Circular or oval? Molecular phylogenetic analysis revealed variations in middorsal spots of Chloeia flava (Pallas, 1766)

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Abstract

Chloeia flava (Pallas, 1766) is characterized by having circular middorsal spots on median segments, and researchers have different opinions on whether circular- and oval-shaped middorsal spots on median segments are intraspecific morphological variations of *C. flava*. However, molecular data are lacking to resolve this problem. Based on specimens of *C. flava* collected from Fujian, China, we conducted morphological and molecular genetic analyses, and the results clarified that middorsal spots were circular in live specimens, while they varied from circular to oval in shape among the fixed specimens. Morphological analyses also indicated that *C. flava* could be distinguished from its morphologically similar species *C. pulchella* by having different-sized middorsal spots and types of chaetae, but molecular data are still needed to test this hypothesis in future studies.

Introduction

Chloeia flava (Pallas, 1766) is one of the most well-known fireworms, probably due to its remarkable circular middorsal pigmentation on each segment (Fig. 1), its wide distribution from the Indian Ocean to the western Pacific and from northern Australia to Japan (Salazar-Vallejo, 2023), and its poisonous harpoon notochaetae (also noted in some other *Chloeia* species) which may cause a painful burning sensation and injuries to human skin (Chaloklum Diving School, 2003; Wildfactsheets, 2022). This species has been widely reported from the Indian Ocean to the western Pacific Ocean (McIntosh 1885; Horst 1912; Salazar-Vallejo 2023; Fig. 2), but researchers had different opinions on whether the oval-shaped middorsal spots are intraspecific morphological variations of *C. flava* (Horst 1912; Imajima & Hartman 1964; Salazar-Vallejo 2023), and it remains to be resolved whether *C. flava* is the senior synonym of its morphologically similar species *C. pulchella* Baird, 1868.

Many studies have indicated that both circular- and oval-shaped middorsal spots should be regarded as intraspecific variations within *C. flava*. However, these studies did not provide detailed morphological comparison between type specimens of *C. flava* and *C. pulchella* from their type localities (Bay of Bengal and northeastern Australia) (Horst 1912; Frickhinger 1916; Monro 1924; Imajima & Hartman 1964). Horst (1912) considered that Baird (1868)'s *C. pulchella* specimens from northeastern Australia agreed well in morphology with McIntosh (1885)'s *C. flava* specimens from Japan, except being smaller. Therefore he regarded *C. pulchella* originally described by Baird (1868) as young individuals of *C. flava*. This implies that oval spots are present in small specimens and during growth, they are modified into circular spots. Horst (1912) also found that some Indonesia specimens were noted with narrow oval spots and considered them as a variety "*Chloeia flava* var. *pulchella*". Frickhinger (1916) also used the variety name "*Chloeia flava* var. *pulchella*" for his Japan specimens. Although no further details of textual description or drawing were given, he was likely referring to some specimens with oval-shaped spots. Monro (1924) regarded the variation in pigmentation pattern as non-diagnostic for *Chloeia* species after seeing a wide variation in middorsal spots "from a narrow ellipse to a circle" among his *C. flava* specimens. But Monro's specimens were collected from a wide range of localities (i.e. Australia, Indonesia, and Japan), and the variation in the shape of the middorsal spots might come from more than one species. Imajima & Hartman (1964) found that their *C. flava* specimens from Japan also varied in the shapes of middorsal spot from a narrow ellipse to a circle, and suspected that Frickhinger's (1916) *Chloeia flava* var. *pulchella* specimens might actually be *C. flava*. Comparing to these studies, a recent study based on a detailed morphological comparison between type specimens of *C. flava* and *C. pulchella*, concluded that circular-/oval-shaped middorsal spots should be treated as diagnostic characteristics for distinguishing the two species because oval spots are not restricted to smaller specimens (Salazar-Vallejo 2023).

Chloeia flava has long been recorded from the southeastern China seas, and researchers have noted both circular- and oval-shaped middorsal spots in their specimens. Monro (1934) and Treadwell (1936) recorded several specimens of *C. flava* from Xiamen (Amoy) and Fuzhou (Foochow) collected by Prof. C. Ping and Prof. T. Y. Chen from Xiamen University, but the records included no further details. Uschakov & Wu (1962) recorded two specimens of *C. flava* collected from the intertidal zone of Dongshan, Fujian, and mentioned that "both specimens with circular and oval dark pigmentation on the dorsum". Yang & Sun (1988) and Sun (2018) briefly described *C. flava* based on specimens deposited in the Marine Biological Museum of the Chinese Academy of Sciences (MBMCAS), and all the *C. flava* specimens they checked were noted with either circular- or oval-shaped middorsal spots.

Since all previous studies were only based on morphological analyses, these viewpoints need tests from molecular analyses. In this study, we conducted both morphological and molecular phylogenetic analyses of 23 specimens of *C. flava* collected from the coastal waters of Dongshan Island, Fujian, China. The results supported the viewpoint that middorsal spots within *C. flava* individuals vary from circular to oval in shape.

Material and methods

Specimen collection and treatment. Twenty-three specimens of *Chloeia flava* were collected by trawling during a benthic survey from the subtidal coastal waters (19–40 m depth) of southeastern Dongshan Island, Fujian, China on 3 Nov 2021 (Table 1). Specimens were fixed and stored in 100% ethanol for further morphological and molecular analyses. Two specimens of *C. flava* (MBM287617, MBM287618) are deposited in MBMCAS, and the other 18 specimens are deposited in the specimen collections of the Marine Benthic Invertebrates Group, College of Ocean and Earth Sciences, Xiamen University (XMU-Pol-2021-302, XMU-Pol-2021-303, XMU-Pol-2021-304, XMU-Pol-2021-305, XMU-Pol-2021-306, XMU-Pol-2021-307, XMU-Pol-2021-309, XMU-Pol-2021-310, XMU-Pol-2021-311, XMU-Pol-2021-312, XMU-Pol-2021-313, XMU-Pol-2021-314, XMU-Pol-2021-315, XMU-Pol-2021-316, XMU-Pol-2021-317, XMU-Pol-2021-318, XMU-Pol-2021-319, XMU-Pol-2021-321, XMU-Pol-2021-322, XMU-Pol-2021-323, XMU-Pol-2021-324).

Table 1
Morphological data of *Chloeia flava* specimens collected from Dongshan coastal waters.

Catalog No.	Shape of dorsal spots in median segments	Length (mm)	Width without chaetae (mm)	Wet weight (g)	Total No. of chaetigers	Collection date	Preservation
MBM287617	circular	59	17	5.80	35	2021.11.3	Ethanol
MBM287618	long oval	64	13	3.75	33	2021.11.3	Ethanol
XMU-Pol-2021-302	circular	64	15	3.88	32	2021.11.3	Ethanol
XMU-Pol-2021-303	circular	55	17	4.43	34	2021.11.3	Ethanol
XMU-Pol-2021-304	circular	55	14	2.90	34	2021.11.3	Ethanol
XMU-Pol-2021-305	circular	58	15	3.64	33	2021.11.3	Ethanol
XMU-Pol-2021-306	circular	57	13	2.94	30	2021.11.3	Ethanol
XMU-Pol-2021-307	circular	68	16	4.95	34	2021.11.3	Ethanol
XMU-Pol-2021-309	circular	35	12	1.20	32	2021.11.3	Ethanol
XMU-Pol-2021-310	circular	37	15	2.08	33	2021.11.3	Ethanol
XMU-Pol-2021-311	circular	48	14	2.23	34	2021.11.3	Ethanol
XMU-Pol-2021-312	circular	49	15	3.05	34	2021.11.3	Ethanol
XMU-Pol-2021-313	oval	68	14	4.60	32	2021.11.3	Ethanol
XMU-Pol-2021-314	circular	60	13	3.47	32	2021.11.3	Ethanol
XMU-Pol-2021-315	almond	65	14	3.65	33	2021.11.3	Ethanol
XMU-Pol-2021-316	oval	67	13	3.67	33	2021.11.3	Ethanol
XMU-Pol-2021-317	oval	56	15	3.50	33	2021.11.3	Ethanol
XMU-Pol-2021-318	oval	75	18	8.01	37	2021.11.3	Ethanol
XMU-Pol-2021-319	oval	45	11	1.59	33	2021.11.3	Ethanol
XMU-Pol-2021-321	oval	71	15	5.14	34	2021.11.3	Ethanol
XMU-Pol-2021-322	oval	55	13	2.77	33	2021.11.3	Ethanol
XMU-Pol-2021-323	oval	54	13	3.03	34	2021.11.3	Ethanol
XMU-Pol-2021-324	oval	65	12	3.08	33	2021.11.3	Ethanol

Morphological analysis. Specimens were observed under a Leica EZ4W stereoscope and a Leica M165C light microscope (LM). Photographs of the whole worm were taken using a Canon EOS 6D Camera with EF 24-105mm Lens (Supplementary Fig. 1). Selected parapodia of chaetiger 2 and chaetiger 10 of XMU-Pol-2021-307 were dissected with iris scissors and mounted on slides for observation. Chaetae of chaetigers 2, 4, 5, 6, 10, 15, 16, 17, and 30 of XMU-Pol-2021-307 were removed with fine-pointed forceps and mounted on slides for observation. Photographs of the mounted parapodia and chaetae were taken using a Leica ICC50 W camera mounted on the Leica M165C light microscope. Photographs of an anterior part of a *Chloeia* specimen (SMNH95025) collected from Tanabe Bay, Japan were taken by Dr. Lena Gustavsson from the Swedish Museum of Natural History (Fig. 3D). This specimen was previously taken as *C. flava* in several studies (Table 2), but it showed consistent middorsal pigmentation with that of a *C. amphora* specimen collected from the Philippines (Fig. 3C, cited from Salazar-Vallejo, 2023: Fig. 10G), while it differed in the dorsal pigmentation in anterior segments from that of our *C. flava* specimens with oval dorsal spots in anterior segments collected from Fujian, China (Fig. 3A, B). Therefore, this specimen as well as its sequences

were treated as *C. amphora* in this study. Mophological data of more specimens from the other sea areas such as the Indian Ocean, tropical Pacific, Australia and Japan, as well as the morphological terminology used in this study follow Yáñez-Rivera & Salazar-Vallejo (2022) and Salazar-Vallejo (2023).

