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Morphological analysis of *Rhynchospio* aff. *asiatica* (Annelida: Spionidae) and comments on the phylogeny and reproduction of the family Spionidae*

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Abstract The genus *Rhynchospio* has fronto-lateral horns on prostomium, paired branchiae from chaetiger 2 to near the posterior end, capillary notochaetae only, and more than two pairs of pygidial cirri. *Rhynchospio* species are common in coastal soft bottom communities; nevertheless, many recorded *Rhynchospio* specimens around the world are currently undescribed. Here we described a *Rhynchospio* species based on specimens collected from Qingdao, China. Comparison with the reported DNA sequences of four gene markers (16S rRNA, 18S rRNA, 28S rRNA, and Histone H3) and brief morphological description of specimens collected from Jinhae Bay, South Korea, previously reported as *Rhynchospio* aff. *asiatica*, indicated that they are conspecific. Morphologically, specimens of *R.* aff. *asiatica* from Qingdao are characterized by having neuropodial hooded hooks from chaetigers 14–17 (vs. 10–23 in *R. asiatica*) to near pygidial chaetigers, sperm from chaetiger 11 to 14 (vs. from chaetiger 11 to 21–22 in *R. asiatica*), oocytes from chaetigers 16–17 to 26–39 (vs. from 22–24 in *R. asiatica*), and 4–6 (vs. up to 6 in *R. asiatica*) pygidial cirri. Genetically, *Rhynchospio* aff. *asiatica* is most closely related to *R. arenincola* Hartman, 1936 from California, USA with the interspecific distances of 20.02% (16S rRNA), 4.50% (18S rRNA), 8.44% (28S rRNA), 2.74% (Histone H3), and 6.10% (concatenated sequences). Water flow across the dorsum created by ciliary beating of the branchiae and nototrochs, observed on live specimens, may help transport gametes from reproductive segments in anterior and middle parts to the posterior brooding segments. Phylogenetic trees based on concatenated sequences of four gene markers of 54 spioniform species in 25 genera revealed two clades, covering the two subfamilies Spioninae and Nerininae respectively. Two families (i.e., Poecilochaetidae and Trochochaetidae) in the order Spionida were clustered within Spionidae, supporting a morphology-based proposal that these families bearing a pair of prehensile, grooved palps should be grouped within a more broadly defined family Spionidae. Mapping morphological and reproductive characteristics to the phylogenetic trees indicated that the ancestor of spionids might lack branchiae, broadcast spawn thick-envelop oocytes and ect-aquasperm, and produce planktotrophic larvae.

Keyword: Annelida; phylogeny; taxonomy; Yellow Sea

1 INTRODUCTION

Spionidae Grube, 1850 is a large family of annelids containing 39 valid genera and over 630 valid species (Read and Fauchald, 2021). There have been

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Fig.1 Type localities of *Rhynchospio* species (modified from Radashevsky et al. (2014))

●: the sampling site of *R.* aff. *asiatica* specimens in Qingdao Bay, Shandong Province, China (36°3′39.6″N, 120°19′15.6″E) used in this study. 1: *R. glutaea* (Ehlers, 1897), Punta Arenas, Strait of Magellan, Chile; 2: *R. arenincola* (Hartman, 1936), San Mateo County, California, USA; 3: *R. asiatica* (Chlebovitsch, 1959), Shikotan Island, South Kurile Islands; 4: *R. inflata* (Foster, 1971), Bimini Islands, Bahamas; 5: *R. glycera* (Blake and Kudenov, 1978), Burwood Beach, New South Wales, Australia; 6: *R. australiana* (Blake and Kudenov, 1978), Perth, West Australia, Australia; 7: *R. tuberculata* (Imajima, 1991), Sagami Bay, Honshu Island, Japan (35°18′42″N, 139°24′0″E); 8: *R. foliosa* (Imajima, 1991), Usujiri Bay, Hokkaido Island, Japan; 9: *R. nhatrangi* (Radashevsky, 2007), Nha Trang Bay, Vietnam (12°16′0″N, 109°12′18″E); 10: *R. darwini* (Radashevsky, 2015), Fannie Bay, Northern Territory, Australia (12°26′8.16″S, 130°49′56.28″E); 11: *R. mzansi* (Simon et al., 2019b), Gansbaai, South Africa (34°36′S, 19°19′E); 12: *R.* aff. *asiatica*, Jinhae Bay, South Gyeongsang, South Korea. Map review No. GS(2016)1563.

morphological (Mackie, 1996; Blake and Arnofsky, 1999) and molecular (Abe et al., 2016) studies of the family, providing insights into the systematic relationships of the genera. However, the phylogenetic placement of some genera (e.g., *Aonides*, *Carazziella*, *Microspio*, *Dispio*, *Tripolydora*, and *Pygospiopsis*) has not been clarified, and in most genera the relationships among species have not been studied. This lack of phylogenetic studies is evident for spionids along the Chinese coasts: although there have been records of 17 genera and 69 species of spionids (Institute of Oceanology, Chinese Academy of Sciences and Liu, 2008; Zhou, 2008; Zhou and Li, 2009; Zhou et al., 2010b), only 9 species in 4 genera (*Scolelepis*, *Polydora*, *Boccardiella*, and *Pseudopolydora*) have been examined with molecular tools (Zhou et al., 2010a; Sato-Okoshi et al., 2013; Ye et al., 2015, 2017, 2019a, b).

Rhynchospio Hartman, 1936 is a genus of Spionidae characterized by a prostomium with fronto-lateral horns, paired branchiae from chaetiger 2 to near the posterior end, notochaetae all capillary and pygidium with more than two pairs of cirri (Foster, 1971; Radashevsky et al., 2014). A total of 13 species of *Rhynchospio* has been described, but only 11 of species are considered valid (Fig.1); *R. microcera* (Dorsey, 1977) and *R. harrisae* (Delgado-Blas and Díaz-Díaz, 2010) might belong to *Microspio* (Radashevsky et al., 2014). All adult *Rhynchospio* are considered hermaphrodites, and currently 5 species, i.e., *R. glutaea* (Ehlers, 1897), *R. arenincola* (Hartman, 1936), *R. asiatica* (Chlebovitsch, 1959), *R. mzansi* (Simon et al., 2019b), and an undescribed species *R.* aff. *asiatica* collected from South Korea are classified as belonging to the *Rhynchospio glutaea* complex characterized by having hooded hooks in neuropodia from chaetigers 9–18, tridentate both in juveniles and adults and pygidium with 2–5 pairs of cirri (Radashevsky, 2007a; Radashevsky et al., 2014). The other 7 species have quite distinct charateristics comparing to the species in *Rhynchospio glutaea* complex (See Taxonomic key to *Rhynchospio* species).

There have been several records of *Rhynchospio* along the Chinese coast of the Yellow Sea, i.e., "*R. arenincola*" and "*R. glutaea*" from Qingdao Bay (Wu et al., 1993; Ji, 2012), and "*R. glutaea*" from Shidao Bay (Zhou, 2008). These *Rhynchospio* records are likely to be conspecific due to the short distance between Qingdao Bay and Shidao Bay (~210 km), but neither detailed morphological or DNA barcoding data were available to determine their taxonomic statuses. At about the same latitude across the Yellow Sea from Jinhae Bay (35°N) of South Korea, roughly 760 km from Qingdao, Radashevsky et al. (2014) found a species of the *R. glutaea* complex (temporally named "*R.* aff. *asiatica*") based on 5.15% K2P genetic distance of the concatenated sequences of four genes (i.e., 16S rRNA, 18S rRNA, 28S rRNA, and Histone H3) from *R. arenincola*. Further studies are needed to check whether the *Rhynchospio* specimens from Qingdao and Jinhae are conspecific or not.

Brooding activities on the body of female spionids is currently known only in *Streblospio* and *Rhynchospio*, but whether the two genera use the same mechanism to transport their gametes to the dorsal brood region is unknown. In *Streblospio*, the gametes were suggested to be transported through coelom to the dorsal brood pouches formed by thin-walled, dorsolateral extensions of the coelom in middle segments (Collier and Jones, 1967). In *Rhynchospio*, Radashevsky (2007a) speculated that the elongated capillaries forming a "hatchery" in posterior chaetigers could keep the elongated introsperm there, thus improving fertilization efficiency. However, it is unknown how *Rhynchospio* transport mature gametes from the anterior and middle fertile segments to the posterior "hatchery".

Morphological and reproductive variations among spioniform genera had been used for uncovering their phylogenetic relationships (Sigvaldadóttir et al., 1997; Blake and Arnofsky, 1999). According to similar reproductive characteristics such as the occurrence of thick-enveloped oocyte, ect-aquasperm, and absence of egg capsule/mass in tube, twenty-one genera, including four spioniform genera (i.e., *Poecilochaetus*, *Trochochaeta*, *Heterospio*, and *Uncispio*) previously treated as independent families (i.e., Poecilochaetidae, Trochochaetidae, Longosomatidae, and Uncispionidae), had been grouped within the clade Nerininae in a cladistic analysis, which suggested that these four spioniform families should be considered as junior synonyms of Spionidae (Blake and Arnofsky, 1999). However, such analyses did not rule out the possibility of homoplasy in morphological and reproductive characteristics among genera of spionids, which may influence their clustering. A recent phylogenetic analysis conducted based on 16S rRNA and 18S rRNA gene sequences of spioniform genera, showed that *Poecilochaetus* and *Trochochaeta* were nested within the clade of subfamily Nerininae, which confirmed previous suggestions to place them into Spionidae (Abe and Sato-Okoshi, 2021). However, phylogenetic analysis based on more gene sequences, together with morphological characteristics, are still needed to make better understanding of their phylogenetic placements within Spionidae.

