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PHYLOGENY FOR GENERA OF NEMATODIRINAE (NEMATODA: TRICHOSTRONGYLINA)

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ABSTRACT: Monophyly for the Nematodirinae, with 5 genera, Murielus, Rauschia, Nematodiroides, Nematodirus, and Nematodirella was confirmed based on comparative morphology and phylogenetic analysis of structural characters. This concept for the nematodirines excludes the monotypic Lamanema chavezii, but otherwise corroborates generic-level diversity as defined in prior studies. Exhaustive analysis resulted in 1 most parsimonious tree (36 steps; consistency index [CI] = 0.93; excluding phylogenetically uninformative characters, CI = 0.92). As an inclusive or monophyletic group, Nematodirinae was diagnosed by 8 synapomorphies (7 are unequivocal): (1) large eggs, (2) long filiform spicules, (3) basal division of the dorsal ray, (4) symmetrical membrane enveloping the spicule tips, (5) fused structure of the spicule tips, (6) absence of the gubernaculum, (7) development of the third-stage larva within the egg, and (8) ornamentation in the form of discrete bosses on the bursa. Exclusion of Lamanema will require new assessments of historical biogeography and the evolution of host associations for the nematodirines.

Nematodirine nematodes globally represent serious pathogens in wild mammals and domestic stock. The speciose genus Nematodirus Ransom, 1907, contains an array of helminth pathogens that infect ruminants throughout the world, and species such as Nematodirus battus Crofton and Thomas, 1951, continue to be regarded as among the most economically significant parasites in domestic sheep, Ovis aries Linnaeus, across the Northern Hemisphere (e.g., Thomas, 1956; Hoberg, Zimmerman, and Rickard, 1985; Hoberg, 1997). Although there has been considerable effort and research to define epidemiology, and the host–parasite interface for species of Nematodirus (e.g., Thomas, 1959; Rickard et al., 1989), our understanding of the broader evolution and history for these and related nematodes remains to be fully elucidated.

Current taxonomy and concepts for the relationships of the Nematodirinae Skrjabin and Orlov, 1937, were established by Durette-Desset and Chabaud (1977, 1981a) and Durette-Desset (1983). Durette-Desset and Chabaud (1977, 1981b) elevated the Molineinai Skrjabin and Shul'ts 1937 to establish the family Molineidae (Trichostrongyloidea), which contained the Nematodirinae, Molineinae Skrjabin and Shul’ts, 1937, and several other subfamilies. Durette-Desset and Chabaud (1977, 1981a) and Durette-Desset (1983, 1985) refined the concept for Nematodirinae and recognized 6 inclusive or monophyletic genera: Nematodirus (type); Nematodirella Yorke and Mapleton, 1926; Murielus Dikmans, 1939; Lamenema Becklund, 1963; Nematodiroides Bernard, 1967; and Rauschia Durette-Desset, 1979. Subsequent proposals for taxonomic revision within the Trichostrongyloidea resulted in elevation of the superfamaly to a subordinal rank within Strongyliida. Concurrently, 3 taxa of superfamaly rank, Molineoeida, Trichostrongyloidea, and Heligmosomoidea, were established to recognize putative monophyletic lineages within the Trichostrongylinia (Durette-Desset and Chabaud, 1993). Although this concept for elevated taxonomic rank has not been universally accepted, the Nematodirinae is thus contained within the Molineoeida and Molineidae (see Durette-Desset et al., 1994).

Durette-Desset and Chabaud (1981a) presented the first explicit hypothesis for relationships within the Trichostrongyloidea and indicated, among the molineids, a putative sister-group relationship for Nematodirinae and Molineinae + Anoplostrongyloidea; Molineidae was regarded as the putative sister of the Trichostrongylidae (Durette-Desset, 1985). Subsequent to establishment of the Trichostrongylina, Durette-Desset et al. (1994) modified these concepts to recognize 3 lineages with unspecified relationships linking the Trichostrongyloidea, Molineoidea, and Heligmosomoidea in a basal polytomy within the Trichostrongylia. Gouy de Bellocq et al. (2001) provided resolution among these taxa, suggesting monophyly for the respective lineages based on a single-locus analysis. Significantly, relatively robust phylogenetic support for 2–3 major clades, representing the Heligmosomoidea + Molineoidea and the Trichostrongyloidea, has been inferred based on partial sequences from 28S rDNA (Gouy de Bellocq et al., 2001). The latter, in part, provides the foundation for selection of potential outgroups suitable for phylogenetic analyses within the Nematodirinae in the current study.

