

Nematodes as evolutionary commuters between marine, freshwater and terrestrial habitats

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Nematodes are the only major metazoan group which is persistently abundant and diverse across marine, freshwater and terrestrial ecosystems. This could be the result of a few major habitat transitions followed by extensive diversification, or numerous habitat transitions followed by moderate diversification. To pinpoint habitat transitions, we superposed nematode habitat associations on an available phylum-wide phylogenetic tree based on small subunit ribosomal DNA sequences (≈ 2730 SSU rDNA sequences covering ≈ 1750 nematode taxa). Our analysis revealed at least 30 major habitat transitions within the phylum Nematoda. These transitions as well as their directionality were unevenly spread over the 12 major clades. Most transitions reside in Clades 1–6, and these transitions are bidirectional. Members of Clades 8–12 showed five full transitions, and these took place exclusively from terrestrial to marine systems. We relate our results to the distinct secretory–excretory systems in Clades 1–6 and Clades 8–12, as well as to differences in water permeability of the nematode cuticle. Hence, the phylum Nematoda is characterized by a relatively large number of habitat transitions followed by moderate diversification. The identification of multiple habitat transitions at a low taxonomic level will facilitate future investigations into the mechanisms underlying this unusual ecological flexibility.

ADDITIONAL KEYWORDS: ecological adaptability – free-living nematodes – habitat transition – phylogenetic analysis – secretory–excretory (S-E) system – SSU rDNA.

INTRODUCTION

Nematodes arose during the early Cambrian (525 Mya) in marine habitats (Rota-Stabelli *et al.*, 2013), and bacterivory is the probable ancestral feeding type (Erwin *et al.*, 2011). One question is whether the high nematode diversity and ubiquitous distribution in sediments and soils worldwide today (Heip *et al.*, 1985; Bongers & Ferris, 1999) are the result of a small number of major habitat transitions followed by extensive diversification, or the outcome of a relatively large number of evolutionary transitions followed by a lower degree of further diversification.

Generally, transitions between marine and terrestrial habitats are rare. This holds for microbes

such as bacteria, Archaea and unicellular eukaryotes. Phylogenetic overviews of these organism groups have revealed a limited number of early branching and highly biodiverse clades that harbour exclusively marine or terrestrial taxa (e.g. Logares *et al.*, 2009). It is also the case for macroscopic groups such as flowering plants (Angiosperms) that made the transition from terrestrial to marine habitats only two or three times (Dittami *et al.*, 2017). With over one million extant species, insects are another example of a highly diversified group that rarely invaded marine habitats. Only about 0.14 % of insects live in marine habitats, and, in most cases, this involves only specific life stages that inhabit estuarine, saltmarsh or mangrove habitats (Vermeij & Dudley, 2000). Within the primarily marine crustaceans and molluscs, transitions from marine to freshwater and terrestrial environments occurred

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multiple times, but transitions from land to the sea have not been documented (Vermeij & Dudley, 2000). So even for successful and highly diverse invertebrate groups, transitions from marine to terrestrial habitats and vice versa are rare, and apparently require far-reaching physiological adaptations.

Nematodes are positioned close to the base of the Ecdysozoa, a superphylum that unites Cephalorhyncha (Kinorhyncha, Priapulida, Loricifera), Nematoda, Nematomorpha, Onychophora, Tardigrada and Arthropoda. The immediate sister group of the nematodes are the Nematomorpha. Among the approximately 350 described Nematomorpha species, only five live in marine habitats (Schmidt-Rhaesa *et al.*, 2013). With 2200 extant species, the phylum Tardigrada is more diverse. A single class of Tardigrades, the Eutardigrada, harbours predominantly terrestrial species, whereas the other class, the Heterotardigrada, includes the exclusively marine order Arthrotardigrada and the partially marine Echiniscoidea (see e.g. Fujimoto *et al.*, 2017). Tardigrade phylogenies suggest a small number of splits between marine and terrestrial clades (Guil *et al.*, 2019). The other basal Ecdysozoan phyla such as Kinorhyncha, Priapulida and Loricifera are species-poor and exclusively marine, whereas the Onychophorans are exclusively terrestrial. Hence, among the basal Ecdysozoa, nematodes show exceptionally high species diversification as well as a more-or-less equal distribution over marine and terrestrial environments (Table 1).

Previously, a number of major habitat transitions were pinpointed for basal Chromadoria, a subclass within the phylum Nematoda, using 125 small subunit ribosomal DNA (SSU rDNA) sequences (Holterman *et al.*, 2008). Here, we used a substantially larger data set (≈ 2730 SSU rDNA sequences covering ≈ 1750 nematode taxa, Table S1) with fairly good phylum-wide coverage to provide insights into the distribution and directionality of major habitat transitions. We aimed to (1) pinpoint and quantify habitat transitions between marine and terrestrial environments, (2) investigate the distribution of major transition events within the phylum Nematoda, and (3) study the nature and ecological background of major habitat transitions. Based on these insights, we formulated a number of hypotheses regarding morphological and physiological attributes that are associated with the transition from marine to terrestrial habitats and vice versa.