Table 2
Accession numbers and specimen voucher/isolate information of *Chloeia* species used in the molecular analysis. Accession numbers first used in this study will be provided once the manuscript is accepted.

Taxon	Collection Locality/Source	Specimen Voucher/Isolate	*COI*	*16S rRNA*	*18S rRNA*	*28S rRNA*	*histone H3*	Reference
**Amphinome rostrata*	Queensland, Australia	SIO-BIC A2385	JN086544	JN086560	JN086534	JN086524	—	Borda et al. 2012
**Amphinome rostrata*	Quintana Roo, Mexico	ECOSUR-OH-P0382	JN223395	JN223399	JN223397	JN223401	—	Borda et al. 2012
**Amphinome rostrata*	Northern Mariana Islands	UF_Annelida 427	JN223394	JN223398	JN223396	JN223400	—	Borda et al. 2012
**Archinome jasoni*	Lau Basin, Pacific Ocean	SIO-BIC A2375	JX028092	JX028027	KM055050	JX028131	—	Borda et al. 2013; Borda et al. 2015
**Archinome storchi*	East Pacific Rise	SIO-BIC A2389	JN086543	JN086552	JN086533	JN086523	—	Borda et al. 2012
**Cryptonome conclava*	Nile Deep Sea Fan, Egypt	SIO-BIC A2383	JN086545	JN086553	JN086535	JN086525	—	Borda et al. 2012
**Euphrosine foliosa*	Banyuls, France	SIO-BIC A2381	JN086547	JN086556	JN086538	JN086528	—	Borda et al. 2012
**Eurythoe complanata*	Bocas del Toro, Panama	SIO-BIC A2380	JN086548	JN086557	JN086539	JN086529	—	Borda et al. 2012
**Eurythoe* sp. A	Linyuan, Taiwan, China	XMU-Pol-2022-521	xxx	xxx	xxx	xxx	—	This study
Eurythoe sp. A	Linyuan, Taiwan, China	XMU-Pol-2022-522	xxx	xxx	xxx	xxx	—	This study
**Hermodice carunculata*	Carrie Bow Cay, Belize	SIO-BIC A2382	JN086549	JN086558	JN086540	JN086530	—	Borda et al. 2012
**Hipponoa gaudichaudi*	San Diego, CA, USA	SIO-BIC A2384	JN086551	JN086561	JN086542	JN086532	—	Borda et al. 2012
**Notopygos caribea*	Panama	PAN343	KM055018	KM055046	KM055064	KM055032	—	Borda et al. 2015
**Notopygos ornata*	Mexico	SIO-BIC A5399	KM055010	KM055038	KM055056	KM055024	—	Borda et al. 2015
**Paramphinome jeffreysi*	GenBank	—	AY838875	AY838840	AY838856	AY838865	—	Struck et al. 2006
**Pareurythoe borealis*	Trondheimsfjord, Norway	SIO-BIC A2379	JN086550	JN086559	JN086541	JN086531	—	Borda et al. 2012
*Amphinomidae sp. A	South China Sea	XMU-Pol-2021-027	—	xxx	xxx	xxx	OL546302	This study
*Amphinomidae sp. B	South China Sea	XMU-Pol-2021-028	OL693262	xxx	xxx	xxx	OL546303	This study
*Amphinomidae sp. C	Shandong, China	XMU-Pol-2021-344	OL964293	xxx	xxx	xxx	xxx	This study
Amphinomidae sp. C	Shandong, China	XMU-Pol-2021-345	OL964294	xxx	xxx	xxx	xxx	This study
**Chloeia amphora* (as *C. flava*)	Tanabe Bay, Japan	SMNH95025	JN852944	JN852917	EF076780	EF076781	—	Wiklund et al. 2008; Norlinder et al. 2012
Chloeia amphora (as *C. flava*)	Tanabe Bay, Japan	CHFL11_119	—	JN086554	JN086536	JN086526	—	Borda et al. 2012
**Chloeia bimaculata*	Hong Kong, China	SWIMS-ANN-19-001	MK696607	MK696609	—	MK696611	—	Wang et al. 2019
Chloeia bimaculata	Hong Kong, China	SWIMS-ANN-19-002	MK696608	MK696610	—	MK696612	—	Wang et al. 2019

* represents the taxa or specimens used in the tree of Fig. 4A; # represents the taxa or specimens used only in calculating K2P genetic distances.

Taxon	Collection Locality/Source	Specimen Voucher/Isolate	*COI*	*16S rRNA*	*18S rRNA*	*28S rRNA*	*histone H3*	Reference
Chloeia flava (as *C. viridis*)	West coast of India	GP0175	—	—	KT900279	—	—	Rengaiyan & Ingole, 2018
Chloeia flava	Dongshan, Fujian, China	MBM287617	—	xxx	xxx	xxx	OL830438	This study
Chloeia flava	Dongshan, Fujian, China	MBM287618	—	xxx	—	xxx	OL830450	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-302	OL693306	xxx	—	xxx	—	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-303	—	xxx	xxx	xxx	OL830433	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-304	—	xxx	xxx	xxx	OL830434	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-305	—	xxx	xxx	xxx	OL830435	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-306	—	xxx	—	xxx	OL830436	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-307	—	xxx	xxx	xxx	OL830437	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-309	OL693307	xxx	—	xxx	OL830439	This study
**Chloeia flava*	Dongshan, Fujian, China	XMU-Pol-2021-310	OL693308	xxx	xxx	xxx	OL830440	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-311	OL693309	xxx	xxx	xxx	OL830441	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-312	—	—	—	—	OL830442	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-313	—	xxx	—	xxx	OL830443	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-314	—	xxx	—	xxx	OL830444	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-315	—	xxx	—	xxx	OL830445	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-316	—	xxx	—	—	OL830446	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-317	OL693310	xxx	—	xxx	OL830447	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-318	—	xxx	xxx	xxx	OL830448	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-319	—	—	—	—	OL830449	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-321	—	xxx	—	xxx	OL830451	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-322	—	xxx	xxx	xxx	xxx	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-323	OL693311	xxx	xxx	xxx	xxx	This study
Chloeia flava	Dongshan, Fujian, China	XMU-Pol-2021-324	—	xxx	—	xxx	xxx	This study
**Chloeia incerta* (as *C. parva*)	Hong Kong, China	SWIMS-ANN-19-003	MK696601	MK696603	—	MK696605	—	Wang et al. 2019
Chloeia incerta (as *C. parva*)	Hong Kong, China	SWIMS-ANN-19-004	MK696602	MK696604	—	MK696606	—	Wang et al. 2019
Chloeia incerta (as *C. parva*)	Dongshan, Fujian, China	XMU-Pol-2021-325	—	xxx	—	xxx	xxx	This study

* represents the taxa or specimens used in the tree of Fig. 4A; # represents the taxa or specimens used only in calculating K2P genetic distances.

