Two species of Thoracostomopsidae (Nematoda: Enoplida) from Jeju Island, South Korea

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During a survey of intertidal zones at beaches on Jeju Island, two species belonging to the family Thoracostomopsidae were discovered. One new species, *Enoploides koreanus* sp. nov. and one known species, *Epacanthion hirsutum* Shi & Xu, 2016 are reported. Along with morphological analysis, mitochondrial cytochrome oxidase c subunit 1 (mtCOI) sequences and 18S rRNA sequences of the species were also obtained and used to check relative p-distance and phylogenetic positions. While most species of *Enoploides* have long spicules, the new species belongs to a group of *Enoploides* with short spicules (< 150 μm). Of the seven species with short spicules, the new species is most closely related to *E. disparilis* Sergeeva, 1974. They both have similar body length, fairly similar sized and shaped spicules with small gubernaculum running parallel to distal end of spicule, and index value of b. The new species can be distinguished from *E. disparilis* by having pre-anal supplementary organ with short conical tail, while *E. disparilis* lacks pre-anal supplementary organ and has a long conico-cylindrical tail. Along with the description of the new species, the genus *Enoploides* Ssaveljev, 1912 is bibliographically reviewed and revised. Of 45 species described to date, 27 are now considered valid, 16 species inquirendae due to inadequate descriptions and ambiguity of the material examined, along with two cases of nomen nudum. With this review, we provide an updated diagnosis and list of valid species, a tabular key comparing diagnostic characters of all valid species, and a new complete key to species. One known species, *Epacanthion hirsutum* Shi & Xu, 2016, is reported in Korea for the first time. The morphology agrees well with the original description provided by Shi & Xu (2016). As they had already reviewed the genus at the time of reporting four *Epacanthion* species, we provide only a description, depiction, and measurements for comparison purposes.

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2 **from Jeju Island, South Korea**

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Abstract

During a survey of intertidal zones at beaches on Jeju Island, two species belonging to the family Thoracostomopsidae were discovered. One new species, *Enoploides koreanus* sp. nov. and one known species, *Epacanthion hirsutum* Shi & Xu, 2016 are reported. Along with morphological analysis, mitochondrial cytochrome oxidase c subunit 1 (mtCOI) sequences and 18S rRNA sequences of the species were also obtained and used to check relative p-distance and phylogenetic positions. While most species of *Enoploides* have long spicules, the new species belongs to a group of *Enoploides* with short spicules (< 150 μm). Of the seven species with short spicules, the new species is most closely related to *E. disparilis* Sergeeva, 1974. They both have similar body length, fairly similar sized and shaped spicules with small gubernaculum running parallel to distal end of spicule, and index value of b. The new species can be distinguished from *E. disparilis* by having pre-anal supplementary organ with short conical tail, while *E. disparilis* lacks pre-anal supplementary organ and has a long conico-cylindrical tail. Along with the description of the new species, the genus *Enoploides* Ssaveljev, 1912 is bibliographically reviewed and revised. Of 45 species described to date, 27 are now considered valid, 16 species inquirendae due to inadequate descriptions and ambiguity of the material examined, along with two cases of nomen nudum. With this review, we provide an updated diagnosis and list of valid species, a tabular key comparing diagnostic characters of all valid species, and a new complete key to species. One known species, *Epacanthion hirsutum* Shi & Xu, 2016, is reported in Korea for the first time. The morphology agrees well with the original description provided by Shi & Xu (2016). As they had already reviewed the genus at the time of reporting four *Epacanthion* species, we provide only a description, depiction, and measurements for comparison purposes.

Introduction

The family Thoracostomopsidae Filipjev, 1927 consists of three subfamilies: Thoracostomopsinae Filipjev, 1927 (2 genera), Trileptiinae Gerlach & Riemann, 1974 (1 genus), and Enoplolaiminae De Coninck, 1965 (19 genera). They are distinguished by the presence or absence of mandibles (Enoplolaiminae or Trileptiinae respectively) or by the presence of a long and eversible spear (Thoracostomopsinae). *Enoploides* Ssaweljev, 1912, belonging to Enoplolaiminae, was first erected with type species *Enoploides typicus* Ssaweljev, 1912 from Russia. The genus is characterized by its high lips with striation; Y-shaped mandibles consisting of two lateral bars converging into one solid bar with a claw-like distal end, curving inwards to the lumen; onchia that are usually shorter than the mandibles; and spicules that are typically long armed with gubernaculum. Multiple revisions and updates of the genus have taken place. The most notable revision was made by Wieser & Hopper (1967), who questioned validity of many existing species. They argued that classification in this genus is only possible according to male genital armature (gubernaculum), and that all description based only on females and juveniles be considered species inquirenda. Aside from this, species have continuously been transferred from and to closely related genera such as *Enoplus* Dujardin, 1845; *Enoplolaimus* de Man, 1893 and *Epacanthion* Wieser, 1953. Most recently, Smol et al. (2014) listed 28 valid species including

59 three transferred from *Epacanthion* by Greenslade & Nicholas (1991). By the number, it is clear
60 that Smol et al. (2014) considered and applied the list of species inquirendae provided by Wieser
61 & Hopper (1967), but a full list of species was not supplied. A species list provided by NeMys
62 (Bezerra et al., 2019) still lists some of the species considered invalid by Wieser & Hopper
63 (1967) as valid species; contains erroneous species such as "*Enoploides uniformis* Pavljuk,
64 1984" (discussed further in results); and is missing two of three species transferred from
65 *Epacanthion* by Greenslade & Nicholas (1991). Our most recent list of valid species consists of
66 27 species, including the new species being reported. The last report on *Enoploides* species dates
67 back to 1993, with a new report on *Enoploides stewarti* Nicholas, 1993 from a freshwater lake in
68 South Australia. Most species of the genus are from marine habitats with the exception of two
69 freshwater species (*Enoploides fluviatilis* Micoletzky, 1923 and *E. stewarti*). Of the 27 valid
70 species, 63% (17) were initially reported from Europe; 14.8% (4) from North America; 7.4% (2)
71 from Asia (including the new species); with 3.7% (1) each from South America, Africa,
72 Australia and the Arctic.
73   The aim of this study was to review and revise the genus *Enoploides* while reporting a
74 new species, *Enoploides koreanus* sp. nov., found from Jeju Island, Korea. *Epacanthion*
75 *hirsutum* originally reported from East China Sea, is also reported in Korea for the first time.
76 Their respective 18S rRNA and COI genes were sequenced and used to check p-distances and
77 phylogenetic positions. We also agree that all future description of the genus be from a sound
78 male as first proposed by Wieser & Hopper (1967).
79

Materials and Methods

1) Sampling and morphological study

82 Three seemingly natural and undisturbed beaches of Jeju Island were sampled in September 11,
83 2018. Two sub-samples of sediment from the intertidal zone were obtained qualitatively using a
84 mini-shovel. Of the subsamples, one was fixed in 5% neutralized formalin solution for
85 morphological analysis and the other was fixed in 70% ethanol for molecular analysis. Samples
86 were brought back to the laboratory and meiofauna were extracted using the Ludox method
87 (Burgess, 2001). Individual specimens were transferred by hand to a Petri dish filled with 10%
88 glycerin. Specimen-containing Petri dishes were placed for a day in a dry oven preset to 40°C for
89 a day to achieve complete dehydration as described by Seinhorst (1959) with the glycerin-
90 ethanol method. A single specimen was mounted in a drop of glycerin on a slide glass as
91 conferred in the wax-ring method (Hooper, 1986). Specimens were examined and identified
92 using Olympus BX51 and Leica DM2500 microscopes. For scanning electron microscopy,
93 specimens were removed from the slide glass and placed in a drop of glycerin. Drops of distilled
94 water were added gradually to the drop of glycerin to rehydrate the specimen. Hydrated
95 specimens underwent ethanol series for dehydration (20%, 40%, 50%, 70%, 80%, 90%, 95%,
96 100%, for 10 min each) to be placed in hexamethyldisilazane (HMDS), with slightly altered
97 concentration and duration compared to a method used by Phillips et al. (2016). A pool of
98 HMDS containing the specimen was placed in drying oven to be completely dried overnight.

99   Dried specimens were mounted on a stub to be sputter-coated, then observed with a COXEM
100 EM-30 scanning electron microscope.

101 **2) DNA extraction and amplification**

102 Each specimen of interest was dissected into head, body, and tail. Heads and tails were retained
103 for morphological analysis and made into permanent slides following Hooper's (1986) wax-ring
104 method. The slides were submitted to the National Institute of Biological Resources
105 (NIBR, Korea). Bodies of each specimen were transferred to a well of distilled water for 20 min
106 to be washed of any remaining ethanol. Washed bodies were moved to individual tubes
107 containing 25 µl of worm lysis buffer, prepared prior to extraction following Williams et al.
108 (1992). The tubes were then placed in PCR-thermo cycler (Takara, Japan) preset to 65°C for 15
109 min, 95°C for 20 min, and 15°C for 2 min. Two gene loci commonly used for marine nematodes
110 were sequenced: mitochondrial cytochrome oxidase C subunit I (COI) gene and 18S small
111 subunit ribosomal rRNA. All genes were amplified using PCR premix (Bioneer Co., Daejeon,
112 Korea) with 5 µl DNA template, 15 µl distilled water, 1 µl of each primer. COI genes were
113 amplified using primer sets (JB3/JB5) amplifying approximately 300 base pairs (bp) as described
114 by Derycke et al., (2010). PCR cycling conditions were: 94°C for 5 min, 35 cycles of (94°C for
115 30 s; 50°C for 30 s; 72°C for 30 s), and 72°C for 10 min. 18S rRNA was amplified using primer
116 sets (MN18F/22R), amplifying approximately 300 bp. PCR cycling conditions were: 95°C for 5
117 min, 37 cycles of (95°C for 30 s, 56°C for 1 min, 72°C for 1 min 30 s), followed by 72°C for 5
118 min, as described by Bhadury et al. (2006). Success of amplification was determined by
119 electrophoresis on 1% agarose gel. If amplification was successful, DNA templates were sent to
120 Macrogene (Korea), to be sequenced on an ABI3730XL sequencer.

121 **3) Molecular data analysis**

122 Sequenced forward and reverse strands were visually checked for signal quality using FinchTV
123 (ver. 1.4.0). Two strands were aligned with ClustalW (Thompson et al., 1994) implemented into
124 MEGA (ver. 7.0.26) (Kumar et al., 2016) with default parameters. All aligned sequences were
125 confirmed with BLAST search (Altschul et al., 1990) on GenBank to check that the sequences
126 were those of nematodes. Pairwise distances between mtCOI and 18S rRNA sequences were
127 calculated using the K2P model (Kimura, 1980) using MEGA 7. The best fit-model for 18S
128 rRNA datasets were assessed using default parameters implemented in MEGA 7.0 (Kumar et al.,
129 2016). Tamura 3-parameter model (Tamura, 1992) with gamma distribution of rates across sites
130 was found to be optimal and used with MEGA 7.0 to build a maximum likelihood (ML) tree with
131 complete deletion and 1,000 bootstrap repetition. Completed tree was exported to FigTree (ver.
132 1.4.4) (Rambaut, 2009) and visually modified. Phylogenetic tree was not constructed using the
133 obtained mtCOI sequences, as only few mtCOI sequences of *Enoploides* were available on
134 GenBank.

