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Radula and mandible of *Biomphalaria* (Planorbidae) of São Paulo, Brazil

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Abstract: The radular apparatus of *Biomphalaria occidentalis* Paraense, 1981, *Biomphalaria oligoza* Paraense, 1971, *Biomphalaria peregrina* (d'Orbigny, 1835), *Biomphalaria schrammi* (Crosse, 1864) and *Biomphalaria tenagophila* (d'Orbigny, 1835) from the Metropolitan Region of São Paulo, Brazil were analyzed from Scanning Electron Microscope (SEM) micrographs. The data support the usage of characters of central, lateral and intermediate teeth in the species identification of *Biomphalaria*. The use of SEM provided additional quantitative and structural details to radula descriptions, which are extremely important for elucidating the taxonomy of *Biomphalaria* species.

Key words: radular apparatus, scanning electron microscope, Planorbidae, Mollusca

Planorbidae are freshwater gastropods that constitute an important taxonomic group due to their wide geographic distribution, abundance and epidemiological importance. This group includes species of the genus *Biomphalaria* Preston, 1910, responsible for the transmission of schistosomiasis in South America, Africa, Asia and the Antilles. The helminth *Schistosoma mansoni* Sambon, 1907 is the etiological agent of mansoni schistosomiasis, a serious and chronic disease, which is endemic in Brazil and has an important socioeconomic role (Katz and Dias 1999, Rey 2008).

Studies on the morphology of *Biomphalaria* are of fundamental importance for this group's systematics, as they allow a correct identification of snails, besides helping in the identifying areas likely to be hot spots of schistosomiasis (Paraense 1961). Although morphological features of the radula are known to be important in taxonomic studies of mollusks in general (Martin and Negrete 2007), Scanning Electron Microscopy (SEM) has been little used for the genus *Biomphalaria*; to date most studies have used light microscopy (Paraense 1970, 1972, 1974, 1981; Paraense *et al.* 1964). SEM allows the observation of small structural details of the radula, which cannot be seen through light microscopy (Runham and Thornton 1967, Solem 1972, Gotting 1985, Simison and Lindberg 1999, Matthews-Cascon and Rabay 2003, Colley 2012, Venkatesan *et al.* 2016). Fuentealba and Figueroa (2012), for example, use radula characters based on SEM images to complement the description of a new species of *Biomphalaria* from the Chilean Patagonia. These authors recommend using radular characters to separate genera or groups of higher taxonomic level, agreeing with Paraense (1970, 1972, 2008). Other authors suggest these details can be used for separating species (Martín and Negrete 2007).

In this study we used SEM at high magnification to analyze and describe radulae and produce complement descriptions of the radular apparatus of *B. occidentalis* Paraense, 1981, *B. oligoza* Paraense, 1975, *B. peregrina* (d'Orbigny, 1835), *B. schrammi* (Crosse, 1864) and *B. tenagophila* (d'Orbigny, 1835).

MATERIALS AND METHODS

Snails were collected from several freshwater bodies in municipalities of the Metropolitan Region of São Paulo-SP/Brazil (Table 1). The collections were manual, with the aid of tweezers and perforated metal shell and coupled to a wooden handle, according to the techniques used in the Ministry of Health Schistosomiasis Surveillance and Control Program (Ohlweiler and Rossignoli 2016). A total of 43 snails were anesthetized with menthol crystals for 4 hours, fixed in a Ralliet-Henry solution for 24 hours, identified at species level according to criteria stipulated by Deslandes (1951) and Paraense (1975), cataloged and deposited in the Malacological Collection of the Division of Special Programs of the Superintendence of Control of Endemic Diseases of the State of São Paulo - SUCEN (Table 1). Collection points were georeferenced with a Garmin Etrex 10 GPS.

Maximum shell diameters of the specimens used in this study were: 14.6 mm for *B. tenagophila*, 7.8 mm for *B. occidentalis*, 5.0 mm for *B. oligoza*, 8.4 mm for *B. peregrina* and 5.4 mm for *B. schrammi*. Shells were measured with an analog DE MEO caliper with scales in millimeters (0.05 mm). For the morphological study of radula and mandible, both parts were placed on depression slides containing sodium hypochlorite diluted in distilled water (1:1), in order to dissolve adjacent tissue. They were washed in distilled water and dehydrated in a series of graded ethanol (70%, 90%, 96% and 100%). Both parts were then placed

Table 1. *Biomphalaria* collected in municipalities of the Metropolitan Region of São Paulo, Brazil, and deposited in the malacological collection of the Division of Special Programs of Sucen.

Lot	Species	Municipalities	N	Geographical Coordinates
888	***Biomphalaria occidentalis***	Mogi das Cruzes	3	23°33'14.3"S; 46°8'56.08"W
902			3	23°31'49.4"S; 46°15'56.5"W
3035			2	23°33'08.1"S; 46°16'51.7"W
2939	***Biomphalaria oligoza***	Biritiba Mirim	2	23°36'38.9"S; 46°05'43.9"W
2989			1	23°36'09.1"S; 46°05'13.4"W
2927		Salesópolis	4	23°35'40.6"S; 45°58'41.2"W
1102	***Biomphalaria peregrina***	Biritiba Mirim	4	23°33'22.9"S; 45°59'41.2"W
2926		Salesópolis	2	23°32'51.1"S; 45°50'28.7"W
2991			1	23°32'51.1"S; 45°50'26.1"W
2971	***Biomphalaria schrammi***	Pirapora do Bom Jesus	5	23°23'22"S; 46°59'56"W
3000	***Biomphalaria tenagophila***	Barueri	1	23°30'38.9"S; 46°51'51.2"W
1095		Biritiba Mirim	4	23°34'3.91"S; 46°2'21.62"W
1109			3	23°33'10.49"S; 46°0'0.29" W
2968		Mogi das Cruzes	5	23°33'25.9"S; 46°15'05.7"W
3001		Suzano	3	23°33'08.1"S; 46°16'51.7"W
TOTAL			43	

N: number of specimens

on stubs using tweezers and absolute alcohol. No material was employed to attach radula and mandible to the stubs. After drying, the stubs were taken to a vacuum evaporator for gold coating.

Forty-three radulae and mandibles were prepared: eight of *B. occidentalis*, seven of *B. oligoza*, seven of *B. peregrina*, five of *B. schrammi* and sixteen of *B. tenagophila*. Morphological features of radulae and mandibles of each species were established based on the analysis of micrographs obtained in a QUANTA 250 FEI Scanning Electron Microscope at the Cell Biology Laboratory of the Butantan Institute, city of São Paulo, São Paulo, Brazil. The features observed on central, lateral, intermediate and marginal teeth were related to the cusps, subcusps, teeth fixation base and radular formula. The formula can be expressed as (M:I:L) T:C:T (L:I:M), where C is the central bicuspid tooth and T are the tricuspid teeth. The latter comprises lateral (L), intermediate (I) and marginal teeth (M).

The teeth of the whole radular surface were observed. However, to compare the radular characters of the five species studied, only intact teeth were taken into account, which are located in the middle region of the radula. The newly formed teeth of the anterior region of the radula, whose morphological details may not be well represented, were not included in the morphological analysis. Teeth of the posterior region of the radula, which suffer wear and tear, and which normally present morphological alterations were also not included. The radulae's teeth lines were not quantified, because, in the anterior region of the radulae, were often observed areas with absence of lines.

