

# Paraphyly of Marimermithida refines primary routes of transition to parasitism in roundworms

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Parasitic life-strategies in the phylum Nematoda (roundworms) are remarkably diverse and intricate in terms of evolution and taxonomy. By analysing novel rDNA data obtained on rare host-associated groups with unusual biology, we reveal paraphyly of the last major taxon with uncertain higher-rank classification that united solely parasitic nematodes (Marimermithida) to show that primarily marine parasitism only emerged independently and repeatedly in a few free-living lineages. We report secondary seaward ingressions of land-based parasites (Mermithida) via invading hosts in the subtidal zone to illustrate the host-borne scenario of oceanic fish and mammal colonization by primarily terrestrial parasites (Spiruria). We also present the first molecular data on marine nematodes from unicellular hosts (foraminiferan protozoans) to demonstrate the independent origins of exploitative nematode associations at a microscopic scale. We argue that, in contrast with primarily intestinal associations arising from saprotrophy and commensalism, non-intestinal host capture (colonization of host body cavity or internal organs) is likely to be a primary route of transition to truly exploitative parasitism in roundworms. Predispositions to host capture in nematode morphology, ecology and life cycles imply its evolution as part of innate pre-adaptations to crossing environmental boundaries to enable multiple successful transitions to parasitism in the phylum history.

**ADDITIONAL KEYWORDS:** evolutionary potential – molecular phylogeny – parasitoid – phylogenetic position – rRNA.

## INTRODUCTION

Nematodes (roundworms) are ubiquitous animals dominating ecological communities and networks.

While the majority are free-living inhabitants of marine sediments and soils, many species comprise parasites and pathogen vectors that have colonized a wide range of hosts at all scales from whales to protozoan cells, in many cases having major economic and medical impacts. Parasitism in nematodes is a

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richly diverse phenomenon in terms of biology, ecology and the evolutionary routes of transition to a parasitic lifestyle. The knowledge of phylogeny of successful free-living-to-parasitic transitions is necessary to study the genomic, structural and environmental bases of parasitism in general biology, medical science and pest control. In the currently embraced phylogenomic framework (Blaxter *et al.*, 1998, 2016; Bik *et al.*, 2010; Smythe *et al.*, 2019; Ahmed *et al.*, 2022; Hodda, 2022), a series of independent transitions to plant- (at least three), non-vertebrate- (ten) and vertebrate (five) parasitism of various forms and extents across the entire phylum have been proposed (Dorris *et al.*, 1999; Blaxter & Koutsovoulos, 2015; Tchesunov & Ivanenko, 2021). A more diversified scenario of at least 20 independent transitions to insect parasitism alone is also suggested (Sudhaus, 2008). Among the three stem nematode lineages, the richest repertoire of parasitic lifestyles is observed among the Chromadoria clade, followed in ranking by Dorylaimia and only a few known cases in Enoplia. The overwhelming majority of parasitic nematodes belong within lineages of terrestrial or limnic ancestry (Blaxter & Koutsovoulos, 2015; Holterman *et al.*, 2019; Okamura *et al.*, 2022), while their descendent marine parasite species are accordingly classed as secondary invaders to the sea. Current knowledge derives roundworms from a marine ancestor inhabiting the sea sediments of the Ediacaran–Cambrian at about 600–500 Mya (e.g. Malakhov, 1994; Rota-Stabelli *et al.*, 2013), which is also supported by solid phylogenomic evidence (e.g. Dunn *et al.*, 2008) of their kinship with well-documented Mid-Cambrian marine tardigrades (e.g. Müller *et al.*, 1995; Labandeira, 2005). Out of all the nematodes, the Enoplia stem achieves a basal position in phylogenetic analyses of protein-coding (a representative sampling in: Blaxter *et al.*, 2016; Smythe *et al.*, 2019; Ahmed *et al.*, 2022), SSU rDNA data (large-scale phylum-inclusive assays, e.g. Holterman *et al.*, 2019), as well as in a comparative framework of early embryogenesis (Malakhov, 1994; Voronov *et al.*, 1998; Schierenberg, 2005), nervous system architecture (Malakhov, 1994), gonad and sperm cell morphology and ultrastructure (Justine, 2002; Yushin & Malakhov, 2014; Yushin *et al.*, 2014). Enoplians comprise diverse, mainly marine, free-living forms abundant in sediments towards deep-sea waters, where they also contribute to the core of nematode communities (Muthumbi *et al.*, 2004; Leduc & Rowden, 2018). However, the only commonly recognized enoplian parasites belong to the Triplonchida clade, which inhabits terrestrial, brackish or freshwater environments. This seemingly definite mapping of parasitic associations onto the nematode phylogeny implies their exclusive on-land ancestry, at the same time highlighting the importance

of identifying and exploring cases of primarily marine parasitism in roundworms to pose hypotheses on the routes, bases and predispositions of parasitism in this most species-rich and abundant group of metazoan life.

The most widespread and impactful marine nematode parasite species belong to the chromadorian Spirurina clade inferred as clade III in Blaxter *et al.* (1998), which infect a wide diversity of mostly vertebrate hosts (bony fish, sharks and marine mammals). Marine spirurians are generally thought to derive from on-land parasitic ancestors via invertebrate vector transmission to definitive hosts in a freshwater–anadromous–marine sequence (e.g. Malakhov, 1994; Li *et al.*, 2018) between the Devonian–Carboniferous and Early Mesozoic (origin of modern-type spirurians; Rota-Stabelli *et al.*, 2013; Li *et al.*, 2018). A major life cycle trait shared with other nematode parasites is the free-to-host phase transition at juvenile/larva stage 3 (J3, third of four pre-adult stages in the nematode life cycle), which may also carry a phoretic or diapausing function (Sudhaus, 2010). Most chromadorian parasites invade their hosts at J3, while for dorylaimian parasitoids (early-cycle larval parasites) this is the stage of host egress. Meanwhile, definitive host habitat is a major distinguishing feature between spirurian and non-spirurian marine parasites. The latter, which are sparsely instantiated across Enoplia, Dorylaimia and Chromadoria stems, never invade the gut but exclusively the body cavity and its various structures across a wide range of mostly benthic non-vertebrate hosts – sea urchins, starfishes, annelids, molluscs, priapulids, other nematodes or even testate gigantic protozoans (Rubtsov & Platonova, 1974; Rubtsov, 1977; Tchesunov & Spiridonov, 1985; Tchesunov, 1997a; Miljutin, 2003). As with their on-land equivalents, marine parasitoids dwell as juveniles in the body cavity or internal organs of the host. After exhausting the host individual to death, they abandon the corpse to become free, non-feeding, reproductive adults. Spirurian, enoplian and dorylaimian parasites may achieve gigantic sizes (up to tens of cm vs. about 1–20 mm in their free-living relatives) and exhibit strong fecundity associated with adaptations in the female reproductive system (Tchesunov & Spiridonov, 1985; Miljutin, 2003). Although these characteristics are traditionally assigned evolutionary significance, this assignation lacks a clear appraisal in a molecular phylogenetic context. Thus, in the present study, we integrated original sequences (including those of rare primarily marine parasites) with available rDNA data from various sources (including environmental metagenomic and amplicon surveys) to combine host-associated and free-living nematode groups into a representative phylogeny. It was then used to interpret evidence of life traits, morphology and