135 **4) Bibliographical revision of the genus**

136 The Bremerhaven Checklist of Aquatic Nematodes by Gerlach & Riemann (1974) was initially
137 used to collect original descriptions and references. Original erection of the genus, as well as
138 other previous revisions and diagnosis of the genus was checked (Wieser, 1953; Wieser &

139 Hopper, 1967; Platt & Warwick, 1983; Smol et al., 2014). Upon collecting all required
140 references: 1) validity and synonymy of species were examined and determined; 2) a table
141 comparing diagnostic characters of all valid species was compiled; 3) locality and distribution of
142 original descriptions were determined; 4) a new complete key to the genus was compiled.
143   **5) Nomenclatural Acts**
144 The electronic version of this article in Portable Document Format (PDF) will represent a
145 published work according to the International Commission on Zoological Nomenclature (ICZN),
146 and hence the new names contained in the electronic version are effectively published under that
147 Code from the electronic edition alone. This published work and the nomenclatural acts it
148 contains have been registered in ZooBank, the online registration system for the ICZN. The
149 ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed
150 through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The
151 LSID for this publication is: urn:lsid:zoobank.org:pub:6F60918D-9DE1-4B75-A251-
152 C01E0694D01F. The online version of this work is archived and available from the following
153 digital repositories: PeerJ, PubMed Central and CLOCKSS.
154
155 **Abbreviations**
156
157 a: body length / maximum body diameter
158 abd: anal body diameter
159 b: body length / pharynx length
160 c: body length / tail length
161 calc: calculated or measured from published measurements and/or figures
162 cs: cephalic setae
163 c': tail length / anal body diameter
164 ils: inner labial setae
165 ols: outer labial setae
166 scs: subcephalic setae
167
168 **Results**
169 **Systematics**
170
171 **Order Enoplida Filipjev, 1929**
172 **Family Thoracostomopsidae Filipjev, 1927**
173 **Subfamily Enoplolaiminae de Coninck, 1965**
174 **Genus Enoploides Ssaveljev, 1912**
175
176 **Generic diagnosis:** (Updated from Wieser, 1953; Wieser & Hopper, 1967; Platt & Warwick,
177 1983; Smol et al., 2014)

178 Enoplolaiminae. Lips high and striated. Buccal cavity with three well-developed solid mandibles
179 with claw-like anterior; mandible not extremely slender (ratio length/width <6); Three onchia
180 shorter than the mandibles. Some species showing sexual dimorphism with pilosity either along
181 the body or within the head region. Spicules usually long, some short, armed with either complex
182 s-shaped/simple non s-shaped gubernaculum. Some species with pre-cloacal supplementary
183 organ/papillae or postanal papillae/cuticular element of different form, at varying distances from
184 the cloacal opening. Terminal setae observed at tail tip in some species. Mostly marine, with two
185 freshwater species (*E. fluviatilis* and *E. stewarti*).

186

187 Type species: *Enoploides typicus* (Ssaweljev, 1912)

188

189 **Notes on generic diagnosis:** *Enoploides* can be easily distinguished from other genera such as
190 *Enoplolaimus* de Man, 1893 and *Mesacanthion* Filipjev, 1927 by the morphology of its
191 mandibles. Mandibles of *Enoploides* are described as "solid", signifying that the two lateral bars
192 converge/fuse together to form a single rod for most of its length. This means that from lateral
193 view, the mandible resembles the letter Y, with its distal end of two lateral bars claw-like,
194 curving inward to the lumen (Fig. 1A). *Enoploides* are sometimes mistaken for *Epacanthion*
195 Wieser, 1953 (a closely related genus), and vice versa. Mandibles of *Epacanthion* consist of two
196 lateral bars separated by a thin sheet of cuticles, meaning the space between bars is not solid as
197 in *Enoploides* (Fig. 1B). This subtle difference is significant enough to separate the two genera,
198 yet easily missed in many diagnoses. Greenslade & Nicholas (1991) transferred three species
199 previously regarded as members of *Epacanthion* to the genus *Enoploides* (*E. crassum*, *E.*
200 *filicaudatum* and *E. incurvatus*) after examining their mandibles.

201

202 **List of valid species**

203

204 1. ***Enoploides amphioxi* Filipjev, 1918** (Filipjev, 1918: 92–92, Table 2, 3, fig. 12A–E; one
205   male and several females, Sevastopol, Black Sea, Russia. Stekhoven, 1950: 52, fig. 19A–C;
206   one female, Mediterranean, Villefranche, grey mud, 80 m, deep).
207 2. ***Enoploides bisulcus* Wieser & Hopper, 1967** (Wieser & Hopper, 1967: 252–253, fig. 1i,
208   10A–D; description based on several males and females, Key Biscayne, Florida, USA,
209   shallow water close to submerged patch, fine sand and debris).
210 3. ***Enoploides brunettii* Gerlach, 1953** (Gerlach, 1953: 527–529, Abb. 4, fig. 4A–E;
211   description based on two males and one female, Mediterranean. Warwick, 1971: 444–451;
212   Exe estuary, England).
213 4. ***Enoploides caspersi* Riemann, 1966** (Riemann, 1966: 186–188, Abb. 49A–F; description
214   based on three male and one female, Elbe estuary, North Sea, Germany).
215 5. ***Enoploides cephalophorus* (Ditlevsen, 1918) Filipjev, 1927** [Ditlevsen, 1918: 207–208, Pl.
216   14, fig. 1, 5, 6, Pl. 15, fig. 1; (=*Enoplolaimus cephalophorus*), description based on several
217   males and females, Limfjord, off Snoghøj, off Hellebæk, Denmark. Filipjev, 1927: 142; (as

218   *Enoploides cephalophorus*), several males and females, Kara Sea, yellow sand, 20 m deep.
219   Allgén, 1946: 5; (as *Enoplolaimus* (*Enoploides*) *cephalophorus* Ditlevsen), several males
220   and females, Norway].
221   6. ***Enoploides cirrhatus* Filipjev, 1918** [Filipjev, 1918: 101–103, Table 3, fig. 15A–D;
222   description based on one male, Shimit Bay, Sevastopol, Black Sea, Russia, saccocirrous
223   sand. Allgén, 1940: 263, fig. 1A–C; (as *Enoplolaimus* (*Enoploides*) *cirrhatus* Filipjev),
224   Norway].
225   7. ***Enoploides crassum* (Ditlevsen, 1926) Greenslade & Nicholas, 1991** [Ditlevsen, 1926:
226   39–40, Pl. 15, figs. 3, 6, 7, 8; (=*Enoplolaimus crassus*), description based on one male and
227   several females, Iceland, Faroe Island, Jan Mayen. Wieser, 1953: 79; (=*Epacanthion*
228   *crassus*), transfers the species to the genus *Epacanthion*. Greenslade & Nicholas, 1991:
229   1041; (=*Enoploides crassus*), transfers the species to the genus *Enoploides*, explaining that
230   the original drawing and description of the mandible does not agree with character of
231   *Epacanthion*. Smol et al., 2014: 202; lapsus *crassum*].
232   8. ***Enoploides delamarei* Boucher, 1977** (Boucher, 1977: 746–748, fig. 6A–G; description
233   based on three males, four females and three juveniles, Pierre Noire (Western Channel),
234   France, infralittoral fine sands).
235   9. ***Enoploides disparilis* Sergeeva, 1974** (Sergeeva, 1974: 122, fig. 2A–B; description based
236   on one male, Black Sea, fine sand, 10m deep).
237   10. ***Enoploides fluviatilis* Micoletzky, 1923** [Micoletzky, 1923: 13–15, fig. 1A–C; description
238   based on four males, five females and three juveniles, Volga river (freshwater), Russia.
239   Also from brackish-water Caspian Sea (ca 13‰) – data of Tchesunov, A.V.].
240   11. ***Enoploides gryphus* Wieser & Hopper, 1967** (Wieser & Hopper, 1967: 235, Pl. 3, fig.
241   11C, Pl. 5, fig. 11A–B; description based on several males, Virginia Key, Florida, USA).
242   12. ***Enoploides harpax* Wieser, 1959** (Wieser, 1959: 21–22, fig. 16A–C; description based on
243   one male and one female, Alki Point, Golden Gardens, Richmond Beach, Seattle
244   Washington, USA).
245   13. ***Enoploides hirsutus* Filipjev, 1918** (Filipjev, 1918: 97–100, Table 3, fig. 13; description
246   based on one male, Sevasotopol, Black Sea, Russia, mud).
247   14. ***Enoploides incurvatus* (Ditlevsen, 1926) Greenslade & Nicholas, 1991** [Ditlevsen, 1926:
248   37, Pl. 14, figs. 4, 5, 8, 9, Pl. 15, fig. 5; (=*Enoplolaimus incurvatus*), description based on
249   male and female, Hanstholm, Denmark. Schuurmans Stekhoven, 1946: 44, fig. 13 A–O;
250   (=*Enoploides incurvatus*), two males, two females and one juvenile, Langesundsfjord,
251   Skageraks, Sweden, rocky bottom, 150–200 m deep. Wieser, 1953: 79; (=*Epacanthion*
252   *incurvatus*). Greenslade & Nicholas, 1991: 1034; mentioned that Schuurmans Stekhoven's
253   (1946) redescription was of different species, renames the species *Epacanthion stekhoveni*,
254   on account of a space between the mandibular columns].
255   15. ***Enoploides labiatus* (Bütschli, 1874) Filipjev, 1918** [Bütschli, 1874: 41, fig. 36A–B;
256   (=*Enoplus labiatus*), description based on female, North Sea. Filipjev, 1918: 91;
257   (=*Enoploides labiatus*). Wieser, 1953: 87; the species is considered as *Enoploides*, but also

258   synonymous with *E. spiculohamatus* Schulz, 1932. Wieser & Hopper, 1967: 251; mentions
259   the species as a doubtful species and adds that synonymy of *E. labiatus* and *E.*
260   *spiculohamatus* cannot be proven and thus should be abandoned. Bouwman, 1981: 63, fig.
261   26; (=*Enoploides cf labiatus* Bütschli, 1874), 6 males, 10 females and dozens of juveniles,
262   the Ems Estuary, mentions that there is high probability that *E. labiatus* is synonymous with
263   *E. spiculohamatus*].

264   16. ***Enoploides labrostriatus* (Southern, 1914) Filipjev, 1921** [Southern, 1914: 53–54, Pl. 8,
265   fig. 24A–F; (=*Enoplus labrostriatus*), description based on males, females and juveniles,
266   Clew Bay, Ireland, sand and shells, 44m deep (converted from fathoms). Filipjev, 1921:
267   565–567; transfers the species to the genus *Enoploides*. Filipjev, 1927: 141; acknowledges
268   the species as the genus *Enoploides*].

269   17. ***Enoploides longispiculosus* Vitiello, 1967** (Vitiello, 1967: 407–410, fig. 3A–G; description
270   based on two males, one female and two juveniles, English Channel).

271   18. ***Enoploides mandibularis* Coles, 1977** (Coles, 1977: 25–27, fig. 8A–C; description based
272   on seven males and ten females; Saldanha Bay, False Bay, South Africa. The mandible is
273   described as "solid" and with broad central expansion on the side facing the buccal cavity.
274   The original figures on the mandibles raise some questions regarding its placement in the
275   genus. Types will have to be examined for further determination).

276   19. ***Enoploides polysetosus* Jensen, 1986** (Jensen, 1986: 93–94, fig. 1A–G; description based
277   on seven males, seven females and thirteen juveniles, East Flower Garden, NW Gulf of
278   Mexico).

279   20. ***Enoploides ponticus* Sergeeva, 1974** (Sergeeva, 1974: 122, fig. 3A–B; description based
280   on one male, Black Sea, silt, 82m deep).

281   21. ***Enoploides rimiformis* Pavljuk, 1984** [Pavljuk, 1984: 1145–1146, fig. 1D–Z (in Russian
282   alphabet); description based on males and females. Sea of Japan (East Sea), sand].