Nematodirines have been diagnosed by a suite of attributes of the anterior esophagus (neodont), synloph, bursa, and ovejectors that encompass both putative pleisiomorphic and apomorphic character states (e.g., Durette-Desset, 1985). Rickard and Hoberg (2000) suggested, in conjunction with preliminary outgroup comparisons relative to the Trichostrongyloidea and Molineidae, that 7 characters may constitute synapomorphies for the Nematodirinae: (1) presence of perioral denticles, (2) long filiform spicules, (3) fusion of spicule tips, (4) symmetrical membrane enveloping spicule tips, (5) dorsal ray divided and separated at base, (6) absence of gubernaculum, and (7) large eggs. Based on an assessment of these criteria, it was proposed that the monotypic Lamanema, with Lamanema chavezii Becklund, 1963, be excluded from the subfamily (referred to Molineinae), leaving Nematodirinae with 5 genera (Rickard and Hoberg, 2000).

Explicit phylogenetic studies among the genera of the nematodirines have not been presented, although scenarios for some aspects of evolution, host relationships, and historical biogeography within the subfamily have been summarized (e.g., Durette-Desset, 1978, 1979, 1985; Rossi, 1983; Durette-Desset et al., 1994). A comprehensive phylogeny for species of Nematodirella was completed by Lichtenfels and Pilitt (1983b). Nematodirus has been the subject of phylogenetic studies emphasizing those species in domestic ruminants (e.g., Lichtenfels and Pilitt, 1983a). Nematodirus was also the focus of analyses...
exploring the relationships of representative species parasitizing cameldids, cervids, and caprines and the putative origin and history for host association and geographic distribution for the pathogen *Nematodirus battus* (Hoberg, 1997). Additionally, taxonomically limited comparisons based on data from nuclear ribosomal sequences were conducted to reveal relationships for populations of *Nematodirus battus* in domestic sheep and the contemporary determinants of geographic distribution in the Holarctic (Audebert et al., 2000; Nadler et al., 2000).

Although monophyly has been assumed, there has been no universal test of this hypothesis for the Nematodirinae. The remaining subfamilies of the Molineidae also lack an unequivocal phylogenetic basis; and synapomorphies for constituent taxa, including Anoplostrongylinae, Molineinae, and Ollulaninae, have not been defined (Rickard and Hoberg, 2000). In the current study, we explore the applicability of characters derived from comparative morphology in (1) providing a phylogenetic diagnosis for the Nematodirinae, (2) testing the hypothesis for exclusion of *Lamanema*, and (3) development of hypotheses for relationships among genera of the subfamily.

**MATERIALS AND METHODS**

Phylogenetic analysis of morphological characters was used to examine the putative relationships among adult nematodes representing 5 genera of the Nematodirinae (Table I). Characters were derived from examination of specimens of exemplar species in adult to data in descriptions and redescriptions (e.g., Travassos, 1937; Skrjabin, Shikholovalova, and Shul'ts, 1954; Durette-Desset, 1979; Lichtenfels and Pilitt, 1983a, 1983b; Sutton and Durette-Desset, 1985). Standard methods for comparative morphology among the putative genera of the Nematodirinae and outgroups are outlined in Chabaud et al. (1967), Durette-Desset (1974, 1979), Durette-Desset and Chabaud (1981a), and Lichtenfels and Pilitt (1983a, 1983b). A putative relationship for *L. chavezii* in the context of the in-group was examined, although previous comparative studies relegated this monotypic genus to the Molineinae (Rickard and Hoberg, 2000).

Characters included morphological attributes typical of male and female nematodes. Polarity was estimated based on taxonomic outgroup comparisons (Watrous and Wheeler, 1981; Maddison et al., 1984); mul-
TABLE II. Character matrix for genera of Nematodirinae.