MATERIAL AND METHODS

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

About 2730 SSU rDNA sequences (Supporting Information, Table S1) were collected and aligned in BioEdit v.5.0.9 (Hall, 1999) as described by Van

Megen *et al.* (2009). Secondary structure information from *Loricica foveata* (http://bioinformatics.psb.ugent.be/webtools/rRNA/secmodel/Lfov_SSU.html; Ben Ali *et al.*, 1999) was used as a backbone for the alignment of SSU rDNA sequences, and mfold (Zuker, 2003) was applied to refine the alignment. A maximum-likelihood tree with 1000 bootstraps was constructed using the program RAxML (Stamatakis, 2014) on the CIPRES Science Gateway cluster (Miller *et al.*, 2010). Sequence data were partitioned according to the secondary structure, and the GTRCAT substitution model was preferred over Γ because of its shorter inference times, and the slightly better Γ likelihood values of the resulting trees (Stamatakis, 2006).

NEMATODE NOMENCLATURE

Commonly 12 major clades are distinguished within the phylum Nematoda (Holterman *et al.*, 2006). Clades 1–6 and 8–12 correspond to classes formerly referred to as the ‘Adenophorea’ and ‘Secernentea’ (Chitwood, 1958), respectively (Fig. 1). Clade 7 consists of a single monogenic terrestrial nematode family, Teratocephalidae. The name ‘Adenophorea’ (=gland bearers) refers to the presence of caudal glands, whereas ‘Secernentea’ means secretors, a term used to indicate the presence of a secretory–excretory (S-E) system with lateral canals. Although they do not constitute monophyletic groups, we used the old class names ‘Adenophorea’ and ‘Secernentea’ when meaningful in the context of this paper.

MAJOR HABITAT TRANSITIONS

Major habitat transitions among nematodes are not directly observable. A neutral marker (i.e. unrelated to habitat preference) was used to generate a phylum-wide phylogenetic tree, and preferred habitats were superposed on this tree. To assess the robustness of the resulting pattern, the support for the relevant branching events was checked (bootstrap values $>70\%$ are considered to be robust). Nematode species isolated from salt marshes and tidal zones were labelled as marine. Hence, not all ‘marine nematodes’ mentioned here live permanently in submerged conditions. Notably there is only a gradual difference between terrestrial and freshwater nematodes. Nematode communities from wet terrestrial habitats are often similar to assemblages in shallow lake sediments (Traunspurger *et al.*, 2006). For this reason, transitions from terrestrial to freshwater habitats and vice versa were not scored separately. When estimating the number of habitat transitions, we operated according to the parsimony principle, using the minimum number of transitions to explain the observed data (collapsing unsupported branches). It is assumed that

Table 1. Estimated number of described terrestrial and marine nematode species

Terrestrial free-living	Terrestrial parasitic	Marine free-living	Marine parasitic	Total number of described species
7500	4000 (animal) 4100 (plant)	6900	4500 (animal) ≈10 (plant)	
Total terrestrial: 15 600 (52% of species parasitic)		Total marine: 11 400 (40% of species parasitic)		27 000

Historically more research attention has been paid to parasitic nematodes than to free-living ones, so the number of free-living nematodes in particular is presumably a substantial underestimation of the actual biodiversity. Sources: Hugot *et al.* (2001), Appeltans *et al.* (2012).

the ancestor of all nematodes was a marine species (Maggenti, 1963).

RESULTS AND DISCUSSION

A phylum-wide molecular framework comprising ≈2730 (nearly) full-length SSU rDNA sequences covering ≈1750 nematode taxa (species may be represented by multiple SSU rDNA sequences) was used to identify major habitat transitions among nematodes. Superposition of preferred habitats of nematodes on an SSU rDNA phylogenetic tree revealed at least 30 transitions between marine and terrestrial environments and vice versa (Fig. 1; for details see Supporting Information, Fig. S1). At first glance, marine nematodes appear to be reasonably well represented among ‘Adenophorea’ (Clades 1–6) and ‘Secernentea’ (Clades 8–12) (Fig. 1). However, the majority of Secernentean marine nematodes are concentrated in Clade 8, and these are parasites of marine fish, sea mammals and marine piscivorous birds. It is questionable whether these parasites can be considered as truly marine nematodes as most are exposed to marine conditions for only limited periods throughout their life cycle (Anderson, 2000). ‘True’ marine nematodes are most prevalent in Clades 1–6, whereas terrestrial nematodes dominate in Clades 8–12 (Fig. 1). Most marine free-living nematodes can be found in Clades 1, 3, 4 and 5. In contrast to Clades 8–12, animal parasitic nematodes in Clades 1–6 are mostly found in terrestrial environments with parasites of both vertebrates and invertebrates represented in Clade 2 (Dorylaimia).

Transitions are found in Clades 1, 3, 5, 6, 8, 9 and 12 (Fig. 1). The occasionally poor support for the branching pattern of the phylogenetic tree prevented an exact determination of the number of transitions. This can be illustrated by two examples: *Contracecum* spp. are parasites of marine fish, and poor backbone resolution among the Anisakidae prevents the pinpointing of a possible transition event (Supporting Information, Fig. S1). Similarly, the marine invertebrate parasites *Trophomera* and *Cynura* (Benthimermithidae, Leduc & Zhao, 2019) may have evolved from *Domorgamus* (a genus harbouring both marine and terrestrial species), but due to poor backbone tree resolution (Fig. S1) it was not scored as a transition event. For the Secernentean nematodes (Clades 8–12), 11 out of the 16 transitions involve animal parasites with limited direct exposure to the marine environment. For free-living (=non-parasitic) Secernentea, transitions were always from a terrestrial to a marine environment. For Adenophorean nematodes (Clades 1–6), a more balanced directionality of habitat transitions was found.

