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Callinera emiliae sp. nov. (Nemertea: Palaeonemertea) from Negros Island, the Philippines

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Abstract

Callinera emiliae sp. nov., the ninth member of the genus, is described based on three specimens collected in Dumaguete, Negros Island, Republic of the Philippines. The new species can be distinguished from its congeners by the following characteristics: lateral sensory organs present; sub-epidermal glandular cells absent; blood vascular system without a ventral cephalic connective; nervous system with two dorsal cerebral commissures; and foregut nerves fused to form a ganglion in front of the mouth. In living specimens, epidermal constrictions were observed in the intestinal region; the presence of intestinal sphincters was confirmed in sectioned material and these correspond with the epidermal constrictions.

Key words: Nemertea, palaeonemertean, Philippines, taxonomy, new species

Introduction

The nemertean fauna of the Philippines is virtually unknown; only two species have previously been reported. One of these is a parasitic species to decapod crustaceans, *Carcinonemertes mitsukurii* Takakura, 1910, reported by Humes (1942) based on a museum specimen of the crab, *Charybdis miles* de Haan, collected in 1908 from San Andreas Island (between Marinduque Island and Luzon Island); the other is a terrestrial species, *Geonemertes philippinensis* Gibson & Moore, 1998, described from a cave 'Cueva Santa,' Quezon National Park, Luzon Island (Gibson & Moore 1998). A faunal survey carried out in the Philippines yielded an undescribed palaeonemertean species, which is fully described and illustrated in the present paper.

Material and methods

Sampling was made on 25 October 2005 at an exposed tidal flat (9º19'56.6''N, 123º18'35.2''E) in front of the Marine Laboratory, Silliman University, Dumaguete, Negros Oriental, Negros Island, Republic of the Philippines (Fig. 1), where the fiddler-crabs, *Uca tetragonon*, were also observed. Nemerteans were dug by a hand shovel from the sediment. Specimens were anaesthetized in $MgCl_2$ solution isotonic with seawater (about 25 psu), fixed in Bouin's solution for 24 hr, embedded in 56–57°C m.p. paraffin wax and sectioned at 8 µm. Sections were subsequently stained by the Mallory trichrome method. Type specimens are deposited in the Zoology Section (New Series), Museum of Natural History, University of the Philippines Los Baños, Laguna, Republic of the Philippines (UPLB-MNH-Z-NS), and the Hokkaido University Museum, Sapporo, Japan (ZIHU). Terminology follows that used by Kajihara (2006); *E* (*b*) and *E* (*i*) correspond Sundberg and Hylbom's (1994: table 1) '5. Height of epidermis/body diameter in brain region' and '6. Height of epidermis/body diameter in midgut region,' respectively.

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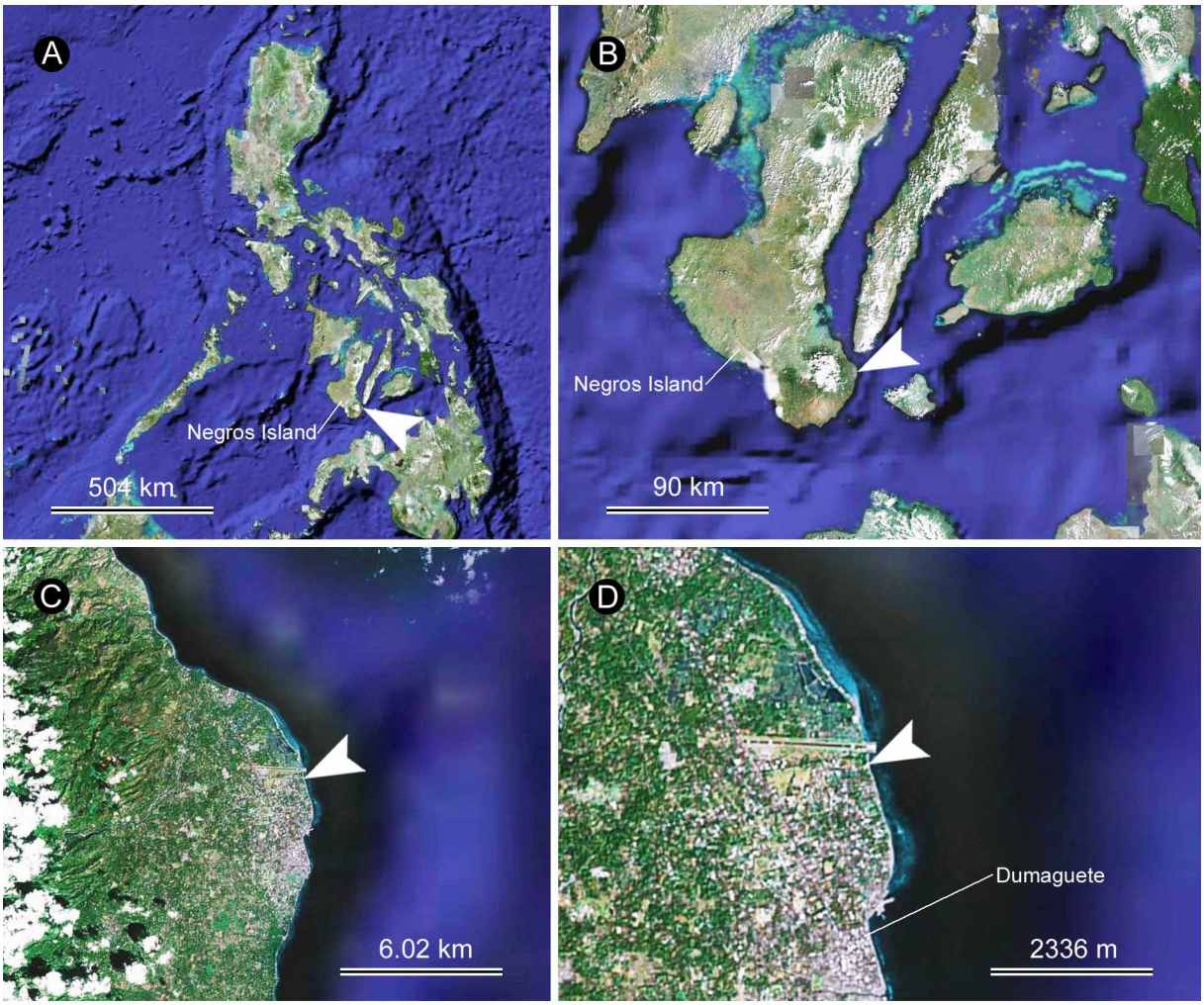