RESULTS

Radular teeth are arranged in bilaterally symmetric transverse rows. Each row has one central bicuspid tooth and several other tricuspids, which can be lateral, intermediate or marginal (Fig. 1A). Radular formulas do not provide any criteria that contribute to specific differentiation (Table 2).

Central tooth (Figs. 1B-3B and Table 3)

Both cusps of the central teeth are triangular, with pointed tips and of approximately equal sizes, although one specimen of *B. occidentalis* with a unicuspid central tooth was found (Fig. 2A). The fixation base of these teeth has a trapezoid form, the larger base being wider than the smaller base. Important characters in the differentiation of the five species of *Biomphalaria* are: angle formed by the main cusps, number of subcusps between the main cusps, number of subcusps external to the tooth, height of the fixation base and width of the smaller fixation base.

Lateral and intermediate teeth (Figs. 1B, 2A-3A, 4A-5A, 6A, 7A, 8A and Table 4)

Lateral teeth change in morphology as they approach the marginal teeth, becoming longer and with more subcusps. At this stage, they are referred to as intermediate teeth, as they are located in the transition area from lateral to marginal teeth. The lateral and intermediate teeth have rectangular mesocones with pointed tips, and triangular endocones and ectocones. The ectocone tends to be smaller

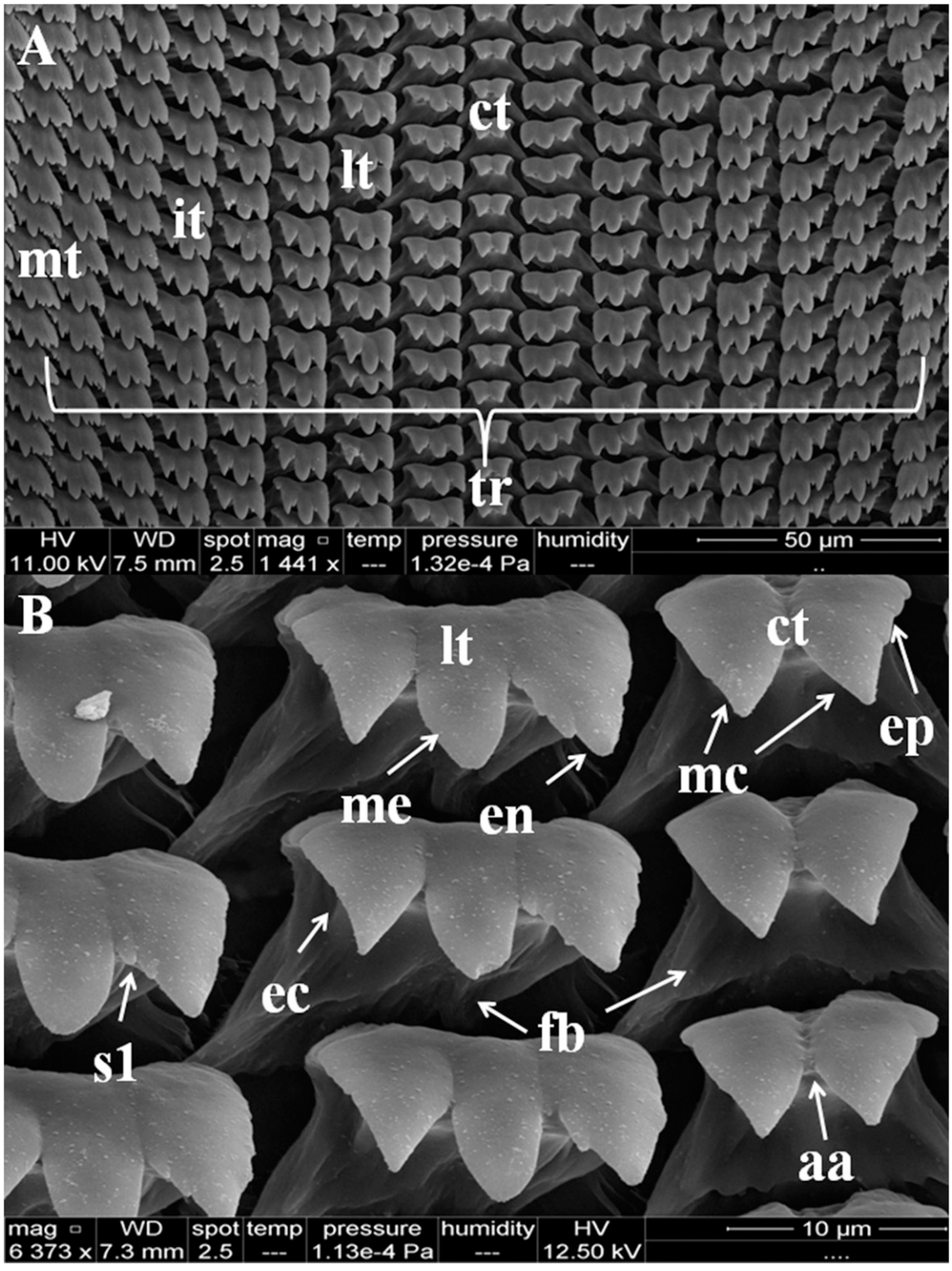

Figure 1. Partial view of the radular ribbon of *Biomphalaria peregrina* A, and central and lateral teeth of *Biomphalaria occidentalis* B, aa, acute angle; ct, central tooth; ec, ectocone; en, endocone; ep, external protuberances of central tooth; fb, tooth fixation base; it, intermediate tooth; lt, Lateral tooth, mc- main cusps; me, mesocone; mt, marginal tooth; s1, subcusps between the mesocone and endocone of the lateral tooth; tr, transversal row of teeth.

Table 2. Variations of the radular formulas of *Biomphalaria* species.

Radular formulas: (M:I:L)T:C:T(L:I:M)

Biomphalaria occidentalis	*Biomphalaria oligoza*	*Biomphalaria peregrina*	*Biomphalaria schrammi*	*Biomphalaria tenagophila*
(18:3:5) 26:1:26 (5:3:18)	(13:2:4) 19:1:19 (4:2:13)	(16:2:3) 21:1:21 (3:2:16)	(10:3:3) 16:1:16 (3:3:10)	(17:4:7) 28:1:28 (7:4:17)
(17:3:6) 26:1:26 (6:3:17)	(13:2:3) 18:1:18 (3:2:13)	(15:2:3) 20:1:20 (3:2:15)	(10:2:3) 15:1:15 (3:2:10)	(20:2:5) 27:1:27 (5:2:20)
(17:4:4) 25:1:25 (4:4:17)	(12:2:2) 16:1:16 (2:2:12)	(13:3:4) 20:1:20 (4:3:13)	(9:2:3) 14:1:14 (3:2:9)	(17:3:7) 27:1:27 (7:3:17)
(16:3:5) 24:1:24 (5:3:16)	(11:2:2) 15:1:15 (2:2:11)	(14:2:3) 19:1:19 (3:2:14)	(8:2:3) 13:1:13 (3:2:8)	(16:3:6) 25:1:25 (6:3:16)
(10:3:5) 18:1:18 (5:3:10)	(10:2:2) 14:1:14 (2:2:10)			(15:3:6) 24:1:24 (6:3:15)

than the endocone and mesocone. Some specimens of *B. occidentalis*, *B. peregrina*, *B. oligoza* and *B. schrammi* had some lateral and intermediate teeth with a blunt or cut-tipped mesocone (Figs. 2B, 3A). Also, some specimens of *B. occidentalis* and *B. tenagophila* had small bifurcations in some endocones and ectocones (Fig. 8A-detail). The number of subcusps external to the lateral and intermediate teeth ranges from 1 to 4, and they are sometimes absent. The fixation bases of these teeth are narrow and high. An important character in the differentiation of the five species of *Biomphalaria* is the number of subcusps present between the three main cusps.

