

EVOLUTION OF PLANT PARASITISM AMONG NEMATODES

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Key Words Aphelenchida, convergent evolution, Dorylaimida, phylogeny, Triplonchida, Tylenchida

■ **Abstract** Despite extraordinary diversity of free-living species, a comparatively small fraction of nematodes are parasites of plants. These parasites represent at least three disparate clades in the nematode tree of life, as inferred from rRNA sequences. Plant parasites share functional similarities regarding feeding, but many similarities in feeding structures result from convergent evolution and have fundamentally different developmental origins. Although Tylenchida rRNA phylogenies are not fully resolved, they strongly support convergent evolution of sedentary endoparasitism and plant nurse cells in cyst and root-knot nematodes. This result has critical implications for using model systems and genomics to identify and characterize parasitism genes for representatives of this clade. Phylogenetic studies reveal that plant parasites have rich and complex evolutionary histories that involve multiple transitions to plant parasitism and the possible use of genes obtained by horizontal transfer from prokaryotes. Developing a fuller understanding of plant parasitism will require integrating more comprehensive and resolved phylogenies with appropriate choices of model organisms and comparative evolutionary methods.

INTRODUCTION

Nematodes are of relatively ancient origin, probably arising in the early Cambrian (4, 79) although some investigators have suggested they may be nearly twice that age (103). Free-living nematodes, ubiquitous in terrestrial and aquatic environments, have evolved parasitic relationships with other eukaryotes on

several independent occasions (15, 16), leading to the suggestion that the nematode bauplan has a proclivity for parasitism.

Current molecular and paleontological evidence suggests that land plants originated between 425 and 490 mya (81), indicating that plants and terrestrial nematodes have coexisted in the earth's soils for an extensive period of time, and given this ecological resource it is unremarkable that the parasitism of plants by nematodes evolved independently and at different times during evolutionary history.

Although nematodes are the most abundant multicellular animals on earth, their contribution to global biodiversity is virtually unknown (18). Even if algorithms used to estimate other invertebrate diversity (61) result in an overestimate (by several orders of magnitude) when applied to nematode species diversity, there is still no other metazoan phylum with a greater disparity between estimated biodiversity and the number of taxonomists working to understand this diversity. However, based on current knowledge of nematode systematics, including abundant morphological, physiological, and ecological diversity among members of the phylum, a comparatively small fraction of nematodes are parasites of animals or plants. Most nematode diversity is represented by species that are free-living in freshwater, marine, or soil ecosystems.

Given the wide ecological spectrum of nematode associations with other organisms, partitioning nematode diversity into free-living and parasitic groups can be problematic, and definitions describing which ecological, life history, physiological, or morphological attributes of the nematode make it a plant parasite have been challenged (55, 70, 107, 108). For example, many nematodes considered to be plant parasites have numerous morphological and physiological traits specific to feeding on plant parts in the rhizosphere, yet cause no discernable pathology in the plant. How is this different from megafauna that feed on the aboveground parts of the plant, for example, grazing herbivores? However, for purposes of this review, we address plant parasitism as broadly and inclusively as possible, and represent all nematodes that feed on plants during a portion of their life cycle as "plant parasites."

Several hypotheses concerning the origin of plant parasitism by nematodes have been promoted, but there is little agreement among them. For example, Maggenti (66) posited that plant parasitism was established independently three times in the Pennsylvanian period by dorylaimids (Ironidae), aphelenchids (Aphelenchoidea), and tylenchids (Tylenchoidea), concomitant with the rise of gymnosperms approximately 310 million years before present (MYBP). From Maggenti's narrative, it is difficult to interpret whether he envisioned the establishment of successive groups of plant parasites as independent events, but plant-parasitic dorylaimids (Dorylaimoidea) were hypothesized to have arisen in the Triassic (230 MYBP), followed by neotylenchids and diptherophoroids (Neotylenchidae, Diptherophoroidea/*Trichodorus*) in the Jurassic (181 MYBP). Maggenti (66) also depicted the major radiation of the tylenchids (Tylenchida) as occurring in the Jurassic, followed by longidorids (*Xiphinema* + *Longidorus*) in the Cretaceous (135 MYBP), when the major radiation of angiosperms began.

Maggenti's hypotheses can be contrasted with Poinar's (74), who suggested a more serial, and earlier, origin of plant parasitism. Poinar infers five independent events, with parasitism accomplished first by the Aphelenchida in the Devonian (375 MYBP), followed by Neotylenchoidea in the Carboniferous (300 MYBP), Tylenchida in the Permian (223 MYBP), Longidoridae in the Triassic (185 MYBP), and Trichodoroidea in the Jurassic (160 MYBP).

Siddiqi (87) suggested that in the Silurian period (420 MYBP) it was the dorylaimids that first developed the protrusible stylet required for plant parasitism, but that parasitism of plants occurred later. Siddiqi also advocated a Cretaceous origin of the diphtherophoroids (Diphtherophorina; in contrast to Maggenti's Jurassic origin) and posits a Devonian genesis of the Secernentean stylet from a cephalobid-oxyurid ancestor. Independently, but also during the Devonian, Siddiqi suggested that a diplogasterid ancestor gave rise to modern aphelenchids. Both Maggenti (66) and Siddiqi (87) provide extended narratives concerning possible evolutionary scenarios for higher taxa. However, the preceding authors (Maggenti, Poinar, and Siddiqi) acknowledge that their hypotheses are hunches made in the absence of paleontological evidence [the earliest nematode fossil is known from Cretaceous amber (73)]. With the advent of modern analytical tools for peering ever more carefully into morphological and genetic legacies of extant plant-parasitic nematodes, many long-standing questions concerning the origin and maintenance of plant parasitism are now poised for new levels of resolution.

MULTIPLE ORIGINS OF PLANT PARASITISM

Dorylaimida, Triplonchida, Tylenchida

Plant parasitism is polyphyletic, an acquired lifestyle evolved independently in each of three major clades in the phylum (15, 28, 36), and among three traditional nematode orders, Dorylaimida Pearse 1942, Triplonchida Cobb 1920, and Tylenchida Thorne 1949 (Secernentea) (Figure 1). In some cases, and specifically in Tylenchida, ordinal status obscures that these plant parasites have evolved within clades that include many free-living nematodes (16).