<table>
<thead>
<tr>
<th>Characters (1–23)</th>
<th>Genus</th>
<th>12345678911</th>
<th>111111112222</th>
<th>01</th>
<th>234567890123</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichostrongyloidea*</td>
<td>000004000000φ</td>
<td>0000040001100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molinoidea*</td>
<td>000000000000</td>
<td>0000000000000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murielas</td>
<td>000000011100s</td>
<td>111100000011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematodirella</td>
<td>11001002220</td>
<td>211111000011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematodiroides</td>
<td>1111200310</td>
<td>211212110011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematodirus</td>
<td>1100112020k1</td>
<td>111111100011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rauschia</td>
<td>11111000020</td>
<td>111110100011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamanema†</td>
<td>20000000300</td>
<td>000000011221</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamanema‡</td>
<td>10000000010</td>
<td>000000011111</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Taxonomic outgroups; see Table I for exemplar species examined. Coding represents estimation of ancestral states for species in Trichostrongyloidea and Molinoidea, respectively. φ = inapplicable character for Trichostrongyloidea.
† Coding is consistent with independent derivation (convergence) in L. chavezii.
‡ Alternative coding consistent with hypothesis for homology in L. chavezii, nematodirines, and outgroups. These decisions influence characters 1, 10, 21, and 22 and apply HAP.

Taxonomic outgroups were coded as polymorphic. Outgroup selection was based on phylogenetic analyses summarized in Durette-Desset et al. (1994) and Gouy de Bellcrocq et al. (2001). Multiple outgroups included species that exemplify Trichostrongyloidea and Molinoidea (Table I). Outgroups are presented as an estimation of ancestral states based on examination of multiple exemplars representing each of the superfamilies. Descriptions for all present in Table II. Alternatives for coding are presented for characters 1, 10, 21, and 22 in L. chavezii and involve either suspension, consistent with putative convergence, or application of Hennig’s Auxiliary Principle (Wiley et al., 1991). Hennig’s Auxiliary Principle specifies that similar characters are considered to be homologous unless there is compelling evidence for convergence or parallelism (Brooks and McLennan, 2002).

 Parsimony analysis was conducted with PAUP 4.0b10 for Macintosh (Swoford, 2001). Searches were completed in the exhaustive option, with optimization by accelerated transformation; characters were unordered and with equal weights. Results are shown as a phylogenetic tree with associated statistics, including length, consistency index (CI), and retention index (RI). Characters and changes were mapped directly onto the most parsimonious tree (MPT) for evaluation with MacClade 4.0 (Maddison and Maddison, 2000). Tree topology and support for relationships among nematodirines were evaluated based on jackknife resampling and calculation of Bremer decay indices (Bremer, 1994). Jackknife analysis was implemented in PAUP with 10,000 replicates, branch and bound, and presentation as a strict consensus tree.

Character descriptions

1. Development of neodont formation (Durette-Desset, 1983): Two states: 0 = dorsal esophageal gland orifice opening in buccal capsule, maybe associated with tooth or tooth is absent; 1 = neodont formation present. The neodont was designated by Durette-Desset (1983, 1985) to recognize a prominent cuticularized tooth arising from the dorsal aspect of the esophageal lumen; presence of the neodont is apomorphic. Such structures are not considered to be homologous across the strongyles and are represented in the Nematodirinae (all genera excluding Murielas) and a limited number of Molinoidea and Trichostrongyloidea. The neodont is present in the molineid, L. chavezii, which has multiple teeth, 1 dorsal and 2 lateroventral; among the trichostrongyloids, including genera and species of the Haemonchinae, there may be single or multiple teeth. In contrast, among Ostracospinae, such as Teladorsagia and Mazamstrongylus, the dorsal esophageal tooth is present, but the neodont is absent. The ancestral condition is estimated as absence of the neodont, and the Molinoidea and Trichostrongyloidea are coded accordingly. In testing the placement of Lamanema, the presence of the neodont was either coded as present (=1), or present and independently derived, representing a hypothesis for convergent acquisition of this attribute (=2); see alternative coding in Table II.