Taxon	Collection Locality/Source	Specimen Voucher/Isolate	*COI*	*16S rRNA*	*18S rRNA*	*28S rRNA*	*histone H3*	Reference
Chloeia incerta (as *C. parva*)	Dongshan, Fujian, China	XMU-Pol-2021-326	—	xxx	—	xxx	xxx	This study
**Chloeia pocicola*		—	MT822294	MT822294	MT827205	MT827204	—	Barroso et al. 2021
Chloeia sp. A (as *C.* sp. r)	Salas y Gomez Ridge, Chile	UCNSCB-6857	—	—	OM135245	OM135245	—	Canete et al. 2022 (DirectSubmission)
Chloeia sp. B (as *C. viridis*)	West coast of India	GP0169	—	—	KT900273	—	—	Rengaiyan & Ingole, 2018
Chloeia sp. B (as *C. viridis*)	West coast of India	GP0170	—	—	KT900274	—	—	Rengaiyan & Ingole, 2018
#Chloeia sp. C (as *Chloeia* sp.)	Pacific Ocean: CCZ	EBS61o-Po21	KJ736485	—	—	—	—	Janssen et al. 2015
#Chloeia sp. C (as *Chloeia* sp.)	Pacific Ocean: CCZ	NB-Po157	KJ736484	—	—	—	—	Janssen et al. 2015
#Chloeia sp. D (as *C. flava*)	Oahu, Hawaii, USA	UF:Invertebrate Zoology:5474-Annelida	MW278384	—	—	—	—	Paulay et al. 2020 (Direct Submission)
#Chloeia sp. D (as *C. flava*)	Oahu, Hawaii, USA	UF:Invertebrate Zoology:5360-Annelida	MW277801	—	—	—	—	Paulay et al. 2020 (Direct Submission)
**Chloeia viridis*	Florida Straights	UF_Annelida 478	JN086546	JN086555	JN086537	JN086527	—	Borda et al. 2012

* represents the taxa or specimens used in the tree of Fig. 4A; # represents the taxa or specimens used only in calculating K2P genetic distances.

DNA extraction, PCR amplification and DNA sequencing. All the 23 specimens of *Chloeia flava* examined for morphological analysis were used for DNA extraction (Table 1). A small part of body wall or several branchial filaments were dissected from each specimen, and genomic DNA was extracted with a DNeasy Blood & Tissue Kit (QIAGEN). Five primer pairs were used in PCR reactions to amplify the target gene fragments, i.e. PolyLCO and PolyHCO for the mitochondrial *COI* gene (Carr et al. 2011), 16SAR-L and 16SBR-H for the mitochondrial *16S rRNA* gene (Palumbi et al. 1991), 1F and 9R for the nuclear *18S rRNA* gene (Giribet et al. 1996); NLF184/21 and D3aR for the nuclear *28S rRNA* gene (Lenaers et al. 1989; Van der Auwera et al. 1994) and H3af and H3ar for the *histone H3* gene (Colgan et al. 1998). PCR products were sequenced on a ABI 3730XL DNA Sequencer at Xiamen Borui Biological Technology Co., Ltd.

Genetic distances and phylogeny. The sequences of the five genes from 24 selected amphinomids including 23 specimens of *C. flava* analyzed in this study deposited in GenBank (https://www.ncbi.nlm.nih.gov/) were downloaded for calculating genetic distances within *Chloeia* and analyzing phylogenetic relationships (Table 2). Several species names of *Chloeia* sequences used in the phylogenetic analyses were relabeled according to existing sequences or by comparing morphological characteristics with known species (Table 2; Fig. 3). The five gene sequences were respectively aligned using MUSCLE implemented in the software MEGA X v. 10.1.7 (Kumar et al. 2018), and poorly aligned positions were removed with Gblocks implemented in the software PhyloSuite v. 1.2.2 (Zhang et al. 2020). The K2P genetic distances (Kimura 1980) among *Chloeia* species were estimated based on each gene sequence (i.e. 590-bp *COI*, 315-bp *16S rRNA*, 1284-bp *18S rRNA*, 736-bp *28S rRNA*, and 242-bp *histone H3*) using MEGA X.

Phylogenetic trees were constructed using the Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The best fitting nucleotide-substitution models were chosen using the software jModelTest v.2.1.1 (Darriba et al. 2012). The ML analysis of the concatenated 3,556 bp dataset was conducted using the "GTR + I + G" model and the thorough bootstrap method for 1,000 pseudoreplicates via raxmlGUI v.1.5b2 (Silvestro & Michalak 2012). For the BI analysis, the same model as in the ML analysis was used and Markov Chains were run for 10, 000, 000 generations with topologies being sampled every 1,000 generations using MrBayes v.3.2.0 (Ronquist & Huelsenbeck 2003). The first 25% trees were discarded as "burn-in" and software Tracer v.1.7.1 (Rambaut et al. 2018) was used to check for the convergence of the trees.

Results

Genetic distances. Table 3 showed the average K2P genetic distances for five genes among *Chloeia* species. *COI* showed higher average K2P genetic distances than the other four gene markers. The average interspecific distances ranged from 16.4% (*C. conspicua* & *C. incerta*) to 28.4% (*C. conspicua* & *Chloeia* sp. D) for *COI* (590 bp), from 6.56% (*C. incerta* & *C. conspicua*) to 16.2% (*C. pocicola* & *C. conspicua*) for *16S rRNA* (315 bp), from 0.0% (*Chloeia* sp. A & *C. viridis*) to 1.28% (*C. pocicola* & *C. conspicua*) for *18S rRNA* (1284 bp), from 0.48% (*C. conspicua* & *C. incerta*) to 3.95% (*C. pocicola* & *C. conspicua*) for *28S rRNA* (736 bp), and for *histone H3* (242 bp), the K2P distance was 13.06% between *C. incerta* and *C. flava.* The average intraspecific K2P genetic distances of *C. flava* (0.48% for *COI*, 0.13% for *16S rRNA*, 0.01% for *18S rRNA*, 0.00% for *28S rRNA*, and 0.96% for *histone H3*), *C. conspicua* (1.03% for *COI*,

1.00% for *16S rRNA*, and 0.00% for *28S rRNA*), *C. incerta* (2.44% for *COI*, 0.39% for *16S rRNA*, 0.09% for *28S rRNA*, and 1.25% for *histone H3*), *Chloeia* sp. B (0.00% for *18S rRNA*), *Chloeia* sp. C (0.86% for *COI*), and *Chloeia* sp. D (0.51% for *COI*) are much smaller than the respective interspecific distances for each gene sequence among *Chloeia* species as listed in Table 3.

Table 3
Pairwise average K2P genetic distances for *COI* (590 bp), *16S rRNA* (315 bp), *18S rRNA* (1284 bp), *28S rRNA* (736 bp) and *histone H3* (242 bp) gene sequences of *Chloeia* species.

Species	N	Species							
		1	2	3	4	5	6	7	8
COI(590 bp)									
1. *Chloeia bimaculata*, Hong Kong, China)	2	0.0103							
2.Chloeia flava(Dongshan, Fujian, China)	6	0.2214	0.0048						
3. *Chloeia incerta* (as *C. parva*, Hong Kong, China)	2	0.1640	0.2307	0.0244					
4. *Chloeia pocicola* (Southern Brazilian coast)	1	0.2343	0.2371	0.2478	—				
5. *Chloeia amphora* (as *C. flava*, Tanabe Bay, Japan)	1	0.2251	0.2374	0.2005	0.2364	—			
6. *Chloeia* sp. A (Pacific Ocean: CCZ)	2	0.2516	0.2362	0.2295	0.2314	0.2291	0.0086		
7. *Chloeia* sp. C (as *C. flava*, Oahu, Hawaii, USA)	2	0.2840	0.2388	0.2506	0.2626	0.2458	0.2784	0.0051	
8. *Chloeia viridis* (Gulf of Mexico)	1	0.2162	0.2177	0.2193	0.2672	0.2307	0.2398	0.2455	—
16S rRNA(315 bp)									
1. *Chloeia bimaculata*, Hong Kong, China)	2	0.0100							
2.Chloeia flava(Dongshan, Fujian, China)	21	0.0933	0.0013						
3. *Chloeia incerta* (as *C. parva*, Hong Kong, China)	4	0.0656	0.0878	0.0039					
4. *Chloeia pocicola* (Southern Brazilian coast)	1	0.2420	0.2276	0.2312	—				
5. *Chloeia amphora* (as *C. flava*, Tanabe Bay, Japan)	2	0.0986	0.0767	0.0709	0.2237	0.0000			
6. *Chloeia viridis* (Gulf of Mexico)	1	0.0764	0.0915	0.0968	0.2108	0.0988	—		
18S rRNA(1284 bp)									
1. *Chloeia flava* (As *C. viridis*, West coast of India)	1	—							
2.Chloeia flava(Dongshan, Fujian, China)	10	0.0008	0.0001						
3. *Chloeia pocicola* (Southern Brazilian coast)	1	0.0128	0.0120	—					
4. *Chloeia amphora* (as *C. flava*, Tanabe Bay, Japan)	2	0.0048	0.0040	0.0112	0.0000				
5. *Chloeia* sp. A (as *C.* sp. r, Salas y Gomez Ridge, Chile)	1	0.0040	0.0032	0.0088	0.0024	—			
6. *Chloeia* sp. B (As *C. viridis*, West coast of India)	2	0.0032	0.0024	0.0096	0.0016	0.0008	0.0000		
7. *Chloeia viridis* (Gulf of Mexico)	1	0.0040	0.0032	0.0088	0.0024	0.0000	0.0008	—	
28S rRNA(736 bp)									
1. *Chloeia bimaculata* (Hong Kong, China)	2	0.0000							
2.Chloeia flava(Dongshan, Fujian, China)	20	0.0152	0.0000						
3. *Chloeia incerta* (as *C. parva*, Hong Kong, China)	4	0.0048	0.0201	0.0009					
4. *Chloeia pocicola* (Southern Brazilian coast)	1	0.0395	0.0526	0.0416	—				
5. *Chloeia amphora* (as *C. flava*, Tanabe Bay, Japan)	2	0.0083	0.0124	0.0103	0.0453	0.0000			
6. *Chloeia* sp. A (as *C.* sp. r, Salas y Gomez Ridge, Chile)	1	0.0251	0.0294	0.0272	0.0424	0.0280	—		
7. *Chloeia viridis* (Gulf of Mexico)	1	0.0223	0.0294	0.0215	0.0424	0.0251	0.0252	—	
Histone H3(242 bp)									
1.Chloeia flava(Dongshan, Fujian, China)	22	0.0096							
2. *Chloeia incerta* (as *C. parva*, Hong Kong, China)	2	0.1306	0.0125						