ecology in a non-formal approach due to the limited statistical power of formal reconstruction methods for a few complex characters. In the ensuing discussion, we attempt to explain the combination of traits in more basal primarily marine vs. more derived limnic-terrestrial lineages to: (1) suggest the ancestral mode of host capture, life cycle and habitat type; (2) propose the evolutionary scenario that produced the diversity of modern parasitic lifestyles; (3) assess the evolutionary significance of important parasitism-related traits; and (4) identify putative pre-adaptations to successful parasitism in baseline nematode organization and ecology.

Historically, marine non-spirurian parasites were classified in morphology-based systems within the orders Mermithida (an established on-land taxon of Dorylaimia), Marimermithida, Benthimermithida and Rhaptothyreida (all *incertae sedis*; Malakhov, 1994; Miljutin, 2014a–c). Despite their apparent rarity, these groups are widespread across the world oceans, from the tidal zone down to depths of 5.2 km (Miljutin, 2014a). Due to being inherently hard to collect, they are observed only occasionally, typically upon laboratory examination of formalin-fixed hosts. The first obtained molecular data on selected individuals were used to fit the aberrant taxa into a mature phylogenetic context. Thus, the spectacular giant parasite of sea urchins *Echinomermella matsi* Jones & Hagen, 1987, initially subsumed into the mermithids, was relocated to another stem lineage, in the crown of Enoplida (Poinar *et al.*, 2011). Another aberrant marine parasite *Nematimermis enoplivora* Tchesunov & Spiridonov, 1993, a minute-scale dweller of the body cavity of other nematodes (Tchesunov & Spiridonov, 1993), is verified as a member of Dorylaimia for the first time on the basis of molecular data examined in the present study. The affinity of rare deep-sea, trophosome-feeding benthimermithids to the chromadorian stem in larval morphology (Tchesunov, 1997b) was confirmed in molecular phylogenetic trees (Tchesunov *et al.*, 2009; Holovachov *et al.*, 2013; Leduc & Zhao, 2019). Upon discovery in deep-sea core samples, the enigmatic Rhaptothyreida were considered alternatively in kinship with Mermithida (Hope, 1977a), Marimermithida or Benthimermithida (Inglis, 1983) based on vestigial mouth and the trophosome character, an organ derived from alimentary tract. Although rhaptothyreids have never been found inside a host to date, they have a juvenile morphology strikingly different from putative adults (e.g. minute cephalic sensilla, the absence of feather-like amphids and presence of a stylet-like structure in the buccal cavity), which may suggest their larval parasitism, i.e. free-living adults and in-host juveniles (Leduc, 2014; noting that latest stages observed in this group so far may still be immature individuals;

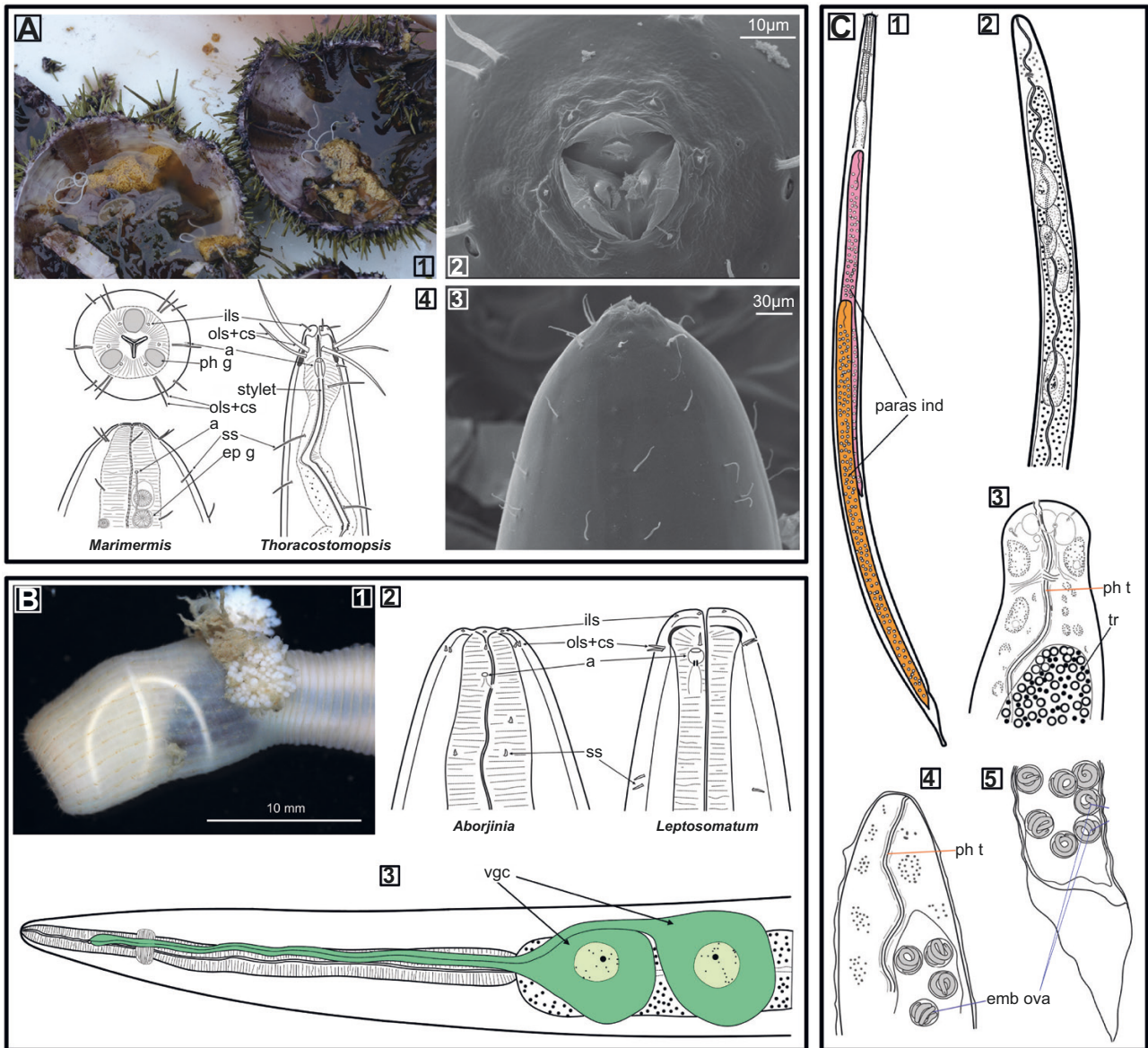
A.V.T., unpublished). The phylogenetic placement of the rhaptothyreids has been recently confined within the order Enoplida in rDNA trees, although with a fuzzier resolution (Leduc *et al.*, 2018). The final major traditional group of marine parasitic nematodes is Marimermithida. The genus *Marimermis* was described by Rubtzov and Platonova (Rubtzov & Platonova, 1974) based on three novel species extracted from the body cavities of starfish or found living freely in inter- to upper subtidal sediments. There the marimermithids were surmised to represent larval parasites (parasitoids) of marine invertebrates, which display an unusual combination of typically parasitic (e.g. up to 15 cm-long body, trophosome-transformed gut) and marine free-living traits (e.g. cephalic and abundant body sensilla). United with the superficially similar benthimermithid *Trophomera* and another three genera, *Marimermis* established the aberrant taxon Marimermithida (Rubtzov, 1980), which until 2021 remained in essence the last stronghold of higher-rank nematode systematics in terms of its uncertainty in phylogenetic placement among roundworms. The proposed affinity of marimermithids to free-living marine Enoplida (Malakhov, 1994) has met with no confident appraisal due to the unavailability of biological samples for molecular analyses. The first molecular data on the marimermithid genus *Aborjinia* recently revealed its membership within the Enoplia stem, although without a reappraisal of whole taxon monophyly (Westerman *et al.*, 2021; Zograf *et al.*, 2021). In the present study, we report molecular evidence on two representatives of Marimermithida, as well as on other rarely captured marine nematode parasites, and discuss the nematode life-strategies and evolutionary adaptations to parasitism within the framework of the resulting phylogeny.