2. Perioral denticle: Two states: 0 = absent; 1 = present. The perioral denticle is present in many other molineids and trichostrongyloids (Durette-Desset and Chabaud, 1981a, 1981b; Durette-Desset, 1983; Durette-Desset et al., 1999), and their presence in nematodirines is considered to represent the apomorphic condition (see Durette-Desset, 1985; Rickard and Hobberg, 1990).

3. Synlophe orientation: Two states: 0 = perpendicular to body surface; 1 = oblique to body surface. Where the synlophe is present among Trichostrongyloidea and Molinoidea, the ridges are usually perpendicular to the surface of the body and bilaterally symmetrical, which is designated as the plesiomorphic condition (Durette-Desset, 1983, 1985).

4. Synlophe orientation: Three states: 0 = perpendicular; 1 = lateral and ventral ridges directed ventrally; 2 = lateral ridges directed dorsally. The orientation of the ventral ridges in species of Rauschia and Nematodiroidea varies independently from the plesiomorphic condition, although a sagittal axis is apparent (Durette-Desset, 1985); orientation in Nematodiroidea and Rauschia is defined as lateroventral and ventral, respectively (Durette-Desset, 1983).

5. Synlophe gradient in size: Two states: 0 = absence of gradient, ridges of equal height; 1 = gradient present, ridges vary in height along axis.

6. Synlophe, maximum numbers of ridges at midbody in reference to Molinoidea: Three states: 0 = midbody ridges ≤ 18; 1 = midbody ridges ≥ 52; 2 = midbody ridges ≤ 60. Although there is minimal overlap, the coding for this character assumes distinct differences among taxa, and a general increase in the numbers of ridges characteristic for species of Nematodirus and Nematodirella (e.g., Lichtenfeld and Lichtenfeld, 1983a; Durette-Desset, 1983a). This character is inapplicable and is designated as φ in the matrix (Table II).

7. Synlophe, hypertrophy in dorsal and ventral ridges: Two states: 0 = ridges never hypertrophied as fins; 1 = ridges hypertrophied and fililike. Hypertrophy of dorsal and ventral ridges is limited to some species of Nematodirus, including Nematodirus davtoni (Grigorion, 1949) and Nematodirus filicolis (Rudolph, 1802) and is considered a putative synapomorphy for the genus, as it is a unique character among the nematodirines, although this attribute has not been evaluated in all inclusive species (see Lichtenfelds and Pillett, 1983a).

8. Vulva, position: Three states: 0 = posterior third or fourth of body; 1 = posterior eighth of body; 2 = midbody or anterior to midbody.

9. Uterus, development of anterior and posterior branches: Four states: 0 = didelphic, both branches fully functional; 1 = monodelphic (acquired presence of 1 branch); 2 = apomorphic; 3 = posterior branch atrophied and vestigial. The monodelphic condition is observed only among Murielas, whereas the posterior or anterior branch is present but nonfunctional in Nematodiroidea and Rauschia.

10. Eggs, maximum length: Three states: 0 = length usually ≤100 μm; 1 = length usually ≥150 μm and ≤180 μm; 2 = length ≥180 μm and generally ≥200 μm. Large eggs constitute a putative synapomorphy for Nematodirinae, although homoplasy is evident in a broader comparative context in the Strongylida. In testing the placement of Lamanema, this character was coded as apomorphic (= 1) or apomorphic and independently derived, consistent with a hypothesis for convergent acquisition (=3); see alternative coding in Table II.

11. Tail form in adult female in reference to Molinoidea: Two states: 0 = tail truncate, with terminal spine; 1 = conical, with spine absent. The truncate tail with spine is considered plesiomorphic (e.g., Lichtenfeld and Lichtenfelds, 1994; Durette-Desset et al., 1999). Multistate taxa are represented in the Nematodirinae, with both conical and truncate tails being observed in Nematodirus and Murielas. It is postulated that the derived state was independently acquired in some species of these respective genera and represents transformation of the truncate tail. For Trichostrongyloidea, φ = inapplicable (Table II).

12. Dorsal ray, structure: Two states: 0 = dorsal ray not bifurcated
at base; 1 = dorsal ray bifurcated at base. This attribute constitutes a putative synapomorphy for Nematodirinae.