Previous phylogenetic studies had provided useful information on the selection of ingroups and outgroups in constructing credible phylogeny among spionids using nucleotide data. Besides Abe and Sato-Okoshi (2021), Rousset et al. (2007) and Struck et al. (2007, 2008) had also indicated close relationships between Spionidae and the two spioniform families Poecilochaetidae and Trochochaetidae, although these relationships were not well resolved. Also in these studies, several families (i.e., Sabellariidae, Sabellidae, and Serpulidae) of the order Sabellida, showed sister group relationships with Spionidae comparing to all other annelid families.

Another two families, i.e., Apistobranchidae and Longosomatidae, once believed to be a part of Spioniformia, however, had been found to be distantly related to Spionidae (Struck et al., 2008; Zrzavý et al., 2009; Blake and Maciolek, 2019; Blake and Petti, 2019). For Longosomatidae, morphological similarities had been found between its single genus *Heterospio* and cirratulids (Blake and Maciolek, 2019). Therefore, we followed these results to include Poecilochaetidae and Trochochaetidae as ingroups, and Sabellariidae, Sabellidae, and Serpulidae as outgroups in our phylogenetic analysis.

To explain the evolution of the morphological types of oocytes and sperm in spionids, two opposing hypotheses had been proposed. Söderström (1920) considered thin and smooth enveloped oocytes as a plesiomorphic characteristic and thick and sculptured envelope as a derived characteristic evolved in Spionidae. Hannerz (1956), on the contrary, suggested that thick-envelope oocytes might have evolved in a primary spionid and thin-envelope oocytes evolved in Spioninae due to reduction of the original thick envelope in connection with the evolution of brooding. Although the latter hypothesis had been supported by a comprehensive cladistic analysis of morphological and reproductive characteristics among genera of Spionidae (Radashevsky, 2007b), such analysis could not avoid the possible influences of homoplasy, and phylogenetic analysis using molecular data are needed to assess these hypotheses.

In this study, we presented the morphological features for *Rhynchospio* specimens collected from Qingdao Bay, China. Through observation of the ciliary beating in live specimens, we proposed a mechanism for transporting gametes from anterior and middle fertile segments to the brooding hatchery in posterior segments. By including DNA barcoding sequences for all available genera of spioniform annelids in our phylogenetic analyses, we aimed to determine the taxonomic statuses and phylogenetic placement of the *Rhynchospio* specimens from Qingdao Bay, and to study the evolution of reproduction of the family Spionidae. We also aimed to use nucleotide data, morphological and reproductive characteristics to make better estimates of the phylogenetic placements of two spioniform families (i.e., Poecilochaetidae and Trochochaetidae) recently included within Spionidae.

2 MATERIAL AND METHOD

2.1 Sample collection and preservation

Specimens of *Rhynchospio* were collected from the high intertidal zone of Qingdao Bay (36°3′39.6″N, 120°19′15.6″E), China, on November 2, 2014, June 18, 2015 and August 31, 2018. The sampling site was close to one of the two outfalls along the coast, located at the eastern side of Zhanqiao (the Pier, Supplementary Fig.S1). Sediments were washed through a 0.5-mm sieve and the retained specimens were fixed either with 10% formaldehyde in seawater and later transferred into 75% ethanol for morphological analysis or directly in 95% ethanol for DNA extraction.

2.2 Morphological analysis

Parapodia and chaetae of chaetigers 1, 2, 4, 10, 20, 30, and a posterior one were dissected with iris scissors and mounted on slides. Photographs of parapodia and chaetae, taken using a Sony DSC-WX350 digital camera mounted on an Olympus CX31RTSF microscope, were used for line-drawings. Scanning electron microscopy (SEM) was used for detailed observation of cilia on palps and branchiae. The anterior part of specimen #17 (Catalog No.: XMU-Pol-2021-050) was dehydrated in pure ethanol, dried with the critical point drying method, mounted on conductive carbon adhesives, sputter coated with gold and observed with a LEO 1530 FESEM scanning electron microscope.

2.3 Molecular-based phylogenetic analyses

2.3.1 DNA extraction, PCR amplification, and sequencing

Two specimens (XMU-Pol-2021-049, XMU-Pol-2021-050) preserved in 95% ethanol were used for DNA extraction. The posterior segments were dissected, and the genomic DNA was extracted using a DNeasy blood & tissue kit (QIAGEN, Hilden, Germany). Four primer pairs were used to amplify corresponding genes, i.e., 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). The PCR protocol followed Zhang et al. (2018). PCR products were purified using a Zymoclean™ Gel DNA Recovery Kit and sequenced using Sanger sequencing at BGI Hong Kong.

2.3.2 Phylogenetic analyses

The sequences of these four genes of 24 spioniform genera, including *Trochochaeta* and *Poecilochaetus*, as well as outgroups (i.e., Serpulidae, Sabellidae, and Sabellariidae) deposited in GenBank (https://www.ncbi.nlm.nih.gov/) were downloaded for phylogenetic analyses (Table 1). Taxa with no DNA sequences available, such as the genera *Amphipolydora*, *Carazziella*, *Laubieriellus*, *Lindaspio*, *Pygospiopsis*, *Scolecolepides*, and *Tripolydora* in Spionidae and the family Uncispionidae, were not included in the analyses. Two families previously considered as members of Spioniformia, Apistobranchidae and Longosomatidae were excluded from the phylogenetic analyses due to their distant relationships from the spionid clade (Struck et al., 2008; Zrzavý et al., 2009; Blake and Maciolek, 2019; Blake and Petti, 2019). The four gene sequences were aligned using the MUSCLE algorithm implemented in the software Mesquite (Edgar, 2004), and poorly aligned positions were removed with the Gblocks Server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The four trimmed genes were concatenated using

Table 1 GenBank accession numbers and specimen voucher/isolate information of used spionids in this study

Taxon	Origin	Specimen voucher/isolate	16S rRNA	18S rRNA	28S rRNA	Histone H3	Reference
Amphicorina mobilis	Australia	AM E3576	–	EF116206	EF116217	EF192921	Kupriyanova and Rouse, 2008; Kupriyanova et al., 2006
Gunnarea capensis	South Africa	SAM E3360	–	DQ317111	EU256544	EF192937	
Sabellaria alveolata	Brittany, France	–	AY340479	AY340442	AY340416	DQ779763	Rousset et al., 2007
Galeolaria caespitosa	Sydney, Australia	AM W24394	–	AB106257	AF185151	AF185233	Brown et al., 1999; Hall et al., 2004
Chitinopoma serrula	Iceland		–	DQ779643	DQ779681	DQ779722	Rousset et al., 2007
Aonidella cf. *dayi*	NE Atlantic	SMF 23398	KF434508	KF434504	–	–	Meißner et al., 2014
Aonides oxycephala	France	Isolate 18577.42; 18577	MG878896	MG913226	MG878926	MG874410	Radashevsky et al., 2020
Aurospio sp.	eVFZ, Atlantic; CCZ, Pacific	Isolate PVT603	MN441729	MN447187	–	–	Guggolz et al., 2020
Aurospio dibranchiata	Pacific Mn nodule province	Isolate KP342	EU340087	EU340091	–	–	Mincks et al., 2009
Boccardia pseudonatrix	South Africa	NCBI:txid1964544	–	KY677894	–	–	Williams et al., 2017
Boccardia polybranchia	South Africa	MB-A082638	–	KY677890	–	–	Williams et al., 2017
Boccardia proboscidea	Bodega Bay, USA	MIMB 28080	KJ546323	KJ546254	KJ546204	KJ546181	Radashevsky et al., 2014
Boccardiella hamata	Sasuhama, Japan	Isolate 5–8	LC595727	LC107608	–	–	Abe et al., 2016; Abe and Sato-Okoshi, 2021
Dipolydora bidentata	Peter the Great Bay, Russia	Isolate 87	JX228112	JX228072	JX228092	–	Radashevsky and Pankova, 2013
Dipolydora cardalia	Russia	Isolate 111(1)	JX228121	JX228080	JX228098	–	
Dipolydora cardalia	Russia	NCBI:txid1276541	JX228121	JX228080	JX228098	–	
Dispio remanei	Brazil	473	–	KU900474	KU900467	–	Direct submission
Glandulospio orestes	NE Atlantic	ZSRO-P2318; SMF 23355	KF434511	KF434505	–	–	Meißner et al., 2014
Laonice cirrata	Russia	Isolate 925	–	KM998752	–	–	Direct submission
Laonice sp. 1	Bohuslän, Sweden	SMNH 81634	DQ779619	DQ779655	DQ779693	–	Rousset et al., 2007
Laonice sp. 2	whale fall∕California borderland basins, USA	Isolate WW26	EU340088	EU340089	–	–	Mincks et al., 2009
Laonice sp. 3	Pacific Ocean: CCFZ	181-Ifr-0670	MK971124	MK971152	–	–	Bonifácio et al., 2019
Malacoceros fuliginosus	St. Efflau, France	–	–	AY525632	–	–	Struck and Purschke, 2005
Marenzelleria viridis	–	–	–	EU418860	EU418868	–	Struck et al., 2008
Marenzelleria arctia	Yenisei Gulf, Russia	WS 1813	KJ546306	KJ546264	KJ546214	KJ546164	Radashevsky et al., 2014
Microspio granulata	Queensland, Australia	AM W44480	KP636514	KP636515	–	–	Meißner and Götting, 2015
Paraprionospio cordifolia	India?	Isolate pac201	–	KT900305	–	–	Paraprionospio cordifolia
Paraprionospio patiens	Eastern Arabian Sea	Isolate 1.M1(a)-Pp; isolate pap203	KY704330	KT900307	KY704327	KY751312	Vijapure et al., unpublished data; Rengaiyan and Ingole, 2018
Poecilochaetus serpens	Arcachon, France	C. Bleidorn 01POE001 (P)	AY569680	AY569652	–	–	Bleidorn et al., 2005
Poecilochaetus sp.	Banyuls, France	SMNH 81615	DQ779630	DQ779667	DQ779705	DQ779754	Rousset et al., 2007
Polydora haswelli	Beibu Gulf, Xuwen, China	Isolate J2	KF562235	KF562242	KF562249	–	Ye et al., 2015