Convergence of plant parasitism between non-Secernentea (e.g., Adenophorea) and Secernentea long has been inferred by phylogenetic interpretation of morphology (23). More recent insight from molecular phylogenies based on the nuclear small subunit ribosomal RNA (SSU rRNA; Figure 1) underscores that non-Secernentean Dorylaimida and Triplonchida lack a unique common ancestor (15). Secernentean Tylenchida, which includes the largest number of described plant parasites, shares a most recent common ancestor with Cephalobina (primarily bacteriovore Cephalobioidea), whereas Triplonchida is the sister clade to the Enoplida Filipjev, 1929 (primarily marine omnivores and bacteriovores), and Dorylaimida are embedded within a currently unresolved clade that includes insect parasites (Mermithida), vertebrate parasites (Trichinellida), and predators (Mononchida,

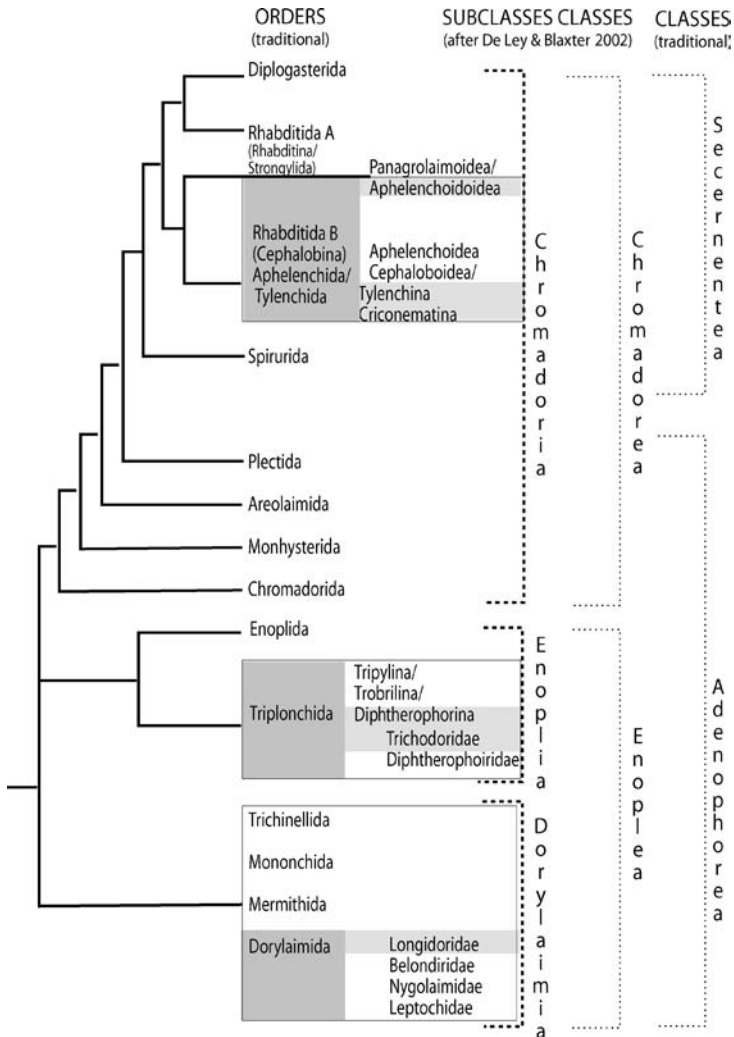


Figure 1 A broad overview of phylogeny of Nematoda based on small subunit ribosomal RNA (15, 28). Clades in which plant parasitism occurs are depicted by shaded boxes.

many Dorylaimida). Since only a few representatives of most orders have been considered in deep-level phylogenies, monophyly of Dorylaimida, Triplonchida, and Tylenchida is not yet well substantiated. In addition to plant parasitism, each order encompasses a range of other trophic specializations. It cannot be assumed that plant parasitism arose only once in each order.

Tylenchida and Aphelenchida

The notion that Tylenchida Thorne, 1949 including aphelenchids comprise a single clade based on inclusion of all stylet-bearing Secernentea (94) was disputed by Siddiqi (86, 87) in separating Aphelenchida as an order distinct from Tylenchida. Siddiqi (85) argued that the stylet (and therefore plant parasitism) arose independently in Aphelenchida (from diplogasterid-like ancestors) and Tylenchida (from cephalobid/oxyurid-like ancestors) (87). Phylogenies based on SSU rRNA (but with limited taxon sampling) suggest that Aphelenchida is polyphyletic, with the fungus-feeder *Aphelenchus* (Aphelenchoidea, a superfamily with few species and putatively no plant parasites) resolved within a Tylenchida/Cephaloboidea clade whereas the plant parasite, *Bursaphelenchus* (Aphelenchoideoidea, a superfamily with many species including plant parasites), is resolved within a distinct clade sharing more recent common ancestry with representative Panagrolaimoidea (Figure 1) (15, 33). Thus, phylogenies based on SSU rRNA, although preliminary, suggest that Aphelenchida may be polyphyletic, and that within Secernentea, plant parasitism evolved at least twice, once within Aphelenchida (Aphelenchoideoidea) and separately within Tylenchida.

MORPHOLOGICAL SPECIALIZATIONS LINKED TO PLANT PARASITISM

Similar morphological characteristics, rather than reflecting a shared evolutionary history and monophyly, may conversely result from shared functional selection pressures on independent evolutionary lineages (6, 110). Among nematodes, exploiting plants as food always is associated with a needle-like protrusible stylet that withdraws cytoplasm; this stylet often functions in conjunction with specialization and enlargement of pharyngeal glands (e.g., the capacity for extracorporeal digestion) relative to reduced pharyngeal muscular development (see below). Repeated independent evolution of a stylet in nematodes suggests functional and perhaps developmental constraints on alternative mechanisms to feeding on plants. Yet despite their general functional similarities, the stylets associated with plant parasites representing Tylenchida, Aphelenchida, Dorylaimida, and Triplonchida differ fundamentally in underlying homologies, development, and evolutionary history.