14. Spicule, membrane: Two states: 0 = symmetrical membrane not enveloping spicule tips; 1 = symmetrical membrane enveloping spicule tips. This attribute represents a putative synapomorphy for Nematodirinae.

15. Spicule tip: Two states: 0 = spicule tips, bifurcate or trifurcate, not fused; 1 = symmetrical and fused; 2 = asymmetrical and fused. This constitutes a putative synapomorphy for Nematodirinae, with an autapomorphic state in Nematodiorides.

16. Gubernaculum: Two states: 0 = present; 1 = absent. This attribute is a putative synapomorphy for Nematodirinae.

17. Bursal rays, relative size of rays 4 and 5 in reference to Mollineoida: 3 states: 0 = ray 4 < 5; 1 = ray 4 = 5; 2 = ray 4 > 5. In Trichostrongyloidea, \( \phi = \) inapplicable (Table II). 18. Bursal rays, structure of ray 4: Two states: 0 = ray 4 narrow; 1 = ray 4 robust.

19. Enterohypertic migration and development: Two states: 0 = development of adult nematodes entirely within small intestine; 1 = larval migration and development in liver and bile ducts of definitive host. Lamanema chavezii completes a migration through the liver prior to completion of the molt to adult in the small intestine (Guerrero et al., 1975; Guerrero, Rojas, and Alva, 1981). Cooperia hepatica Ortlepp, 1938, is apparently the only species among the Trichostrongyloidea or the Molineoidae that has a hepatic phase, and in this case, adult nematodes reproduce in the liver and bile ducts of ruminants (Anderson, 1992).

20. Accessory bursal membrane: Two states: 0 = absent; 1 = present. In L. chavezii, the presence of a membrane surrounding well-developed dorsal or “7” papillae is considered the apomorphic condition, consistent with the structure of this attribute among Trichostrongyloidea (see Hoberg and Lichtenfels, 1994).

21. Bursal rays 2 and 3: Two states: 0 = rays 2 and 3 parallel, generally narrow; 1 = rays 2 and 3 highly divergent, robust. Rays 2 and 3 are parallel in all nematodirines and most Mollineoidae and consistent with hypotheses of Durette-Desset et al. (1999), narrow and parallel rays represent the plesiomorphic condition. In contrast, divergence of rays 2/3 is characteristic in L. chavezii and some Trichostrongyloidea, such as Haemonchinae, but this pattern may not be homologous. Coding for L. chavezii is either =1 or =2, the latter to denote convergence and independent derivation for similar structures in these taxa; see alternative coding in Table II.

22. Development of third larval stage within the egg: Two states: 0 = first larval stage hatches from egg; 1 = third larval stage develops and hatches from egg. The apomorphic state is limited to nematodirines and has been documented in species of Nematodirus, Nematodirella, and Rauschia, but is currently undetermined, but predicted to occur, among species of Murielus or Nematodiroides. Coding for L. chavezii is either =1 or =2. The latter in recognition of putative convergence and independent derivation of this pattern of development; see alternative coding in Table II.

23. Bursal lobes, ventral structure and ornamentation: Two states: 0 = markings absent, or present only as irregular projections, spines, or raised plates, often with reticulate striations along bursal margin; 1 = markings always composed of discrete, raised cuticular bosses disposed on lateral lobes. Sarwar (1956) discussed and reviewed the possible significance of a variety of cuticular markings observed on the internal lateral lobes of the bursa among the Trichostrongyloidea (=Trichostrongylina). Stringfellow (1968) concluded that the bursal bosses typical among species of Nematodirus could have utility in taxonomy and in the unequivocal identification of otherwise similar congenerous. Among the Nematodirinae, bursal bosses are discrete and prominent, disposed in characteristic patterns on the internal surface of the lateral lobes but never arranged as plates. Such discrete bosses are typical among all genera of the Nematodirinae, but are absent in Lamanema. Among other Molineids, ornamentation of the bursal lobes is represented by spines in some Molineinae, small triangular spines in Anoplostrongylinae, or small projections and a bursa with distinctly scalloped margins in the Ollulaninae (Sarwar, 1956). Where bursal ornamentation has been observed among Trichostrongyloidea, it is composed of discrete large plaques or plate-like, continuous and irregular bosses among some Ostertaginae, minute bosses with marginal reticulated striations among Cooperinae, rows of superimposed bosses among the Haemonchinae, and by reticular striations among the Amidostomatinae (Travassos, 1937; Sarwar, 1956). Consequently, the condition observed among nematodirines is considered as a putative synapomorphy for the subfamily.