## MATERIAL AND METHODS

### MATERIAL COLLECTION AND SAMPLING SITES

Specimens of *Marimermis maritima* Rubtzov & Platonova, 1974 were discovered in the coelomic cavity of the sea urchin *Strongylocentrotus polyacanthus* A.Agassiz & H.L.Clark, 1907 (Fig. 1A-1). The host specimens were diver-collected in the north-west Pacific Ocean near the coast of the island of Matua (middle Kurile Islands, Russia; 48.1° N, 153.2° E) in August 2016. The *Aborjinia* sp. specimen was found in a priapulid *Priapulius caudatus* Lamarck, 1816 (Fig. 1B-1). The host organism was collected at a depth of 5349–5352 m in the north-west Pacific Ocean, Kurile-Kamchatka Trench, using an Agassiz trawl during the KuramBio deep-sea expedition (R/V *Sonne*, SO-223) conducted in August 2012. *Nematimermis enoplivora* was discovered inside the nematode *Enoplus communis*





**Figure 1.** Cavitory marine nematodes. A, *Marimermis maritima*: 1, live specimens exposed in open host, sea urchin *Strongylocentrotus polyacanthus*; 2, 3, cephalic end apically and laterally, respectively, scanning electron microphotograph; pharyngeal gland outlets on lips, abundant irregular somatic setae; 4, generalized anterior ends juxtaposed in *M. maritima* [modified from Tchesunov (1997a)] and *Thoracostomopsis barbata* (Enoplida: Thoracostomopsidae) [modified from Lorenzen (1994)]. B, *Aborjinia* sp.: 1, juvenile occupying introvert of *Priapulus caudatus* (photograph courtesy of Dr A. S. Maiorova, NSCMB FEB RAS); 2, generalized anterior ends juxtaposed in *Aborjinia* sp. and *Leptosomatium* sp. (Enoplida: Leptosomatidae); 3, anterior body with two-celled ventral secretory-excretory gland, a distinctive genus feature [redrawn and modified from Miljutin (2003)]. C, *Nematimermis enoplivora*: 1, two mature parasitoid individuals within host body of nematode *Enoplus communis* (Enoplida: Enoplidae); 2, 3, anterior body and head of immature parasitoid retrieved from body cavity of *E. communis*; 4, 5, anterior and tail regions of mature parasitoid from *E. communis*; trophosome resorbed, embryos with larval stylet inside egg cases, nested retained exuvia (old moulted cuticles) [all drawings modified from Tchesunov & Spiridonov (1993)]. (See also Fig. S1.) Key: a, amphid; emb ova, embryonated eggs; ep g, epidermal glands; ils, inner labial sensilla; ols + cs, joint crown of outer labial and cephalic setae; paras ind, parasitoid individuals; ph g, pharyngeal glands; ph t, pharyngeal tube; ss, somatic setae; stylet, spear-shaped armature; tr, trophosome; vgc, ventral gland cells.

Bastian, 1865 (Fig. 1C-1). Hosts were collected along with muddy intertidal-to-subtidal sediments near the White Sea Biological Station of Moscow State University (Kandalaksha Bay, White Sea) during summer 2009. Individuals of phanodermatid K2 (Supporting Information, Fig. S1) and *Camacolaimus* spp. were found in the foraminifer *Reophax curtus* Cushman, 1920 collected by subtidal trawling during the summers 2010–15 (Kandalaksha Bay, White Sea). The parasitic specimens' isolation and processing are detailed in the Supporting Information, Material S1. The free-living species *Paracanthonus caecus* (Bastian, 1865) Micoletzky, 1924, *Thoracostomopsis barbata* Ditlevsen, 1918, *Setostephanolaimus* sp., *Thoracostoma* sp. and *Metachromadora vivipara* (de Man, 1907) were sampled from marine subtidal sandy sediments during the summers 2010–18; *Tripyla* aff. *glomerans* Bastian, 1865 was isolated from freshwater detrital debris of the Chernaya river bed in August 2018 (Kandalaksha Bay, White Sea); *Anticoma possjetica* Platonova, Belogurov & Sheenko, 1979 was obtained from mussel clumps of the bivalve *Crenomytilus grayanus* (Dunker, 1853) collected at a 10 m depth near the Vostok Biological Station of A. V. Zhirmunsky National Scientific Centre of Marine Biology in August 2004 (Vostok Bay, Sea of Japan). The methods for nematode extraction, as well as DNA/RNA isolation and sequencing, are detailed in the Supporting Information, Material S1.

