RESEARCH REPORT

DESCRIPTION OF *HALIPLECTUS MEXICANUS* SP. N. (NEMATODA: HALIPLECTIDAE) FROM MEXICO

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ABSTRACT

Cid del Prado-Vera, I., E. Sosa-Perez, A. Michaud, H. Ferris, and S. A. Subbotin. 2026. Description of *Haliplectus mexicanus* sp. n. (Nematoda: Haliplectidae) from Mexico. Nematropica 56: 57–65.

Haliplectus mexicanus sp. n. is described from rhizosphere soil of the breadnut tree, *Brosimum alicastrum* growing in the tropical forest at La Mancha Ecological Reserve of the Ecology Institute of Jalapa, Veracruz State, Mexico. The new species is characterized by females, males, and juveniles having a short conical tail with rounded terminus less than 38 µm long, body less than 500 µm long, anterior region of the body wider than the posterior one, cuticle with conspicuously ornamented annuli, and pores along the lateral and submedian regions of the body. The cephalic region has four fine annulations and is separated from the rest of body by a constriction. The amphidial fovea are very conspicuous, post-labial and circular. The stoma is tubular, curved in the posterior region. The pharynx has a short twisted procorpus, muscular bipartite basal bulb rounded with a transverse constriction. The female reproductive system is amphidelphic with reflexed ovaries. Males lack a bursa and have four contiguous midventral precloacal papilliform supplements. Spicules slender 27 µm long average, the gubernaculum wedge-shaped, broad anteriorly part, and tapering to a blunt tip. Phylogenetic relationships of new species and *Haliplectus* sp. from California with some representatives of this genus are given as inferred from the analysis of 18S, D2-D3 of 28S rRNA and *COI* gene sequences.

Key words: Mexico, new species, phylogeny, SEM, taxonomy, Veracruz State.

RESUMEN

Cid del Prado-Vera, I., E. Sosa-Perez, A. Michaud, H. Ferris, and S. A. Subbotin. 2026. Description of *Haliplectus mexicanus* sp. n. (Nematoda: Haliplectidae) from Mexico. Nematropica 56: 57–65.

Haliplectus mexicanus sp. n. fue colectada del suelo alrededor del árbol *Brosimum alicastrum* en el bosque tropical en la estación Reserva Ecológica La Mancha, del Instituto de Ecología de Jalapa, Veracruz. La nueva especie se caracteriza porque las hembras, machos y juveniles tienen una cola corta, cónica con término redondeado menor a 38 µm promedio, el tamaño del cuerpo menor a 500 µm, la región anterior del cuerpo más ancha que la posterior, la cutícula ornamentada a lo largo del cuerpo con poros en la parte lateral

media del cuerpo. La región cefálica compuesta de cuatro finos anillos y separada del cuerpo por una constricción. Anfidios circulares muy conspicuos posteriores a la región labial. Estoma tubular con una flexión en su parte posterior, procorpus del esófago corto y con una flexión, bulbo basal del esófago esférico y con una línea transversal que lo divide en dos áreas. Sistema reproductor femenino amfidelfica, con ovarios reflejados. Machos sin bursa y con cuatro papilas precloacales en la parte ventral, inmediatas a la cloaca. Espículas 27 µm promedio de largo, gubernaculum en forma de cuña en su parte anterior y con término redondeado. Se presentan las relaciones filogenéticas de nuevas especies y de *Haliplectus* sp. de California con algunos representantes de este género, tal como se infiere del análisis de las secuencias de los rRNA genes 18S, D2-D3 of 28S y *COI*.

Palabras clave: Estado de Veracruz, filogenia, México, nueva especie, SEM, taxonomía.

INTRODUCTION

During a nematology survey at the La Mancha Ecological Reserve of the Ecology Institute of Jalapa, Veracruz State, Mexico, nematodes of the genus *Haliplectus* were collected from rhizosphere soil of the breadnut tree, *Brosimum alicastrum* (Moraceae) growing in the jungle area. Based on morphological and molecular characteristics, these nematode specimens were identified as a new species described herein as *Haliplectus mexicanus* sp. n.