**RESULTS**

Phylogeny for Nematodirinae

Exhaustive analysis, based on a matrix where Hennig's Auxiliary Principle was suspended for specific characters in *Lamanema* (Table II), resulted in 1 MPT (36 steps; CI = 0.94; RI = 0.93; excluding phylogenetically uninformative characters, CI = 0.92) consistent with monophyly for the Nematodirinae (Fig. 1). As a monophyletic group, Nematodirinae was diagnosed by 8 synapomorphies (7 unequivocal; Fig. 1): (1) large eggs (character 10), (2) long filiform spicules (character 12), (3) basal division of the dorsal ray (character 13), (4) symmetrical membrane enveloping the spicule tips (character 14), (5) fused structure of the spicule tips (character 15), (6) absence of the gubernaculum (character 16), (7) development of the third-stage larva within the egg (character 22), and (8) ornamentation in the form of bosses on the copulatory bursa of males (character 23). Additionally, the neodont (character 1) and corona radiata or perioral denticles (character 2) are well developed in all nematodirines except among species in the basal *Murielus*. In the context of parsimony analysis, 7 characters were uninformative; characters 4, 7, 9, 18, 19, and 21 represented apomorphies for respective genera at this level of universality (see Wiley, 1989), whereas character 11 (structure of female tail) is polymorphic within *Nematodirus* and *Murielus*. Jackknife resampling and Bremer decay indices revealed strong justification for monophyly of the Nematodirinae (jackknife = 100%); moderate support for putative relationships for *Rauschia* + *Nematodiroides* and *Nematodirus* + *Nematodirella* was evident (Fig. 1). Instability is apparent at the node for crown taxa (jackknife = 80%) and for *Rauschia* + *Nematodiroides* (63%) (Fig. 1); a tree 1 step longer than the MPT places *Rauschia* basal to *Nematodiroides* and *Nematodirus* + *Nematodirella*. The genus *Lamanema* is clearly excluded from the Nematodirinae in the context of coding that defines apomorphic but convergent states for characters 1, 10, 21, and 22 (Fig. 1; Table II). The topology of the trees resulting from both the primary analysis and jackknife resampling was congruent.

Analysis of a matrix specifying alternative coding for *Lamanema* (coding consistent with the Hennig's Auxiliary Principle, where similar structures are considered homologous) resulted in 2 MPTs (35 steps; CI = 0.86; RI = 0.81; excluding phylogenetically uninformative characters, CI = 0.81). These trees (not shown) placed (1) Trichostrongyloidea and *Lamanema* in a basal polytomy with the Molineoida as the sister group of the nematodirines or (2) *Lamanema* as the sister for the nematodirines. A strict consensus of these hypotheses placed Trichostrongyloidea, Molineoida, and *Lamanema* in a basal polytomy and supported monophyly for the Nematodirinae (tree not shown). Jackknife resampling resulted in a strict consensus tree placing *Lamanema* as the sister group for the inclusive Nematodirinae, although the node was supported in only 51% of the replicates and the Bremer decay value = 0. In contrast, the values for Bremer decay and jackknife were 4 steps and 98%, respectively, in defining a monophyletic Nematodirinae with 5 genera; topology and support for relationships of these
inclusive taxa were largely identical to that revealed in the primary analysis.

Analyses did not refute the exclusion of the monotypic _Lamanema_ from the Nematodirinae. _Lamanema chavezi_ lacks putative synapomorphies that constitute phylogenetic diagnosis for the Nematodirinae (Fig. 1). Characters 1 (neodont), 10 (large eggs), 21 (divergence of rays 2/3), and 22 (development of the L3) are ambiguous with respect to placement of _L. chavezi_ based on either reconstruction resulting from alternatives in coding. This suite of characters also exhibits homoplasy relative to a restricted number of unrelated taxa within the Strongylida and Trichostrongylina. _Lamanema_ possesses large eggs and a neodont but lacks unequivocal characters that are phylogenetically informative for demonstrating a relationship to the nematodirines.

