

# A new troglobite species of *Habeastrum* Simone, 2019 from Brazil, and support for classification in Diplommatinidae (Mollusca, Caenogastropoda)

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## Abstract

The genus *Habeastrum* Simone, 2019 was recently described based on empty shells, counting with two troglobite species. Conchological features allowed a preliminary classification in the caenogastropod family Diplommatinidae, but this family allocation was left open to future studies. Herein, we present a detailed anatomical study of newly acquired specimens, confirming the classification in Diplommatinidae. These new specimens, from Minas Gerais state, SE Brazil, belong to a new troglobite species described herein, *Habeastrum strangei* sp. nov. The present records extend the genus distribution ca. 1,100 km east-northeast.

## Key Words

Cyclophoroidea, Gastropoda, *Habeastrum strangei* sp. nov., Minas Gerais, troglofauna

## Introduction

The genus *Habeastrum* Simone, 2019, has been recently described to allocate two unique and previously unknown species collected in caves in Mato Grosso do Sul state, central Brazil. It presently includes two terrestrial troglobite species: the type species *H. parafusum* Simone, 2019 and *H. omphalium* Simone, 2019.

The original description of the genus was based solely on empty shells (the operculum was also unknown) and the following conchological features allowed a tentative classification in Diplommatinidae (Simone 2019): the minute size, the delicate axial sculpture, and the rounded profile of the shell whorls. However, unlike most New World diplommatinids, the shell of *Habeastrum* is dextral, with a simple non-deflected aperture, and uniform shell growth (i.e., the whorls increase regularly and slowly in size during growth).

The latter features are unusual for the family (Thiele 1929; Wenz 1938) and Simone (2019) recognized that the familiar attribution of the new genus should be left open for future studies counting with specimens with preserved soft parts.

Such specimens were recently sent to us by two different sources: Prof. Dr. Maria Elina Bichuette, a researcher from the Universidade Federal de São Carlos (UFSCar, Brazil) specialized in Brazilian troglofauna, and the environmental consulting company Carste Ciência e Meio Ambiente (CARSTE, Brazil). These new specimens were collected in caves in various different municipalities in Minas Gerais state, southeastern Brazil. We present here an anatomical study of the specimens, confirming the diplommatinid affinity of the genus. Furthermore, they are deemed to belong to a new species, described herein as *Habeastrum strangei* sp. nov.

## Material and methods

The new material from Minas Gerais contained both whole specimens (preserved in 70% ethanol) and empty shells. It was donated by Dr. Bichuette to the malacological collection of the Museu de Zoologia da Universidade de São Paulo (MZSP, São Paulo, Brazil). Selected specimens (four females and three males) were dissected in ethanol following standard techniques (Simone 2011), aided by a stereomicroscope equipped with a camera lucida and a digital camera. The shell was lightly crushed with the aid of forceps and the soft parts were extracted without being damaged. All dissection steps were photographed and all drawings were made with the help of the camera lucida. The radula was extracted using standard techniques and examined under SEM in the Laboratory of Electronic Microscopy of the MZSP. SEM images of the shell were captured in the Staatliches Museum für Naturkunde Stuttgart (SMNS, Stuttgart, Germany) and of the radula in the MZSP. The list of examined material, as well as collection details, can be found below.

Two specimens were randomly selected for DNA extraction, with two samples acquired from each. Unfortunately, we could not acquire good quality DNA for amplification and sequencing. Even though the specimens seemed fresh, we suspect they might have stayed too long in the field sample before being fixed in ethanol and/or not have been thoroughly fixed: the snails were deeply retracted into their shells and thus, the ethanol might not have reached them properly given the presence of the operculum. After these four trials, using two different extraction kits and protocols, we opted for not spending more specimens.

List of abbreviations used in the figures: **aa**, anterior aorta; **an**, anus; **ap**, aperture of pallial gonoduct; **au**, auricle; **bg**, buccal ganglion; **ce**, cerebral ganglion; **cm**, columellar muscle; **cv**, pulmonary (ctenidial) vein; **dg**, digestive gland; **di**, diaphragm septum; **eg**, esophageal gland; **es**, esophagus; **ey**, eye; **fs**, foot sole; **ft**, foot; **go**, gonad; **in**, intestine; **jw**, jaw; **ki**, kidney; **m1** to **m6**, extrinsic and intrinsic odontophore muscles; **mb**, mantle border; **mj**, jaws and perioral muscles; **mo**, mouth; **oa**, opercular pad; **oc**, odontophore cartilage; **od**, odontophore; **op**, operculum; **ot**, oral tube; **ov**, pallial oviduct; **pa**, penis distal aperture; **pc**, pericardium; **pd**, penis duct; **pe**, penis; **pl**, pleural ganglion; **pn**, pedal ganglion; **pt**, prostate; **py**, pallial cavity (lung); **rn**, radular nucleus; **rs**, radular sac; **rt**, rectum; **sn**, snout; **st**, stomach; **te** cephalic tentacle; **tg**, integument; **vd**, vas deferens; **ve**, ventricle; **vo**, visceral oviduct.