Marginal teeth (Figs. 4B, 5B, 6B, 7B, 8A and Table 5)

The following numbers of subcusps were observed in the marginal teeth: up to 8 external, up to 3 between mesocone and ectocone, and 1 to 7 between mesocone and endocone. The fixation base of the marginal teeth was narrow in all species. No relevant morphological differences were observed among marginal teeth of the five species studied.

Mandible

In all specimens of all species the brown-colored mandible is formed by a single longitudinal T-shaped mandibular plate with two long and thin attachment rods (Fig. 8B).

DISCUSSION

The most relevant characters in taxonomic and phylogenetic studies of *Biomphalaria* are from organs of the reproductive and renal systems, because they have reliable characters for separating species (Fuentealba and Figueroa 2012). The radula of *Biomphalaria* has been seldom used for taxonomic purposes. The radular formulas of *B. occidentalis*, *B. oligoza*, *B. peregrina*, *B. schrammi* and *B. tenagophila* herein described are similar to the ones presented by Paraense (1974, 1981) and Paraense *et al.* (1964), and do not contribute to specific differentiation. Nevertheless, a variation in number of tricuspid teeth (lateral, intermediate and marginal) was observed in the analyzed specimens, as can be verified in their radular formulas. According to Paraense (1972), this variation occurs due to the snails` different stages of development. Studies carried out with other groups of mollusks indicate that the snail development stage, although influencing the number of radular teeth, does not affect its morphology (Martín and Negrete 2007). The number, shape and size of the main cusps in central, lateral and intermediate teeth did not vary among the studied species, presenting a pattern similar to the described by Paraense (1970) for the genus *Biomphalaria*.

Central teeth

The specimen of *B. occidentalis* with a radula containing unicuspid central teeth was herein considered an anomaly, as described in Paraense (1981), due to the fact that the general pattern of the genus is having central bicuspid teeth (Paraense 1970). The subcusps present between cusps of the central teeth of *B. oligoza*, *B. peregrina* and *B. schrammi* may occasionally be absent in some of the central teeth, contrary to what is observed in *B. tenagophila* and *B. occidentalis* radulae. In these latter two species, these subcusps are absent, as described by Paraense (1981) and Paraense *et al.* (1964). Only a few central teeth of *B. tenagophila* may, occasionally, present subcusps between the main cusps. The presence of subcusps between cusps of *B. oligoza* central teeth had already been mentioned by Paraense (1974); however, he did not mention their presence in *B. peregrina*, which differs from our results. This is likely because our observations were performed using SEM, which is capable of producing images at high magnification without losing resolution, therefore allowing a thorough analysis of radular characters.

The angle formed between central teeth cusps is associated with the presence or absence of subcusps. In specimens of *B. oligoza*, *B. peregrina* and *B. schrammi* the angle formed is obtuse, as the central teeth mostly contain subcusps between the cusps. On the other hand, in *B. occidentalis* and in most central teeth of *B. tenagophila* the angle is acute, due to the absence of subcusps. The presence of subcusps or external

Table 3. Morphological characteristics of the central teeth of *Biomphalaria* radulae.

Character	*Biomphalaria occidentalis*	*Biomphalaria oligoza*	*Biomphalaria peregrina*	*Biomphalaria schrammi*	*Biomphalaria tenagophila*
Number of cusps	2	2	2	2	2
Form of cusps	Triangular, pointed tips	Triangular, pointed tips	Triangular, pointed tips	Triangular, pointed tips	Triangular, pointed tips
Angle formed by cusps	Acute	Obtuse	Obtuse	Obtuse	Acute
Subcusps between cusps	Absent	1 or 2 (*occasionally absent)	1 or 2 (*occasionally absent)	1 or 2 (*occasionally absent)	Absent (*occasionally 1 or 2)
Subcusps or external protuberance	Occasional	Present	Present	Present	Occasional
Shape of tooth fixation base	Trapezoid	Trapezoid	Trapezoid	Trapezoid	Trapezoid
Height of tooth fixation base	High	High	High	Short	High
Width of larger tooth fixation base	Wide	Wide	Wide	Wide	Wide
Width of smaller tooth fixation base	Does not exceed tooth limits	Does not exceed tooth limits	Does not exceed tooth limits	Exceeds tooth limits	Does not exceed tooth limits

*Some teeth of the radula

protuberances in central teeth of *B. oligoza*, *B. peregrina* and *B. schrammi* is different from *B. occidentalis* and *B. tenagophila*, in which protuberances appear only occasionally. Rumi *et al.* (2017) mention a fold and a groove between the cusps of the central teeth were observed when analyzing the *B. peregrina* radula from Argentina, these characteristics were not observed in our material.

Lateral and intermediate teeth

According to Paraense (1966), the lateral teeth of *B. peregrina* have blunt or cut-tipped mesocones, which was not verified in the integer teeth of the radulae of our specimens. In the current study, this mesocone aspect was observed in few lateral and intermediate teeth of *B. peregrina*, *B. occidentalis*, *B. oligoza* and *B. schrammi*, mainly, the posterior region of some radulae, where the teeth of greater use are found. Such feature, as well as the small bifurcations found in some endocones and ectocones of *B. occidentalis* and *B. tenagophila*, may be attributed to wear caused by the continuous use of the teeth, especially in adult individuals, or eating habit of snails (Martín and Negrete 2007). When analyzing *B. peregrina* radulae, Paraense (1974) did not make reference to the presence of subcusps between cusps of lateral and intermediate teeth. These were only absent in some teeth of specimens of *B. peregrina*, *B. oligoza* and *B. schrammi* in this study. The *B. occidentalis* radula described by Paraense (1981: Fig. 2) does not show evidence of the presence of subcusps between lateral and intermediate teeth cusps, which were occasionally found in our material. Also, regarding *B. tenagophila*, Paraense *et al.* (1964) mentioned the presence of subcusps only in the first lateral teeth, referring to these as interstitial denticles. These subcusps were occasionally found in some lateral and intermediate teeth of the *B. tenagophila* specimens we examined.

We consider the presence of subcusps between cusps of lateral and intermediate teeth of *B. oligoza*, *B. peregrina* and *B. schrammi* to be common, whereas in *B. occidentalis* and *B. tenagophila* subcusps between cusps of lateral and intermediate teeth are only occasionally present, and only in some teeth. The number of external subcusps in lateral and intermediate teeth did not vary, among the five species. Although Paraense (1966) mentioned the presence of external subcusps in lateral and intermediate teeth of *B. peregrina*, he did not quantify them.