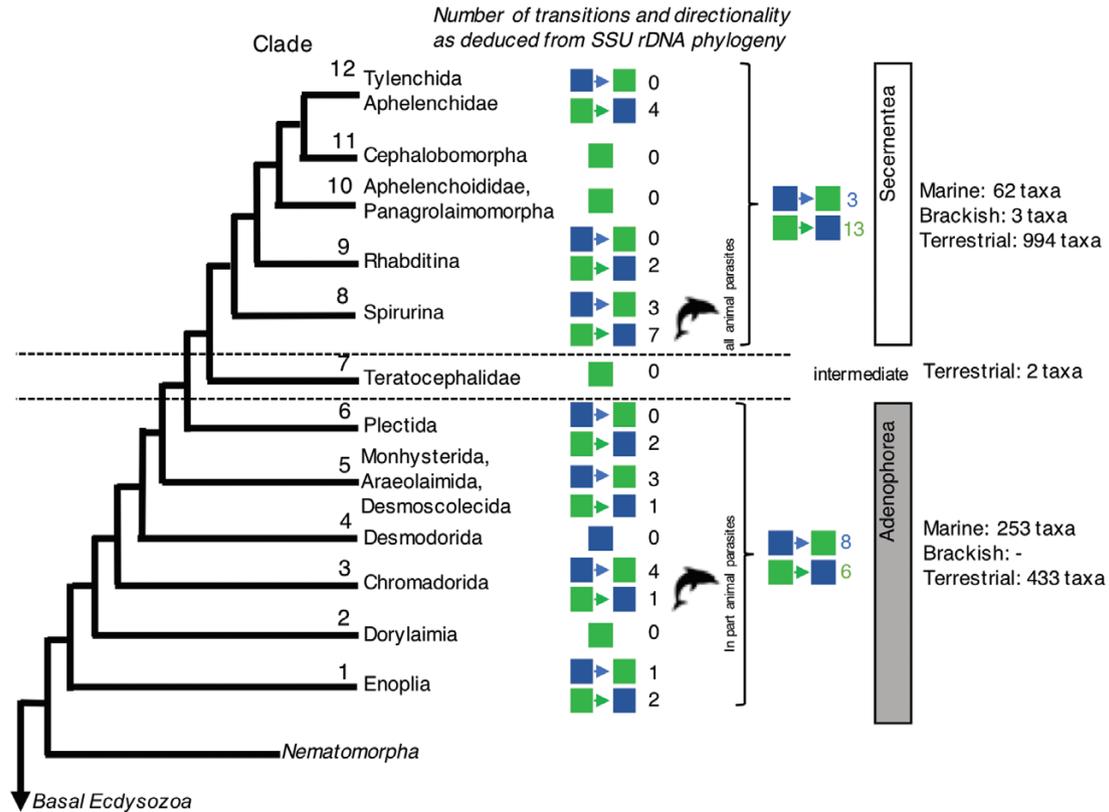


Figure 1. Distribution of major habitat transition across the 12 nematode clades. For clade numbers, we adhered to the system proposed by Holterman *et al.* (2006). Blue squares refer to marine habitat, whereas green squares represent terrestrial or freshwater habitats. Arrows between blue or green squares are used to indicate major habitat transitions. Dolphin icons refer to major habitat transitions among animal-parasitic nematodes. Note that major habitat transitions cannot be scored directly, and were rather deduced from the distribution of preferred habitats over the phylum-wide, SSU rDNA-based phylogenetic tree. The no longer current class names ‘Adenophorea’ and ‘Secernentea’ as proposed by Chitwood (1937, 1958) are included here as they appear to be remarkably appropriate in this particular context.

Habitat transitions of free-living and parasitic nematodes may occur at low taxonomic levels: within the animal parasitic genera *Philometra* and *Procamallamus* (Fig. 2A, members of Clade 8), within the genus *Theristus* (Fig. 2B, a member of Clade 5), and within one family, Ironidae (Fig. 2C, a member of Clade 1). In the sections below, we discuss the nature and ecological background of major habitat transitions for three groups of nematodes.

MAJOR HABITAT TRANSITIONS AMONG ANIMAL PARASITIC NEMATODES (CLADE 8, TRANSITIONS 10, 11, 12)

The distal part of the Philometridae tree (Fig. 2A) is dominated by parasites of marine and brackish water fish. Habitat transition 10 comprises the freshwater nematode *Philometra obturans*. It parasitizes on the gill arteries of northern pike (*Esox lucius*) (Käll *et al.*, 2004), while copepods function as intermediate hosts.