## Systematics

### Family Diplommatinidae L. Pfeiffer, 1856

#### Genus *Habeastrum* Simone, 2019

**Type species.** *Habeastrum parafusum* Simone, 2019; Recent, Brazil.

#### *Habeastrum strangei* sp. nov.

<http://zoobank.org/3956C96C-C6EB-4044-93C3-52180331AB08>

Figs 1–4

**Type material.** Holotype MZSP 151626 (Fig. 1F). Paratypes: MZSP 151628 (Fig. 1G), MZSP 137432, MZSP 137475, MZSP 147862, MZSP 147863 (all from type locality).

**Type locality.** Brazil, Minas Gerais state, Presidente Olegário municipality, Lapa da Fazenda São Bernardo (18°16'37"S, 46°06'46"W).

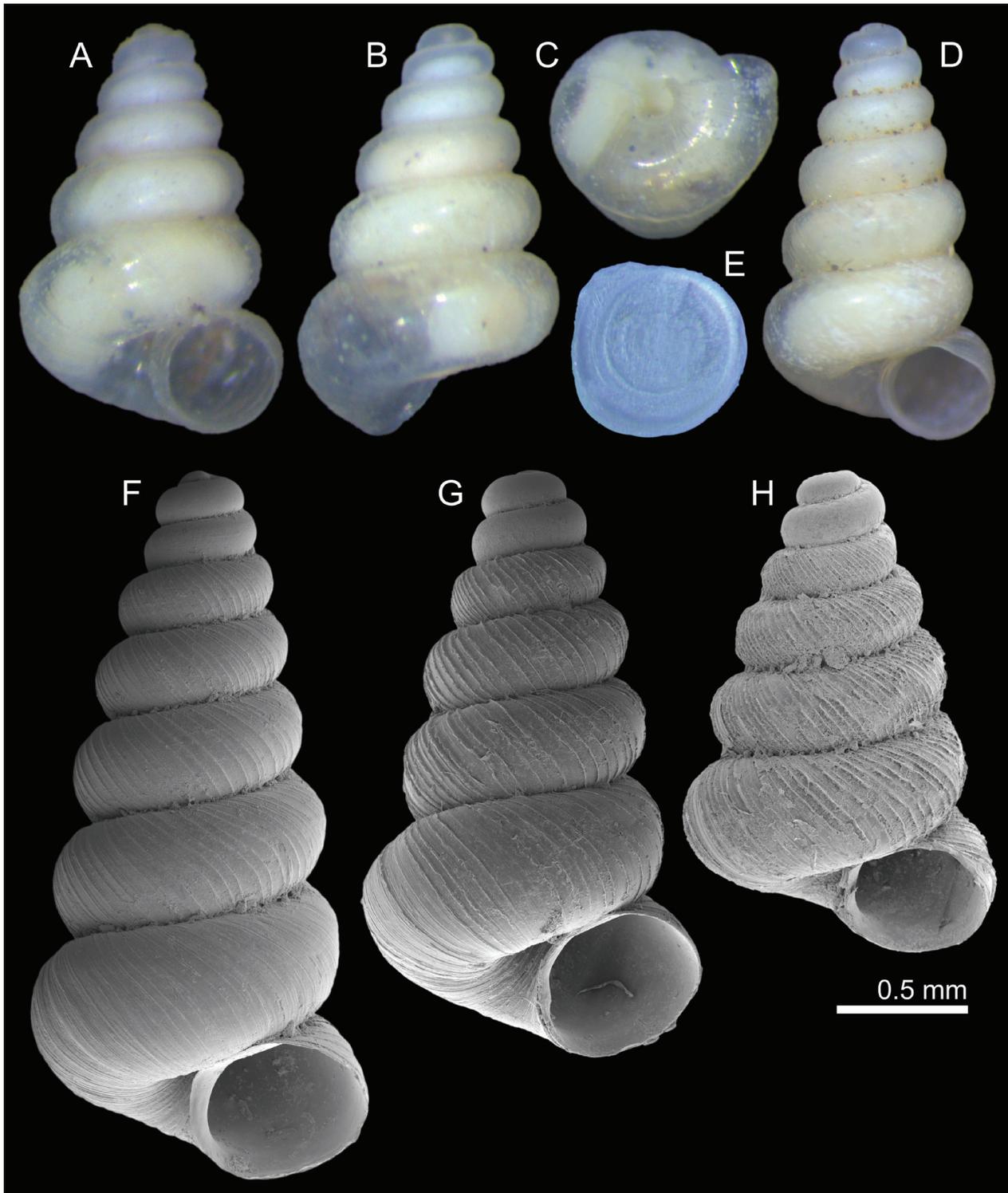
**Additional material.** BRAZIL. Minas Gerais. Pains municipality: Gruta dos Coralóides (20°20'33.4"S, 45°46'45.7"W), MZSP 106471; Caverna CBA (20°17'43.8"S, 45°47'18.0"W), MZSP 106481. Pedro Leopoldo municipality: unnamed cave (19°37'50"S, 44°00'25"W): MZSP 147121, MZSP 147130, MZSP 147134. Piumhi municipality: unnamed caves (20°20'45"S, 45°50'55"W) MZSP 147445; (20°20'46"S, 45°50'56"W), MZSP 147442, MZSP 147433, MZSP 147462; (20°20'51"S, 45°50'54"W), MZSP 147464; (20°20'57"S, 45°50'45"W), MZSP 147437, MZSP 147438, MZSP 147439, MZSP 147446; (20°20'59"S, 45°50'45"W), MZSP 147467; (20°21'05"S, 45°50'28"W), MZSP 147441; (20°21'06"S, 45°50'21"W), MZSP 147671. Presidente Olegário municipality: Lapa da Juruva (18°19'19"S, 46°04'53"W), MZSP 147864; Lapa do Moacir (18°11'10"S, 46°09'34"W), MZSP 137054, MZSP 137145; Lapa Vereda da Palha (18°15'19"S, 46°07'34"W), MZSP 137181, MZSP 137256, MZSP 150023; Lapa Zé de Sidinei (18°18'06"S, 46°05'41"W), MZSP 137132, MZSP 137143, MZSP 147865.