The genus *Haliplectus*, first described by Cobb in 1913, comprises a group of free-living nematodes found in marine, brackish, freshwater, and terrestrial environments that have distinctive morphological features. Species of the genus are globally distributed, reported from a wide range of coastal and benthic ecosystems. Taxonomic identification of species of the genus relies on detailed morphological analysis. Diagnostic characteristics include the configuration of the pharyngeal basal bulb (monopartite vs. bipartite), the form and dimensions of the stoma, and the presence or absence of preanal supplementary structures in males. The descriptions of most of the species are rather incomplete and often based on only a few specimens (Chitwood, 1956; Cobb, 1913; Gharakhani et al., 2022; Shahina et al., 2014; Swart & Heyns, 1993; Zhou et al., 2022).

Nearly 30 species have been described in the genus *Haliplectus* (Gharakhani et al., 2022; Zhou et al., 2022). Identification keys for species of the genus have been provided by several authors (Gharakhani et al., 2022; Swart & Heyns, 1993; Zhou et al., 2022). The position of the genus within the class Chromadorea is not well defined and is considered *incerta sedis*. Chitwood (1951) placed *Haliplectus* in the family Plectidae Örley, 1880, and the subfamily Haliplectinae Chitwood, 1951. However, recent phylogenetic analyses indicated that sequences of the genus *Haliplectus* form an independent lineage, branching off early within the Chromadorea (Holovachov et al., 2012), and do not support for classification of the genus within the order Plectida (Gharakhani et al., 2022).

The goals of this study were to: i) provide a morphological description of *Haliplectus mexicanus* sp. n., ii) report rRNA and *COI* gene sequences for this species from Mexico and *Haliplectus* sp. from California, and iii) investigate phylogenetic relationships within the genus *Haliplectus*.

MATERIALS AND METHODS

Nematode samples and morphological study

Soil samples with *H. mexicanus* sp. n. were collected from five different points around a *Brosimum alicastrum* tree growing in the jungle area of the La Mancha Ecological Reserve. Nematodes were extracted from soil using centrifugal flotation, killed by heating to 70°C and fixed in Golden fixative (Ayoub, 1977; Hooper, 1986). Fixed nematodes were stored at room temperature for 30 days and then transferred to a small Petri dish placed in a desiccator containing 95% ethanol. Samples were incubated at 40°C for 1–2 days, processed to glycerin using a modification of Seinhorst's (1959) method (Cid del Prado-Vera & Subbotin, 2012). Nematodes were mounted in anhydrous glycerin containing 1–2 drops of fast green stain on glass slides using the paraffin wax ring method (Hooper, 1970). Measurements and drawings were made using a drawing tube on an

American Optical compound microscope. For scanning electron microscopy, some specimens were placed in phosphate buffer for 15 min and dehydrated in an alcohol series (10–100%) for 15 min at different concentrations. The specimens were critical point dried and coated with gold palladium before observation under a Jeol JSM6390 scanning electron microscope at 10 kV (Cid del Prado-Vera & Subbotin, 2012). Several nematode specimens were fixed in 70% ethanol for molecular analysis.

Specimens of *Haliplectus* sp. from California were extracted from a sample collected at sand dunes of Dillon Beach, Marin County, CA (N 38º14".895; W 122º58".061).

Molecular analysis

DNA was extracted from several specimens, using proteinase K, PCR, and cloning protocols as described by Subbotin (2021a). Several primer sets were used for PCR amplification in this study: i) G18SU (5′ - GCT TGT CTC AAA GAT TAA GCC - 3′) and R18Tyl1 (5′ - GGT CCA AGA ATT TCA CCT CTC - 3′) primers amplifying the partial 18S rRNA gene; ii) F18Tyl2 (5′ - CAG CCG CGG TAA TTC CAG C - 3′) and R18Tyl2 (5′ - CGG TGT GTA CAA AGG GCA GG - 3′) primers amplifying the partial 18S rRNA gene; iii) D2A (5′ - ACA AGT ACC GTG AGG GAA AGT TG - 3′) and reverse D3B (5′ - TCG GAA GGA ACC AGC TAC TA - 3′) primers amplifying the D2-D3 expansion segments of 28S rRNA gene; iv) JB3 (5′ -TTT GGG CAT CCT GAG GTT TAT - 3′) and JB5 (5′ - AGC ACC TAA ACT TAA AAC ATA ATG AAA - 3′) primers amplifying the partial *COI* gene. Sequencing was conducted at Azenta (California, USA). Sequences were submitted to the GenBank database under accession numbers: PX417920 (18S rRNA gene), PX418374, PX586626 (D2-D3 of 28S rRNA gene), and PX417912, PX455431 (*COI* gene). New sequences of the 18S rRNA, D2-D3 of 28S rRNA and *COI* genes were aligned using ClustalX 1.83 (Chenna et al., 2003) with corresponding published sequences (De Ley et al., 2005; Gharahkhani et al., 2022; Macheriotou et al., 2019; Tytgat et al., 2019; van Megen et al., 2009; Zhou et al., 2022). The alignments were analyzed with Bayesian inference (BI) using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) under the GTR + G + I model as described by Subbotin (2021b). The trees were visualised with the program TreeView 1.6.6 and drawn with Adobe Illustrator CC. Sequence analyses of alignments were performed with PAUP 4.0b 10 (Swofford, 2003). Pairwise divergences between taxa were computed as absolute distance values and as percentage mean distance values based on whole alignment, with adjustment for missing data.