283   22. ***Enoploides spiculohamatus* Schulz, 1932** (Schulz, 1932: 341–344, fig. 5A–K; description
284   based on males and female, Kiel Bay. Benwell, 1981: 177–181, fig. 1A–D; two males and
285   two juveniles examined, Scotland, states that Bresslau & Stekhoven (1940) may be *E.*
286   *spiculohamatus*, but the description is poor; also states that Stekhoven (1935) is not *E.*
287   *spiculomahatus*, but lacks details to consider it a new species).

288   23. ***Enoploides stewarti* Nicholas, 1993** (Nicholas, 1993: 167–170, fig. 2E–F, 3A–E;
289   description based on several males and females, Lake Alexandrina, South Australia, sand at
290   water edge of freshwater).

291   24. ***Enoploides typicus* Ssaweljev, 1912** (Ssaweljev, 1912: 115; description based on one male,
292   no depiction, Kolafjord, Russia, mud, 70–80m deep).

293   25. ***Enoploides tyrrhenicus* Brunetti, 1949** (Brunetti, 1949: 42–44; Mediterranean. Gerlach,
294   1953: 526–527, fig. 3A–E; three males, Mediterranean).

295   26. ***Enoploides vectis* Gerlach, 1957** [Gerlach, 1957: 426, fig. 4C–G; (=*Enoploides brunettii*
296   var. *vectis* var. n.), description based on a male, Rio de Janeiro, Brazil, middle sand. Wieser
297   & Hopper, 1967: 252; raised to species level].

298

Species inquirendae

1. *Enoploides brattstroemi* Wieser, 1953 (Wieser, 1953: 88–89, fig. 47A–B; description based on two juveniles, Gulf of Corcovado and Boca del Guafo, Chile, littoral and sublittoral, sheltered algae and coarse bottom, 25 m deep, lapsus *brattströmi*. Wieser & Hopper, 1967: 251; argues that classification within this genus is only possible using the male genital armature, and classifies any existing descriptions based on only juveniles and females as *species inquirendae*).

2. *Enoploides brevis* Filipjev, 1918 (Filipjev, 1918: 100–101, Table 3, fig. 14; description based on immature female, Sevastopol, Black Sea, Russia. Wieser & Hopper, 1967: 251; argues that classification within this genus is only possible using the male genital armature, and classifies any existing descriptions based on only juveniles and females as *species inquirendae*).

3. *Enoploides filicaudatum* (Mawson, 1956) Greenslade & Nicholas, 1991 [Mawson, 1956: 64, fig. 27A–B; (=*Epacanthion Filicaudatum*), description based on two juveniles, Antarctic. Greenslad & Nicholas, 1991: 1041, fig. 6; transfers the species to the genus *Enoploides* after examining a juvenile kept in South Australian Museum collection (SAMA V3267), which they designated as lectotype. According to them, the mandibles are distinctly solid with mandibular rods joined medially, fitting of mandibles found in the genus *Enoploides*. This species is placed as species inquirenda due to the following reasons: 1) The original description was based on two juveniles, which Greenslade & Nicholas (1991) later could only locate one. We agree with Wieser & Hopper (1967) whom insisted descriptions based on only females and juveniles are insufficient to distinguish between species; 2) While Greenslade & Nicholas (1991) confirmed that the mandibles of this species resemble ones found in the genus *Enoploides*, it is still difficult to distinguish it from other species within the genus. For instance, its species defining characteristic as according to Mawson (1956) is for its tail shape. This characteristic alone is too ambiguous to define a species].

4. *Enoploides italicus* (Steiner, 1921) Filipjev, 1918 [Steiner, 1921: 54, fig. A[1]; (=*Enoplolaimus italicus*), no locality, no measurements. Filipjev, 1927: 141; (=*Enoploides italicus*). Wieser, 1953: 88; mentions the species being doubtful, reasoning original description only being provided with a figure of a head with 12 setae without description].

5. *Enoploides kerguelensis* Mawson, 1958 (Mawson, 1958: 345, fig. 27A–C; description based on one female and three juveniles, Kerguelen Island, Antarctica. Wieser & Hopper, 1967: 252; argues that classification within this genus is only possible using the male genital armature, and classifies any existing descriptions based on only juveniles and females as *species inquirendae*).

6. *Enoploides longicaudatus* Wieser, 1953 (Wieser, 1953: 91, fig. 51A–B; description based on two juveniles, Golfo de Ancud, Chile, coarse sand, small stones and a few boulders,

338   much detritus, 30–40 m deep. Wieser & Hopper, 1967: 252; argues that classification
339   within this genus is only possible using the male genital armature, and classifies any
340   existing descriptions based on only juveniles and females as *species inquirendae*).

341   7. *Enoploides longisetosus* Schuurmans Stekhoven, 1943 (Schuurmans Stekhoven, 1943:
342   338–339, fig. 10A–B; description based on a juvenile, Chile. Wieser, 1953: 88; mentions
343   that the species is doubtful because only one juvenile is known and cervical and body
344   setae are very long. Based on the fact that the description is based on one juvenile, the
345   species is considered species inquirenda).

346   8. *Enoploides macrochaetus* (Allgén, 1929) De Coninck & Schuurmans Stekhoven, 1933
347   (species inquirenda) [Allgén, 1929: 15–16, fig. 5A–B; (=*Enoplolaimus macrochaetus*),
348   description based on a juvenile, Skagerrak, Sweden. Allgén, 1953: 555; transfers the
349   species to the genus *Enoploides*. De Coninck & Schuurmans Stekhoven, 1933: 39; labels
350   it a doubtful species due to the original description lacking sufficient figures].

351   9. *Enoploides oligochaetus* Mawson, 1956 (Mawson, 1956: 67–68, fig. 31–33; description
352   based on three juvenile females, Antarctica, no mud, 163 m deep. Wieser & Hopper,
353   1967: 252; argues that classification within this genus is only possible using the male
354   genital armature, and classifies any existing descriptions based on only juveniles and
355   females as *species inquirendae*, lapsus *oligotricha*).

356   10. *Enoploides paralabiatus* Wieser, 1953 (Wieser, 1953: 89, fig. 49A–C; description based
357   on four juveniles and three females, Seno Reloncavi, Chile, sand and mud with terrestrial
358   plant detritus. Wieser & Hopper, 1967: 252; argues that classification within this genus is
359   only possible using the male genital armature, and classifies any existing descriptions
360   based on only juveniles and females as *species inquirendae*).

361   11. *Enoploides pterognathus* Mawson, 1956 (Mawson, 1956; 68–69, fig. 32A–B; description
362   based on juvenile females, Antarctica. Wieser & Hopper, 1967: 252; argues that
363   classification within this genus is only possible using the male genital armature, and
364   classifies any existing descriptions based on only juveniles and females as *species*
365   *inquirendae*).

366   12. *Enoploides reductus* Wieser, 1953 (Wieser, 1953: 91, fig. 50A–B; description based on
367   one juvenile, Golfo Corcovado and Boca del Guafo, Chile, coarse sand with some stones,
368   25 m deep. Wieser & Hopper, 1967: 252; argues that classification within this genus is
369   only possible using the male genital armature, and classifies any existing descriptions
370   based on only juveniles and females as *species inquirendae*).

371   13. *Enoploides sabulicola* (Allgen, 1933) Wieser, 1953 [Allgen, 1933: 24–25, fig. 8A–B;
372   (=*Enoplolaimus sabulicola*), description based on one juvenile, Norway. Wieser, 1953:
373   88; transfers the species to the genus *Enoploides*, but lists it as a doubtful species, likely
374   due to the original description being based on a single juvenile and insufficient
375   description].

376   14. *Enoploides suecicus* De Coninck & Stekhoven, 1933 [Allgén, 1929: 13–14, fig. 3A–B;
377   (=*Enoplolaimus savaljevi*), description based on one juvenile, Sweden. De Coninck &

378   Stekhoven, 1933: 25; transfers the species to the genus *Enoploides*. Wieser, 1953: 88;
379   lists *Enoplolaimus saveljevi* as synonymous to *E. balticus* Stekhoven, 1935, while listing
380   *E. balticus* as a doubtful species. Also mentions they are most likely juveniles of *E.*
381   *labiatus*. De Coninck & Stekhoven, 1933: 25; nomen novum *Enoploides suecicus* nom.
382   nov. for *Enoplolaimus saveljevi*].
383   15. *Enoploides tridentatus* Ssaweljev, 1912 (Ssaweljev, 1912: 116; description based on one
384   female, no depiction, Kolafjord, Russia. Wieser & Hopper, 1967: 252; argues that
385   classification within this genus is only possible using the male genital armature, and
386   classifies any existing descriptions based on only juveniles and females as *species*
387   *inquirendae*).
388
389   **Nomen nudum**
390
391   1. *Enoploides tyrannis* Bussau, 1993 (nomen nudum) (Bussau, 1993: 465–469, fig. 198A–
392   C, 199A–D; description based on one male and two females. Bussau, 1993 is considered
393   nomen nudum as it does not conform to Article 13 of ICZN (1999).
394   2. *Enoploides uniformis* Pavljuk, 1984 [Pavljuk, 1984: 1145–1146, fig. 1D–Z (in Russian
395   alphabet); (=*Enoploides rimiformis*), description based on males and females. Sea of
396   Japan (East Sea), sand. This species name is an accepted name on NeMys, however,
397   Pavljuk, 1984 does not include a description of a species with such name. It is likely a
398   genuine mistake confusing the name of *rimiformis* as *uniformis*].
399
400   ***Enoploides koreanus* sp. nov.**
401   Figs. 1A, 2, 3, Table 1
402   urn:lsid:zoobank.org:act:87CC02F7-3A2E-4137-84E5-C8E7A97DA6D2
403
404   **Description:** Males (Fig. 2; holotype n=1, paratype n=2). Cuticle smooth above cephalic
405   capsule, strongly striated below cephalic capsule until tail tip (Fig. 4A, B). Three lips high with
406   its border striated heavily with grooves, each lip with two inner labial setae. Six inner labial
407   setae, long and thin (11 μm long), at base of lips in one crown. Six longer outer labial setae (43
408   μm long) and four shorter cephalic setae (15 μm long) in one crown, situated at anterior portion
409   of cephalic capsule. ~20 subcephalic/cervical setae immediately after, some near second crown
410   of setae near outer labial and cephalic setae, some near region of cephalic capsule end, in random
411   lengths, some short, some as long as cephalic setae (Fig. 4A). Buccal cavity short and funnel
412   shaped, wide at the anterior end, narrowing gradually towards the base. Buccal cavity armed
413   with three equally sized and shaped "solid" mandibles and teeth. Mandible Y-shaped, two lateral
414   bars converging into one solid bar, with distal end of each lateral bars claw-like, curving inwards
415   to the lumen. Three onchia of equal size, posterior to each base of mandibles. Amphid not
416   observed. Somatic setae irregularly scattered along the cervical region, in random lengths.
417   Pilosity denser from level of buccal cavity until the nerve ring region. Pharynx long with grooves