Its closest relative (residing in the same SSU rDNA-based branch (see also Černotíková *et al.*, 2011) is the ovarian parasite *Philometra floridensis*. Its principal host is the red drum (*Sciaenops ocellatus*), found along the east coast of the USA. The widespread presence of the intermediate host in both marine and freshwater habitats might have contributed to the habitat transition of *Philometra obturans*. The next transition (11) gave rise to a small cluster of parasites of gonads of marine fish, namely *Philometra lateolabracis*, *P. madai*, *P. nemipteri*, *P. saltatrix* and *P. lagocephali*. Several members of this cluster parasitize on a wide range of perciform families (Quiazon *et al.*, 2008). This cluster arose from a freshwater clade harbouring representatives of the genera *Nilonema*, *Alinema* and *Rumai*. *Nilonema* and *Rumai* are blood-feeding tissue dwellers in arapaima (*Arapaima gigas*), a freshwater fish from the Amazon delta. Cyclopid copepods are possible intermediate hosts (Santos & Moravec, 2009). It is unknown whether members of

this marine cluster have an intermediate host as well. In contrast to the Philometridae members mentioned above, habitat transition 12 comprises intestinal parasites of perciform fishes (Moravec & Justine, 2011). Species including *Procamallanus monotaxis* and *Procamallanus annulatus* have probably evolved from more basal freshwater, intestinal parasitic Camallanidae (Gagne *et al.*, 2018; Muzzall & Hessenauer, 2018). We are not aware of intermediate hosts for these intestinal parasites.

MAJOR HABITAT TRANSITIONS AMONG FREE-LIVING BACTERIVOROUS MONHYSTERIDA (CLADE 5, TRANSITIONS 20, 21, 22)

At least four major habitat transitions took place in the bacterivorous families Monhysteridae and Xyalidae (Fig. 2B). Members of the Monhysteridae have been labelled as extreme colonizers [cp 1 on the colonizer–persister scale of Bongers (1990), whereas Xyalidae were classified in cp 2]. Monhysteridae are non-selective deposit feeders in wet (freshwater or marine), nutrient-rich environments. Xyalidae are generally known as marine bacterivores (Bongers, 1994).

Seven of the 17 Monhysteridae genera inhabit marine habitats, namely *Cryonema*, *Diplolaimella*, *Diplolaimelloides*, *Halomonhystera*, *Hieminema*, *Monhystrella* and *Thalassomonhystera* (Fonseca & Decraemer, 2008). For representatives of three genera, full-length SSU rDNA data were available, and these sequences could be aligned with other, non-marine Monhysteridae. Transition 20 comprises *Diplolaimella* and *Diplolaimelloides*, nematodes that are known to contribute to the decomposition of organic material by bioturbation (Alkemade *et al.*, 1992). Members of both genera are epibenthic in coastal thalassic waters, and they are thought to feed on microbes present on the surface of sediment particles (Coomans & Eyualem-Abebe, 2006). These marine Monhysteridae probably evolved from freshwater genera such as *Monhystera*, *Eumonhystera* and *Tridentulus*. *Halomonhystera*, a genus that can be found in very high numbers in marine biotopes with high organic contents, arose independently. However, poor support for the relevant bifurcations makes it difficult to determine if the ancestor of the Monhysteridae was terrestrial and *Halomonhystera* reverted to a marine habitat, or whether the ancestor to Monhysteridae was marine and the other Monhysterids switched to a terrestrial habitat independently.

The Xyalidae genus *Theristus* is unusual as it harbours both freshwater and marine species. Whereas *Theristus agilis*, *T. athesinus* and *T. ruffoi* are examples of freshwater species (e.g. Gagarin & Naumova, 2012), most other species including *Theristus acer* are

marine bacterivores. From the marine Xyalidae, four genera have been reported from estuarine sediments at the boundary between marine and freshwater environments: *Daptonema*, *Theristus*, *Trichotheristus* and *Zygonemella*. It has been suggested that their presence in such habitats could have facilitated the transition towards freshwater habitats (Venekey *et al.*, 2014). In our analysis, *Theristus* was the only Clade 5 genus for which SSU rDNA data were available from both freshwater and marine representatives. Transition 21 comprises the putative evolution of *Theristus agilis*, a species found in freshwater and brackish water systems, from marine relatives.

All *Geomonhystera* are terrestrial bacterivorous nematodes, and they can be found on mosses, on the bark of trees or on epiphytes (Prado-Vera *et al.*, 2018). Our analysis suggests that *Geomonhystera* (transition 22) – and in fact all members of the families Monhysteridae and Xyalidae – arose from the marine family Comesomatidae. Members of the genus *Sabatieria* are found in both brackish and marine habitats. *Sabatieria pulchra* is found in the redox potential discontinuity layer, and is relatively tolerant of anoxic conditions. Moreover, this species is attracted to the root tips of *Potamogeton perfoliatus* (Jensen, 1984). These Comesomatidae and *Geomonhystera* both have loose relationships with higher plants (Prado-Vera *et al.*, 2018).

MAJOR HABITAT TRANSITIONS AMONG FREE-LIVING BACTERIVOROUS ENOPLIDA (CLADE 1, TRANSITIONS 28, 29, 30)

In our SSU rDNA-based analysis, the Trefusiidae arose within the family Trischistomatidae (Fig. 2C), and this observation corresponds with previous findings by Bik *et al.* (2010). *Tripylina* and *Trischistoma* are referred to as predators feeding on other nematodes. Although *Rhabdocoma* is often found on kelp and cordgrass (*Spartina*), it is not a plant parasite. Kelp is known to provide shelter for large numbers of nematodes, copepods, mites, polychaetes and ostracods (Arroyo *et al.*, 2004), and it seems plausible that marine *Rhabdocoma* feed on other Metazoans. Hence habitat transition 28 might have been facilitated by the similarity in trophic ecologies between the terrestrial and marine representatives in this branch.