**Etymology.** The specific epithet is given in honor of Dr. Stephen V. Strange, a fictional character from Marvel Comics. Besides being an acknowledgement of one of the greatest Marvel characters, the name also alludes to the weirdness of this dextral diplommatinid species.

**Diagnosis.** Spire conical and tall. Teleoconch sculpture consisting of strongly prosocline, markedly raised ribs, more widely spaced than in congeners.

**Description.** Shell (Figs 1, 2A). Shell minute (adult ~2 mm high, ~1 mm wide), conical, ~7 to 7½ whorls; spire elongated. Shell walls translucent, whitish; white soft body easily visible inside. Protoconch (Fig. 2A) rounded, smooth, ~2 whorls. Teleoconch sculptured by widely-spaced raised ribs, strongly prosocline; space between ribs ~3 to 5 times the width of a rib; ribs can be more closely-spaced in first teleoconch whorls. Whorl profile rounded, strongly convex. Suture deep, well-marked. Whorls increase steadily in size towards aperture; body whorl does not bulge or change direction of coiling. Aperture circular. Peristome complete, simple and sharp, with only faint deflection over umbilical region. Umbilicus narrow, deep. Operculum (Fig. 1E). Thin, corneous, translucent, rounded, paucispiral. Nucleus central. Slightly projected in inner-superior quadrant. Flexiclaudent. Occupying entire aperture.

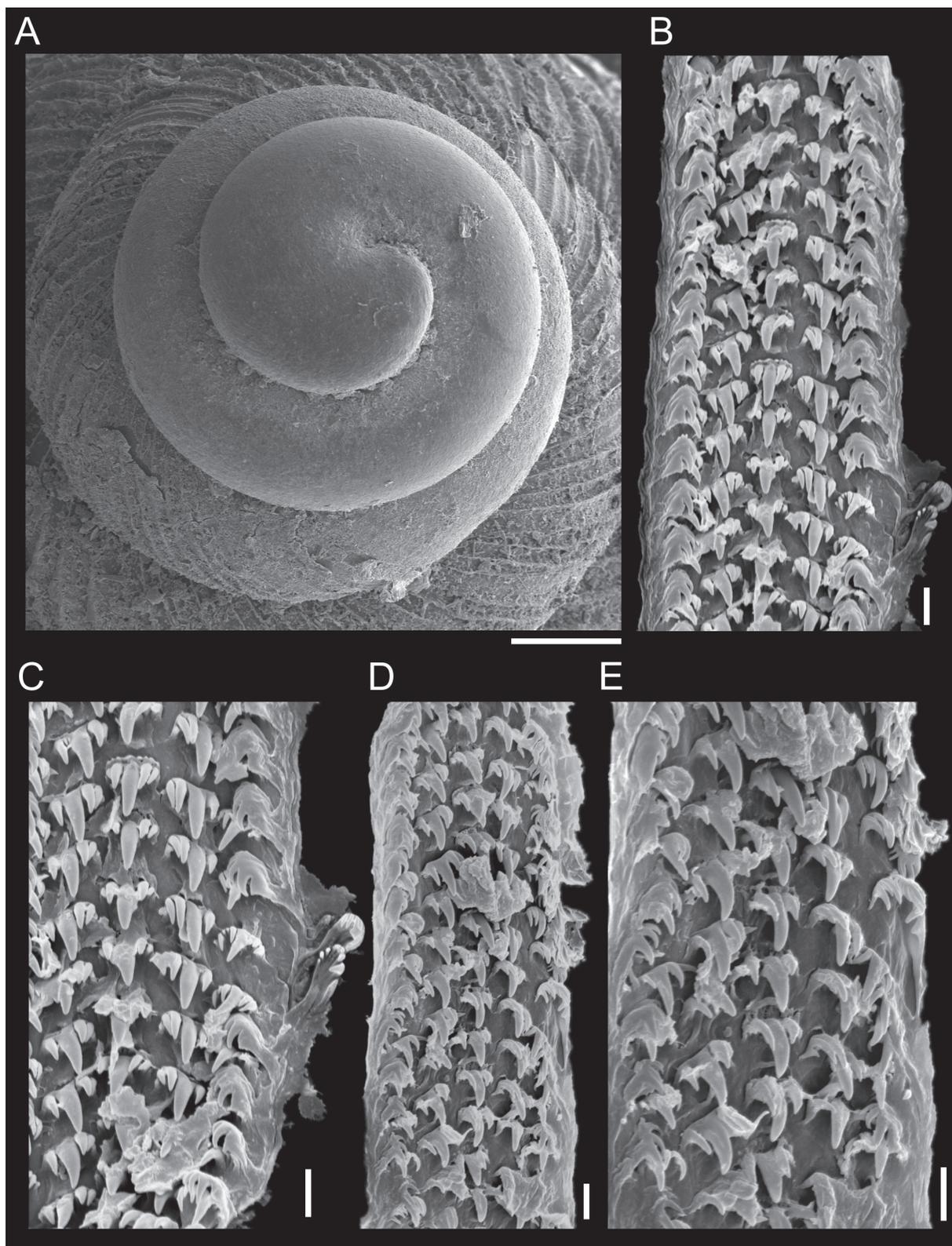
Head-foot (Fig. 3A, C). Totally unpigmented (Fig. 1A–D), ~1/3 whorl in length. Head protruded, size ~1/4 of



