Description of *Atherospio aestuarii* sp. nov. (Annelida: Spionidae) from Japan ~~with note on the phylogenetic position of the enigmatic genus *Atherospio*~~

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Background. There are currently two species within the small enigmatic genus *Atherospio* Mackie & Duff, 1986, which belongs to the *Pygospiopsis-Atherospio* group in the family Spionidae Grube, 1850. The taxonomic relationship of the genus *Atherospio* with other spionid or spioniform genera is currently not well understood due to its unusual morphological characteristics. **Methods.** Here, we describe a new *Atherospio* species, *Atherospio aestuarii* **sp. nov.**, based on materials collected from three localities in Japan: Hirota Bay (Iwate Prefecture), Ago Bay (Mie Prefecture), and Yakushima Island (Kagoshima Prefecture). We have also evaluated the possible systematic position of this new species by conducting molecular phylogenetic analyses using the nuclear 18S, 28S, and mitochondrial 16S rRNA gene sequences. **Results.** The morphology of *A. aestuarii* **sp. nov.** resembles that of *A. disticha* Mackie & Duff, 1986 and *A. guillei* (Laubier & Ramos, 1974) in having branchiae fused to the notopodial lamellae on a restricted number of segments from chaetiger 7, modified neurochaetae on chaetiger 5, and at least some bidentate neuropodial hooks with the secondary tooth below the main fang. The form and arrangement of the modified aristate neurochaetae in double vertical rows closely resemble those found on chaetigers 4 and 5 of *A. disticha*. The new species lacks the occipital antenna present in *A. disticha*. In this respect it resembles *A. guillei*, however, that species differs in having robust neuropodial spines on chaetiger 5 and peristomial papillae, and a preponderance of unidentate neurochaetae. Both *A. guillei* and the new species have slender needle-like notochaetae in their posteriormost chaetigers. *Atherospio aestuarii* **sp. nov.** is distinguished from both congeneric species by its branchial and neuropodial hook distributions. The new species is also unique in that it was recorded at relatively shallow depths, which included intertidal zones. The results of our molecular phylogenetic analysis indicate that the new species was included in a clade that included the genera of the *Polydora* complex, *Pygospio* Claparède, 1863, *Glandulospio* Meißner, Bick, Guggolz & Götting, 2014, *Spio* Fabricius, 1785, *Microspio* Mesnil, 1896, *Marenzelleria* Mesnil, 1896, *Rhynchospio* Hartman, 1936, *Scolelepis* Blainville, 1828, *Dispio* Hartman, 1951, and *Malacoceros* Quatrefages, 1843 with robust statistical support. The new species formed a clade with *Dispio* and *Scolelepis*, however, statistical support for the node was not ~~high of~~ significant.

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Abstract

Background. There are currently two species within the small enigmatic genus *Atherospio* Mackie & Duff, 1986, which belongs to the *Pygospiopsis-Atherospio* group in the family Spionidae Grube, 1850. The taxonomic relationship of the genus *Atherospio* with other spionid or spioniform genera is currently not well understood due to its unusual morphological characteristics.

Methods. Here, we describe a new *Atherospio* species, *Atherospio aestuarii* **sp. nov.**, based on materials collected from three localities in Japan: Hirota Bay (Iwate Prefecture), Ago Bay (Mie Prefecture), and Yakushima Island (Kagoshima Prefecture). We have also evaluated the possible systematic position of this new species by conducting molecular phylogenetic analyses using the nuclear 18S, 28S, and mitochondrial 16S rRNA gene sequences.

Results. The morphology of *A. aestuarii* **sp. nov.** resembles that of *A. disticha* Mackie & Duff, 1986 and *A. guillei* (Laubier & Ramos, 1974) in having branchiae fused to the notopodial lamellae on a restricted number of segments from chaetiger 7, modified neurochaetae on chaetiger 5, and at least some bidentate neuropodial hooks with the secondary tooth below the main fang. The form and arrangement of the modified aristate neurochaetae in double vertical rows closely resemble those found on chaetigers 4 and 5 of *A. disticha*. The new species lacks the occipital antenna present in *A. disticha*. In this respect it resembles *A. guillei*, however, that species differs in having robust neuropodial spines on chaetiger 5 and peristomial papillae, and a preponderance of unidentate neurochaetae. Both *A. guillei* and the new species have slender needle-like notochaetae in their posteriormost chaetigers. *Atherospio aestuarii* **sp. nov.** is distinguished from both congeneric species by its branchial and neuropodial hook distributions. The new species is also unique in that it was recorded at relatively shallow depths, which included intertidal zones. The results of our molecular phylogenetic analysis indicate that the new species was included in a clade that included the genera of the *Polydora* complex, *Pygospio* Claparède, 1863, *Glandulospio* Meißner, Bick, Guggolz & Götting, 2014, *Spio* Fabricius, 1785, *Microspio* Mesnil, 1896, *Marenzelleria* Mesnil, 1896, *Rhynchospio* Hartman, 1936, *Scolelepis* Blainville, 1828, *Dispio* Hartman, 1951, and *Malacoceros* Quatrefages, 1843 with robust statistical support. The new species formed a clade with *Dispio* and *Scolelepis*, however, statistical support for the node was not ~~high of~~ significant.

Introduction

Atherospio Mackie & Duff, 1986 is a small genus in the family Spionidae Grube, 1850 that currently consists of two species: *A. disticha* Mackie & Duff, 1986 and *A. guillei* (Laubier & Ramos, 1974). The genus is closely related to *Pygospiopsis* Blake, 1983 (including the recently synonymized genus *Pseudatherospio* Lovell, 1994: Blake and Maciolek 2018) as it has similar prostomial shapes, an occipital antenna, modified anterior neurochaetae, branchiae that are either basally or entirely fused to the notopodial lamellae, and unusual bidentate neuropodial hooks. In other spionids, the small tooth (teeth) of the neuropodial hooded hooks is (are) superior to the main fang on the convex side, while for *Atherospio* and *Pygospiopsis* the neuropodial hooded or unhooded hooks have a small tooth or knob on the concave side, which is subapical to the terminal shaft or main fang (Blake & Maciolek 2018). *Atherospio*, *Pygospiopsis*, and a recently established genus, *Aciculaspio* Blake & Ramey-Balci, 2020, are collectively called the *Pygospiopsis*-*Atherospio* group (Blake & Ramey-Balci 2020) and currently consist of nine species. *Atherospio* and *Pygospiopsis* are distinguishable as the former have their first branchiae on chaetiger 7, while the latter ~~having~~ simple or partially fused branchiae anterior to chaetiger 7 in a variety of patterns (Blake & Maciolek 2018). *Aciculaspio* differs from both *Atherospio* and *Pygospiopsis* as it has branchiae from ~~setiger~~ 2 and simple, unidentate-hooded hooks with curved and pointed fangs (Blake & Ramey-Balci 2020).

Blake et al. (2020) divided the spionid genera into four clades following Blake & Arnofsky (1999) and Blake (2006): (1) Subfamily Nerininae Söderström, 1920; (2) Subfamily Spioninae Söderström, 1920; (3) Clade ~~consisted~~ of *Pygospiopsis*, *Atherospio*, and *Pseudatherospio* (= *Pygospiopsis*-*Atherospio* group); and (4) five monotypic genera with no strong affinity for other spionids (*Glandulospio* Meißner, Bick, Guggolz & Götting, 2014; *Glyphochaeta* Bick, 2005; *Spiogalea* Aguirrezabalaga & Ceberio, 2005; *Spiophanella* Fauchald & Hancock, 1981; and *Xandaros* Maciolek, 1981). Species belonging to the *Pygospiopsis*-*Atherospio* group are superficially similar to species in subfamily Spioninae (including the *Polydora* complex and the genera *Pygospio* Claparède, 1863, *Microspio* Mesnil, 1896, and *Spio* Fabricius, 1785), some of which were originally classified as separate genera within Spioninae. *Pygospiopsis dubia* (Monro, 1930) was originally described as *Pygospio*, and Blake (1983) later established the genus *Pygospiopsis* for this species. *Atherospio guillei* was originally described as *Polydora* Bosc, 1802 in the *Polydora* complex, and later, Meißner & Bick (2005) transferred this species to *Atherospio*. *Atherospio guillei* and the species belonging to the *Polydora* complex both have heavy spines in the fifth segment. However, this is not considered to be evidence of a close relationship between the two taxa as these heavy spines are not homologous sensu stricto, as in *A. guillei* they are neuropodial, while in polydorins they are notopodial (Mackie & Duff 1986, Radashevsky & Fauchald 2000, Radashevsky 2012).