Members of the genus *Syringolaimus* are predators living in the top layer of marine sediments (Jensen, 1992), and a number of species have been found far off the coast in abyssal ecosystems (Tchesunov, 2017). Bik *et al.* (2010) showed a sister relationship between *Syringolaimus*, and *Rhabdolaimus* and *Campydora*. Addition of more representatives of this group changed this sister relationship to one that points at *Syringolaimus* as being derived from the supposedly

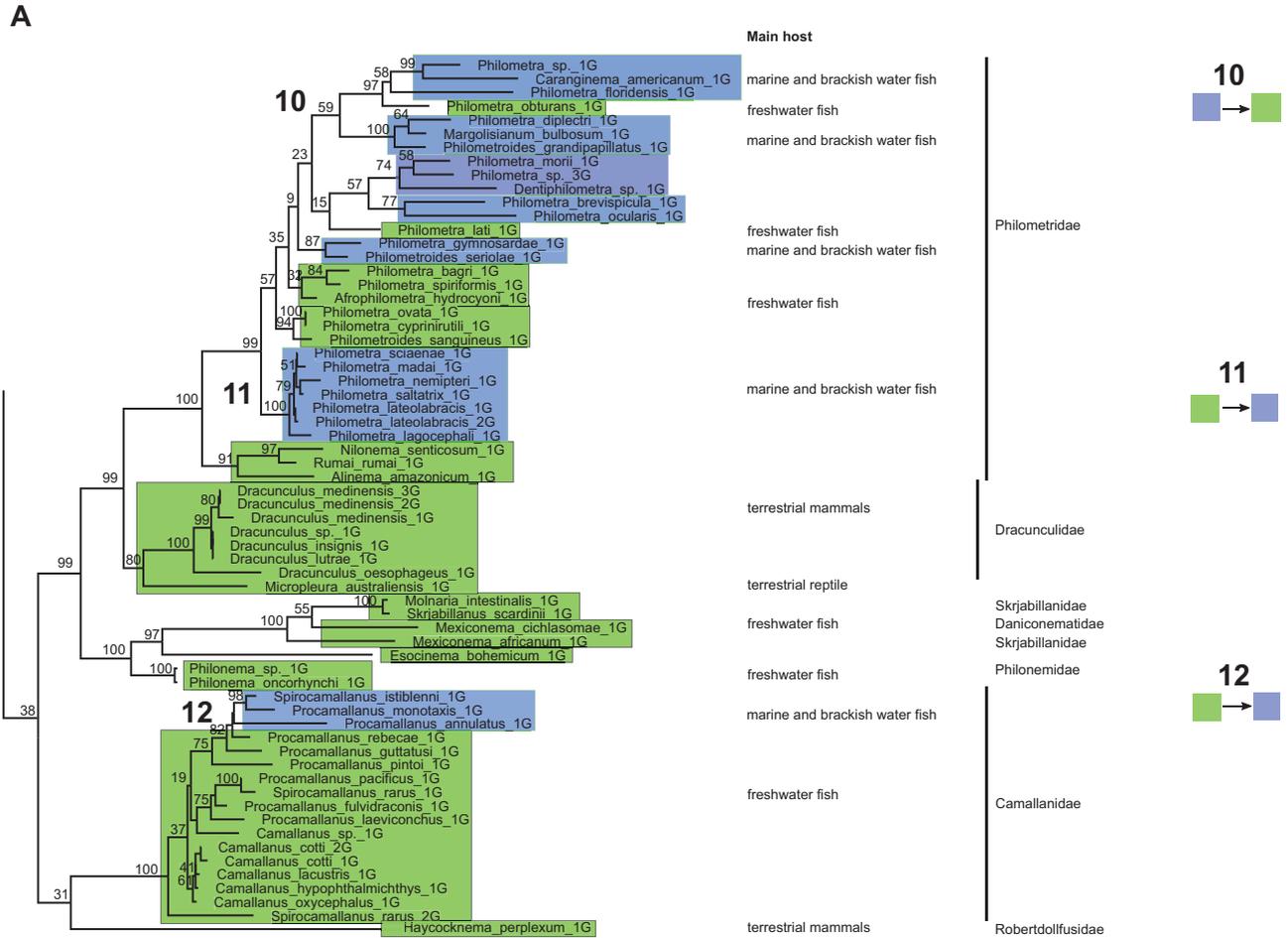


Figure 2. Excerpts from an RAxML tree based on (nearly) full-length SSU rDNA sequences from individual nematodes (for full tree see Supporting Information, Fig. S1). Next to each of the nodes, bootstrap values are given. A branching event is considered to be well supported if the bootstrap value is >70%. Colours are used to indicate terrestrial (and freshwater) nematodes (green) and marine nematodes (blue). Major habitat transitions are numbered, and these numbers correspond to the habitat transitions marked in Fig. S1. Part A focuses on a specific part of Clade 8, and includes members of the order Spirurina. B concentrates on a specific part of Clade 5, and includes members of the orders Monhysterida, Araeolaimida and Desmoscolecida. C focuses on a specific part of Clade 1, and includes members of the order Enoplida.

predacious genus *Campydora*, and bacterivorous Rhabdolaimidae (transition 29).