FIGURE 1. A–D, maps showing the sampling locality at increasing enlargements; arrowheads indicating sampling site (modified from ©2007 TerraMetrics and ©2006 GoogleEarth images).

Family Callineridae Bergendal, 1901
Genus *Callinera* Bergendal, 1900
***Callinera emiliae* sp. nov. (Figs 2–6)**

Diagnosis: A *Callinera* having lateral sensory organs; sub-epidermal glandular cells absent; blood vascular system without cephalic ventral connective; nervous system with double dorsal and single ventral cerebral commissures; foregut nerves fusing to form ganglion in front of mouth; epidermis with constrictions in intestinal region; and intestinal sphincters present.

Etymology: The specific name is dedicated to Dr Emilia S. Yap, a Filipino food scientist who guided my collecting trip in the Philippines.

Material examined: Holotype, ZIHU-3187, male, serial transverse sections of complete specimen, 77 slides. Paratypes: UPLB-MNH-Z-NS-0389, serial transverse sections of the anterior region of the body, 27 slides; UPLB-MNH-Z-NS-0390, serial transverse sections of the anterior region of the body, 7 slides.

External features: Body about 7 cm long and 1.8 mm wide when anaesthetized (Fig. 2A). Head translucent, dorsoventrally flattened, and demarcated from succeeding body; white rhynchodaeum visible through cephalic surface; with neither eyes nor cephalic furrows (Fig. 2B). Foregut region cross-sectionally rounded; foregut cream-white. Epidermis translucent except in posterior foregut region, where epidermal hue suddenly changing into light flesh colour; from there on backward, epidermis gradually resuming translucent appearance; pair of lateral organs present, recognized as horizontally elongated epidermal indentation, located on lat-

eral side of body on each side just posterior to light flesh colour epidermal band (Fig. 2C). Proboscis white in colour. In intestinal region six constrictions present (Fig. 2A, D); gonads arranged in row on each side.