The close relationship between the *Pygospiopsis*-*Atherospio* group and the subfamilies Spioninae and Nerininae has not been consistently supported in previous studies. The first phylogenetic analysis of the Spionidae genera using morphology by Sigvaldadóttir et al. (1997) indicated that there were four clades in ~~spionid~~: (1) *Aonidella* López-Jamar, 1989 and *Xandaros*; (2) *Prionospio* complex, *Laonice* Malmgren, 1867, *Spiophanes* Grube, 1860, and *Aonides* Claparède, 1864; (3) a large unresolved assemblage of genera including the *Polydora* complex, *Scolelepis* Blainville, 1828, *Malacoceros* Quatrefages, 1843, and *Spio*; and (4) *Atherospio*, *Pseudatherospio*, and *Pygospiopsis*, but the support for these clades was weak and the selection of outgroups was subsequently deemed unfortunate (Blake et al. 2020). Mackie (1996) re-examined the intergeneric relationships within ~~the~~ Spionidae examined by Sigvaldadóttir et al. (1997) by adding several new taxa and eight taxa with questionable generic attribution using the same outgroups and indicated ~~that~~ generally ~~consistent~~ with the previous results, but ~~were~~

~~characterized by the separation~~ ~~a large~~ unresolved assemblage ~~of genera in Sigvaldadóttir et al. (1997) into~~ a 'polydorid' group (~~but~~ ~~include~~ *Pygospio muscularis* Ward, 1981 and ~~exclude~~ *Tripolydora* Woodwick, 1964 and *Pseudopolydora primigenia* Blake, 1983) and a large group including 11 genera. The third phylogenetic analysis of the spionid genera using morphological, reproductive, and developmental characteristics from Blake & Arnofsky (1999) indicated that there were three clades: two major clades consisting of the subfamily Spioninae and a larger clade consisting of all remaining spionid genera and the genera *Heterospio* Ehlers, 1874 (now considered to be a taxon closely related to cirratuliform polychaetes rather than spioniforms: Blake & Maciolek 2019), *Poecilochaetus* Claparède in Ehlers, 1875, *Trochochaeta* Levinsen, 1884, and *Uncispio* Green, 1982, and a ~~minor third~~ clade consisting of the enigmatic genus *Pygospiopsis* (including *Atherospio*). At present, because of several unusual morphological characteristics of the *Pygospiopsis-Atherospio* group, its taxonomic relationship with other spionids or spioniforms is not well understood. However, Blake & Maciolek (2018) noted that the large recurved hooded hooks of *P. profunda* Blake & Maciolek, 2018 have ~~some~~ similarities with the giant modified neuropodial hooks or spines of some *Uncispio* species.

To date, there are no available molecular data on the *Pygospiopsis-Atherospio* group or *Uncispio* deposited in the DNA Data Bank of Japan (DDBJ), the European Nucleotide Archive (ENA), or GenBank databases. Therefore, these taxa were not included in the first and recent comprehensive molecular phylogenetic analyses of the spionid genera, which was conducted by Abe & Sato-Okoshi (2021) and Wang et al. (2022), respectively. Our field surveys have identified several specimens of the genus *Atherospio*, which have never been recorded from Japan before, from several study sites. In this study, we report the morphology of the specimens and compare it with that of other species of the genus, and describe a new species, *Atherospio aestuarii* **sp. nov.** We also evaluate the phylogenetic position of *Atherospio* by conducting the first molecular phylogenetic analysis including the genus, whose phylogenetic position has remained a question until now.

Materials & Methods

Specimen collection

Specimens of the *Atherospio* species were collected from bottom sediments in the intertidal zone of Otomo-ura (38°59′45″N, 141°40′54″E), Hirota Bay, Iwate Prefecture on August 6, 2017, August 18, 2018, and August 4, 2020; subtidal zones < 1 m in depth in a nameless small inlet of Ago Bay (34°17′55″N, 136°49′52″E), Mie Prefecture on October 8, 2021; and a small fishing port at the mouth of the Kurio River (30°16′27″N, 130°25′17″E) on Yakushima Island, Kagoshima Prefecture, Japan on November 6, 2021 (Figs. 1 and 2). The water areas where the specimens were collected in this study are not protected, and no permission of any kind is required to collect the organisms. In the field survey of this study, we did not collect any commercially marine species and did not use any collection method that violated the prefectural fishery regulation, so we did not need any permission for the survey.

Morphological observation

Specimens were observed and photographed in a live condition and then fixed in 10% neutral formalin seawater or 70% ethanol for morphological and molecular analyses. The morphology of the living and fixed *Atherospio* species was observed under a stereomicroscope (Wraymer LW-820T, Osaka, Japan) and phase-contrast microscope (Nikon Eclipse 80i, Tokyo, Japan). Light micrographs were obtained using a digital camera (Sony α6000, Tokyo, Japan) attached to the microscope. Live specimens were anesthetized in a 7% magnesium chloride solution if required. Four specimens were stained with a solution of methyl green in ethanol for light microscopy analysis. The type materials were deposited in the National Museum of Nature and Science (NSMT), Tsukuba, Japan, under the following museum registration numbers: NSMT-Pol H-858 and P-859–866.

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Molecular analysis

Nuclear 18S, 28S, and mitochondrial 16S rRNA gene analyses were performed on the holotype and the six paratypes. Genomic DNA was extracted from 70% ethanol-preserved tissue by grinding and heating at 95°C for 20 min in 50 µl TE buffer (pH 8.0) with 10% Chelex 100 (Bio-Rad), according to Richlen & Barber (2005). Ten-fold diluted extracted DNA in TE buffer was used as a template for polymerase chain reaction (PCR). Partial sequences of the nuclear 18S, 28S, and mitochondrial 16S rRNA genes were amplified by PCR using the primer pairs 18S-1F1/18S-1R632, 18S-2F576/18S-2R1209, and 18S 3F1129/18S-R1772 for 18S (Nishitani et al. 2012), D1R/D2C for 28S (Scholin et al. 1994), and 16Sar/16Sbr for 16S (Palumbi et al. 1991). PCR was performed in a 10 µL reaction mixture containing 0.5 µL of template DNA, 4 µL of sterilized water, 5 µL of 2 × KOD One PCR Master Mix (TOYOBO, Osaka, Japan), and 0.05 µM of 50 µM forward and reverse primers. The PCR cycling conditions were 36–40 cycles of denaturation at 98°C for 10 s, annealing at 54°C or 56°C (16S), or 60°C (18S and 28S) for 5 s, and extension at 68°C for 1 s. PCR products were purified using Enz-Sap (Edge BioSystems, San Jose, CA, USA) and sequenced by Eurofins Genomics (Tokyo, Japan). Forward and reverse complementary sequences and contigs were assembled using GeneStudio ver. 2.2.0.0 (GeneStudio, Inc. Suwanee, GA, USA). All sequences generated in this study have been deposited in the DDBJ/ENA/GenBank nucleotide sequence database under accession numbers LC685029–LC685049 (Table 1).

To reconstruct the molecular phylogeny, sequences of the 18S, 28S, and 16S rRNA genes were aligned with the sequences of other spionid species and outgroups obtained from GenBank (Table 1) using the MAFFT online service ver. 7 (Katoh et al. 2017) and the L-INS-i algorithm. The gene sequences of the sabellid species *Amphicorina mobilis* (Rouse, 1990) and *Sabella pavonina* Savigny, 1822, obtained from DDBJ/ENA/GenBank, were used as the outgroup taxa (Table 1). Ambiguously aligned regions were eliminated using the Gblocks server ver. 0.91b with the least stringent settings (Castresana 2000; Talavera & Castresana 2007). The final lengths of the aligned sequences were 1703, 663, and 434 bp for the 18S, 28S, and 16S rRNA gene sequences, respectively (Supplementary file S1). A phylogenetic tree was constructed based on the concatenated sequences of the 18S, 28S, and 16S rRNA gene regions using maximum likelihood (ML) analyses performed with IQ-TREE (Nguyen et al. 2015) implemented in PhyloSuite v.1.2.2 (Zhang et al. 2020) under an edge-linked partition model. The TNe+I+G4, TIM3+F+I+G4, and TIM2+F+I+G4 models were selected as the best substitution models for the 18S, 28S, and 16S rRNA gene regions, respectively, by ModelFinder (Kalyaanamoorthy et al. 2017) as implemented in IQ-TREE under the Bayesian information criterion (BIC). We evaluated the robustness of the ML trees using the Shimodaira–Hasegawa–like approximate likelihood-ratio test (SH-aLRT) with 5,000 replicates (Guindon et al. 2010), the approximate Bayes (aBayes) test (Anisimova et al. 2011), and ultrafast bootstraps (UFBoot) with 5000 replicates (Hoang et al. 2018). An SH-aLRT ≥ 80%, aBayes ≥ 0.95, and UFBoot ≥ 95% were defined as robust statistical supports.

Results

Systematics

Family Spionidae Grube, 1850
Genus *Atherospio* Mackie & Duff, 1986

Type-species: *Atherospio disticha* Mackie & Duff, 1986

Diagnosis (Emended from Meißner & Bick 2005). Prostomium deeply incised, longer than wide, posteriorly tapered and not extended into a distinct caruncle; occipital antenna present or absent or minute process at the position of this antenna present. Nuchal organs small or indistinct. Dorsal branchiae from chaetiger 7; branchiae with distal digitate process, outer branchial margin completely fused with notopodial postchaetal lamella. Parapodia biramous with well developed postchaetal lamellae and alimbate mostly hirsute capillaries in noto- and neuropodia. Chaetigers 4 and 5 or solely chaetiger 5 with modified chaetae in the neuropodium being falcate and pointed or aristate spines, modified chaetae in a irregular short row superior to several capillary chaetae. Neuropodial hooks alongside capillaries; hooks uni-or bidentate, secondary tooth below main fang; hook distally with closely applied sheath. Notopodial hooks absent. Posterior spine-like notochaetae present or absent. Sabre chaetae absent but several capillaries in inferiormost position throughout the body. Genital pouches absent. Pygidium surrounded by several pairs of lateral cirri.

