

*Phylogeny of Nemertea with special interest
in the placement of diversity from Far East
Russia and northeast Asia*

**Sebastian Kvist, Alexei V. Chernyshev &
Gonzalo Giribet**

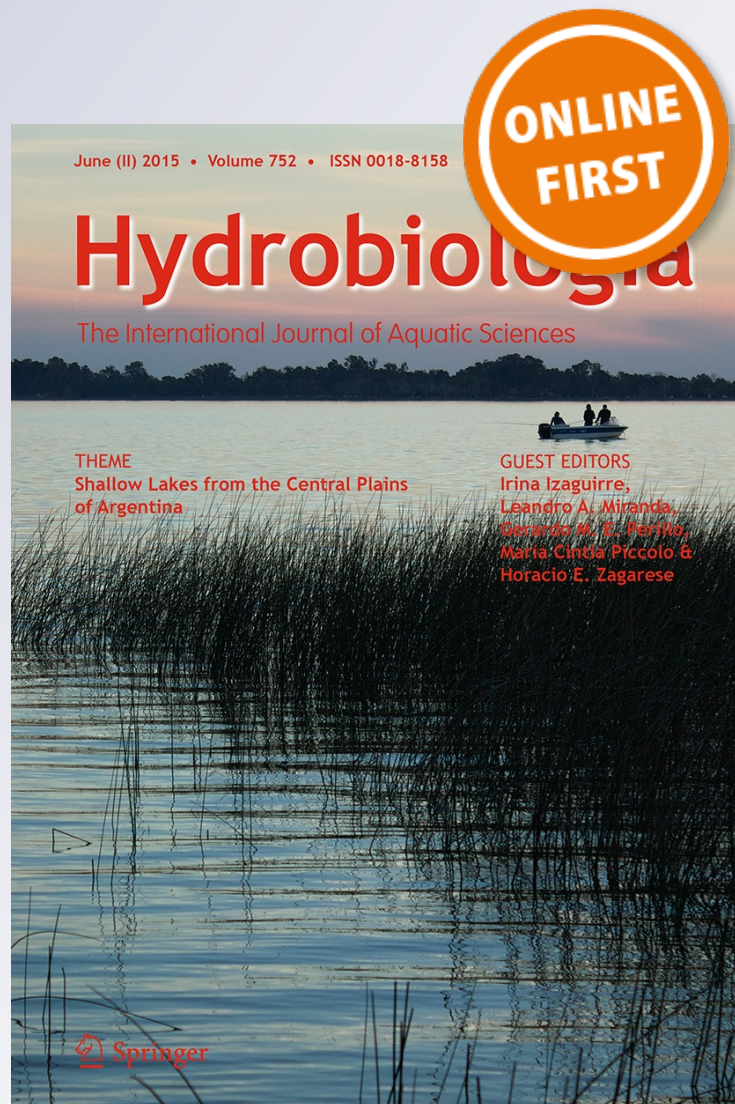
Hydrobiologia

The International Journal of Aquatic
Sciences

ISSN 0018-8158

Hydrobiologia

DOI 10.1007/s10750-015-2310-5



Your article is protected by copyright and all rights are held exclusively by Springer International Publishing Switzerland. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Phylogeny of Nemertea with special interest in the placement of diversity from Far East Russia and northeast Asia

Sebastian Kvist · Alexei V. Chernyshev · Gonzalo Giribet

Received: 13 February 2015 / Revised: 23 April 2015 / Accepted: 29 April 2015
© Springer International Publishing Switzerland 2015

Abstract Recent investigations, based mostly on molecular data, have unraveled the evolutionary history of several common ribbon worm (phylum Nemertea) species and solidified the taxonomic status of many higher taxa within the group. However, a large proportion of enigmatic species have yet to be placed in a phylogenetic framework. We investigated the phylogenetic positions of 26 novel and/or perplexing nemertean species from the Sea of Okhotsk, the Sea of Japan, the Kuril–Kamchatka Trench, and Vietnam (including the first record of a reptant nemertean from the Far East seas of Russia). We conducted both maximum likelihood and parsimony analyses, utilizing four molecular loci—mitochondrial

COI and 16S rRNA, as well as nuclear 18S rRNA and 28S rRNA—finding that the current classification for most of these species is corroborated by their phylogenetic placement. We then discuss the evolution of some unique morphological traits possessed by some of these species, using the molecular phylogeny as a backbone for our general conclusions.

Keywords Nemertea · Phylogeny · Sea of Okhotsk · Sea of Japan · Vietnam · Kuril–Kamchatka Trench

Introduction

Notwithstanding the utility of morphological characters in delimiting and diagnosing ribbon worm

Handling editor: Christian Sturmbauer

S. Kvist (✉) · G. Giribet
Museum of Comparative Zoology, Department of
Organismic and Evolutionary Biology, Harvard
University, 26 Oxford Street, Cambridge, MA 02138,
USA
e-mail: skvist@rom.on.ca

Present Address:

S. Kvist
Department of Natural History, Royal Ontario Museum,
100 Queen's Park, Toronto, ON M5S 2C6, Canada

S. Kvist
Department of Ecology and Evolutionary Biology,
University of Toronto, 25 Willcocks Street, Toronto,
ON M5S 2B4, Canada

A. V. Chernyshev
A.V. Zhirmunsky Institute of Marine Biology, Far East
Branch, Russian Academy of Sciences,
Vladivostok 690059, Russia

A. V. Chernyshev
Far Eastern Federal University, Vladivostok 690600,
Russia

(phylum Nemertea) species, molecular data seem to currently provide the most effective measure for inferring phylogenies (Schwartz & Norenburg, 2001; Strand & Sundberg, 2005a; Sundberg et al., 2010; Sundberg & Strand, 2010; Strand et al., 2014; Leasi & Norenburg, 2014; see Turbeville, 2002 for a comprehensive review of nemertean phylogenetics). For several nemertean taxa, this is due partly to the homoplastic nature of morphological characters, which impelled Sundberg et al. (2010) and Sundberg & Strand (2010) to propose that molecular data should accompany any modern species description within Nemertea. In reply to the need for molecular approaches, especially regarding more problematic taxa with few distinguishing morphological features, contemporary nemertean systematics has seen a surge in contributions employing molecules to shed light on long-reigning morphological phylogenetic hypotheses and taxonomic classification systems (Sundberg et al., 2001; Thollesson & Norenburg, 2003; Andrade et al., 2012, 2014; Kvist et al., 2014). Taken together, these studies have allowed for robust tests of species affiliations and evolutionary relationships among the most commonly sampled nemertean taxa. It remains, however, that several, perhaps more enigmatic, taxa have yet to be placed in a phylogenetic framework and that the body of undescribed diversity is likely large within Nemertea, much like most other phyla (e.g., May, 1988; Blaxter, 2003). Beyond the imbalance in the distribution of scientific funding opportunities in opposing parts of the world, one of the principal reasons for the idleness towards groups of taxa is likely the logistic difficulty inherent in taxonomic sampling efforts in remote geographic regions. As a result, a disproportionate amount of the known nemertean diversity that has been firmly placed in a phylogenetic context inhabits the same general area around the western and southern European coastlines, as well as parts of the Americas.

Although over 300 nemertean species have been recorded from northeastern Asia, including about 120 species from the Far East seas of Russia [the Sea of Okhotsk and the northern part of the Sea of Japan (also known as the East Sea)] (Crandall et al., 2002; see also Gibson, 1995; Kajihara et al., 2008; Chernyshev, 2014), the full diversity of nemertean taxa in this geographic region is still largely unappreciated (e.g., Korotkevich, 1971; Chernyshev, 2013; Chernyshev et al., 2015), and most of these taxa have yet to be solidly placed in a

phylogeny. The oceanographic properties of these semi-enclosed bodies of water are in stark contrast; deep basins mainly represent the Sea of Japan, whereas the Sea of Okhotsk also holds more shallow regions (Preller & Hogan, 1998). Furthermore, palaeoenvironmental studies have shown that the seas have undergone major geomorphological shifts since the late Pleistocene (Ryu et al., 2005). These events have led to differences not only in oceanic currents (e.g., Hidaka, 1966; Kitamura et al., 2001), but also uniqueness in micro-organismal diversity (e.g., Kojima, 2002) and benthic faunal compositions (e.g., Sahling et al., 2003). Indeed, the Sea of Okhotsk and the Sea of Japan have jointly been suggested as one of Earth's most biologically valuable ecoregions, placing among the top polar and sub-polar marine ecosystems in terms of biological value (Olson & Dinerstein, 1998). Despite of this importance, several major invertebrate groups remain unsurveyed in this area of the Pacific Ocean. To make matters worse, the depth and geomorphological complexity of the Kuril–Kamchatka Trench (>10,000 m at the deepest), positioned slightly east of the Sea of Okhotsk, makes sampling efforts within this area particularly difficult. To partially alleviate this situation, the present study aims to elucidate the general evolutionary relationships of several nemertean specimens collected from the Sea of Okhotsk, the Sea of Japan and the Kuril–Kamchatka Trench, as well as a few specimens from Vietnam.