418   and sinuous external contours. Cardia inverse triangular shaped seemingly embedded into the
419   intestine. Somatic setae sparsely distributed along the body in singles until tail region.
420   Metanemes not observed. Testes paired and opposed, anterior testis slightly right of the intestine
421   and posterior testis also to right of the intestine. Precloacal supplementary organ, 8 μm long, 82
422   μm above cloacal opening, roughly 2.6 anal body diameters above the anus. Spicules paired,
423   simple, thin and curved at an obtuse angle, proximal end with a knob (more distinct in some
424   specimens than others) and distal end blunt and rounded. Spicule width equivalent throughout its
425   length. Gubernaculum simple, rod-like shape, running parallel to distal half of spicule from level
426   of spicule curvature to its distal end. Distal end with slightly rounded head (Fig. 4C). Tail region
427   with some somatic setae in singles with no patterns observed. Tail conical, inconspicuously
428   cylindrical at distal end. Caudal glands just below distal end of the spicules, at level of anus,
429   running until a well-developed spinneret. Several caudal setae observed (Fig. 4B) along the tail
430   with no terminal setae present at tail tip.
431   Female (Fig. 3; allotype n=1, paratype n=5). Female generally longer and larger in size.
432   Short sub-cephalic setae below outer labial and cephalic setae (Fig. 4D). Cervical setae in singles
433   and less frequent compared to male at posterior end of cephalic capsule. Reproductive system
434   didelphic-amphidelphic, both ovaries reflexed, positioned left of intestine (Fig. 3C). Tail region
435   with some caudal setae in singles with no visible patterns. No terminal setae observed at tail tip.
436
437   **Type locality:** Intertidal zone at coast of Shinyang Seopjikoji Beach (West), Jeju Island, South
438   Korea (33º26'05"N 126º55'15"E), in sandy beach with some algae, collected 11 September
439   2018.
440
441   **Materials examined:** All specimens deposited in the National Institute of Biological Resources
442   (South Korea). Holotype 1♂ (NIBRIV0000858255) on one slide, Allotype 1♀
443   (NIBRIV0000858256) on one slide, Paratypes 1♂, 4♀♀ all on separate slide
444   (NIBRIV0000858257–NIBRIV0000858261), 1♂ and 2♀♀ dried, mounted on two separate
445   stubs, each sex on separate stubs and coated with gold for SEM (NIBRIV0000858262,
446   NIBRIV0000858263).
447
448   **Additional materials examined:** All specimens deposited in the National Institute of Biological
449   Resources (South Korea). 1♀, and 3 juveniles all on separate slide but with only head and tails
450   retained, with segment of body used for molecular analysis. (NIBRIV0000858264–
451   NIBRIV0000858267).
452
453   **Diagnosis:** *Enoploides*. Male. Body length 1851–2307 μm. Cuticle finely striated along the
454   body, smooth only in cephalic capsule region. Six inner labial setae 11–19 μm. Six longer outer
455   labial setae 30–48 μm, four shorter cephalic setae 11–20 μm long sharing one crown. Many
456   subcephalic and cervical setae in head region in case of males, less dense in case of females.
457   Buccal cavity 18–30 μm long. Males with testis paired and opposed. Spicule short (34–39 μm),

458   thin, curved at obtuse angle, its width equal throughout the length. Distal end rounded with knob
459   on proximal end. Gubernaculum simple rod-shaped, running parallel to distal end of spicules.
460   Distal end with subtle round head. Precloacal supplementary organ present. Tail conical,
461   inconspicuously cylindrical at distal end. a=58.5–67.1, b=4.1–4.4, c=16.2–18.8, c'=3.7–4.2.
462
463   **Differential diagnosis:** This diverse genus consisting of 27 valid species can be divided into two
464   groups by the length of their spicules: 1) a group with short spicules (< 150 μm); 2) a group with
465   long spicules (>150 μm). Instead of comparing just the spicule length, it would have been ideal
466   to separate the group by spicule length/abd. Many species unfortunately lack measurement of
467   anal body diameter in their description, making this a difficult task. Total of seven species have
468   spicules shorter than 150 μm: *E. caspersi*, *E. cirrhatus*, *E. disparilis*, *E. fluviatilis*, *E. stewarti*, *E.*
469   *tyrrhenicus*, and *E. koreanus* sp. nov. When Nicholas (1993) first acknowledged this group and
470   created a key for *Enoploides* with short spicules, he included *E. polysetosus*, but even then it was
471   distinguished for having the longest spicules in the group. We defined this group to consist of
472   those bearing spicules shorter than 150 μm. *E. polysetosus* is therefore no longer considered to
473   have short spicules. Species such as *E. caspersi* and *E. tyrrhenicus* can easily be distinguished
474   from the other by the former's unique post-anal organ and the latter's complex gubernaculum. Of
475   the seven species, the new species is most closely related to *E. disparilis* in terms of general
476   morphology: 1) they both have simple and short spicules (34–39 μm vs. 35 μm) with a knob on
477   its proximal end; 2) they both have simple and short gubernaculum (12 μm vs. 19 μm) parallel to
478   distal end of the spicule; and 3) they share similar body lengths (2107–2307 μm vs. 2250 μm)
479   and index value of b (4.1–4.4 vs. 4.4). They can be differentiated from one another by the
480   following characteristics: 1) the new species has a pre-anal supplementary organ, while *E.*
481   *disparilis* does not; 2) the new species has a shorter conical tail (121–130 μm), while *E.*
482   *disparilis* has a longer conico-cylindrical tail (254 μm); 3) the new species and *E. disparilis* have
483   different index values of a (58.5–67.1 vs. 44.7) and c (16.2–18.8 vs. 9.8). Another species similar
484   to the new species is *E. cirrhatus*. which also bears a small spicule with a knobbed proximal end
485   and similarly shaped gubernaculum (adjacent to the spicules, small and curved at upper end).
486   Aside from those characteristics, the new species differ from *E. cirrhatus* in the body lengths
487   (2107–2307 μm vs. 4350 μm), index values (a=58.5–67.1 vs. 26; b=4.1–4.4 vs. 5.25) all differ
488   greatly, and *E. cirrhatus* does not have a pre-anal supplementary organ, but bear seven pairs of
489   pre-anal papillae instead.
490   Group of species with long spicules (> 150 μm) can be further divided into subgroups by
491   the morphology of the gubernaculum: 1) S-shaped gubernaculum; 2) complex gubernaculum; 3)
492   gubernacula that are short, simple, small, plated, arcuate, or weak. The term "S-shaped" has been
493   used by several authors, including Filipjev (1918), Wieser & Hopper (1967), and Pavljuk (1984),
494   to describe certain shapes of the gubernaculum. Wieser & Hopper (1967) conveniently grouped
495   species with this S-shaped gubernaculum (*E. cephalophorus*, *E. gryphus*, *E. spiculohamatus*, *E.*
496   *amphioxi*, *E. labrostriatus* and *E. bisulcus*) and provided a figure showing different gubernacula
497   of several species within the genus. The S-shape can be extremely general and almost any shape

498   can be regarded as S-shapes given the curving nature of gubernaculum's contours. For instance,
499   gubernaculum of *E. spiculohamatus* was considered S-shaped by Wieser & Hopper (1967), while
500   gubernaculum of *E. vectis* was not. Wieser & Hopper (1967) even referred to this group as "more
501   or less S-shaped" confirming its ambiguous nature. To reduce ambiguity, any gubernaculum
502   described as complex in the original description or consisting of multiple parts is not considered
503   S-shaped here. There is certainly some uniformity of morphology of these "complex"
504   gubernacula, which consist of multiple parts, bluntly shaped, anterior to the spicules. This
505   removes *E. spiculohamatus* from the S-shaped group initially assigned by Wieser & Hopper
506   (1967), as its gubernaculum consists of multiple parts and more closely resembles other
507   complex-gubernaculum bearing species such as *E. brunettii, E. labiatus, E. longispiculosus*, and
508   *E. vectis*. Refer to Table 2 for comparison of diagnostic morphological characters and
509   gubernaculum type of all valid *Enoploides* species.
510
511   **Etymology:** The species name refers to its occurrence in Korea.
512
513   **Key to all valid species of the genus *Enoploides***
514
515   1. Spicules less than 150 µm long… 2
516   -Spicules more than 150 µm long… 7
517   2. Presence of a prominent post-anal supplementary organ … *E. caspersi*
518   -Absence of a prominent post-anal supplementary organ … 3
519   3. Pre-anal supplementary organ absent … 4
520   -One pre-anal supplementary organ present … 5
521   -Seven to eight pre-anal supplementary papillae in midline … *E. cirrhatus*
522   4. index c~9, spicules ~35 µm long and gubernaculum without apophysis … *E. disparilis*
523   -index c~12–16, spicules ~90 µm long and gubernaculum with weak apophysis… *E. tyrrhenicus*
524   5. Tail shorter than 100 µm with two post-anal papillae … *E. fluviatilis*
525   -Tail longer than 100 µm … 6
526   6. Spicule ~100 µm long with plate-like gubernaculum with weak apophysis and three terminal
527   setae at tail tip … *E. stewarti*
528   -Spicule ~30–40 µm long with rod-like gubernaculum with a rounded head at distal end and no
529   terminal setae at tail tip … *E. koreanus* sp. nov.
530   7. Gubernaculum S-shaped … 16
531   -Gubernaculum not S-shaped … 8
532   8. Gubernaculum complex with multiple parts … 9
533   -Gubernaculum short/small, weak, arcuate, plate … 12
534   9. Pre-anal supplementary organ less than 1 abd away from cloacal opening … *E. vectis*
535   -Pre-anal supplementary organ 1.5–1.7 abd away from cloacal opening … *E. spiculohamatus*
536   -Pre-anal supplementary organ more than 2 abd away from cloacal opening … 10
537   10. Spicule length less than 200 (< 4 abd) … *E. brunettii*

538 -Spicule length greater than 200 (> 4 abd) … 11
539 11. Post-anal cuticular element characteristically S-curved … *E. labiatus*
540 -Post-anal cuticular element not s-curved … *E. longispiculosus*
541 12. Cephalic setae shorter than 10 µm and buccal cavity extremely short (9 µm) … *E. typicus*
542 -Cephalic setae longer than 20 µm and buccal cavity average … 13
543 13. Mandible with unique broad central expansion facing the buccal cavity … *E. mandibularis*
544 -Mandible generic in characteristic to the genus … 14
545 14. Proximal end of spicule funnel shaped … *E. incurvatus*
546 -Proximal end of spicule not funnel shaped, normally curved … 15
547 15. Gubernaculum embracing the distal end of spicule, forming a short plate on each side … *E.*
548 *crassum*
549 -Gubernaculum triangular, unpaired, flat, covering the spicules above and below … *E. hirsutum*
550 -Gubernaculum arcuate with wide distal end and narrow and curved proximal end … *E ponticus*
551 16. Head globular … *E. cephalophorus*
552 -Head non-globular … 17
553 17. Presence of cracks on mandibles … *E. rimiformis*
554 -Absence of cracks on mandibles … 18
555 18. Dorsal tooth missing … *E. delamarei*
556 -Dorsal tooth present … 19
557 19. Distal end of spicule with mobile spine … *E. amphioxi*
558 -Distal end of spicule without mobile spine … 20
559 20. Gubernaculum with characteristic ventral knob … *E. gryphus*
560 -Gubernaculum without characteristic ventral knob … 21
561 21. Spicule with diagonal reinforcement … 22
562 -Spicule without diagonal reinforcement … 23
563 22. Spicule longer than 400 µm … *E. bisulcus*
564 -Spicule shorter than 300 µm … *E. harpax*
565 23. Spicule ~490 µm long and smooth, proximal end funnel shaped and distal end slightly
566 expanded and pointed, index c ~20 … *E. labrostriatus*
567 -Spicule ~160–170 µm long, almost straight, index c ~11–16 … *E. polysetosus*
568
569 **Order Enoplida Filipjev, 1929**
570 **Family Thoracostomopsidae Filipjev, 1927**
571 **Subfamily Enoplolaiminae de Coninck, 1965**
572 **Genus *Epacanthion* Wieser, 1953**
573 ***Epacanthion hirsutum* Shi & Xu, 2016**
574 Fig. 5, Table 3
575
576 **Description:** Male (Fig. 5; holotype n=1). Cuticle smooth. Lips high with heavy striation and
577 grooves, each lip bearing two inner labial setae. Six inner labial setae, fairly long and thin at base