In transition 30, the predacious freshwater genus *Ironus* (Hunt, 1977) arose from marine genera. Based on a comparison of the anterior feeding apparatus of *Ironus*, *Dolicholaimus* and *Trissonchulus*, the current authors that all members of these three genera are predators. Hence, this habitat transition may have been facilitated by the availability of similar food (i.e. prey) in freshwater and marine environments.

GENERAL PATTERNS REGARDING MAJOR HABITAT TRANSITIONS AMONG NEMATODES

There are several ecologically relevant aspects that are associated with major habitat transitions among

nematodes. These include the common presence of an intermediate host (in the case of some animal parasitic nematodes), exposure to a mixture of marine and freshwater conditions such as in estuarine sediments, and/or preference for food sources that are present in both wet terrestrial and aquatic habitats. In the case of animal parasites, shifts from terrestrial to marine hosts and vice versa are not considered as full habitat transitions because of the very limited direct exposure of nematodes to marine conditions. If we leave out transitions that involve animal parasites, major habitat transitions among ‘Secernentea’ are relatively rare (five transitions, see also Supporting Information, Fig. S1). Isolated events were pinpointed in Clade 12 such as *Meloidogyne spartinae* (transition 1) parasitizing

B

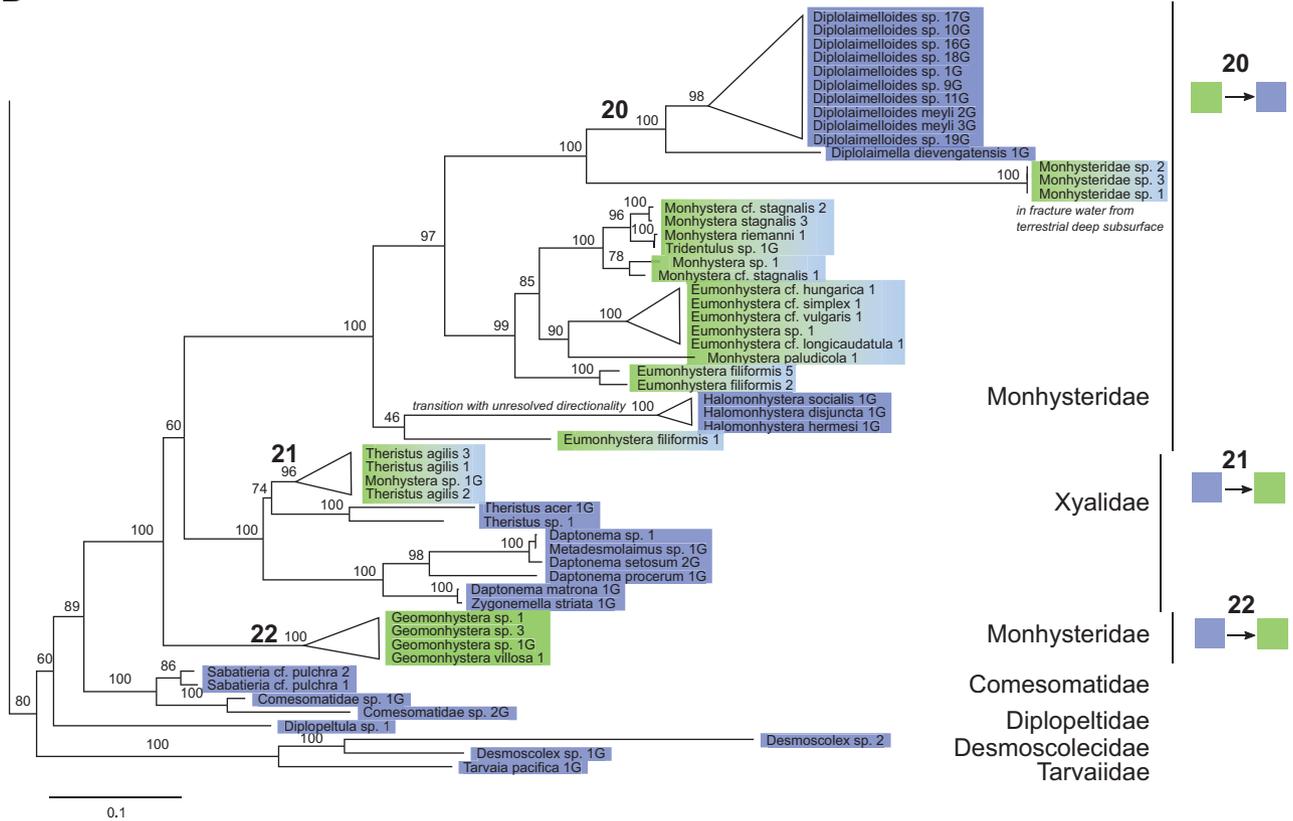


Figure 2. Continued

on saltwater cordgrass (*Spartina alterniflora*) (Elmer & LaMondia, 2014), *Meloidoderita salina* (transition 3) feeding on the roots of the halophytic plant *Atriplex portulacoides* (Ashrafi *et al.*, 2012) and *Halenchus fusicola* (transition 4) found on galls of *Ascophyllum nodosum*, a common cold-water seaweed (Siddiqi, 2000). Overall, the few full habitat transitions pinpointed among members of Clades 8–12 are unidirectional, namely transitions from terrestrial to marine habitats (Fig. S1), and led to low levels of diversification (i.e. small number of taxa arising from such transitions). A number of putative transition events in Clades 8–12 could not be verified because no DNA was available from the relevant species. This includes the marine *Aphelenchoides* species *A. gynotylurus* and *A. marinus*, species that belong to the predominantly terrestrial family Aphelenchoididae (Clade 10) (Timm & Franklin, 1969), *Filenchus marinus* (Timm, 1956), as well as *Mesocriconema maritimum*. If we hypothesize that the SSU rDNA sequences of these individual species would align among representatives of the corresponding genera, then these species would exemplify additional isolated cases of terrestrial-to-marine habitat transitions.