The information will be finalized when the article is accepted for publication. – means data not available.

To be continued

Table 1 Continued

Taxon	Origin	Specimen voucher/isolate	16S rRNA	18S rRNA	28S rRNA	Histone H3	Reference
Polydora hoplura	Saldanha, South Africa	Isolate SDH05	LC101880	LC101849	LC101862	–	Sato-Okoshi et al., 2017
Polydora lingshuiensis	Xincun Bay, Hainan, China	Isolate MT2	KF562232	KF562236	KF562243	–	Ye et al., 2015
Prionospio cf. *ehlersi*	Abyssal Southern Ocean	Isolate CP284	EU340081	EU340095	–	–	Mincks et al., 2009
Prionospio cf. *cirrifera*	Abyssal Southern Ocean	Isolate CP94	EU340079	EU340093	–	–	
Pseudopolydora ushioni	Uranouchi Bay, Japan	Isolate 13–7	LC107854	AB973926	AB973934	–	Simon et al., 2019a
Pseudopolydora uphondo	Sodwana Bay, South Africa	Isolate 32–2	LC107865	LC107847	LC107851	–	
Pygospio elegans	Bodega Bay, USA	MIMB 28090	KJ546313	KJ546272	KJ546227	KJ546171	Radashevsky et al., 2014
Pygospio sp.	Oregon, USA	MIMB 28101	KJ747087	KJ747077	KJ747067	KJ747058	Radashevsky et al., 2016b
Rhynchospio arenincola	Oregon, USA	MIMB 28096	KJ546318– KJ546322	KJ546286– KJ546290	KJ546236– KJ546240	KJ546176– KJ546180	Radashevsky et al., 2016a
Rhynchospio arenincola	British Columbia, Canada	MIMB 18893	KJ546328– KJ546331	KJ546291– KJ546294	KJ546232– KJ546235	KJ546186– KJ546189	
Rhynchospio darwini	Darwin, Australia	NTM W025648	KP986492	KP986493	KP986494	KP986498	
Rhynchospio darwini	Darwin, Australia	MIMB 28105	KP986495	KP986496	KP986497	KP986499	
Rhynchospio cf. *foliosa*	Oregon, USA	MIMB 28104	KP986488	KP986489	KP986490	KP986491	
Rhynchospio cf. *foliosa*	Oregon, USA	MIMB 28104	KR607514	KR607515	KR607516	KR607517	
Rhynchospio glutaea	Golfo Nuevo, Argentina	MIMB 28131	KJ546332– KJ546336	KJ546281– KJ546285	KJ546241– KJ546245	KJ546190– KJ546194	
Rhynchospio aff. *asiatica*	Jinhae Bay, South Korea	MIMB 28097	KJ546344– KJ546347	KJ546295– KJ546298	KJ546246– KJ546249	KJ546198– KJ546200	Radashevsky et al., 2014
Rhynchospio aff. *asiatica*	Qingdao Bay, China	Isolate XMU-Pol-2021-049	MW646557	MW646561	MW646559	MW654180	This study
Rhynchospio aff. *asiatica*	Qingdao Bay, China	Isolate XMU-Pol-2021-050	MW646558	MW646562	MW646560	MW654181	
Rhynchospio mzansi	Western Cape, South Africa	Isolate 1–5	MF625254– MF625257	MF625258– MF625261	MF625262– MF625265	MF625266– MF625269	Simon et al., 2019b
Rhynchospio nhatrangi	Nha Trang Bay, Vietnam	MIMB 18026	KJ546342– KJ546343	KJ546299– KJ546300	KJ546250– KJ546253	KJ546201– KJ546203	Radashevsky et al., 2014
Scolelepis laonicola	Kandalaksha Bay, Russia	–	–	EF569206	–	–	Vortsepneva et al., 2008
Scolelepis squamata	Sylt, Germany	–	–	AF448164	–	–	Bleidorn et al., 2003
Scolelepis sp.	Eastern Arabian Sea	Isolate sco206	–	KT900310	–	–	Rengaiyan and Ingole, 2018
Spio filicornis	West Greenland	Isolate GL1.6	FR823435	FR823430	–	–	Meißner et al., 2011
Spio sp.	Koni Peninsula, Russia	MIMB 28132	KT200126	KT200135	KT200143	KT200118	Radashevsky et al., 2016b
Spiophanes hakaiensis	British Columbia, Canada	CMNA 2019-0105	MN193557	MN186820	MN193549	MN193948	Radashevsky et al., 2020
Spiophanes cf. *kroeyeri*	abyssal Southern Ocean	Isolate CP160	EU340080	EU340094	–	–	Mincks et al., 2009
Spiophanes uschakowi	Peter the Great Bay, Russia	MIMB 28164	MG878915	KM998760	MG878949	MG874433	Radashevsky et al., 2020
Streblospio sp.	Arabian sea	V2(a)-Sg3	KY704328	KY704336	–	KY751310	Direct submission
Trochochaeta multisetosa	North Sea, Norway; Askeröfjord, Sweden	ZMBN 126365; SIO BIC A6333	MN193553	MN296517	MN193541	MN193941	Radashevsky et al., 2020
Trochochaeta sp.	–	THS-2006	–	DQ790097	DQ790070	–	Struck et al., 2007

SequenceMatrix v.1.7.8 (Vaidya et al., 2011). The K2P genetic distances (Kimura, 1980) between *Rhynchospio* species was estimated based on each gene sequence (i.e., 246-bp 16S rRNA, 1 493-bp 18S rRNA, 292-bp 28S rRNA, 289-bp Histone H3) and their concatenated sequence (2 320 bp) using MEGA X (Kumar et al., 2018). Two phylogenetic analyses were conducted using the Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. One analysis focused on the phylogeny among *Rhynchospio* species, which were carried out based on the concatenated sequences (2 444 bp) of partial 16S rRNA (294 bp), 18S rRNA (1 502 bp), 28S rRNA (323 bp), Histone H3 (325 bp) genes of *Rhynchospio*, *Boccardia*, and *Pygospio* rooted with *Marenzelleria* sequences. The other analysis focused on the phylogeny among spioniform genera, which were constructed based on the concatenated sequences (2 483 bp) of partial 16S rRNA (302 bp), 18S rRNA (1 535 bp), 28S rRNA (321 bp) and Histone H3 (325 bp) genes of Spionidae, Sabellidae, and Sabellariidae rooted with Serpulidae sequences. The best fitting nucleotide-substitution model was evaluated using jModelTest v.2.1.1 (Darriba et al., 2012). Specifically, the ML analysis was conducted using the "thorough bootstrap" option with 1 000 bootstrap replicates via raxmlGUI v.2.0.0 (Edler et al., 2021). The BI analysis was conducted using MrBayes v.3.2.0 with corresponding models used in the ML analyses and Markov Chains were run for 10 000 000 generations with topologies being sampled every 1 000 generations (Ronquist and Huelsenbeck, 2003). The first 25% trees were discarded as "burn-in" and software Tracer v.1.7.1 was used to check for the convergence of the trees (Rambaut et al., 2018).