578   of lips in one crown. Six longer outer labial setae and four shorter cephalic setae in one crown.
579   Cervical setae scattered randomly at posterior end of cephalic capsule, as long as cephalic setae.
580   Buccal cavity funnel shaped, wide at the anterior end, gradually narrowing towards the base.
581   Buccal cavity armed with three equally sized and shaped mandibles and teeth, respectively.
582   Mandibles with two lateral bars diverging away from one another distally. Distal end of each
583   lateral bars "claw-like", curving towards the lumen like hooks. Mandibles widening near the
584   base, each armed with fairly weak, narrow looking onchia. Mandibular columns divided by a
585   sheet of cuticle. Pharyngeal glands not readily visible. Pharynx fairly long and muscular, its
586   width consistent throughout its length, except the swollen anterior end. Fairly long somatic setae
587   in singles, randomly distributed along the head. Pilosity intense until level of nerve ring, scarcer
588   throughout. A little below the level of nerve ring, a ring of densely arranged cervical setae.
589   Cardia inverse triangular, seemingly embedded in the intestine. Testes paired and opposed, both
590   ends positioned left of intestine. Spicules slightly curved with small gubernaculum at distal end
591   of spicules. No precloacal supplementary organ observed. Caudal glands after cloacal opening,
592   well-developed. Tail conical-cylindrical, two long sub-terminal setae observed and two terminal
593   setae at tail tip.
594
595   Type species: *Epacanthion buetschlii* (Southern, 1914) (28 valid species) (Refer to Shi & Xu,
596   2016)
597
598   **Locality:** Intertidal zone at coast of Shinyang Seopjikoji Beach (East), Jeju Island, South Korea
599   (33°26'09.6"N 126°55'29.3"E), in rocky with dark coarse sandy beach, collected 11 September
600   2018.
601
602   **Materials examined:** All specimens deposited in the National Institute of Biological Resources
603   (South Korea). 1♂ (NIBRIV0000858270).
604
605   **Additional materials examined:** All specimens deposited in the National Institute of Biological
606   Resources (South Korea). 1♀, and 1 juvenile all on a separate slide but with only head and tails
607   retained, with segment of body used for molecular analysis. (NIBRIV0000858271,
608   NIBRIV0000858272).
609
610   **Remarks:** The morphology agrees well to the description provided by the original authors, Shi
611   & Xu (2016). Mandibles clearly resemble those seen in *Epacanthion* species, consisting of two
612   lateral bars (parallel to one another and the space in between not solid) separated by a thin sheet
613   of cuticle. Its distinguishing characteristic, a single row or ring of densely arranged setae below
614   the level of the nerve ring is quite distinct. All measurements are also within the range of the
615   original (Table 3)
616
617   **Molecular analysis**

618 **Mitochondrial cytochrome oxidase C subunit 1 (mtCOI)**

619 We successfully amplified and sequenced DNA of four *Enoploides koreanus* sp. nov. and two
620 *Epacanthion hirsutum*. Despite the JB3/JB5 primers being commonly used in molecular studies
621 of nematodes (Derycke et al. 2005, 2010, 2016, and Avó et al. 2017) few *Enoploides* sequences
622 were available on GenBank to produce a meaningful phylogenetic analysis. Instead, the pairwise
623 distance of all available *Enoploides* mtCOI sequences was calculated using K2P-substitution
624 model using MEGA 7.0. There was no genetic divergence between the new species, while in
625 comparison to other congeners, 19% to 24% divergence was seen (Table 4). This is well within
626 range to genetic divergence seen between congeners using mtCOI sequences (Derycke et al.
627 2010).

628

629 **18s rRNA**

630 We successfully amplified and sequenced DNA of four *Enoploides koreanus* sp. nov. and two
631 *Epacanthion hirsutum*. To test how our sequences group with existing sequences on GenBank,
632 we rebuilt 18S rRNA ML tree by Pereira et al. (2010) with most *Enoploides* 18S sequences
633 available on GenBank (listed in Table 5). The reason for selecting their tree was because their
634 study dealt with a number of free-living marine nematodes (especially Thoracostomopsidae) and
635 used the same primer sets utilized in the present study. The ML tree was more or less similar to
636 the one provided by Pereira et al. (2010), with Thoracostomopsidae forming monophyletic clade
637 with 100% bootstrap. *Enoploides koreanus* and *Epacanthion hirsutum* sequences obtained from
638 this study both formed a clade with their respective congener species, with 83% and 78%
639 bootstraps respectively (Fig. 6). One 18S rRNA sequence of *Epacanthion hirsutum* (MG599065)
640 on GenBank was also retrieved to examine similarities. Although this sequence is considerably
641 longer than ours (1671 bp vs. 310–311 bp), for regions which do overlap, they showed no
642 differences at any of the sites. Sequences obtained in this study have been submitted to GenBank
643 and their accession numbers are MN514234–MN514242 (mtCOI) and MN512311–MN512318
644 (18S).

645

646 **Discussion**

647 One matter unresolved from this revision is the synonymy and confusion of species *Enoploides*
648 *spiculohamatus*, *E. labiatus* and *E. longispiculosus*. Both Wieser & Hopper (1967) and Benwell
649 (1981) suggested abandoning the issue as it cannot be proven. The main problem is that the
650 original descriptions (Bresslau & Stekhoven, 1940; Stekhoven, 1935) are poor and in the case of
651 Stekhoven (1935), it may not even be *E. spiculohamatus* according to Benwell (1981). As
652 nothing can be done about these species, they are left as valid species, but the fact that diagnostic
653 features of *E. labiatus* and *E. longispiculosus* are nearly identical to one another remains
654 problematic (Table 2).

655 The genus *Enoploides* currently consists of 27 valid species. The most recent review by
656 Smol et al. (2014) reported 28 valid species. It is unclear which exact species were listed as valid
657 during their report, as a full species list was not provided. Aside from a list of doubtful species

658 provided by Wieser & Hopper (1967), one more species was transferred to species inquirenda
659 through this revision. *Enoploides filicaudatum* is one of three species transferred from
660 *Epacanthion* by Greenslade & Nicholas (1991). While mandibles may resemble those seen in the
661 genus *Enoploides*, the fact that the original description was based on two juveniles is
662 problematic. Mawson (1956) pointed out that the distinguishing characteristic of this species is
663 the shape of its tail. Such an ambiguous characteristic should not be used as sole reason to
664 discern species, and thus it is regarded as species inquirenda.
665

Conclusion

667 Our work reports *Enoploides koreanus* sp. nov., and *Epacanthion hirsutum* from Jeju Island,
668 Korea. The new species belongs to a group with short spicules (< 150 μm) and is closely related
669 to *E. disparilis* and *E. cirrhatus* based on simplicity and similarity of the spicules and
670 gubernaculum. However, these two species lack the pre-anal supplementary organ present in the
671 new species. Their body ratios also differ to certain degree. Amplification success with the 18S
672 rRNA gene allowed us to visualize the phylogenetic position of our species, which formed clades
673 with their respective congeners with acceptable bootstraps. From compiling and comparing valid
674 species of the genus, we also agree with Wieser & Hopper (1967), that basing descriptions on
675 females and juveniles is ill-advised for this genus, as both lack the distinguishable morphological
676 characteristics evident in males, such as pilosity of the head, morphology of the spicule and
677 gubernaculum, and presence or absence of the pre/post-anal supplementary organ. A
678 bibliographic review of the genus has updated the diagnosis and list of valid species to 27, and a
679 new key to the genus as well as tabular key comparing diagnostic characters of all valid species
680 within the genus have been provided. *Epacanthion hirsutum* reported in Korea agrees with the
681 original description provided by Shi & Xu (2016). Its distinguishing character, a single row of
682 densely arrange setae below the nerve ring, is easily discernible and there were no discrepancies
683 with any of the measurements when compared with the original (Table 3).
684

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687

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883
884

885 **Tables**
886

887 Table 1. Measurement of major morphological characters of *Enoploides koreanus* sp. nov.
888   Measurements are in μm where applicable, and morphometric values are rounded.
889 Table 2. Comparison of diagnostic morphological characters of all *Enoploides* species. Males
890   only, morphometric values are rounded. Species with spicules shorter than 150 μm are
891   marked with asterisk.
892 Table 3. Comparison of diagnostic morphological characters between *Epacanthion hirsutum*
893   found from Korea and from original description. Males only, morphometric values are
894   rounded.
895 Table 4. Kimura 2-parameter distance between all available sequences of *Enoploides* species
896   based on mtCOI alignment.
897 Table 5. Sequences retrieved from GenBank for phylogenetic analysis of this study.
898

899 **Figures**
900

901 Figure 1. Head region, showing different type of mandibles. **(A)** *Enoploides koreanus* sp. nov.,
902   male. **(B)** *Epacanthion hirsutum* Shi & Xu, 2016., male. Scale bars: 20 μm (A and B).
903 Figure 2. *Enoploides koreanus* sp. nov. male. **(A)** head, lateral view. **(B)** total view. **(C)** tail, with
904   spicules, gubernaculum and preanal supplementary organ. Scale bars: 30 μm (A and C)
905   and 100 μm (B). Figure credit: Raehyuk Jeong.
906 Figure 3. *Enoploides koreanus* sp. nov. female. **(A)** head, lateral view. **(B)** tail region with caudal
907   glands. **(C)** reproductive system with vulva. **(D)** total view. Scale bars: 20 μm (A and B),
908   40 μm (C) and 100 μm (D). Figure credit: Raehyuk Jeong.
909 Figure 4. Scanning electron micrograph of *Enoploides koreanus* sp. nov. **(A)** male, head region,
910   lateral view, arrow showing seemingly three setae (presumed to be ols, cs, and scs)
911   tangled to one another. **(B)** male, tail region, arrows showing several caudal setae. **(C)**
912   male, cloacal opening with arrow showing round head of gubernaculum peeking out,
913   behind it two spicules extending outwards. **(D)** female, head region, lateral view, with
914   arrows showing short subcephalic setae below outer labial and cephalic setae. **(E)** female,
915   mouth region, subapical view, showing lip configuration. **(F)** female, cloacal region,
916   ventral view. Scale bars: 10 μm.
917 Figure 5. *Epacanthion hirsutum* Shi & Xu, 2016. male. **(A)** head, lateral view. **(B)** tail region
918   with spicules and gubernaculum. Scale bars: 30 μm (A and B). Figure credit: Raehyuk
919   Jeong.
920 Figure 6. Maximum likelihood tree of 18S rRNA gene based on TPM3+G (with bootstrap values
921   shown at each nodes; 1000 replicates).
922

Figure 1

Head region, showing different type of mandibles.

