***Carinoma hamanako* sp. nov. (Nemertea: Palaeonemertea), the First Representative of the Genus from the Northwest Pacific**

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Carinoma hamanako sp. nov., the ninth member of the genus, is described based on three specimens collected in Lake Hamana, Shizuoka Prefecture, Pacific coast of Honshu, Japan. The new species can be distinguished from its congeners by the blind-ending rhynchocoelic villar vessels, the lateral rhynchocoel vessels rejoining the lateral vessels, the unbranched excretory canals, the situation of the posterior part of the mid-dorsal nerve within the longitudinal muscle layer of the body wall, and the absence of a vascular plexus in the foregut region. A phylogenetic analysis based on the nuclear 28S rRNA and mitochondrial cytochrome *c* oxidase subunit I genes shows that *C. hamanako* sp. nov. forms a clade with two congeners, *C. mutabile* Griffin, 1898 and *C. tremaphoros* Thompson, 1900, thus supporting the generic placement of the new species.

Key Words: Nemertea, palaeonemertean, Japan, taxonomy, anatomical description, molecular phylogeny, new species.

Introduction

Palaeonemerteans, or Palaeonemertea s. lat., comprise a likely paraphyletic assemblage of basal clades within the phylum Nemertea, with one group, the hubrechtellids, being a sister group of the Heteronemertea, and the latter two together constituting a monophyletic Pilidiophora (Thollessen and Norenburg 2003). So far, 110 species in Palaeonemertea s. str., i.e. excluding the 15 species of Hubrechtellidae, are known worldwide (Gibson 1995; Kajihara 2006; Kajihara *et al.* 2008).

The palaeonemertean (s. lat.) fauna of Japanese waters has been studied by Takakura (1898, 1922), Yamaoka (1940), Iwata (1951, 1952, 1954a, b, 1957), Senz (1997), and Kajihara (2006); 14 species in seven genera are currently known, including hubrechtellids (reviewed by Kajihara 2007), but no member of the genus *Carinoma* Oudemans, 1885 has been reported from this region. In the present paper we describe a new species of *Carinoma*, the ninth member of the genus, representing the first record of *Carinoma* from the Northwest Pacific. In addition, its phylogenetic position is evaluated based on DNA sequence data from two molecular markers

commonly used in phylogenetic studies, 28S ribosomal RNA (28S rRNA) and cytochrome *c* oxidase subunit I (COI).

Material and Methods

Nemerteans were collected at ebb tide on 30 March 2009 from a sandy to muddy tidal flat on Ikarise Island, 34°41'04"N, 137°35'59"E, located in the channel forming the outlet to the Pacific Ocean of Lake Hamana, Shizuoka Prefecture, Honshu, Japan. They were anaesthetized in a MgCl₂ solution isotonic to seawater. The anterior half of each worm was fixed in 10% formalin seawater for 24 hours and preserved in 70% EtOH for morphological observation; the posterior halves were fixed and preserved in RNAlater® (Ambion, Inc., Austin, Texas, USA) for nucleotide extraction. The anterior halves were dehydrated in 100% EtOH, cleared in xylene, and embedded in paraffin wax (m.p. 56–57°C) before being sectioned at 8 µm thickness. Sections were stained by the Mallory trichrome method (Gibson 1994). The description of morphological characters and their states is largely based on Sundberg *et al.* (2009). The slides of the type series are deposited in the Hokkaido University Museum, Sapporo, Japan (ZIHU).

Total genomic DNA was extracted from the posterior halves of the specimens, using the DNeasy kit (Qiagen, Inc., Valencia, California, USA) according to the manufacturer's protocol. A fragment of the 5' end of 28S rRNA was amplified using the primer set 28rd1a/28rd5b (Edgecombe and Giribet 2006). The COI fragment was amplified using the primer pair LCO1490/HCO2198 (Folmer *et al.* 1994). PCR reactions were performed using AmpliTaq DNA polymerase (Perkin-Elmer, Waltham, Massachusetts, USA). Thermal cycling was initiated with 2 min of denaturation at 94°C followed by 35 cycles of 30 s at 94°C, 1 min at 36–44°C, and 1 min at 72°C. After cycling, the reaction was completed with an extension phase at 72°C for 10 min. The PCR products were visualized in a 1% agarose gel and purified through enzymatic reaction with ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA). For both 28S rRNA and COI, sequencing reaction products were analyzed using an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Chromatograms were edited and overlapping sequence fragments were assembled using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Blast searches (Altschul *et al.* 1997), as implemented in the NCBI website (<http://www.ncbi.nlm.nih.gov/>), were conducted to check for putative contaminations. Sequences from the holotype (ZIHU 3819) are analysed in the present study (Table 1); those from one of the paratypes (ZIHU 3820) are also deposited at GenBank (accession numbers for 28S rRNA and COI, respectively, HQ856864 and HQ848629).