Marginal teeth

No obvious differences were observed in the number of subcusps present in the marginal teeth of the studied snails. When analyzing the *B. peregrina* radula, Paraense (1974) did not mention the number of subcusps of the marginal teeth, referring to these only as multicusp teeth. According to Paraense (1970, 1972), subcusps between the three cusps of the marginal teeth appear, initially, due to the fragmentation

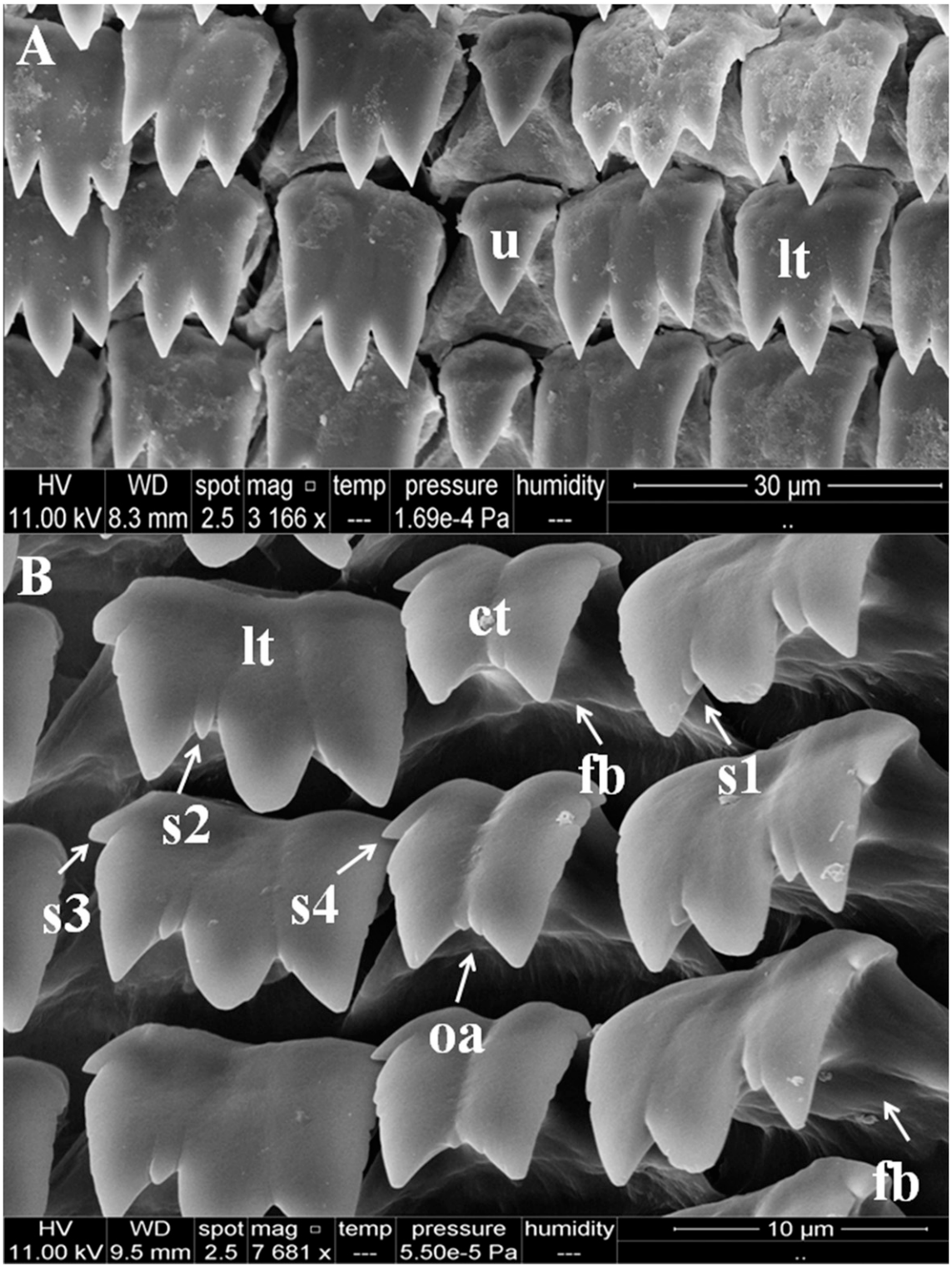

Figure 2. Anomalous radula of *Biomphalaria occidentalis* A, and central and lateral teeth of *Biomphalaria oligoza* B, ct, central tooth; fb, tooth fixation base; lt, lateral tooth; oa, obtuse angle; s1, subcusps between the mesocone and endocone of the lateral tooth; s2, subcusps between the mesocone and ectocone of the lateral tooth; s3, external subcusps of the lateral tooth; s4, external subcusps of the central tooth; u, unicuspid central tooth.