Remarks. The morphology of the new species described below is generally consistent with the diagnosis for the genus *Atherospio* by Meißner & Bick (2005). Since the description of "Dorsal branchiae on chaetiger 7 and following 4–6 chaetigers" and "Postbranchial neuropodial hooks" in the diagnosis provided by Meißner & Bick (2005) does not apply to the new species, we emended these parts of the diagnosis as "Dorsal branchiae from chaetiger 7" and "Neuropodial hooks", respectively, to include the new species. We also added the diagnosis ~~about~~ posterior needle-like notochaetae found in *A. guillei* and the new species.

Atherospio aestuarii sp. nov.

Japanese name: Irie-nogi-supio
LSID. urn:lsid:zoobank.org:act:287692C4-C105-41BC-8718-37C6BBE10B7C
(Figs. 3 and 4)

Type material. Holotype: NSMT-Pol H-858, small fishing port at the mouth of the Kurio River, 30°16′27″N, 130°25′17″E, Yakushima Island, Kagoshima Prefecture, subtidal, < 1 m depth, muddy sand, November 6, 2021 (complete specimen). Paratypes: NSMT-Pol P-859, Otomo-ura, 38°59′45″N, 141°40′54″E, Hirota Bay, Iwate Prefecture, intertidal, gravelly muddy sand, Aug. 6, 2017 (incomplete 1 specimen); NSMT-Pol P-860, Otomo-ura, 38°59′45″N, 141°40′54″E, Hirota Bay, Iwate Prefecture, intertidal, gravelly muddy sand, August 18, 2019 (incomplete 1 specimen); NSMT-Pol P-861, Otomo-ura, Hirota Bay, 38°59′45″N, 141°40′54″E, Iwate Prefecture, intertidal, gravelly muddy sand, August 4, 2020 (incomplete 1 specimen); NSMT-Pol P-862 (incomplete 1 specimen), NSMT-Pol P-863 (incomplete 7 specimens), NSMT-Pol P-864 (incomplete 1 specimen), nameless small inlet in Ago Bay, 34°17′55″N, 136°49′52″E, Mie

Prefecture, subtidal, < 1 m depth, gravelly muddy sand, October 8, 2021; NSMT-Pol P-865 (incomplete 1 specimen), NSMT-Pol P-866 (incomplete 2 specimens), small fishing port at the mouth of the Kurio River, 30°16′27″N, 130°25′17″E, Yakushima Island, Kagoshima Prefecture, subtidal, < 1 m depth, muddy sand, November 6, 2021.

Description. Holotype complete (pygidium damaged) with 64 chaetigers, measuring 9.5 mm long and 1.2 mm wide at chaetiger 5 (Fig. 3); paratypes incomplete up to 14.4 mm long, 1.5 mm wide for 40 chaetigers. Body wide, dorsoventrally flattened for first 6 chaetigers (Figs. 3B and 4A), then gradually narrower and becoming cylindrical in cross-section. Body white to light tan in preserved specimen (Fig. 3A), translucent white to light tan when alive with red blood vessels and pale orange to brown digestive tract internally (Figs. 3C, 3E, and 4A); body and palp pigmentation absent.

Prostomium longer than wide, anteriorly incised; posteriorly extending to middle of chaetiger 1 (Figs. 3B and 4A). Eyes dark red, two pairs arranged in trapezoidal shape, lateral pair situated anteriorly, kidney-shaped, larger than medial ones (Fig. 4A and 4B). Caruncle and occipital antenna absent. Nuchal organs U-shaped with outward curving posterior part, located just behind prostomium and between notopodial lamellae of chaetiger 1 (Figs. 3B and 4B). Palps arising from lateral to prostomium (Fig. 4A). Peristomium extending lateral to prostomium, forming upper lip of mouth and extending ventrally forming ventral lip of mouth; thick everted proboscis or pharynx present; oral lips relatively smooth; peristomial papillae (see Blake & Maciolek 2018) absent.

Chaetigers 1–6 abranchiate (Figs. 3B, 3C, and 4A). Notopodial postchaetal lamellae long, digitiform or lanceolate on chaetiger 1 (Figs. 3B and 4C), broader on chaetiger 2, and becoming broad triangular or oval on chaetigers 3–6 (Fig. 4E). Neuropodial lamellae digitiform or lanceolate on chaetiger 1, broad triangular on chaetiger 2, and oval to triangular on chaetigers 3–6. Chaetiger 5 of same size as neighboring chaetigers. Midventral series of white rectangular pads in anterior chaetigers, indistinct in fixed specimens.

Branchiae from chaetiger 7 to 18–23, long and cirriform, with digitiform process at distal end (Fig. 4F); overlapping mid-dorsal or not, full-sized from chaetigers 10–12; fully fused with notopodial postchaetal lamellae in outer margin (Fig. 4F); ciliation along inner margin, extending to a nototroch across the whole width of the chaetiger. In branchial chaetigers, notopodial postchaetal lamellae foliated and often wavy, especially when alive (Fig. 3C and 3E); neuropodial postchaetal lamellae rounded, larger dorsoventrally than that of chaetigers 1–6 (Fig. 4F). In postbranchial chaetigers, both postchaetal lamellae smaller, rather more subtriangular.

Notochaetae in most chaetigers long slender capillaries without limbations; some posterior notopodia with bundles of needle-like capillaries raised dorsally (Fig. 3F); notopodial hooks absent. Neurochaetae capillaries without limbations in anterior chaetigers. Neuropodia of chaetiger 5 double vertical rows of aristate spines dorsal to small bundle of capillaries; spines in posterior row slightly thicker than those of closely applied anterior row, taper steeply towards tip with short aristae part; spines in the anterior row taper gradually towards tip with long aristae part (Fig. 4D and 4E). Hooded hooks in neuropodia from chaetigers 16–19 to the posterior-most chaetiger, accompanied by capillaries in all chaetigers; numbering up to 6 in a series, reduced in posterior chaetigers, S-curve on the shaft, hooks bidentate with secondary tooth on concave side at right angle to and below main fang (Fig. 4G). Neuropodial sabre chaetae absent.

Pygidium without anal cirri probably due to damage.

Methyl green staining. Anterior half of the prostomium deeply stained (Fig. 3B). Peristomium stained with vertical stripes (Figs. 3D and 4C). Tips of some post-chaetal lamellae deeply stained. Chaetigers 1–6 diffusely stained with scattered deeply stained cells on both dorsal and ventral sides; chaetiger 7 onward more strongly stained than chaetigers 1–6 on dorsal ventral, and lateral sides (Fig. 3B, 3D, and 4C). Unstained ventral large white spots, one pair per chaetiger, present from chaetiger 2 to posterior middle-body chaetigers (Fig. 4C).

Remarks. *Atherospio aestuarii* **sp. nov.** closely resembles *A. disticha* and *A. guillei* and ~~has an~~ intermediate morphology ~~of~~ these species. *Atherospio aestuarii* **sp. nov.** is similar to *A. disticha* and differs from *A. guillei* in having branchiae fused to the notopodial lamellae on a restricted number of segments from chaetiger 7, modified neurochaetae on chaetiger 5, and at least some bidentate neuropodial hooks with the secondary tooth below the main fang (Table 2). The form and arrangement of the modified aristate neurochaetae in double vertical rows closely resemble those found on chaetigers 4 and 5 of *A. disticha*. The new species lacks the occipital antenna present in *A. disticha*. In this respect it resembles *A. guillei*, however, that species differs in having robust neuropodial spines on chaetiger 5 and peristomial papillae, and a preponderance of unidentate neurochaetae. Both *A. guillei* and the new species have slender needle-like notochaetae in their posteriormost chaetigers. *Atherospio aestuarii* **sp. nov.** is distinguished from both congeneric species by its branchial and neuropodial hook distributions; as the last branchial chaetiger and the first chaetiger with neuropodial hook are more posterior in the former species. The other two nominal *Atherospio* species were collected from ≥ 27 m depths in the subtidal zone (Table 2), whereas the new species was unique in that it was recorded at relatively shallow depths, which included intertidal zones.