RESULTS

Haliplectus mexicanus *sp. n. (Table 1, Figures 1-3)*

Female. Body less than 500 µm long, straight, or slightly ventrally arcuate upon fixation, anterior end of body wider than the posterior. Body width varying, at basal bulb level 29 µm, anterior to vulva 25–35 µm, and at anus level 14–17 µm. Cuticle with conspicuous annules, each with fine longitudinal striae, the anterior annules of body are 2.0 µm high and mid body and in tail region, 1.0 µm; anastomoses in anterior, midbody, and mainly anterior to anus and in tail, cuticular pores along the body, abundant in lateral and submedian areas, fewer on ventral and dorsal side. Cephalic region with four annuli separated from the rest of body by a constriction. Labial disc hexagonal shape, lips very small and not projecting. Labial papillae pore-like. Amphidial fovea conspicuous circular 2–3 µm in diameter and about one head-width or 4–5 µm from anterior end. Stoma a narrow, cuticularised tube, curved in the posterior end and 8–19 µm long. Procorpus muscular and twisted in the anterior part, 10–20 µm long. Wedge-shaped isthmus short, 4–12 µm long. Median bulb absent. Basal bulb spherical bipartite with transverse separation, 14–18 µm long by 10–16 µm wide. Nerve ring 29–31 µm from the anterior end, excretory pore not observed. Cardia cylindrical, small 5.0 µm long. Intestine granulated with wide lumen. Female reproductive system amphidelphic, anterior gonad 20% of the body length and posterior 15%; ovaries reflexed, oocytes arranged in more than three rows in the germinal zone, oviduct simple, and spermatheca not differentiated as from the uterus. Vagina simple, not muscular 4–10 µm long, vulva lips thin and not protruding. Anterior gonad to vulva 62–82 µm and posterior 52–65 µm long. Vulva

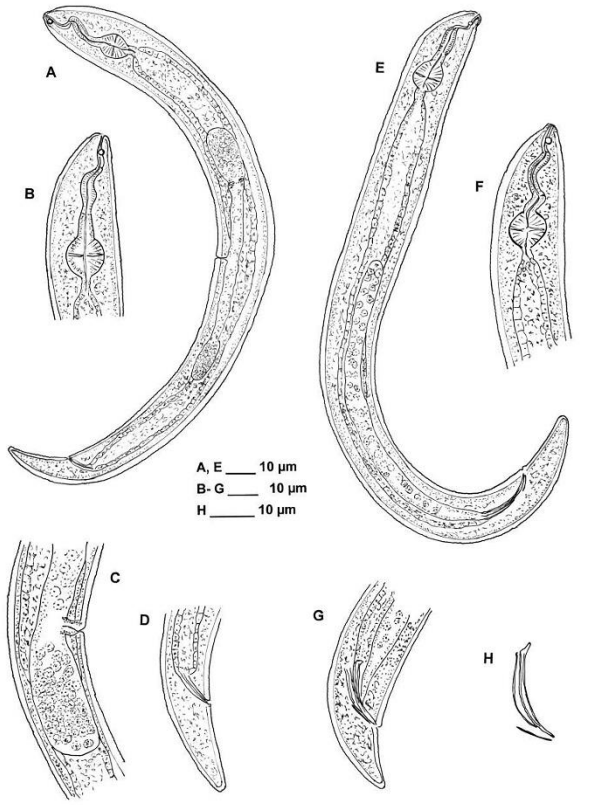

Figure 1. *Haliplectus mexicanus* sp. n. Female – A–D. A. Whole body; B: Anterior end of body lateral view; C. Vulva region, lateral view; D. Posterior end body, anus, and tail. Male – E–H. E: Whole body; F: Anterior end, body lateral view; G: Posterior end body, cloaca and tail; H: Spicules and gubernaculum.