**Figure 1.** Shells of *Habeastrum strangei* sp. nov. **A–E.** specimens before shell crushing for anatomical study (MZSP 137145); **A.** spm #1 apertural view, soft body seen by translucency (1.6 mm high); **B.** same, dorsal view; **C.** same, umbilical view (1.1 mm wide); **D.** spm #2, apertural view (1.9 mm high); **E.** operculum of spm #1, outer view (0.4 mm wide); **F–H.** SEM images of shells, all in same scale for comparison; **F.** holotype, adult (MZSP 151626); **G.** paratype, juvenile (MZSP 151628); **H.** juvenile (MZSP 150023).

head-foot. Snout (sn) cylindrical, projected downwards; mouth central in ventral surface, longitudinal; length ~1/8 of head foot. Pair of cephalic tentacles (te), narrow, ~1.5 times longer than snout; positioned laterally to slightly dorsally in relation to snout base; gradually tapering up to pointed tip. Eyes present (ey), dark-pigmented; located

away from tentacles base, slightly ventrally positioned; each eye mostly duplicated, but with both components very close to each other, so that some specimens (~40%) apparently possess only a single elongated eye. Foot (ft) occupying ~1/2 of head-foot; sole (fs) with thick edges; longitudinal, lateral sulcus separating sole (mesopodium)

