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Description of A New Species and Re-description of A Species of Fireworms (Annelida: Amphinomidae: *Chloeia*) from Hong Kong

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A new fireworm species and a poorly described species of the genus *Chloeia* are described on the basis of specimens collected from Hong Kong. The new species *C. bimaculata* n. sp. is characterized by having two distinct mid-dorsal dark spots one behind the other on each segment. It is similar to its sympatric species *C. parva*, which is characterized by having a mid-dorsal "Y"-shaped dark pigment on each segment. *C. bimaculata* n. sp. diverges from *C. parva* by 16.8% for the COI gene, 4.6% for the 16S rRNA gene and 0.6% for the 28S rRNA gene. Other *Chloeia* species with the corresponding gene sequences available in the GenBank show greater divergences.

Key words: Divergence, Polychaete, Taxonomy, South China Sea.

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BACKGROUND

Members of the polychaete worm family Amphinomidae Lamarck, 1818 are common in tropical and subtropical shallow-water ecosystems. They are often called fireworms because their

chaetae, commonly known as bristles, contain neurotoxins that produce a painful burning sensation around the area of contact with human skin. *Chloeia* Lamarck, 1818 is a genus of Amphinomidae. The type species of *Chloeia* was originally named *Aphrodita flava* Pallas, 1766 after Aphrodite (Venus) (McIntosh 1885). Lamarck (1818) established *Chloeia* which was characterized by a fusiform body and bipinnate branchiae (Barroso and Paiva 2011).

Morphological characteristics used to distinguish species of *Chloeia* include dorsal pigmentation pattern, distribution and development of branchiae, types of noto- and neurochaetae, length ratio of middle antenna/caruncle, and types of pygidial cirri (Kudenov 1995). However, as noted by previous researchers, some of these characteristics cannot always be applied in species identification. For instance, the pigmentation may fade quickly in ethanol (*e.g.*, *C. pinnata* Moore, 1911 in Kudenov 1995), and the fragile chaetae are easily broken (*e.g.*, *C. furcigera* Quatrefages, 1866 and *C. nuda* Quatrefages, 1866). To date, 42 species of *Chloeia* have been named, but only 27 species are considered valid (Read and Fauchald 2019). The original descriptions of most of these *Chloeia* species, which were published in the late 18th to early 20th century, were usually very brief or vague, therefore the validity of some of the species had been questioned by a number of researchers. For instance, McIntosh (1885) doubted the statement by Quatrefages, 1866 "that *C. furcigera* is distinguished by having bifid bristles in both dorsal and ventral series" and considered it "only shows that the true nature of these organs in the group was misunderstood, since all are morphologically bifid". Horst (1910) considered two species, *C. bengalensis* Kinberg, 1867 and *C. malaica* Kinberg, 1867, as *nomen nudum* because they were only briefly mentioned, without description nor figures. He also questioned the validity of *C. furcigera* Quatrefages, 1866, *C. inermis* Quatrefages, 1866 and *C. nuda* Quatrefages, 1866 because the species were described based on specimens that were not well preserved. McIntosh (1885) recognized *C. rupestris* Risso, 1826 and stated that "Risso afterwards described a new form (*C. rupestris*) from the Mediterranean, a fact which escaped the notice of some of his successors"; but Hartman (1959) considered this species as "indeterminable" without giving any reasons. *C. ancora* Frickhinger, 1916, characterized by the anchor-shaped marking on dorsum, was described based on specimens collected from Japan. Hartman (1959) considered this species valid, but Imajima and Hartman (1964) considered the variation in body color had "no special meaning" and treated *C. ancora* as a junior synonym of *C. flava* (Pallas, 1766).

Borda et al. (2015) conducted a phylogenetic study of Amphinomidae based on morphological and molecular (COI, 16S, 18S and 28S) characters. They found that the family could be broadly divided into two monophyletic subfamilies: Amphinominae included the genera *Amphinome*, *Hipponoa*, *Cryptonome*, *Pareurythoe*, *Hermodice*, *Eurythoe* and *Paramphinome*; and Archinominae included the genera *Notopygos*, *Archinome* and *Chloeia*. While this study has clarified the phylogenetic relationships among the genera of this family, for each of these genera there are very few sequences available in public databases allowing analyses of species-level relationships. Specifically, a search of the GenBank on 15 July 2019 revealed only 31 accessions for the genus *Chloeia*, which covered a total of eight genes (COI, 16S, 18S, 28S, extracellular globin, hemerythrin, small subunit rRNA, EF-1-alpha-1) from 5 species. Among them, only two of the species (*i.e.*, *Chloeia flava* and *Chloeia viridis*) have sequences of at least two genes.

This study was prompted by an outbreak of fireworms in Hong Kong waters in the summer of 2018, which caused concern by local swimmers (SCMP 2018). While trying to identify the fireworm specimens collected from local swimming beaches and shallow-water sandy bottoms, we discovered an undescribed species and a species that had not been described in detail. It aimed to provide morphological description for these two species. In addition, due to the recent potential utility of DNA sequences in fireworm species delimitation, the following genes sequenced are COI, 16S rRNA and 28S rRNA, and the molecular divergences are calculated among *Chloeia* species with the corresponding sequences available in the GenBank.

MATERIALS AND METHODS

Specimens

Chloeia specimens from Hong Kong were collected from either beaches or shallow waters (Table 1, Supplementary materials Fig. S1). Specimens of *C. bimaculata* n. sp. were collected from subtidal sandy bottom (~ 4 m deep) in Port Shelter off Sharp Island during night diving in 2012 and 2017. Specimens of *C. parva* were collected from Lido Beach and Anglers' Beach in Tsuen Wan during low tide in June 2018, and subtidal waters of Tolo Harbour by bottom trawling in August 2018.

Table 1. Major morphological characteristics and sampling information for two specimens of *Chloeia bimaculata* n. sp. (SWIMS-ANN-19-001, SWIMS-ANN-19-002) and twelve specimens of *C. parva* (SWIMS-ANN-19-003 to SWIMS-ANN-19-014) collected from Hong Kong, along with the holotype (BMNH 1962.3.43a) of *C. parva* and two other specimens labelled as *C. parva* in BMNH

Catalog No.	Total length (mm)	Width of chaetiger 18 (mm)	Total no. of chaetigers	Pharynx extension	Caruncle reaching chaetiger	No. of folds on central crest	First chaetiger with harpoon notochaetae	Collection date	Locality	Preservation
SWIMS-ANN-19-001	28	7	30	N	4	15	5	2017.9.30	Port Shelter	Ethanol
SWIMS-ANN-19-002	39	14	34	N	4	20	6	2013.6	Port Shelter	Ethanol
SWIMS-ANN-19-003	55	17	35	N	3	12	6	2018.6.21	Lido Beach	Ethanol
SWIMS-ANN-19-004	58	17	38	N	4	10	7	2018.6.21	Lido Beach	Ethanol
SWIMS-ANN-19-005	69	18	36	N	4	14	6	2018.7.17	Anglers' Beach	Formalin
SWIMS-ANN-19-006	97	20	39	N	5	29	6	2018.7.23	Tolo Harbor	Ethanol
SWIMS-ANN-19-007	83	20	38	N	4	24	6	2018.7.23	Tolo Harbor	Ethanol
SWIMS-ANN-19-008	65	14	37	N	4	23	7	2018.7.23	Tolo Harbor	Ethanol
SWIMS-ANN-19-009	56	16	37	Y	4	25	6	2018.8.17	Tolo Harbor	Ethanol
SWIMS-ANN-19-010	82	18	37	Y	5	21	7	2018.8.17	Tolo Harbor	Ethanol
SWIMS-ANN-19-011	41	8	34	N	4	19	6	2018.8.17	Tolo Harbor	Ethanol
SWIMS-ANN-19-012	38	8	33	N	5	25	8	2018.8.17	Tolo Harbor	Ethanol
SWIMS-ANN-19-013	53	11	35	N	5	22	6	2018.8.17	Tolo Harbor	Ethanol
SWIMS-ANN-19-014	72	20	35	N	4	24	6	2018.8.17	Tolo Harbor	Ethanol
BMNH 1962.3.43a	25	-	26	-	-	-	-	-	-	In Slide
BMNH 1933.3.2.7	72	16	38	N	5	27	6	1933.3.2	Xiamen	Ethanol
BMNH 1938.5.7.13	21	6	30	N	5	12	5	1938.5.7	Vizagapatam	Ethanol