POSSIBLE PHYSIOLOGICAL EXPLANATIONS FOR THE ASYMMETRIC DISTRIBUTION OF TRANSITIONS OVER CLADES 1–12

The difference in the incidence of full major habitat transitions and their directionality – unidirectional and relatively rare for Clades 8–12, and bidirectional and relatively frequent for Clades 1–6 – might be attributable to two major differences between ‘Adenophorea’ and ‘Secernentea’, i.e. the functionality of the S-E system and the water permeability of their cuticle (Wharton, 1986). Most Adenophoreans have a relatively simple S-E system consisting of a single ventral gland cell, a renette, usually with a non-cuticularized terminal duct (for a few groups the S-E system might be absent). Secernenteans have a derived S-E system, namely a tubular H-shaped system with a cuticle-lined duct. In the past, the structural variability of the S-E system has been used as a key feature defining a class-level subdivision of the phylum Nematoda (Decraemer *et al.*, 2014). The more elaborate S-E system in Secernenteans is mainly involved in osmotic regulation, but it might also serve a secretory function similar to that in Adenophoreans. These glands synthesize and secrete glycoproteins that coat the cuticle surface, and this coating is

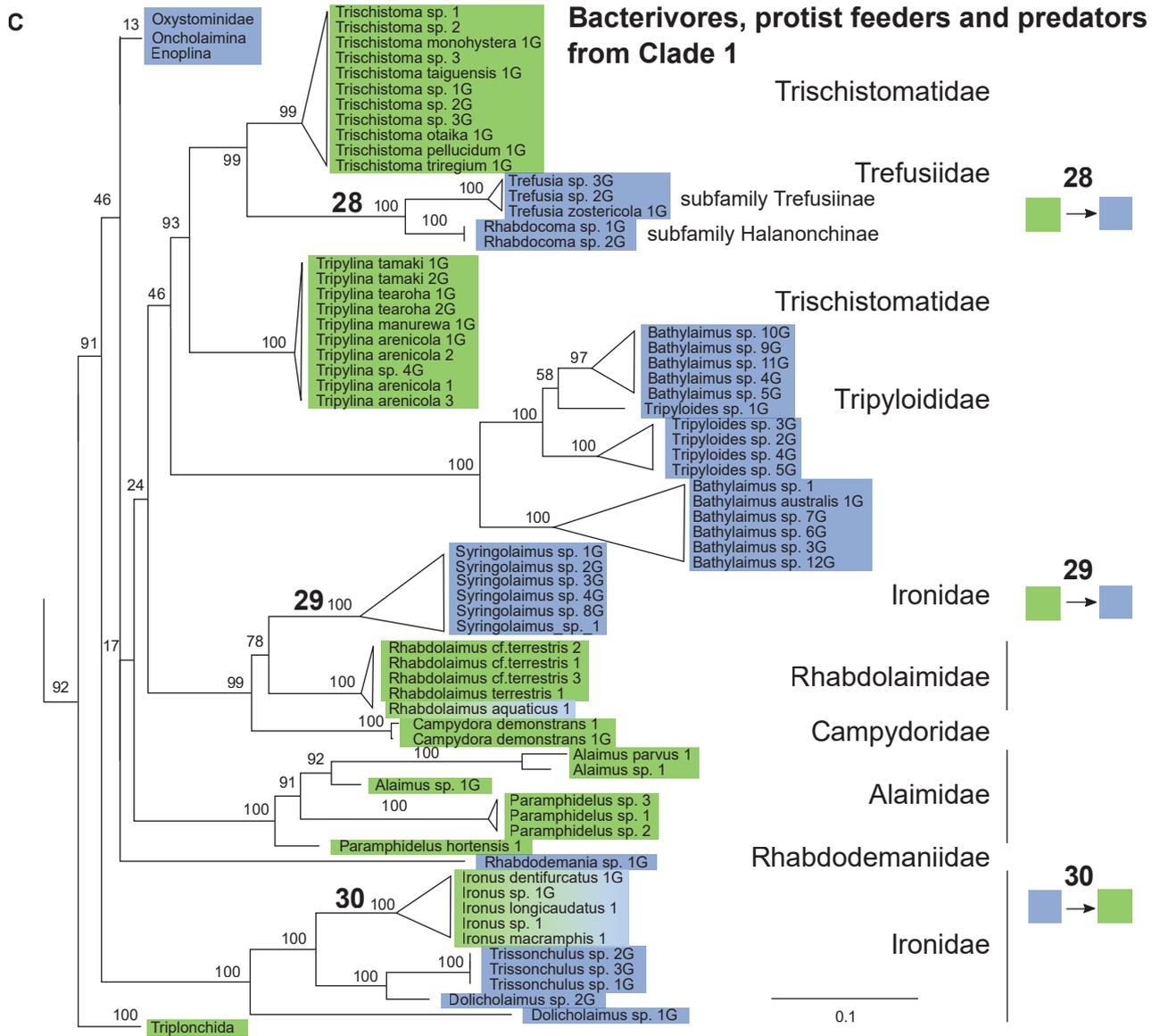


Figure 2. Continued

thought to act as a lubricant to assist movement of free-living nematodes (Bird & Bird, 1991). Secretions from the Adenophorean S-E system have also been demonstrated to assist marine nematodes in attaching themselves to sediment particles as well as playing a role in tube-building (van de Velde & Coomans, 1987; Turpeenniemi & Hyvärinen, 1996).

The higher complexity of the S-E system in Secernenteans might relate to the relatively low permeability of the cuticle to water as compared to the Adenophoreans (Wharton, 1986). We might therefore hypothesize that the low water permeability of the cuticle of free-living Secernenteans has reduced their flexibility to migrate between terrestrial and marine conditions. Conversely, free-living Adenophoreans with

their readily water-permeable cuticle and their relatively simple single-celled S-E system show greater ecological flexibility. This is confirmed by Lee (1961) and Nkem *et al.* (2006) who reported high osmotic stress survival rates of some Adenophorean species despite their simple S-E systems and limited osmoregulatory capacity.

Dorylaimida, a highly speciose order residing in Clade 2, has often been labelled as fully terrestrial (Blaxter & Koutsovoulos, 2015). However, a few exceptions have been described including *Halodorylaimus marinus* and *H. micramphis* (Andrássy, 1988), and well as the *Eudorylaimus* species *E. maritimus* (Ditlevsen, 1913). No amplifiable DNA was available from these species and, hence, we could not confirm these potential terrestrial-to-marine habitat transitions.

CONCLUSIONS AND HYPOTHESES FOR FUTURE RESEARCH

For most micro- and macro-organismal groups present in both terrestrial and marine habitats such as bacteria, Archaea and molluscs, phylogenetic analyses have revealed large and diversified habitat-specific clusters, suggesting a small number of major habitat transitions (Vermeij & Dudley, 2000; Logares *et al.*, 2009). Among the currently described nematode species, 58% are terrestrial and 42% marine (Table 1). Here we superposed the habitat preferences of nematodes on a large, phylum-wide phylogenetic tree. A conservative analysis revealed 30 transitions, which may take place at a very low taxonomic level: genera such as *Philometra*, *Theristus* and *Procamallanus* harbour both marine and terrestrial–freshwater species (Fig. 2A, 2B).