Materials and methods

Specimen collection

The specimens used in the present study were collected by different methods during several expeditions to the Far East seas of Russia and northeastern Asia. Intertidal nemerteans were collected without any equipment; deeper dwelling species of nemerteans (between 6 and 400 m depth) were collected by different kinds of dredges; the deepest-dwelling (abyssal) nemerteans were collected by Agassiz trawl and epibenthic sledges during the German–Russian deep-sea expedition to the abyssal plain adjacent to the Kuril–Kamchatka Trench on board the R/V “Sonne” in the summer of 2012 (see Figs. 1, 2, 3 for some representative taxa). Tissue samples from live nemerteans were fixed in 95% ethanol for DNA

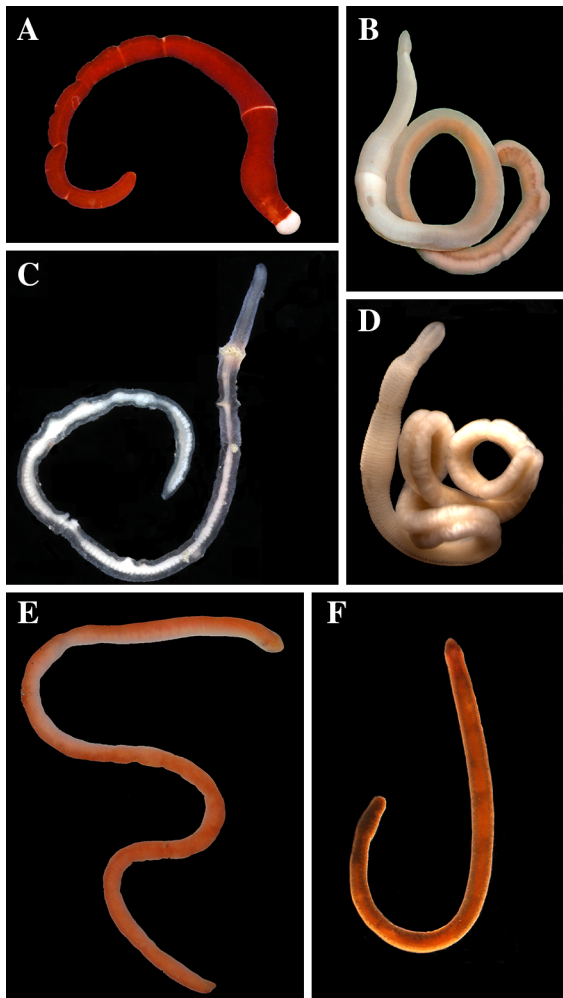


Fig. 1 Live habitus of selected specimens. Photos by A. Chernyshev. **A** *Tubulanus* sp. IZ-45552 (Sea of Japan, Vostok Bay, Aug. 2012). **B** *Callinera kasyanovi* Chernyshev, 2008 IZ-45551 (Sea of Japan, Peter the Great Bay, Aug. 2012). **C** *Cephalothrix iwatai* Chernyshev, 2013 IZ-45650 (Sea of Japan, Nov. 2013). **D** *Parahubrechtia* sp. IZ-45554 (abyssal plain adjacent to the Kuril–Kamchatka Trench, Aug. 2012). **E** *Monostilifera* sp. IZ-45641 (Sea of Okhotsk, Kuril Islands, Jul. 2011). **F** *Sacconemertidae* sp. IZ-45649 (Sea of Okhotsk, near Magadan, Oct. 2008). Additional specimen details can be found in MCZbase (<http://mczbase.mcz.harvard.edu/>)

extraction; in some instances, worms were fixed in 2% formalin for 2 h then transferred to 95% ethanol (DNA sequencing was still successful for these specimens). For investigation of internal morphology, the nemertean worms were anaesthetized in 7% magnesium chloride, dehydrated in absolute ethanol, cleared in xylene,

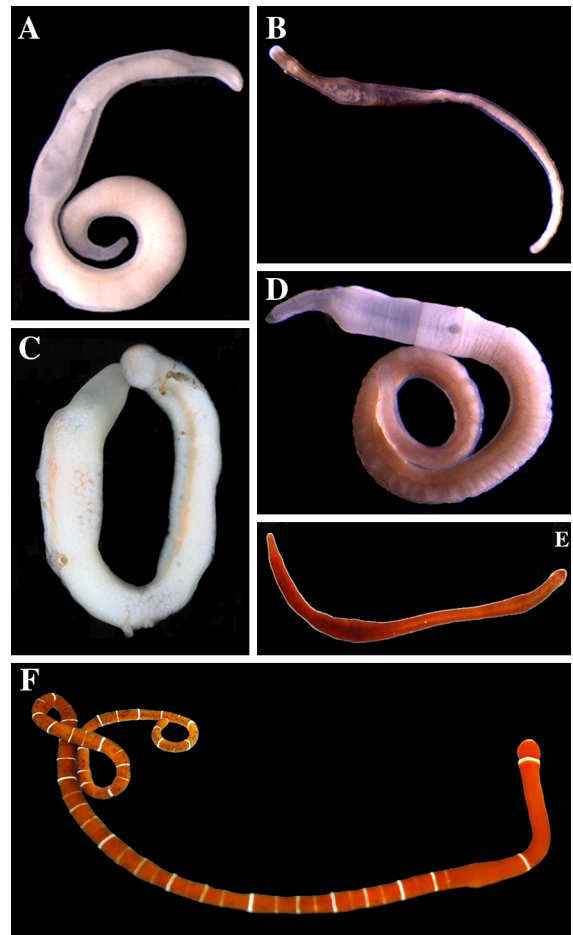
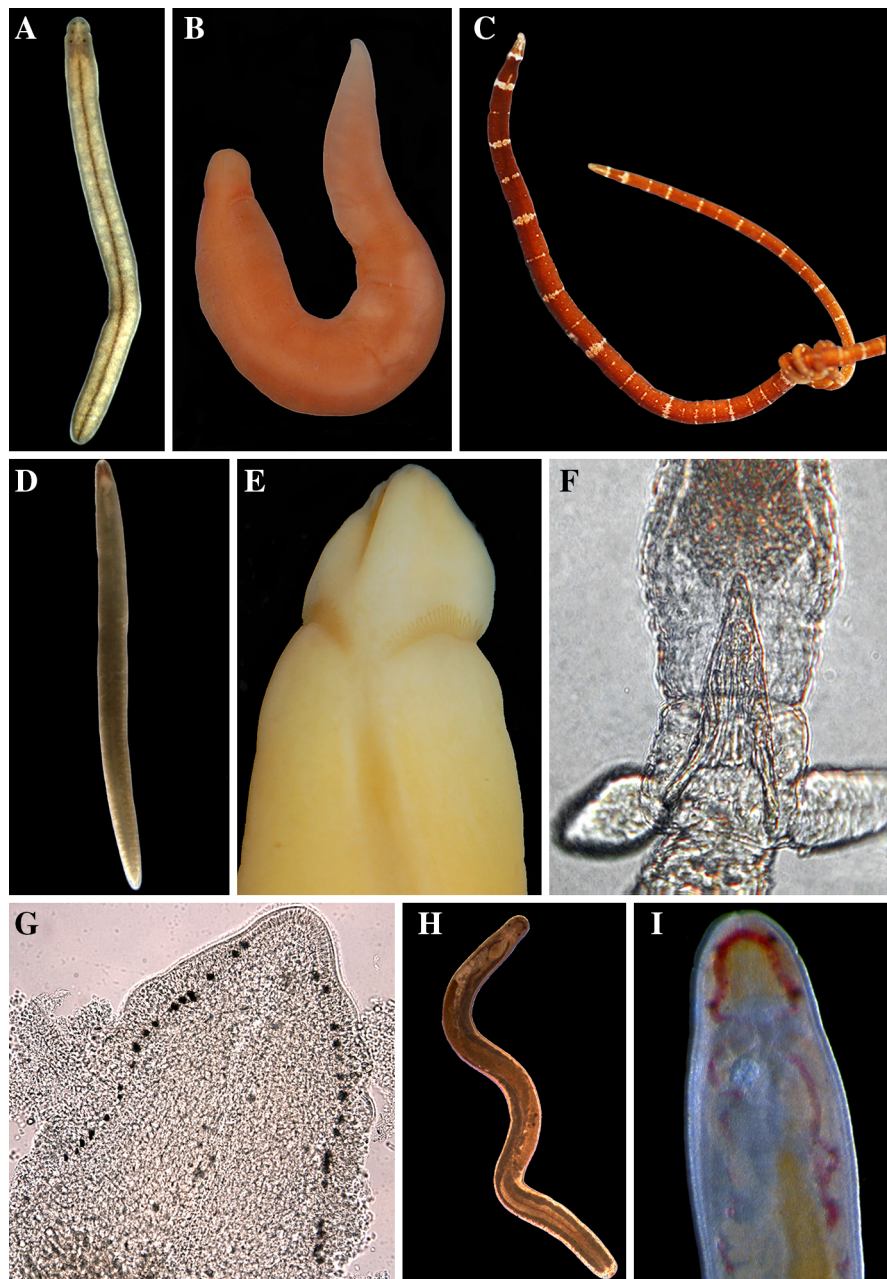


Fig. 2 Live habitus of selected specimens. Photos by A. Chernyshev. **A** *Hubrechtella juliae* Chernyshev, 2003 IZ-45553 (Sea of Japan, Vostok Bay, Jul. 2013). **B** *Carinina* sp. IZ-45550 (Sea of Japan, Vostok Bay, Jul. 2013). **C** *Gononemertes* sp. IZ-45558 (abyssal plain adjacent to the Kuril–Kamchatka Trench, Aug. 2012). **D** *Callinera* sp. IZ-45635 (Sea of Japan, Vostok Bay, Aug. 2007). **E** *Tortus* sp. IZ-45645 (Sea of Okhotsk, Iturup Island, Jul. 2011). **F** *Tubulanus* sp. IZ-45559 (Sea of Okhotsk, Kuril Islands, Jul. 2011). Additional specimen details can be found in MCZbase (<http://mczbase.mcz.harvard.edu/>)

and embedded in paraffin wax (m.p. 56–57°C). Serial sections of 6–8 μm thickness were stained with Mallory's trichrome method. Collecting details and other information for selected specimens is available in the MCZ online database (<http://mczbase.mcz.harvard.edu/>) and can be accessed through their voucher number (Table 1).