Mackie et al. (1995) and Mackie & Garwood (1995) reported two provisionally unnamed spionid taxa closely related to *A. disticha* from Cardigan Bay in the Irish Sea as ‘Spionidae gen. A’ and ‘Spionidae gen B’ and mentioned that ‘Spionidae gen. B’ is morphologically similar to *A. guillei* (as *Polydora*). Several *Atherospio* related taxa collected from Europe and Hong Kong including ‘Spionidae gen. A’ and ‘Spionidae gen B’ were referred as ‘Genus A’ and ‘Genus B’ (include *A. guillei*, but may also involve two separate taxa) in Mackie (1996). In his character matrices which provided the main characteristics of the morphology of these two groups (Mackie 1996: Tables 2 & 3), 'Genus A' and 'Genus B' are distinguished by the former lacking and the latter having posterior modified notochaetae and the former having one type of anterior modified neurochaetae but the latter having two types. *Atherospio aestuarii* **sp. nov.** does not fall into either group because it has posterior needle-like notochaetae and one type of anterior modified neurochaetae.

Etymology. The specific name *aestuarii* is from the Latin word *aestuarium*, which means the estuary, inlet, and intertidal zone, thus referring to the habitat of this species.

Habitat. Muddy and gravelly muddy sand sediment in the intertidal to subtidal zone, < 1 m in depth.

Distribution. Currently identified in Otomo-ura, Hirota Bay (Iwate Prefecture), Ago Bay (Mie Prefecture), and Yakushima Island (Kagoshima Prefecture), Japan.

Molecular phylogeny

The intraspecific p-distances in the 18S, 28S, and 16S rRNA gene sequences of the seven *A. aestuarii* **sp. nov.** specimens were 0%, 0%–0.26%, and 0%–2.20%, respectively. In the molecular phylogenetic analyses based on the concatenated sequences, the *Polydora* complex + *Pygospio*, subfamily Spioninae sensu Blake et al. (2020) + *Glandulospio*, and that plus *Marenzelleria* Mesnil, 1896, *Rhynchospio* Hartman, 1936, *Atherospio*, *Dispio*, *Scolelepis*, and *Malacoceros* were recovered as clades with robust statistical support (SH-aLRT ≥ 80%, aBayes ≥ 0.95, UFBoot ≥ 95%) (Fig. 5). *Atherospio aestuarii* **sp. nov.** formed a clade with *Dispio* Hartman, 1951 and *Scolelepis* Blainville, 1828, however, the support value for the node was not robust (SH-aLRT = 76.3, aBayes = 0.99, UFBoot = 54).

Discussion

In contrast to the ~~previous views from~~ phylogenetic analyses by Sigvaldadóttir et al. (1997) and Blake & Arnofsky (1999), ~~the results of~~ our molecular phylogenetic analysis indicated that *Atherospio aestuarii* **sp. nov.** did not form a clade distinct from the subfamilies Spioninae and Nerininae, but rather could be included within a clade that included the genera of the subfamily Spioninae sensu Blake et al. (2020) plus *Glandulospio*, *Marenzelleria*, *Rhynchospio*, *Scolelepis*, *Dispio*, and *Malacoceros* (Fig. 5). This clade corresponds to that referred to as the subfamily Spioninae in the alternative classification of the subfamily suggested by Wang et al. (2022) based on the results of molecular phylogenetic analysis. Monophyly of Spioninae sensu Wang et al. (2022) was supported also by Abe & Sato-Okoshi (2021) and the present study. However, the alternative subfamily classification suggested by Wang et al. (2022) has the following problems: (1) Nerininae sensu Wang et al. (2022) has been recovered as either monophyletic with low support (Wang et al. 2022) or as paraphyletic (Abe & Sato-Okoshi 2021, This study) and (2) if Nerininae does not include *Scolelepis*, then this subfamily is not valid because the type-genus is *Nerine* which is a junior synonym of *Scolelepis*. The paraphyly of Nerininae sensu Blake et al. (2020) is also clearly indicated by the previous (Abe & Sato-Okoshi 2021, Wang et al. 2022) and the present study. The subfamily classification of the Spionidae should be revisited with more comprehensive and robust molecular phylogenetic tree. Nevertheless, our molecular phylogenetic analysis supports previous recognitions by Mackie & Duff (1986), Radashevsky & Fauchald (2000), and Radashevsky (2012) which indicate that the members belonging to the *Pygospiopsis-Atherospio* group are not closely related to the superficially similar taxa, that is, *Polydora* and *Pygospio*, and that the heavy spines in the fifth segments of *Polydora* and *Atherospio* are not homologous sensu stricto. The possibility of a close relationship between *Atherospio* and *Dispio*/*Scolelepis* is worth further investigation through molecular phylogenetic analysis with the addition of potential closely related taxa such as *Australospio* Blake & Kudenov, 1978 (Sigvaldadóttir et al. 1997), *Lindaspio* Blake & Maciolek, 1992 (Mackie 1996), and *Pygospiopsis*, as the statistical support for the clade was not robust in the present study.

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Table and figure legends

Table 1. Terminal taxa of spionid species and outgroups (Sabellidae) used in the phylogenetic analyses and the DDBJ/EMBL/GenBank accession numbers. The classifications defined by Blake et al. (2020) and Wang et al. (2022) are also provided. The gene sequences obtained in this study are highlighted in boldface type, together with the museum registration number of the specimens.

Table 2. Taxonomic characteristics of three species in *Atherospio* Mackie and Duff, 1986 (based on Meißner & Bick 2005, Blake & Maciolek 2018).

Fig. 1. Maps of the sampling localities of *Atherospio aestuarii* **sp. nov.** (A) Japan. (B) Hirota Bay. (C) Ago Bay. (D) Yakushima Island.

Fig. 2. Photos of the sampling localities of *Atherospio aestuarii* **sp. nov.** (A) Otomo-ura in Hirota Bay, Iwate Prefecture. (B) A nameless small inlet in Ago Bay, Mie Prefecture. (C) A small fishing port at the mouth of the Kurio River in Yakushima Island, Kagoshima Prefecture.

Fig. 3. *Atherospio aestuarii* **sp. nov.** Stereomicrographs showing the morphology of preserved (A, B, D) and live (C, E, F) specimens (holotype: NSMT-Pol H-858). (A) Entire body. (B) Anterior chaetigers, dorsal view (methyl green stained). (C) Anterior chaetigers, lateral view. (D) Anterior chaetigers, lateral view (methyl green stained). (E) Chaetigers 4–11, lateral view. (F) Pygidium, lateral view. Scale bars: (A) = 2 mm; (B, D) = 1 mm; (C, E, F) = 500 µm.

Fig. 4. *Atherospio aestuarii* **sp. nov.** Light micrographs showing the morphology of living (A) and fixed (B–G) specimens (paratypes). (A) Anterior chaetigers, dorsal view (NSMT-Pol P-866). (B) Anterior chaetigers, dorsal view (methyl green stained, NSMT-Pol P-862), arrowheads indicate the nuchal organs. (C) Anterior chaetigers, ventral view (methyl green stained, NSMT-Pol P-862). (D) Neurochaetae in left parapodium from chaetiger 5, anterior view (NSMT-Pol P-866), black and white arrowheads indicate the aristate spines in the anterior and posterior row, respectively. (E) Left parapodium from chaetiger 5, anterior view (NSMT-Pol P-866). (F) Right parapodium from chaetiger 7, anterior view (NSMT-Pol P-866), arrowhead indicates the digitiform process at the distal end of the branchia. (G) Neuropodial hooded hooks from chaetiger 34 (NSMT-Pol P-860). Scale bars: (A, C) = 500 µm; (B) = 300 µm; (D–G) = 10 µm.

Fig. 5. Maximum likelihood tree inferred from concatenated sequences of nuclear 18S and 28S and mitochondrial 16S rRNA gene sequences of spionid species obtained in the present study and from the DDBJ/EMBL/GenBank database (Table 1). The gene sequences obtained in this study are highlighted in boldface. The subfamily classifications defined by Blake et al. (2020) and Wang et al. (2022) are shown in the colored bars on the right side and black, blue, red, green, and yellow bars indicate the family Spionidae, subfamilies Spioninae and

Nerininae, *Polydora* complex, and *Prionospio* complex, respectively. SH-aLRT/approximate Bayes support/ultrafast bootstrap support values of ≥ 80% / ≥ 0.95 / ≥ 95%, respectively are given beside the respective nodes. Nodes with red circles indicate triple high support values of SH-aLRT ≥ 80, approximate Bayes support ≥ 0.95, and ultrafast bootstrap support ≥ 95. The scale bar represents the number of substitutions per site. Sequences of *Amphicorina mobilis* and *Sabella pavonina* are used for outgroup rooting.

Supplementary file S1. Multiple sequence alignment of concatenated 16S, 18S, and 28S rRNA gene sequences used for molecular phylogenetic analysis.

Supplementary file S2. Raw data for morphological measurements.

Supplementary file S3. Gene sequences obtained in this study and their DDBJ/EMBL/GenBank accession numbers (As soon as the paper is published, we will publish the gene sequences deposited in DDBJ/EMBL/GenBank).

Table 1(on next page)

Terminal taxa of spionid species and outgroups (Sabellidae) used in the phylogenetic analyses and the DDBJ/EMBL/GenBank accession numbers.

The classifications defined by Blake et al. (2019) and Wang et al. (2022) are also provided. The gene sequences obtained in this study are highlighted in boldface type, together with the museum registration number of the specimens.

Table 1.