Stylet

Unlike Tylenchida and Aphelenchida, not all Dorylaimida or Triplonchida have a stylet, yet all Dorylaimida have a tooth. In the relatively small subset of Dorylaimida specialized for plant parasitism the tooth is expressed as a stylet (odontostylet). Variation in expression of feeding apparatus within Dorylaimida provides a point of comparison to interpret homologies including specializations specific to plant parasitism as well as putative stylet morphoclines suggestive of evolutionary patterns (87). In some predatory Dorylaimida the tooth, positioned

subventrally in the stoma, lacks a lumen; when prey are punctured, liquid food passes along the tooth (23). In other Dorylaimida, including plant parasites, the tooth fills the stoma and food passes through a tooth lumen; this lumen is derived from a longitudinal infolding on the tooth, evident as a dorsal seam (77, 105). In the stylet of a plant-parasitic dorylaimid, the anterior end (odontostyl) is derived from a hollow tooth (odontia) that is supported posteriorly by the odontophore. The odontophore is a specialization of, and continuous with, the cuticle-lined pharyngeal lumen; the lumen is continuous with that of the tooth (23, 104). The dorylaimid stylet comprised of odontostyle and odontophore protracts and retracts respectively by sets of eight and four muscles (77, 93, 104, 105).

The feeding apparatus of Triplonchida includes a simple open stoma characteristic of microbivores, a stoma lined with complex sets of stomal plates of yet unknown function, and stylet-like onchiostyles adapted for plant parasitism. The onchiostyle differs in structure and ontogeny from the odontostyle of Dorylaimida and the stomatostyle of Tylenchida and Aphelenchida (51). The curved onchiostyle is a modified solid dorsal tooth that extends from the wall of the anterior end of the pharynx; posteriorly it intersects with an extension to which protractor muscles attach. As a puncturing tool, the onchiostyle lacks a lumen; food passes from the puncture directly into the stoma that surrounds the stylet (51). Within Triplonchida, resolving questions of homologies and evolution of feeding structures specialized for plant parasitism relative to microbivorous members of this order must await more detailed phylogenetic hypotheses (28).

We have noted that presence of a stylet (stomatostylet) in Tylenchida and Aphelenchida is the primary morphological basis to propose a unique shared history (2, 65, 67). However, fine structural studies, although not yet designed to resolve homologies of the Aphelenchida versus Tylenchida stylet, nevertheless demonstrate that “. . . the stylet apparatus in *Aphelenchoides*. . . is very different from that described for species of (Tylenchida)” (84, p. 325); specifically, the stylet differs in structure of the shaft, the number and orientation of protractors, and innervations (49, 84). Although it has been suggested that the protractor muscle arrangement in these representatives of aphelenchids is primitive relative to that of tylenchids (84), there is not sufficient context to interpret either homology or character polarity of these and other key stylet components for plant parasitism. Because of these limitations, as presently understood, differences in stylet morphology are insufficient to resolve relationships between Tylenchida and Aphelenchida, or to address relationships among Aphelenchida.

We observed that Dorylaimida and Triplonchida include nonplant parasites and nonstylet-bearing taxa with morphologies that are consistent with a morphocline of feeding structures and invite investigations into specializations linked to plant parasitism. Within Secernentea, a morphocline of the stoma was proposed (Figure 2), suggesting evolution of the stomatostyle of Tylenchida (2, 3, 65). In this series, the stomatostyle was hypothesized to have evolved from the complex stomas of a series of Diplogasterida including *Tylopharynx*, but De Ley et al. (29) demonstrated that presumed homologies between the stomas of *Tylopharynx* and

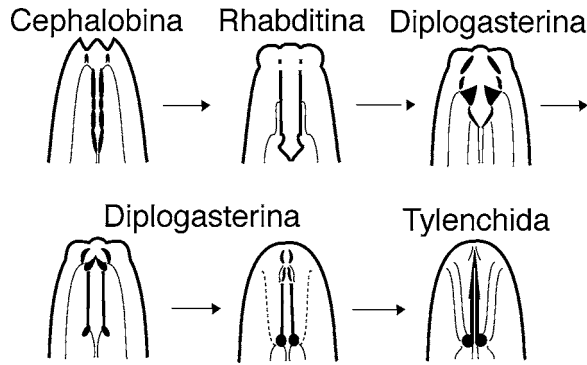


Figure 2 Morphocline of the stomas of Secernentea suggesting a historical view of evolution of the stylet of Tylenchida [redrawn from Andrassy (2)].

Tylenchida were untenable, a discovery that is consistent with the SSU rRNA phylogeny that rejects a previously broadly held Diplogasterida/Tylenchida clade (2, 47, 65, 90, 94). One of the puzzles of phylogenetic trees placing Tylenchida as a sister of Cephaloobioidea is the implication for stylet evolution, considering the nonstylet-like open stoma adapted for microbivory in most Cephaloobioidea and apparently the absence of morphological transformations that might provide a starting point for interpreting homologies of feeding structures and the evolution of some tools of plant parasitism: e.g., a stylet and highly glandular basal bulb.

Homologies of feeding structures in Secernentea have been the topic of considerable comparative morphology (7, 8, 30, 32, 34, 95, 97), but generally the more detailed comparative studies have not been specifically extended to Tylenchida and particular adaptations associated with plant parasitism. Although highly variable among Secernentea, the basic stoma pattern includes three chambers: anteriorly a cheilostom associated with hypodermis, followed by a gymnostom surrounded by two arcade syncytia, and posteriorly a stegostom that is enclosed by the anterior end of the pharynx (Figure 3) (8, 30, 34). This stoma-associated region of the pharynx typically includes an anterior-posterior stack of four sets of radial cells. The radial cells of each set are positioned dorsally and subventrally and each set is interrupted by a marginal cell in the ventral and subdorsal positions. The radial cells may be expressed as epithelia or muscle. In Diplogasterina and Rhabditina, the anterior two sets of radial cells are epithelial whereas in Cephalobina and outgroups all four sets are muscular (Figure 3) (8, 34). Molecular phylogenies do not support relationships consistent with a morphocline from diplogasterids to the tylenchid stylet, and in the absence of obvious morphological intermediates, homologies and possible character transformations of the open stoma of Cephalobina to the specialized stylet of Tylenchida/Aphelenchida remain unclear. Re-examination of