Fig. 3 Live habitus of selected specimens. Photos by A. V. Chernyshev. **A** *Monostilifera* sp. IZ-45646 (Sea of Japan, Vostok Bay, Jul. 2008). **B** *Reptantia* sp. IZ-45643 (Sea of Okhotsk, Kuril Islands, Jul. 2011). **C** *Balionemertes* sp. IZ-45637 (Vietnam, Van-Phong Bay, May 2010). **D** *Sacconemertopsis belogurovi* Chernyshev, 1991 IZ-45634 (Sea of Japan, Vostok Bay, Aug. 2013). **E** Cratnemertidae sp. IZ-45644 (Sea of Okhotsk, Kuril Islands, Jul. 2011). **F** *Callinera* sp. IZ-45635 (Sea of Japan, Vostok Bay, Aug. 2007). **G** *Cephalotrichella alba* Gibson & Sundberg, 1992 IZ-45638 (Vietnam, Thus Island, Oct. 2010). **H**, **I** *QuasitetraSTEMMA stimpsoni* (Chernyshev, 1992) (Sea of Japan, Vostok Bay, Aug. 2013). Additional specimen details can be found in MCZbase (<http://mczbase.mcz.harvard.edu/>)



DNA sequencing and phylogenetic analyses

The methods used in the present study for extraction, amplification, purification, and sequencing of DNA are identical to those used by Kvist et al. (2014), but the present study did not target histones H3 and H4, but instead only the mitochondrial cytochrome *c* oxidase subunit I (COI) and 16S rRNA, as well as nuclear 28S rRNA and 18S rRNA. The resulting DNA

sequences (Table 1) were assembled and edited using Sequencher ver. 5.1 (Gene Codes Corporation, Ann Arbor, MI) and subsequently BLASTed against GenBank nr to identify potential contaminations within the samples. Clean, uncontaminated sequences were then joined with those used by Andrade et al. (2012) and Kvist et al. (2014) (these studies targeted the same loci), and each locus was separately aligned using MAFFT ver. 7 (Katoh & Standley, 2013). For

Table 1 Newly sequenced specimens used in the present study with Museum of Comparative Zoology (Harvard University) voucher numbers, locality data, and corresponding GenBank accession numbers

Identification	Voucher number	Locality	18S rRNA	28S rRNA	16S rRNA	COI
<i>Palaeonemertea</i> sp.	IZ-45654	Russia, abyssal plain adjacent to the Kuril–Kamchatka Trench	KP270787	KP270813	–	–
<i>Carinina</i> sp.	IZ-45550	Russia, Sea of Japan, Vostok Bay	KP270788	KP270814	KP270838	KP270863
<i>Callinera</i> sp.	IZ-45635	Russia, Sea of Japan, Vostok Bay	KP270789	KP270815	KP270839	KP270864
<i>Callinera kasyanovi</i>	IZ-45551	Russia, Sea of Japan, Peter the Great Bay	KP270790	KP270816	KP270840	KP270865
<i>Balionemertes</i> sp.	IZ-45637	Vietnam, Van-Phong Bay	KP270791	KP270817	KP270841	KP270866
<i>Cephalotrichella alba</i>	IZ-45638	Vietnam, Thu Island	KP270792	KP270818	KP270842	KP270867
<i>Tubulanus</i> sp.	IZ-45552	Russia, Sea of Japan, Vostok Bay	KP270793	KP270819	KP270843	KP270868
<i>Parahubrechtia</i> sp.	IZ-45554	Russia, Sea of Japan, Vostok Bay	KP270794	KP270820	KP270844	KP270869
<i>Tubulanidae</i> sp.	IZ-45651	Russia, Sea of Japan	KP270795	KP270821	KP270845	–
<i>Tubulanidae</i> sp.	IZ-45557	Russia, abyssal plain adjacent to the Kuril–Kamchatka Trench	KP270796	KP270822	KP270846	–
<i>Tubulanus</i> sp.	IZ-45559	Russia, Sea of Okhotsk, Kuril Islands	KP270797	KP270823	KP270847	KP270870
<i>Parahubrechtia</i> sp.	IZ-45633	Vietnam, Nam Zu Islands	KP270798	–	KP270848	KP270871
<i>Tubulanus punctatus</i>	IZ-45636	Russia, Sea of Okhotsk, Kuril Islands	KP270799	KP270824	KP270849	KP270872
<i>Cephalothrix iwatai</i>	IZ-45650	Russia, Sea of Japan	KP270800	KP270825	KP270850	KP270873
<i>Heteronemertea</i> sp.	IZ-45556	Russia, abyssal plain adjacent to the Kuril–Kamchatka Trench	KP270801	KP270826	KP270851	KP270874
<i>Hubrechtella juliae</i>	IZ-45553	Russia, Sea of Japan, Vostok Bay	KP270802	KP270827	KP270852	KP270875
<i>Reptantia</i> sp.	IZ-45643	Russia, Kuril Islands	KP270803	KP270828	KP270853	KP270876
<i>Cratenemertidae</i> sp.	IZ-45644	Russia, Kuril Islands	KP270804	KP270829	KP270854	KP270877
<i>Gononemertes</i> sp.	IZ-45558	Russia, abyssal plain adjacent to the Kuril–Kamchatka Trench	KP270805	KP270830	KP270855	KP270878
<i>Poseidonemertes maslakovae</i>	IZ-45640	Russia, Sea of Japan, Vostok Bay	KP270806	KP270831	KP270856	KP270879
<i>Monostilifera</i> sp.	IZ-45646	Russia, Sea of Japan, Vostok Bay	KP270807	KP270832	KP270857	KP270880
<i>Tortus</i> sp.	IZ-45645	Russia, Sea of Okhotsk, Iturup Island	KP270808	KP270833	KP270858	KP270881
<i>Monostilifera</i> sp.	IZ-45641	Russia, Kuril Island	KP270809	KP270834	KP270859	KP270882
<i>Sacconemertidae</i> sp.	IZ-45649	Russia, Sea of Okhotsk, near Magadan	KP270810	KP270835	KP270860	KP270883
<i>Sacconemertopsis belogurovi</i>	IZ-45634	Russia, Sea of Japan, Vostok Bay	KP270811	KP270836	KP270861	KP270884
<i>Quasitetrastemma stimpsoni</i>	IZ-45648	Russia, Sea of Japan, Vostok Bay	KP270812	KP270837	KP270862	KP270885

Note that the sequences were joined with those of Andrade et al. (2012) and Kvist et al. (2014) to form the final data matrix. Dashes indicate non-sequenced regions

this purpose, the L-INS-i strategy was employed for the COI and 16S rRNA datasets, whereas the 28S rRNA and 18S rRNA datasets used the E-INS-i strategy (a gap opening penalty of 3.0 was employed for 18S rRNA and 28S rRNA, and all remaining parameters remained as default for each of the

alignment processes). Thereafter, Mesquite ver. 2.5 (Maddison & Maddison, 2010) was used to compile the final data matrix.

Both maximum likelihood (ML) and maximum parsimony (MP) approaches were employed to estimate the phylogenetic relationships among the

terminals and to calculate clade support by multiparametric bootstrapping. The ML analysis was carried out using RAxML ver. 7.6.3 (Stamatakis, 2006) on the CIPRES Science Gateway platform (Miller et al., 2010) under optimal partitioning schemes as suggested by PartitionFinder ver. 1.1.1 (Lanfear et al., 2012). The analysis utilized a GTR+ Γ model of nucleotide evolution for all partitions and consisted of 1000 iterations with 25 initial GAMMA rate categories and final optimization with four GAMMA shape categories. Multiparametric bootstrap support values were calculated from 1000 pseudoreplicates with a different starting tree for each iteration.