Figure 2. *Haliplectus mexicanus* sp. n. Light microscope photographs. Female – A: Anterior end body; B: Vulva region, lateral view; C: Posterior end body, lateral view. Male – D: Whole body; E. Posterior end body, lateral view.

aperture difficult to see, and 28–35 µm width of body level. Rectum 6–18 µm long. Tail conical with rounded terminus, anus conspicuous rounded. Caudal glands and spinneret absent.

Male. Body slender with anterior extremity wider than the posterior end of body, curved ventrally in posterior region, similar in size to females. Cuticle with conspicuous annuli that have fine longitudinal striae; numerous pores along the body. Cephalic region conspicuous and separated from labial disc and the rest of body by a constriction and four annuli. Amphidal apertures conspicuous circular 3.0 µm in diameter, and 4–8 µm from anterior end. Stoma long cuticularised, curved in the posterior end, and 9–19 µm long. Procorpus muscular and twisted in the anterior part, 11–25 µm long. Isthmus short 2–9 µm long. Basal bulb bipartite as in females with a transverse break. Diorchic with paired and opposed testes. Spicules slender, curved ventrally 20–30 µm long, gubernaculum wedge-shape and thin 6–11 µm long. Four contiguous precloacal supplements present. Tail conical with rounded end. Caudal glands and spinneret absent.

Juveniles. Similar to females and males with the anterior part of body wider than the posterior. Cuticle with conspicuous annuli, each ornamented with fine longitudinal striae along the body and with numerous submedian pores along the body. The cephalic region conspicuous of 4–6 µm high and 7–8 µm wide, separated from the rest of body by a constriction and with four fine annuli. Amphidal apertures conspicuous, circular, 4–6 µm from anterior end; stoma tubular curved in the posterior end and 12–20 µm long. Procorpus muscular and twisted in the anterior part, 12–19 µm long. Isthmus short 2–10 µm long. Terminal bulb

Table 1. Measurements of females, males, and juveniles of *Haliplectus mexicanus* sp. n. All measurements are in µm and in the form: mean ± s.d. (range).