To assess the phylogenetic affinities of the new species, we included 14 species of palaeonemerteans, including two species of *Carinoma*, for which sequences were available in GenBank (Table 1). DNA sequence alignment was performed by MAFFT version 6 using the strategy G-INS-i (Katoh *et al.* 2005). The phoronid *Phoronis vancouverensis* Pixell, 1912 and the brachiopod *Terebratalia transversa* (Sowerby, 1846) were chosen as outgroups. Palaeonemerteans are often found as a basal group within Nemertea (Thollessen and Norenburg 2003), and the phyla Brachiopoda and Phoronida appear as the closest groups to nemerteans according to

Table 1. List of species included in the phylogenetic analysis, with GenBank accession numbers.

Species	28S rRNA	COI
<i>Callinera grandis</i> Bergendal, 1903	–	EU489491 ^a
<i>Carinina plecta</i> Kajihara, 2006	–	EU489493 ^a
<i>Carinina ochracea</i> Sundberg <i>et al.</i> 2009	–	EU489492 ^a
<i>Carinoma hamanako</i> sp. nov.	HQ856863 ^b	HQ848628 ^b
<i>Carinoma mutabile</i> Griffin, 1898	AJ436887 ^c	AJ436942 ^c
<i>Carinoma tremaphoros</i> Thompson, 1900	AJ436888 ^c	AJ436943 ^c
<i>Cephalothrix filiformis</i> (Johnston, 1828)	AJ436890 ^c	AJ436944 ^c
<i>Cephalothrix simula</i> (Iwata, 1952)	AJ436891 ^c	GU733830 ^d
<i>Cephalothrix spiralis</i> Coe, 1930	AJ436892 ^c	AJ436946 ^c
<i>Hubrechtella dubia</i> Bergendal, 1902	AJ436889 ^c	EU489495 ^a
<i>Tubulanus annulatus</i> (Montagu, 1804)	AY210473 ^e	EU489497 ^a
<i>Tubulanus lutescens</i> Cantell, 2001	–	EU489498 ^a
<i>Tubulanus punctatus</i> (Takakura, 1898)	AJ436893 ^c	AJ436947 ^c
<i>Tubulanus rhabdotus</i> Corrêa, 1954	AJ436894 ^c	AJ436948 ^c
<i>Tubulanus sexlineatus</i> (Griffin, 1898)	AJ436895 ^c	AJ436949 ^c
Outgroups		
<i>Phoronis vancouverensis</i> Pixell, 1912	AF342797 ^f	EU484462 ^g
<i>Terebratalia transversa</i> (Sowerby, 1846)	AF342802 ^f	FJ196085 ^h

Sources: ^aSundberg *et al.* (2009), ^bAndrade *et al.* (in press), ^cThollessen and Norenburg (2003), ^dChen *et al.* (2010), ^ePassamaneck and Halanych (2006), ^fMallatt and Winchell (2002), ^gSantagata and Cohen (2009), ^hFuchs *et al.* (2009).

recent molecular phylogenies (Dunn *et al.* 2008). jMODELTEST 0.1.1 (Posada 2008) was used to choose the best-fit model of molecular evolution for our data set under the Akaike Information Criterion (Akaike 1974). This test suggested the general time-reversible model with a discrete Gamma distribution and rate heterogeneity (GTR+G+I) as best fitting to the data for both the nuclear and mitochondrial genes. Maximum likelihood analyses were performed with RAxML version 7.0.4 (Stamatakis 2006; Stamatakis *et al.* 2008) using the sequences obtained for *C. hamanako*, together with those of other palaeonemerteans and the outgroups from GenBank (Table 1). 28S rRNA and COI sequences were analysed independently; a concatenated dataset was also analysed. The search for the optimal maximum likelihood (ML) trees was performed in the cluster computing facility of the Faculty of Arts and Sciences, Harvard University. The ML tree search was conducted by performing 300 distinct runs using the default algorithm of the program for random trees (option -d) as a starting tree for each run. The final tree was determined by a comparison of likelihood scores under the GTR+G+I model among suboptimal trees obtained per run. One thousand fast bootstrap replicates were conducted to evaluate nodal support.