Classification by Blake et al. (2019)	Classification by Wang et al. (2022)	Genus	Species	Locality	Museum registration number	Accession number			Reference
						18S	28S	16S	
Pygospiopsis-Atherospio Group	-	*Atherospio*	*Atherospio aestuarii* **sp. nov.**	Japan (Otomo-ura)	NSMT-Pol P-861	LC685029	LC685036	LC685043	This study
				Japan (Ago Bay)	NSMT-Pol P-862	LC685030	LC685037	LC685044	This study
				Japan (Ago Bay)	NSMT-Pol P-863	LC685031	LC685038	LC685045	This study
				Japan (Ago Bay)	NSMT-Pol P-864	LC685032	LC685039	LC685046	This study
				Japan (Kurio River)	NSMT-Pol H-858	LC685033	LC685040	LC685047	This study
				Japan (Kurio River)	NSMT-Pol P-865	LC685034	LC685041	LC685048	This study
				Japan (Kurio River)	NSMT-Pol P-866	LC685035	LC685042	LC685049	This study
Subfamily Nerininae	Subfamily Nerininae	*Aonidella*	*Aonidella* cf. *dayi* Maciolek in López-Jamar, 1989	NE Atlantic		KF434504	-	KF434508	Meißner et al. (2014)
		Aonides	*Aonides oxycephala* (Sars, 1862)	France		MG913226	MG878926	MG878895	Radashevsky et al. (unpubl.)
		Aurospio	*Aurospio dibranchiata*	Kaplan, Pacific		EU340091	-	EU340087	Mincks et al. (2009)

	Maciolek, 198	Mn nodule province				
	Aurospio foodbancsia Mincks, Dyal, Paterson, Smith & Glover, 2009	West Antarctic Peninsula shelf	EU340097	-	EU340078	Mincks et al. (2009)
Laonice	*Laonice* sp. VR-2006	Sweden	DQ779655	DQ779693	DQ779619	Rousset et al. (2007)
Paraprionospio	*Paraprionospio coora* Wilson, 1990	Japan	LC545859	-	LC595689	Abe & Sato-Okoshi (2021)
	Paraprionospio patiens Yokoyama, 2007	Japan	LC545861	-	LC595691	Abe & Sato-Okoshi (2021)
Poecilochaetus	*Poecilochaetus serpens* Allen, 1904	France	AY569652	-	AY569680	Bleidorn et al. (2005),
	Poecilochaetus sp. VR-2006	France	DQ779667	DQ779705	DQ779630	Rousset et al. (2007)
Prionospio	*Prionospio dubia* Day, 1961	USA	EU418859	EU418867	-	Struck et al. (2008)
	Prionospio sp. C sensu Guggolz et al. (2020) (as *Prionospio* sp. 29 PB)	Clarion–Clipperton Fracture Zone	MK971148	-	MK971035	Bonifácio et al. (2020)
	Prionospio sp. E sensu Guggolz et al. (2020) (as *Prionospio ehlersi*)	CROZEX	EU340095	-	EU340081	Mincks et al. (2009)

		Prionospio sp. KJO-2005	USA	DQ209226	DQ209246	-	Osborn et al. (2007)
	Spiophanes	*Spiophanes* cf. *convexus* Delgado-Blas, Díaz-Díaz & Viéitez, 2019	France	MG913229	MG878931	MG878902	Radashevsky et al. (2020a)
		Spiophanes uschakowi Zachs, 1933	Russia	KM998760	MG878949	MG878915	Radashevsky et al. (2020a)
	Streblospio	*Streblospio* sp.	India	KY704336	KY704324	KY704328	Vijapure et al. (unpubl.)
	Trochochaeta	*Trochochaeta multisetosa* (Örsted, 1844)	Norway	MN296517	-	MN193552	Radashevsky et al. (2020a)
		Trochochaeta sp. THS-2006	-	DQ790097	DQ790070	-	Struck et al. (2007)
Subfamily Spioninae	*Dispio*	*Dispio remanei* Friedrich, 1956	Brazil	KU900474	KU900467	-	Rebelo & Schettini (unpubl.)
	Malacoceros	*Malacoceros fuliginosus* (Claparède, 1868)	France/Germany	AY525632	-	EF431961	Struck & Purschke (2005), Blank & Bastrop (2009)
		Malacoceros cf. *indicus* (Fauvel, 1928)	Japan	LC545857	-	LC595687	Abe & Sato-Okoshi (2021)
		Malacoceros sp. V040	Germany	MN215953	MN215954	-	Surugiu et al. (2022)
	Marenzelleria	*Marenzelleria arctia* (Chamberlin, 1920)	Russia	KJ546264	KJ546214	KJ546306	Radashevsky et al. (2014)
		Marenzelleria viridis (Verrill, 1873)	USA/Danmark	EU418860	EU418868	DQ309252	Struck et al. (2008), Bastrop & Blank (2006)

	Rhynchospio	*Rhynchospio arenicola* Hartman, 1936	USA	KJ546286	KJ546236	KJ546318	Radashevsky et al. (2014)
		Rhynchospio cf. *foliosa* Imajima, 1991 (as *Rhynchospio foliosa*)	USA	KP986489	KP986490	KP986488	Radashevsky et al. (2016a)
	Scolelepis	*Scolelepis squamata* (Müller, 1806)	Spain	MN215944	MN215960	-	Surugiu et al. (2022)
		Scolelepis texana Foster, 1971	Japan	LC545882	-	LC595712	Abe & Sato-Okoshi (2021)
Incertae sedis	*Glandulospio*	*Glandulospio orestes* Meißner, Bick, Guggolz & Götting, 2014	NE Atlantic	KF434505	-	KF434511	Meißner et al. (2014)
Subfamily Spioninae	*Boccardia*	*Boccardia proboscidea* Hartman, 1940	Japan	LC107607	AB973944	LC595721	Abe et al. (2016), Simon et al. (2019), Abe & Sato-Okoshi (2021)
		Boccardia pseudonatrix Day, 1961	France	LC682681	LC682702	LC682725	Sato-Okoshi et al. (unpubl.)
	Boccardiella	*Boccardiella hamata* (Webster, 1879)	France	LC682684	LC682705	LC682727	Sato-Okoshi et al. (unpubl.)
	Dipolydora	*Dipolydora bidentata* (Zachs, 1933)	Russia	JX228065	JX228085	JX228103	Radashevsky & Pankova (2013)
		Dipolydora giardi (Mesnil, 1893)	France	LC682685	LC682706	LC682728	Sato-Okoshi et al. (unpubl.)

Microspio	*Microspio granulata* Blake & Kudenov, 1978	Australia	KP636515	-	KP636514	Meißner & Götting (2015)
Polydora	*Polydora cornuta* Bosc, 1802	Japan	LC541483	LC541485	LC541484	Abe & Sato-Okoshi (2020)
	Polydora hoplura Claparède, 1868	Japan	LC101841	LC101854	LC101870	Sato-Okoshi et al. (2017)
	Polydora onagawaensis Teramoto, Sato-Okoshi, Abe, Nishitani & Endo, 2013	Japan	AB691768	LC682719	LC595745	Teramoto et al. (2013), Abe & Sato-Okoshi (2021), Sato-Okoshi et al. (unpubl.)
Polydorella	*Polydorella dawydoffi* Radashevsky, 1996	Vietnam	-	MG460975	MG460900	Radashevsky et al. (2020b)
Pseudopolydora	*Pseudopolydora paucibranchiata* (Okuda, 1937)	Japan	LC019991	LC019995	LC595758	Abe et al. (2016), Abe & Sato-Okoshi (2021)
	Pseudopolydora tsubaki Simon, Sato-Okoshi & Abe, 2017	Japan	AB973929	AB973937	LC107857	Simon et al. (2019)
Pygospio	*Pygospio elegans* Claparède, 1863	Russia	KJ747074	KJ747064	KJ747084	Radashevsky et al. (2016b)
	Pygospio sp. VVP-2014	USA	KJ747077	KJ747067	KJ747087	Radashevsky et al. (2016b)
Spio	*Spio filicornis* (O. F.	Greenland	FR823431	-	FR823436	Meißner et al. (2011)

			Müller, 1776)						
			Spio sp. 2573	Russia		KT200135	KT200143	KT200126	Radashevsky et al. (2016b)
Sabellidae (Outgroup)	Sabellidae (Outgroup)	*Amphicorina*	*Amphicorina mobilis* (Rouse, 1990)	Japan/Australia		AB646767	AB646766	HM800966	Yoshihara et al. (2012), Capa et al. (2011)
		Sabella	*Sabella pavonina* Savigny, 1822	-/Sweden/France		U67144	AY612632	AY340482	Nadot & Grant (unpubl.), Persson & Pleijel (2005), Rousset et al. (2007)

Table 2(on next page)

Taxonomic characteristics of three species in *Atherospio* Mackie and Duff, 1986 (based on Meißner & Bick 2005, Blake & Maciolek 2018).

Table 2.