Characters	Holotype Female	Lectotype Male	Paratype Females	Paratype Males	Juveniles
n	1	1	14	11	12
L	371.0	340.0	400.0 ± 5.0 (340.0–500.0)	380.0 ± 5.0 (330.0–460.0)	340 ± 4.0 (280.0–420.0)
a	12.4	12.1	13.8 ± 1.6 (10.9–16.5)	13.0 ± 1.5 (11.6–15.4)	12.7 ± 1.3 (10.9–15.1)
b	7.4	7.1	8.3 ± 1.2 (6.8–10.5)	7.5 ±0.8 (6.3–9.1)	7.1 ± 0.7 (6.2–8.8)
c	13.25	12.1	13.4 ± 2.2 (9.1–16.7)	13.7 ± 1.5 (11.2–15.9)	11.6 ± 1.1 (9.2–13.3)
c'	1.9	1.4	1.9 ± 0.4 (1.3–2.5)	1.4 ± 0.2 (1.1–1.7)	1.9 ± 0.3 (1.6–2.6)
Stoma length	15.0	17.0	14.4 ± 2.7 (10.0–19.0)	14.5 ± 3.2 (9.0–19.0)	14.8 ± 1.8 (12.0–19.0)
Procorpus length	15	14.0	14.7 ± 2.5 (10.0–20.0)	17.8 ± 4.0 (11.0–25.0)	17.4 ± 4.1 (12.0–25.0)
Isthmus length	5.0	5.0	6.7 ± 2.4 (4.0–12.0)	4.5 ± 1.9 (2.0–9.0)	4.1 ± 2.5 (2.0–10.0)
Basal bulb length	15.0	14.0	15.1 ± 0.9 (14.0–18.0)	13.7 ± 1.0 (12.0–15.0)	14.0 ± 0.9 (13.0–15.0)
Basal bulb width	14.0	14.0	14.2 ± 1.8 (10.0–16.0)	14.0 ± 1.0 (12.0–16.0)	13.7 ± 0.8 (13.0–15.0)
Body width	30.0	28.0	29.2 ±1.6 (27.0–32.0)	29.0 ± 1.1 (27.0–31.0)	26.9 ± 2.2 (23.0–30.0)
V% or T%	51.5	52.3	51.8 ± 3.0 (46.9–58.1)	47.0 ± 9.2 (40.0–65.5)	–
Tail length	28.0	28.0	30.6 ±5.0 (21.0–38.0)	27.6 ± 3.7 (24.0–36.0)	29.7 ± 4.2 (22.0–37.0)
Tail width	15.0	20.0	15.9 ± 1.1 (14.0–17.0)	19.8 ± 1.0 (19.0–22.0)	15.2 ± 1.3 (13.0–18.0)
Distance from amphid to head	5.0	5.0	5.6 ± 1.5 (4.0–9.0)	5.6 ± 1.2 (4.0–8.0)	4.9 ± 0.7 (4.0–6.0)
Rectum	7.0	–	12.8 ± 4.0 (6.0–18.0)	–	7.4 ± 3.8 (5.0–17.0)
Spicules	–	26.0	–	26.6 ± 2.7 (20.0–30.0)	–
Gubernaculum		8.0	–	8.6 ± 1.9 (6.0–11.0)	–

well-developed, bipartite with transverse break, same length and width 13–15 µm. Nerve ring at 35 µm from the anterior end; excretory pore not observed. Cardia small 10 µm long. Intestine granulated with wide lumen. Tail conical with rounded end.

Type locality. Sandy soil around roots of the breadnut tree, *Brosimum alicastrum* in the jungle region of the La Mancha Ecological Reserve of the Ecology Institute of Jalapa, Veracruz State, Mexico. Global coordinates: (N 19°35'.820; W 96°22'.550), 2 m above sea level.

Type specimens. Slides with holotype female (CNHE12440), allotype male (CNHE12441), and paratype females (CNHE12442) were deposited in the Laboratorio de Helmintología del Instituto de Biología, UNAM, Mexico. Other paratype materials in the Colegio de Postgraduados Nematode Collection (A-130).

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Haliplectus mexicanus sp. n. belongs to group II of the genus: species with bipartite basal bulb. The new species is similar to *H. minor* Shahina, Siddiqi & Nasira, 2014, and *H. minimus* Gerlach, 1967, in having body length less than 500 µm, pharyngeal basal bulb spherical shape bipartite with transverse separation, index c, position of vulva and stoma without denticles. It differs from the first species by index a 14 (10.9–16.5) vs 17.5–21.5 and tail length 31.7 (21–38) vs 40–47 µm. It differs from the second species in the index c 13.2 (9.1–16.7) vs 10.7 and by the soil habit vs marine. *Haliplectus mexicanus* sp. n. is also similar to the species with bipartite pharyngeal basal bulb *H. mayor* Zhou et al., 2022, and *H. iranicus* Gharahkhani et al., 2022, but differ from these species in females in length of the body 400 ± 5.0 (340–500) µm long vs 1726 (1998) and 602 (546–707) µm long, respectively, and by the tail length 31.7 (21–38) vs 91–106 and 57 (54–63) µm long, respectively. It differs from *H. futianisus* Zhou et al., 2022, by pharyngeal basal bulb unstriated vs striated bipartite and body length of females 0.41 (0.35–0.50) vs 0.564–0.655 µm and tail length 30.6 ± 5.0 (21–38) vs 46–52 µm. *Haliplectus mexicanus* sp. n. also differs to *H. dorsalis* Chitwood, 1956, in the V% 51.8 (46.9–58.1) vs 44–46% and tail length, 31.7