previous (9, 20, 39) and unpublished micrographs suggests that the hexaradiate cephalic framework of Tylenchida is surrounded by hypodermal tissue, equivalent to the cheilostom of other Secernentea including Cephalobina. Furthermore, the vestibule extension and cone are enclosed by syncytial tissue that appears to be homologous with the arcade syncytia, equivalent to the gymnostom of Cephalobina (Figure 3). It is noteworthy that it is the portion of the stylet (i.e., cone) and stoma lining (i.e., cephalic framework, vestibule, and vestibule extension) associated with this region that is shed during molts, whereas the pharynx apparently reabsorbs and then reconstitutes the stylet shaft and knobs, during molts (80). Between two arcade syncytia, junctional complexes (=guide ring) form with gymnostom near the junction with the pharyngeal tissue (stegostom) surrounding the shaft and stylet knobs. Three stylet protractors extend as anteriorly directed protrusions of the pharynx and at their posterior end are equivalent in position to interradiar radial cells of Cephaloidea (Figure 3). Narrow processes of marginal cells occur ventrally and subdorsally between the protractors (J.G. Baldwin, unpublished). It is not clear what underlying cells produce specific stylet components, and indeed one difficulty of making this interpretation is that elaborations of the stoma lining, including teeth and probably stylets, may project far beyond their point of attachment, and in adults the underlying cellular relationship and presumed point of developmental origin may be obscured (8). Attempts to follow cell lineages to the point of embryonic formation of the stylet have been unsuccessful, because for those Tylenchida where it has been attempted, embryos are generally too optically dense for successful 4D microscopy (32; C.M. Dolinski, personal communication). Alternately, postembryonic development of the stylet relative to development in Cephaloidea may prove to be significant in determining homologies and interpreting stylet evolution. Indeed, new comparative tools including specific stains, confocal and electron tomography could be particularly valuable in addressing these issues and are likely to provide new characters for mapping on phylogenies that more clearly resolve the precise cephalobid sister taxa of tylenchids. Notable cephalobid candidates include *Medibulla* Siddiqi, 1993; this, like several tropical cephalobid species, has an elongate narrow stoma. Furthermore, the pharynx of *Medibulla* has a prominent median bulb and glandular basal bulb, reminiscent of Tylenchida.

Pharynx

The stylet is a key adaptation associated with plant parasitism but this structure cannot be considered apart from evolutionary specializations of the pharynx. In contrast to plant parasites, microbivores, often essentially filter feeders (35), require a pharynx with considerable muscular pumping power; this is usually expressed as a triradiate lumen dilated by radial muscles (1, 110, 111). Similarly, predators with large open stomas armed with teeth may depend on mechanical breakdown of food and pharyngeal peristalsis and this is reflected in the muscular structure of the pharynx (21). Plant parasites, however, and particularly those feeding through

a small stylet orifice, may have relatively reduced pumping requirements; often this is expressed as more extensive and specific development of pharyngeal glands and reduction and concentration of pumping musculature. Gland products for plant parasitism may include the capacity to extracorporeally liquefy cytoplasm, penetrate plant tissue, and induce and maintain specialized modifications of host feeding sites (37, 52) (see below).

Whereas the pharynx of predatory Mononchida are almost completely muscular, the pharynx of Dorylaimida, including plant-parasitic Longidoridae, comprises a narrow nonmuscular anterior region with a circular lumen and broad muscular posterior region with a triradiate lumen. The posterior region encloses four or five glands (51); through ducts, these glands open into the cuticle-lined pharynx lumen; in this case, glands are not known to differ in size or morphology relative to nonplant parasite outgroups. The pharynx of Triplonchida has a narrow but muscular anterior region and broader nonmuscular posterior expansion that includes five pharyngeal glands (50, 51).

Adaptations of the pharynx associated with plant parasitism, developed based on comparison with nonparasitic Secernentea, have been most thoroughly investigated in the Tylenchida. Typically, Secernentea have a three-part pharynx including a corpus, isthmus, and basal bulb; often the corpus is comprised of a distinct procorpus and metacarpus, and the basal bulb includes a heavily cuticularized grinder that functions in mechanically crushing microorganisms. Three to five glands are embedded among muscles in the basal bulb, typically the dorsal gland opening is in the procorpus or stegostom, and a pair of subventral glands open into the metacarpus. Where present, a second pair of subventral glands typically open within the grinder (1, 110). Ultrastructural analysis of representatives indicates that the basal bulb of Rhabditina and Cephalobina includes four sets of radial muscle cells, whereas in Diplogasterida, radial muscles are reduced to the anterior two sets in conjunction with loss of the grinder (1, 109–111).

In many agricultural plant parasites representing Tylenchida and Aphelenchida, the pharyngeal basal bulb (or a lobe of the bulb) is almost completely comprised of glands and lacks muscle (5, 10, 38, 54, 58, 83, 84, 89). In part, it is the lack of a muscular grinder and prominence of glands in the basal bulb that previously contributed to the argument for Diplogasterida and Tylenchida as sister taxa (65). More recently, detailed investigations of the basal bulb of *Basiria*, putatively a representative of the most morphologically conserved members of the Tylenchida (11), revealed that although greatly reduced relative to the glands, a full complement of muscle cells was present, and they are identical in number and position to cells and nuclei in the Cephaloidea. Whereas these similarities may be symplesiomorphies, the significance is support for the hypothesis that loss of musculature in the basal bulb occurred within certain Tylenchida rather than basal to a putative Tylenchida/Diplogasterida clade; in this regard, the morphological findings are consistent with the alternative validated by SSU rRNA gene phylogeny that the Tylenchida/plant parasitism arose within Cephaloidea.

EXPLOITING THE PARASITIC LIFESTYLE

Convergent Specializations of Feeding Styles

Specializations in plant-parasitic lifestyles include migratory ectoparasitism and burrowing endoparasitism as well as various types of independently evolved (see below) sedentary ecto- and endoparasitism and a full range of intermediates. These lifestyles relate to divergent parasite-specific host reactions and in each case the major pathology is the result of secretion products of pharyngeal glands (see section on Genetic and Biochemical Aspects of Evolution) injected into the host cell (52). Sedentary parasites are associated with modifying and regulating host cell function to yield feeding sites acting as a metabolic sink and sustaining the parasite through its life. These feeding sites include various types of nurse cells (56, 68, 69), often specific to the nematode species. Nurse cells include single uninucleate giant cells as in *Sarisodera* sp., some *Meloidodera* (Heteroderinae) and *Rotylenchulus* sp. (Hoplolaimidae), multinucleate giant cells as in *Meloidogyne* or multinucleate syncytia as in many other Heteroderidae, and some other *Rotylenchulus* (22, 68, 69, 92).