The samples were fixed either in 95% ethanol or with 10% formaldehyde in seawater and later transferred into 75% ethanol. Type and non-type specimens of *C. parva* were loaned from the Natural History Museum in London (BMNH) for comparison.

Morphological analysis

Whole specimens were photographed using a Canon EOS 5D Mark IV camera with a Canon EF 100mm macro lens. More detailed morphological structures of the body, such as the prostomium, caruncle, dorsal pigmentation, parapodia and pygidium, were photographed using a Canon 700D camera attached to an Olympus SZX9 stereoscope through a photo tube. Parapodia of selected specimens were dissected with iris scissors and mounted on slides for observation and photography. Chaetae of selected chaetigers were mounted on slides and photographed using a True Chrome II camera attached to a Motic BA210 compound microscope. The photographs of thick materials were taken at different foci and stacked to enhance the field depth using the software Helicon Focus 6 as described in Wang et al. 2018.

Molecular analysis

Specimens of *Chloeia bimaculata* n. sp. (Catalog No.: SWIMS-ANN-19-001, SWIMS-ANN-19-002) and *C. parva* (Catalog No.: SWIMS-ANN-19-003, SWIMS-ANN-19-004), preserved in 95% ethanol, were used for DNA extraction. For each specimen, a small piece of tissue was dissected from the ventral body wall, and the genomic DNA was extracted using a DNeasy blood & tissue kit (QIAGEN). The primers LCO1490 and HCO2198 were used for amplifying the mitochondrial cytochrome oxidase I (COI) gene (Folmer et al. 1994). The primers 16SAR-L and 16SBR-H were used to amplify the mitochondrial 16S rRNA gene (Palumbi et al. 1991). The primers NLF184/21 and D3aR were used to amplify the nuclear 28S rRNA gene (Lenaers et al. 1989; Van der Auwera et al. 1994). A Zymoclean™ Gel DNA Recovery Kit was used to purify the PCR products, and the samples were then sent to BGI Hong Kong for sequencing on an ABI 310 Genetic Analyzer. DNA sequences of three genes of COI, 16S rRNA and 28S rRNA from species of *Chloeia* and three other genera of family Amphinomidae used as the outgroup were downloaded from the GenBank; all these sequences were published in previous studies (Table 2). Alignment of the sequences was conducted using the Mesquite software (Edgar 2004) based on the Muscle algorithm, and the unaligned sequences and highly

divergent regions were removed using the online Gblocks Server. The software jModeltest2 was used to evaluate the molecular evolution models for the three genes and their concatenated sequences based on the Akaike Information Criterion (AIC) (Darriba et al. 2012). The GTR+I model was the best model for the 28S rRNA gene, whereas the GTR+I+G model was as the best model for the COI, 16S rRNA gene and the concatenated sequences of the three genes. Phylogenetic analyses were conducted using the Maximum Likelihood (ML) method implemented in the software RaxmlGUI 1.5 beta based on 1,000 replicates.

Table 2. GenBank accession numbers of DNA sequences used in phylogenetic analyses

Taxa	Collection Locality/Source	Accession Number			References
		COI	16S	28S	
Amphinome rostrata (Pallas, 1766)	Uracas Island, Northern Mariana Islands	JN223394	JN223398	JN223400	Borda et al. 2012
Eurythoe complanata (Pallas, 1766)	Bocas del Toro, Panama	JN086548	JN086557	JN086529	Borda et al. 2012
Notopygos caribea Yáñez-Rivera & Carrera-Parra, 2012	Belize and Panama	KM055018	KM055046	KM055032	Borda et al. 2015
Notopygos ornata Grube, 1856	Eastern Pacific Ocean (Mexican coast)	KM055016	KM055044	KM055030	Borda et al. 2015
Chloeia flava (Pallas, 1766)	Tanabe Bay, Japan	JN852944	JN852917	EF076781	Wiklund et al. 2008
Chloeia viridis Schmarda, 1861	Florida Straights, USA	JN086546	JN086555	JN086527	Borda et al. 2012
Chloeia bimaculata **n. sp.** Holotype	Hong Kong, China	MK696607	MK696609	MK696611	This study
Chloeia bimaculata **n. sp.** Paratype	Hong Kong, China	MK696608	MK696610	MK696612	This study
Chloeia parva	Hong Kong, China	MK696601	MK696603	MK696605	This study
Chloeia parva	Hong Kong, China	MK696602	MK696604	MK696606	This study

RESULTS

Morphological Description

Order Amphinomida

Family Amphinomidae Lamarck, 1818

Subfamily Archinominae Kudenov, 1991

Genus *Chloeia* Lamarck, 1818

Type species: *Chloeia flava* (Pallas, 1766).

Type locality: Ambon Island, Indonesia.

***Chloeia bimaculata* n. sp.**

(Figs. 1, 2, 5; Tables 1, 2)

urn:lsid:zoobank.org:act:208AE0F8-1ED0-4876-80C3-92A2826FACDE

Materials examined: Two specimens collected from a subtidal sandy bottom in Port Shelter, Hong Kong (Table 1). Holotype: SWIMS-ANN-19-001, 30 chaetigers, 28 mm long, 7 mm wide excluding chaetae, collected on 30 September 2017. Paratype: SWIMS-ANN-19-002, 34 chaetigers, 39 mm long, 14 mm wide excluding chaetae, collected in June 2013.

Diagnosis: Body fusiform, with around 30 segments. Dorsum with two dark spots arranged one behind the other in each chaetiger. Prostomium with anterior and posterior lobes. Anterior lobe with a pair of palpal antennae. Posterior lobe with 2 pairs of eyes, a pair of lateral antennae, and an unpaired median antenna. Caruncle well developed, with a wider central crest and two narrower lateral crests. Branchiae first present from chaetiger 4, bipinnate. Notopodial cirri numbering one or two per anterior segment. Notochaetae thick bifurcate, harpoon or spinose. Neurochaetae bifurcate only.

Description of holotype: Fusiform in shape (Fig. 1A), both live and fixed specimen pale in color, with a unique pigmentation pattern on dorsal surface: two mid-dorsal dark spots arranged one behind the other on each chaetiger starting from the fifth; an oblique guard line present on left and right side of posterior dark spot (Fig. 1I, L, M). A pair of dark pigmented lines present on anterior margin of branchiae and notopodial chaetae fascicles in each segment, connecting guard lines in its anterior ends (Fig. 1L, M); a pair of inner pigmented lines present on posterior margin of notopodial chaetae fascicles; a pair of outer pigmented lines present on anterior margin of neuropodial chaetae fascicles (Fig. 1B, I, M).