(A) *Enoploides koreanus* sp. nov., male. **(B)** *Epacanthion hirsutum* Shi & Xu, 2016., male. Scale bars: 20 µm (A and B).
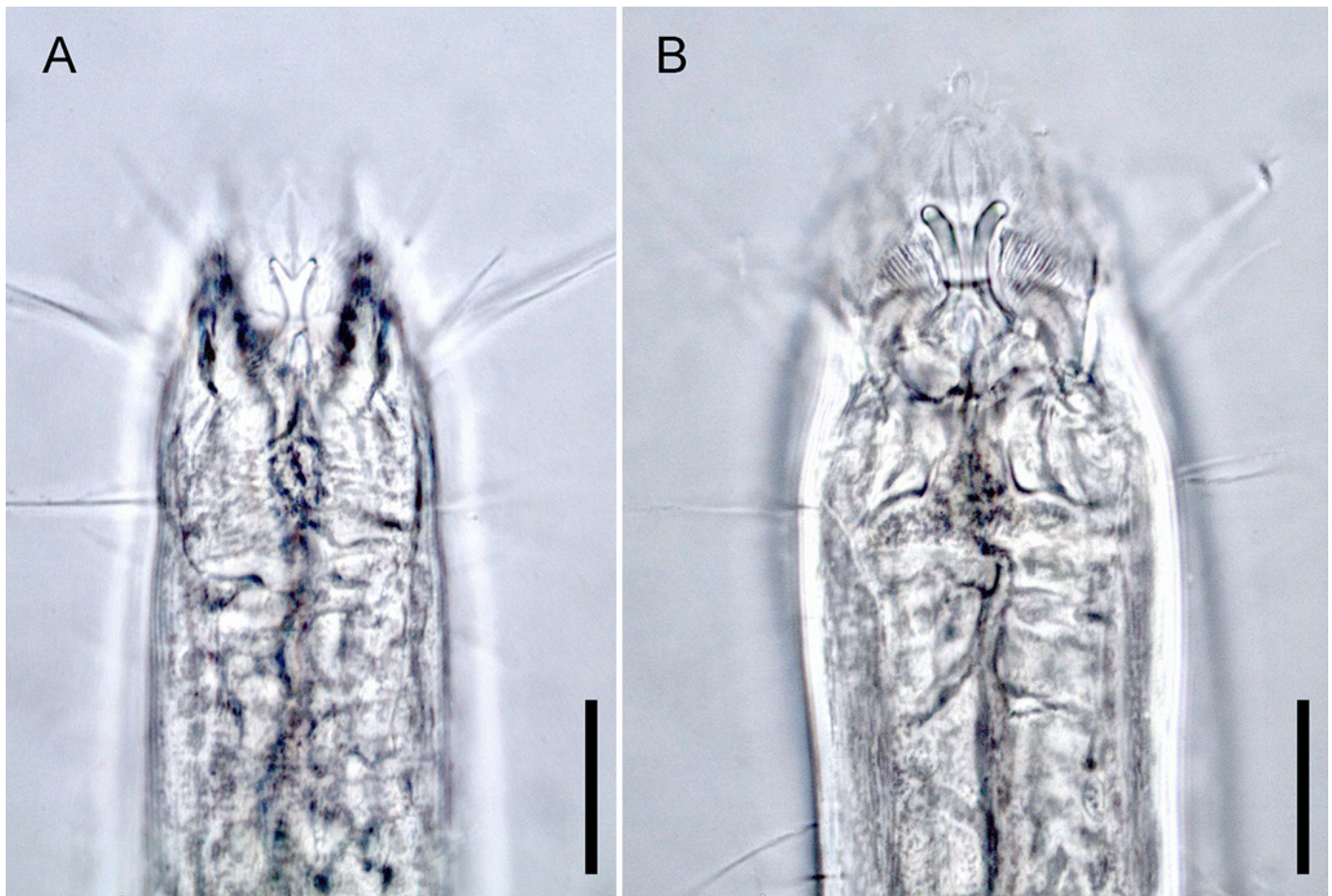
Figure 2

Enoploides koreanus sp. nov. male.

(A) head, lateral view. **(B)** total view. **(C)** tail, with spicules, gubernaculum and preanal supplementary organ. Scale bars: 30 μm (A and C) and 100 μm (B). Figure credit: Raehyuk Jeong.

ils

ols

scs   cs

A

B

C
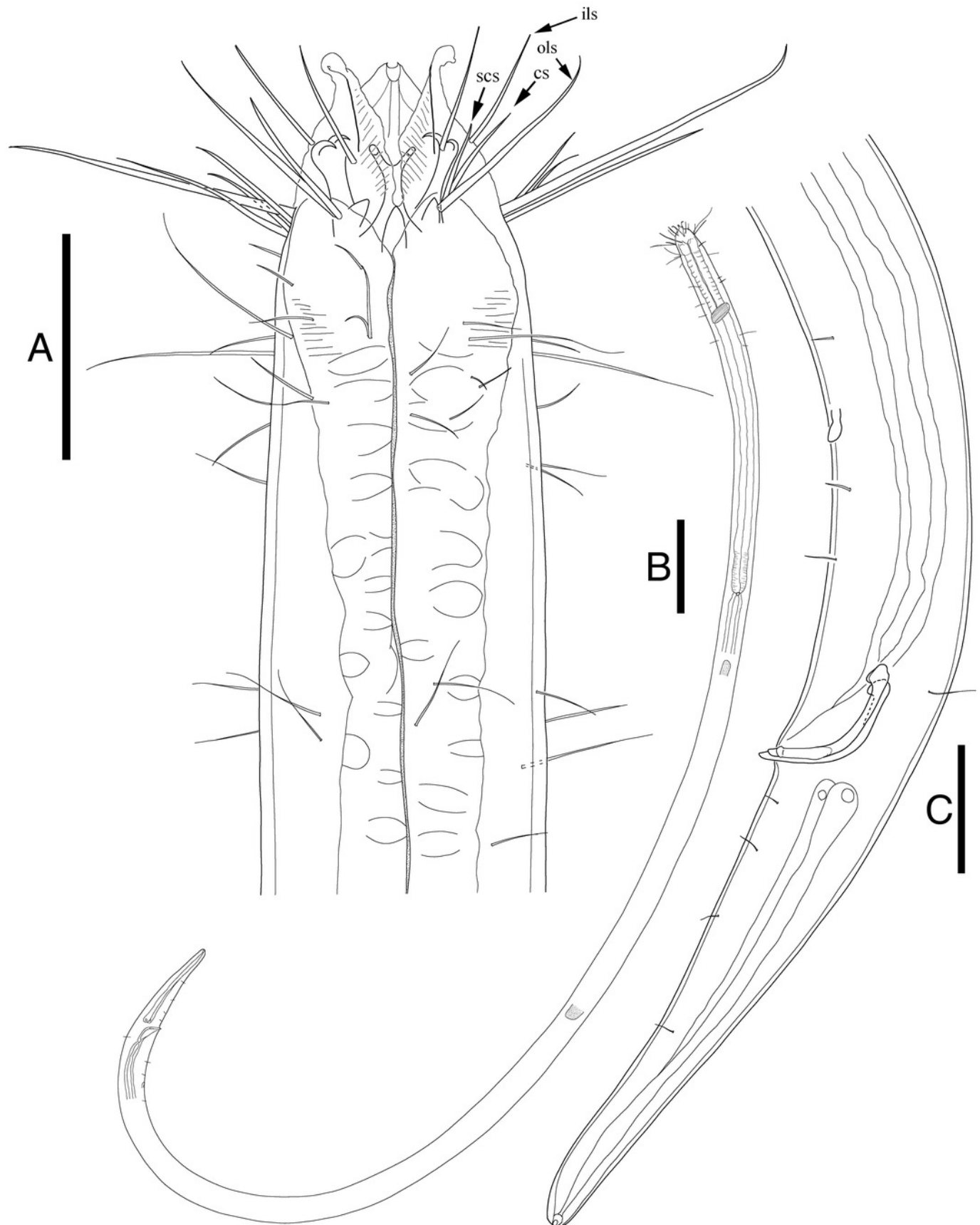
Figure 3

Enoploides koreanus sp. nov. female.

(A) head, lateral view. **(B)** tail region with caudal glands. **(C)** reproductive system with vulva.

(D) total view. Scale bars: 20 μm (A and B), 40 μm (C) and 100 μm (D). Figure credit:

Raehyuk Jeong.
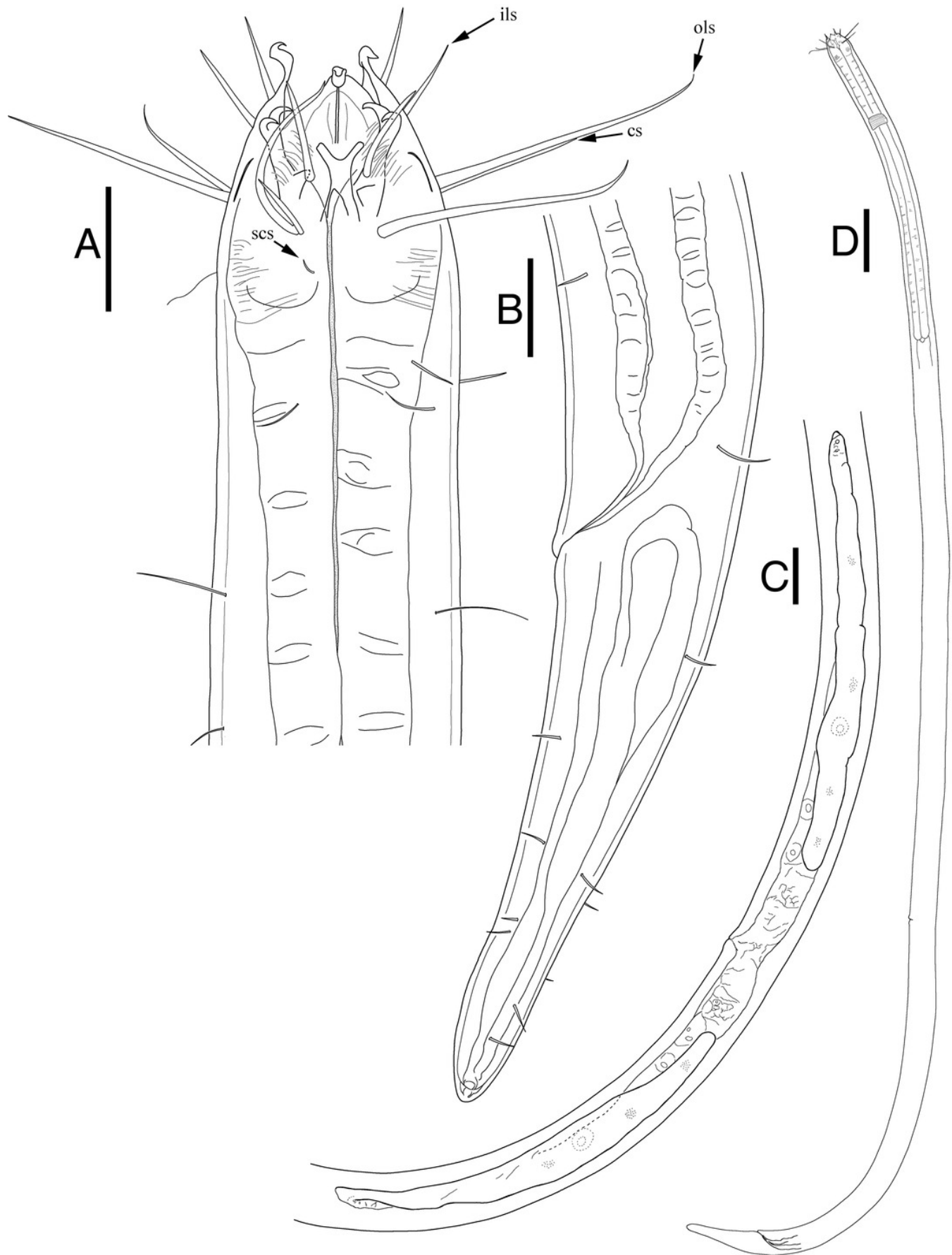
Figure 4

Scanning electron micrograph of *Enoploides koreanus* sp. nov.