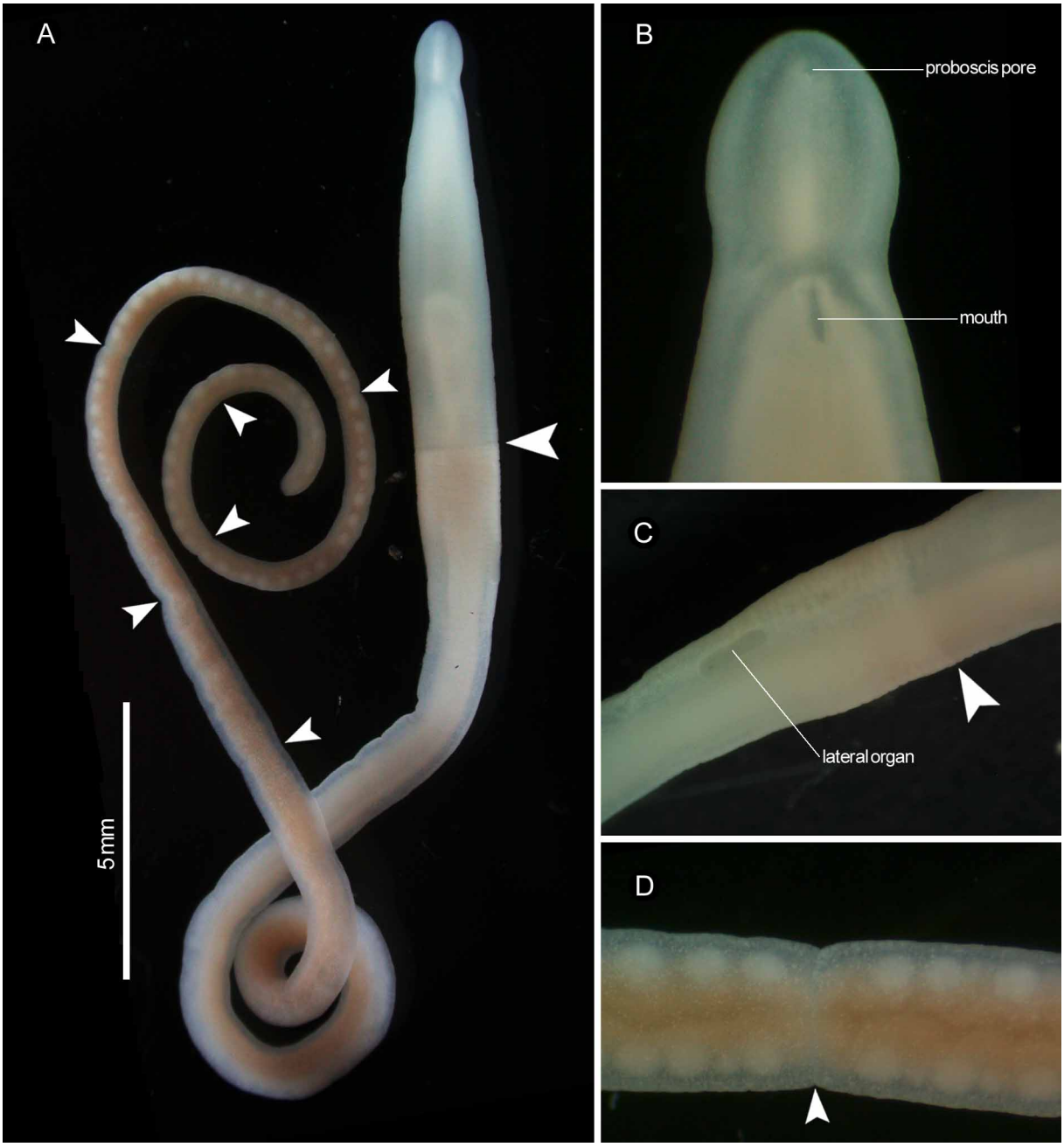

FIGURE 2. *Callinera emiliae* sp. nov. Holotype, ZIHU-3187, photographs taken in life. A, general appearance of complete specimen; large arrowhead indicating anterior border of epidermal band, small arrowheads indicating epidermal constrictions. B, enlargement of head, viewed ventrally. C, enlargement of lateral organ, viewed ventro-laterally, head to the right; arrowhead indicating anterior border of epidermal band. D, enlargement of intestinal region; arrowhead indicating epidermal constriction.

Body wall, musculature, and parenchyma: Ciliated epidermis up to 100 µm thick in brain region, gradually thinner posteriorly, 5–30 µm thick in intestinal region; basophilic glandular cells predominant in pre-cerebral region (Fig. 3A); in foregut region, acid fuchsin staining glandular cells predominant before being abruptly replaced by Orange G staining acidophilic cells in flesh-coloured epidermal band (Fig. 3B); posteriorly, epidermis containing basophilic and neutrophilic glandular cells, in addition to acidophilic glandular cells; *E* (*b*) = 0.08 (holotype ZIHU-3187 and paratype UPLB-MNH-Z-NS-0389) and 0.06 (paratype UPLB-MNH-Z-NS-0390); *E* (*i*) = 0.04 (holotype). Dermis up to 10 µm thick in foregut region; processes of connective tissue into epidermis not found; innermost side of dermis not forming mesh-like structure. Body-wall

musculature composed of outer circular and inner longitudinal muscle layers; inner circular muscle layer present (Fig. 3B) from mouth through rhynchocoel posterior chamber; thin diagonal layer present between outer circular and longitudinal muscle layers (Fig. 3B). Pre-cerebrally, transverse muscle fibres running below rhynchodaeum and cephalic vessels (Fig. 3A). Longitudinal muscle plate present between rhynchocoel and alimentary canal (Fig. 3C), terminating before rhynchocoel posterior chamber. Intestine with six sphincters (Fig. 3D), corresponding with epidermal constrictions.