	Species		
Character	*A. disticha* Mackie & Duff, 1986	*A. guillei* (Laubier & Ramos, 1974)	*A. aestuarii* Abe & Kan, **sp. nov.**
Prostomium: anterior margin	2 rounded lobes	2 lobes, deeply incised	2 lobes, deeply incised
Occipital antenna	Short	Absent	Absent
Peristomial papillae	Absent	Present	Absent
Anterior notopodial lamellae[1]	1–2: digitiform; 3–6: broad, triangular	1: digitiform; 3–6: broad, triangular	1: digitiform; 3–6: broad, triangular or oval
Anterior neuropodial lamellae[1]	1–2: broad, triangular; 3–6: elliptical	1: digitiform; 3–6: broadly rounded	1: digitiform; 3–6: oval to triangular
Branchial distribution[1]	7 to 11/12: broad, fully fused to dorsal lamellae	7 to 11–13: long, thick, fully fused to dorsal lamellae	7 to 18–23: long, thick, fully fused to dorsal lamellae
Modified anterior neurochaetae	Chaetigers 4–5 with double vertical row of aristate spines	Chaetiger 5 with 2–3 heavy spines and 3+ thin spines	Chaetiger 5 with double vertical row of aristate spines
Posterior neuropodial hooks	Bidentate hooded hooks with narrow, curved shaft from chaetiger 13–15	Uni- and bidentate with straight or curved shaft; hood absent; from chaetiger 15–16	Bidentate hooded hooks with narrow, curved shaft from chaetiger 16–19
Posterior needle-like notochaetae	Absent	Present	Present
Pygidium	6–9 cirri	8 cirri	Unknown
Methyl green staining	Not tested	No pattern	Prostomium, peristomium, and posterior to 7th chaetiger are clearly stained
Distribution	West coast of Scotland: 27 m, Celtic Deep: >100 m, Kattegat: 50 m	North Sea: 38–41 m, Mediterranean Sea: 44–99 m	Japan, intertidal to subtidal shallower than 1 m depth

References	Mackie & Duff (1986), Mackie et al. (1995)	Laubier & Ramos (1974), Meiβner & Bick (2005)	This study

[1] Numbers refer to the chaetigers on which the character appears.

Figure 1

Maps of the sampling localities of *Atherospio aestuarii* sp. nov.

(A) Japan. (B) Hirota Bay. (C) Ago Bay. (D) Yakushima Island.

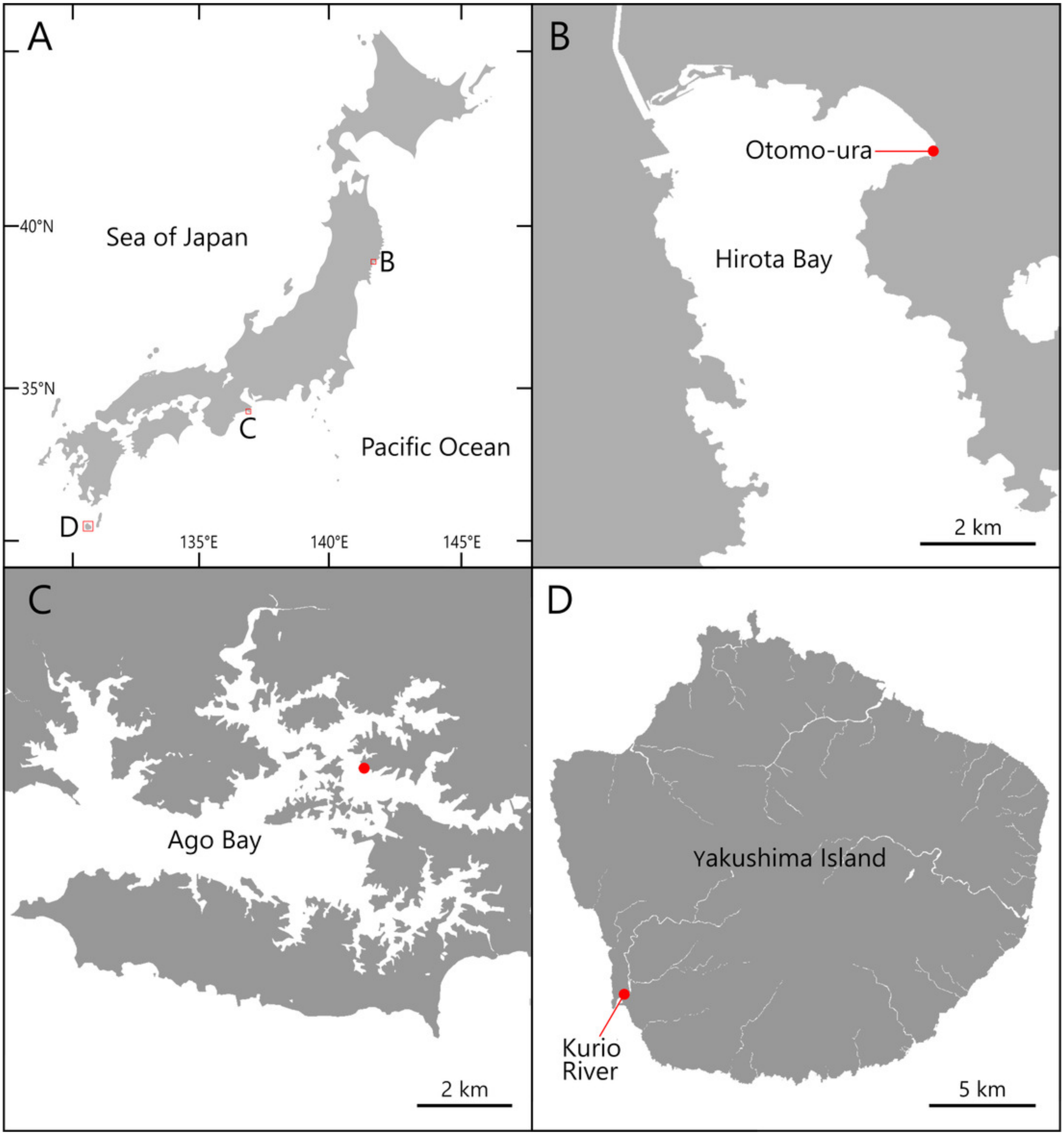

A
40°N
35°N
135°E
140°E
145°E
Sea of Japan
Pacific Ocean
B
C
D
B
Otomo-ura
Hirota Bay
2 km
C
Ago Bay
2 km
D
Yakushima Island
Kurio River
5 km

Figure 2

Photos of the sampling localities of *Atherospio aestuarii* sp. nov.

(A) Otomo-ura in Hirota Bay, Iwate Prefecture. (B) A nameless small inlet in Ago Bay, Mie Prefecture. (C) A small fishing port at the mouth of the Kurio River in Yakushima Island, Kagoshima Prefecture.