#### DATASET CONSTRUCTION AND PHYLOGENETIC ANALYSIS

Data processing, phylogenetic pipeline, sequence origins and accession IDs are detailed in the Supporting Information, Material S1. Original data were obtained by Sanger or NGS sequencing [depending on the object; NCBI BioProject PRJNA772260; NCBI accession IDs (for genes, full cistrons or raw Sequence Read Archive entries) provided in Fig. 2 and the Supporting Information, Table S1]. Full rDNA cistrons were assembled from the original (four species) or third-party (five species) NGS data. The latter included transcriptomic reads (Ahmed *et al.*, 2021) and metagenomic eDNA surveys (D'haeseleer *et al.*, 2017; Woehle *et al.*, 2018). The original Sanger sequences and assemblies are marked with asterisk (\*) on Figure 2. The rDNA contigs on three species (*Pontonema* sp., *Symplocostoma* sp. and *Tobrillus* sp.) were fetched, as is, from third-party transcriptomic assemblies (Smythe *et al.*, 2019; *figshare* data without NCBI accession IDs) and trimmed for inclusion in the alignment. The third-party NGS-based original assemblies and pre-fetched contigs are provided in a separate datafile (see Data Availability and Supporting Information, Table

S1). Full-amplicon rDNA cistrons were identified and retrieved from a PacBio eDNA survey (Jamy *et al.*, 2020) for several anonymous species to further balance taxon sampling. The study-specific dataset thus constructed accommodates a representative cross-phylum diversity of host-associated and free-living groups (Supporting Information, Table S1). The rDNA gene alignments were constructed using RNA secondary structure-aware algorithms, concatenated and used for maximum likelihood (ML) and Bayesian (BI) tree inference. Phylogenetic hypotheses were verified in appropriate ML-based statistical tests of topologies (detailed in the Supporting Information, Material S1).

## RESULTS

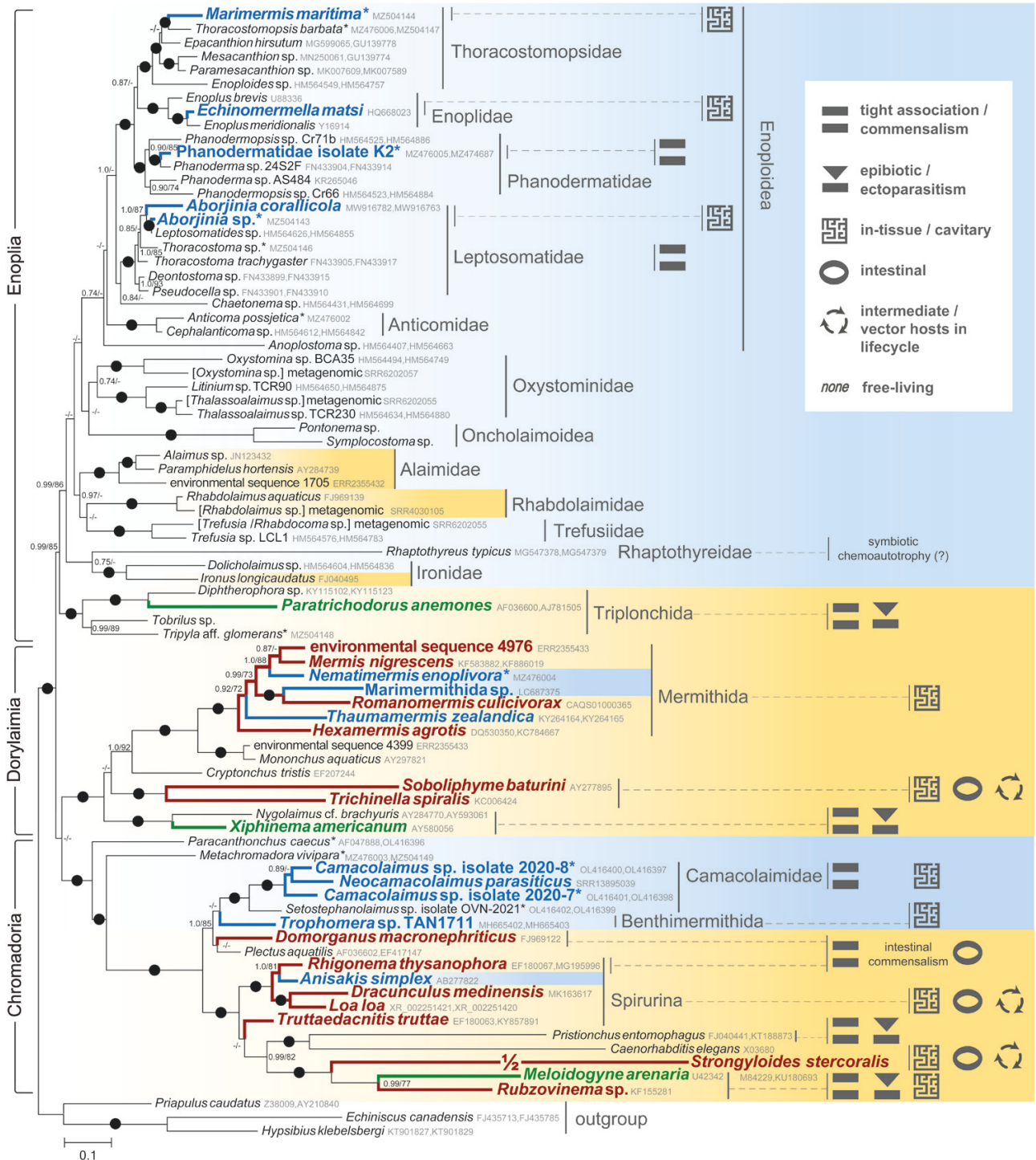
### SPECIMEN IDENTIFICATION

Individuals of *Marimermis maritima* (Fig. 1A), *Aborjina* sp. (Fig. 1B) and *Nematimermis enoplivora* (Fig. 1C) were expert-identified in this study to species or genus level based on morphometry and descriptive data in light microscopy. The phanodermatid K2 specimen was selected from among a few juveniles extracted from foraminiferan hosts captured in a single sample and identified with certainty to family level as Phanodermatidae (Supporting Information, Fig. S1). Detailed morphology data and verification are available for expert evaluation in the Supporting Information, Material S1. Other nematode specimens used for DNA sequencing originally in the study were also expert-identified to species or genus level.