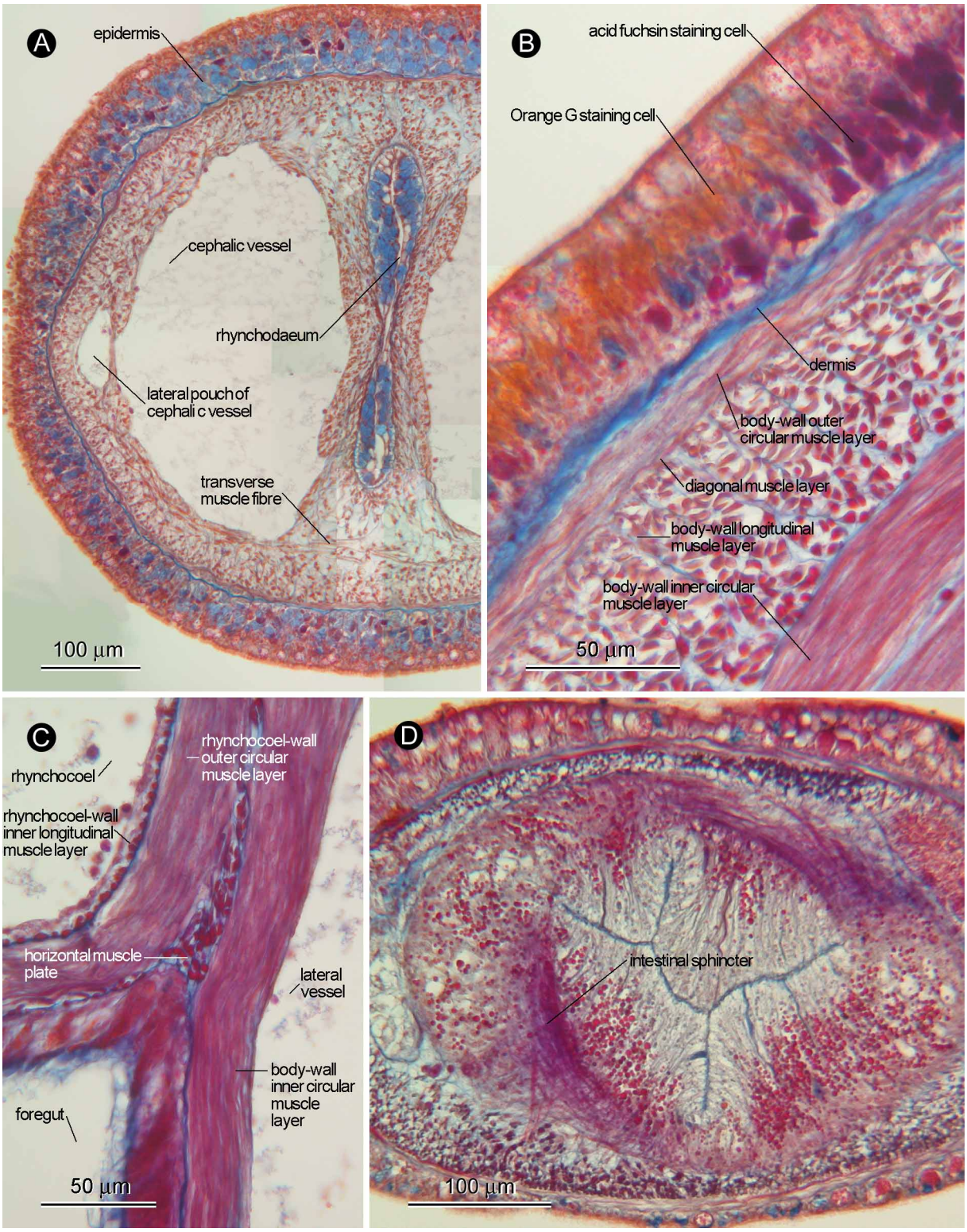

FIGURE 3. *Callinera emiliae* sp. nov. Holotype, ZIHU-3187, photomicrographs of transverse sections. A, rhynchodaeum. B, epidermal band. C, posterior portion of foregut region, showing horizontal muscle plate. D, intestinal sphincter.