For MP, the software TNT (Goloboff et al., 2008) was used with the following settings: gaps treated as missing data (to use the same information employed in the probabilistic analyses), 1000 initial addition sequences, five rounds of ratcheting, and three rounds of tree fusing after the initial Wagner tree builds and requiring that the minimum length tree be found a total of ten times. The resulting trees were then returned to TNT for TBR branch swapping (using the command bbreak). Bootstrap support values were calculated from 1000 pseudoreplicates with the same settings as mentioned above.

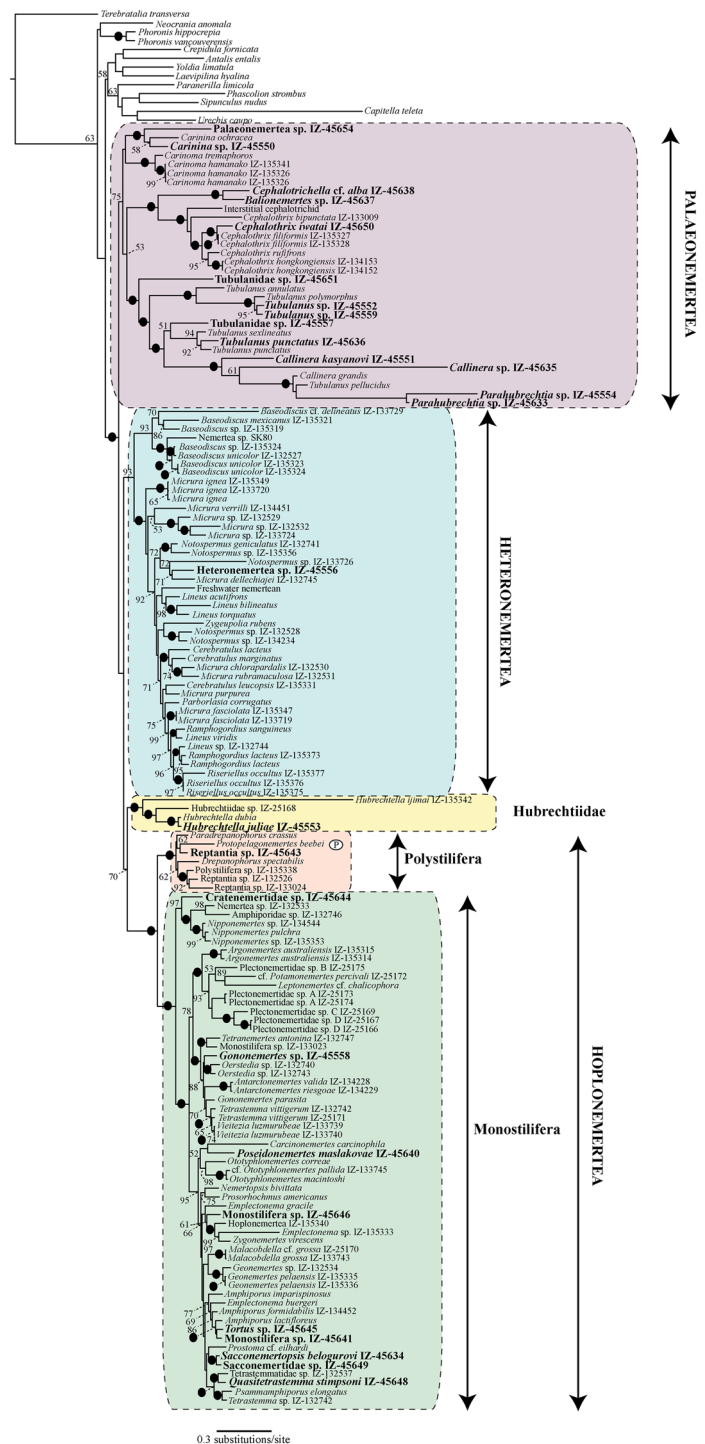
Results

The final concatenated matrix occupied 8763 aligned sites (28S rRNA: 5126 sites; 18S rRNA: 2329 sites; COI: 663 sites; 16S rRNA: 645 sites) for a total of 158 terminals. Out of these sites, 3973 were parsimony informative and 2918 were constant. During the course of this study, it became clear that *Prosorhochmus nelsoni*, a species that has previously been used in a phylogenetic context (Andrade et al., 2012; Kvist et al., 2014), likely has been misidentified. Sequences for this taxon were therefore removed from the final data matrix prior to alignment. Note that the phylogenetic position of this species in previous assessments is therefore likely erroneous and should be viewed with appropriate caution.

The ML analysis of the partitioned dataset resulted in a final GAMMA-based $\ln L$ score of -212574.653315 for the best tree (Fig. 4). Except for some minor re-arrangements, the new tree is identical to that reported by Kvist et al. (2014); all major clades within Nemertea [monophyletic with 100% likelihood

bootstrap support (LBS)] were recovered as monophyletic, including Palaeonemertea (75% LBS), Heteronemertea (93% LBS), Hoplonemertea (100% LBS), Polystilifera (100% LBS), and Monostilifera (100% LBS). However, much like Kvist et al. (2014), we did not recover a monophyletic Piliophora but, instead, representatives of Hubrechtidae s.l. placed as the sister group to Hoplonemertea (Fig. 4). Because of the likeness between the present ML tree and that described by Kvist et al. (2014), the following account will focus mainly on the placement of the new east Russian and northeast Asian taxa, which are indicated by IZ numbers in the tree description below. Within Palaeonemertea, a taxonomically unidentified specimen from abyssal depths (IZ-45654) places as the sister group (58% LBS) to two specimens pertaining to the genus *Carinina* Hubrecht, 1885, including a new specimen of an undescribed species with an atypical olive-brown body pigmentation (IZ-45550). This group places as sister group (100% LBS) to a clade containing *Carinoma tremaphoros* Thompson, 1900 and *Carinoma hamanako* Kajihara, Yamasaki & Andrade, 2011, and this clade, in turn, is the sister group to the remaining palaeonemerteans. Interestingly, the newly sequenced Vietnamese *Balionemertes* Sundberg, Gibson & Olsson, 2003 species (IZ-45637) forms a clade (100% LBS) with *Cephalotrichella alba* Gibson & Sundberg, 1992 (IZ-45638; also from Vietnam), as the sister clade to the remaining cephalotrichids (100% LBS). Within the latter clade, the only newly sequenced specimen, the deep-sea dwelling *Cephalothrix iwatai* Chernyshev, 2013 (IZ-45650), groups tightly with two specimens of the intertidal species *Cephalothrix filiformis* (Johnston, 1828) (100% LBS). The remaining tubulanids form the sister group to the cephalotrichids (100% LBS) with an unknown, unpigmented bathyal tubulanid (IZ-45651) placing as the sister to the remaining taxa (100% LBS). Furthermore, *Tubulanus* sp. (IZ-45552) nests with a newly sequenced specimen of *Tubulanus* (IZ-45559) with unknown species-level affiliation (95% LBS). The closest relative to these specimens is *Tubulanus polymorphus* Renier, 1804 (100% LBS). An unknown abyssal tubulanid (IZ-45557) nests as the sister group (94% LBS) to a clade containing *Tubulanus sexlineatus* (Griffin, 1898) and two specimens of *Tubulanus punctatus* (Takakura, 1898), one of which was newly sequenced (IZ-45636). Further, *Callinera kasyanovi* Chernyshev, 2008 (IZ-45551) places as the sister taxon

Fig. 4 Best scoring tree from the maximum likelihood analysis of the four-marker dataset (lnL = -212574.653315). Likelihood bootstrap values >50% are shown to the left of each node, and *solid circles* indicate full bootstrap support. The *encircled "P"* indicates the only included specimen of Pelagica, which is further discussed in the text. Specimens sequenced for the present study are denoted in *bold font*, and IZ numbers refer to the morphological voucher ID deposited in the Department of Invertebrate Zoology collection of the MCZ



(61% LBS) to a clade containing the remaining palaeonemerteans, composed of an unknown *Callinera* species (IZ-45635), which is the sister taxon (100% LBS) of a larger clade containing *Callinera grandis*

Bergendal, 1903, *Tubulanus pellucidus* (Coe, 1895), and two unknown species of the genus *Parahubrechtia* (IZ-45554 and IZ-45633) from the Sea of Japan and Vietnam.

Within Heteronemertea, the only new specimen, an unidentified abyssal species (IZ-45556), nests with *Micrura dellechiajei* (Hubrecht, 1879) (71% LBS) and these are, in turn, the sister group of a specimen of *Notospermus* with unknown species-level affiliation. Probably, *Micrura dellechiajei* and Heteronemertea IZ-45556 are closely allied to the genus *Notospermus*. Moving down the tree, *Hubrechtella juliae* Chernyshev, 2003 (IZ-45553) nests with the remaining hubrechtiiids (100% LBS), as sister to *Hubrechtella dubia* Bergendal, 1902 (100% LBS) with relatively short branch lengths between the two taxa.