### PHYLOGENETIC ANALYSIS

Maximum likelihood (ML) and Bayesian inference (BI) multi-gene rDNA-based reconstructions produced congruent topologies in most areas, with the exception of selected deeper nodes of the nematode tree (Fig. 2; Supporting Information, Fig. S2; result reproducible with SSU and LSU partitions separately, details not provided). Due to the greater susceptibility of the ML method to model misspecification and heterogeneity-associated biases, we depict the BI tree on Figure 2 and leave the mismatched nodes unlabelled for node support. The two topologies are not discriminated in ML-based statistical hypothesis testing (Supporting Information, Table S2), have an overall high support and conform to the established phylum phylogeny (Bik *et al.*, 2010; Smythe *et al.*, 2019; Ahmed *et al.*, 2022). With the exception of *Rhaptothyreus typicus* Hope & Murphy, 1969, the host-associated species of the study are confidently distributed between different nodes of the tree, not forming a direct relationship. The former





**Figure 2.** Bayesian tree of Nematoda based on concatenated rDNA data. Gene sampling maximizes the available coverage of SSU, 5.8S and LSU genes (accession IDs for each taxon correspond either to individual genes available, full cistrons or eDNA samples). The data covers the three major clades (Enoplia, Dorylaimia and Chromadoria), with extended sampling of marine Enoplia, and a selection of representative host-associations (in bold) for the clades. Parasitism-relevant life traits are mapped as pictograms, explained in the legend and addressed in the following discussion section. Traits apply either to a particular sampled species or more broadly to the subclade it represents (according to general knowledge of typical large subclades). Pictogram series denote the co-occurrence of traits either within subclade or individual life cycle. The habitat is colour-coded to generally typify subclades (e.g. as per Holterman *et al.*, 2019), blue – marine; yellow – brackish/

branch is long and, therefore, may be consistently misplaced across the phylogeny due to associated inference artefacts (Leduc *et al.*, 2018). Of key relevance to the discussion is the absence of its validated monophyly with any of the host-associated lineages in statistical tests. Other study species of Enoplia were unambiguously placed in different traditional taxonomic families united within single superfamily (Enoploidea) of the order Enoplida. The traditional order Marimermithida is paraphyletic: *Marimermis maritima* groups within Thoracostomopsidae, while *Aborjina* sp. is placed within Leptosomatidae. The partial SSU and LSU genes of *Leptosomatides* sp. from Bik *et al.* (2010) are nearly identical with those of *Aborjina* sp. from this study. Since this genetic material was obtained from a free-living (off-host) individual obtained from marine sediments near the coast of California at a 2694 m depth, it probably represents a misidentified specimen of *Aborjina* that had abandoned its host. *Echinomermella matsi* falls inside the genus *Enoplus* as a member of the family Enoplidae, the closest clade to Thoracostomopsidae. The foraminifer-associated phanodermatid K2 groups within Phanodermatidae in sistership with *Phanoderma* sp. Outside Enoplia, the same-habitat *Camacolaimus* spp. specimens form a confident clade with the cavitary annelid parasite *Neocamacolaimus parasiticus* Holovachov & Boström, 2014 within the monophyletic assemblage of Camacolaimidae (a family of Chromadoria), which also includes the deep-sea cavitary invertebrate parasite *Trophomera* sp. The confident inference of *Nematimermis enoplivora* in the crown of the order Mermithida within Dorylaimia corroborates an earlier morphology-based view (Tchesunov & Spiridonov, 1993) to reveal its primary on-land ancestry and secondary intrusion to the sea.

## DISCUSSION

Our phylogenetic analyses of rDNA cistron genes and rigorous hypothesis testing unequivocally resolve the relationships of several non-spirurian marine host-associated taxa (Fig. 2) and clearly demonstrate multiple origins of primarily marine parasitism in roundworms. The benthimermithid *Trophomera* finds

its place within Chromadoria as a deviant parasitic marine representative of the freshwater-terrestrial-marine plectid lineage (in accord with: Tchesunov *et al.*, 2009; Holovachov *et al.*, 2013; Leduc & Zhao, 2019) in sistership with a peculiar assemblage of minute-size nematodes (Camacolaimidae) associated with foraminiferan protozoans or parasitising coelom of microscopic annelids. With Enoplia, it was unexpected that all zones of emergent parasitism within the stem are confined to the single traditional superfamily Enoploidea, otherwise consisting entirely of free-living forms. In agreement with the original study, the position of Rhaptothyreida is established outside Enoploidea within the order Enoplida (Leduc *et al.*, 2018). *Rhaptothyreus typicus* appears long-branched in rDNA trees, which requires a focused systematic analysis, exploring more taxon sampling and parameter variation until additional molecular markers are available. ML-based hypothesis testing reveals its undefined placing with no confident affinity to mutually non-monophyletic host-associated lineages (Supporting Information, Table S2). Despite within life-cycle morphological disparity, suggesting larval parasitism in this enigmatic worm, in-host findings of rhaptothyreids are presently unknown. The stuffing of their trophosome cells with putative bacterial particles (Miljutin *et al.*, 2006) may suggest a non-parasitic but aberrant chemoautotrophic lifestyle, similar to vestimentiferan and pogonophoran annelids, as well as the mouthless nematodes *Astomonema* and *Parastomonema* (Miljutin, 2014c).