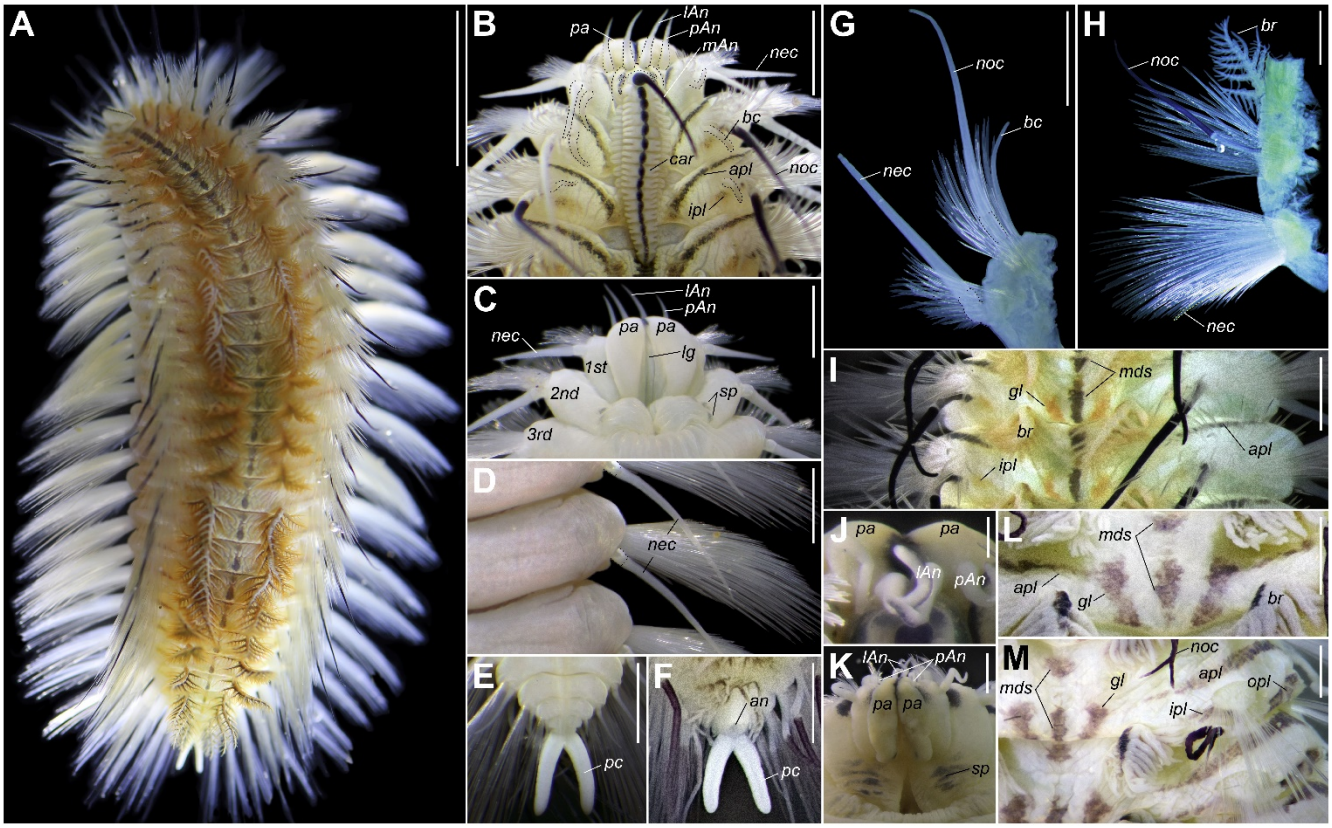

Fig. 1. *Chloeia bimaculata* n. sp. A-I, holotype (SWIMS-ANN-19-001); J-L, paratype (SWIMS-ANN-19-002). (A) living specimen, dorsal view; (B) anterior part, dorsal view; (C) anterior part, ventral view, showing two small pigment spots on chaetiger 2; (D) chaetiger 9-11, ventral view, left side; (E) pygidium, ventral view, showing two digitiform pygidial cirri; (F) pygidium, dorsal view, showing anus; (G) parapodium of chaetiger 2, left side, posterior view; (H) parapodium of chaetiger 10, left side, posterior view; (I) chaetiger 6-7, dorsal view, showing dorsal cirri and the pattern of dorsal pigmentation; (J) prostomium, showing antennae and palps; (K) anterior part, ventral view, showing black spots on chaetiger 2; (L) chaetiger 6, dorsal view, showing mid-dorsal pigmentation; (M) chaetiger 7-8, dorsal view, showing dorsal pigmentation pattern. Abbreviations: *an*, anus; a*pl*, anterior pigmented line; *bc*, branchial cirrus; *br*, branchia; *car*, carucle; *gl*, guard line; *ipl*, inner-posterior pigmented line; *lAn*, lateral antenna; *lg*, longitudinal groove; *mAn*, median antenna; *mds*, mid-dorsal spots; *nec*, neuropodial cirrus; *noc*, notopodial cirrus; *pa*, palp; *pAn*, palpal antenna; *pc*, pygidial cirrus; *opl*, outer-posterior pigmented line; *sp*, spots. Scale bars: A = 10 mm; B-I, K-M = 1 mm; J = 250 μm.

Prostomium with an anterior lobe and a posterior lobe (Fig. 1B). Anterior lobe wider than long, with a pair of cirriform and pale palpal antennae. Posterior lobe smaller, with a pair of lateral antennae arising from its anterior margin (Fig. 1B, J), stouter and slightly longer than palpal antennae. A median antenna, dark purple, arising from anterior margin of caruncle, stouter than and about twice as long as lateral antennae and ¾ as long as caruncle (Fig. 1B). Palps fused, forming a shallow mid-dorsal groove with a dark purple line on bottom (Fig. 1B, J), and a pale mid-ventral longitudinal groove extending to mouth (Fig. 1C, K). Two pairs of black eye spots trapezoidally arranged on posterior prostomial lobe (Fig. 1B). Caruncle with one wider central crest and two narrower lateral crests, each with ~15

transverse grooves; a chain of oval black spots present along entire mid-central crest (Fig. 1B). Caruncle fused to dorsum of first two chaetigers, with a free end extending posteriorly to middle of chaetiger 4 (Fig. 1B). Mouth surrounded by ventral palps and ventral side of first three chaetigers (Fig. 1C).

Parapodia biramous with widely separated dorsal and ventral rami (Fig. 1G, H). Branchial cirri inarticulate, pale in color, more dorsal to, and slender and shorter than notopodial cirri, only present in first three parapodia (Fig. 1B, G). Notopodial cirri biarticulate, located posteriorly to notopodial chaetal fascicles (Fig. 1B, G, H); cirrophore pale or blackish (Fig. 1G-I), cirrostyle pale or blackish in first two parapodia, and black in all other parapodia (Fig. 1B, G, H); cirrostyle more than 5 times as long as cirriphore in first two parapodia, about 3-4 times in all other parapodia (Fig. 1G, H). Neuropodial cirri biarticulate, located posteriorly to chaetae fascicles, pale in color; cirrophore short (Fig. 1C, D, G), cirrostyle more than 10 times the length of cirrophore from fifth parapodia (Fig. 1D).