2.4 Morphological and reproductive characteristics of spionids

To study the evolution of morphological and reproductive characteristics with reference to the molecular-based phylogenetic analyses, the following characteristics were compiled for all the 54 analyzed spioniform species. There are morphological groups: Group A: heavy spines present in chaetiger 5 and full-sized branchiae starting from chaetiger 7, with exceptions in four genera: branchiae in *Boccardia* and *Boccardiella* starting from chaetiger 7 in juveniles but chaetiger 2 in adults; branchiae in *Dipolydora* starting from chaetiger 7–10; though not included in the phylogenetic analysis, adult *Tripolydora* has full-sized branchiae from chaetiger 7, but short ones in chaetigers 2–6. Group B: without heavy spines and branchiae starting from chaetiger 1 or 2 to near the end. Group C: branchiae starting from chaetiger 1, 2, or 3, on a certain number of anterior segments. Group D: without branchiae in adults. Types of sperm (i.e., Ect: ect-aquasperm; and Int: introsperm). Types of oocyte (i.e., type I: oocytes with thick envelopes bearing numerous vesicles; type II: oocytes with thick envelopes without vesicles; and type III: oocytes with thin envelopes without vesicles). Reproductive patterns (i.e., Bro: brooding; and Nbro: non-brooding). Egg protection modes (i.e., cap I: hollow cylinder attached to tube; cap II: beadlike string capsules attached to the wall of tube; cap III: series of single capsules; intub: egg cocoon/egg mass in tube; onfem: on the body of female; eem: external egg masses/external jellylike cocoon; and Brsp: broadcast spawning into water column). Types of larval development (i.e., Pla: planktotrophic; Lec: lecithotrophic; and Dir: direct development). The morphological groups were created by the authors in this study and the other groups mentioned here were present on different studies (Jamieson and Rouse, 1989; Blake and Arnofsky, 1999; Radashevsky et al., 2016a, 2018; Blake et al., 2020).

3 RESULT

3.1 Systematics

Family Spionidae Grube, 1850

Genus *Rhynchospio* Hartman, 1936

Type species: *Rhynchospio arenincola* Hartman, 1936, by original designation. Type locality: San Mateo County, central California, USA.

Diagnosis (emended from Blake et al., 2020): Prostomium with fronto-lateral horns, caruncle variously developed; eyespots two pairs; occipital antenna absent. Branchiae from chaetiger 2 to near the end, free from dorsal lamellae or only fused basally. Notochaetae all capillary. Neurochaetae capillaries, hooded hooks, and sabre chaetae. Pygidium with cirri or lobes.

Remark: Blake et al. (2020) introduced the eyespots of *Rhynchospio* as present or absent, but we found that 10 of the 11 *Rhynchospio* species have 4 eyespots, while *R. glutaea* has only 2 after the original description.

Rhynchospio* aff. *asiatica

(Figs.1–5, Supplementary Fig.S1, Tables 1–3, Supplementary Table S1; Supplementary Videos S1–S4)

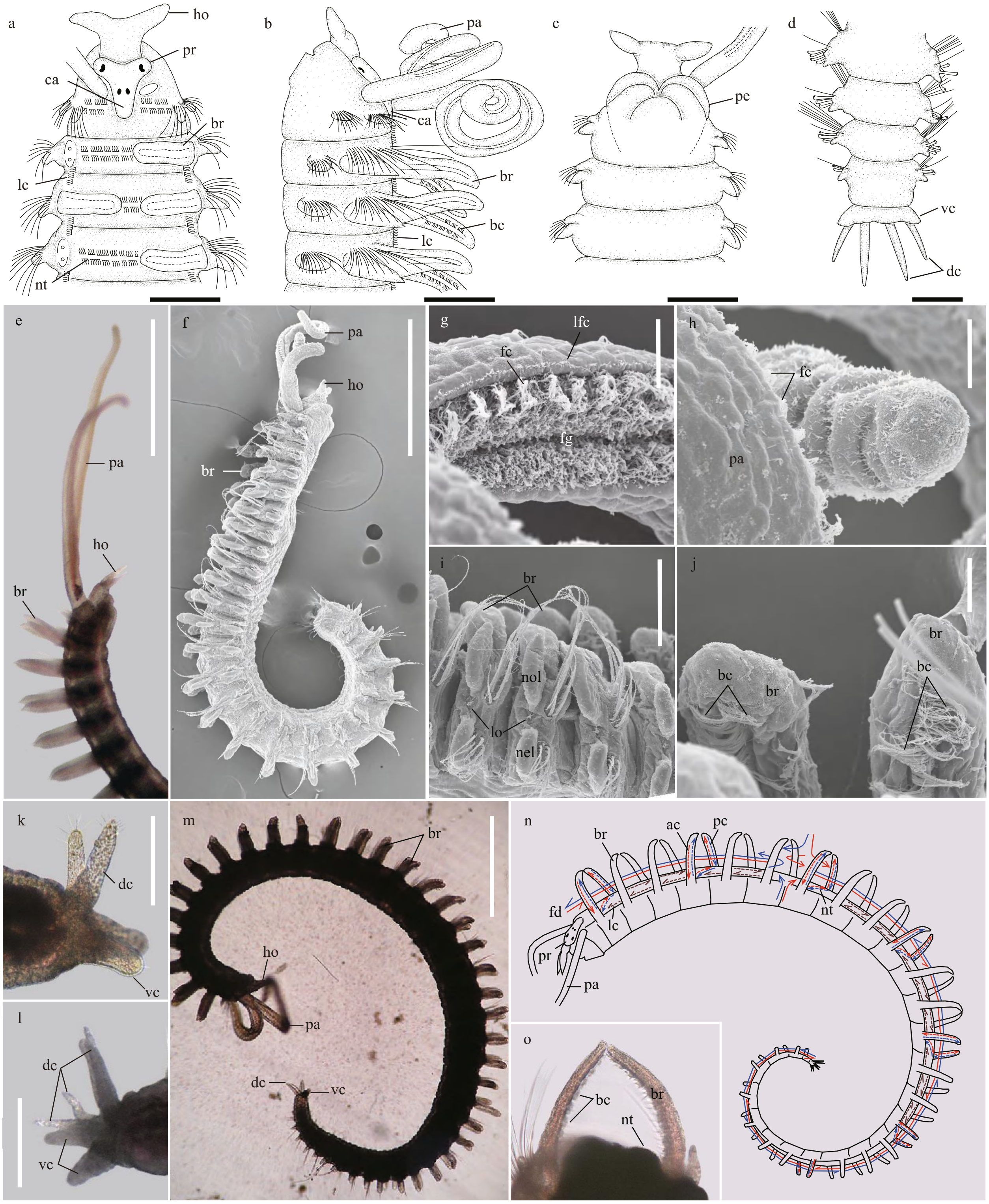

Fig.2 Morphology of *Rhynchospio* aff. *asiatica*

a. anterior end, dorsal view; b. same, lateral view; c. same, ventral view; d. posterior end, ventral view; e–j. specimen #17; e. anterior end, lateral view; f. same, SEM; g. middle part of palp, frontal view; h. distal end of palp; i. segments 4–7, lateral view; j. branchiae of segments 3–4; k. pygidium with 2 ventral cirri and 2 dorsal cirri; l. pygidium with 2 ventral cirri and 4 dorsal cirri; m. lateral view of a whole worm; n. schematic drawing showing the water flow direction (solid line) and cilia wave direction (dashed line); o. a dorsal cilia ring formed by a pair of branchia and the dorsum in anterior segments. Scale bars: g, h, j: 20 µm; d, i, k, l: 100 µm; a–c: 200 µm; e, f, m: 500 µm. Abbreviations: ac: anterior row of cilia; bc: branchial cilia; br: branchiae; ca: caruncle; dc: dorsal cirri; fc: frontal cilia; fd: water flow directions; fg: food groove; ho: fronto-lateral horns; lc: intersegmental longitudinal cilia; lfc: lateral-frontal cilia; lo: lateral ciliated organ; nel: neuropodial postchaetal lamellae; nol: notopodial postchaetal lamellae; nt: double-row nototrochs; pa: palps; pc: posterior row of cilia; pe: peristomium; pr: prostomium; vc: ventral cirri.