Taxonomy

Family **Carinomidae** Bergendal, 1900

Carinomidae Bergendal, 1900: 741; Bürger 1897–1907: 402, 414.
Statolitonemertidae Korotkevich, 1982: 17. Synonymized by Chernyshev (1999).

Remarks. Chernyshev (1999: 1275) ascribed the family name to Bürger (1897–1907), which, however, was published in six different parts, and the part containing the establishment of the family name Carinomidae was published in 1905. Therefore, Bergendal's (1900) usage of the name precedes that of Bürger (1897–1907). The formally correct spelling of the family name is Carinomatidae, given that the type genus *Carinoma* is taken to be neuter in gender (see Remarks for the genus below); however, the spelling Carinomidae is in prevailing use and Carinomatidae has never been used. Carinomidae should thus be retained under Article 29.5 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999).

Genus **Carinoma** Oudemans, 1885

Carinoma Oudemans, 1885: 7. Type species: *Valencinia armandi* McIntosh, 1875, fixed by monotypy. Gender: neuter.

Remarks. Although Oudemans (1885) did not mention its etymology, the generic name *Carinoma* is apparently derived from *Carinina*, the palaeone-mertean genus which was frequently referred to in the original description of *Carinoma* in conjunction with remarks on their mutual morphological similarity and/or dissimilarity. Because the suffix *-oma* is neuter in gender, the three specific epithets *caraibica* Stiasny-Wijnhoff, 1925, *mutabilis* Griffin, 1898, and *patagonica* Bürger, 1895, which were originally published and have widely been used in feminine form, should be *caraibicum*, *mutabile*, and *patagonicum*, respectively, when combined with *Carinoma*.

Carinoma hamanako sp. nov. (Figs 1–5)

Material examined. Holotype: ZIHU 3819, serial transverse sections of anterior half of body, 19 slides. Paratypes: ZIHU 3820, serial transverse sections of anterior half of body, 10 slides; ZIHU 3821, serial transverse sections of anterior half of body, 8 slides.

Comparative material. *Carinoma mutabile*: ZIHU 3974, serial transverse sections of anterior fragment of body, sectioned at 9 μ m thickness, 20 slides, collected and identified by Svetlana A. Maslakova on 19 October 2009 from a mudflat in Charleston, Oregon, USA; ZIHU 3975, same data as 3974, serial transverse sections from tip of head to intestinal region of a complete specimen, sectioned at 10 μ m thickness, 20 slides.

Fig. 1. *Carinoma hamanako* sp. nov., photographs in living state. A, Holotype, ZIHU 3819, general shape; B, paratype, ZIHU 3821, tail.

Diagnosis. A *Carinoma* having 1) rhynchocoelic villar vessels terminating blindly posteriorly, 2) lateral rhynchocoel vessels posteriorly connected to the lateral vessels, 3) unbranched excretory canals, 4) the mid-dorsal nerve shifted downward into the body-wall longitudinal muscle layer in the posterior part of the body, and 5) lateral blood vessels that do not form a vascular plexus in the foregut region.

Description. *External features.* Body about 8 cm long and 1 mm wide (Fig. 1A). Head white, dorsoventrally flattened, with neither eyes nor cephalic furrows. Foregut region white, round in cross section. Intestinal region cream-white or yellowish-white, somewhat flattened dorsoventrally. Tail flattened, translucent, devoid of intestinal lateral diverticula (Fig. 1B).

Body wall. Epidermis without non-cellular inclusions. Epidermis of anterior body with intra-epithelial muscle fibre network (Fig. 2A). In brain region, thickness of epidermis/lateral body diameter <1. Dermis forming distinct zone between epidermis and body-wall circular muscle layer. Thickness of dermis less than half of epidermal height. Muscle processes (or radial muscles) extending from circular muscle layer into epidermis in cephalic region (Fig. 2B). Muscle layers consisting of outer circular and inner longitudinal muscle layers; anteriorly, three additional muscle layers present: 1) outer longitudinal muscle layer between dermis and outer circular muscle layer (Fig. 2A); 2) diagonal muscle layer beneath outer circular muscle layer (Fig. 2C); and 3) inner circular muscle layer interlaced with longitudinal muscle fibres and continuous with rhynchocoel wall, enormously thickened anterior to, but disappearing posterior to, foregut-intestine junction (Fig. 2D, E). Pre-cerebrally, dorsoventral and transverse muscles present, originating from within epidermis; some dorsoventral muscles piercing body-wall outer circular muscle layer, and running through cephalic blood lacunae; transverse muscles running along dorsal and ventral sides of body-wall outer circular muscle layer. Dorsal and ventral muscle crosses present between body wall circular muscle layers. Central (medial) muscle plate present between foregut and rhynchocoel only in oral region. Parenchyma barely distinguishable other than as membranes enclosing various body organ systems. Transverse muscle fibres present in mouth region (commencement of body-wall inner circular muscle layer).