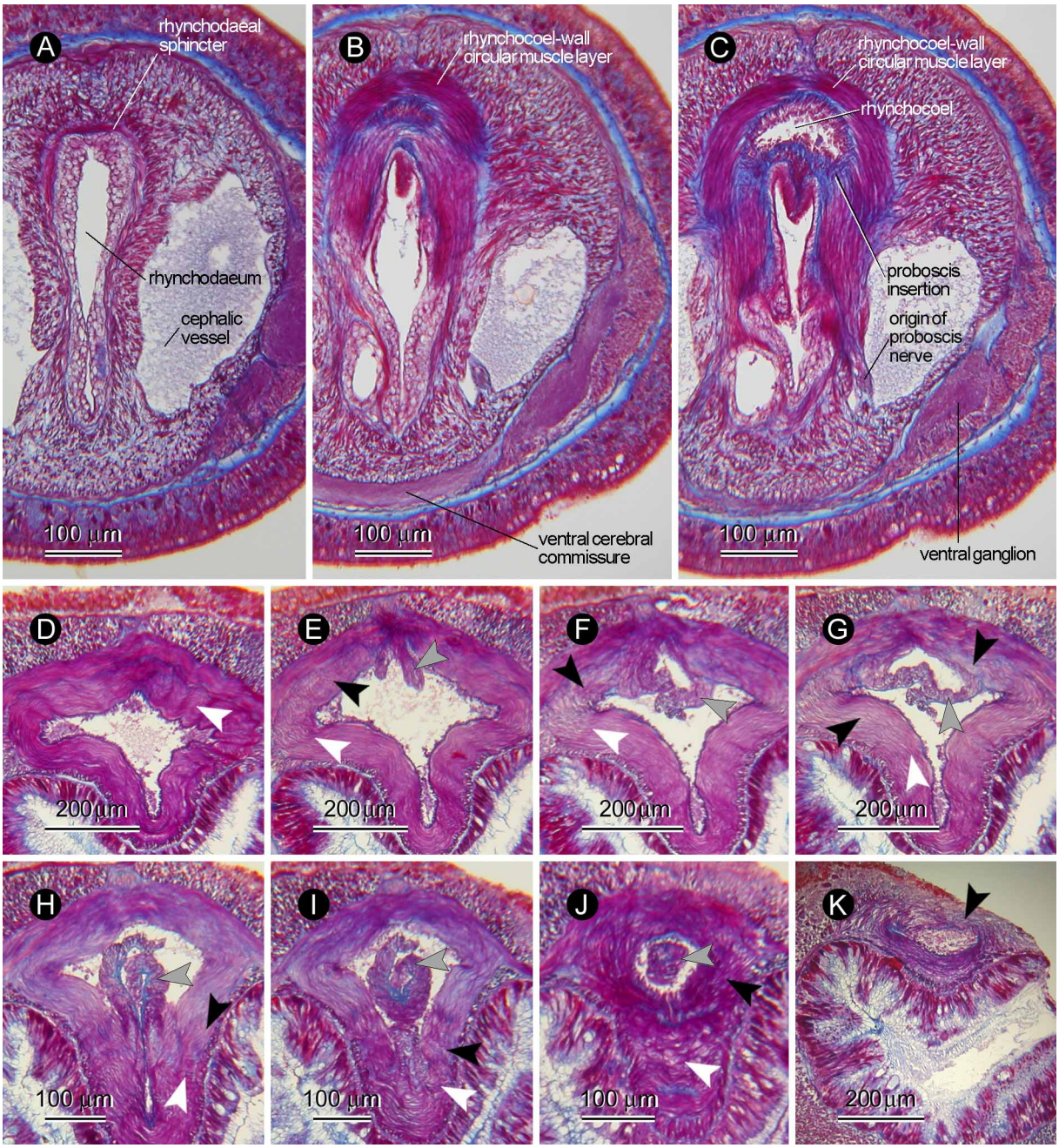

FIGURE 4. *Callinera emiliae* sp. nov. Holotype, ZIHU-3187, photomicrographs of transverse sections. A, rhynchodaeal sphincter. B, ventral cerebral commissure. C, proboscis insertion. Paratype, UPLB-MNH-Z-NS-0389, D–K, junction between anterior (indicated by white arrowheads) and posterior (indicated by black arrowheads) rhynchocoel chamber; grey arrowheads indicating 'pleats' of posterior rhynchocoel chamber wall, originating from body-wall inner and outer circular muscle layers.

Proboscis apparatus: Proboscis pore opening mid-ventrally near tip of head, leading to rhynchodaeum; rhynchodaeal wall containing basophilic glandular cells (Fig. 3A) except just in front of proboscis insertion (Fig. 4A). Rhynchodaeal sphincter present just in front of proboscis insertion, composed of circular muscles derived from body-wall longitudinal muscle layer (Fig. 4A, B). Proboscis insertion situated near brain ring, composed of fibres from rhynchodaeal sphincter; pair of proboscis nerves originating from ventral ganglia, running below cephalic vessel and entering proboscis via insertion (Fig. 4C). Rhynchocoel extending about 60% of body length, with wall composed of outer circular and inner longitudinal muscle layers (Fig. 3C). Rhynchocoel posterior chamber (or 'muscular sac') present, its wall mid-dorsally derived from body-wall inner and outer circular muscle layers; pair of 'pleats' present between anterior and posterior rhynchocoel junction, also originating from body-wall inner and outer circular muscle layers (Fig. 4D–K). Proboscis histo-