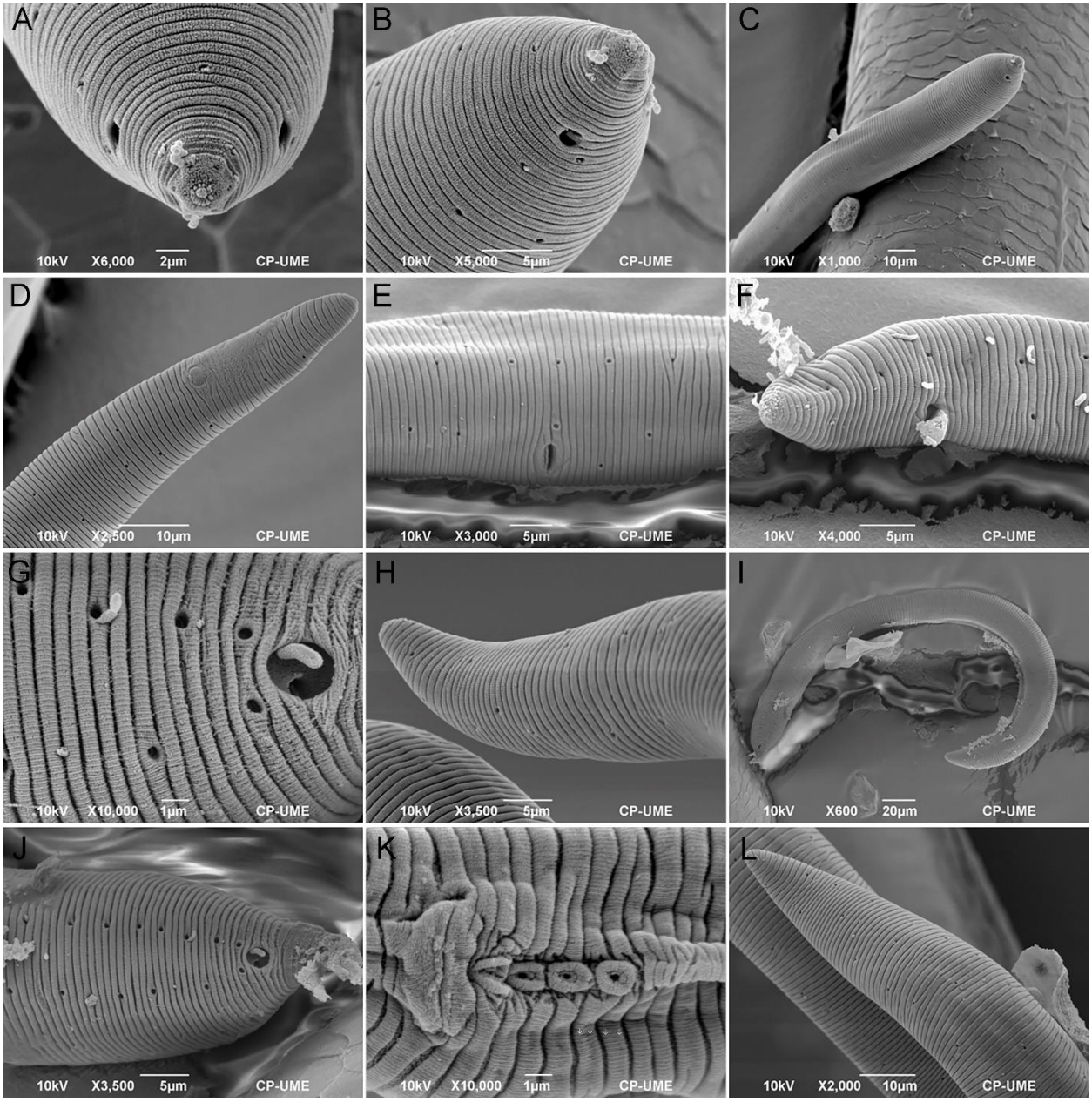

Figure. 3. *Haliplectus mexicanus* sp. n. Scanning electron photomicrographs. Female – A: Anterior end frontal view; B: Anterior end, lateral view; C: Cervical area of body; D: Posterior end body, anus, and tail; E: Vulva ventral view; F: Posterior end body: tail and anus; G: Amphid and body pores, lateral view; H: Posterior end body, dorsal view. Male – I: Whole body; J: Amphid and body pores, lateral view; K: Genital papillae or supplements, ventral view; L: Posterior end body, ventral view.