Branchiae pale-yellowish in live specimen, bipinnate, present from fourth parapodia to posterior end (Fig. 1A); with 12-20 alternating branches arising from primary stem, each terminating in smaller branches or digitiform terminal filaments (Fig. 1H). Branchiae smaller in anterior chaetigers, best developed from middle chaetigers to near end and decreasing in size in last few chaetigers (Fig. 1A). Most branchiae with pigmentation on inner side of primary stem (Fig. 1I, L, M).

Notochaetae three types: (1) bifurcate chaetae (Fig.2A, B), with distal teeth varying in length and width, only present in first four chaetigers; (2) harpoon chaetae (Fig. 2C-G), with serrations on unilateral side of spinous stem; number of lateral serrations from ~11-12 in anterior chaetigers to 22 in middle and posterior chaetigers, present from chaetiger 5 to posterior end, most numerous in the three types; (3) spinose chaetae (Fig. 2H), without serrations, located in superior chaetal fascicle, present from anterior to posterior parapodia, several, least numerous among the three types of notochaetae. Neurochaetae thinner, longer, and more numerous than neuropodial chaetae in majority of chaetigers (Fig. 1H). Neurochaetae bifurcate chaetae only (Fig. 2I-L), similar in morphology with bifurcate notochaetae (Fig. 2A, B), distributed in all parapodia; distal teeth short and blunt in anterior parapodia, becoming longer and sharper in posterior parapodia. All notopodial and neuropodial chaetae with tubular cavity extending into teeth in bifurcate notochaetae and neurochaetae.

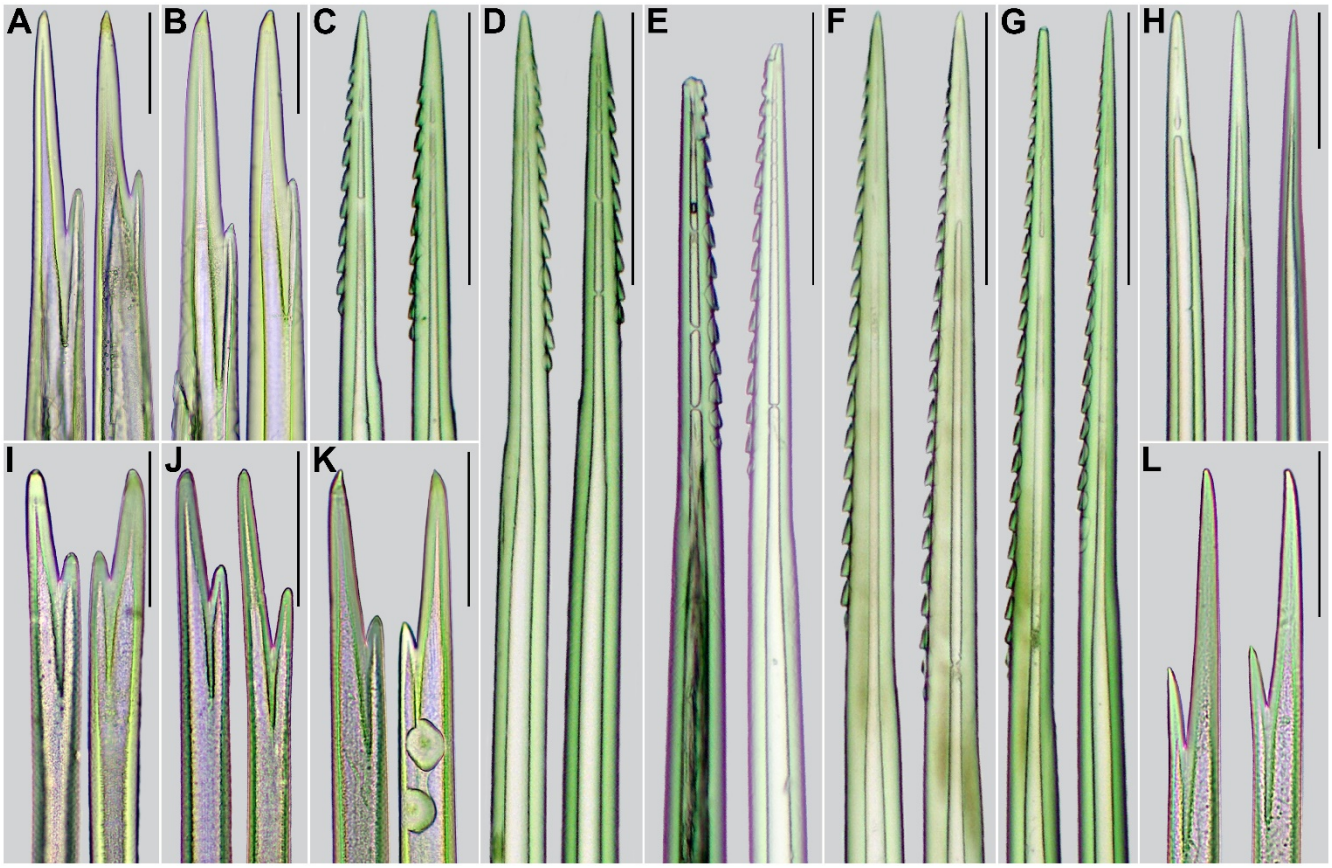

Fig. 2. *Chloeia bimaculata* n. sp. chaetae. A-L: holotype (SWIMS-ANN-19-001). (A) bifurcate notochaetae, chaetiger 2, right side; (B) bifurcate notochaetae, chaetiger 4, left side; (C) harpoon notochaetae, chaetiger 5, right side; (D) harpoon notochaetae, chaetiger 6, left side; (E) harpoon notochaetae, chaetiger 10, left side; (F) harpoon notochaetae, chaetiger 17, left side; (G) harpoon notochaetae, chaetiger 23, left side; (H) spinose notochaetae, chaetiger 5, 17 and 23, respectively; (I) bifurcate neurochaetae, chaetiger 2, right side; (J) bifurcate neurochaetae, chaetiger 10, right side; (K) bifurcate neurochaetae, chaetiger 17, left side; (L) bifurcate neurochaetae, chaetiger 23, left side. Scale bars: A-B, I-L = 50 μm; C-G = 200 μm; H = 100 μm.

Pygidium with a terminal anus on dorsal side (Fig. 1F). A pair of pygidial cirri digitiform, as long as 5 posterior chaetigers (Fig. 1E, F).

Etymology: The specific epithet *bimaculata* refers to the two mid-dorsal dark spots in each chaetiger.

Habitat: Subtidal sandy bottom, depth less than 20 meters.

Distribution: The type specimens were collected from Sharp Island in Port Shelter (Fig. S1). Based on photographs of fireworms posted on the Internet by local SCUBA divers, this species has been recorded from other locations of eastern Hong Kong waters, including Tung Ping Chau.

***Chloeia parva* Baird, 1868**

(Figs. 3-5; Tables 1, 2)

Chloeia parva, Baird 1868, p.233, pl. IV, fig. 8, a-b; McIntosh 1885, p.15; Horst 1886, p.167; Beddard 1889, p. 259; Horst 1910, p. 171; Horst 1912, p. 19, pl. VII, fig. 4, pl. VIII, figs. 1-3; Fauvel 1932, p. 56; Fauvel 1953, p. 96, fig. 46, f; Hartman 1959, p. 131; Yang and Sun 1988, p. 165, fig. 69, B-F; Liu 2008, p. 441; Barroso and Paiva 2011, p. 422, tab. 1.