Phylogenetic trees. Taking *Euphrosine foliosa* as the outgroup (Wiklund et al. 2008), two phylogenetic trees were reconstructed based on 23 and 54 specimens of amphinomids respectively, and both trees showed similar phylogenetic topologies (Fig. 4A, B). Two subfamilies Amphinominae Savigny *in* Lamarck, 1818 and Archinominae Kudenov, 1991 were classified with high support values (A, BS/BPP = 89/1, 100/1; B, BS/BPP = 90/1, 100/1). Five *Chloeia* species (i.e. *C. viridis*, *C. conspicua*, *C. incerta*, *C. amphora*, and *C. flava*) were clustered in a monophyletic clade in the left tree (Fig. 4A), and seven *Chloeia* species (i.e. *Chloeia* sp. A, *C. viridis*, *C. conspicua*, *C. incerta*, *Chloeia* sp. B, *C. amphora*, and *C. flava*) were clustered in a monophyletic clade in the right tree (Fig. 4B). The monophyletic clades in both trees were here regarded as *Chloeia* (restricted). In both trees, two deep-sea species, *C. pocicola* from the Southern Brazilian coast (745 m to 775 m) and Amphinomidae sp. B from the South China Sea (1167 m) were first clustered in a single clade and then this clade was sister to the genus *Archinome*. A *Chloeia* specimen (GP0175) originally regarded as *C. viridis* from western coast of India was clustered within the clade of *C. flava*; another two specimens of *Chloeia* (GP169, GP170), which were also regarded as *C. viridis*, were here taken as *Chloeia* sp. B clustered with the clade including *C. amphora* and *C. flava* (Fig. 4B).

Systematics

Family Amphinomidae Savigny in Lamarck, 1818

Subfamily Archinominae Kudenov,

Genus Chloeia Savigny in Lamarck, 1818

Type species: *Amphinome capillata* Bruguière, 1789, by monotypy (junior synonym of *Aphrodita flava* Pallas, 1766).

Type locality: Pallas indicated (1766: 98) that he had one specimen from the Bengal Bay and another one from Amboina. His specimens would be syntypes and the type locality would involve both places (ICZN 1999: Art. 76.1).

Chloeia flava (Pallas, 1766**)**

(Figs. 1–6, Tables 1–3)

Aphrodita flava Pallas, 1766a: 97–102, Pl. 8, Figs. 7–11.

Amphinome capillata Bruguière, 1789: 45–46 (unnecessary repl. name).

Chloeia capillata: Savigny 1822: 58–59; Milne-Edwards 1837, Pl. 9, Fig. 1.

Terebella flava: Milne-Edwards 1837:31.

Chloeia flava: de Quatrefages 1866: 386–388, Pl. 17, Fig. 4; Willey 1905: 244–245, Pl. 1, Figs. 1, 2 (syn.); Kinberg (1910: 33, Pl. 11, Fig. 1); Augener 1926: 436; Fauvel 1932: 55; Fauvel 1953: 96, Fig. 46d, i; Hartman 1959: 131; Amoureux et al. 1978: 73; Barroso & Paiva 2011: 422, Table 1 (*partim*); Yáñez-Rivera & Salazar-Vallejo 2022: 516–517, Fig. 6; Salazar-Vallejo 2023: 45–49, Figs. 1D, 19, 20.

Chloeia ceylonica Grube, 1874: 326.

Chloeia flava var. *pulchella* Horst, 1912: 19, Plate VII Fig. 3; Frickhinger, 1916: 233.

Chloeia flava pulchella: Sabith et al. 2022: 21–24, Figs. 2, 3 (*non* Baird, 1868).

Material examined. Twenty-three specimens (voucher numbers see Table 1), all complete, 35–75 mm long, 11–18 mm wide, 30–37 chaetigers, Dongshan, Fujian (23°30'N, 117°19'E ~ 23°42'N, 117°35'E), bottom trawl, 19–40 m, sandy and muddy sand, 3 Nov 2021, coll. Zhi Wang.

Diagnosis (modified from Yáñez-Rivera & Salazar-Vallejo 2022). Body fusiform. Middorsal pigmentation in median segments circular, progressively oval-shaped anteriorly and posteriorly. Middorsal spots oval to circular, surrounded by a thin pale margin in live specimens, pale margin expanded in posterior half of each segment in fixed specimens. Bipinnate branchiae from chaetiger 4. Ventral cirri of similar size throughout body. Notochaetae thick bifurcate, harpoon or spinose. Short tine of harpoon notochaetae very short, like a spur. Neurochaetae bifurcate only.

Description (based on XMU-Pol-2021-307, unless otherwise stated). Specimen complete, long, fusiform (Fig. 5A), pale, 68 mm long, 16 mm wide without chaetae, 34 chaetigers.

Dorsum with black spots middorsally, one per segment, oval in chaetigers 4–12, circular in chaetigers 13–21, oval from chaetigers 22 to posterior end, displaced posteriorly on each segment (Fig. 5A). Middorsal spots circular in live specimens (Fig. 1), circular or oval in median segments in fixed specimens (Supplementary Fig. 1). Each black spot surrounded by a pale thin margin in live specimens, pale margin expanded in posterior half of each segment in fixed specimens (Fig. 5A, D-H). Anterior edge of pale margin surrounded by a semi-circular obscure dark pigmentation (guard lines) in each segment, connecting a pair of wide pigmented lines on anterior margin of branchiae and notopodial chaetal fascicles on each segment (Fig. 5D-H); a pair of inner pigmented lines present on posterior margin of notopodial chaetal fascicles; a pair of outer pigmented lines present on anterior margin of neuropodial chaetae fascicles (Fig. 5B, H, J).

Prostomium with anterior and posterior lobes (Fig. 5B). Anterior lobe with cirriform palps, pale; posterior lobe with lateral antennae arising from its anterior margin, pigmented dorsally, as long as palps; median antenna fully pigmented, arising from anterior margin of caruncle, slightly stouter and about as long as lateral antennae, and 2/5 as long as caruncle (Fig. 5B). Two pairs of black eyes, trapezoidally arranged on posterior prostomial lobe (Fig. 5B). Caruncle with one wider median lobe (crest) and two narrower lateral lobes (crests), each with ~ 35 transverse folds; a chain of black spots present along mid-central lobe (Fig. 5B). Caruncle fused to dorsum of first two chaetigers, with a free end extending posteriorly to middle of chaetiger 4 (Fig. 5B). Lips fused, forming a shallow middorsal groove, and a mid-ventral longitudinal groove extending to mouth (Fig. 5B, C). Mouth surrounded by ventral lips and ventral side of first three chaetigers (Fig. 5B).

Parapodia biramous with widely separated dorsal and ventral rami (Fig. 5I, J). Cirriform branchiae pale, more dorsal to, and slender and shorter than notopodial cirri, only present along chaetigers 1–3 (Fig. 5B). Notopodial cirri biarticulate, dorsally pigmented, located posteriorly to notopodial chaetal fascicles (Fig. 5B, I, J); cirrophore with several rings (Fig. 5I, J), cirrostyle more than 5 times longer than cirrophore in middle parapodia in live specimens. Neuropodial cirri biarticulate, located below neurochaetal fascicles, pale; cirrophore short, cirrostyle more than 10 times longer than cirrophore in middle parapodia (Fig. 5L).

Branchiae bright or dark red in live specimens, bipinnate, present from chaetiger 4 to posterior end (Fig. 5A); with 12–20 alternating branches arising from primary stem, each terminating in smaller branches or digitiform terminal filaments (Fig. 5A, H-G). Branchiae smaller in anterior chaetigers, best developed from median chaetigers to near end and decreasing in size in last few chaetigers (Fig. 5A, H-G). All branchial stem pale, smaller branches pale or yellowish-brown (Fig. 5A, H-G).

Notochaetae three types: (1) bifurcates (Fig. 6A, B), longer tines 3.5-6.0 times longer than shorter tines, only present in first four chaetigers; (2) harpoon chaetae (Fig. 6C-G), with serrations on one side and a very small tine (or "spur") opposite to serration side; 5–10 denticles in anterior chaetigers to ~ 30 in middle and posterior chaetigers, present from chaetiger 5 to posterior end; (3) spinose chaetae (aciculars) (Fig. 4H), without serrations, located in superior chaetal fascicle, present from anterior to posterior parapodia, several in number, with or without tiny tine ("spur"). Subterminal region of some notochaetae yellowish, throughout body (Fig. 5J, K; Fig. 6B, C, E-H). All notochaetae with tubular cavity extending into longer tines (Fig. 6A-H). Neurochaetae thinner, longer, and more numerous than notopodial chaetae in most chaetigers (Fig. 5J). Neurochaetae bifurcates (Fig. 4I–L), similar to bifurcate notochaetae (Fig. 6A, B), tines short, blunt in anterior parapodia, becoming longer and sharper in posterior parapodia (Fig. 6I-L).