Molecular characterisation and phylogenetic position of the new species within the genus

18S rRNA gene. Two 18S rRNA gene alignments were generated. The first alignment included sequences from the 5′-end of 18S rRNA gene for 24 *Haliplectus* and two outgroup taxa accessions and was 813 bp in length. The second alignment included sequences from the 3′-end of *18S* rRNA gene for 21 *Haliplectus* and two outgroup taxa accessions and was 792 bp in length. Phylogenetic relationships of *H. mexicanus* sp. n. with other *Haliplectus* species as inferred from the analysis of this gene are given in Figure 4. The

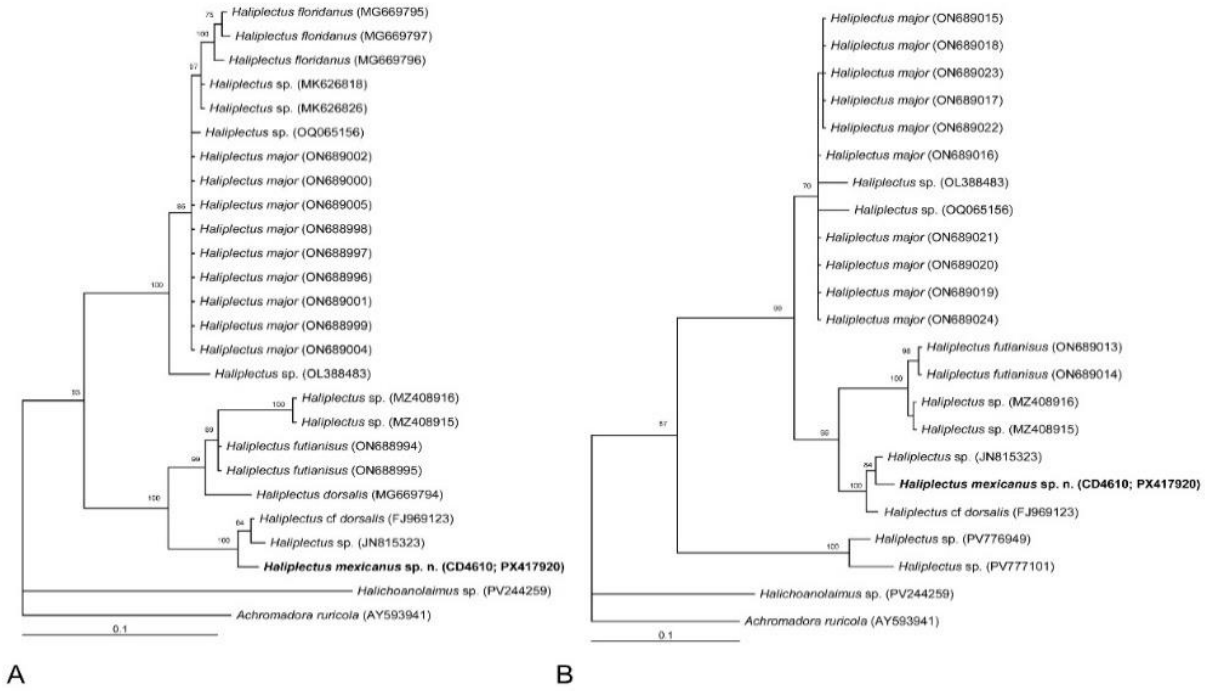

Figure 4. Phylogenetic relationships within the genus *Haliplectus* inferred from Bayesian analysis using the partial 18S rRNA gene sequences (A: 5′-end of 18S rRNA gene, nchar = 813; B: 3′- end of 18S rRNA gene, nchar = 792) under the GTR + I + G model. Posterior probability values more than 70% are shown near the corresponding nodes. Sequences generated in this study are indicated in bold.