Host responses specific to particular nematode taxa have proven useful as characters in phylogenetic analysis of Heteroderinae (6), but a broader understanding of the evolution of the mode and direction of plant parasitism has been largely speculative. For example, a common perspective is that feeding sites among sedentary root-knot and cyst nematodes reflect the most elaborate and putative derived adaptations known among plant parasites (26), a hypothesis testable in the context of phylogenetic trees. While acknowledging the limitations of preliminary data (taxon sampling, alignment issues, information content), some clades of plant parasites emerge with enough support to allow us to address several taxonomic and evolutionary hypotheses with a modicum of confidence (Figure 4). Emerging understanding of the patterns of these clades also provides a framework for mapping modes of parasitism, including the evolution of sedentary endoparasitism.

Well-resolved portions of trees of Tylenchida based on the SSU rRNA gene, uphold both root-knot (*Meloidogyninae* Skarbilovich, 1959) nematodes and cyst (plus closely related noncyst) nematodes (*Heteroderinae* Filipjev & Schuurmans Stekhoven, 1941) as monophyletic clades (Figure 4). They reject, however, a widely held view that these two groups of sedentary endoparasites collectively share a unique common ancestor or even that they are "closely related" within Tylenchida. This view is reflected in classifications that include subfamilies Heteroderinae and *Meloidogyninae* within family Heteroderidae Filipjev & Schuurmans Stekhoven, 1941 (63, 65). Rather, well-supported aspects of the analyses uphold an alternate hypotheses that Heteroderinae share a clade with Hoplolaimidae Filipjev, 1934 (60, 102; for review see 6) and affirm that evolution of the capacity to induce complex multinucleate host nurse cells by Heteroderinae and *Meloidogyne* is convergent. With rapidly developing understanding of genes and pathways for induction and maintenance of host nurse cells, faulty assumptions

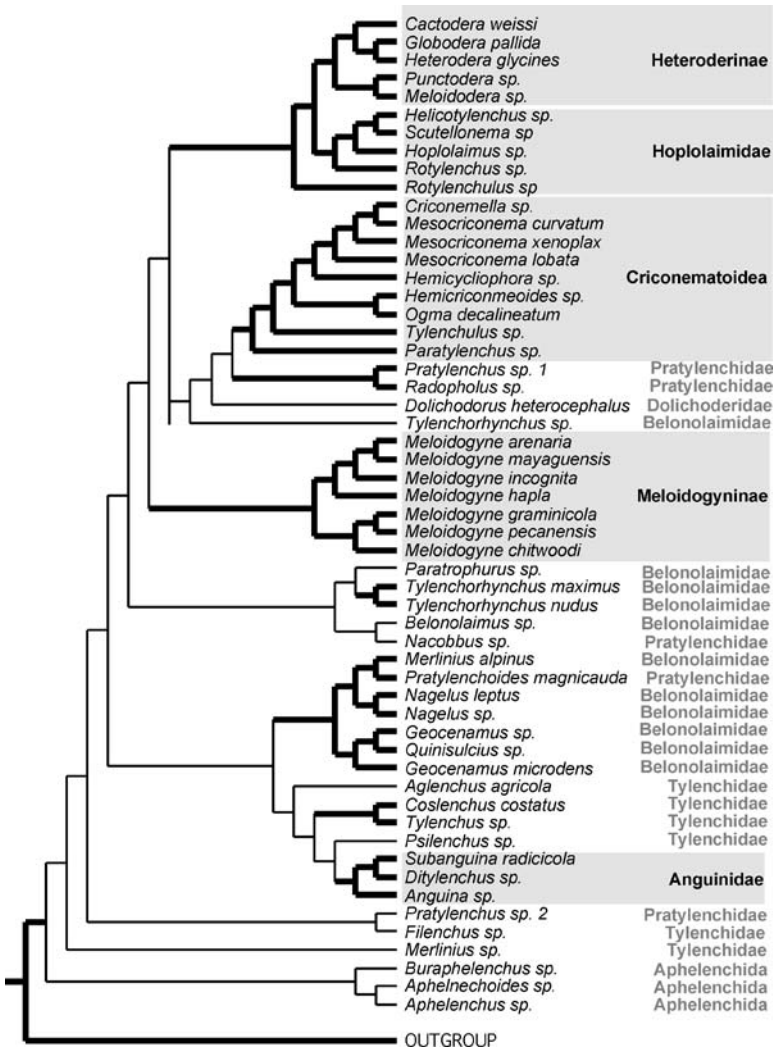


Figure 4 Preliminary phylogenetic tree of Tylenchida based on 650 bp of nuclear small subunit ribosomal RNA inferred using maximum likelihood quartet puzzling with the best fit model as selected by *Modeltest*. Bold lines depict clades with stronger support as assessed by parsimony jackknifing (>75%). Full-length SSU sequences from subsampled taxa reveals improved support for Meloidogyninae and Hemicyclophoridae as sister taxa, forming a monophyletic clade with respect to the Hoplolaimidae. Right column represents species position in classical taxa as primarily proposed by Luc et al. (64). Well-supported higher taxa are shaded. Depiction of Aphelenchida as monophyletic varies with outgroup choice, suggesting the need for additional taxa and genes to resolve relationships among members of this taxon.

of monophyly of Heteroderinae and Meloidogyninae versus convergence has critical implications for extrapolating from models in one group (i.e., *Meloidogyne*) to make inferences about the other (i.e., *Heterodera*, *Globodera*). New phylogenetic insight further suggests that understanding the evolution of complex host responses in Heteroderinae will benefit from comparative analysis with sister-clade Hoplolaimidae Filipjev, 1934, a group primarily, but not exclusively, of migratory ectoparasites and a point of potentially informative genetic comparison (Figure 4).

Classification systems often include the sedentary parasite, *Rotylenchulus*, within Hoplolaimidae (42) but phylogenies based on the SSU rRNA gene indicate that although *Rotylenchulus* is a sister taxon to Heteroderinae + Hoplolaimidae, it is paraphyletic with Hoplolaimidae (Figure 4). Sedentary parasite *Tylenchulus* is supported by SSU rRNA gene phylogeny as within a distinctive clade corresponding to Criconematidae; whereas the assigned ranks vary, this structure is consistent with classifications of Raski & Luc (75) and Siddiqi (85, 86). Similarly, as anticipated from extant classifications, the sedentary parasite *Anguina* forms a clade with migratory *Ditylenchus* (43) (Figure 4). Conversely, the Tylenchidae and Pratylenchidae, including the sedentary *Nacobbus*, are not resolved by SSU rRNA as clades within Tylenchida. This cannot be entirely attributed to a flaw in the resolution of this gene, since monophyly of Tylenchidae and Pratylenchidae can be questioned regardless, as classically defined by a context of morphological character sets clearly understood to be ambiguous or plesiomorphic.