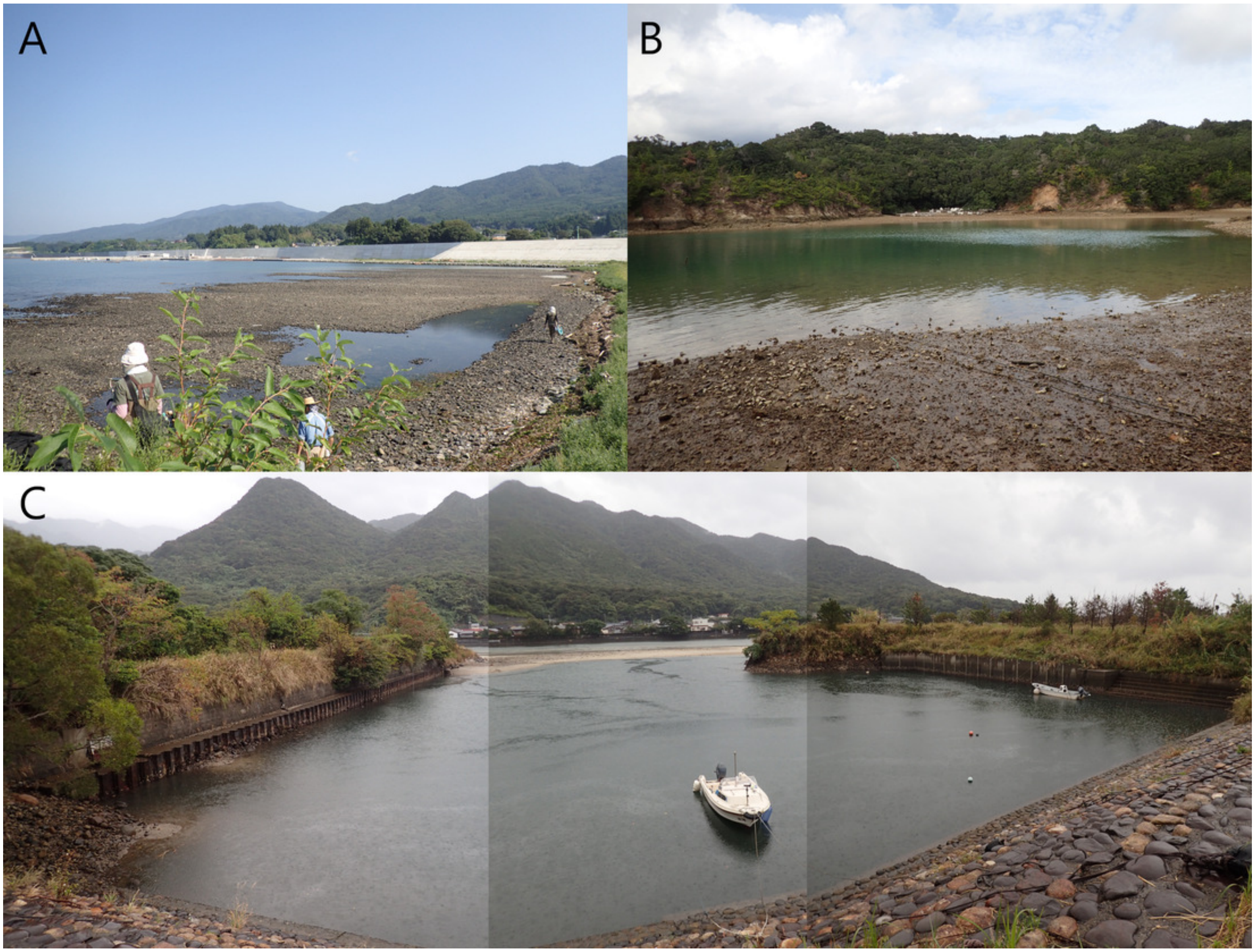


Figure 3

Atherospio aestuarii sp. nov. Stereomicrographs showing the morphology of preserved (A, B, D) and live (C, E, F) specimens (holotype: NSMT-Pol H-858).

(A) Entire body. (B) Anterior chaetigers, dorsal view (methyl green stained). (C) Anterior chaetigers, lateral view. (D) Anterior chaetigers, lateral view (methyl green stained). (E) Chaetigers 4–11, lateral view. (F) Pygidium, lateral view. Scale bars: (A) = 2 mm; (B, D) = 1 mm; (C, E, F) = 500 µm.

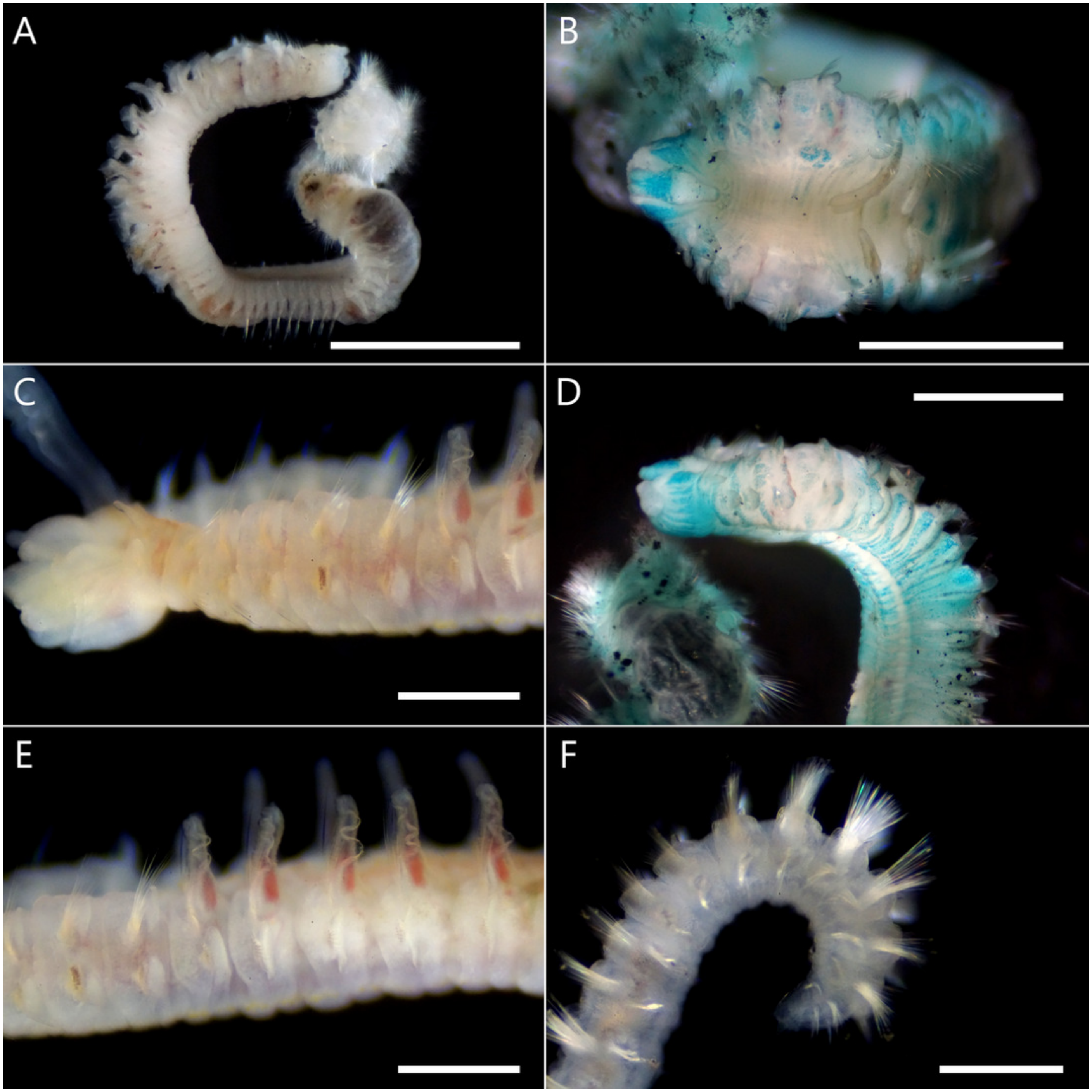

A
B
C
D
E
F

Figure 4

Atherospio aestuarii sp. nov. Light micrographs showing the morphology of living (A) and fixed (B–G) specimens (paratypes).

(A) Anterior chaetigers, dorsal view (NSMT-Pol P-866). (B) Anterior chaetigers, dorsal view (methyl green stained, NSMT-Pol P-862), arrowheads indicate the nuchal organs. (C) Anterior chaetigers, ventral view (methyl green stained, NSMT-Pol P-862). (D) Neurochaetae in left parapodium from chaetiger 5, anterior view (NSMT-Pol P-866), black and white arrowheads indicate the aristate spines in the anterior and posterior row, respectively. (E) Left parapodium from chaetiger 5, anterior view (NSMT-Pol P-866). (F) Right parapodium from chaetiger 7, anterior view (NSMT-Pol P-866), arrowhead indicates the digitiform process at the distal end of the branchia. (G) Neuropodial hooded hooks from chaetiger 34 (NSMT-Pol P-860). Scale bars: (A, C) = 500 µm; (B) = 300 µm; (D–G) = 10 µm.