Pygidium with anus dorsal (Fig. 3M); anal cirri digitiform, four times longer than wide (Fig. 5M, N).

Variation. The 23 *C. flava* specimens collected from Dongshan coastal waters had 30–37 chaetigers, were 35–75 mm long and 11–18 mm wide. Middorsal black spots varied in the ratio of longitudinal length/width (from 1.0 to 3.2) among chaetigers and among specimens. In some specimens (e.g. XMU-Pol-2021-302, 64 mm long; XMU-Pol-2021-307, 68 mm long; and XMU-Pol-2021-310, 37 mm long), circular black spots are present in middle segments; while in some other specimens (e.g. MBM287618, 64 mm long; XMU-Pol-2021-316, 67 mm long; and XMU-Pol-2021-319, 45 mm long), only oval black spots are present throughout the worm dorsum. The sizes of these *C. flava* specimens as shown in Table 1 indicated that the shape of middorsal spots in median segments were not size-dependent, i.e. circular and oval middorsal spots could be noted in both large- and small-sized specimens.

According to previous studies, 12 specimens of *C. flava* originally collected from India, Myanmar, Thailand, Singapore, Indonesia, China and Australia have much wider morphological variations in total number of chaetigers (28–42), body length (30–150 mm) and body width (6–26 mm) (Yáñez-Rivera & Salazar-Vallejo 2022; Salazar-Vallejo 2023). Dorsal pigmentation of two of those specimens (i.e. neotype MNHN IA-TYPE 247 and paraneotype MNHN IA-TYPE 252) collected from eastern coast of India had faded seriously, with only middorsal spots left visible.

Remarks. According to the phylogenetic analyses in this study, the 23 specimens of *C. flava* form a single clade (Fig. 4B), and these specimens have varied-shaped middorsal spots, such as circular, oval, almond, or even blunt rectangular (Fig. 5D-H; Supplementary Fig. 1). The molecular results confirmed Monro (1924)'s opinion that shapes of middorsal spots in *C. flava* specimens may have a wide variation. Due to the lack of molecular data on *C. pulchella* specimens, the phylogenetic relationships between *C. pulchella* and *C. flava* remain to be solved.

Morphological differences between *Chloeia flava* and *C. pulchella* could be noted by checking the type materials. The syntype (BMNH 1971.238) of *C. pulchella* have oval-shaped but apparently smaller middorsal spots, i.e. shorter than 1/2 of the length of each segment (Salazar-Vallejo 2023: Fig. 45), than either *C. flava* from India, Thailand, Singapore, Japan, and China (McIntosh 1885: 11; Salazar-Vallejo 2023: Fig. 20; this study, Fig. 5D-H; Supplementary materials) or those specimens previously identified as *C. pulchella* from India, Indonesia, Thailand and Japan (Horst 1912: Pl. VII, Fig. 3; Izuka 1912: Pl. II, Fig. 4; Salazar-Vallejo 2023: Figs. 46D-F, 47), which have middorsal spots as long as about 2/3 of the length of each segment. Besides, *C. pulchella* bears acicular neurochaetae and its harpoon notochaetae bear no spurs (Salazar-Vallejo 2023: Fig. 45F, G); while in *C. flava*, harpoon notochaetae bear tiny spurs, and neurochaetae with only bifurcates (McIntosh 1885: Pl. IA. Figure 7, 9; Salazar-Vallejo 2023: Fig. 20E-G; this study: Fig. 6C-G, I-L). Therefore, *C. pulchella* is obviously a distinct species from *C. flava*, and *C. pulchella* should be restricted to only include those specimens that have smaller-sized oval middorsal spots.

The suggested Chinese name of *Chloeia flava* is " ". Some other Chinese names such as " " or " " for this species were also used, which refers to the remarkable yellowish colour along subdistal areas of notochaetae. But since yellowish notochaetae also present in *C. fusca*, we suggest using " " for this species.

Habitat. Subtidal sandy, sandy mud and muddy sand sediment, in shallow water.

Distribution. Currently known from India (neotype), Indian Ocean, Myanmar, Thailand, Singapore, Indonesia, New Guinea, China, Japan, and Australia. In the China Seas, this species was only collected from the South China Sea and the East China Sea.

Discussion

Phylogenetic analyses in this study have confirmed that middorsal spots may vary in different shapes, such as circular, oval, and almond-shaped in fixed specimens of *C. flava*. Besides, we consider that *C. flava* and *C. pulchella* could be distinguished by having different sizes of middorsal spots (occupying about 2/3 *vs*. 1/2 of segment length) and different morphology of harpoon notochaetae (spurred vs. not spurred) in median segments, instead of using shapes of middorsal spots as diagnostic characteristics. Morphological variations and molecular evidences could provide a better understanding of identification and distribution of the two species, especially those recorded in historical references.

Frickhinger (1916) regarded his Japan specimens as a variety "*Chloeia flava* var. *pulchella*", but he did not give any descriptions. These specimens, however, is likely referring to specimens of *C. flava* with oval-shaped spots, considering that *C. flava* had been found from Japan (McIntosh 1885: 8–13, Plate IA Fig. 7; Salazar-Vallejo 2023: Fig. 47A-F) and *C. pulchella* were currently only found from northeastern Australia and Malaysia (the latter locality comes from Internet videos, see Supplementary materials). A single specimen collected from the Andaman Islands, Bay of Bengal, which was identified as *Chloeia flava pulchella* Baird, 1868 (Sabith et al. 2022), actually matches the characteristics of *C. flava* by having the middorsal spots occupying about the posterior 2/3 of each segment and bearing spurred harpoon notochaetae and bifurcate neurochaetae. Another three *Chloeia* specimens collected from the west coast of India were identified as *C. viridis* (Rengaiyan & Ingole 2018), while the phylogenetic tree in Fig. 4 indicated that they actually belonged to two distinct species, i.e. one specimen (GP0175) belonged to *C. flava* (Fujian, China), and the other two specimens (GP0169-GP0170) forming a single clade (*Chloeia* sp. B (as *C. viridis*)) grouped with *C. amphora* (Tanabe Bay, Japan; Fig. 4D, E) and *C. flava* (Fujian, China). Localities of *C. flava* specimens from the west coast of India and the southeast coast of China have a long actual distance of around 7000 km, together with the localities from Japan, Indonesia, New Guinea, and Australia (Salazar-Vallejo 2023: 46, 47), *Chloeia flava* has a wide distribution in at least two biogeographic provinces (i.e. Indomalayan Realm & Australasian Realm). Grube (1877: 509) mentioned a distribution of *Chloeia* in the middle Atlantic (Cape Verde) and this was also followed by McIntosh (1885: 8, 9). However, both morphological and molecular analyses based on Atlantic specimens are currently lacking to confirm this even wider distribution in future studies. Videos and images from websites, some had been introduced in Salazar-Vallejo (2023), show that live *Chloeia* specimens from Australia (GMT 2021), from an unknown locality (Senja 2021, 0:28), and Malaysia (Senja 2021, 1:27) fit the morphology of *C. pulchella* by having apparently smaller middorsal spots than those of *C. flava* (Iromongara 2009; Japan Marine Club 2018; Senja 2021, 0:00), which indicates that *C. pulchella* might have a wider distribution apart from its type locality in northeastern Australia.

Declarations

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Conflict of interest

The authors declare no competing interests.

Ethical approval

No animal testing was performed during this study.

Sampling and field studies

All necessary permits for sampling and field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements.

Data availability

The datasets supporting the conclusions of this article are included within the article and the online resources.

Author contribution

ZW and CK conceived and designed research. ZW, DY, JWQ conducted field observations. ZW wrote the manuscript. ZW and DY conducted molecular analyses. JWQ and SISV revised the manuscript. All authors read and approved the manuscript.