Evolution of Virus Transmission

Unlike Tylenchida and Aphelenchida, plant-parasitic Longidoridae and Triplonchida are exclusively migratory ectoparasites, with little apparent diversity or radiation in feeding approaches within these groups. Nevertheless, ectoparasitism does not preclude a sophisticated host-parasite relationship; Longidoridae use long stylets to feed deeply for extended periods (87), and feeding is often associated with root swellings and modification of host cells (17, 40). Furthermore, some species of Longidoridae and Triplonchida have evolved the capacity to transmit specific viruses, but unfortunately, lack of well-developed species-level phylogenies for these groups precludes producing detailed hypotheses for the evolution of virus transmission patterns. On the scale of deep phylogenetic branches, clearly virus transmission evolved independently in the separate clades of Triplonchida and Longidoridae; the former exclusively transmits tobnaviruses (31) whereas the latter transmit primarily nepoviruses (19). Genus-level phylogeny of plant-parasitic Longidoridae (24, 25) based on morphology and phylogeography indicate that *Xiphinema* and *Longidorus*, the two genera with species known to transmit viruses, are not collectively monophyletic, suggesting either that the capability to transmit viruses arose independently in these two genera, or conversely that virus transmission is ancestral to Longidoridae but that it was secondarily lost or has not yet been discovered in other genera including *Xiphidorus*, *Paralongidorus*, and *Longidoroides*. Virus transmission is surprisingly specific between nematode species

vector and serologically distinctive virus strains and is apparently determined by recognition between the virus protein coat and the lining of the nematode feeding apparatus (19, 78), but the coevolution of this specificity remains unexplored.

Dormant, Dispersal, and Infective Stages

Beyond the preadaptations of feeding structures, the evolution of plant parasitism also includes specializations for synchronizing the life cycles of nematodes with the availability of suitable hosts in space and time. Nematodes representing disparate clades and divergent trophic groups can survive extreme conditions through mechanisms of suspended development including anhydrobiosis, cryobiosis, and dauer juvenile stages (101); however, the details of these capabilities are variable and for plant parasitism they often involve some special twists. The extent of homologies among the full range of dormant conditions and various parasitic dispersal and infective stages remains largely unknown; yet recognition of some degree of commonality, and perhaps evolutionary conservation, between the thoroughly studied dauers of microbivorous *Caenorhabditis elegans* (a facultative shunt to the third juvenile stage) and infective or dispersal stages of certain plant parasites is compelling (13, 76).

The second-stage juveniles of many species of plant-parasitic Tylenchida have survival capabilities in conditions unfavorable for development, but environmental tolerance is further specialized in some clades. For example, some species of Anguinidae and Heteroderidae have life cycles that exploit a relatively narrow host range and are largely dependent on dormant stages for dispersal. In Anguinidae, depending on species, the second-, third- or fourth-stage juvenile may survive for years in dried plant material or seed, pending conditions favoring development. In some Heteroderidae, second-stage juveniles may survive in eggs for years within the dried remnant of the female, hatching only in the presence of a suitable host. In each case the life cycle resumes due to exogenous environmental triggers, often involving signals from suitable developing host plants, and/or temperature changes (57, 101). It is noteworthy that within Tylenchida, clades with the most striking examples of dormant infective stages are not closest relatives (Figure 4), suggesting independent evolutionary modifications, but perhaps building upon much more broadly conserved developmental pathways including components also expressed in nonparasitic nematodes (13).

Understanding the evolution of dormant stages, and specializations of these stages for plant parasitism in Tylenchida, Dorylaimida and Triplonchida is currently limited due to taxonomic undersampling of these pathways throughout Nematoda. Is the capacity for dormancy broadly convergent, or conversely, are fundamental pathways conserved (plesiomorphic) within Secernentea (Figure 1) or even more broadly throughout Nematoda? Answering these questions is prerequisite to applying extensive developmental, genetic, and molecular knowledge concerning dauers of *C. elegans* to the understanding of similar processes in plant-parasitic nematodes. A starting point for expanding knowledge of dauer pathways

in *C. elegans* is to use organismal phylogenies to select taxa for comparative analysis. SSU rRNA trees strongly support a clade including Rhabditina, Diplogasterida, and vertebrate parasitic Strongylida (Figure 1), and phylogenetic comparisons of representative species should provide a powerful comparative model. Such evolutionary approaches might include mapping presence/absence of dauer character-states on organismal phylogenies, and comparison of species trees with gene trees for loci in the dauer pathway. Similarly, plant-parasitic Tylenchida is more closely related to Cephaloibeida (primarily microbivorous) rather than to *C. elegans* (Table 1). Therefore, questions of conservation of dormancy pathways with microbivores versus independent evolution of dormancy in plant-parasitic Tylenchida should initially be tested by comparative phylogenetic approaches involving Cephalobina, which will first require development of cephalobid model systems for investigation of dormancy and dauer pathways. Beyond Secernentea little is known of specific dormant stages in plant-parasitic Dorylaimida and Triplonchida; in the absence of adequate sampling of representative taxa, questions concerning conserved versus convergent evolution of characters related to dormancy and their specializations for plant parasitism will remain untested.

Responses to hatching factors and dormancy induction and recovery as well as mate and food recognition are often dependent on sensory systems, but to a large degree these capabilities are not specifically evolved for plant parasitism; rather they are more generally shared with nonplant parasites. Physical signals (e.g., temperature) as well as gradients of end products of metabolism, including carbon dioxide, have long been known to attract plant parasites and other nematodes across considerable distances (59, 72). More specific signals include "hatching factors" from host diffusates (57) and largely unidentified short-range gradient signals that mediate orientation of a parasite to host roots, recognition of preferred invasion sites, highly specific hosts, and suitable mates (72). Where capabilities to respond to components of such specialized signals occur as derived characters across taxa, they may be phylogenetically informative toward interpreting the evolution of groups of plant parasites; furthermore, they may indeed prove to be a consideration in evolutionary processes of isolation and speciation. Extending knowledge from more fully understood sensory responses in nonparasitic models to plant parasites could provide a beginning point for how attractants work in parasitic systems (72) and could reveal patterns relevant to the evolution of plant parasitism.