Materials examined: Twelve specimens (Catalog No.: SWIMS-ANN-19-003 to SWIMS-ANN-19-014), collected from two beaches in Tsuen Wan during low tide, and subtidal waters of Tolo Harbour by trawling in 2018 (Table 1); a non-type specimen (BMNH 1933.3.2.7) collected from Xiamen (Amoy), China; a non-type specimen (BMNH 1938.5.7.13) collected from Vizagapatam, India; the holotype (BMNH 1962.3.43a) collected from unknown locality in the Indo-Pacific.

Diagnosis: Body fusiform, with around 30 segments. Dorsum with mid-dorsal "Y"-shaped dark pigmentation on each segment. Prostomium with anterior and posterior lobes. Anterior lobe with a pair of palpal antennae. Posterior lobe with 2 pairs of eyes, a pair of lateral antennae, and an unpaired median antenna. Caruncle well developed, with a wider central crest and two narrower lateral crests. Branchiae first present from chaetiger 4, bipinnate. Notopodial cirri numbering one or two per anterior segment. Notochaetae thick bifurcate, harpoon or spinose. Neurochaetae bifurcate only.

Description: Fusiform in shape, measuring 38 mm to 97 mm long, 8 mm to 20 mm wide excluding chaetae, and 33 to 39 chaetigers. Live specimens faint yellow in dorsum; chaetal fascicles similar in color with the dorsum. Fixed specimens pale, with a distinct dorsal surface pigmentation pattern: a broad dark purple line in first few chaetigers; gradually turning to the shape of Greek "Y" (upsilon) in other segments (Fig. 3A, I, K). A slightly curved guard bands present on left and right side and joint its hinder end in each segment (Fig. 3A, I, K). A pair of wide pigmented lines present on anterior margin of branchiae and notopodial chaetal fascicles in each segment, connecting guard lines in its anterior ends; a pair of inner pigmented lines present on posterior margin of branchiae and connecting guard lines; a pair of outer pigmented lines present on anterior margin of neuropodial chaetae fascicles (Fig. 3B, I).

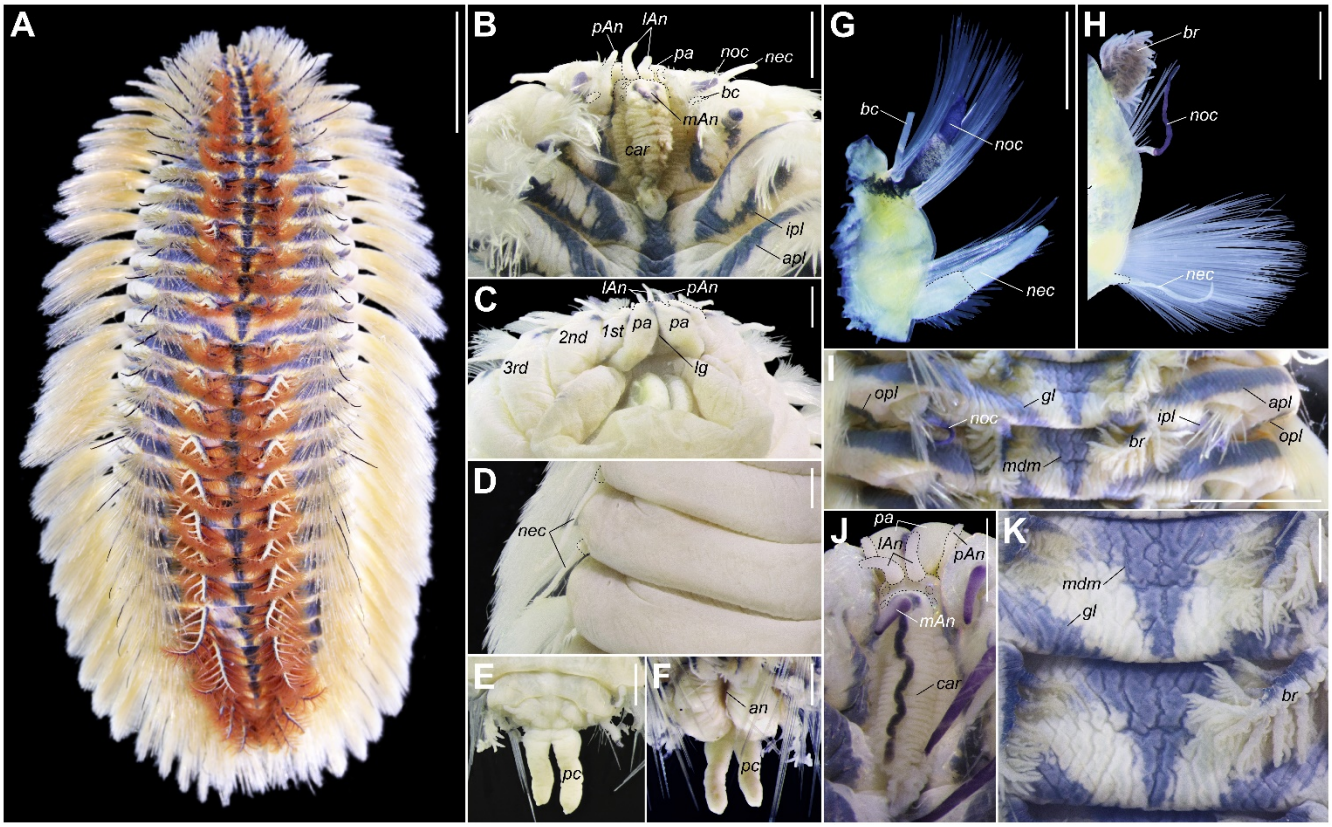

Fig. 3. *Chloeia parva*. A, J, specimen SWIMS-ANN-19-005; B-I, K, specimen SWIMS-ANN-19-003. (A) living specimen, dorsal view; (B) anterior part, dorsal view; (C) anterior part, ventral view; (D) chaetiger 7-9, ventral view, right side; (E) pygidium, ventral view, showing two finger-shaped pygidial cirri; (F) pygidium, dorsal view, showing anus (black arrow); (G) parapodium of chaetiger 2, right side, posterior view; (H) parapodium of chaetiger 10, right side, posterior view; (I) chaetiger 15-16, dorsal view, showing the pattern of dorsal pigmentation; (J) caruncle, dorsal view; (K) chaetiger 15-16, dorsal view, showing branchiae and the pattern of dorsal pigmentation. Abbreviations: *an*, anus; *apl*, anterior pigmented line; *bc*, branchial cirrus; *br*, branchia; *car*, carucle; *gl*, guard line; *ipl*, inner-posterior pigmented line; *lAn*, lateral antenna; *lg*, longitudinal groove; *mAn*, median antenna; *mdm*, mid-dorsal mark; *nec*, neuropodial cirrus; *noc*, notopodial cirrus; *opl*, outer-posterior pigmented line; *pa*, palp; *pAn*, palpal antenna; *pc*, pygidial cirrus. Scale bars: A = 10 mm; I = 5 mm; H = 2 mm; B-G, J, K = 1 mm.