An unknown reptant nemertean (IZ-45643) places well within the clade containing polystiliferan hoplonemerteans, as the sister taxon (62% LBS) to *Protopelagonemertes beebei* Coe, 1936. Within Monostilifera, the newly sequenced unknown cratenemertid species (IZ-45644) places as the sister group (100% LBS) to a clade containing an unknown “amphiporid,” “Nemertea sp.,” and three species of *Nipponnemertes*. An unknown species of *Gononemertes* (IZ-45558) places as the sister group (100% LBS) to a clade containing two unknown species of *Oerstedtia*. *Poseidonemertes maslakovae* Chernyshev, 2002 (IZ-45640) nests with *Carcinonemertes carcinophila* (Kölliker, 1845) (74% LBS) and these, in turn, form a monophyletic group with three species of *Ototyphlonemertes* (52% LBS). An unknown monostiliferan with three pairs of the eyes (IZ-45646) is recovered as the sister taxon (100% LBS) to a clade containing an unknown hoplonemertean, an unidentified species of *Emplectonema* and *Zygonemertes virescens* (Verrill, 1879). The included species of *Tortus* (IZ-45645) form a monophyletic group (<50%) with an unknown monostiliferan (IZ-45641) and these are, in turn, the sister group (100% LBS) to *Amphiporus lactifloreus* (Johnston, 1828). Finally, the brackish water species *Sacconemertopsis belogurovi* Chernyshev, 1991 (IZ-45634) groups with (100% LBS) an unidentified marine sacconemertid species (IZ-45649), and these are the sister group to the freshwater species *Prostoma* cf. *eilhardi*. This clade, in turn, is the sister group of a clade containing an unidentified tetrastemmatid, the newly sequenced *Quasitetrastemma stimpsoni* (Chernyshev, 1992) (IZ-45648) (these are monophyletic with 100% LBS), an unknown species of *Tetrastemma* and *Psammamphiporus elongatus* (Stephenson, 1911) (the latter two also form a clade with 100% LBS).

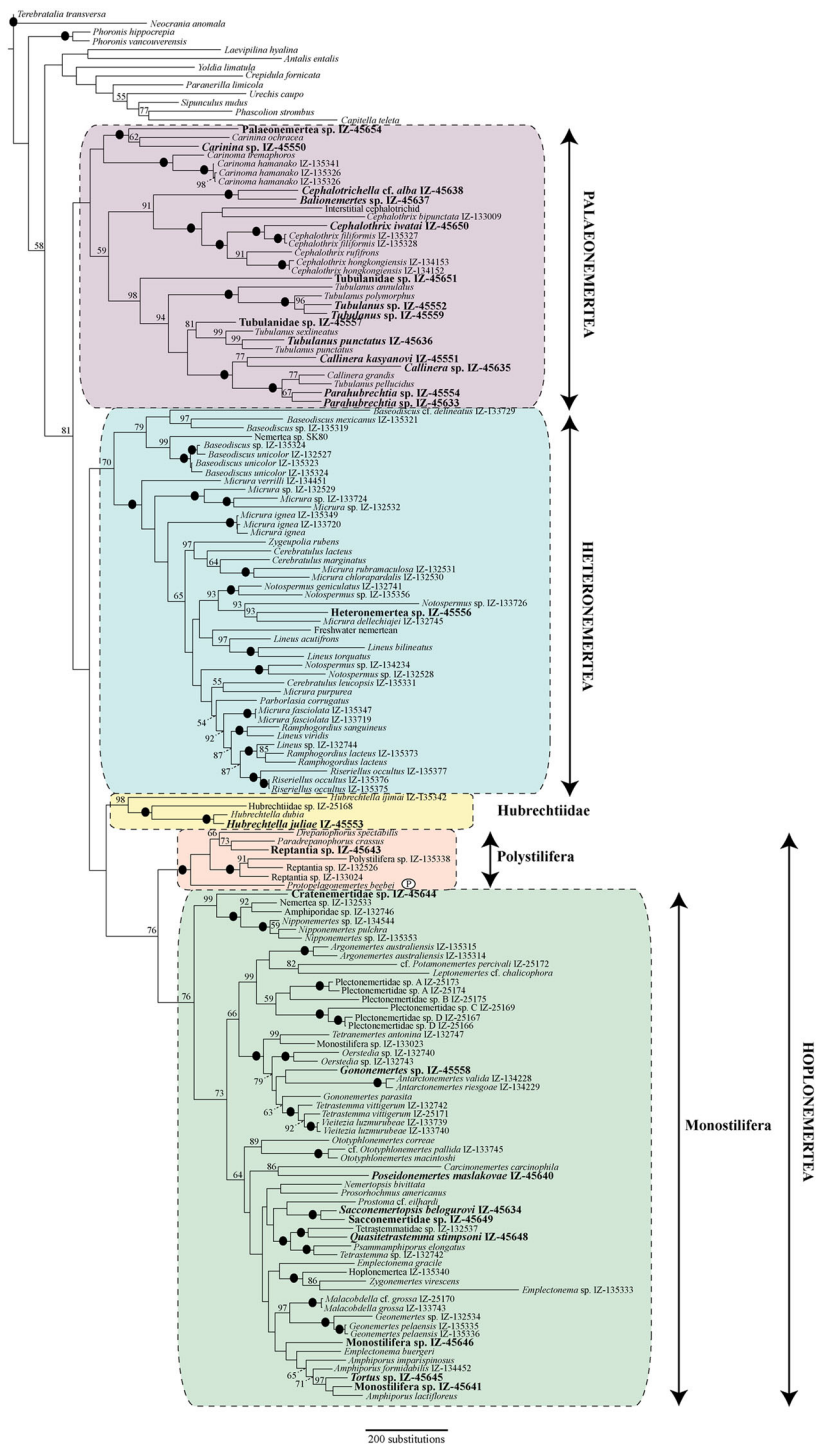
The parsimony analysis in TNT recovered four most parsimonious trees, of 47332 steps, and with a compound consistency index of 0.243 and retention index of 0.609. The strict consensus of these is shown in Fig. 5. By and large, the tree is congruent with the ML tree with the exception of mostly minor rearrangements described below, yet the overall bootstrap support values are lower for the parsimony tree. As opposed to the ML tree, *Callinera kasyanovi* (IZ-45551) groups with *Callinera* sp. (IZ-45635) (77% parsimony bootstrap support; PBS) in the MP tree, and these constitute the sister group of *Callinera grandis*, *T. pellucidus*, and the two species of *Parahubrechtia* (IZ-45554 and IZ-45633). Apart from this, the relationships within Palaeonemertea are identical between the ML and MP trees. The grouping of Heteronemertea sp. (IZ-45556) with *Micrura dellechiajei* is also recovered in the MP analysis (93% PBS), although some discrepancies do exist between the trees regarding the detailed relationships of several genera within Heteronemertea (see Figs. 4, 5). The position of *Hubrechtella juliae* (IZ-45553) is the same in both trees.

Within Hoplonemertea, the unknown reptant nemertean (IZ-45643) nests with *Paradrepanophorus crassus* (Quatrefages, 1846) (73% PBS) as opposed to *Protopelagonemertes beebei* in the ML tree. In addition, the placement of the unknown monostiliferan (IZ-45646) is discrepant between the two analyses; in the MP tree, it places as the sister taxon (>50% PBS) of a larger group containing *Emplectonema buergeri* Coe, 1901 (= *Neesia buergeri*), *Amphiporus imparispinosus* Griffin, 1898, *Amphiporus formidabilis* Griffin, 1898, *Tortus* sp. (IZ-45645), Monostilifera sp. (IZ-45641), and *Amphiporus lactifloreus*. Beyond this, the ML and MP trees agree on the immediate placement of the remaining newly sequenced taxa.

Discussion

The present phylogenetic hypotheses illustrate the relative positions of 19 specimens of nemerteans collected from the Sea of Okhotsk and the Sea of Japan, four specimens from the Kuril–Kamchatka Trench, and three specimens collected in Vietnam, considerably expanding the nemertean data sets to include Asian diversity. Several of these represent species that have not previously been placed within a

Fig. 5 Strict consensus of four equally parsimonious trees (length: 47332 steps; consistency index: 0.243; retention index: 0.609) produced by TNT for the full four-marker dataset. Legends as in Fig. 4



phylogenetic framework, and many of them are entirely new to science—the formal descriptions of these are currently in preparation (A. Chernyshev).

Beyond exposing the general evolutionary relationships of the group, the analyses presented here also serve as a baseline for future morphological studies of

these enigmatic eastern Russian and northeast Asian taxa, and as a first control of the novelty of some of the species included in the present study. It should be noted that the lack of species-level identifications of several of the new taxa is a direct result of their suspected novelty. Below, we discuss some interesting aspects of the proposed phylogenetic placements of some of the newly sequenced taxa.

Our addition of several new taxa to the phylogeny does not circumvent the high levels of para- and polyphyly, mainly at the generic level, reported by previous studies (Sundberg et al., 2001; Strand & Sundberg, 2005a, b; Andrade et al., 2012; Kvist et al., 2014; Strand et al., 2014). The *Carinina* + *Carinoma* group is consistently recovered as monophyletic in contemporary phylogenetic analyses yet the sister group relationship of the remaining tubulanids (*Tubulanus* + *Callinera*) and Cephalotrichidae renders Tubulanidae paraphyletic. The position of the two *Carinina* species confirms the relevance of establishing the separate family Carininidae Chernyshev, 2011. In contrast with other tubulanid genera, all *Carinina* species have an intraepidermal brain and lateral nerve cords and lack lateral organs. We here propose that lateral organs are a synapomorphy of the clade containing *Tubulanus* + *Callinera* + *Parahubrechtia* (i.e., Tubulanidae s.str.).