GENETIC AND BIOCHEMICAL ASPECTS OF EVOLUTION

Foregoing sections have detailed structural, physiological, and behavioral specializations that have occurred during the evolution of plant-parasitic nematodes from their free-living ancestors. Microscopic observations of active pharyngeal secretion during juvenile migration and feeding-site establishment led early researchers to hypothesize that host feeding cells, particularly of sedentary parasites, formed in response to substances secreted by the nematodes (12, 62). More recently, hypotheses about plant-nematode interactions have been framed in the context of

potential genetic specializations and protein expression by plant parasites. For example, according to Gao et al. (44, p. 720), “the most evolutionarily advanced adaptations for plant parasitism by nematodes are the products of parasitism genes expressed in their pharyngeal gland cells and secreted through their stylet (feeding spear) into host tissue and cells.” These specialized secreted proteins mediating parasitism (including pharyngeal proteins) have been coined the “parasitome” (48, 53); pharyngeal secretions injected into host cells are also responsible for inducing plant pathology (52).

Genetic specializations in cases of particularly intimate host-parasite interactions (e.g., Heteroderinae and Meloidogynine) have been the subject of many recent studies using current tools of molecular biology and genomics. In contrast, the nature of genetic specializations in nematodes from Tylenchida and Aphelenchida with seemingly less intimate host associations (e.g., migratory ectoparasitism and burrowing endoparasitism, sedentary ecto- and endoparasitism, and a full range of intermediate associations) has rarely been investigated (37). Some molecular investigations have revealed that plant parasites and their nonparasitic relatives (typically the comparison is *C. elegans*) share certain genes in patterns that suggest a long history of gene continuity, for example, common guanylyl cyclase chemoreceptor genes [see references 141 and 145 in (26)]. Of the estimated 15,000–20,000 protein-coding genes in plant parasite genomes, most are expected to have homologues in free-living nematode relatives, whereas only a small fraction are expected to play a direct role in parasitism. Finding and elucidating the functions of these parasitome genes has become a major focus of applied plant nematology, and comparative phylogenetic methods can play key roles in these investigations.

The parallel evolution of plant parasitism and the repeated evolution of parasitic lifestyles should be reflected in the evolution of parasitome genes, including the biochemical preadaptations for the specializations of plant parasitism. Although most plant parasites are migratory, it is mainly specialized sedentary nematodes forming nurse cells (e.g., *Heterodera* and *Meloidogyne*) that have been examined for genes acquired during the evolution of plant parasitism; this lack of information precludes broad testing for parallelism of parasitome genes. This research focus on *Heterodera* and *Meloidogyne* reflects both their economic impact and interest in their biology; sedentary parasites modify and regulate plant cell function yielding feeding sites (nurse cells, including single and multinucleate giant cells, and syncytia) that act as metabolic sinks and sustain the nematode through its remaining life (56, 68, 69). Because pharyngeal secretions have a major role in establishing nurse cells, molecular methods that specifically target unique gland cell secretions are a promising approach for defining the pharyngeal components of the parasitome. Gene (cDNA) libraries established from pharyngeal gland cell cytoplasm and selected using subtractive hybridization (45) or signal peptide methods (98) have provided profiles of expressed gland-specific parasitism genes. The signal peptide method has revealed 53 candidate parasitism genes expressed (and likely secreted) in *Heterodera glycines* pharyngeal glands, of which 38 were novel proteins as inferred from similarity-based database searches (46). This surprisingly

large number of unique parasitome genes from pharyngeal glands leads directly to questions regarding their origins.

Some likely mechanisms for acquisition of parasitome genes include: (a) adaptation of pre-existing genes to encode new functions, (b) gene duplication and divergence of paralogs, and (c) horizontal gene transfer (82). Several parasitism genes isolated from sedentary plant-parasitic nematodes have been identified as putative candidates for horizontal gene transfer from bacteria including β -1,4-endoglucanases (cellulases), pectinases, chorismate mutase, glutamine and polyglutamate synthetases, L-threonine aldolase, and Nod factors (14, 26, 46, 82, 96). Interestingly, in bacteria, several of these genes are located on plasmids, which might play some as yet unknown role in facilitating horizontal transfer. For the genes just noted, inferences of horizontal transfer were based on BLAST searches wherein nematode proteins were found to have greatest similarity to bacterial proteins, and in many cases had no apparent homologues in other eukaryotes (26, 88, 106). Among these genes, those coding for proteins involved in degradation of plant celluloses and pectins have been discovered in a phylogenetically diverse group of nematodes; they are expressed in subventral glands and are known to be used during juvenile root penetration and intracellular migration in *H. glycines* (27, 99). Genes encoding cellulases have also been reported from *Paratrichodorus minor*, *Bursaphelenchus xylophilus*, *Rotylenchus reniformis*, *Ditylenchus dipsaci*, *Pratylenchus agilis*, *Meloidogyne* spp., *Globodera rostochiensis*, and *Globodera tabacum*. *H. glycines* has been reported to have six different cellulase genes expressed in the subventral glands (46). The phylogenetic distribution of nematodes known to have cellulase genes (Figures 1 and 4) suggests that either horizontal gene transfer of β -1,4-endoglucanases occurred early in nematode evolution (but note this gene is absent from *C. elegans*), or that this event was not a singularity. For cyst and root-knot nematodes, the conserved positions of introns in genomic clones of known cellulase genes argue against independent transfers since these taxa last shared a common ancestor (26). In contrast, a phylogenetic tree of family 5 hydrolase amino acid sequences is not readily reconciled with the disputed (e.g., Figure 4) hypothesis of common ancestry for cyst and root-knot nematode cellulases (26). Although cellulase genes in plant parasites would appear to be a convincing case of horizontal gene transfer from prokaryote to eukaryote, additional research is required to resolve the full history of this gene complex/nematode association. Understanding this history will require phylogenetic approaches, not only to screen for the presence and absence of genes among nematodes as part of a rigorous comparative investigation (82), but also for detailed phylogenies of the putative genes themselves, in order to assess if, for example, cellulase genes resident in plant parasites are orthologous, or conversely, if the gene trees are best explained by multiple horizontal transfer events in combination with gene losses in certain nematode lineages.