The morphology-based affinity of *Nematimermis enoplivora* (Fig. 1C) is confirmed within the Dorylaimia stem in a traditional order (Mermithida) uniting on-land invertebrate parasitoids (Tchesunov & Spiridonov, 1993). This strongly suggests its relatively recent invasion to the sea from an on-land ancestor parasitizing a terrestrial or brackish-water host. This trajectory may be explicated by several case findings of mermithids in the supra- to intertidal zone [*Thaumamermis zealandica* Poinar, Latham, David & Poulin, 2002 of the sandhopper (Poinar *et al.*, 2002), with molecular evidence in Tobias *et al.*, 2017], tidal- to truly subtidal habitats [*Agamomermis* sp. of the hairy shore crab (Dusto, 2020), molecular evidence unpublished; Mermithidae sp. of a benthic tanaidacean

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limnic/terrestrial. Marine animal parasites/associates are listed in dark blue, brackish/limnic/terrestrial in red, while plant parasites/associates are shown in green. Nodes mismatched in BI and ML topologies are unlabelled. Otherwise, labels contain BI posterior probabilities (left) and ML bootstrap support (right). Values < 0.7/70 are replaced with dashes (-). Bipartitions with support >0.95/95 dotted (●). BI posterior probabilities are calculated across GTR+ $\Gamma$  parameter space in 3 M generations, ML bootstrap support estimated under GTR+F+G16 model in 100 replicates. NCBI accessions appended to taxon names, original sequences and assemblies marked with asterisk (\*). Scale bar: substitutions per site. See also Supporting Information, Figure S2 (for an ML tree) and Supporting Information, Table S1 (for details on gene sampling, data origin and accession IDs).

crustacean (Kakui & Shimada, 2022); both attributed to Mermithidae in phylogenetic trees] towards the deep sea (*Thalassomermis megamphis* Tchesunov & Hope, 1997 of an unknown host, molecular data unavailable). We supplement this evidence with a molecular-verified report of truly marine parasitism in Mermithida, which represents a separate evolutionary lineage of independent seaward ingression that exploits other-nematode hosts (Fig. 2). This extant event of host-borne crossing of environmental boundaries supports the hypothetical scenario of vector-borne transition to marine vertebrate hosts realized early in the chromadorian evolution by ancestral spirurians (Malakhov, 1994; Li *et al.*, 2018).

An unusual instance of primarily marine parasitic-like nematode associations reported in this study is phanodermatid K2. According to our molecular analyses, it represents an unidentified species of the family Phanodermatidae and, therefore, comprises the first report of cavitory association in this otherwise free-living enoplid group. Phanodermatid K2 complements a cohort of peculiar associates of the large (about 1 mm size) unicellular testate agglutinated foraminifer *Reophax curtus* predominating in meiobenthic communities of the White Sea (Tchesunov, 2006; Miljutin *et al.*, 2012). Among other benthic foraminifers, *Reophax curtus* is known to provide a niche for miniature-scale parasitism in the chromadorian family Camacolaimidae (Hope & Tchesunov, 1999; Tchesunov & Milyutin, 2007; Holovachov, 2015) that invades about 10% of the foraminifer population. These worms, which dwell within a vacuole or interstitially in a cavity between cytoplasm and the test, can probably leave or reinvade the host at variant stages via the ostium or other wall pores. The non-mutualistic nature of this association is evident from a relatively low invasion rate, cytoplasm lesion and defensive host response of enclosing the intruder within a proteinaceous capsule. As with camacolaimids, some phanodermatid specimens in our samples exhibited peculiar exaggerated pharyngeal glands, perhaps due to enhanced secretion in-test of the foraminifer (observations by D.I.G., unpublished; see Supporting Information, Fig. S1, Material S1). Nutrients are likely absorbed in both solid (alimentary) and solute (transcuticular) form, as is entailed from a poor residual gut content under microscopy, which also suggests that this alliance has an exploitative nature. Among the discovered invasive camacolaimid species, only a few are obligately associated with the foraminifer and not found in sediments (A.V.T., unpublished); this reveals the transient nature of this cavitory association between the free-living state toward facultative and permanent host capture. An independent instance of the same association in a marine enoplid illustrates the capacity of

cavitory parasitism as a universal transition route that is being exploited in evolution multiple times successfully at a minute-dimension scale as well. The realization of this scenario, even at a relatively short evolutionary timescale, gains support in our present finding of a confident compact assemblage uniting protist-associated forms (*Camacolaimus* spp. isolates) and a true coelomic parasite of microscopic benthic polychaetes (*Neocamacolaimus parasiticus*; Ahmed *et al.*, 2021).

The position of the giant sea urchin parasite *Echinomermella matsi* within the otherwise free-living genus *Enoplus* (Poinar *et al.*, 2011) is re-confirmed. This species has no direct relationships with mermithids or other known marine parasitic nematodes. Instead, it appears the closest kin of an *Enoplus* species, thus rendering this traditional genus paraphyletic. An in-genus sistership of radically diverged lifestyles and phenotypes is unprecedented and represents a natural model system to study genomic predispositions to parasitism, as well as generating insights into particular mechanisms underlying host capture and in-host survival. This type of knowledge is key to understanding evolutionary transition pathways and a prerequisite to developing drug and vaccine candidates against known or potential disease agents. Commonly, free-to-host transitions occur over long timescales and accumulate genomic disparity, which obscures comparisons between the parasitic and free-living model counterparts. Recent research in this field has benefited from two narrow systems of emerging or facultative parasitism in three strongyloidid genera (Chromadoria clade IV; Hunt *et al.*, 2016) and the genus *Caenorhabditis* (Chromadoria clade V; Stevens *et al.*, 2020). Two different evolutionary scenarios have been exposed, an evident genomic reduction in the strong commensal *Caenorhabditis bovis* (Kreis, 1964) Andr assy, 1983 (at the expense of G-coupled receptor-coding genes involved in sensory systems and cell-to-cell communication) and a negligible loss but massive gene family expansion (mostly in astacin-like and SCP/TAPS immunomodulatory proteins), as well as their invasion-specific upregulation, in facultative and obligate strongyloidid parasites. A close relatedness to a free-living genome enabled strong inference of selective gene family duplications in both models and transcriptomic/proteomic evidence of their direct association with in-host survival towards a proposal of operonic control of parasitism-associated genes in *Strongyloides* species. Despite rarity of the *Echinomermella matsi* parasite, the *Echinomermella-Enoplus* complex may represent a promising model on which to direct future research in this field owing to its unique phylogenetic compactness, which is combined with biological and structural disparity that far surpasses the mentioned systems. *Echinomermella*



*matsi* is a cavitory parasite and obviously represents a separate, primarily marine route of emergent parasitism to complement evidence for the two different scenarios in strongly commensal, primarily free-living, soil rhabditids and intestinal strongyloidid parasites adopting a more intricate and probably descended vector-lost strategy of percutaneous J3 invasion and in-gut migration inside the host. A free-living component of the model is already available with genomic and transcriptomic data on *Enoplus brevis* Bastian, 1865 (NCBI BioProject Database 2014). Meanwhile, this instance of outbreaking parasitism, entailing radical change in phenotype and lifestyle within a narrow lineage, is not unique among nematodes. As we exemplify below with our findings, similar cases of explosive adaptation are discovered within the Enoploidea superfamily and may, in fact, be more common across the phylum. This observation suggests that such life-trait plasticity during adaptive evolution is inherent in roundworms as a phenomenon endemic among other Metazoa.