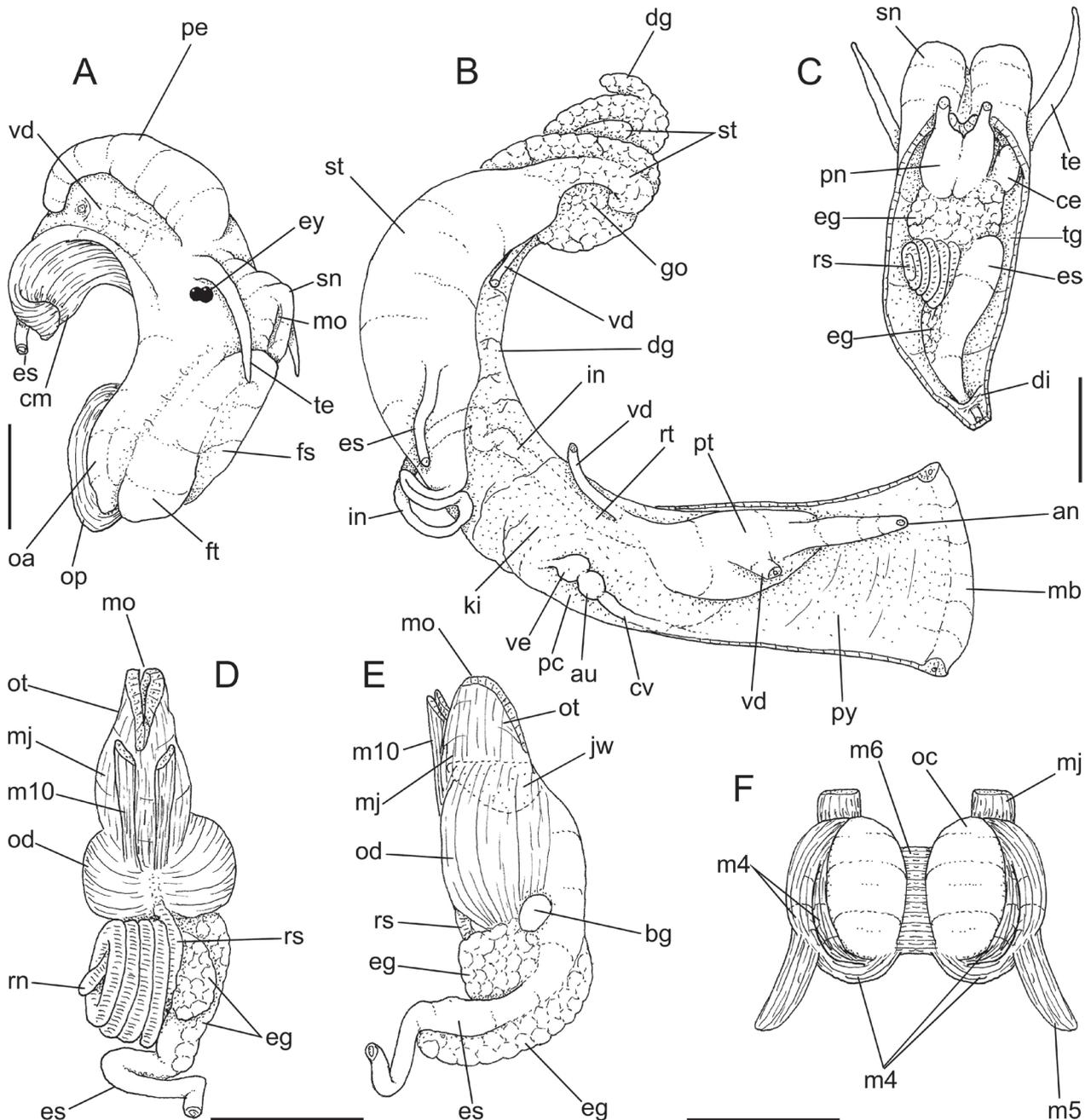


**Figure 2.** SEM images of *Habeastrum strangei* sp. nov. **A.** protoconch detail (MZSP 150023); **B–E.** Radula (MZSP 137145), in different views. Scale bars: 100 µm (**A.**); 5 µm (**B–E.**).

from dorsal epipodium, flap-like, continuous with opercular pad (oa); epipodium wider, slightly thinner than mesopodium. Columellar muscle (cm) relatively thick, occupying  $\sim 1/2$  of head-foot, tapering gradually up to blunt, thin tip. Haemocoel (Fig. 3C) almost as wide as

long, occupying  $\sim 3/4$  of head-foot; mostly filled by nerve ring and foregut structures. Diaphragm septum (di) small, positioned very posteriorly.

Pallial organs (Figs 3B, 4A). Mantle border (mb) unpigmented, relatively thick, simple. Pallial cavity (py)



**Figure 3.** Anatomy of *Habeastrum strangei* sp. nov. **A.** head-foot, male, right-slightly anterior view; **B.** pallial cavity, ventral view and visceral mass partially uncoiled, some structures seen by translucency; **C.** head and haemocoel, ventral view, foot and columellar muscle removed; **D.** foregut, ventral view; **E.** same, left view; **F.** odontophore, dorsal view, superficial layer of structures removed, cartilages deflected. Scale bars: 250 μm.

occupying ~1/2 whorl, no vestige of gill or osphradium; no visible vessels. Genital pallial structures bulging in posterior-right quadrant. Rectum (rt) flanking left edge of genital pallial glands. Anus (an) long, siphoned, ending at some distance posterior to border. Kidney (ki) and pericardium (pc) located on posterior end of cavity.

Visceral mass (Fig. 3B). Length 4 to 5 whorls, stomach (st) occupying most of last whorl, ending ~1/3 whorl posterior to pallial cavity. Digestive gland (dg) and gonad white, ~3 whorls posterior to stomach; gonad (go) occupying columellar surface, ~1/3 of digestive gland width.

Circulatory and excretory systems (Fig. 3B and Fig. 4A). Heart (pc) relatively small, ~1/20 pallial cavity's volume; located in anterior-left side of visceral mass' anterior end, partly bulging inside pallial cavity. Pulmonary vein (cv) gradually individualizing in postero-left region of pallial cavity, connecting in small auricle, anteriorly located; ventricle of same size as auricle. Kidney (ki) white, entirely solid; length ~1/3 whorl, about as wide as local whorl; nephrostome not seen.