Proboscis apparatus. Proboscis pore subterminal. Gland cells of rhynchodaeum completely absent in holotype, but sparsely present in one paratype (ZIHU 3820). Rhynchocoel composed of three regions. First region continuous with body-wall inner circular muscle layer, anteriorly consisting of circular muscle fi-

Fig. 2. *Carinoma hamanako* sp. nov., holotype, ZIHU 3819, photomicrographs of transverse sections. A, Brain region, showing body-wall musculature (arrowheads indicating intra-epithelial muscle fibres); B, pre-cerebral region, showing muscle processes from body-wall inner circular muscle layer penetrating dermis and extending to epidermis, indicated by arrowheads; C, foregut region, showing diagonal muscles beneath body-wall outer circular muscle layer, indicated by arrowheads; D–H, nephridial region, showing three different regions of rhynchocoel (white arrowhead indicating the first region, black the second, and gray the third). Abbreviations: DE, dermis; EP, epidermis; FG, foregut; IC, body-wall inner circular muscle layer; IL, body-wall inner longitudinal muscle layer; LD, intestinal lateral diverticulum; OC, body-wall outer circular muscle layer; OL, body-wall outer longitudinal muscle layer; RC, rhynchocoel.

Fig. 3. *Carinoma hamanako* sp. nov., photomicrographs of transverse sections; A, B, D, holotype, ZIHU 3819; C, paratype, ZIHU 3820. A, Proboscis, showing non-overlapping muscle crosses, indicated by arrowheads; B, proboscis, showing diagonal muscle layer and delicate inner circular muscles, indicated by arrowheads; C, foregut region; D, pre-cerebral region, showing four main channels of cephalic blood lacunae. Abbreviations: CL, cephalic blood lacuna; DM, diagonal muscles; FG, foregut; LV, lateral blood vessel; PE, proboscis epithelium; PN, proboscis nerve; RD, rhynchodaeum; RH, rhabdoid; RL, lateral rhynchocoel blood lacuna; RV, rhynchocoelic villar vessel; SP, mid-dorsal sensory pit.

bres, thickened posteriorly in posterior stomach region, there consisting of interwoven longitudinal and circular muscle fibres (Fig. 2D, E). Second region short, consisting of circular muscles only, opening to dorsal wall of third region (Fig. 2E–G). Third region starting close to stomach-intestine junction, with thin wall composed of interwoven longitudinal and circular muscle fibres (Fig. 2F–H). Posterior end of rhynchocoel not included in all specimens examined, so rhynchocoel length unknown. Rhynchocoelic caecum not found. Proboscis insertion situated at cerebral ring. Proboscis showing no distinct antero-posterior histological differentiation; epithelium not developed into papillae; rhabdoids mostly found in dorsal side of epithelium (Fig. 3A). Proboscis musculature in everted state consisting of inner circular, longitudinal, diagonal, and outer circular muscle layers (Fig. 3B); two muscle crosses of non-overlapping type (cf. Chernyshev 2010) present between

diagonal layer and outer circular muscle layer (Fig. 3A). Proboscis with two nerves (Fig. 3A); peripheral neural sheath indistinct. Proboscis armature absent. Proboscis not used for locomotion.

Alimentary system. Mouth located just behind brain. Oesophagus absent. Stomach histologically differentiated into anterior basophilic (Fig. 3C) and posterior acidophilic regions (Fig. 2D). Intestinal caecum absent. Intestinal diverticula (Fig. 2F) simple, unbranched pouches.