A solid statement of this study is paraphyly of the traditional order Marimermithida and its confident redistribution on nematode phylogeny. This inference implies a higher-rank taxonomic restructuring of a part of the nematode system and dismisses the last major taxonomic division that united marine parasites alone. Our analyses reject the hypotheses of marimermithid affinity with the order Mermithida or anywhere close within the Dorylaimia stem [as assumed, e.g. in Inglis (1983) and later in De Ley & Blaxter (2004)] but distribute them between the two distinct traditional families among the groups within Enoploidea superfamily of Enoplia. Accordingly, the peculiar life traits uniting the former taxon and shared with dorylaimian mermithids (larval cavitory parasitism in invertebrates, enlarged size and intense fecundity), thus need to be reinterpreted as potentially convergent. Although the enoplian origin of marimermithids has been grounded in pharyngeal and sensory morphology (Rubtsov & Platonova, 1974; Rubtsov, 1980; Tchesunov, 1997b; Figs 1A-2,3,4; 1B-2), the inference of *Marimermis maritima* within the ramified crown of Enoplia in family Thoracostomopsidae is unexpected and was not previously anticipated. Thoracostomopsids are marine free-living nematodes known as predators and scavengers (Moens *et al.*, 2014). They retain normal nematode anatomy with some particular structures in buccal armoury, evidently used to grip food particles, for example, smaller nematode prey. None of the thoracostomopsid species was known as parasitic or associated with any host. One particular character in free-living thoracostomopsids is, however, remarkable to functionally resemble that in a cohort of non-related parasitic nematodes. *Thoracostomopsis barbata* Ditlevsen, 1918, the closest free-living relative

of *M. maritima* in the current dataset, represents the sole thoracostomopsid genus possessing a long eversible spear in the mouth cavity (Fig. 1A-4). This spear, which comprises a joint structure compounding elements along the entire buccal cavity (Inglis, 1964), is apparently used for piercing food objects, albeit the genus diet is unknown. This spear structure is unique among free-living nematodes (Lorenzen, 1994). Spear-like structures or stylets of various origin used for penetrating the host are known from the invasive larvae of on-land dorylaimian vertebrate parasites Dioctophymatida and Trichinellida, invertebrate parasitoids Mermithida, on-land chromadorian insect- and plant-parasitizing Tylenchina and certainly from marine chromadorian invertebrate-infesting Benthimermithida (Tchesunov, 1997a) as part of the association-rich camacolaimid assemblage, as well as nematomorphs (hairworms) outside Nematoda (Schmidt-Rhaesa, 2014). These structures are not homologous across taxa. Although invasive larvae in *Marimermis* remain undiscovered, we posit them to possess a spear-like structure resembling that observed in *Thoracostomopsis*. Another noticeable character of Thoracostomopsidae consists of the pronounced, sophisticated epidermal glands in some genera. The glands in *T. barbata* have a fine fringe radial striation (Lorenzen, 1994) resembling the elaborate rosette-like epidermal organs in *Marimermis* (Rubtsov & Platonova, 1974; Tchesunov, 1997a). Although their functional load is unstudied, active levels of epidermal secretion implied by their presence are likely to be inherent in transcuticular transmission and permeability processes critical in parasitic in-host survival and feeding, and/or prerequisite to crossing environmental boundaries. Cuticular and secretion lability is considered, according to some views, as among the baseline characters that enable the spectacular potential of nematodes to conquer new niches (Holterman *et al.*, 2019).

*Aborjinia* sp. is inferred separately from *M. maritima* within Enoploidea as an internal branch of the family Leptosomatidae, in line with Zograf *et al.* (2021) and Westerman *et al.* (2021). It also shares with its relatives some elaborate glandular structures in the tail region that suggest enhanced secretion (Miljutin, 2003, 2014a) typical in marine free-living nematodes, as well as a peculiar two-celled ventral secretory-excretory gland or renette (Miljutin, 2003; Fig. 1B, 3). This organ is mono-celled in the majority of free-living and parasitic forms, with a rare two-celled deviation described in two *Leptosomatium* species (Bongers, 1983a). In contrast to *M. maritima* and its relatives, leptosomatid nematodes possess more plain, uniform morphology that is less transformed in *Aborjinia*: minute nearly papilloid sensory sensilla, normal muscular pharynx, no elaborate armature in the mouth cavity and normal cellular midgut

allowing food ingestion and alimentary feeding. This uniformity probably lies behind the misidentification of an adult *Aborjinia* specimen for *Leptosomatides* sp. in the samples by [Bik et al. \(2010\)](#) (see Results). Leptosomatids are non-predacious, large-bodied (up to 20 mm and more) forms considered giants among marine free-living nematodes. With this complex of features, Leptosomatidae is the sole enoplidan family reported to have strong associations with cnidarian anthozoans ([Hope, 1977b](#)) to the extent of true in-tissue parasitism of *A. corallicola* [Westerman et al., 2021](#) recently discovered inside the stolon body of a cold-water octocoral and placed on molecular trees ([Westerman et al., 2021](#)). The association with coral is clearly epibiotic in emergence and exemplifies a pertinent case of gradually transforming lifestyle to capture the host. Some leptosomatids are known to facultatively dwell within sponges and even feed from these hosts ([Bongers, 1983b](#)), thus revealing prerequisites to the adoption of a parasitic lifestyle. While the in-sponge environment is close to marine, its compartmentalization may predispose physiological and behavioural adaptations to facultative towards permanent cavitory parasitism.

The confident allocation of primarily marine parasitic and parasitic-like associations on nematode phylogeny demonstrates their independent emergence to allow comparison of structural and life traits in these vs. free-living and more derived groups. Permanent host capture can be observed to be attained via transient associations of various form and extent realized in dense communities transforming into epibiotic toward occupancy of internal spaces in sourcing nutrients and protection. Such communities are likely to emerge and maintain themselves temporally over the entire life cycle of the animal, predisposing infestation early in the cycle at a larval stage. The direct colonization of the gut proceeded naturally in this scenario via adaptations to strongly commensal connections with hosts. Conversely, truly exploitative associations of surviving on the host's resources emerged in an alternative evolutionary pathway. We surmise that cavitory larval host capture in a direct life cycle was a primary route of transition to true parasitism in roundworms, and extant parasitoids (larval parasites) infesting the host body cavity or internal organs realize the primary parasitic lifestyle. More complex strategies involving intermediate- or vector hosts and definitive intestinal stages can be derived via natural extension of within-lifetime capture beyond one host along contemporary environmental food chains towards untapped resources and more effective dispersal. Such transmissions have evolved in advanced nematode parasites of the trichinellid-dioctophymatid lineage in *Dorylaimia* and the historic Secernentea group in Chromadoria, especially in