Notably, major habitat transitions and their directionality are not evenly distributed across the phylum Nematoda. Differences in both the frequency and the directionality of these transitions co-vary with a major subdivision of the phylum Nematoda in Clades 1–6 ('Adenophorea') and Clades 8–12 ('Secernentea'). This subdivision correlates with the distribution of two major types of S-E systems, as well as with differences in water permeability of the cuticle. We hypothesize a causal relationship between these two attributes and the frequency and directionality of major habitat transitions. However, both the functioning and the diversification of the two S-E systems, and differences in water permeability of the cuticle among nematode clades, remain poorly studied. Our findings provide a starting point for future studies aimed at identifying the mechanisms that determine the ecological flexibility of nematodes. The marine plant-parasitic genus *Halenchus* (*H. fucicola* in Supporting Information, Fig. S1, transition 4) presents a useful model organism. This genus arose within the terrestrial family Anguinidae, and genomes of two closely related family members were recently sequenced, *Ditylenchus destructor* (Zheng *et al.*, 2016) and *Ditylenchus dipsaci* (J. Helder, unpublished data). Given that biological material is scarce, an RNA-Seq-based characterization of mixed stages of this marine brown alga parasite could be most informative. Comparison with the predicted transcriptome of these two *Ditylenchus* species could reveal physiological changes that were realized as a result of this habitat transition.

Litoditis (previously *Pellioiditis*) *marina* (Derycke *et al.*, 2005), a member of the speciose bacterivorous family Rhabditidae (Supporting Information, Fig. S1, transition 6), could prove equally useful. The transcriptome of mixed stages of this species could be compared with the transcriptome of *Caenorhabditis elegans* or *C. briggsae*, two very well-characterized

members of this family. Here too, comparisons may lead to the identification of pathways that enabled the transition of terrestrial bacterivores to marine and brackish water environments.

Our superposition of habitat preferences on a phylum-wide phylogenetic tree of nematodes revealed a relatively large number of transitions unevenly spread over the individual major clades. This research revealed a few major habitat transitions at low taxonomic levels, i.e. within a genus or a family. This will greatly facilitate the unravelling of the mechanisms that underlie the remarkable ecological flexibility of members of the phylum Nematoda.

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SUPPORTING INFORMATION

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

Fig. S1. RAxML tree based on (nearly) full-length SSU rDNA sequences from 2730 individual nematodes. Branching events are considered to be well supported if the bootstrap value is >70%. Colours are used to indicate habitat: terrestrial (and freshwater) nematodes are labelled with a green background, marine nematodes with a blue background.