Circulatory system. Cephalic vasculature forming blood lacunae in front of cerebral ganglia, consisting of four main channels connected by dorsal and ventral anastomoses (Fig. 3D). Pair of rhynchocoelic villar vessels present (Fig. 3C), terminating blindly in anterior foregut region. Pair of lateral rhynchocoel blood lacuna present, originating from lateral vessels anteriorly, then running dorsolaterally just outside rhynchocoel wall (Fig. 3C), and posteriorly rejoining lateral vessels at foregut-intestine junction; lateral rhynchocoel blood lacuna becoming very narrow before rejoining lateral vessel (Fig. 4A–H). Lateral blood vessel (=lateral lacuna) outside body-wall inner circular muscle layer (Fig. 3C). Mid-dorsal vessel absent. Extra vascular pouches absent. Vascular plexus absent in foregut region.

Nervous system. Cerebral ganglia and anterior part of lateral nerve cords external to body-wall outer circular muscle layer, posterior parts of lateral nerve cords found among inner longitudinal muscle fibres. Dorsal cerebral ganglia with one commissure. Outer neurilemma of cerebral ganglion absent, but inner neurilemma present. No statocysts in brain tissue. Large nerves absent in head region. Mid-dorsal nerve and mid-ventral nerve situated outside body-wall outer circular muscle layer anteriorly, but embedded in longitudinal muscle layer posteriorly. Posterior junction of lateral nerve cords unknown. Pair of neurochord cells present posterior to dorsal cerebral ganglion on each side (Fig. 5A). Neurochords present in lateral nerve cords (Fig. 4A). Myofibrillae absent in lateral nerve cords. Buccal nerves absent.

Excretory system. Excretory canal situated below lateral blood vessel on each side (Fig. 4A), extending backward from posterior foregut region to anterior intestinal region, turning anteriorly, then bending slightly posterodorsad to lead to excretory pore near foregut-intestine junction (Fig. 5B). Excretory canal unbranched, situated outside body-wall inner circular muscle layer. Nephridial gland inconspicuous. Glandular components not found in excretory tubules.

Reproductive system. Sexes presumably separate; among examined specimens, only holotype (a mature male) including posterior gonadal region. Single testis alternating with intestinal diverticula (Fig. 5C). Gonoduct dorsolateral. Reproductive strategy unknown.

Sensory organs. Apical organ absent. Typical basophilic cephalic glands extending to level of brain, but not behind it, situated between body-wall inner circular muscle layer and connective tissue basement membrane, opening through numerous independent ducts. Cerebral organ absent. Side organs (=lateral sensory organs) absent. Ten mid-dorsal sensory pits in head region present in all specimens examined (Figs 3D, 5D).

Etymology. The specific epithet, a noun in apposition, is the Japanese name for Lake Hamana, the type locality of the new species.

Remarks. The anatomical features of the present species, including intra-epithelial muscle fibres in the epidermis of the anterior body, the rhynchocoel wall

Fig. 4. *Carinoma hamanako* sp. nov., holotype, ZIHU 3819, photomicrographs of transverse sections (A–H, anterior to posterior), showing lateral rhynchocoel blood lacuna (indicated by arrowheads) rejoining lateral vessel; anterior-most gonadal sac indicated by asterisk. Abbreviations: EX, excretory canal; LV, lateral blood vessel; NE, neurochord.

being partially composed of interwoven longitudinal and circular fibres, the blood system with six post-cerebral vessels in the foregut region, and the absence of statocysts, agree well with the diagnoses of *Carinoma* given by Gibson (1979) and Chernyshev (1999). This genus currently includes eight species: *C. armandi* (McIn-

Fig. 5. *Carinoma hamanako* sp. nov., holotype, ZIHU 3819, photomicrographs of transverse sections. A, Pair of neurochord cells, indicated by arrowheads; B, excretory pore, indicated by arrowhead; C, intestinal region, showing arrangement of testis; D, pre-cerebral region, showing mid-dorsal sensory pit. Abbreviations: CL, cephalic blood lacuna; DE, dermis; EP, epidermis; GD, gonoduct; IN, intestine; LD, intestinal lateral diverticulum; RC, rhynchocoel; SP, mid-dorsal sensory pit; TE, testis.

tosh, 1875) from the UK (McIntosh 1875; Bergendal 1902, 1903a, b); *C. caraibicum* from Curaçao, in the Caribbean Sea (Stiasny-Wijnhoff 1925); *C. mutabile* from the Pacific coast of the USA (Griffin 1898; Coe 1901, 1904, 1905, 1940, Corrêa 1964; Okazaki *et al.* 1988; Hochberg and Lunianski 1998; Thollessen and Norenburg 2003); *C. patagonicum* from Punta Arenas, Chile (Bürger 1895a); *C. patriciae* Gibson, 1979 from Magnetic Island, Australia (Gibson 1979); *C. renieri* Senz, 2000 from the Arabian (or Persian) Gulf (Senz 2000); *C. tremaphoros* Thompson, 1900 from the Atlantic coast of the USA (Thompson 1900; Sumner *et al.* 1913; Coe 1943, 1951; McCaul 1963; Turbeville and Ruppert 1983; Thollessen and Norenburg 2003; Maslakova *et al.* 2004a, b); and *C. uschakovi* Chernyshev, 1999 from the White Sea and the Barents Sea (Chernyshev 1999). The new species differs from its congeners by the com-

Table 2. Comparison of anatomical features in the genus *Carinoma*.