Other putative examples of horizontal gene transfer, including candidate donor bacteria are recognized for several genes in the root-knot nematode, *Meloidogyne*. Within *Meloidogyne*, some genes are shared by congeners and thus appear to be conserved within the genus, whereas others, being specific to a particular

species, may have been acquired more recently (82). Certain *Meloidogyne* genes, including *nodL*, are intriguingly similar at the amino acid level to those of putative donor bacteria, plant-associated *Rhizobium* that are sympatric with the root-knot nematode. Other candidates being explored as sources of transferred genes include bacterial symbionts or parasites of nematodes (14, 82). Although it is not known at what points within the evolutionary history of nematodes particular genes now employed for parasitism might have been acquired, it is tempting to speculate that certain genes were present prior to divergence of the Tylenchida from their free-living sister taxa (Cephalochoidea), and that horizontal gene transfer events to free-living nematodes were preadaptive for parasitism. Such scenarios would favor gene transfer due to close associations between bacterivores such as cephalobids and plant-associated bacteria including nitrogen-fixing nodules (100).

Not all putative parasitism genes in nematodes have patterns of phylogenetic distribution consistent with long histories of association. For example, the polyglutamate biosynthesis gene (*Mt-pgsA*) of *Meloidogyne artiellia*, which is not found in database searches of other eukaryotes (including *C. elegans*), appears to be absent from *G. rostochiensis*, and hybridizes only weakly to *Meloidogyne javanica*. Although the function of this gene in *M. artiellia* is unknown, it is expressed exclusively in the intestine throughout the life cycle (96). Other parasitism genes have characteristics suggesting they have evolved by adaptive molecular mimicry rather than by gene transfer events. A dorsal gland polypeptide (HgCLE) reported from *H. glycines* has regions of amino acid similarity to a plant ligand involved in intercellular signaling (71); it is feasible that this nematode "pseudoligand" is used for parasite modification of plant cells. The simplicity of features shared by plant (CLE) and nematode (HgCLE) sequences at the amino acid level suggests that convergent evolution rather than gene transfer from plant to nematode may explain the similarity between these polypeptides (71).

Thus competing hypotheses include conservation, with loss in many clades (e.g., *C. elegans*), from a more ancient acquisition (26, 82) in Nematoda versus independent acquisition in conjunction with the three or more independent lines of plant parasitism. These possibilities can be tested by broader recognition of patterns of distribution of these genes throughout Nematoda and resolving congruence with species trees.

PERSPECTIVES AND CONCLUSIONS

Understanding the evolution of plant parasitism cannot progress without improved phylogenetic resolution of phylum Nematoda. Existing molecular phylogenetic trees for the phylum provide a valuable yet preliminary insight into the origins and diversity of plant parasites. However, a common theme of this review is that many fascinating morphological, biochemical, genetic, and behavioral specializations of plant parasites cannot be interpreted in an evolutionary context because of limited information on distribution of these features among nematodes and lack of a phylogenetic framework for interpreting character evolution.

Producing robust phylogenetic hypotheses requires increased taxonomic representation of plant parasites; however, additional nonparasitic relatives of plant parasites also must be included to develop comprehensive trees that permit reliable reconstruction of ancestral states for understanding the evolution of specializations involving plant parasitism. These trees will also serve to identify nematode clades that include taxa most appropriate for use as comparative model organisms (e.g., Cephalochoidea for Tylenchida). Development of broad-scale phylogenies for Nematoda has classically been problematic due to the isolation of nematologists along ecological lines, despite evidence for widespread convergence and paraphyly or even polyphyly (28, 41, 91). The recently established NSF Tree of Life project (<http://nematol.unh.edu/>) on phylogeny of Nematoda has the potential to overcome these difficulties by leveraging worldwide expertise and coupling molecular and morphological phylogenetic methods with bioinformatics tools to provides new opportunities to build upon previous noteworthy inroads into nematode phylogeny.

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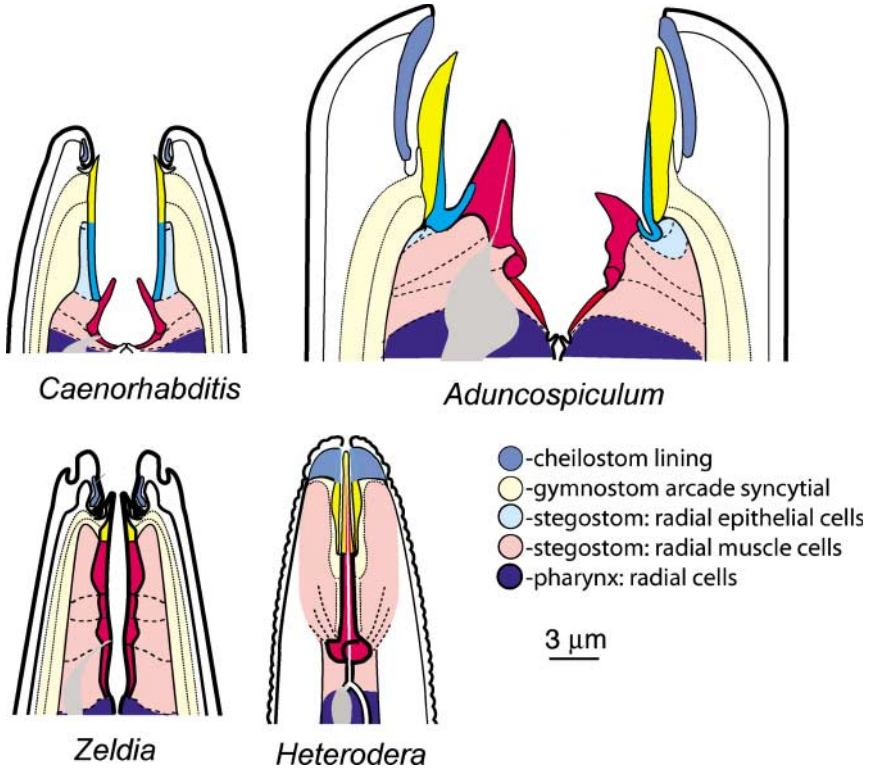


Figure 3 Inferred homologies of stoma structures based on transmission electron microscope reconstruction of representatives of Rhabditina (*Caenorhabditis*), Diplogasterina (*Aduncospiculum*), Cephalobina (*Zeldia*), and Tylenchida (*Heterodera*). Cuticular linings of stoma are depicted by deeper shades that correspond to underlying gymnostom, stegostom epithelial, or stegostom muscle cells (shown in key). Adapted from Baldwin et al. (8).



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