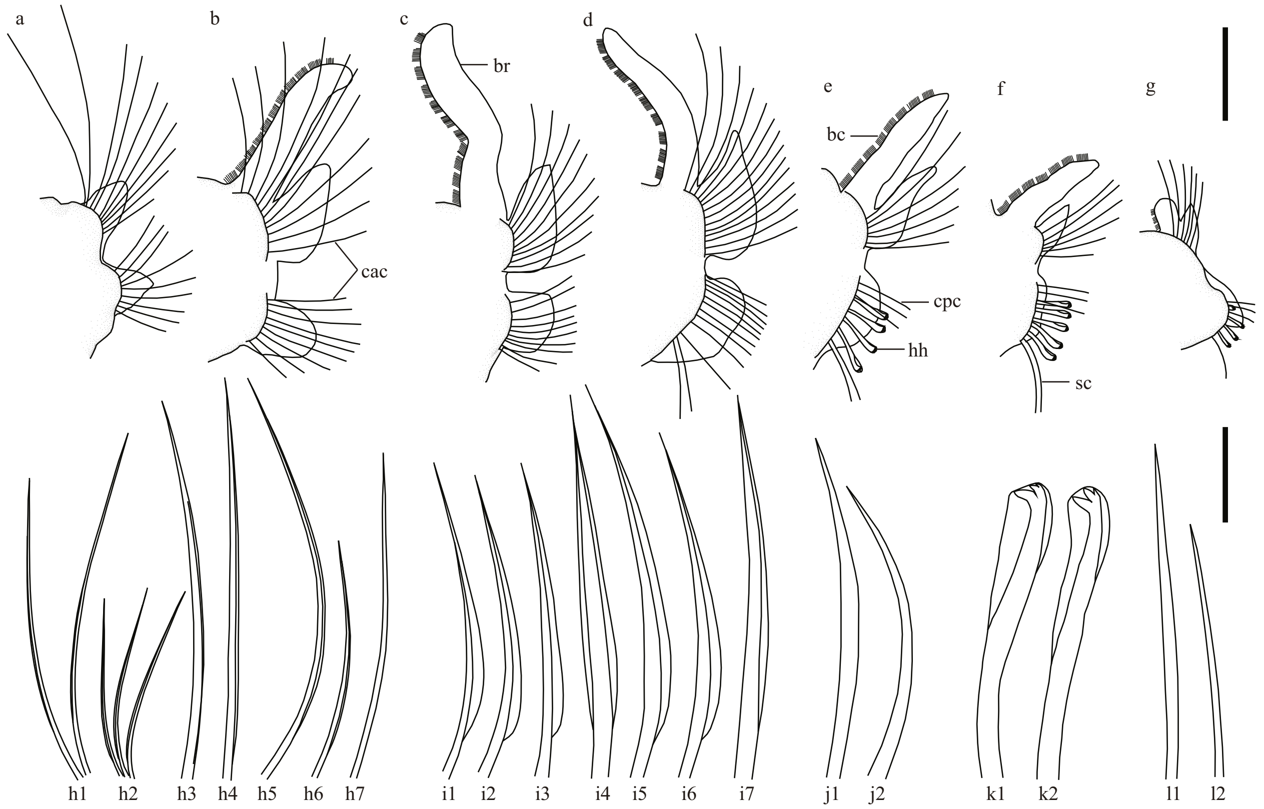

Fig.3 Parapodia and chaetae of *Rhynchospio* aff. *asiatica*

a–g. parapodia; a. chaetiger 1, anterior view; b. chaetiger 2, anterior view; c. chaetiger 4, anterior view; d. chaetiger 10, anterior view; e. chaetiger 20, anterior view; f. chaetiger 30, anterior view; g. posterior chaetiger, anterior view; h–l. chaetae; h. capillary chaetae in posterior rows of chaetigers; h1. chaetiger 1, notopodium; h2. chaetiger1, neuropodium; h3. chaetiger 2, notopodium; h4. chaetiger 2, neuropodium; h5. chaetiger 5, notopodium; h6. chaetiger 20, notopodium; h7. chaetiger 30, notopodium; i. chaetae in anterior rows of chaetigers; i1. chaetiger 1, notopodium; i2. chaetiger 1, neuropodium; i3. chaetiger 2, neuropodium; i4. chaetiger 3, notopodium; i5. chaetiger 5, notopodium; i6. chaetiger 10, neuropodium; i7. chaetiger 15, neuropodium; j. sabre chaetae; j1. chaetiger 20; j2. posterior chaetiger; k. hooded hooks in the posterior rows of neuropodia; k1. chaetiger 16; k2. chaetiger 20; l. companion chaetae in the anterior rows of neuropodia; l1. chaetiger 17; l2. chaetiger 20. Scale bars: h3, h4, i–l: 20 μm; h1, h2, h5–h7: 50 μm; a–g: 100 μm. Abbreviations: bc: branchial cilia; br: branchiae; cac: capillary chaetae; cpc: companion chaetae; hh: hooded hooks; sc: sabre chaetae.

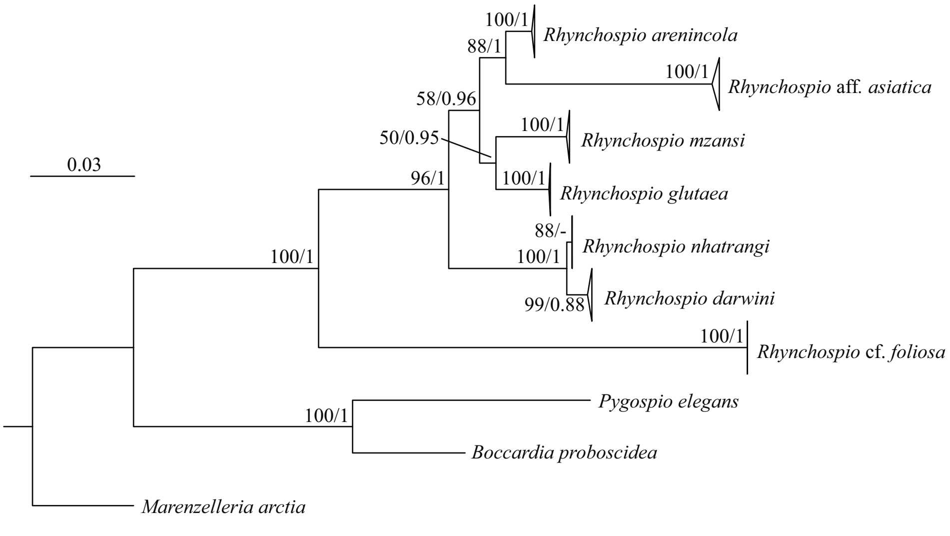

Fig.4 Phylogenetic tree reconstructed with Maximum Likelihood (ML) and Bayesian Inference (BI) methods

The tree was constructed based on the concatenated sequences (2 444 bp) of partial 16S rRNA (294 bp), 18S rRNA (1 502 bp), 28S rRNA (323 bp), and Histone H3 (325 bp) genes of *Boccardia*, *Pygospio*, and *Rhynchospio* rooted with *Marenzelleria* sequences. Values of robustness were calculated from ML and BI analyses. Only bootstrap (BS) values ≥50 and Bayesian posterior probabilities (BPP) values ≥0.7 are shown at nodes. -: node absent in BI. Information of spionids used is listed in Table 1. Scale bar indicates the number of substitutions per site.

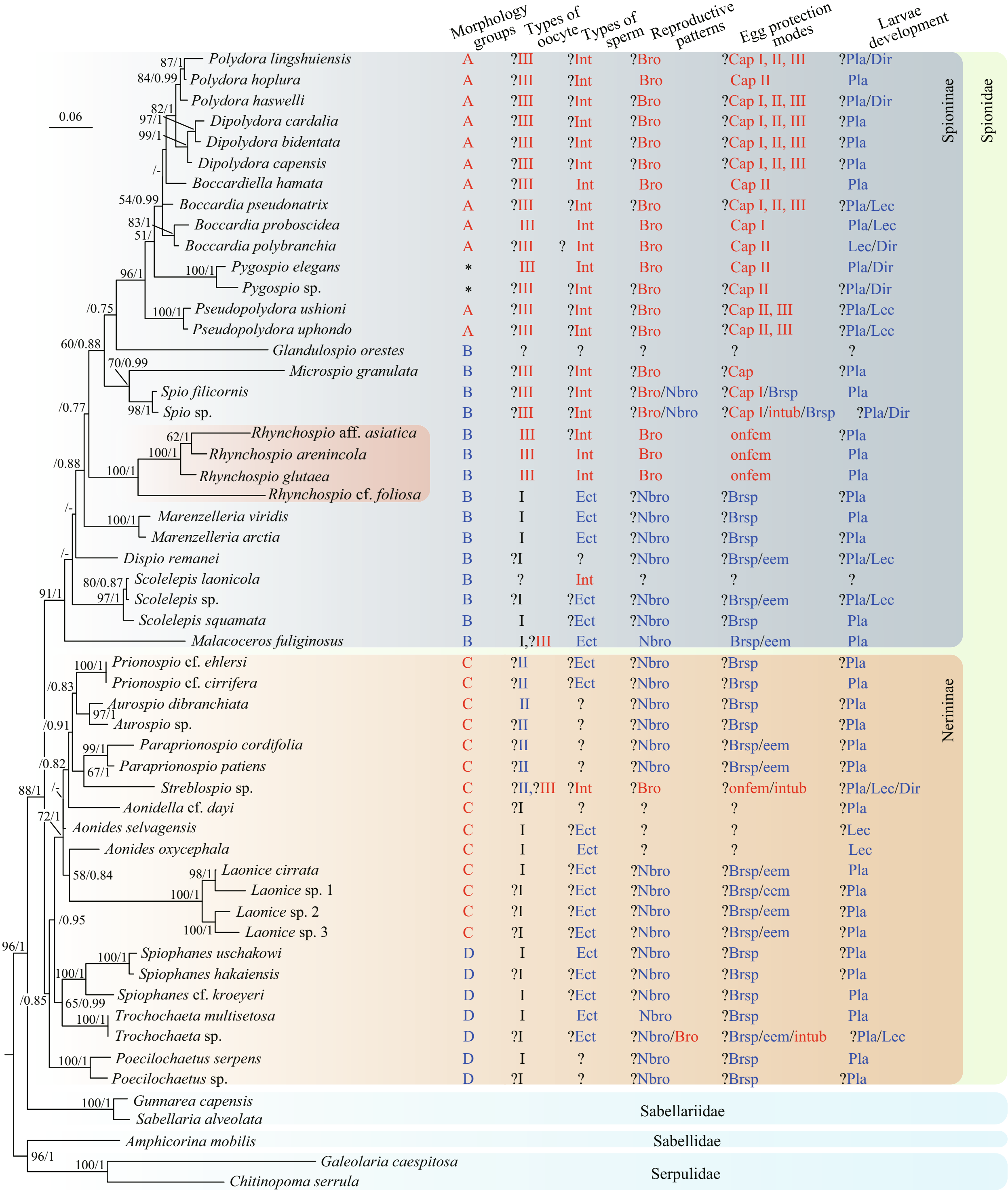

Fig.5 Phylogenetic tree reconstructed with Maximum Likelihood (ML) and Bayesian Inference (BI) methods