(A) male, head region, lateral view, many subcephalic setae on head region tangled to one another. **(B)** male, tail region, arrows showing several caudal setae. **(C)** male, cloacal opening with arrow showing round head of gubernaculum peeking out, behind it two spicules extending outwards. **(D)** female, head region, lateral view, with arrows showing short subcephalic setae below outer labial and cephalic setae. **(E)** female, mouth region, subapical view, showing lip configuration. **(F)** female, cloacal region, ventral view. Scale bars: 10 μm.
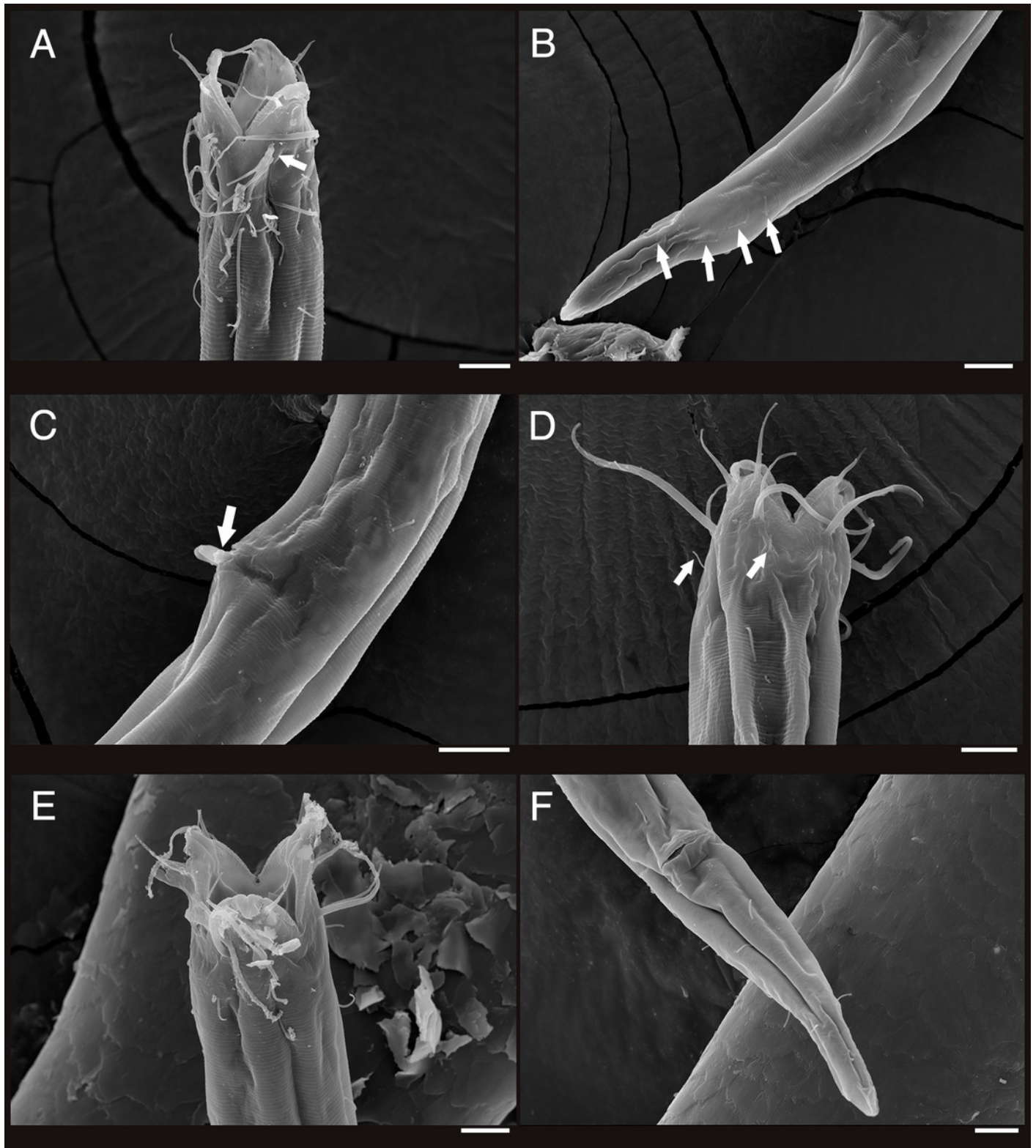
Figure 5

Epacanthion hirsutum Shi & Xu, 2016. male.

(A) head, lateral view. **(B)** tail region with spicules and gubernaculum. Scale bars: 30 μm (A and B). Figure credit: Raehyuk Jeong.
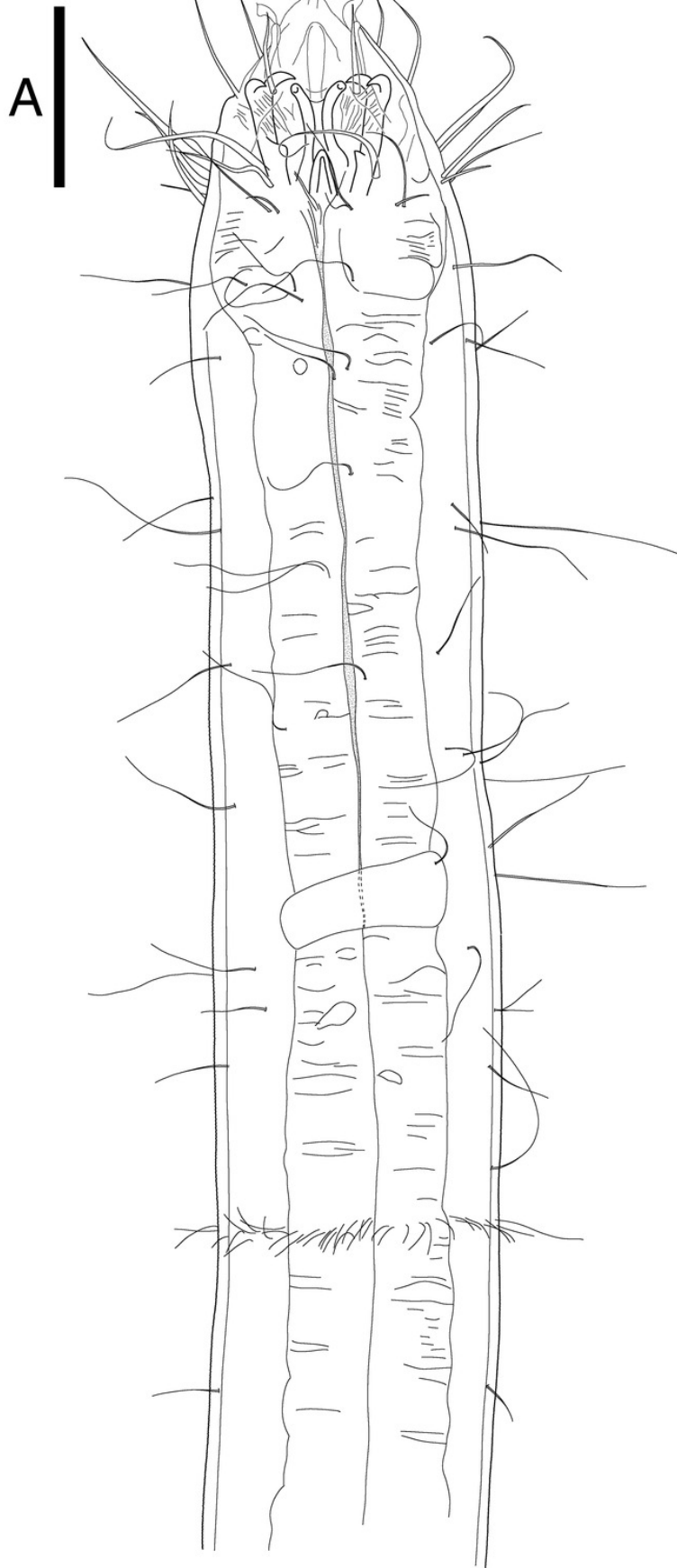
Figure 6

Maximum likelihood tree of 18S rRNA gene based on TPM3+G.

(with bootstrap values shown at each nodes; 1000 replicates).

Enoploides koreanus **MN512313**
Enoploides koreanus **MN512314**
Enoploides koreanus **MN512312**
Enoploides koreanus **MN512311**
Enoploides **sp. MG599078**
Enoploides **sp. GU139765**
Enoploides delamarei **MG599079**
Enoploides longispiculosus **MG669767**
Enoploides brunettii **AY854193**
Mesacanthion **sp. 1 GU139757**
Mesacanthion **sp. 2 GU139756**
Mesacanthion **sp. 3 GU139758**
Epacanthion **sp. GU139759**
Epacanthion longicaudatum **MG599066**
Epacanthion sparsisetae **MG599068**
Epacanthion hirsutum **MN512317**
Epacanthion hirsutum **MN512318**
Epacanthion hirsutum **MG599065**

Thoracostomopsidae

Bathylaimus **sp. GU139751**   **Tripyloididae**

Viscosia **sp. GU139749**
Metoncholaimus **sp. GU139748**
Oncholaimus **sp. GU139747**

Oncholaimidae

0.050

Measurement of major morphological characters of *Enoploides koreanus* sp. nov.

Measurements are in μm where applicable, and morphometric values rounded.

Characters	♂ holotype	♂ (n=3) mean ± sd (range)	♀ (n=6) mean ± sd (range)
body length	2107	2209±100 (2107–2307)	2136±159 (1851–2292)
maximum body diameter	36	35±2 (33–36)	42±3 (39–47)
diameter at the level of cephalic setae	29	29±1 (29–30)	29±6 (18–34)
length of inner labial setae	11	14±4 (11–18)	17±1 (16–19)
length of outer labial setae	43	37±7 (30–43)	42±4 (39–48)
length of cephalic setae	15	15±4 (11–18)	17±3 (13–20)
distance from anterior to cephalic setae	13	18±5 (13–23)	23±3 (19–27)
width at cephalic capsule end	34	34±0 (34–34)	37±2 (32–38)
mandible length	12	11±1 (10–12)	12±1 (10–13)
tooth length	5	5±0 (5–5)	6±1 (5–6)
buccal cavity length	18	24±6 (18–29)	29±2 (26–30)
distance from nerve ring from anterior end	127	127±10 (117–137)	133±9 (119–143)
pharynx (oesophagus) length	510	527±17 (510–543)	524±30 (488–558)
corresponding body diameter at pharynx	36	35±2 (33–36)	40±2 (36–42)
cardia length	13	10±3 (8–13)	9±2 (7–12)
tail length	130	125±5 (121–130)	114±8 (100–122)
anal body diameter	31.0	31±2 (29–33)	33±3 (29–36)
c'	4	4±0 (3.7–4.2)	3.5±0 (3.3–3.8)
spicule length as arc	39.0	36±3 (34–39)	n/a
spicule length as arc / anal body diameter	1	1.2±0 (1.1–1.3)	n/a
length of gubernaculum	12	12±1 (11–12)	n/a
supplementary organ length	8	9±1 (8–9)	n/a
distance from cloacal opening to supplementary organ	82.0	85±3 (82–87)	n/a
distance from anterior end to vulva	n/a	n/a	1355±111 (1170–1456)
corresponding body diameter at vulva	n/a	n/a	42±3 (39–47)
distance from anterior end to vulva as percentage of total body length	n/a	n/a	63±2 (60–64)
a	58.5	63.8±4.6 (58.5–67.1)	50.6±5 (47.5–58.8)
b	4.1	4.2±0.2 (4.1–4.4)	4.1±0 (3.8–4.3)
c	16.2	17.8±1.4 (16.2–18.8)	18.7±1 (17.5–20.4)

2   Table 1. Measurement of major morphological characters of *Enoploides koreanus* sp. nov.
3   Measurements are in μm where applicable, and morphometric values are rounded.