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Figures

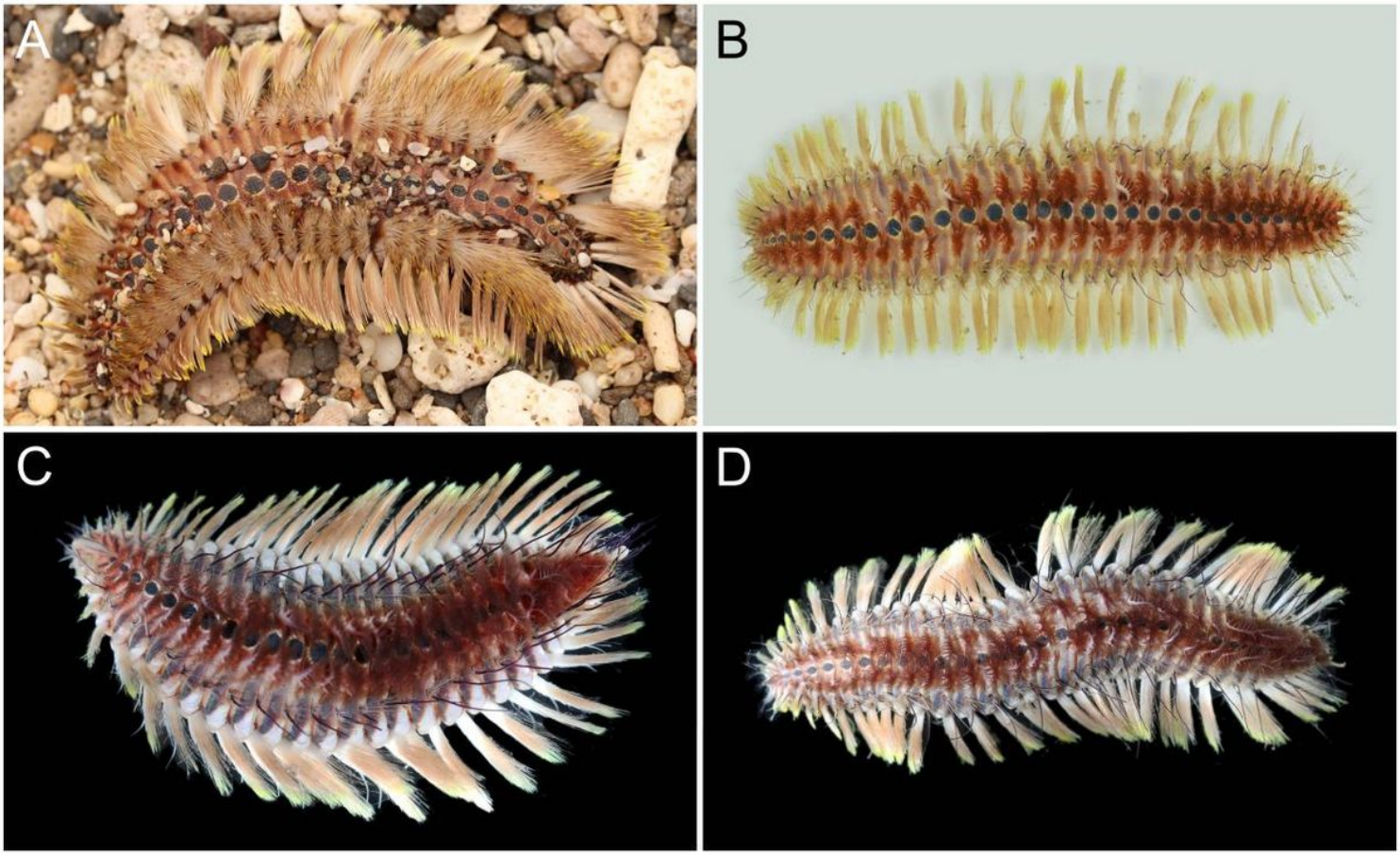

Figure 1

Living specimens of *Chloeia flava* (Pallas, 1766) from China. (A), A specimen from intertidal of Weizhou Island (photo: heimi), specimen about 60 mm long, dorsal view. (B), A specimen from Weizhou Island (photo: Juhao Wang), specimen about 60 mm long, dorsal view. (C), (D), Two specimens from Hainan coastal waters (photo: Yanjie Zhang), specimen in C about 30~40 mm long, specimen in D about 70 mm long, dorsal view.

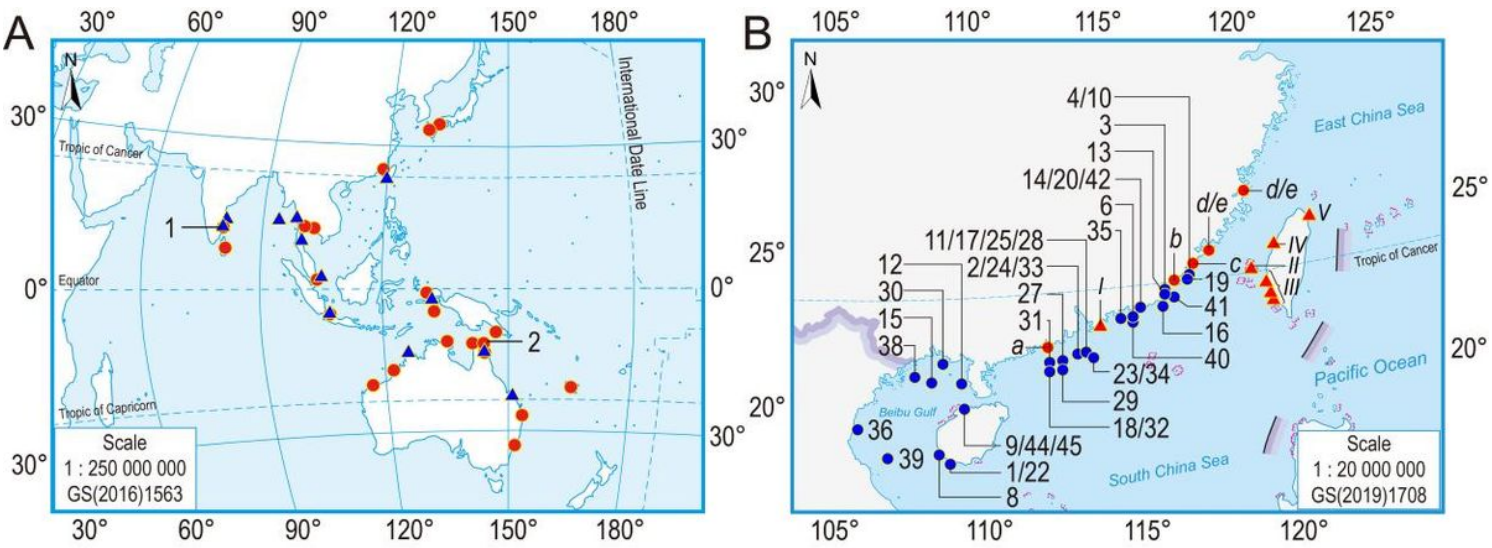

Figure 2

Sampling localities of *Chloeia flava* and*C. pulchella* specimens checked by Salazar-Vallejo (2023) (A) and localities of *C. flava* recorded from the China Seas (B). (A), Black numbers represent the type localities of *C. flava* (Punducherry, India) and *C. pulchella* (Raine Island reefs, northeastern Australia); red circles represent localities of some of the *C. pulchella* specimens; blue triangles represent localities of some of the *C. flava* specimens. (B), Red circles (*a-e*) represent localities of *C. flava* records from published references, i.e. *a*: Zhenhai Bay, *b*: Zhelin Bay, *c*: Jiuzhen Bay, *d/e*: Xiamen, and Fuzhou (Foochow); red triangles (*I-V*) represent *C. flava* localities in the China Seas from the internet, i.e. *I*: Hong Kong, *II*: Penghu, *III*: Zhongyun, Mituo, Mashagou, *IV*: Taizhong, *V*:

northeast coast of Taiwan; blue circles with black numbers represent localities of some of the MBMCAS-stored *C. flava* specimens checked by Sun (2018). The other references (*a-e*, *I-V*) with sampling sites data used in Figure 2B are included in Supplementary Materials.