Prostomium with an anterior lobe and a posterior lobe (Fig. 3B, J). Anterior lobe wider than long, with a pair of cirriform and pale palpal antennae. Posterior lobe smaller, with a pair of lateral antennae arising from its anterior margin, stouter and slightly shorter than palpal antennae (Fig. 3B, J). One median antenna arising from the anterior base of the caruncle, stouter and similar in length with lateral antennae, and about one fifth the length of the caruncle, with purple pigmentation only on posterior side (Fig. 3B, J). Palps fused, forming a shallow mid-dorsal groove with a dark purple line on bottom, and a pale mid-ventral longitudinal groove extending to mouth (Fig. 3C, J). Two pairs of dark eye spots arranged trapezoidally on posterior prostomial lobe, anterior two eyes slightly larger (Fig. 3B, J; the dark pigment of the eye spots in some specimens faded after preservation in ethanol). Caruncle

consists of a wider central crest and two narrower lateral crests, each covered with ~12 transverse folds (Fig. 3B). In specimens with an intact crest, summit of caruncle with an undulated bead-like purple line (Fig. 3J). Base of caruncle fused on the first two chaetigers and extends posteriorly to third chaetiger (Fig. 3B). Mouth surrounded by ventral palps and ventral side of first three chaetigers (Fig. 3C).
All parapodia biramous with widely separated doral and ventral rami (Fig. 3G, H). Branchial cirri inarticulate, pale in color, only present in the first three parapodia, more dorsal to, and slender and shorter than notopodial cirri (Fig. 3B, G). Notopodial cirri biarticulate, cirrophore partially marked with dark purple pigmentation, but pigmentation fades in preserved specimens; cirrostyle marked with slight purple in the first three parapodia, and in dark purple from the fourth parapodia onwards; cirrostyle about 3-4 times the length of cirrophore; all notopodial cirri located posteriorly to the chaetal fascicles (Fig. 3G, H). Neuropodial cirri biarticulated; cirrophore shorter than cirrostyle; cirrophore and cirrostyle in all parapodia pale (Fig. 3D, G, H).

Branchiae bright red in living worms; bipinnate, present from fourth parapodia to posterior end (Fig. 3A); with 10-20 alternating branches arising from primary stem, each terminating in smaller branches or digitiform terminal filaments (Fig. 3H, I, K). Branchiae smaller in anterior chaetigers, best developed in middle chaetigers to near end and decreasing in size in last few chaetigers (Fig. 3A). All branchiae with purple pigmentation marked on inner side of primary branchial stems (Fig. 3A, I, K). Notochaetae three types: (1) bifurcate chaetae (Fig. 4A-C), distal teeth varying in length and width, only present in the first five chaetigers; (2) harpoon chaetae (Fig. 4D-G), with harpoon-shaped serrations on unilateral side of the spinous stem below the apex, with ~ 7 lateral serrations in anterior chaetigers to larger than 20 lateral serrations in middle and posterior chaetigers, present from chaetiger 6 to posterior end, most numerous among the three types of chaetae; (3) spinose chaetae without serrations, located in superior chaetal fascicles, present from anterior to posterior chaetigers, several, least numerous among the three types (Fig. 4H). Neurochaetae thinner, longer, and more numerous than neuropodial chaetae in the majority of chaetigers (Fig. 3H). Neurochaetae bifurcate chaetae only (Fig. 4I-L), similar in morphology with bifurcate notochaetae (Fig. 4A-C), distributed in all parapodia; tips of distal teeth in anterior chaetiger amber, short and blunt, but longer and sharper in posterior chaetigers (Fig. 4I-L).

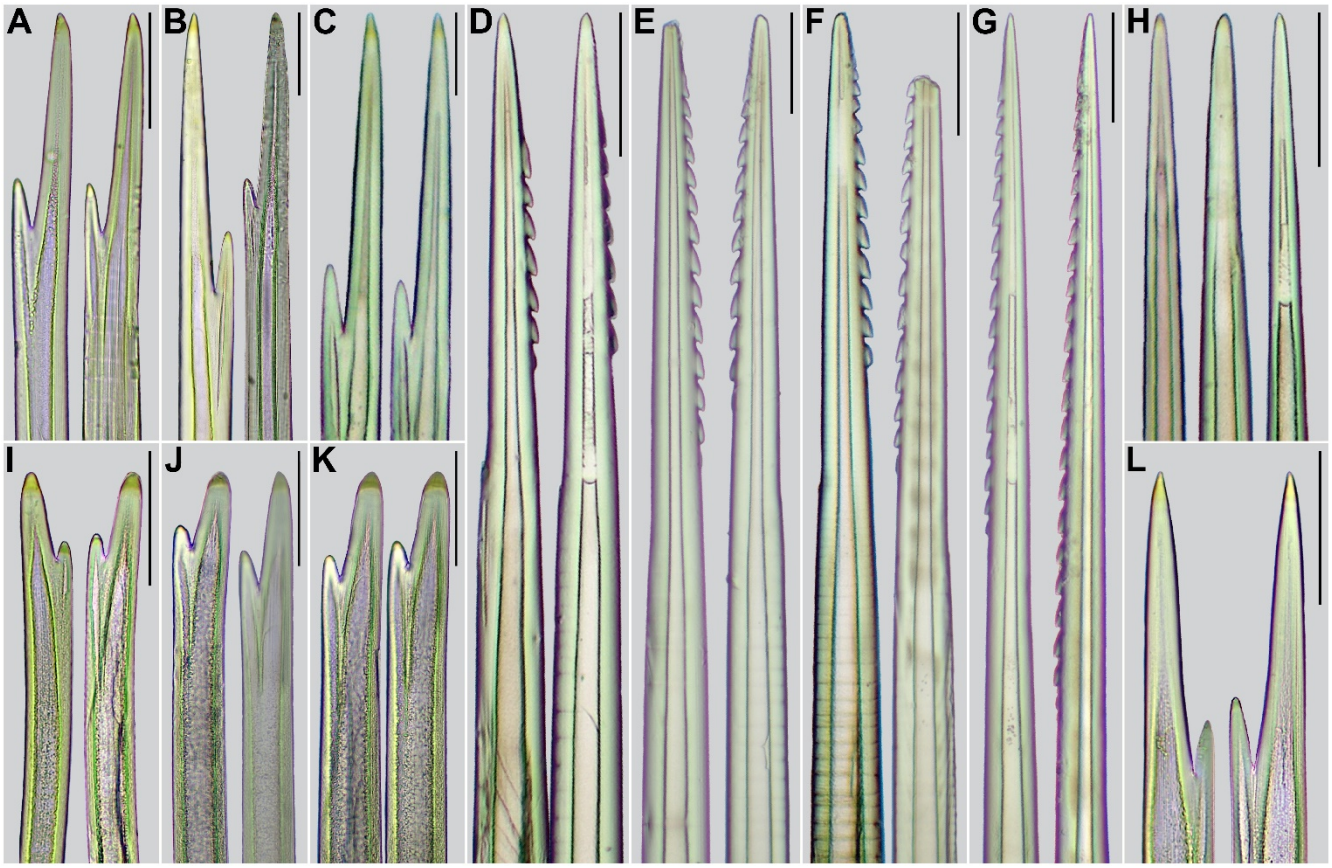

Fig. 4. *Chloeia parva* chaetae. A-L: specimen SWIMS-ANN-19-003. (A) bifurcate notochaetae, chaetiger 2, left side; (B) bifurcate notochaetae, chaetiger 4, left side; (C) bifurcate notochaetae, chaetiger 5, right side; (D) harpoon notochaetae, chaetiger 6, left side; (E) harpoon notochaetae, chaetiger 10, left side; (F) harpoon notochaetae, chaetiger 17, left side; (G) harpoon notochaetae, chaetiger 30, left side; (H) spinose notochaetae, chaetiger 5, 17 and 30, respectively; (I) bifurcate neurochaetae, chaetiger 2, left side; (J) bifurcate neurochaetae, chaetiger 10, left side; (K) bifurcate neurochaetae, chaetiger 17, left side; (L) bifurcate neurochaetae, chaetiger 30, left side. Scale bars: A-C, I-L = 50 μm; D-H = 100 μm.