Digestive system (Fig. 3B–F). Mouth and snout described above. Buccal mass (Fig. 3D, E) occupying ~1/5 of haemocoel volume; located just posteriorly to mouth, in-

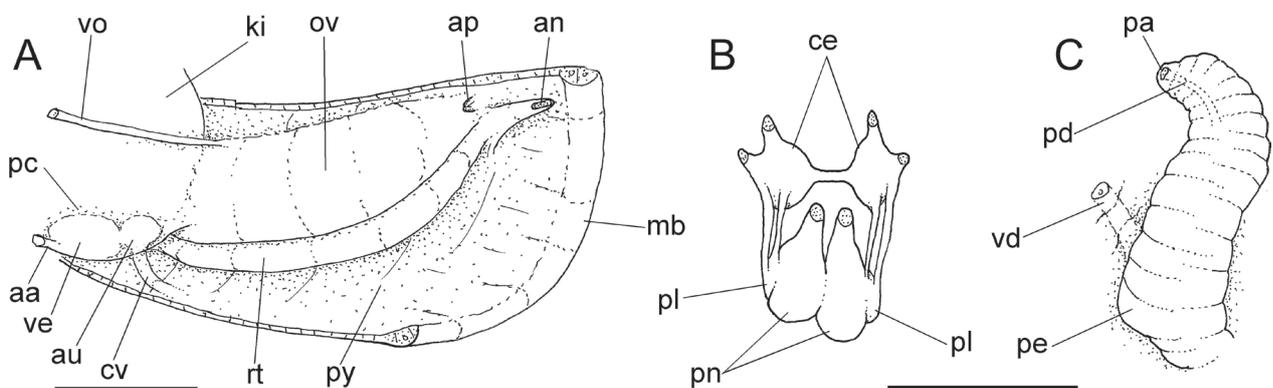
side snout (Fig. 3C). Oral tube (ot) with thick muscular walls of mostly longitudinal fibers (mj); its dorsal portion occupies  $\sim 1/3$  of buccal mass length (Fig. 3E), while ventral portion occupies  $\sim 1/2$  (Fig. 3D). Pair of jaw plates (jw) very thin, yellowish, simple; each plate semispherical, located dorso-laterally in transition to oral tube and odontophore, occupying  $\sim 1/2$  of oral tube's inner surface. Odontophore (od) occupying  $\sim 1/2$  of buccal mass volume. Odontophore muscles (Fig. 3D–F): **mj**, pair of jaw and peribuccal muscles, originating in antero-external surface of cartilages, surrounded by m4, running towards anterior region, splaying in oral tube; **m4**, main pair of dorsal tensor muscles of radula; longitudinal fibers, externally cover cartilages; composed of two layers, internal layer  $\sim 1/2$  the size of outer layer (Fig. 3F); **m5**, pair of secondary dorsal tensor muscles of radula, narrow and thin; originating in ventral-posterior region of m4, running medially and anteriorly, inserting in ventral surface of radular ribbon, in middle portion of odontophore; **m6**, horizontal muscle, narrow and thin, connecting both cartilages in their ventral edge, with  $\sim 4/5$  of cartilages' length; **m10**, pair of ventral odontophore protractors, originating in ventral region of mouth; running posteriorly, flanking ventral surface of buccal mass at short distance from its median line, inserting in odontophore close to its limit with oral tube (Fig. 3D, E).

Radular sac very long, stored as 4–5 compact whorls located to the right of esophagus in the region preceding buccal mass (Fig. 3C, D: rs). Radular nucleus (rn) simple, faintly bulging. Radula (Fig. 2B–E) taenioglossate, with two marginal teeth and one lateral tooth on each side of central rachidian tooth. Each tooth is composed of a central large claw-like denticle and two smaller denticles on each side (the lateral-most being slightly smaller).

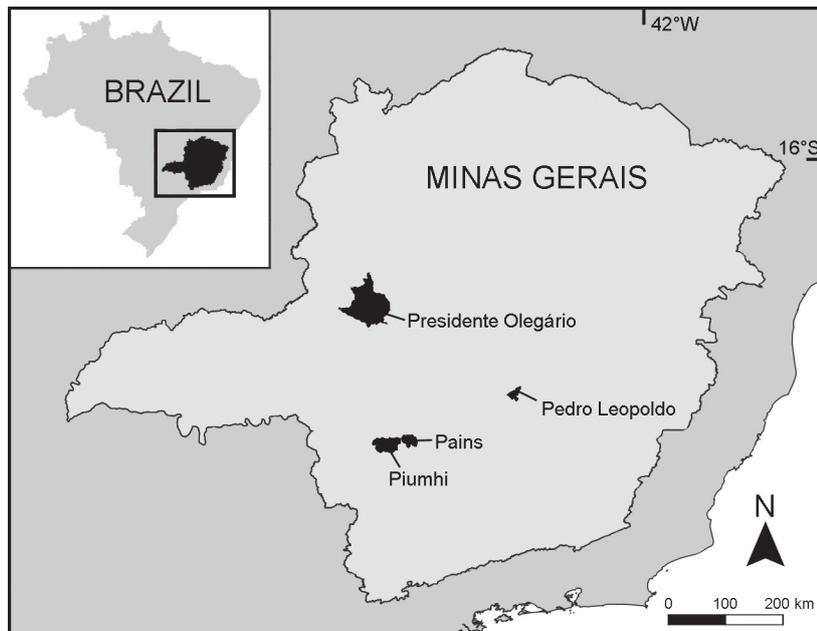
Buccal cavity with pair of wide and low dorsal folds as continuation from jaw plates, gradually diminishing towards esophagus. Pair of buccal ganglia (Fig. 3E: bg) of considerable size, rounded, each one located laterally, in transition between buccal mass and esophagus. Esophagus (es) as simple continuation of buccal cavity, relatively narrow; pair of dorsal folds from buccal cavity gradually disappear in anterior esophagus, keeping a simple and smooth surface. Pair of lateral esophageal glands (Fig. 3C–E: eg); right gland  $\sim 1/3$  haemocoel's length, slightly broader than