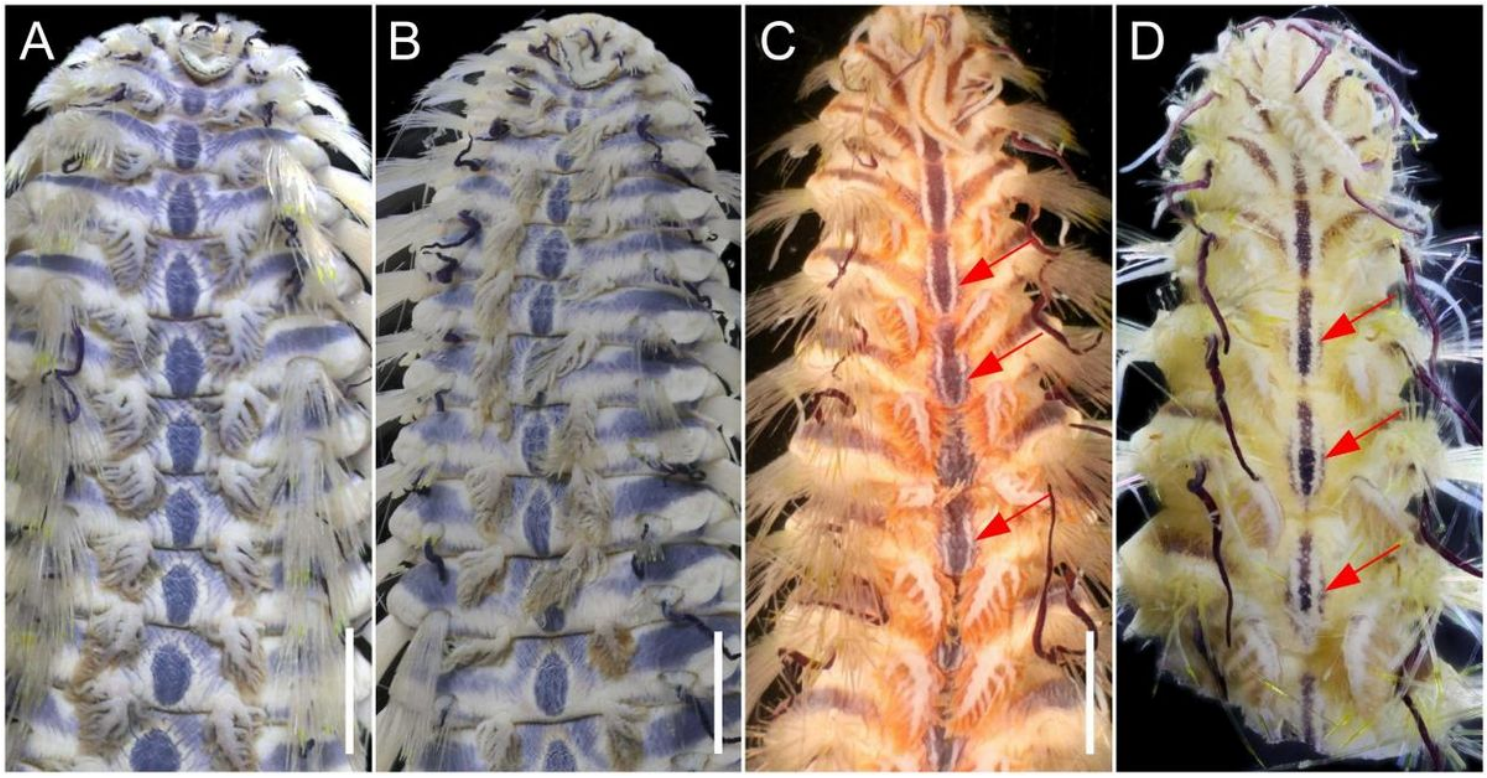

Figure 3

Anterior segments of *Chloeia flava*Baird, 1868 (A, B) and *C. amphora* Horst, 1910 (C, D). (A), XMU-Pol-2021-307, with circular middorsal spots in median segments (see Figure 5A), Fujian, China; (B), XMU-Pol-2021-316, with oval middorsal spots in median segments (see Supplementary Figure 1), Fujian, China; (C), non-type specimen, CAS 218219 (image cited from Salazar-Vallejo, 2023: Fig. 10G), Philippines; (D), non-type specimen, SMNH95025, previously treated as *C. flava*, Tanabe Bay, Japan (Photo: Lena Gustavsson, Swedish Museum of Natural History). Red arrows mark a dark thin guard line on the outer side of the pale thin band. **Scale bars**: (A)-(C) = 2 mm; (D), unavailable.

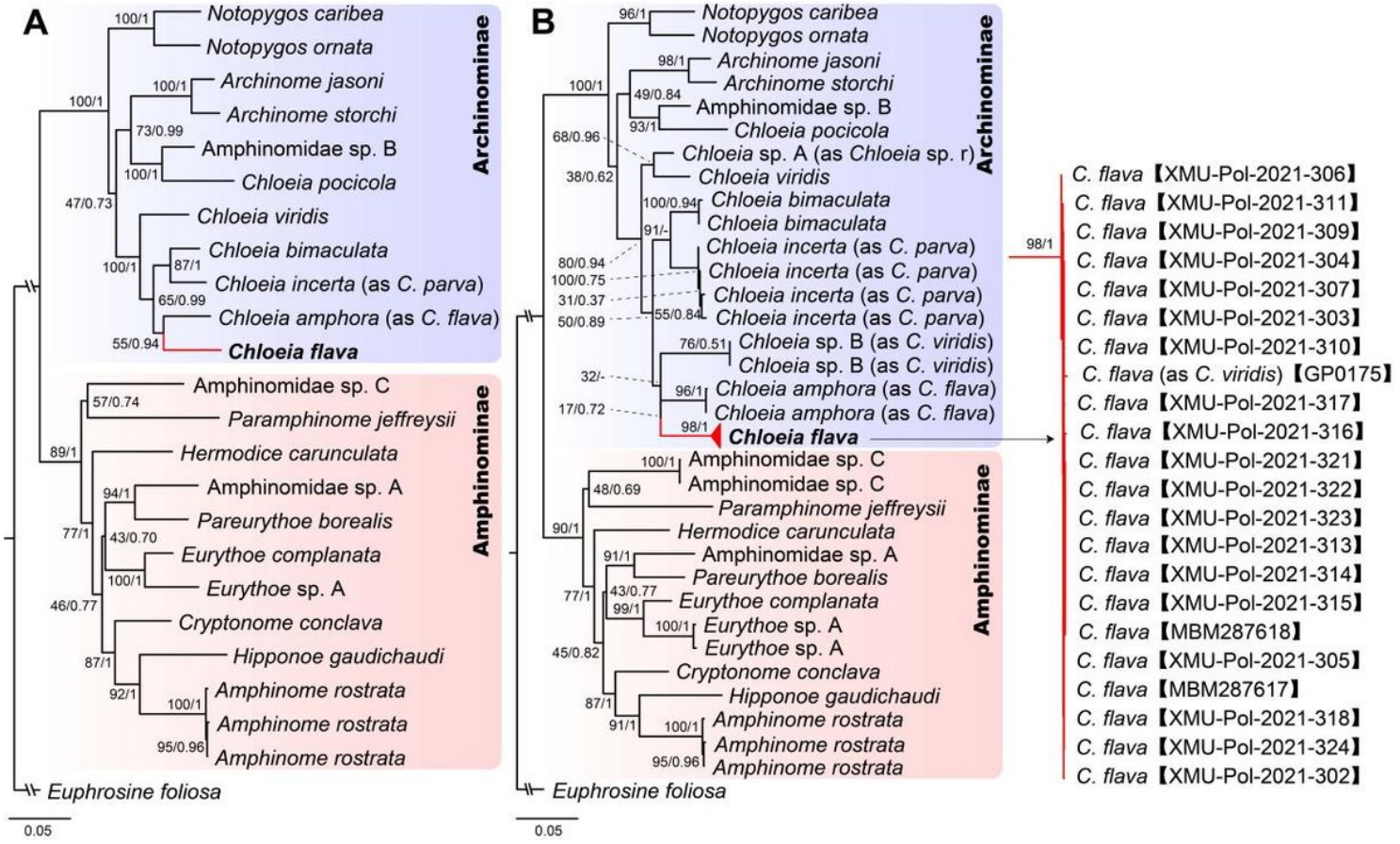

Figure 4

Phylogenetic trees of amphinomids reconstructed with maximum likelihood (ML) and Bayesian (BI) approaches. The trees were reconstructed based on the concatenated sequences (3,556 bp) of partial *COI* (609 bp), *16S rRNA*(303 bp), *18S rRNA* (1,674 bp) and *28S rRNA* (970 bp) genes with 24 (A) and 55 (B) amphinomid specimens. Branch support values refer to bootstrap (BS) and Bayesian posterior probabilities (BPP). –, node absent in BI. Vouchers and GenBank accession numbers of the specimens are listed in Table 2. The scale bar indicates the number of substitutions per site.