logically differentiated into four regions: first region [= Bergendal's region (Kajihara 2006)], 160 µm long, composed of glandular epithelium and longitudinal muscle layer, pair of proboscis nerves present between these two layers (Fig. 5A); second region, 720 µm long, possessing circular muscle layer between glandular epithelium and longitudinal muscle layer, with pair of proboscis nerves situated between glandular epithelium and circular muscle layer (Fig. 5B), glandular epithelium posteriorly becoming thicker and densely ciliated (Fig. 5C); third region, 1.2 mm long, with glandular epithelium containing distinct acidophilic glandular cell mass above proboscis nerves (Fig. 5D); fourth main region, probably accounting for more than 80% of entire proboscis length, possessing epithelium basically composed of cells with neutrophilic cytoplasm, containing acidophilic and basophilic granules (Fig. 5E), followed by proboscis retractor muscle (Fig. 5F); fourth main region and proboscis retractor muscle extending into rhynchocoel posterior chamber (Fig. 5F).

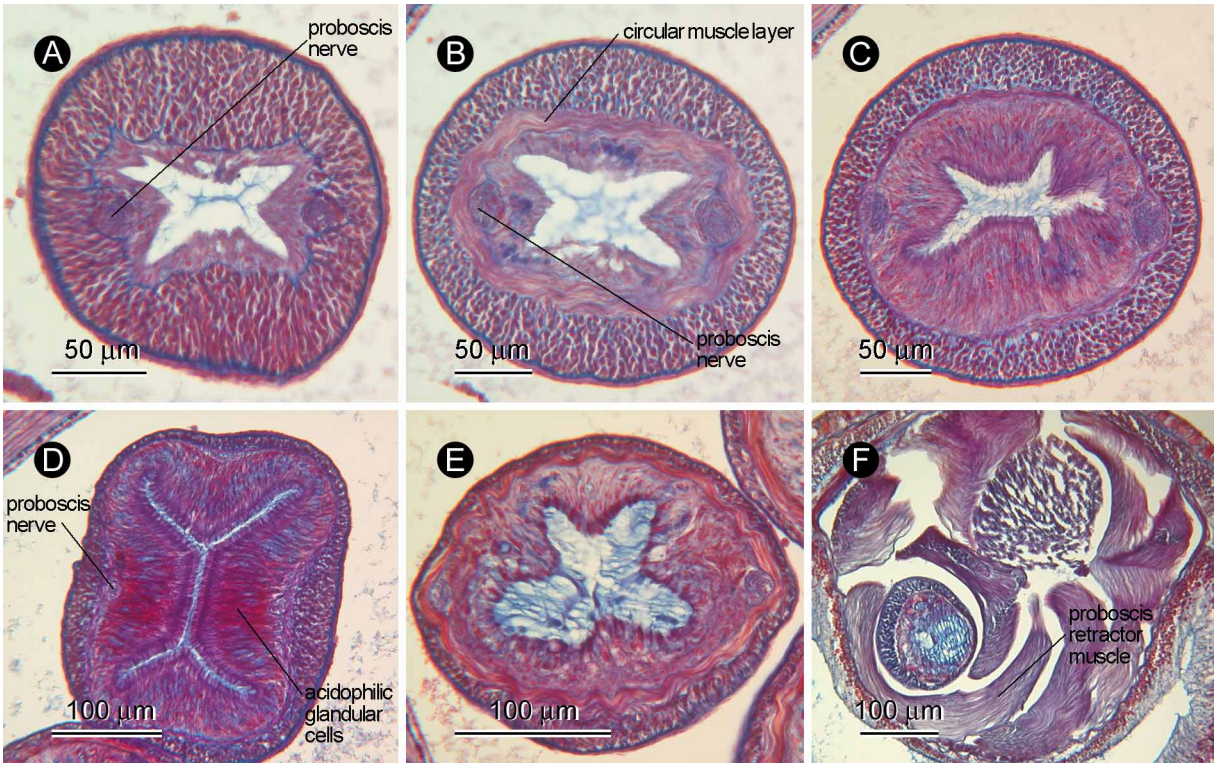

FIGURE 5. *Callinera emiliae* sp. nov. Holotype, ZIHU-3187, photomicrographs of transverse sections of proboscis. A, first region. B, C, second region. D, third region. E, fourth main region. F, proboscis retractor muscles filling rhynchocoel posterior chamber.