Pygidium with a terminal anus on dorsal side (Fig. 3F). A pair of pygidial cirri digitiform, as long as the last 4 chaetigers (Fig. 3E, F).

Habitat: Subtidal soft bottom, depth less than 20 meters; intertidal sandy bottom.

Distribution: Our specimens were collected from beaches of Tsuen Wan, and subtidal waters of Tolo Harbour, Hong Kong during an outbreak of this species. Based on previous literature (Fauvel 1953) as well as our observation of photographs posted by divers onto the Internet, this species should be widespread in South China Sea. However, records from the Indian Ocean (Fauvel 1932) need further study.

Molecular Analysis

Phylogenetic trees (Fig. 5) were built based on partial DNA sequences of three genes [*i.e.*, *COI* (637 bp), 16S rRNA (451 bp) and 28S rRNA (958 bp)] and their concatenated sequences (2046 bp). All of the phylogenetic trees show that the *Chloeia* species form a monophyletic clade. According to the concatenated sequences, *C. bimaculata* n. sp. and *C. parva* are the most closely related within the selected species. Besides, the interspecific divergences within *Chloeia* species are 16.8-23.6% for *COI*, 4.6-10.0% for 16S, 0.6-2.4% for 28S and 6.2-10.7% for the concatenated sequences (Table S1), which is much larger than the maximum intraspecific divergences of corresponding sequences (2.6%, 0.7%; 0, and 0.9%). These analyses; therefore, support *C. bimaculata* n. sp. and *C. parva* as two distinct species.

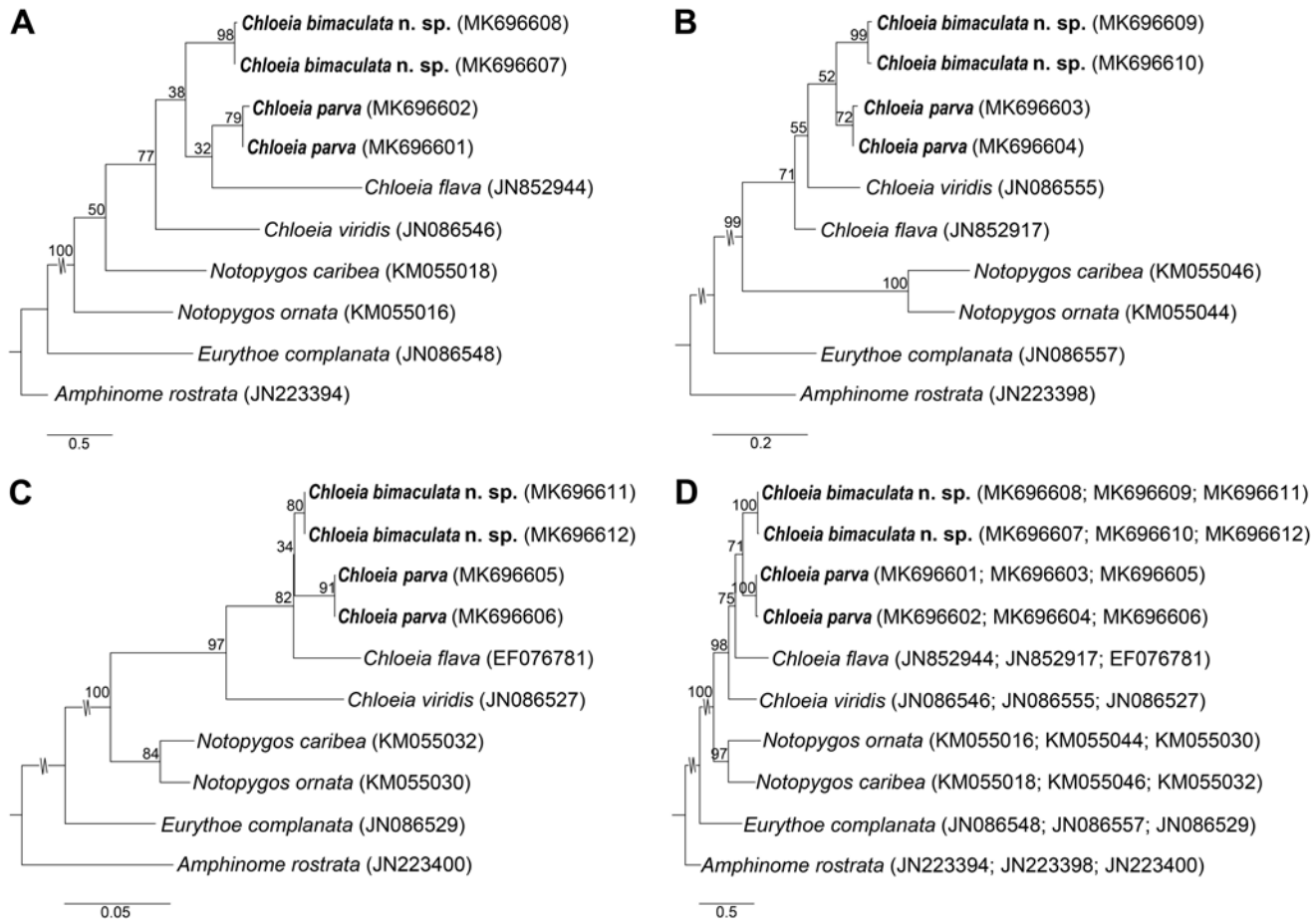

Fig. 5. Maximum likelihood (ML) phylogenetic trees based on COI (A), 16S (B), 28S (C) and their concatenated sequences (D). Numbers on the branches represent ML bootstrap values (maximum: 100) based on 1000 replicates. GenBank accession numbers of the COI, 16S and 28S genes used are shown in parentheses. Scale bar corresponds to the estimated mean number of nucleotide substitutions per site.

DISCUSSION

Chloeia bimaculata n. sp. is distinct from all other *Chloeia* species by its specific pigmentation pattern on the mid-dorsum, *i.e.*, two mid-dorsal dark spots arranged one behind the other. *Chloeia macleayi* Haswell, 1878, collected from Cape Sidmouth, Australia, is similar in dorsal pigmentation in having "in the centre, two obscure dark spots, one behind the other". However, the caruncle reaches the 4th chaetiger in *C. bimaculata* n. sp. but only the 3rd chaetiger in *C. macleayi*. The palpal antennae are thinner than the lateral antennae in *C. bimaculata* n. sp., but they were stouter than the lateral antennae in *C. macleayi*. In *C. bimaculata* n. sp. only the first parapodium has anterior surface spots, but in *C. macleayi* the first two parapodia have anterior surface spots.