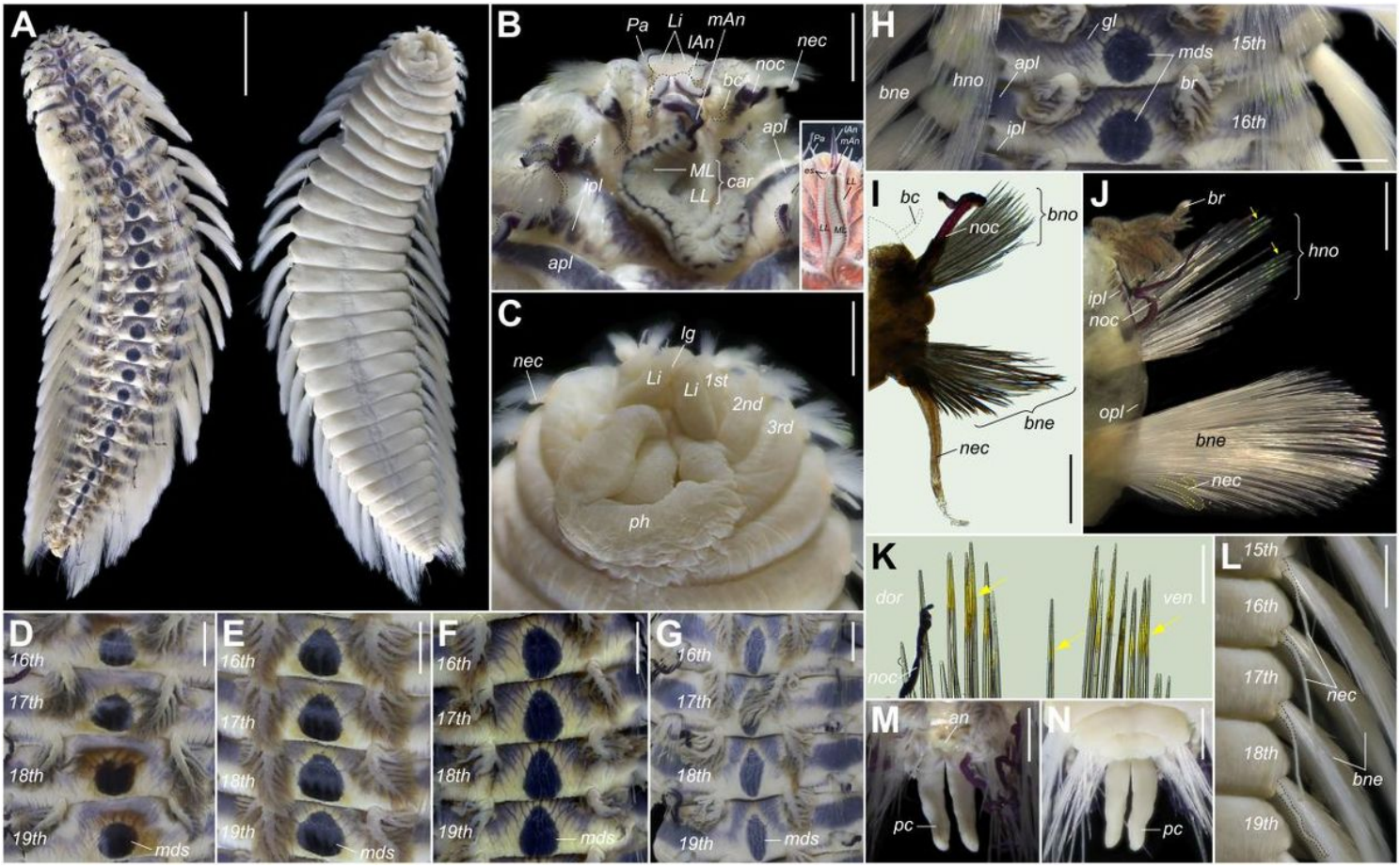

Figure 5

Morphology of *Chloeia flava*. (A)-(C), (H)-(N), XMU-Pol-2021-307. (A), fixed specimen, dorsal view (left), ventral view (right); (B), large image, anterior part, dorsal view; inset, a live specimen from Hainan, China (photo: Yanjie Zhang), dorsal view; (C), anterior part, ventral view; (D)-(G), dorsal view, showing the different shapes of middorsal spots in median segments (chaetigers 16 to 19), (D), XMU-Pol-2021-302, spots circular shaped (E), XMU-Pol-2021-304, spots subcircular shaped, (F), XMU-Pol-2021-315, spots almond shaped, (G), XMU-Pol-2021-316, spots drop shaped; (H), chaetigers 15 and 16, spots circular shaped, dorsal view; (I), parapodium of chaetiger 2, right side, posterior view; (J), parapodium of chaetiger 10, right side, posterior view; (K), ends of harpoon notochaetae in chaetiger 10, with the yellow areas marked in yellow arrows; (L), chaetigers 15-19, ventral view, left side; (M), pygidium, dorsal view, showing anus; (N), ventral view, showing two digitiform pygidial cirri. **Abbreviations**: *an*, anus; *apl*, anterior pigmented line; *bc*, branchial cirrus; *bne*, bifurcate neurochaetae; *bno*, bifurcate notochaetae; *br*, branchia; *car*, caruncle; *dor*, dorsal side; *es*, eye spots; *gl*, guard line; *hno*, harpoon notochaetae; *ipl*, inner-posterior pigmented line; *lAn*, lateral antenna; *lg*, longitudinal groove; *Li*, lips; *LL*, lateral lobes; *mAn*, median antenna; *mds*, middorsal spots; *ML*, median lobe; *nec*, neuropodial cirrus; *noc*, notopodial cirrus; *opl*, outer-posterior pigmented line; *pa*, palp;*pc*, pygidial cirrus; *ph*, pharynx; *ven*, ventral side. **Scale bars**: (A) = 10 mm; (C)-(H), (J), (L) = 2 mm; (B), (I), (M), (N) = 1 mm; (K) = 0.5 mm.

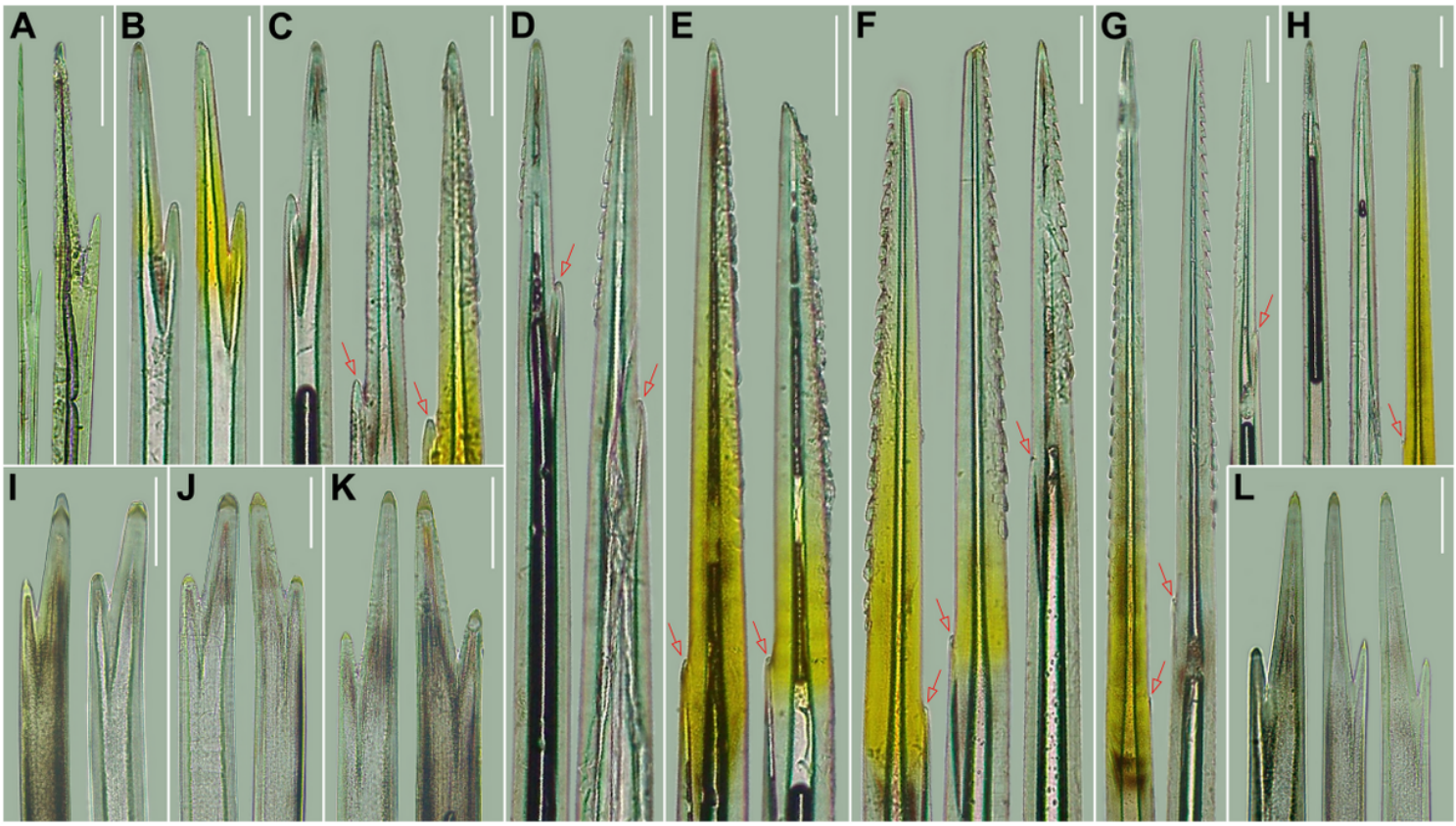

Figure 6

Chaetae of *Chloeia flava*. (A)-(L), XMU-Pol-2021-307. (A), bifurcate notochaetae, chaetiger 2, left side; (B), bifurcate notochaetae, chaetiger 4, left side; (C), bifurcate (left one) and harpoon (middle and right ones) notochaetae, chaetiger 5, left side, red arrows indicate position of short tines (spurs); (D), harpoon notochaetae, chaetiger 6, left side; (E), harpoon notochaetae, chaetiger 10, left side; (F), harpoon notochaetae, chaetiger 15 (left one, left side), chaetiger 16 (middle one, right side), chaetiger 17 (right one, right side); (G), harpoon notochaetae, chaetiger 30 (left one, left side; middle and right ones, right side); (H), spinose notochaetae, chaetiger 6 (left side), 10 (left side) and 30 (right side), 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)-(H) = 0.1 mm; (I)-(L) = 0.2 mm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SypplementaryFig.1.eps
- supplementarymaterials.docx