Species	Rhynchocoelic villar vessels	Lateral rhynchocoel vessels	Excretory canal	Mid-dorsal nerve in posterior body	Foregut vascular plexus
<i>C. armandi</i>	end blindly	end blindly	unbranched	among body-wall longitudinal muscle layer	absent
<i>C. caraiubicum</i>	end blindly	rejoin lateral vessels	?	?	?
<i>C. mutabile</i>	end blindly	rejoin lateral vessels	branched	among body-wall longitudinal muscle layer	absent
<i>C. patagonicum</i>	?	?	?	outside outer circular muscle layer	?
<i>C. patriciae</i>	end blindly	extend to full length of body	unbranched	among body-wall longitudinal muscle layer	absent
<i>C. renieri</i>	end blindly	extend to full length of body	branched	among body-wall longitudinal muscle layer	present
<i>C. tremaphoros</i>	rejoin lateral vessel	rejoin lateral vessels	?	?	?
<i>C. uschakovi</i>	end blindly	?	unbranched	among body-wall longitudinal muscle layer	present
<i>C. hamanako</i> sp. nov.	end blindly	rejoin lateral vessels	unbranched	among body-wall longitudinal muscle layer	absent

Unknown character states are represented by a question mark (?).

Fig. 6. Phylogenetic tree resulting from maximum likelihood analysis of combined 28S rRNA and COI ($\ln L = -14170.39$). Numbers on nodes indicate bootstrap support values $\geq 50\%$.

bination of characters summarised in Table 2 and formalised above in the specific diagnosis.

Molecular Phylogeny

The 28S rRNA dataset comprised 13 sequences, which resulted in a multiple sequence alignment of 1,260 bp. Uncorrected p -distances between the 28S rRNA sequences of *C. hamanako*/*C. mutabile*, *C. hamanako*/*C. tremaphoros*, and *C. mutabile*/*C. tremaphoros* were 4.8%, 5.1%, and 6.0%, respectively. The resulting likelihood tree ($\ln L = -8066.08$) showed monophyly of *Carinoma* with 100% bootstrap frequency (BF hereafter) (data not shown). *Carinoma hamanako* formed a clade with *C. tremaphoros*, with *C. mutabile* as their sister group. The COI dataset comprised 17 sequences and the software package MAFFT yielded a 679 bp alignment. Its resulting likelihood tree ($\ln L = -5609.79$) presented a topology similar to that of 28S rRNA, with minor differences (data not shown). Uncorrected p -distances between the COI sequences of *C. hamanako*/*C. mutabile*, *C. hamanako*/*C. tremaphoros*, and *C. mutabile*/*C. tremaphoros* were 17.2%, 15.0%, and 15.2%, respectively. The maximum likelihood analysis for the concatenated 28S rRNA and COI

sequences gave a tree of $\ln L = -14170.39$, showing monophyly of *Carinoma* with 100% BF (Fig. 6). It also demonstrated the monophyly of *Cephalothrix*, with nodal support of 100% BF. *Hubrechtella dubia* Bergendal, 1902 appeared as the sister taxon to *Cephalothrix* with high nodal support (82% BF). Their joint clade is the sister group of a clade containing the species of Tubulanidae included in the analysis.

Our phylogenetic analysis supports the previous notion that *Carinoma* is a group distinct from other palaeonemerteans (e.g., Bürger 1895b; Bergendal 1900; Friedrich 1935; Hylbom 1956), and also confirms the placement of *C. hamanako* in the genus *Carinoma*. Despite the relative geographical distances, *C. hamanako* from the Western Pacific turned out to be more closely related to the Western Atlantic *C. tremaphoros* than to the Eastern Pacific *C. mutabile*. A comprehensive biogeographic and phylogeographic study of the species of *Carinoma* will likely shed light on the evolution and distribution of these nemerteans.

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