esophagus, bulging relatively uniformly along right esophageal wall; left esophageal gland  $\sim 1/2$  of right gland's length, but circa twice its width; both esophageal glands hollow inside, walls and inner surface thick and glandular. Posterior esophagus narrow, simple (lacking glands or chambers) (Figs 3B–E: es), about as long as anterior esophagus; esophageal insertion in stomach small, ventral,  $\sim 1/3$  of main gastric chamber (Fig. 3B: es). Stomach (Fig. 3B: st) size and location described above; its main chamber very wide and long, occupying most of the whorl posterior to kidney. Main gastric chamber lacking internal fold or subchamber; its posterior end suddenly narrows, running posteriorly as a narrow tube along 1.5 whorl (Fig. 3B: st at right); its posterior end is rounded, unclear if it connects to digestive gland, as it is lying along the gland's middle region. Anterior region of stomach bluntly tapering; intestine originating in rounded anterior gastric end. Intestine (Fig. 3B: in) performing a tight loop anteriorly to stomach, suddenly turning left and posteriorly, performing a wide loop dorsally to anterior gastric region; afterwards intestine runs through renal tissue towards anterior region of pallial cavity. In the pallial cavity, rectum (rt) runs through prostate in males (Fig. 3B) or to the left of visceral oviduct in females (Fig. 4A). Anus (an) long, siphoned (mainly in males), ending posteriorly to mantle border.

Genital system (Fig. 3A, B and Fig. 4C). **Male**. Testis (Fig. 3B: go) described above. Vas deferens surging from testis, individualizing at close to posterior region of stomach (Fig. 3B: superior vd), running close to shell's columella (with no detectable seminal vesicle) up to posterior end of pallial cavity (Fig. 3B: inferior vd), inserting in posterior end of prostate. Prostate gland (pt) elliptical, occupying  $\sim 1/4$  of pallial cavity, mostly solid-glandular; pallial vas deferens (vd) connecting on its ventral surface, penetrating integument on the right side of pallial floor (Fig. 3A: vd), running straightly to penis' base. Penis (Fig. 3A and 4C) wide ( $\sim 1/3$  of head-foot width), slightly dorso-ventrally flattened, almost half of head-foot length; penis with uniform width along its length, except for apical region, tapering bluntly. Penis duct (pd) entirely closed (tubular), running along middle penial region, opening in penis tip (pa). **Female** (Fig. 4A). Visceral components similar to those of males. Visceral oviduct very narrow (vo), inserting in pos-



**Figure 4.** Anatomy of *Habeastrum strangei* sp. nov. **A.** pallial cavity roof, female, ventral view; **B.** central nervous system (nerve ring), ventral view; **C.** penis and adjacent region of nuchal surface, dorsal view. Scale bars: 250  $\mu\text{m}$ .



**Figure 5.** Map of Minas Gerais state showing the municipalities where *Habeastrum strangei* sp. nov. occurs.

tero-right side of pallial oviduct. Pallial oviduct (ov) as single glandular mass, lacking apparent chambers or divisions; walls thick-glandular, ventral and dorsal walls thick. Pallial oviduct occupying  $\sim 1/3$  of pallial cavity: length  $\sim 4/5$  of cavity, width  $\sim 1/2$ . Anterior end of pallial oviduct tapering bluntly onto female aperture (ap); aperture simple, turned anteriorly, located posteriorly and to the right of anal base.

Central nervous system (Fig. 4B). Nerve ring hypsothroid, relatively large, occupying  $\sim 1/6$  of haemocoel (Fig. 3C: pn, ce). Located just posteriorly to buccal mass. Each cerebral ganglion (ce) spherical, with  $\sim 1/4$  of nerve ring volume. Cerebral commissure narrow, about as wide as each cerebral ganglion. Each pleural (pl) and pedal (pn) ganglion fused with each other, separated only by shallow ventral furrow; each pleuro-pedal ganglion slightly larger than each cerebral ganglion. Both commissures very narrow, of similar length on both sides,  $\sim 1.5$  times longer than the width of each ganglion.