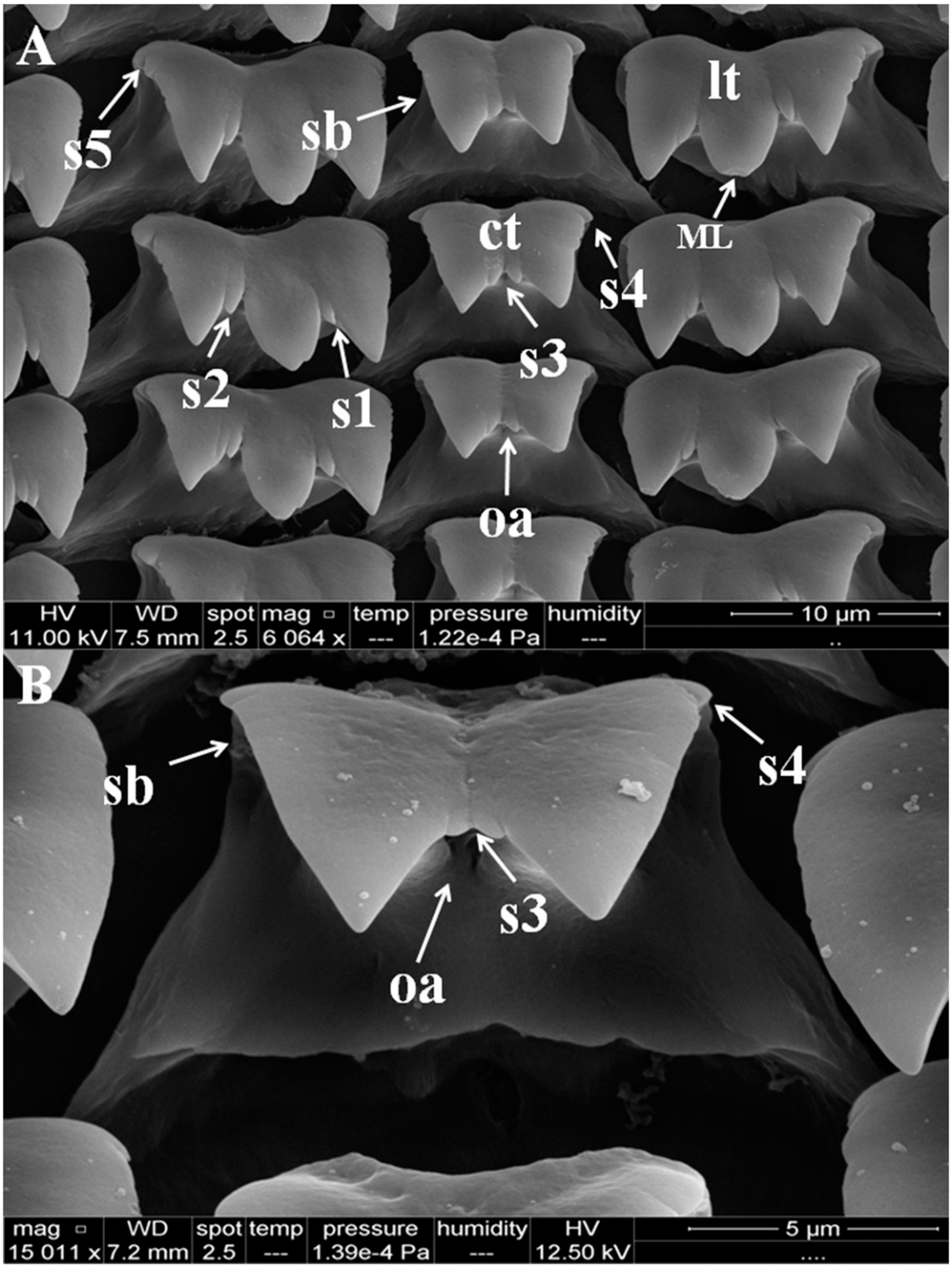

Figure 3. Central and lateral teeth of *Biomphalaria peregrina* A, and central tooth of *Biomphalaria schrammi* B, ct, central tooth; lt, lateral tooth; ml, lateral tooth containing the mesocone with cut edges; oa, obtuse angle; sb, smaller tooth fixation base; s1, subcusps between the mesocone and endocone of the lateral tooth; s2, subcusps between the mesocone and ectocone of the lateral tooth; s3, subcusps between the central tooth; s4, external subcusps of the central tooth; s5, external subcusps of the lateral tooth.

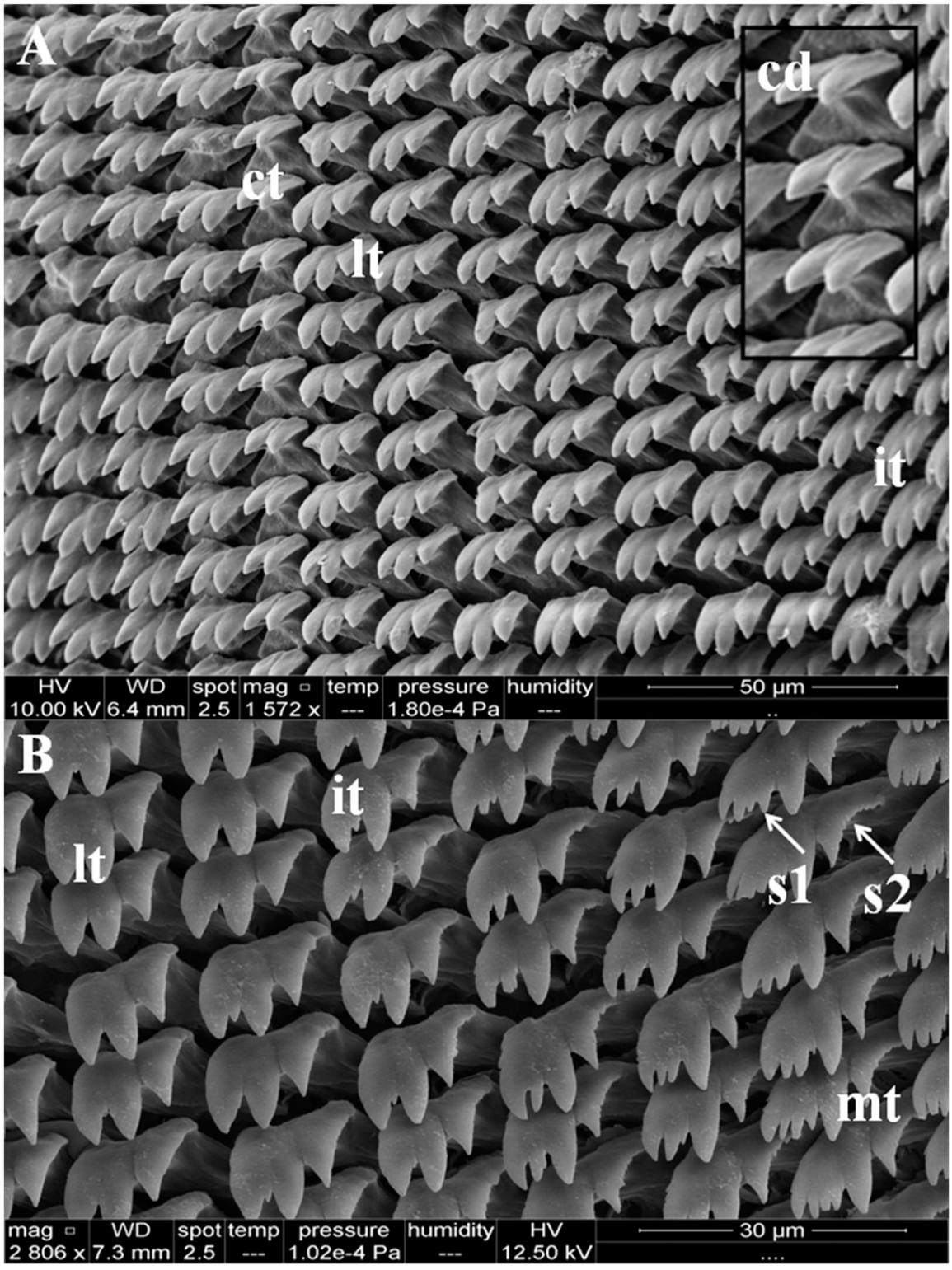

Figure 4. Partial view of the radular ribbon of *Biomphalaria tenagophila* A, and lateral, intermediate and marginal teeth of *Biomphalaria occidentalis* B, cd, central tooth in detail; ct, central tooth; it, intermediate tooth; lt, lateral tooth; mt, marginal tooth; s1, subcusps between the mesocone and endocone of the marginal tooth; s2, external subcusps of the marginal tooth.