spirurians and strongyloidids ([Fig. 2](#)). Nowadays these commonly exhibit in-host migration patterns of the infective larva to reach the gut destination ([Benesh et al., 2017](#)), which may be adequately explained by secondary fallout of the primary cavitory stage in the life cycle. This scenario is well illustrated with Cucullanidae, which comprise a more basal (vs. more derived spirurians and strongyloidids) secernentean lineage parasitizing a range of marine, freshwater, as well as anadromous vertebrate hosts. Thus, *Truttaedacnitis truttae* (Fabricius, 1794) ([Fig. 2](#)) of salmonid fish uses relict jawless vertebrates, lampreys, as intermediate hosts to infect trout in European freshwaters, whilst *T. pybusae* Anderson, 1992 [*T. stelmioides* (Vessichelli, 1910)] was experimentally shown to accomplish its life cycle in resident brook lamprey, avoiding the transmission to trout in North American populations ([Anderson, 1996](#)). With lamprey, infective nematode larvae hatch in the environment and get ingested by filter-feeding lamprey ammocoetes in freshwater. In riverine American trout populations with no lamprey in the food chain, *T. truttae* transmits via invertebrate first hosts (in-tissue of limnic snails, gammarid or copepod crustaceans, etc.), with the infective larvae remaining unhatched at consumption [direct experiments and PCR-based evidence in [Choudhury & Cole \(2019\)](#)]. Such a life-cycle structure suggests that the ancestral first stage of transmission in this nematode lineage was cavitory colonization of a freshwater/soil invertebrate vector host, which has later been extended on to anadromous jawless vertebrates like modern cyclostomates or extinct agnaths (e.g. Ostracodermi) that inhabited coastal freshwaters. Cucullanid parasites with a fully marine life cycle, such as *T. heterodonti* of heterodontid sharks, are thereby assumed to be derived forms that have lost the connection with anadromous vertebrates.

The hypothetic cavitory scenario gains direct support from occasional exquisite Early Devonian nematode fossils preserved in the stomatal chambers of an early land plant ([Poinar Jr, et al., 2008](#)). The finding demonstrates family clusters of worms – including eggs, juveniles and adults – in substomatal and cortical cavities, as well as interstitially inside plant tissue, with indications of on-site reproduction and perhaps a facultative semi-bacterivorous nature of association. The fossil quality allowed its putative assignment close to an extant marine subtaxon of Enoplida due to a barely changed free-living morphology. A grounded speculation is that the primary nematode body plan and physiology already provided the prerequisites for successful colonization of miscellaneous inner environments, with such transitions having been realized multiple times successfully in the phylum history. Transient associations can be facilitated at minor or no morphological adaptation, as exemplified above with strongly commensal soil

rhabditids or by the case of even stronger intestinal commensalism of the debris-inhabiting chromadorian *Domorganus* (von Thun, 1967; Valovaya, 1989), with some of its species foraging in the gut of oligochaetes in the same manner as its closest relatives in soil environments. We observe that a stylet-like armoury in the mouth cavity is often associated with evolutionarily advanced host capture conducing to true parasitism in non-related groups of both animal- and plant-parasitic nematodes. This structural feature distinguishes lineages enriched with parasitic or parasitic-like lifestyles whose associations have a non-gut (in-tissue or cavitory) nature, even at closer evolutionary scales. For instance, the diverse camacolaimid assemblage in Chromadoria unites stylet-bearing forms and includes both minute transient protozoan in-test associates and larger true animal coelomic parasites (including deep-sea benthimermithids), whilst the stylet-less sister *Domorganus* realizes a different strategy of strong intestinal commensalism (Fig. 2). Unlike this morphological precondition of true parasitism, the gigantism, strong fecundity and non-alimentary feeding (truly transcuticular or trophosome-based) typical of parasitic forms can be considered secondary adaptations to permanent host-capture, conferring more advantage in nutrient-rich but temporary habitats requiring dispersal in the life cycle. It can be speculated that roundworms may comprise unique metazoans that originated as a higher-rank lineage already pre-equipped with the necessary toolkit in morphology and physiology to enable effective expansion across environmental niches, including parasitism. We should expect and pursue particular achievements by focusing on the genomic and physiological bases of nematode parasitism to explain its higher incidence within some natural lineages uniting mostly free-living forms (e.g. Enoploidea in Enoplia reported in this study or Camacolaimidae in Chromadoria), as well as to clarify preconditions for the selective occupation of non-intestinal environments at the earliest primary stage of transmission. This line of research is also promising in terms of exploring the limits of animal phenotypic adaptation by capitalising on peculiar natural model systems of compact parasitic-free-living species complexes that begin to be discovered among the diversity of roundworms.

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#### COMPETING INTERESTS

The authors declare no competing interests.

#### DATA AVAILABILITY

Untrimmed and trimmed concatenated alignments of SSU, 5.8S and LSU rDNA (fasta) are available from the *figshare* repository (<https://doi.org/10.6084/m9.figshare.19314137>). Initial molecular data are available from the NCBI specialized repositories ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov); original Sanger and NGS data deposited as BioProject PRJNA772260); third-party contigs and original assemblies of third-party NGS data are available from *figshare* (<https://doi.org/10.6084/m9.figshare.17294216>); the data origin and accession details are provided in the [Supporting Information \(Table S1\)](#).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Figure S1.** Microdrawing of female Phanodermatidae gen. sp.

**Figure S2.** ML tree of Nematoda based on combined SSU, 5.8S and LSU rDNA data.

**Table S1.** Molecular data origin and availability.

**Table S2.** AU and c-ELW statistical tests of topologies.

**Material S1.** Supplementary material.

1. Supplementary material and methods

1.1. Material extraction, processing and free-living nematodes collection

1.2. DNA/RNA extraction and sequencing

1.3. Dataset and phylogenetic pipeline



- 1.4. Statistical ML tests of phylogenetic hypotheses
2. Biological material identification
  - 2.1. Specimen identification in *Marimermis maritima* (Fig. 1A)
  - 2.2. Specimen identification in *Aborjinia* sp. (Fig. 1B)
  - 2.3. Specimen identification in phanodermatid K2 isolate
    - 2.3.1. Female morphology of Phanodermatidae gen. sp. (Fig. S1)
3. Supplementary references