Alimentary canal: Mouth opening just posterior to brain; foregut histologically differentiated into two regions: anterior region possessing epithelium composed of ciliated glandular cells containing basally basophilic and distally neutrophilic contents (Fig. 6A); posterior region possessing epithelium dominated by acidophilic glandular cells, sporadically containing neutrophilic cells (Fig. 6B). No intestinal caecum. Main intestinal canal possessing epithelium composed of tall columnar cells containing neutrophilic cytoplasm with basal acidophilic granules (Fig. 6C); intestine without lateral diverticula.

Blood system: Pair of cephalic vessels meeting anteriorly, dorsoventrally pierced by muscle strands; in rhynchodaeal region, dorsoventral muscle strands more or less sagittally continuous, making cephalic vessel seemingly possess lateral pouch (Fig. 3A); with neither dorsal nor ventral connectives. Post-cerebrally, lateral vessels first situated inside body-wall inner circular muscle layer; posteriorly, near junction between anterior and posterior foregut regions, lateral vessels traversing through body-wall inner circular muscle layer to be situated immediately outside it. Mid-dorsal vessel absent.

Nervous system: Brain and lateral nerves situated between dermis and body-wall outer circular muscle layer; inner neurilemma incompletely developed, outer neurilemma well developed (Fig. 4C). Two dorsal and

one ventral cerebral commissures present. Pair of foregut nerves anteriorly meeting to form ganglion just before mouth (Fig. 6D). Mid-dorsal nerve present between dermis and body-wall outer circular muscle layer, frequently sending fibres downward to rhynchocoel wall.

Sense organs: Pair of lateral organs situated anterior to rhynchocoel posterior chamber, containing basophilic cells (Fig. 6E). Neither cerebral sense organs nor statoliths present. No eyes.

Excretory system: Excretory system composed of thin walled collecting tubule on each side of body above lateral blood vessel (Fig. 6E), opening to exterior at its posterior end (Fig. 6F). In holotype, anterior glandular mass not well developed; anterior end of excretory system not appearing to enter lateral vessel. In one of two paratypes (UPLB-MNH-Z-NS-0389), anterior end of excretory collecting tubule entering lateral blood vessel; the other paratype (UPLB-MNH-Z-NS-0390) lacking posterior portion of body containing excretory system.

Reproductive system: The holotype was a male. Sexes of paratypes are unknown. Testes up to 200 µm in diameter, arranged in row above lateral blood vessel on each side (Fig. 6C).