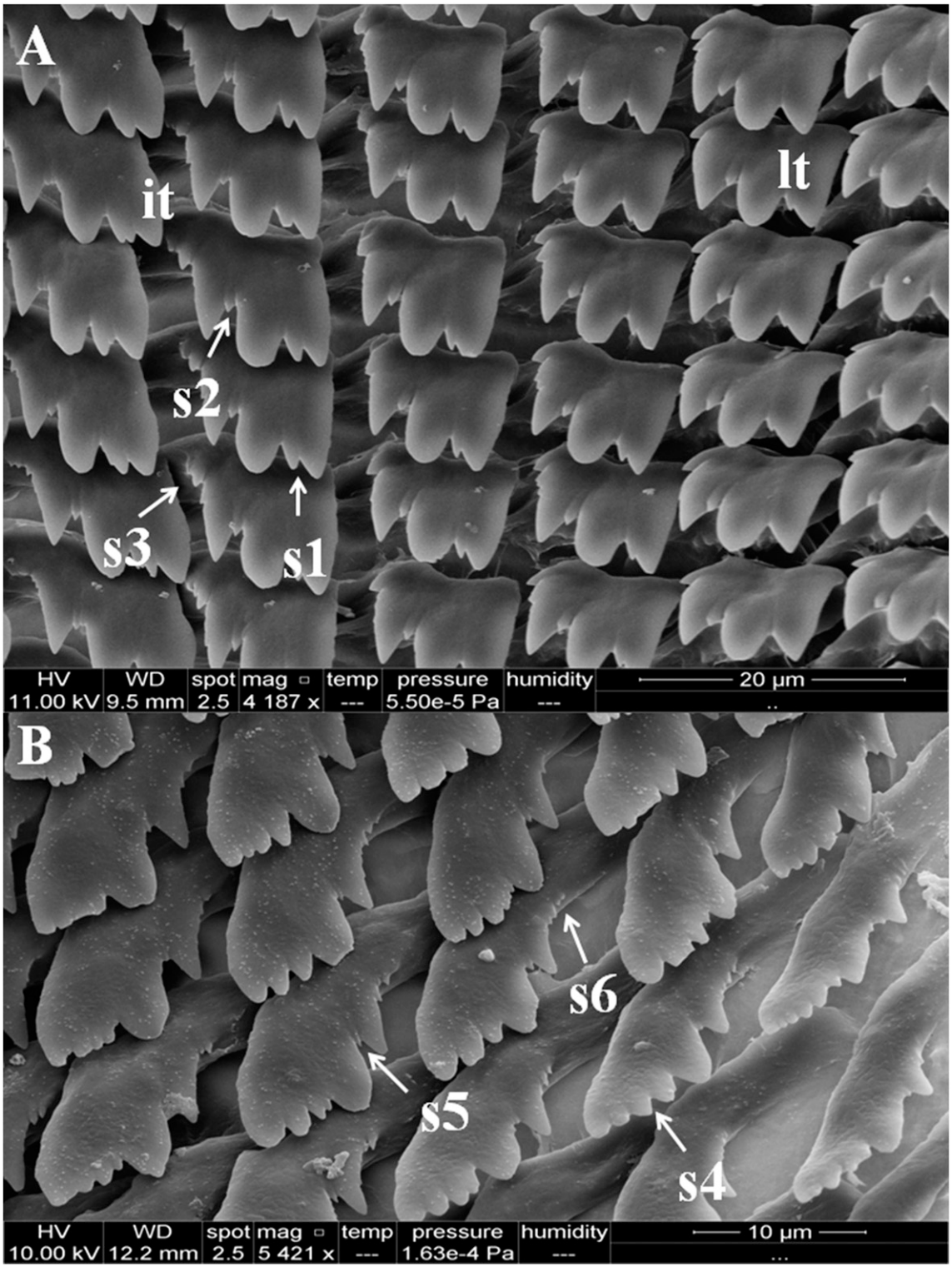

Figure 5. Lateral and intermediate teeth A, and marginal teeth B, of *Biomphalaria oligoza*. it, intermediate tooth; lt, lateral tooth; s1, subcusps between the mesocone and endocone of the intermediate tooth; s2, subcusp between the mesocone and ectocone of the marginal tooth; s3, external subcusps of the marginal tooth; s4, subcusps between the mesocone and endocone of the marginal tooth; s5, subcusp between the mesocone and ectocone of the marginal tooth; s6, external subcusps of the marginal tooth.

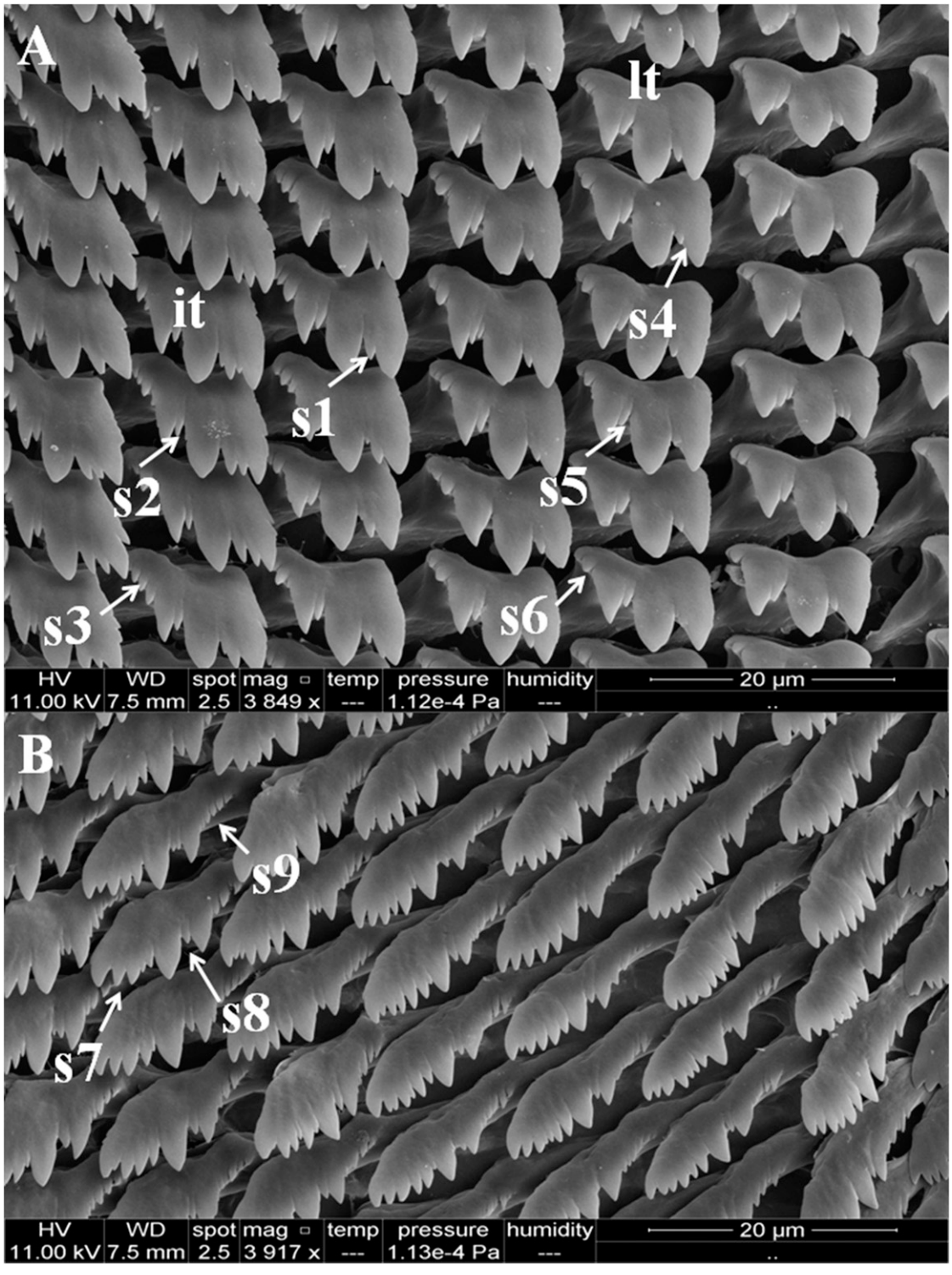

Figure 6. Lateral and intermediate teeth A, and marginal teeth B, of *Biomphalaria peregrina*. it, intermediate tooth; lt, lateral tooth; s1, subcusps between the mesocone and endocone of the intermediate tooth; s2, subcusp between the mesocone and ectocone of the marginal tooth; s3, external subcusps of the marginal tooth; s4, subcusps between the mesocone and endocone of the lateral tooth; s5, subcusp between the mesocone and ectocone of the lateral tooth; s6, external subcusps of the lateral tooth; s7, subcusps between the mesocone and endocone of the marginal tooth; s8, subcusp between the mesocone and ectocone of the marginal tooth; s9, external subcusps of the marginal tooth.