In contrast to the results conveyed by Sundberg et al. (2003), the present phylogenetic hypothesis supports the position of *Balionemertes* + *Cephalotrichella* (Cephalotrichellidae) as the sister group of the remaining cephalotrichids, and this node also receives maximum support in both the ML and MP analyses. Some authors have regarded *Cephalotrichella* as a junior synonym of *Cephalothrix* (see e.g., Sundberg et al., 2003; Leasi & Norenburg, 2014) despite of the following apomorphies for the family Cephalotrichellidae: (i) the presence of an additional outer longitudinal muscle layer in the proboscis (Chernyshev, 2011a, b), (ii) the absence of nephridia (Gibson & Sundberg, 1992; Sundberg et al., 2003), (iii) ganglionic cell tissue of the lateral nerve cords that encloses the inner longitudinal muscle fascicle (Chernyshev, 2011a, b), and (iv) the ability to swim (Chernyshev, 2011b). In addition, species of both *Balionemertes* and *Cephalotrichella* possess numerous epithelial eyes (Chernyshev, 2011b), but this character has also been noted for some tubulanids (see Ritger and Norenburg, 2006). Interestingly, the

placement of *C. iwatai* as the sister taxon of the intertidal *C. filiformis* suggests a secondary invasion of abyssal depths by this species [*C. iwatai* is a deep-sea dwelling species found at depths greater than 1500 m (Chernyshev, 2013)]. The species does not possess any obvious morphological adaptations or apomorphic characters and is very similar to several intertidal species of the genus (A. Chernyshev, pers. obs.). Because of the relatively recent geomorphological changes in the seabeds of the Sea of Okhotsk and the Sea of Japan (Ryu et al., 2005), it is possible that the abyssal fauna of these areas has invaded the deep-sea much later than the deep-dwelling species of the Kuril–Kamchatka Trench, but this remains to be more thoroughly tested. There is no doubt that the bathyal and abyssal fauna of the North Pacific Ocean (and probably other regions) holds numerous undescribed palaeonemerteans. According to Chernyshev et al. (2015), 43% of nemertean specimens collected in the abyssal plain adjacent to the Kuril–Kamchatka Trench belong to Palaeonemertea. Our analyses, for the first time, include sequences of several deep-sea palaeonemerteans: *Palaeonemertea* IZ-45654 (5,000 m), *C. iwatai* IZ-45650 (1,550–3,000 m), Tubulanidae sp. IZ-45651 (300 m), Tubulanidae sp. IZ-45557 (5,200 m), and *Tubulanus* sp. IZ-45559 (300–600 m).

Tubulanidae sp. IZ-45557 is the deepest-dwelling known palaeonemertean—it has the typical internal morphology for the genus *Tubulanus* but lacks cerebral organs—most phylogenetic hypotheses suggest that cerebral organs have been repeatedly lost, not only in Palaeonemertea but also in Tubulanidae s.str., if assuming that the ancestral nemertean possessed such structures. The position of *T. pellucidus* within the clade *Callinera* + *Parahubrechtia* may be the result of a misidentification, because the external morphology of this species, with its uniformly whitish body, is very similar to the species of *Callinera* and *Parahubrechtia*.

It was unexpected that the three species of *Callinera* did not form a clade, as this genus has a unique synapomorphy—a well-developed muscular sac in the posterior rhynchocoel portion. It is notable that both of the included species of *Callinera* from the Sea of Japan, as well as a third species, *Callinera nishikawai* Kajihara, 2006, possess a stylet-like structure in the mid-part of the proboscis, a unique feature within Palaeonemertea (Kajihara, 2006; Chernyshev, 2010).

The type species of the genus *Parahubrechtia* (*P. jillae* Gibson & Sundberg, 1999) was described from Hong Kong and belongs to the family Hubrechtidae by virtue of an early cladistic analysis (Sundberg & Hylbom, 1994). Chernyshev (2003, 2011a) proposed to transfer *P. jillae* to Tubulanidae because this species lacks a mid-dorsal vessel and possesses lateral organs and a glandular ‘ring,’ but the systematic position of *Parahubrechtia* has remained ambiguous. *Parahubrechtia* lacks clear synapomorphies and differs from *Tubulanus* by the absence of the cerebral organs and from *Callinera* by the absence of the muscular sac in the posterior part of the rhynchocoel. Two unknown species were identified as *Parahubrechtia* following features such as unpigmented body, absence of cerebral organs and rhynchocoel muscular sac, presence of inner circular musculature in the body wall and lateral organs, cerebral position of the proboscis insertion, simple blood system with no mid-dorsal vessel, and the uniform construction of the proboscis. Our analyses show that *Parahubrechtia* is closely related to *Callinera* and ‘*Tubulanus pellucidus*.’

Although we did not recover a monophyletic Pilidiphora here, it has been shown that the use of large datasets, with the inclusion of transcriptomic data, recovers Heteronemertea and Hubrechtidae as a monophyletic group (Andrade et al., 2014). The presence of a layer of zigzag-shaped myofibers (according to confocal laser scanning microscopic data, these ‘zigzag’ muscles are not the result of body contraction but consist of thicker and irregularly wriggled longitudinal muscles; Chernyshev, 2011a) in the longitudinal musculature seems to be a synapomorphy shared by *Hubrechtella juliae*, *H. dubia*, *H. malabarensis* Gibson, 1979, *H. alba* Gibson, 1997, and *H. sinimarina* Gibson & Sundberg, 1999 (Chernyshev, 2003). Based on the rather sparse sampling of the present study for hubrechtids, *H. juliae* places as the sister taxon of *H. dubia*. The addition of *H. malabarensis*, *H. alba*, and *H. sinimarina* in a phylogenetic context should shed light on the accuracy of this sister taxon relationship and the level of homoplasy (or non-homoplasy) in the zigzag-shaped myofibrils.

All reptant nemerteans possess eyes, with the exception of *Siboganemertes weberi* Stiasny-Wijnhoff, 1923, the enigmatic ‘*Drepanophorus longiceps* [= *Hirohitonemertes longiceps* (Iwata, 1957)]’ from Sagami Bay (Japan) and three species of the boreal

genus *Uniporus*. The new Russian reptant species included here (IZ-45643) is eyeless and represents the first reptant species recorded from Far East Russia. Beyond this, its external morphology clearly separates it from other eyeless taxa. It has previously been hypothesized that Pelagica is the sister group of Reptantia + Cratenemertidae (Gibson, 1988), but modern molecular phylogenies do not support this relationship. An alternative hypothesis postulates that Pelagica and Reptantia are sister taxa (see Crandall, 1993 and the adapted classification scheme in Sundberg, 1991), and the MP tree recovered here also supports this hypothesis (Fig. 5; this result is also supported by other analyses [e.g., Andrade et al., 2012, 2014], but only one species belonging to Pelagica was used therein and in the present study). However, the ML tree shown here (Fig. 4) as well as other molecular phylogenetic studies (e.g., Thollesson & Norenburg, 2003) supports a third hypothesis, in which Pelagica originates from within Reptantia, resulting in a paraphyletic status of a, therefore, artificial Reptantia. Future studies should focus on expanding the taxon sampling principally for Pelagica, but also for Reptantia, in order to unravel the evolutionary histories within polystiliferan hoplonemerteans.

The included Russian cratenemertid specimen nests within a well-supported Cratenemertidae clade containing the confamilial genus *Nipponnemertes*, as well as an unidentified nemertean and an unidentified “amphiporid”—this suggests that the family level identification of the latter specimen is possibly erroneous. This clade constitutes the sister group of the remaining monostiliferan hoplonemerteans. It has already been shown that some morphological features, such as interwoven fibers in the rhynchocoel musculature, are shared by *Nipponnemertes* and reptant hoplonemerteans (Gibson, 1988), making the phylogenetic separation of ‘Cratenemertidae’ and the remaining monostiliferan hoplonemerteans of particular interest. However, such fibers also exist in other monostiliferan taxa, for example, the terrestrial genera *Argonemertes* and *Leptonemertes*, as well as the freshwater genus *Potamonemertes* (see Moore & Gibson, 1981) and the marine genus *Malacobdella* (see Chernyshev, 2011a); these genera are all included in the present study (Figs. 4, 5). The disparate placement of these taxa within the present trees suggests a high level of convergent evolution of this morphological character (as suggested by Sundberg,

1993) but, more importantly, the trees add another line of evidence towards the monophyletic status of Cratenemertidae, if the identification of “Amphiporidae sp. IZ-132746” is indeed erroneous, as the current taxonomic classification system suggests. Morphologically, the Russian cratenemertid specimen (Cratenemertidae sp. IZ-45644) is very similar to the genus *Valdivianemertes*. The specimen lacks eyes and possesses well-developed secondary cephalic grooves of the anterior cephalic furrows; the latter character is common in Cratenemertidae and most Reptantia (including species IZ-45643) (Crandall, 2001; Chernyshev, 2011a). Similar secondary grooves have been found in some species of the genus *Baseodiscus* (Schwartz, 2009; Chernyshev, 2011a) suggesting that this character may be homoplastic.