The tree was constructed based on the concatenated sequences (2 483 bp) of partial 16S rRNA (302 bp), 18S rRNA (1 535 bp), 28S rRNA (321 bp), and Histone H3 (325 bp) genes of Sabellidae, Sabellariidae, and Spionidae rooted with Serpulidae sequences. Values of robustness were calculated from ML and BI analyses. Only bootstrap (BS) values ≥50 and Bayesian posterior probabilities (BPP) values ≥0.7 are shown at nodes. -: node absent in BI. Information of spionids and the remaining taxa used is listed in Table 1. The scale bar indicates the number of substitutions per site. For morphological groups and reproductive characteristics refer to "Material and Methods". * *Pygospio* bears no spines with its branchiae starting from chaetiger 10. Question marks before characteristics indicate that such classification has not been reported in this species, but it has been noted in other species of the same genus.

Materials examined: One specimen (XMU-Pol-2021-050) collected on 2 November 2014, three specimens (XMU-Pol-2021-034, XMU-Pol-2021-035, and XMU-Pol-2021-036) collected on 18 June 2015,

Table 2 Major morphological characteristics for 17 specimens of *Rhynchospio* aff. *asiatica* (XMU-Pol-2021-034 to XMU-Pol-2021-050) used in this study

Taxa	Catalog No.	Length (mm)	Width (mm)	Total No. of chaetigers	First chaetiger with hooded hooks	Chaetigers with oocytes	Pygidial cirri[†]	Collection date	Preservation
Specimen #1	XMU-Pol-2021-034	7.5	0.5	51	16	17–33	2+3	2015.6.18	Formalin
Specimen #2	XMU-Pol-2021-035	9.1	0.5	59	16	17–39	–	2015.6.18	Formalin
Specimen #3	XMU-Pol-2021-036	8.5	0.5	59	17	17–33	–	2015.6.18	Formalin
Specimen #4	XMU-Pol-2021-037	10.9	0.6	54	15	–	2+4	2018.8.31	Formalin
Specimen #5	XMU-Pol-2021-038	8.3	0.6	51	16	16–26	2+3	2018.8.31	Formalin
Specimen #6	XMU-Pol-2021-039	7.1	0.5	47	15	–	2+4	2018.8.31	Formalin
Specimen #7	XMU-Pol-2021-040	7.3	0.5	40	16	–	2+3	2018.8.31	Formalin
Specimen #8	XMU-Pol-2021-041	10.3	0.5	54	16	–	2+4	2018.8.31	Formalin
Specimen #9	XMU-Pol-2021-042	7.1	0.4	45	14	–	2+4	2018.8.31	Formalin
Specimen #10	XMU-Pol-2021-043	4.3	0.5	38	15	–	2+3	2018.8.31	Formalin
Specimen #11	XMU-Pol-2021-044	7.3	0.5	50	15	–	2+4	2018.8.31	Formalin
Specimen #12	XMU-Pol-2021-045	7.8	0.6	51	15	–	2+4	2018.8.31	Formalin
Specimen #13	XMU-Pol-2021-046	6.3	0.6	44	16	–	2+2	2018.8.31	Formalin
Specimen #14	XMU-Pol-2021-047	6.8	0.7	50	16	–	2+2	2018.8.31	Formalin
Specimen #15	XMU-Pol-2021-048	8.9	0.5	56	16	16–31	2+3	2018.8.31	Formalin
Specimen #16	XMU-Pol-2021-049	8.1	0.5	55	15	–	2+4	2018.8.31	Ethanol
Specimen #17	XMU-Pol-2021-050	6.2	0.5	43	14	–	2+4	2014.11.2	Ethanol

[†]: the formula indicates "the number of ventral cirri" + "the number of dorsal cirri". – means data not available.

and thirteen specimens (XMU-Pol-2021-037, XMU-Pol-2021-038, XMU-Pol-2021-039, XMU-Pol-2021-040, XMU-Pol-2021-041, XMU-Pol-2021-042, XMU-Pol-2021-043, XMU-Pol-2021-044, XMU-Pol-2021-045, XMU-Pol-2021-046, XMU-Pol-2021-047, XMU-Pol-2021-048, and XMU-Pol-2021-049) collected on 31 August 2018 from an intertidal muddy sand beach in Qingdao Bay, Shandong, China (Table 2).

Description: Body slender, anterior part slightly wider and taper off to the end (Fig.2a–d). Prostomium T-shaped, bearing two conical latero-frontal horns (Fig.2a); caruncle not elevated above prostomium, reaching chaetiger 1 (Fig.2a & b); nuchal organs not observed; eyespots two pairs arranged in inverted trapezoid, small, brownish in color, anterior eyes crescent, twice as large as posterior ones (Fig.2a). Occipital tentacle absent (Fig.2a). Palps extending backward to chaetiger 9, with ventral longitudinal food groove along whole length (Fig.2b, e–h).

Branchiae flattened, present from chaetiger 2 to near the posterior end (Fig.2e & m), free from notopodial postchaetal lamellae (Fig.3b–g); branchiae longer, wider in anterior and middle chaetigers, shorter, narrower in posterior chaetigers (Fig.2a, b, e, m). Paired branchiae bending towards each other in each segment, with branchial surfaces oriented parallel to longitudinal body axis (Fig.2a, i, j). Two parallel rows of branchial cilia present along inner surface of each branchia (Fig.2b, j, n), together with double-row nototrochs, forming a ciliary ring on dorsum of each segment (Fig.2a, b, o). Metameric nuchal organs not observed. Short bands of cilia present between segments on dorsolateral edges (Fig.2a & b). Notopodial and neuropodial postchaetal lamellae of chaetiger 1 triangular, small (Fig.3a); notopodial postchaetal lamellae larger from chaetiger 2, subtriangular with terminal tips, becoming smaller from middle to posterior chaetigers (Fig.3b–g). Neuropodial postchaetal lamellae oval shaped from chaetiger 2, becoming wider and shorter in middle chaetigers, and obtuse triangular in posterior chaetigers (Fig.3b–g).

Anterior chaetigers with two rows of capillary chaetae in both notopodia and neuropodia (Fig.2i). Chaetae in anterior row with wide unilateral or bilateral limbation and fine granulation on distal part of shaft (Fig.3i). Chaetae in posterior row longer and thinner, without granulation (Fig.3h). Hooded hooks present only in neuropodia, 3–7 in posterior row (Fig.3e–g), from chaetiger 14–17 to posterior end (Fig.3e–g), with one main fang and two smaller upper teeth situated one above the other (Fig.3k). Sabre

Table 3 Key morphological data of *Rhynchospio* species around the world

No.	Species	Total No. of chaetigers	Length (mm)	Width (mm)	Latero-frontal horns	Eye spots	First chaetiger with hooded hooks	Pygidial cirri	Type locality	Reference
1	*R. inflata*	–	5.3	–	Smooth	4, -, trapezoidally arranged, anterior pair larger	37, tridentate	Posterior end missing	Bimini Islands, Bahamas	Foster, 1971
2	*R. australiana*	31	5	0.3	Smooth, wide	4, -, shallow crescent arranged, anterior pair larger	8, tridentate	3; plate like & knob like	Perth, West Australia, Australia	Blake and Kudenov, 1978
3	*R. glycera*	–	8	0.5	Smooth	4, -, trapezoidally arranged, two pairs of similar size	27, quadridentate	Posterior end missing	Burwood Beach, New South Wales, Australia	Blake and Kudenov, 1978
4	*R. tuberculata*	63	12	1.0	With tubercles	4, black, crescent arranged, two pairs of similar size	17, tridentate	Ventral 1 pair; lateral 4 pairs, long; rudimentary, 3–4	Sagami Bay, Honshu Island, Japan	Imajima, 1991
5	*R. foliosa*	79	18	1.0	Smooth	4, black, -, - (not distinct)	17, tridentate	18; foliaceous or cylindrical	Usujiri Bay, Hokkaido Island, Japan	Imajima, 1991
6	*R.* cf. *foliosa*	90	22	1	Smooth	4, dark red, trapezoidally arranged, anterior pair slightly larger	17–18, tridentate	Ventral 1 pair; dorsal up to 15 pairs, thinner and longer	Newport, Oregon, USA	Radashevsky et al., 2016a
7	*R. nhatrangi*	52–55	7.5–8	0.5	Smooth	4, red, trapezoidally arranged, anterior pair slightly larger	11–14, uni-/ tridentate	Ventral 1 pair; dorsal 2 pairs, thinner, slightly longer	Nha Trang Bay, Vietnam	Radashevsky, 2007a
8	*R. darwini*	33–60	2.5–5	0.2–0.3	Smooth	4, red, trapezoidally arranged, anterior pair slightly larger	11–12, tridentate	Ventral 1 pair; dorsal 2 pairs, thinner, slightly longer	Fannie Bay, Northern Territory, Australia	Radashevsky, 2015
9	*R. asiatica*	45–80	15–20	1.3–1.5	Smooth	4, -, trapezoidally arranged, anterior pair larger	10–23, tridentate	Ventral 1 pair; dorsal up to 5 pairs, thinner, longer	Shikotan Island, Yuri Island, South Kurile Islands	Chlebovitsch, 1959; Radashevsky et al., 2014
10	*R.* aff. *asiatica*	38–59	4–11	0.4–0.7	Smooth	4, brownish, trapezoidally arranged, anterior pair larger	14–17, tridentate	Ventral 1 pair; dorsal 1 to 2 pairs, thinner, slightly longer	Jinhae Bay, South Korea; Qingdao Bay, China	Radashevsky et al., 2014; this study
11	*R. arenincola*	45	7–10	0.6	Smooth	4, black, rectangularly arranged, posterior pair larger	18, tridentate	4 pairs of bilobed cirri	San Mateo County, California, USA	Hartman, 1936
12	*R. glutaea*	90	24	1.3	Smooth	2 in original description (supposed to be 4), -, -, -	18; tridentate	2 pairs; coniform and ball like	Punta Arenas, Strait of Magellan, Chile	Ehlers, 1897
13	*R. mzansi*	47–68	7–13	0.6–0.9	Smooth	4, black, trapezoidally arranged, anterior pair slightly larger	15–18, tridentate	Ventral 1 pair; dorsal 2–4 pairs, thinner, longer	Gansbaai, South Africa	Simon et al., 2019b