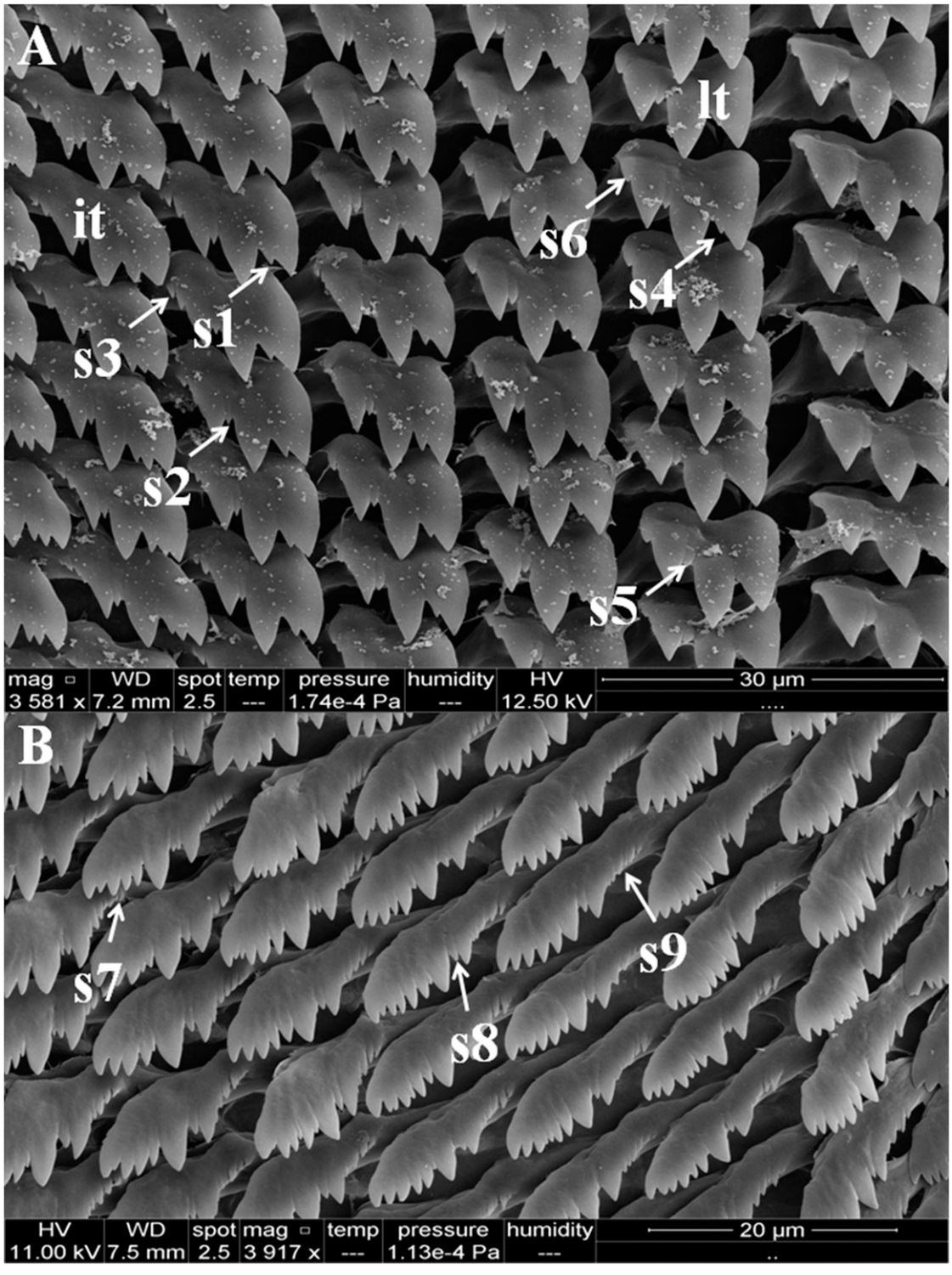

Figure 7. Lateral and intermediario teeth A, and marginal teeth B, of *Biomphalaria schrammi*. it, intermediate tooth; lt, lateral tooth; s1, subcusps between the mesocone and endocone of the intermediate tooth; s2, subcusp between the mesocone and ectocone of the marginal tooth; s3, external subcusps of the marginal tooth; s4, subcusps between the mesocone and endocone of the lateral tooth; s5, subcusp between the mesocone and ectocone of the lateral tooth; s6, external subcusps of the lateral tooth; s7, subcusps between the mesocone and endocone of the marginal tooth; s8, subcusp between the mesocone and ectocone of the marginal tooth; s9, external subcusps of the marginal tooth.