The relative placement of *Gononemertes* sp. (IZ-45558) is curious, as it does not form a monophyletic group with the other included member of the genus, *Gononemertes parasita* Bergendal, 1900. The specimen was collected from 5406 m depth (making it the deepest-dwelling known benthic nemertean), from within the deep-sea ascidian *Culeolus* sp. (Pyruridae). In this regard, its parasitic lifestyle resembles that of *Gononemertes parasita* and, in addition, the species share internal morphology typical for the genus (a strongly developed reproductive system, gonochorism, absence of eyes, a reduced stylet apparatus, and a short rhynchocoel). Our main explanation for the remote positioning of these species is that the similarity between the morphological features of these species is a product of convergent evolution, driven by their parasitic lifestyle, such that *Gononemertes* sp. (IZ-45558) in fact pertains to a new genus. This would mean that, much like most other hoplonemertean genera, *Gononemertes* is an artificial genus in need of taxonomic revision (the positions of *Gononemertes* sp. as the sister taxon of *Oerstedia* spp. in the ML tree and *Antarctonemertes* spp. in the MP tree both receive <50% resampling support). The taxonomic fate of the new enigmatic ‘*Gononemertes*’ species is still under review, as its determination will require the inclusion of data from another species, *Gononemertes australiensis* Gibson, 1974 (*G. australiensis* and *Gononemertes* sp. differ from *G. parasita* by the presence of high rhynchodeal epithelium and absence of the cerebral organs).

Based on a smaller subset of molecular data, *Poseidonemertes* (currently in the family Amphiporidae

s.l. or Poseidonemertidae sensu Chernyshev, 2002) has been recovered as the sister group of each of *Emplectonema* (see Mahon et al., 2010), *Ototyphlonemertes* (see Tholleson & Norenburg, 2003), and *Zygonemertes* (see Kajihara et al., 2011). Based on the larger dataset used here, *P. maslakovae* is found to be the sister taxon of *C. carcinophila* (currently in the family Carcinonemertidae), but this relationship does not receive high bootstrap support in the ML and MP trees (Figs. 4, 5, respectively). The *Poseidonemertes* + *Carcinonemertes* group places as the sister group to a clade composed of species of *Ototyphlonemertes* in the ML tree, but this arrangement, again, does not receive high support. Because of this, it seems necessary to employ larger datasets in order to shed light on the recalcitrant divisions of clades, pertaining to the relationships between these genera. In terms of morphological synapomorphies supporting this arrangement, species of both *Poseidonemertes* and *Carcinonemertes* possess two eyes, whereas species of *Ototyphlonemertes* completely lack eyes in the adult forms (larvae of *Ototyphlonemertes* do possess two eyes; Chernyshev, 2000). Despite of this, Poseidonemertidae sensu Chernyshev, 2002 is recovered as non-monophyletic, as the only other included member of the family, *Tetranemertes antonina* (Quatrefages, 1846), is recovered in a remote part of both the ML and MP trees.

The phylogenetic placement of *Sacconemertopsis belogurovi* is particularly important, yet difficult to corroborate, seeing as neither this species nor any congener has been previously placed in a phylogenetic framework. The genus currently resides in the family Sacconemertidae, including the genera *Sacconemertes*, *Sacconemertopsis*, *Gurjanovella*, *Cyanophthalma*, *Communoporus*, *Arctonemertes*, *Amphiporella*, *Elcania*, and *Potamostoma* (Chernyshev, 2005). The unifying morphological synapomorphy for this group is the presence of bilobed testes (Chernyshev, 2005). Out of its members, only *Gurjanovella littoralis* Uschakov, 1926 and *Cyanophthalma obscura* (Schultze, 1851) have been used in molecular phylogenies (Tholleson & Norenburg, 2003; Strand & Sundberg, 2005b, respectively). In those studies, *G. littoralis* places as the sister taxon of *Emplectonema gracile* (Johnston, 1837) and *C. obscura* places as the sister taxon of a clade containing species of the genus *Prostoma*. Notably, in the present study, the brackish water species *Sacconemertopsis belogurovi* and our unknown marine sacconemertid

species place together, as the sister group of the freshwater taxon *Prostoma* cf. *eilhardi*. Although the family level encompassment of *Prostoma* is problematic (currently in the family Tetrastemmatidae s.l. or Prostomatidae sensu Chernyshev, 2005), a more Sacconemertidae-inclusive study would help evince its relation to this family. The result conveyed here represents the second line of independent evidence (see Strand & Sundberg, 2005b) towards a close relationship between Sacconemertidae sensu Chernyshev, 2005 and *Prostoma*. This is particularly compelling seeing as five genera within Sacconemertidae (*Sacconemertes*, *Sacconemertopsis*, *Sacconemertella*, *Potamostoma*, and *Cyanophthalma*) inhabit brackish water ecosystems, such that any transition from freshwater to marine systems (or vice versa) may have evolved via brackish water (see Gibson, 1972).

The genus *Tortus* was erected by Korotkevitch (1971), who regarded the genus as part of the family Emplectonematidae. Species of *Tortus* also strongly resemble members of the family Amphiporidae but differ in the detailed morphology of the precerebral septum (Chernyshev, 2005). Although the genus was transferred to the family Neesiidae by Chernyshev (2005), the topology of the current tree suggests that the differences in the precerebral septum between *Tortus* sp. (IZ-45645) and *Amphiporus lactiflorens* could be a result of strong developmental changes to the cephalic glands of the former species, such that the precerebral septum character hold less phylogenetic power than previously thought.

Acknowledgements This study was supported in part by a Russian Foundation for Basic Research grants (14-04-01067 and 15-04-01933) to A.V.C. It was also supported by funding from the Wenner-Gren Foundations, Helge Ax:son and Olle Engkvist Byggmästare's Foundation to S.K., as well as by internal funds from the Museum of Comparative Zoology to G.G. We thank the Harvard FAS Center for Systems Biology for providing sequencing support and the Willi Hennig Society for making TNT freely available. Three anonymous reviewers provided comments and ideas that greatly improved earlier versions of the paper.

References

- Andrade, S. C., M. Strand, M. Schwartz, H. Chen, H. Kajihara, J. von Döhren, S. Sun, J. Junoy, M. Thiel, J. L. Norenburg, J. M. Turbeville, G. Giribet & P. Sundberg, 2012. Disentangling ribbon worm relationships: multi-locus analysis supports traditional classification of the phylum Nemertea. *Cladistics* 28: 141–159.
- Andrade, S. C., H. Montenegro, M. Strand, M. Schwartz, H. Kajihara, J. L. Norenburg, J. M. Turbeville, P. Sundberg & G. Giribet, 2014. A transcriptomic approach to ribbon worm systematics (Nemertea): resolving the Piliidophora problem. *Molecular Biology and Evolution* 31: 3206–3215.
- Blaxter, M., 2003. Counting angels with DNA. *Nature* 421: 122–124.
- Chernyshev, A. V., 2000. Nemertean larvae of the Ototyphlonemertidae family in the plankton of Peter the Great Bay, Sea of Japan. *Russian Journal of Marine Biology* 26: 48–50.
- Chernyshev, A. V., 2002. Description of a new species of the genus *Poseidonemertes* (Nemertea; Monostilifera) with establishment of the family Poseidonemertidae. *Zoologicheskii Zhurnal* 81: 909–916. [In Russian].
- Chernyshev, A. V., 2003. New species of the genus *Hubrechtella* (Nemertea, Anopla) from the Sea of Japan and validation of the family Hubrechtellidae. *Russian Journal of Marine Biology* 29: 333–336.
- Chernyshev, A. V., 2005. System of families of enoplan nemerteans of the order Eumonostilifera (Nemertea: Enopla). *Russian Journal of Marine Biology* 31: S27–S33.
- Chernyshev, A. V., 2010. Confocal laser scanning microscopy analysis of the phalloidin-labelled musculature in nemerteans. *Journal of Natural History* 44: 37–40.
- Chernyshev, A. V., 2011a. Comparative Morphology, Systematics and Phylogeny of the Nemerteans. *Dal'nauka, Vladivostok*. [In Russian].
- Chernyshev, A. V., 2011b. Nemertean worms (Nemertea) of the Vietnamese coastal waters. In Lutaenko K. A. (ed) *Proceedings of the Workshop Coastal Marine Biodiversity and Bioresources of Vietnam and Adjacent Areas of the South China Sea, Nha Trang, Vietnam, November 24–25, 2011 Vladivostok-Nha Trang: Dal'nauka: 21–25*.
- Chernyshev, A. V., 2013. Two new species of deep-sea nemerteans from the SoJaBio expedition in the Sea of Japan. *Deep Sea research Part II: Topical Studies in Oceanography* 86–87: 148–155.
- Chernyshev, A. V., 2014. Nemertean biodiversity in the Sea of Japan and adjacent areas. In Sun, S., et al. (eds), *Marine Biodiversity and Ecosystem Dynamics of the North-Western Pacific Ocean*. Publishing House of Science, Beijing: 119–135.
- Chernyshev, A. V., S. Abukawa & H. Kajihara, 2015. *Sonne-nemertes cantelli* gen. et sp. nov. (Heteronemertea)—a new *Oxypotelella*-like nemertean from the abyssal plain adjacent to the Kuril–Kamchatka Trench. *Deep Sea research Part II: Topical Studies in Oceanography* 111: 119–127.
- Crandall, F. B., 1993. Major characters and enoplan systematics. *Hydrobiologia* 266: 115–140.
- Crandall, F. B., 2001. A cladistic view of the Monostilifera (Hoploneurertea) with interwoven rhynchocoel musculature: a preliminary assessment. *Hydrobiologia* 456: 87–110.
- Crandall, F. B., J. L. Norenburg, A. V. Chernyshev, S. A. Maslakova, M. L. Schwartz & H. Kajihara, 2002. Checklist of the nemertean fauna of Japan and northeastern Asia. Smithsonian Institution, Washington D.C.: 1–44. Available from <http://pages.uoregon.edu/svetlana/Crandall2002.pdf>