There is intraspecific variation in morphological characteristics in *Chloeia bimaculata* n. sp. The paratype of *Chloeia bimaculata* n. sp. differs from the holotype in being bigger in body size, with more chaetigers (Table 1). The 6th chaetiger, rather than the 5th chaetiger, is the first chaetiger with harpoon notochaetae. There are more pigments on the lateral antennae (Fig. 1G) and ventral side of the chaetiger 2 in front of each angle of the mouth (Fig. 1K) in the paratype compared to that in the holotype (Fig. 1B, C). Pigments are darker on the guard bands, posterior side of each parapodia and the inner side of the primary branchial stems (Fig. 1L, M) in the paratype compared to that in the holotype (Fig. 1I). There are 20 transverse folds in the caruncle of the paratype, rather than 15 in the caruncle of the holotype. The dark line along the summit of the central crest of caruncle is more continuous for the paratype compared to the more bead-like line in the holotype. There are two notopodial cirri on the 2nd and 3rd chaetiger in the paratype (left side) compared to only one in the holotype.

Chloeia parva has morphological variations among geographical locations. The specimens collected from the three localities (*i.e.*, Hong Kong, unknown locality in the Indo-Pacific for the holotype, and Xiamen for a non-type material) are very similar in mid-dorsal pigmentation pattern (Fig. 3A, K; Baird 1868; Fig. S3A), as well as the color pattern in the notopodial cirri, the inner- and outer-posterior pigmented lines (Fig. 3I; Fig. S2A; Fig. S3A), and morphologies of the bifurcate neurochaetae and harpoon notochaetae (Fig. 4D-G, I-L; Fig. S2B-F; Fig. S3D, E).

Baird (1868) did not report the type locality of *Chloeia parva* Baird, 1868 in his original description of this species. He did not provide a drawing of the body including the pigmentation pattern on the dorsum, although he did clearly state that "along the centre of the back, on each segment, there is a dark mark in the shape somewhat of the Greek Y (upsilon) shaped pigmentation". Baird (1868) only drew a harpoon notochaeta and a bifurcate neurochaeta. An examination of the holotype deposited in the Natural History Museum in London (BMNH) showed that the body is now missing, with only a

parapodium mounted on a slide (BMNH 1962.3.43a) available. Based on arrangements of the chaetae and cirri, this is an anterior parapodium from the right hand side of the body. Its notopodial cirri are biarticulated, with dark purple cirrophore and light purple cirrostyle; cirrostyle about three times the length of cirrophore; neuropodial cirri missing. There are remarkable dark purple pigmentations on the inner- and outer side of the parapodium, which are similar to those of the Hong Kong specimens. This parapodium has both dorsal and ventral chaetal fascicles (Fig. S2A), with two types of chaetae (*i.e.*, bifurcate neurochaetae and harpoon notochaetae; Fig. S2B-F) on it, which agree with our specimens collected from Hong Kong. The bifurcate notochaetae have two distal teeth varying in length and width, tips amber; the notochaetae are shrunk, which might be caused by the mounting media.
BMNH 1933.3.2.7, collected from Xiamen, also agrees well with our specimens in the pigmentation pattern on the dorsum, as well as the chaetal types. Overall, a comparison with the holotype and the non-type specimen collected from Xiamen indicates that our specimens are *C. parva*.

The specimen of *Chloeia parva* BMNH 1938.5.7.13 (Table 1; Fig. S3F-L), collected from Vizagapatam, India, was originally recorded as *C. parva*. This specimen is about half the length of our smallest specimen, but has larger width/length ratio, with both anterior and posterior ends being more tapered. The pigmentation of this specimen is very light. Although it has light purple "Y" shaped marks on the mid-dorsum (Fig. S3F), it has no pigment marks that are present in the Xiamen and Hong Kong specimens of *C. parva* (*i.e.*, anterior, inner- and outer-posterior pigmented lines on the dorsum). The notopodial cirri of the Indian specimen is light purple in color, which is different from the Xiamen and Hong Kong specimens with dark purple notopodial cirri. Moreover, the slender bifurcate neurochaetae present in the Indian specimen are not present from the Xiamen and Hong Kong specimens (Fig. S3K).

CONCLUSIONS

Although fireworms are common polychaetes in tropical and subtropical ecosystems, their diversity is poorly known. In this work we described a new species and redescribed another species of *Chloeia* from Hong Kong. In addition, we provided the partial sequences of three genes (COI, 16S rRNA and 28S rRNA), which will help delimit *Chloeia* species at the molecular level and assess their phylogenetic relationship.

Acknowledgments: This work and the new species name have been registered with ZooBank under urn:lsid:zoobank.org:pub:AAE81751-3EA2-4F35-BF5A-6FD7999A1F77). We thank Tse On Anson Tang from the Hong Kong Government Lifeguard General Union and Kin Chung Jason Yau from the University of Hong Kong for assistance with collecting the *Chloeia* specimens, and Yu Zhao from Hong Kong Baptist University for taking photos. This study was supported by Shenzhen Science and Technology Innovation Committee (Project number JCYJ20170307161326613).

Authors' contributions: JWQ initiated the study. ZW, YZ, YJX and JWQ designed the study and wrote the manuscript.

Competing interests: ZW, YZ, YJX and JWQ declare they have no conflict of interest.

Availability of data and materials: Type and non-type specimens are deposited in the Swire Institute of Marine Science, the University of Hong Kong (SWIMS).

Consent for publication: All of the authors agreed to publish the paper.

Ethics approval consent to participate: Not applicable.

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Supplementary Materials

Fig. S1. Sampling sites of *Chloeia bimaculata* n. sp. from Sharp Island (blue triangle) and *C. parva* from Anglers' Beach, Lido Beach and Tolo Harbour (red circles), Hong Kong. (download)

Fig. S2. *Chloeia parva* holotype (BMNH 1962.3.43a). (A) parapodium, right side, posterior view. B-F, chaetae. (B), (C) bifurcate neurochaetae. (D) notochaetae, marked in red arrow; (E) harpoon notochaetae, teeth may be lost in some chaetae, marked in blue hollow arrow; (F) harpoon notochaetae, marked in black filled arrow. Abbreviations: *ipl*, inner-posterior pigmented line; *opl*, outer-posterior pigmented line. Scale bars: A = 1 mm; B, F = 50 μm; C = 25 μm; D, E = 100 μm. (download)

Fig. S3. Specimens of *Chloeia parva* from Xiamen (Amoy), China and Vizagapatam, India. A-E, specimen (BMNH 1933.3.2.7) from China. (A) whole worm, dorsal view; (B) whole worm, ventral view. C-E, chaetae. (C) bifurcate notochaetae, anterior chaetigers; (D) harpoon notochaetae; (E) bifurcate neurochaetae. F-L, specimen (BMNH 1938.5.7.13) from India. (F) whole worm, dorsal view; (G) whole worm, ventral view. H-L, chaetae. (H) bifurcate notochaetae, anterior chaetigers; (I) harpoon notochaetae; (J) harpoon notochaetae; (K), (L) bifurcate neurochaetae. Scale bars: A, B = 20 mm; C, H-J = 100 μm; D = 400 μm; E, K, L = 50 μm; F, G = 5 mm. (download)

Table S1. Pairwise average divergences (%) for COI (637bp), 16S rRNA (451bp), 28S rRNA (958bp) and their combined (2046bp) sequences. Maximal and minimal values of average divergences between different *Chloeia* species are marked in bold and underlined, respectively. (download)