, 1990; Warén & Hain, 1992; Urgorri & Troncoso, 1994; Warén & Gofas, 1996; Haszprunar & Schaefer, 1997).

*Laevipilina cachuchensis* n. sp. is gonochoristic, like most Tryblidia. The large size of the oocytes of the new species (up to 180 µm in length) agrees with what is known for size of eggs or embryos of *Veleropilina veleronis*, *L. antarctica* and *Micropilina arntzi* (Menzies & Layton, 1963; Haszprunar & Schaefer, 1996, 1997; Schaefer & Haszprunar, 1996).

In the light of the discussed characters, the above-mentioned differences are enough to consider *L. cachuchensis* a new species. Nevertheless, and according to considerations of Warén & Hain (1992: 173) it seems necessary to re-define the genus *Laevipilina* (and other tryblidian genera), and the criteria used to differentiate species, taking into account intraspecific variability and emphasizing characters such as apex and radula. The radula seems to be not so uniform, as pointed out by Warén & Gofas (1996) after a detailed SEM study. Thus, *L. antarctica* has a radula quite different from the other species of this genus, and similar to that of *Veleropilina* sp. (Warén & Gofas, 1996), with a similar central tooth and lateral teeth with few, strong denticles. Also, the globose apex of *L. antarctica* differs from that of the other *Laevipilina* species and resembles the apex of *Veleropilina reticulata*

and *V. zografi* (Warén & Gofas, 1996). The remaining characters defining the genera *Laevipilina* and *Veleropilina* are so similar or ambiguous that they are not clearly diagnostic. The availability of enough well preserved specimens is, of course, the limitation for more detailed studies.

Data on the biology of the different species of Tryblidia are scarce, since the majority are described from empty shells. With the exception of the first two known species (*Neopilina galathea* and *Vema ewingi*), the majority present evident adaptations to life on hard substrata, particularly in the form of the foot and size of the shell. Although the hard substrata on which they were collected varied in character, several species (*Vema levinae*, *L. rolani*, *Monoplacophorus zenkevitchi*, *Veleropilina zografi* and *Micropilina rakiura*) were located on ferromanganese nodules, as was *L. cachuchensis* n. sp. (Cesari, Giusti & Minelli, 1987; Filatova, Sokolova & Levenstein, 1968; Marshall, 1998; Urgorri & Troncoso, 1994; Warén & Gofas, 1996).

As noted by Urgorri & Troncoso (1994) for *L. rolani*, taking into account the similar structure of the radula and the digestive contents, it is possible to deduce that *L. cachuchensis* n. sp. feeds off the layer of organic remains deposited on the substratum on which it lives. We suggest that the cusps of the lateral teeth are used as scraping pallets on the organic-mineral surface layer of the ferromanganese nodules. Narrower cusps, but with a more sturdy denticulation than in *L. antarctica*, may scrape in more consistent layers or on stones with consolidated fauna.

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