Comparison of diagnostic morphological characters of all *Enoploides* species.

Males only, morphometric values rounded. Species with spicules shorter than 150 μm marked with asterisk.

Species	Body length [μm]	a	b	c	c'	Length of Setae		Spicule length [μm] (spicule length as arc/abd) left/right if applicable	Gubernaculum (length [μm])	Gubernaculum type	Supplementary organ/papilla distance from cloacal opening [μm] (supplementary organ distance from cloacal opening/abd)
						Inner labial Setae	Outer labial setae/cephalic setae				
Enoploides amphioxi Filipjev, 1918	5400	51	5.5	26	4.2	24	50/24	500 (12.5)	present (65)	S-shaped	present (3.5)
Enoploides bisulcus Wieser & Hopper, 1967	3500–4200	Not measured	Not measured	~20–21 calc	4	25	45–50/25–28	420–475	present (not measured)	S-shaped	present 120–150
Enoploides brunettii Gerlach, 1953	2060–2045	29–36	4.5–4.6	14.7–16	3–3.5	18–24	45–53	190 (3–3.5)	present (36–39)	Complex with multiple parts	present (1.5–2)
Enoploides caspersi Riemann, 1966*	3900–5150	54–60	5.4	21.4	not measured	27–31.5	90/38	42.5–48 (0.7 calc)	present (62–72)	S-shaped	present 142–148, post-anal supplementary organ also present
Enoploides cephalophorus (Ditlevsen, 1918) Filipjev, 1927	3100	39	4.8	17.9	not measured	not measured	not measured	264	present	S-shaped	present 128
Enoploides cirrhatus Filipjev, 1918*	4350	26	5.25	16	4.5	13	40	60 (1)	present (15)	Small, adjacent to spicule on dorsal side, upper edge curved	not mentioned, 7–8 pre-anal papillae present
Enoploides crassum (Ditlevsen, 1926) Greenslade & Nicholas, 1991	3000	21	3.9	17	not measured	not measured	not measured	"very long"	present	Short plates on distal end of spicule	present (at level of middle of spicule)
Enoploides delamarei Boucher, 1977	2090–2693	37.3–43.1	4.5–5.3	14.8–21.4	3.4–4.6 calc	18–19	45–47/21	413–568 (10–19.6 calc)	present	S-shaped	present (3.9–4.6)

Enoploides disparilis Sergeeva, 1974*	2250	44.7	4.4	9.8	not measured	12.5	not measured	35 (0.9)	present (19)	Small	absent
Enoploides fluviatilis Micoletzky, 1923*	1340–1900	42.5–53	2.4–3.23	25–31	not measured	8.5–10	24–27/10	(1.46–1.84)	present	Unclear	present (2)
Enoploides gryphus Wieser & Hopper, 1967	3000–3700		~3.7–4.1 calc	~15 calc	~4 calc	28–32	60–80/20–27	230–260 (~4.3–5.1 calc)	present	S-shaped	present 80–110 (~1.8 calc)
Enoploides harpax Wieser, 1959	4150	50	4.4	27.7	3.5–4	25	62/28	290 (5.8 calc)	present	S-shaped, with two hooks	present 120 (2.4 calc)
Enoploides hirsutus Filipjev, 1918	2850	24	3.8	19	not measured	8	27/23	425 (8.3)	present (43)	Unpaired, flat triangular	present (3)
Enoploides incurvatus (Ditlevsen, 1926) Greenslade & Nicholas, 1991	4300	30	5	18.7	not measured	not measured	not measured	176 *measured in linear	uncertain "small"	Small	present 160
Enoploides labiatus (Bütschli, 1874) Filipjev, 1918	2800–3700	22–29	4.1–4.7	17–20.5	not measured	0.25 head diameter	1 head diameter/0.5–0.6 head diameter	300–330 (5.0–5.9)	present (1 abd)	Complex with multiple parts	present (3)
Enoploides labrostriatus (Southern, 1914) Filipjev, 1921	5550	42	6.85	20	4.6 calc	not measured	not measured	490 (8.1 calc)	present	S-shaped "tubular"	present (~2.3 calc)
Enoploides longispiculosus Vitiello, 1967	3700–4055	20.9–22.1	4.4	17.6–19.4	not measured	15–18	51–64/42–43	460 (5.4)	present (74)	Complex, massive, strongly cuticularized with multiple parts	present 233 (2.7)
Enoploides mandibularis Coles, 1977	4800–6400	~24–26.6 calc	~4.8–5.8 calc	~15–16 calc	~3.5–4 calc	10	30/20	400–460 (~4.4–4.6 calc)	present	"short, weakly developed"	present 350

Enoploides polysetosus Jensen, 1986	4380–5160	41–60	3.8–4.5	11.7–15.8	5.4 calc	20	45–50/28–30	162–174 (~2.8 calc)	present	S-shape with two teeth	present 132–158 (~2.3 calc)
Enoploides ponticus Sergeeva, 1974	3345	21.3	3.4	19.3	2.7 calc	21	23	343–363	present (54)	Arcuate, consist of two parts: distal wide and proximal narrow/curved	present 175 (2.7)
Enoploides rimiformis Pavljuk, 1984	2200–2300	44–55	4.6–5	19–20.3		20	43/21	188–192 (~7)	present (33)	S-shaped with 2 hooks	present 75 (3)
Enoploides spiculohamatus Schulz, 1932	2340–3030	28.2–34.3 calc	4.1–4.6 calc	15.1–15.9	2.7–3	20	48/24–25	280–356	present (42–45)	Complex structure with multiple parts; pair of plates joined by process at proximal end, distal end grooved with claw-shaped projection on dorsal groove, two rounded projections in ventrolateral position.	present 89–94 (1.5–1.7 calc)
Enoploides stewarti Nicholas, 1993*	1930–3080	28–49	3.9–4.9	17–24	1.7–2.7 calc	8–15	27–37/14–19	107–121 (2.1–2.6)	present	Simple plate with very weak apophysis	present 78–118 (1.5–2.4 calc)
Enoploides typicus Ssaweljev, 1912	2600	40	4.6	14	not measured	not measured	8.2	not measured	present	Similar to *Mesacanthion tenuicaudatus* "median plate with two lateral grooves with loops"	unclear
Enoploides tyrrhenicus Brunetti, 1949*	1776–2044	30–40	3.3–3.6	12.2–16.3		24	70–75/30	87–90	present	"Complicated"	absent
Enoploides vectis Gerlach, 1957	3160	50	4.2	15.8	3.8 calc	27	64/20	340	present (57)	Complex with bent projection at proximal end	present 41 (0.8)

Enoploides koreanus sp. nov.*	2107–2307	58.5–67.1	4.1–4.4	16.2–18.8	3.7–4.2	11–18	30–43/11–18	34–39 (1.1–1.3)	present	Rod-like plate with round head on distal end	present 82–87

2   Table 2. Comparison of diagnostic morphological characters of all *Enoploides* species. Males only, morphometric values are rounded.
3   Species with spicules shorter than 150 μm are marked with asterisk.

Comparison of diagnostic morphological characters between *Epacanthion hirsutum* found from Korea and from original description.

Males only, morphometric values rounded.

Characters	♂ (n=1) from Jeju Island, Korea	♂ (n=10) from original description (Shi & Xu, 2016)
body length	2468	1879–2433
length of inner labial setae	15	13–20
length of outer labial setae	35	30–35
length of cephalic setae	19	12–14
mandible length	18	13–17
mandible length to width, ratio	2	1.7–2
tooth length	8	7–10
distance from nerve ring from anterior end	145	133−145
pharynx (oesophagus) length	723	625–700
tail length	183	167–198
anal body diameter	41	39–43
spicule length as arc	30	21–33
spicule length as arc / anal body diameter	1	0.5–0.8
length of gubernaculum	6	5–9
a	49.4	37.6–52.1
b	3.4	2.9–3.5
c	13.5	10–13.7
c'	4.5	4.1–5

3 Table 3. Comparison of diagnostic morphological characters between *Epacanthion hirsutum*
4 found from Korea and from original description. Males only, morphometric values are rounded.

Kimura 2-parameter distance between six *Enoploides* species from Jeju Island based on COI alignment.

Enoploides koreanus [MN514234]						
Enoploides koreanus [MN514235]	0.000					
Enoploides koreanus [MN514236]	0.000	0.000				
Enoploides koreanus [MN514237]	0.000	0.000	0.000			
Enoploides sp. [HM564984]	0.191	0.191	0.191	0.191		
Enoploides sp. [HM64945]	0.236	0.236	0.236	0.236	0.218	
Enoploides longispiculosus [FN998930]	0.225	0.225	0.225	0.225	0.204	0.184

3 Table 4. Kimura 2-parameter distance between all available sequences of *Enoploides* species
4 based on mtCOI alignment.

Sequences retrieved from GenBank for phylogenetic analysis of this study.

Name	Family	Marker	Accession number	Reference
Metoncholaimus sp.	Oncholaimidae	18S rRNA	GU139748.1	Pereira et al. (2010)
Oncholaimus sp.	Oncholaimidae	18S rRNA	GU139747.1	Pereira et al. (2010)
Viscosia sp.	Oncholaimidae	18S rRNA	GU139749.1	Pereira et al. (2010)
Rhabdodemania sp.	Rhabdodemaniidae	18S rRNA	GU139750.1	Pereira et al. (2010)
Enoploides koreanus	Thoracostomopsidae	18S rRNA	MN512311	This study
Enoploides koreanus	Thoracostomopsidae	18S rRNA	MN512312	This study
Enoploides koreanus	Thoracostomopsidae	18S rRNA	MN512313	This study
Enoploides koreanus	Thoracostomopsidae	18S rRNA	MN512314	This study
Enoploides koreanus	Thoracostomopsidae	mtCOI	MN514234	This study
Enoploides koreanus	Thoracostomopsidae	mtCOI	MN514235	This study
Enoploides koreanus	Thoracostomopsidae	mtCOI	MN514236	This study
Enoploides koreanus	Thoracostomopsidae	mtCOI	MN514237	This study
Enoploides longispiculosus	Thoracostomopsidae	mtCOI	FN998930	Derycke et al. 2010
Enoploides sp.	Thoracostomopsidae	18S rRNA	GU139765.1	Pereira et al. (2010)
Enoploides sp.	Thoracostomopsidae	mtCOI	HM564984	Bik et al. (2010)
Enoploides sp.	Thoracostomopsidae	mtCOI	HM564945	Bik et al. (2010)
Epacanthion hirsutum	Thoracostomopsidae	18S rRNA	MN512317	This study
Epacanthion hirsutum	Thoracostomopsidae	18S rRNA	MN512318	This study
Epacanthion hirsutum	Thoracostomopsidae	18S rRNA	MG599065	Shi, Pu & Xu, 2017, unpublished data
Epacanthion hirsutum	Thoracostomopsidae	mtCOI	MN514241	This study
Epacanthion hirsutum	Thoracostomopsidae	mtCOI	MN514242	This study
Epacanthion sp.	Thoracostomopsidae	18S rRNA	GU139759.1	Pereira et al. (2010)
Mesacanthion sp. 1	Thoracostomopsidae	18S rRNA	GU139757.1	Pereira et al. (2010)
Mesacanthion sp. 2	Thoracostomopsidae	18S rRNA	GU139756.1	Pereira et al. (2010)
Mesacanthion sp. 3	Thoracostomopsidae	18S rRNA	GU139758.1	Pereira et al. (2010)
Bathylaimus sp.	Tripyloididae	18S rRNA	GU139751.1	Pereira et al. (2010)

2
3 Table 5. Sequences retrieved from GenBank for phylogenetic analysis of this study.