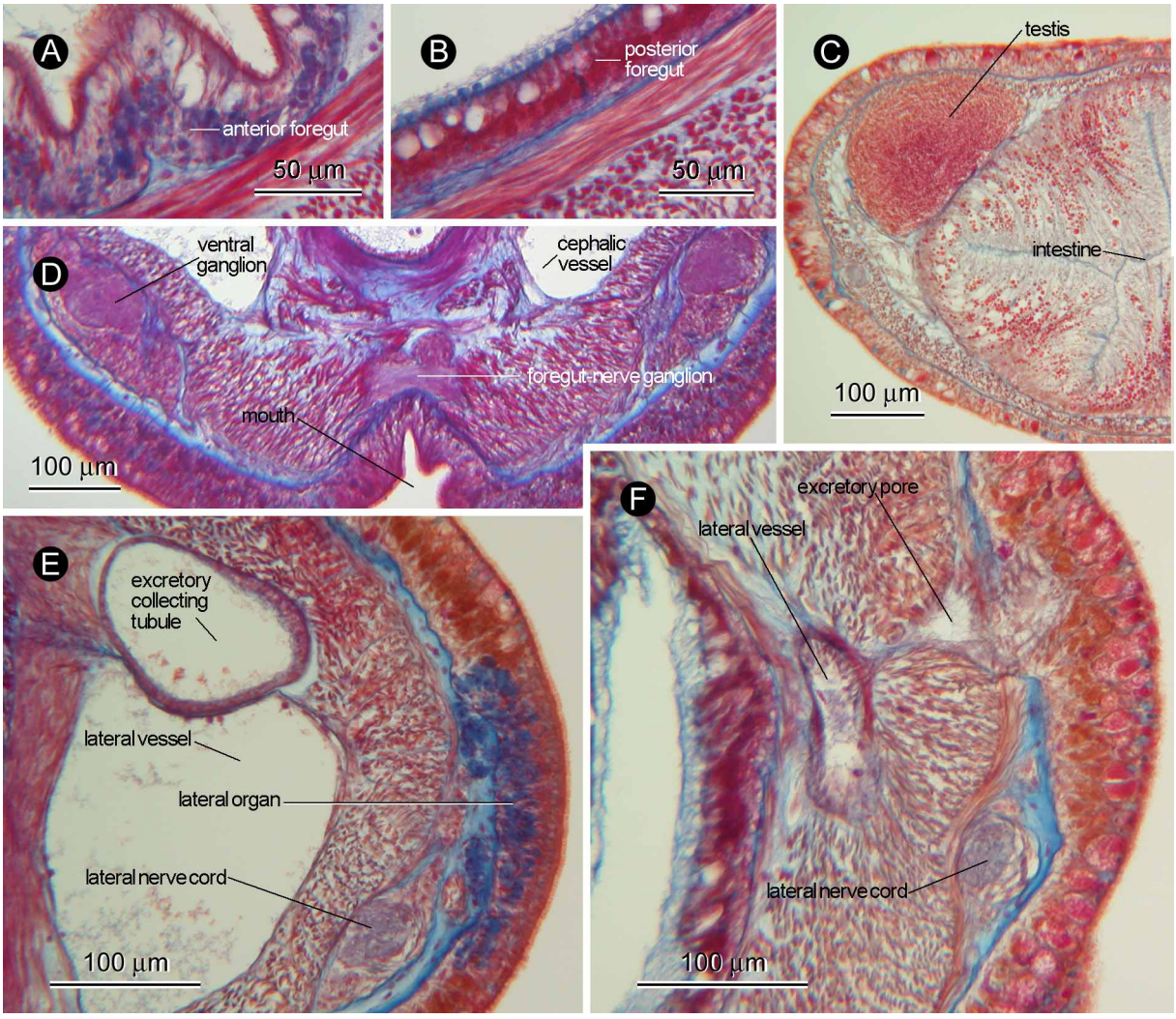

FIGURE 6. *Callinera emiliae* sp. nov. Holotype, ZIHU-3187, photomicrographs of transverse sections. A, anterior region of foregut. B, posterior region of foregut. C, intestinal region showing testis. D, foregut nerve ganglion just before mouth. E, lateral organ. F, excretory pore.

Remarks: Callinera emiliae sp. nov. is identified as the member of the genus *Callinera* by possessing a nervous system situated between the dermis and body-wall outer circular muscle layer, the rhynchocoel posterior chamber (= muscular sac), and no cerebral sense organs. *Callinera emiliae* sp. nov. can be distinguished from the congeners by the characteristics summarized in Table 1.

The morphology of the rhynchocoel posterior chamber in *Callinera emiliae* sp. nov. was most clearly distinguishable in one of the two paratypes that had lost the proboscis. There are thin pleats originating from the body-wall inner and outer circular muscle layers in the junction between the anterior and posterior rhynchocoel chambers. In the holotype, however, the pleats were pressed against the rhynchocoel wall by the proboscis, which made tracing of the morphology difficult. Remarkably, the structure of the rhynchocoel posterior chamber is similar to that reported in *Tubulanus borealis* Friedrich, 1936. The rhynchocoel posterior chamber is regarded as the possible synapomorphy for the genus *Callinera* in the cladistic analysis based on 50 morphological characters performed for 49 palaeonemerteans by Sundberg and Hylbom (1994). However, *T. borealis* was not included in their analysis due to the plethora of unknown character states in this taxon. Anyhow, the two genera *Callinera* and *Tubulanus* appear to be not clearly discriminated by the traditionally used characters, *viz*., the presence/absence of the cerebral sensory organs and rhynchocoel posterior chamber.

TABLE 1. Comparison of six characters among *Callinera* species. Data compiled from Bergendal (1900a, b, c, 1901, 1903), Rogers *et al*. (1992), Gibson and Sundberg (1999), Senz (2000), Chernyshev (2002), and Kajihara (2006).

Taxa	A	B	C	D	E	F
C. bergendali Gibson & Sundberg, 1999	1	0	0	0	1	0
C. blanchardi Senz, 2000	1	0	1	0	1	0
C. buergeri Bergendal, 1900	1	0	1	1	2	0
C. grandis Bergendal, 1903	0	1	1	0	2	1
C. monensis Rogers *et al*., 1992	0	0	0	1	1	0
C. nishikawai Kajihara, 2006	1	0	1	0	1	1?
C. quatrefagesi Senz, 2000	1	0	1	0	2	1
C. zhirmunskyi Chernyshev, 2002	1	0	1	1	2	0
***C. emiliae* sp. nov.**	1	0	1	0	2	0

Characters and character states:
A: Lateral sensory organs: (0) absent; (1) present.
B: Body-wall longitudinal muscle layer: (0) without; (1) with sub-epidermal glandular cells.
C: Proboscis: (0) without; (1) with circular muscle layer in region following Bergendal's region.
D: Cephalic blood vascular system: (0) without; (1) with ventral lacuna.
E: Dorsal cerebral commissure(s): (1) one; (2) two.
F: Foregut nerves: (0) fuse to form a single median nerve before branching anterior to mouth, or remaining as two distinct nerves (1).

Acknowledgements

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