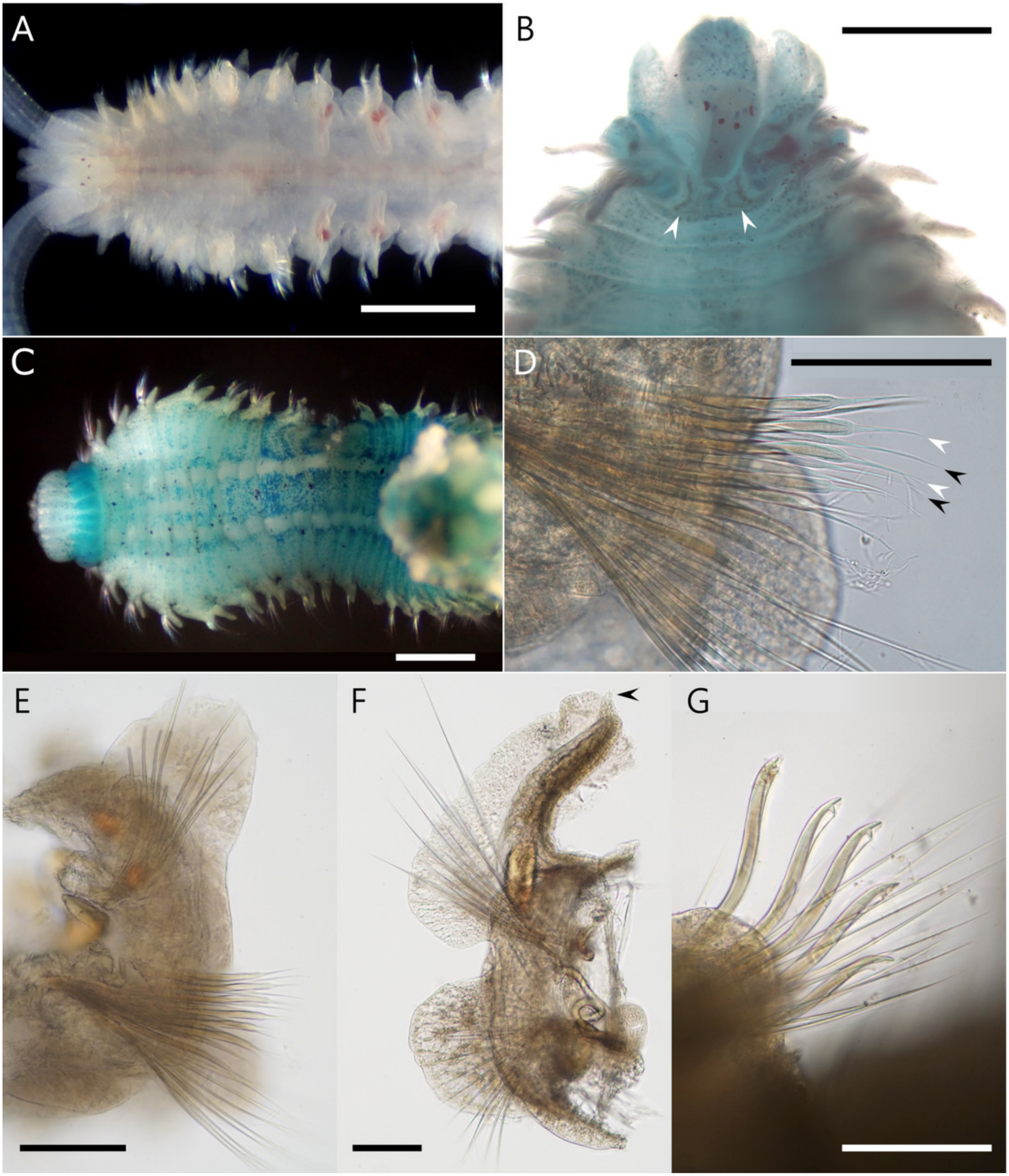
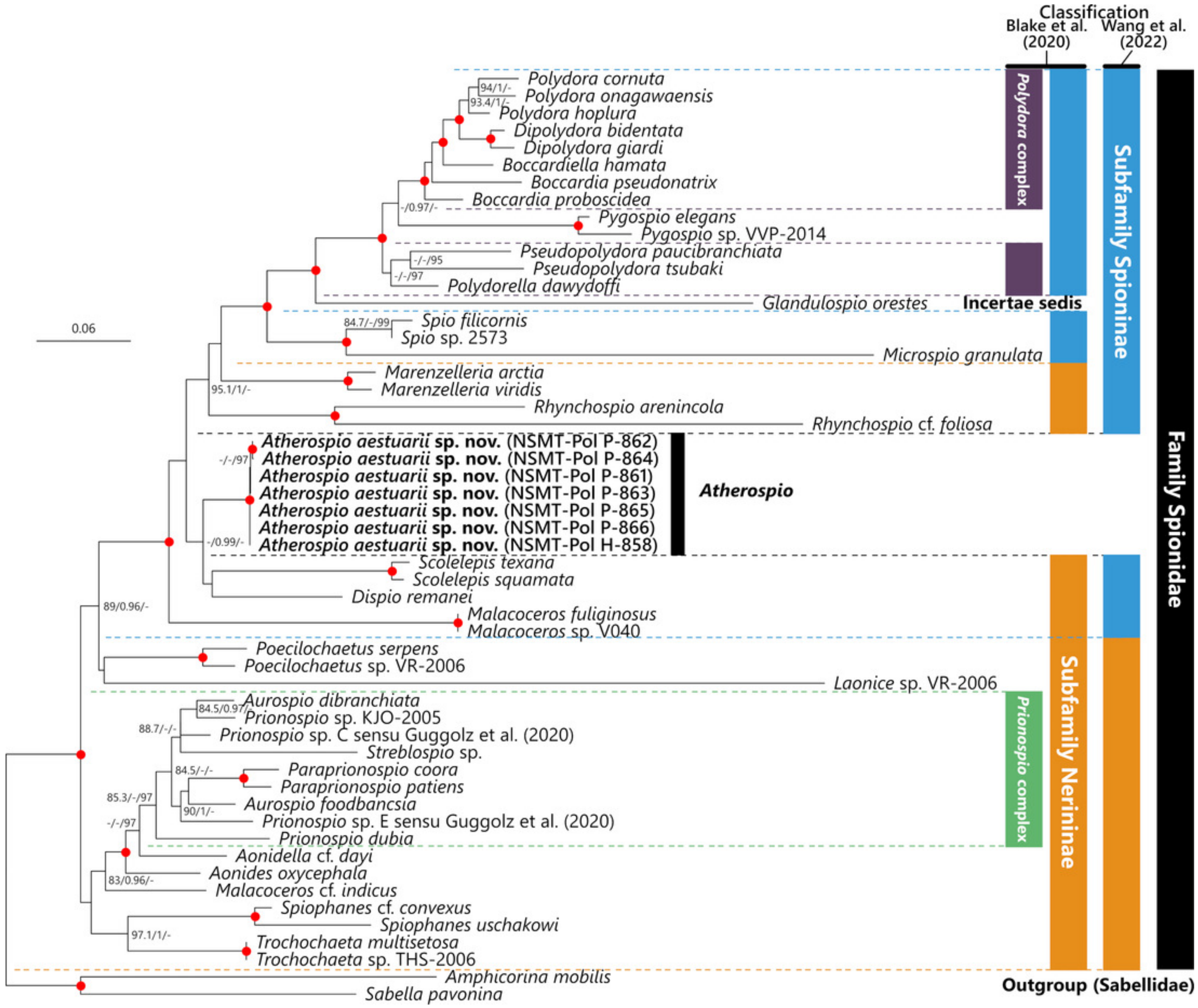

A
B
C
D
E
F
G

Figure 5

Maximum likelihood tree inferred from concatenated sequences of nuclear 18S and 28S and mitochondrial 16S rRNA gene sequences of spionid species obtained in the present study and from the DDBJ/EMBL/GenBank database (Table 1).

The gene sequences obtained in this study are highlighted in boldface. The subfamily classifications defined by Blake et al. (2020) and Wang et al. (2022) are shown in the colored bars on the right side and black, blue, red, green, and yellow bars indicate the family Spionidae, subfamilies Spioninae and Nerininae, *Polydora* complex, and *Prionospio* complex, respectively. SH-aLRT/approximate Bayes support/ultrafast bootstrap support values of ≥ 80% / ≥ 0.95 / ≥ 95%, respectively are given beside the respective nodes. Nodes with red circles indicate triple high support values of SH-aLRT ≥ 80, approximate Bayes support ≥ 0.95, and ultrafast bootstrap support ≥ 95. The scale bar represents the number of substitutions per site. Sequences of *Amphicorina mobilis* and *Sabella pavonina* are used for outgroup rooting.


Classification
Blake et al. (2020)
Wang et al. (2022)
0.06
Polydora cornuta
Polydora onagawaensis
Polydora hoplura
Dipolydora bidentata
Dipolydora giardi
Boccardiella hamata
Boccardia pseudonatrix
Boccardia proboscidea
Pygospio elegans
Pygospio sp. VVP-2014
Pseudopolydora paucibranchiata
Pseudopolydora tsubaki
Polydorella dawydoffi
Glandulospio orestes
Spio filicornis
Spio sp. 2573
Microspio granulata
Marenzelleria arctia
Marenzelleria viridis
Rhynchospio arenincola
Rhynchospio cf. foliosa
Atherospio aestuarii sp. nov. (NSMT-Pol P-862)
Atherospio aestuarii sp. nov. (NSMT-Pol P-864)
Atherospio aestuarii sp. nov. (NSMT-Pol P-861)
Atherospio aestuarii sp. nov. (NSMT-Pol P-863)
Atherospio aestuarii sp. nov. (NSMT-Pol P-865)
Atherospio aestuarii sp. nov. (NSMT-Pol P-866)
Atherospio aestuarii sp. nov. (NSMT-Pol H-858)
Scolelepis texana
Scolelepis squamata
Dispio remanei
Malacoceros fuliginosus
Malacoceros sp. V040
Poecilochaetus serpens
Poecilochaetus sp. VR-2006
Laonice sp. VR-2006
Aurospio dibranchiata
Prionospio sp. KJO-2005
Prionospio sp. C sensu Guggolz et al. (2020)
Streblospio sp.
Paraprionospio coora
Paraprionospio patiens
Aurospio foodbancsia
Prionospio sp. E sensu Guggolz et al. (2020)
Prionospio dubia
Aonidella cf. dayi
Aonides oxycephala
Malacoceros cf. indicus
Spiophanes cf. convexus
Spiophanes uschakowi
Trochochaeta multisetosa
Trochochaeta sp. THS-2006
Amphicorina mobilis
Sabella pavonina
94/1/-
93.4/1/-
-/0.97/-
-/-/95
-/-/97
84.7/-/99
95.1/1/-
-/-/97
-/0.99/-
89/0.96/-
84.5/0.97/-
88.7/-/-
84.5/-/-
85.3/-/97
90/1/-
-/-/97
83/0.96/-
97.1/1/-
Polydora complex
Incertae sedis
Subfamily Spioninae
Atherospio
Family Spionidae
Prionospio complex
Subfamily Nerininae
Outgroup (Sabellidae)