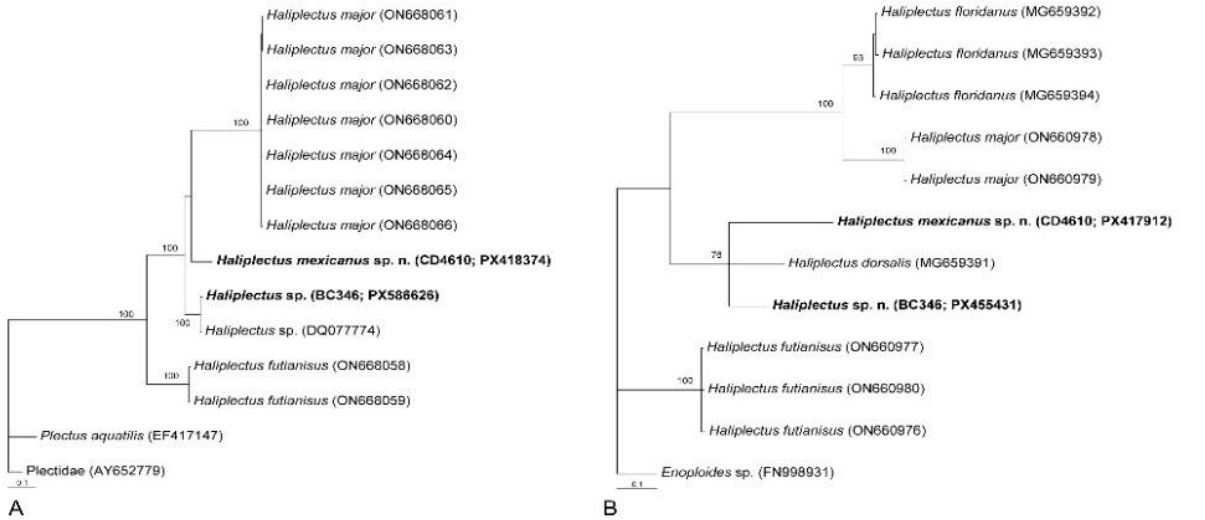

Figure 5. Phylogenetic relationships within the genus *Haliplectus* inferred from Bayesian analysis using (A) D2–D3 expansion segments of the 28S rRNA gene sequences and (B) partial cytochrome c oxidase subunit I (*COI*) gene sequences under the GTR + I + G model. Posterior probability values more than 70% are shown near the corresponding nodes. Sequences generated in this study are indicated in bold.

sequence of *Haliplectus mexicanus* sp. n. formed a highly supported clade with those of *Haliplectus* sp. (JN815323) from Pomponio State Beach, California, and *Haliplectus cf. dorsalis* (FJ969123) from unknown location and differed from them by 1.4%, 0.7%, and 0.8%, 1.2%, respectively.

The D2-D3 of 28S rRNA gene. The alignment contained 12 sequences of *Haliplectus* and two sequences of outgroup taxa and was 770 bp in length. Phylogenetic relationships of *H. mexicanus* sp. n. with other *Haliplectus* species as inferred from the analysis of this gene are given in Figure 5A. The sequence of *Haliplectus mexicanus* sp. n. differed from that of *Haliplectus* sp. from Pomponio State Beach (DQ77774) and Dillon Beach (PX586626), California by 13.0%. Sequences of two *Haliplectus* sp. from California were identical.

COI gene. The alignment contained 11 sequences of *Haliplectus* and one sequence of outgroup taxon and was 333 bp in length. Phylogenetic relationships of *H. mexicanus* sp. n. with other *Haliplectus* species as inferred from the analysis of this gene are given in Figure 5B. The sequence of *Haliplectus mexicanus* sp. n. differed from that of *H. dorsalis* (MG659391) from Viet Nam by 21.6% and from that of *Haliplectus* sp. from Dillon Beach (PX455431), California by 18.6%.

DISCUSSION

Haliplectus mexicanus sp. n. represents a distinct addition to the genus *Haliplectus*, distinguished by its combination of unique morphological and morphometric features across females, males, and juveniles, including its short conical tail, conspicuously ornamented cuticle, and characteristic cephalic and pharyngeal structures. The diagnostic traits of the reproductive systems and male supplementary structures further support its taxonomic separation from known species. Molecular analyses based on 18S, D2-D3 of *28S* rRNA, and *COI* gene sequences corroborate the morphological evidence, confirming the phylogenetic placement of *H. mexicanus* sp. n. and clarifying its relationships with *Haliplectus* species, including the unidentified *Haliplectus* sp. from California. Together, these results enhance our understanding of species diversity within *Haliplectus* and contribute to a more resolved framework for future taxonomic and phylogenetic studies of the group.

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