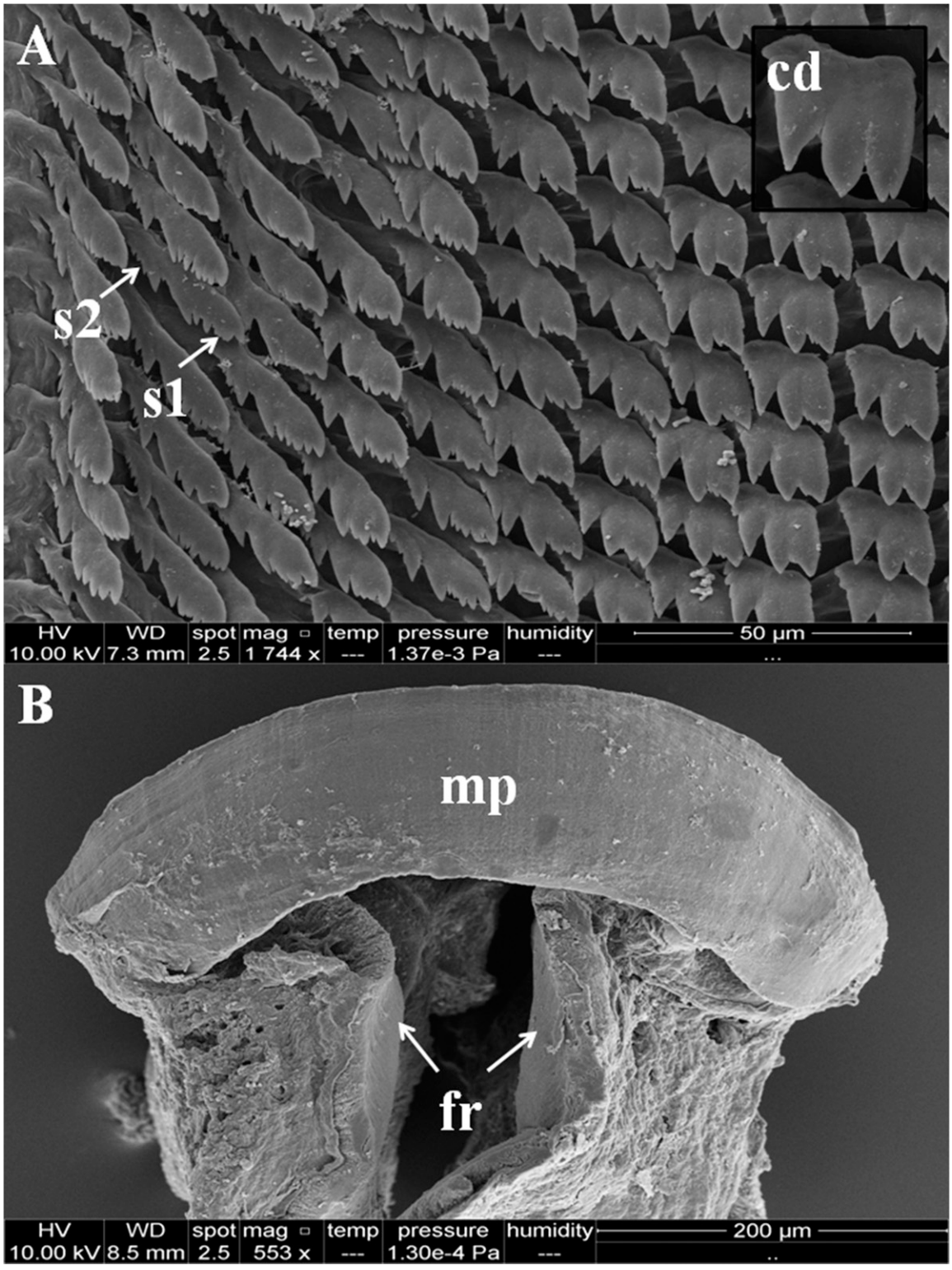

Figure 8. Lateral, intermediate and marginal teeth A, and mandible B, of *Biomphalaria tenagophila*. cd, lateral tooth in detail; fr, fixing rods; it, intermediate tooth; lt, lateral tooth; mp, mandibular plate**;** mt, marginal tooth; s1**,** subcusps between the mesocone and endocone of the marginal tooth; s2**,** external subcusps of the marginal tooth.

Table 4. Morphological characteristics of the lateral and intermediate teeth of *Biomphalaria* radulae.

Characters	*Biomphalaria occidentalis*	*Biomphalaria oligoza*	*Biomphalaria peregrina*	*Biomphalaria schrammi*	*Biomphalaria tenagophila*
Number of cusps	3	3	3	3	3
Form of mesocone	Rectangular, pointed tip	Rectangular, pointed tip	Rectangular, pointed tip	Rectangular, pointed tip	Rectangular, pointed tip
Form of endocone and ectocone	Triangular	Triangular	Triangular	Triangular	Triangular
Size of cusps	Ectocone smaller than endocone and mesocone	Ectocone smaller than endocone and mesocone	Ectocone smaller than endocone and mesocone	Ectocone smaller than endocone and mesocone	Ectocone smaller than endocone and mesocone
Subcusps between cusps	Absent (occasionally 1 or 2)	1 or 2 (occasionally absent)	1 or 2 (occasionally absent)	1 or 2 (occasionally absent)	Absent (occasionally 1 or 2)
Subcusps external to teeth	Up to 3 (occasionally absent)	Up to 4 (occasionally absent)	Up to 4 (occasionally absent)	Up to 3 (occasionally absent)	Up to 3 (occasionally absent)
Tooth fixation base	Narrow, high	Narrow, high	Narrow, high	Narrow, high	Narrow, high

of the endocone into subcusps. Fragmentation of the ectocone and mesocone occur only after this; therefore, it is more difficult to distinguish cusps from subcusps in teeth closer to the margin of the radular ribbon. The subcusps of marginal teeth increase considerably in number due to the subdivisions of endocone, mesocone and ectocone, tending to become equal to the main cusps, generating teeth with a leafy and serrated appearance (Paraense 1970, 1972). Nevertheless, it was possible to distinguish the three main cusps of the marginal teeth in many of the examined specimens.

Fixation bases of the teeth

The fixation base of the central, lateral, intermediate and marginal teeth had the same pattern among the examined specimens, with one exception. In *B. schrammi* the fixation base of the central tooth differed from the remaining species in height and width of the smaller base.

Mandible

The mandible did not reveal any relevant differences among the species herein studied, so we agree with Paraense (1988) in that it follows the pattern of the genus *Biomphalaria*.

Biomphalaria includes several species complexes, including the tenagophila complex which includes *B. tenagophila*, *B. tenagophila guaibensis* Paraense, 1984 and *B. occidentalis* and the straminea complex, which includes *B. straminea* (Dunker, 1848), *B. kuhniana* (Clessin, 1883) and *B. intermedia* (Paraense & Deslandes, 1962). These complexes include species with very similar morphological characters and are difficult to differentiate by the shell and soft parts alone (Spatz *et al.* 1999, Paraense 1988). Also, other species of the genus have very similar morphology: *B. peregrina* and *B. oligoza* (Paraense 1974, 1975) and, *B. cousini* and *B. amazonica* (Paraense, 1966). We believe that studying the radula using SEM can add quantitative or structural details of extreme importance to elucidating differences among these taxa.

Based on the data provided herein, we suggest using the following characters as auxiliaries in the identification of the *Biomphalaria* species addressed in this study: number of subcusps between the cusps of the central teeth; presence and absence of subcusps or external protrusions in the central teeth; number of subcusps between the cusps of the lateral and intermediate teeth; and height of the fixation base of central teeth and the smaller width of the fixation base of the central teeth. We also recommend the analysis of radula of the other species of the genus *Biomphalaria*, in order to confirm the consistency of radular characters.

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Table 5. Morphological characteristics of the marginal teeth of *Biomphalaria* radulae.

Characters	*Biomphalaria occidentalis*	*Biomphalaria oligoza*	*Biomphalaria peregrina*	*Biomphalaria schrammi*	*Biomphalaria tenagophila*
Number of cusps	3	3	3	3	3
Subcusps between mesocone and endocone	Present (up to 7)	Present (up to 5)	Present (up to 7)	Present (up to 5)	Present (up to 6)
Subcusps between mesocone and ectocone	Up to 2 (occasionally absent)	Up to 2 (occasionally absent)	Up to 2 (occasionally absent)	Up to 3 (occasionally absent)	Up to 3 (occasionally absent)
Subcusps external to teeth	Up to 8 (occasionally absent)	Up to 5 (occasionally absent)	Up to 6 (occasionally absent)	Up to 5 (occasionally absent)	Up to 6 (occasionally absent)
Tooth fixation base	Narrow	Narrow	Narrow	Narrow	Narrow

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