- Gibson, R., 1972. Nemerteans. Hutchinson, London.
- Gibson, R., 1988. Evolutionary relationships between mono- and polystyliferous hoplonemerteans: *Nipponemertes* (Cratenemertidae), a “missing link” genus? *Hydrobiologia* 156: 61–74.
- Gibson, R., 1995. Nemertean genera and species of the world: an annotated checklist of original names and description citations, synonyms, current taxonomic status, habitats and recorded zoogeographic distribution. *Journal of Natural History* 29: 271–562.
- Gibson, R. & P. Sundberg, 1992. Three new nemerteans from Hong Kong. In Morton, B. (ed.), *The Marine Flora and Fauna of Hong Kong and Southern China III*, Vol. 1. University Press, Hong Kong: 97–129.
- Gibson, R. & P. Sundberg, 1999. Six new species of palaeone-merteans (Nemertea) from Hong Kong. *Zoological Journal of Linnean Society* 125: 151–196.
- Goloboff, P. A., J. S. Farris & K. C. Nixon, 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24: 774–786.
- Hidaka, K., 1966. Japan Sea. In Fairbridge, R. W. (ed.), *Encyclopedia of Oceanography*. Reinhold Publisher, New York: 417–424.
- Kajihara, H., 2006. Four palaeone-merteans (Nemertea: Anopla) from a tidal flat in middle Honshu, Japan. *Zootaxa* 1163: 1–47.
- Kajihara, H., A. V. Chernyshev, S. C. Sun, P. Sundberg & F. B. Crandall, 2008. Checklist of nemertean genera and species published between 1995 and 2007. *Species Diversity* 13: 245–274.
- Kajihara, H., M. Olympia, N. Kobayashi, T. Katoh, H.-X. Chen, M. Strand & P. Sundberg, 2011. Systematics and phylogeny of the hoplonemertean genus *Diplomma* (Nemertea) based on molecular and morphological evidence. *Zoological Journal of the Linnean Society* 161: 695–722.
- Katoh, K. & D. M. Standley, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Kitamura, A., O. Takano, H. Takata & H. Omote, 2001. Late Pliocene-early Pleistocene paleogeographic evolution of the Sea of Japan. *Paleogeography, Paleoclimatology, Paleoecology* 172: 81–98.
- Kojima, S., 2002. Deep-sea chemoautotrophic communities in the Northwestern Pacific. *Journal of Oceanography* 58: 343–363.
- Korotkevich, V. S., 1971. On the ecology and systematics of the nemerteans of the Possjet Bay of the Sea of Japan. *Issledovaniya Fauny Morei* 8: 109–122. (**In Russian**).
- Kvist, S., C. E. Laumer, J. Junoy & G. Giribet, 2014. New insights into the phylogeny, systematics and DNA barcoding of Nemertea. *Invertebrate Systematics* 28: 287–308.
- Lanfear, R., B. Calcott, S. Y. W. Ho & S. Guindon, 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.
- Leasi, F. & J. L. Norenburg, 2014. The necessity of DNA taxonomy to reveal cryptic diversity and spatial distribution of meiofauna, with a focus on Nemertea. *PLoS one* 9: e104385.
- Maddison, W. P. & D. R. Maddison, 2010. Mesquite: a modular system for evolutionary analysis version 2.5. Available from <http://mesquiteproject.org>.
- Mahon, A. R., D. J. Thornhill, J. L. Norenburg & K. M. Halanych, 2010. DNA uncovers Antarctic nemertean biodiversity and exposes a decades-old cold case of asymmetric inventory. *Polar Biology* 33: 193–202.
- May, R. M., 1988. How many species are there on earth? *Science* 241: 1441–1449.
- Miller, M. A., W. Pfeiffer & T. Schwartz, 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA: 1–8.
- Moore, J. & R. Gibson, 1981. The *Geonemertes* problem (Nemertea). *Journal of Zoology (London)* 194: 175–201.
- Olson, D. M. E. & E. D. Dinerstein, 1998. The global 200: a representation approach to conserving the Earth's most biologically valuable ecoregions. *Conservation Biology* 12: 502–515.
- Preller, R. H. & P. J. Hogan, 1998. Oceanography of the Sea of Okhotsk and the Japan/East Sea. In Brink, K. H. & A. R. Robinson (eds), *the Sea*. Wiley, New York: 429–481.
- Ritger, R. K. & J. L. Norenburg, 2006. *Tubulanus riceae* new species (Nemertea: Anopla: Palaeone-merteata: Tubulanidae), from South Florida, Belize and Panama. *Journal of Natural History* 40: 931–942.
- Ryu, E., S. Yi & S.-J. Lee, 2005. Late Pleistocene-Holocene paleoenvironmental changes inferred from the diatom record of the Ulleung basin, East Sea (Sea of Japan). *Marine Micropaleontology* 55: 157–182.
- Sahling, H., S. V. Galkin, A. Salyuk, J. Greinert, H. Foerstel, D. Piepenburg & E. Suess, 2003. Depth-related structure and ecological significance of cold-seep communities—a case study from the Sea of Okhotsk. *Deep Sea Research Part I: Oceanographic Research Papers* 50: 1391–1409.
- Schwartz, M. L., 2009. Untying a Gordian knot of worms: systematics and taxonomy of the Piliophora (phylum Nemertea). Ph.D. thesis. Washington DC: George Washington University.
- Schwartz, M. L. & J. L. Norenburg, 2001. Can we infer heterone-mertean phylogeny from available morphological data? *Hydrobiologia* 456: 165–174.
- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Strand, M. & P. Sundberg, 2005a. Delimiting species in the hoplonemertean genus *Tetrastemma* (phylum Nemertea): morphology is not concordant with phylogeny as evidenced from mtDNA sequences. *Biological Journal of the Linnean Society* 86: 201–212.
- Strand, M. & P. Sundberg, 2005b. Genus *Tetrastemma* Ehrenberg, 1831 (phylum Nemertea)—a natural group? Phylogenetic relationships inferred from partial 18S rRNA sequences. *Molecular Phylogenetics and Evolution* 37: 144–152.
- Strand, M., A. Herrera-Bachiller, A. Nygren & T. K anneby, 2014. A new nemertean species: what are useful characters for ribbon worm descriptions? *Journal of the Marine Biological Association of the UK* 94: 317–330.

- Sundberg, P., 1991. A proposal for renaming higher taxonomic categories in the phylum Nemertea. *Journal of Natural History* 25: 45–48.
- Sundberg, P., 1993. Phylogeny, natural groups and nemertean classification. *Hydrobiologia* 266: 103–113.
- Sundberg, P., R. Gibson & U. Olsson, 2003. Phylogenetic analysis of a group of palaeonemerteans (Nemertea) including two new species from Queensland and the Great Barrier Reef, Australia. *Zoologica Scripta* 32: 279–296.
- Sundberg, P. & R. Hylbom, 1994. Phylogeny of the nemertean subclass Palaeonemertea (Anopla, Nemertea). *Cladistics*, 10: 347–402.
- Sundberg, P. & M. Strand, 2010. Nemertean taxonomy—time to change lane? *Journal of Zoological Systematics and Evolutionary Research* 48: 283–284.
- Sundberg, P., E. Thuroczy Vodoti & M. Strand, 2010. DNA barcoding should accompany taxonomy—the case of *Cerebratulus* spp (Nemertea). *Molecular Ecology Resources* 10: 274–281.
- Sundberg, P., J. M. Turbeville & S. Lindh, 2001. Phylogenetic relationships among higher nemertean (Nemertea) taxa inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution* 20: 327–334.
- Thollesson, M. & J. L. Norenburg, 2003. Ribbon worm relationships: a phylogeny of the phylum Nemertea. *Proceedings of the Royal Society of London B: Biological Sciences* 270: 407–415.
- Turbeville, J. M., 2002. Progress in nemertean biology: development and phylogeny. *Integrative and Comparative Biology* 42: 692–703.