**Distribution.** Caves in central Minas Gerais state, SE Brazil, over an area of ca. 35,000 km<sup>2</sup> (Fig. 5).

**Habitat.** In the caves in Presidente Olegário municipality, this species was found in epigeal environments and in all cave zones (entrance, twilight, and dark zones). Most specimens collected consisted of empty shells, but the good preservation considering their fragility suggests they were not subjected to transport. No precise information was recorded from other municipalities.

## Discussion

As discussed in the original description of *Habeastrum* (Simone 2019), the following shell features pointed towards a diplommatinid affinity: the minute size, the rounded profile of the whorls, and the delicate axial sculpture (Fig. 1F–H). On the other hand, a series of charac-

ters is uncommon for a diplommatinid (Thiele 1929; Wenz 1938), such as the uniform shell growth (most diplommatinids have uneven growth of the body whorl, sometimes resulting in bizarre shapes), the dextral coiling (most New World diplommatinids are sinistral), the presence of umbilicus (most seal the umbilicus with the last whorl), and the simple peristome (most have expanded, sometimes trumpet-like, peristomes) (Tielecke 1940; Webster et al. 2012). Some genera however, like *Nicida* W.T. Blanford, 1868 from the Indian subcontinent and *Pugnellia* Oppenheim, 1895 from the Italian Eocene, contain dextral species with simple peristomes (e.g., Wenz 1938; Raheem et al. 2014). The existence of these features among the conchological variability in Diplommatinidae strengthens the argument for placing *Habeastrum* within this family. In any event, the set of unique features of *Habeastrum* amongst the diplommatinids reinforces its generic separation.

Webster et al. (2012) suggested that the ancestral state in Diplommatinidae was sinistral coiling, with dextral shells having evolved independently three times. As such, *Habeastrum* would represent a fourth, South American, instance of reversal to dextrality.

Even though the conchological variation in Diplommatinidae is well described and illustrated in the literature, the same is not true for their anatomical features. Most works have focused on other cyclophoroidean families (Barker 2001, and references therein) and studies on diplommatinid anatomy are very scarce, precluding any extensive comparisons. This gap in the knowledge of anatomy and physiology of operculate land snails, especially when compared to pulmonate snails, has long been recognized (e.g., Hunter 1964; Purchon 1977). To date, the most complete account of Cyclophoroidea remains that of Tielecke (1940), who unfortunately did not have South American diplommatinid specimens available for study.

Overall, the shape of the snout, the arrangement of the pallial cavity, reproductive system, and the hypoathroid central nervous system of *Habeastrum* (Figs 3, 4) are all in line with Cyclophoroidea anatomy (Tielecke 1940; Barker 2011). The classification of *Habeastrum* in the cyclophoroidean family Diplommatinidae, besides the above-mentioned shell features, can also be based on its similarities to Latin American *Adelopoma* Doering, 1885.

The only other Latin American diplommatinid species with a detailed anatomy is *Adelopoma paulistanum* Martins & Simone, 2014, from São Paulo state, SE Brazil. *Habeastrum strangei* sp. nov. shares some features with it, such as: the shape of the snout; the duplication of the eyes; the attributes of the pallial cavity (lacking gill and osphradium, with kidney and pericardium disposed in a similar way); the characters of the jaws and odontophore; and the relatively large and similarly-arranged nerve ring. By contrast, *Habeastrum strangei* sp. nov. differs from *A. paulistanum* by: the dextral coiling; the broader width of the pallial genital structures; the presence of esophageal glands; the lack of salivary glands; the stomach bulging posteriorly; and the presence of a penis. The latter is a plesiomorphic state in Diplommatinidae, as some species are aphyallic (Tielecke 1940; Barker 2001; Martins and Simone 2014).

There is more information available on the radulae of other Neotropical diplommatinids than any other anatomical feature (Doering 1885; Baker 1923; Bartsch and Morrison 1942; Martins and Simone 2014). The radula of cyclophoroideans is plesiomorphically taenioglossate (Barker 2001; Ponder et al. 2008): two marginal teeth and one lateral tooth on each side of the central rachidian tooth. This condition seems to be kept in *Adelopoma* (Doering 1885; Baker 1923; Martins and Simone 2014) and also in *Habeastrum strangei* sp. nov. (Fig. 2B–E).

Besides the now three species of *Habeastrum*, there are only six other diplommatinids reported from Brazil, belonging to the genera *Adelopoma* and *Habeas* Simone, 2013 (Simone 2006; Birckolz et al. 2016; Salvador 2019). Nevertheless, the family allocation of the latter is yet to be confirmed, given that they are known only from empty shells and present unusual features for the family, such as a large shell size (> 5 mm overall and >10 mm for *H. corpus* Simone, 2013) and the presence of an anal notch in the aperture (Simone 2013). Most Brazilian diplommatinids have been described in the 2010s, typically from understudied cave environments (Simone 2013, 2019; Martins and Simone 2014; present work). Furthermore, similarities in shell morphology across vast areas might hide a cryptic diversity (Salvador et al. 2018; Salvador 2019). Thus, the true diversity of this group in Brazil and its biogeographical implications are yet to be fully understood.

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