– means data not available.

chaetae without limbation, 2–3 per rami, present below hooded hooks from chaetigers 14–17 (Fig.3e–g, j). Companion chaetae without limbation, 2–4 in number, present in anterior row of hooded hooks (Fig.3e–g, l).

Pygidium with one pair of stumpy ventral cirri and 1 to 2 pairs of thinner and longer dorsal cirri in most specimens (Fig.2d, k, l).

Dorsal tunnel: Observations of live specimens of *Rhynchospio* collected from Qingdao revealed a dorsal tunnel for the first time (Fig.2m–o). On most segments, a somewhat closed circle is formed by paired branchiae and the dorsum (Fig.2o; Supplementary Video S1). All these circles together form a dorsal tunnel. Two rows of parallel cilia are present along the inner side of branchiae and the dorsum, forming two parallel ciliary rings on each segment (Fig.2j & n). The anterior ciliary band beats backward and the posterior ciliary band beats forward, creating a dexioplectic metachronal wave with the effective beating of cilia being to the right of the wave (Supplementary Videos S2 & S3). The same pattern of cilia beating has been widely observed in annelids (Knight-Jones, 1954). When the anterior row of cilia in all segments beats in the same direction from anterior to posterior, it forms a backward water flow; when the posterior row of cilia in each segment beats from posterior to anterior direction, it forms a forward water flow (Supplementary Video S3). By coordinating the beating of the anterior and posterior ciliary bands, the worm can transport small particles along the dorsal tunnel from anterior to posterior, or vice versa (Supplementary Videos S3 & S4).

Ecology: The specimens of *Rhynchospio* aff. *asiatica* examined in this study live in muddy sand sediment in the high intertidal zone of Qingdao Bay, a habitat encountering fluctuations in environmental conditions created by inundation of tides and discharge of domestic sewage (Supplementary Fig. S1). Du et al. (2011) indicated higher sedimentary organic content (1.81% vs. 0.44%) in the studied intertidal zone than that in offshore zone. In addition, as a tourist spot, the study site encounters frequent human disturbances including trampling and clam digging especially in the summer. The abundance of *Rhynchospio* in the study site varied seasonally from high at 21 522 inds./m^2 in August to low at 308 inds./m^2 in May (Wang, 2015).

K2P genetic distances: Among the *Rhynchospio* species compared, the Qingdao Bay sequences are most closely related to those of *R.* aff. *asiatica* from Jinhae Bay, South Korea. The K2P genetic distances between the two populations was only 0.41% for 16S rRNA, 0.12% for 18S rRNA, 0 for 28S rRNA, 0.88% for Histone H3, and 0.19% for the concatenated sequences. These small genetic distances clearly indicated that the Qingdao population is conspecific to the South Korea population, which will be described as a new species (Vasily I. Radashevsky, personal communication). Among the described *Rhynchospio* species, *R. arenincola* has the smallest average K2P distances with *R.* aff. *asiatica*, i.e., 6.10% for the concatenated sequences of four genes, 20.02% for 16S rRNA, 4.50% for 18S rRNA, 8.44% for 28S rRNA, and 2.74% for Histone H3. Other species are more distantly related (Supplementary Table S1).

3.2 Phylogeny and morphology in spionids

Phylogenetic analysis (Fig.4) shows that all *Rhynchospio* aff. *asiatica* specimens collected from Qingdao and Jinhae form a well-supported clade (BS=100; BPP=1) that is sister to *R. arenincola* from California (BS=88; BPP=1); and all seven *Rhynchospio* species included in the analysis form a monophyletic clade (BS=100; BPP=1). All spionids form a single clade (BS=88; BPP=1) that is sister to Sabellariidae (BS=96; BPP=1). Two clades are detected within spionids: clade 1 (here referred to as Spioninae) includes *Polydora*, *Dipolydora*, *Boccardiella*, *Boccardia*, *Pygospio*, *Pseudopolydora*, *Glandulospio*, *Microspio*, *Spio*, *Rhynchospio*, *Marenzelleria*, *Dispio*, *Scolelepis*, and *Malacoceros* (BS=91; BPP=1); clade 2 (here referred to as Nerininae) includes *Prionospio*, *Aurospio*, *Paraprionospio*, *Streblospio*, *Aonidella*, *Aonides*, *Laonice*, *Spiophanes*, *Trochochaeta*, and *Poecilochaetus*, although with low support values (BS=46; BPP=0.85). Morphologically, all taxa within morphology group A, together with *Pygospio*, form a single clade (BS=96; BPP=1), while all taxa within morphology group B showed paraphyletic relationships (excluding *Spio* and *Microspio*). All taxa within morphology group C form a single clade (BS=72; BPP=1), while *Poecilochaetus* within morphology group D showed paraphyletic relationship with the group formed by *Spiophanes* and *Trochochaeta* (BS=65; BPP=0.99).

3.3 Reproductive and larval characteristics in spionids

Despite there are ambiguous or missing information in reproductive and larval characteristics, distinct clusters corresponding to the phylogenetic tree could be noted within each characteristic (Fig.5). Four clusters of spionids according to types of oocytes include a cluster corresponding to type III, a cluster corresponding to type II, and two separate clusters corresponding to type I at the basal positions of Spioninae and Nerininae. Two exceptions in *Malacoceros* and *Streblospio* are noted with both thick- and thin-enveloped oocytes. Three characteristics (i.e., types of sperm, reproductive patterns and egg protection modes) are basically divided into two clusters, i.e., one cluster includes taxa corresponding to those with oocyte type III, while the other cluster including other spioniform taxa excluding some species of *Trochochaeta*, *Streblospio*, and an ectoparasitic spionid *Scolelepis laonicola*. Almost all spioniform taxa have planktotrophic larval development, while lecithotrophic and direct development modes are only found in about 1/4 to 1/3 of the analyzed genera in this study.

4 DISCUSSION

Rhynchospio aff. *asiatica*, originally recorded from Jinhae, South Korea, is characterized by having sperm in chaetigers 11–14, and oocytes starting from chaetiger 16 (Radashevsky et al., 2014). This species described based on individuals from Jinhae will be formally named in a manuscript submitted for publication (Vasily I. Radashevsky, personal communication). Our *Rhynchopsio* individuals from Qingdao are characterized by having smooth latero-frontal horns, four trapezoidally arranged eyespots, neuropodial hooded hooks starting from chaetigers 14–17 to posterior end, sperm in chaetigers 11–14, oocytes from chaetigers 16–17 to chaetigers 26–39, and 2–3 pairs of pygidial cirri. Morphologically, the specimens from Qingdao could be classified as belonging to the *Rhynchospio glutaea* complex, and further identified as *R.* aff. *asiatica* due to their similarity in the distribution of sperm and oocytes along segments (Radashevsky et al., 2014). Molecular results support the two populations of *Rhynchospio* to be conspecific by showing quite small inter-population K2P distances.

Although thirteen *Rhynchospio* species including an unnamed species *R.* cf. *foliosa* have been recorded, molecular data are only available for seven species (Radashevsky et al., 2014, 2016a; Simon et al., 2019b; this study), and the phylogenetic placements of the other six species (i.e., *R. asiatica*, *R. foliosa*, *R. tuberculata*, *R. australiana*, *R. glycera*, and *R. inflata*) are still undetermined. Based on morphological characteristics, three *Rhynchospio* species, i.e., *R. australiana*, *R. glycera*, and *R. inflata*, have apparent divergences with *R. foliosa*/*R.* cf. *foliosa*, the two tropical species (i.e., *R. darwini* and *R. nhatrangi*), and those in the *Rhynchospio glutaea* complex, Specifically, *R. australiana* bears a pair of broad horns (rather than digitiform in the *R. glutaea* complex), lacking (rather than possessing) notopodial capillaries on chaetiger 1, plate-like (rather than cirriform) pygidium, and perpendicularly (rather than parallelly) oriented branchial surface, inner and outer ciliary bands (rather than two parallel inner bands), and lacking (rather than possessing) transverse ciliary dorsal ridges on each segment (Blake and Kudenov, 1978). The branchial orientation and ciliary pattern of *R. australiana* are more similar to those of *Malacoceros* (Hourdez et al., 2006) and *Aonides* (Radashevsky, 2015). *R. glycera* has two posterior pairs of digitiform nuchal lobes (rather than lacking nuchal lobes in the *R. glutaea* complex) on caruncle, and bears quadridentate (rather than tridentate) hooded hooks (Blake and Kudenov, 1978). *R. inflata* was named after its inflated anterior body region and its neuropodial hooded hooks starting from chaetiger 37, far posterior than those in the other *Rhynchpsio* species (Table 3). Not described but showed in figures (Figs. 113, 114 in Foster, 1971), *R. inflata* seems to resemble *R. australiana* in that they both have ciliated branchiae on inner and outer margins. Another species, *R. tuberculata*, however, is similar in morphology to species of the *R. glutaea* complex, except that it has three small tubercles on the underside of its lateral-frontal horns. Further study for *R. australiana*, *R. glycera*, *R. inflata*, and *R. tuberculata*, might uncover their divergences in phylogeny with the remaining *Rhynchospio* species.

Water flow caused by ciliary action of nototrochs may help to transfer the oxygen dissolved in seawater into tubes of some genera of Spioninae (Simon, 1967). In *Rhynchospio* aff. *asiatica*, however, the water flow is stimulated by ciliary beating on the inner side of paired branchiae and nototrochs of each segment, which leads to directional movement of water along the dorsum of the worm. The water flow on the dorsum of *R.* aff. *asiatica* could be observed from directional movement of small particles suspended in the water tunnel, indicating that it may have a function of transporting the gametes from anterior and middle fertile segments to the posterior hatchery formed by elongated capillaries. Given the similarity in the arrangement of ciliary bands, it may be reasonable to infer that perhaps this water flow system exists in all *Rhynchospio* species (perhaps excluding *R. inflata* and *R. australiana* which have perpendicularly oriented branchial surfaces), which can be confirmed by observing live specimens in the future. This feature may cause adaptive changes, given that high fertilization efficiency might be desirable for these spionids that produce only a small amount of sperm and oocytes (Radashevsky, 2007a).

Our study has contributed to a better understanding of the phylogeny of Spionidae. The genera within Spionidae could be basically divided into two clades/subfamilies, i.e., Spioninae and Nerininae (Fig.5). Five genera (i.e., *Dispio*, *Malacoceros*, *Marenzelleria*, *Rhynchospio*, and *Scolelepis*) previously grouped within Nerininae due to their similarities in reproductive characteristics, such as thick-enveloped oocytes, ect-aquasperm, and broadcast spawning (Blake and Arnofsky, 1999), form a single clade with the other genera belonging to Spioninae with relatively high support values (BS=91; BPP=1) in this study. This phylogenetic result is supported by the morphological evidence that all the five genera have branchiae on most of the segments (referring to morphology Group B), similar to that of *Spio* and *Microspio*, which belong to Spioninae (Blake and Arnofsky, 1999). Similar close relationships between these genera (i.e., *Marenzelleria*, *Rhynchospio*, *Malacoceros*, *Dispio*, and *Scolelepis*) and those belonging to Spioninae were also showed in phylogenetic results of Abe and Sato-Okoshi (2021), while they were grouped within Nerininae according to morphological characteristics (i.e., number of eyes, pigmentation, and the position of gastrotrochs). In addition, our phylogenetic results agree with that of Abe and Sato-Okoshi (2021) by showing that the two spioniform genera (i.e., *Trochochaeta* and *Poecilochaetus*) are clustered within the clade of Spionidae. The two phylogenetic analyses thus provided molecular evidences to support the proposal by Blake and Arnofsky (1999) that Trochochaetidae and Poecilochaetidae should be included within a broadly defined Spionidae.

The phylogenetic results (Fig.5) support the suggestion proposed by Hannerz (1956) and Radashevsky (2007b) that the ancestor of spionids might have thick-envelope oocytes, and thin-envelope oocytes is a derived trait in Spioninae. Given that thin-enveloped oocytes and introsperms have widespread occurrence in other annelid families (Jamieson and Rouse, 1989; Blake and Arnofsky, 1999), they might have occurred more than once in the evolutionary history of annelids, and their evolution within Spioninae could be considered as adaptive changes to various brooding activities, such as having capsules/egg mass with brooding in tube or brooding on the dorsum of female. With three spioniform genera, i.e., *Spiophanes*, *Trochochaeta*, and *Poecilochaetus*, at the base of the phylogenetic tree, our results further indicate that the common spionid ancestor might have no branchiae, and various arrangement patterns of branchiae (Groups A, B, C) together with various brooding/egg protection modes might be derived characteristics. This conclusion differs from the scenario proposed by Radashevsky (2007b) that the first spionid might have branchiae on most segments, which might be due to the basal position of the genera such as *Marenzelleria*, *Dispio*, *Scolelepis*, and *Malacoceros* in his cladistic analysis. Overall, our phylogenetic analysis and mapping of reproductive characteristics to the phylogenetic tree revealed a potential evolutionary history of reproductive characteristics in spionids.

Taxonomic key to *Rhynchospio* species (modified from Radashevsky et al., 2014)

1 Anterior body swollen; hooks in neuropodia from chaetiger 37........ *R. inflata* (Bimini Islands, Bahamas)

– Anterior body slender, hooks in neuropodia from earlier than chaetiger 37..2

2 Notopodia from chaetiger 1 lacking capillaries, hooks in neuropodia from chaetiger 8 .... *R. australiana* (West Australia, Australia)

– Notopodia from chaetiger 1 with capillaries, hooks in neuropodia from later than chaetiger 8........3

3 Caruncle with two posterior pairs of digitiform nuchal lobes, hooks in neuropodia from chaetiger 27 quadridentate .... *R. glycera* (New South Wales, Australia)

– Caruncle posteriorly narrow, without lobes, hooks in neuropodia from chaetigers 9–18, tridentate...........4

4 Ventral side of prostomial horns with 3 minute tubercles, medially constructed .... *R. tuberculata* (Honshu, Japan)

– Ventral side of prostomial horns smooth and tapered, without tubercles 5

5 Anterior branchiae large, broad, foliaceous 6

– Anterior branchiae of moderate size, elongate 7

6 Caruncle anteriorly elevated above prostomium; up to 18 pygidial cirri or lobes ....

.... *R. foliosa* (Hokkaido, Japan)

– Caruncle not elevated above prostomium; up to 32 pygidial cirri .... *R.* cf. *foliosa* (Oregon, USA)

7 Mature worm hooks in neuropodia of chaetigers 11–14 unidentate; from chaetiger 15 onwards tridentate .... *R. nhatrangi* (Nha Trang Bay, Vietnam)

– Mature worm hooks in neuropodia all tridentate 8

8 Worm length up to 5 mm for 60 chaetigers; hooks in neuropodia from chaetigers 11–12; sperm in chaetigers 11–14, oocytes from chaetiger 15; pygidial cirri up to 3 pairs .... *R. darwini* (Northern Territory and Queensland, Australia)

– Worm length up to 20 mm for 80 chaetigers; hooks in neuropodia from chaetigers 10–23; sperm in chaetigers 11 to 21–22, oocytes from chaetigers 22–24; pygidial cirri up to 6 pairs .... *R. asiatica* (Kurile Islands)

– Worm length up to 11 mm for 59 chaetigers; hooks in neuropodia from chaetigers 14–17; sperm in chaetigers 11–14, oocytes from chaetiger 16–17; pygidial cirri 2–3 pairs .... *R.* aff. *asiatica* (Yellow Sea, East China Sea and Sea of Japan)

– Worm length up to 20 mm for 80 chaetigers; hooks in neuropodia from chaetiger 18; sperm in chaetigers 11 to 15–17, oocytes from chaetigers 17–19; pygidial cirri 4 pairs .... *R. arenincola* (Pacific North America)

– Worm length up to 24 mm for 90 chaetigers; hooks in neuropodia from chaetiger 18; sperm in chaetigers 12 (rarely 11) to chaetigers 16–17, oocytes from chaetiger 18; pygidial cirri 2 pairs ....*R. glutaea* (Atlantic and Pacific South America)

– Worm length up to 13 mm for 68 chaetigers; hooks in neuropodia from chaetigers 15–18; pygidial cirri 3–5 pairs .... *R. mzansi* (Western Cape, South Africa)

5 DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Electronic supplementary material

Supplementary material (Supplementary Table S1, Supplementary Fig.S1, and Supplementary videos S1–S4) is available in the online version of this article at https://doi.org/10.1007/s00343-021-1068-z.