**Character evolution and relationships**

Relationships among genera of nematodirines appear well resolved (Fig. 1): _Molineus_ is basal; and 2 subclades, _Rauschia_ + _Nematodiroides_ and _Nematodirus_ + _Nematodirella_ are sister groups diagnosed by presence of a neodont and corona radiata
(characters 1, 2). *Rauschia* + *Nematodrioides* is diagnosed by orientation and gradient in the synlophoe (characters 3, 5). *Nematodirus* + *Nematodirella* is diagnosed by a high number of ridges in the synlophoe (character 6), position of the vulva at the midbody or in the anterior (character 8), and bursal rays 4 and 5 of equal size (character 17). Each of the genera is further diagnosed by suites of synapomorphic characters that are shared among congeners. Notably, species of *Nematodirella* are diagnosed by extremely long spicules (character 9) and species of *Nematodirus* may be diagnosed by the presence of finlike ridges (character 7) comprising the synlophoe in the dorsal and ventral field. This latter attribute, however, may occur only among a limited number of species and, thus, may be polymorphic in the genus, and as such, may constitute equivocal support for *Nematodirus*. *Rauschia* lacks unequivocal synapomorphies based on this analysis; a ventrolateral orientation of the synlophoe (character 4) and extremely large eggs (character 10) characterize all species, but the derivation of the apomorphic condition for these attributes is considered to be ambiguous (Fig. 1).

Two genera, *Murielus* and *Nematodirus*, apparently represent multistate taxa, where respective species possess either a conical or truncate tail with spine (character 11). Although the truncate state is a putative synapomorphy for Molineoidea (Gouy de Bellocq et al., 2001), it represents the plesiomorphic state for the Nematodirinae based on the concept of universality (see Wiley, 1979). Thus, polymorphism is attributable to independent origins of the conical tail from the truncate tail with spine in species of *Murielus* and *Nematodirus*.

Homoplasy was represented in 2 attributes, including the spicule length (character 12) and length of eggs (character 10). Extreme length of spicules, ≥4 mm, represents parallel development in *Nematodrioides zembrae* and species of *Nematodirella*. Additionally, species of 3 genera, *Rauschia*, *Nematodirus*, and *Nematodirella*, are characterized by eggs generally ≥200 µm in length (character 10); relatively smaller eggs in *Nematodrioides* represent a putative reversal.

**DISCUSSION**

**Phylogenetic diagnosis for Nematodirinae**

Monophyly for the Nematodirinae, with 5 genera, was confirmed in the current study based on comparative morphology and phylogenetic analysis of structural characters (Fig. 1). This concept for the nematodirines excludes the monotypic *L. chavezii*, but otherwise corroborates generic-level diversity as defined in prior studies (e.g., Durette-Desset and Chabaud, 1977, 1981a; Durette-Desset, 1985). Additionally, results of our phylogenetic analyses in part confirm the preliminary conclusions about character support and recognition of synapomorphies for diagnosis of Nematodirinae (Rickard and Hoberg, 2000).

The morphology of *L. chavezii* has been considered highly divergent and enigmatic with respect to the nematodirines and other molineoids or trichostrongyloids (Becklund, 1963; Durette-Desset, 1978; Sutton and Durette-Desset, 1985; Rickard and Hoberg, 2000). Based on the structure of the synlophoe and esophageal tooth, Durette-Desset (1978) considered *Lamanema* as a transitional form linking Nematodirinae and Molineinae. Subsequently, absence of the corona radiata and the structure of the synlophoe led Durette-Desset and Chabaud (1981a), Durette-Desset (1985), and Sutton and Durette-Desset (1985) to conclude that *Lamanema* represented the most primitive member of the Nematodirinae. These plesiomorphic characters, however, would have no bearing on understanding the putative relationships for *Lamanema* with either the nematodirines or other *Trichostrongylina*; the presence of a neodont represents an independently derived character within the Nematodirinae and Haemonchinae (Durette-Desset, 1983, 1985). Rickard and Hoberg (2000) offered an alternative hypothesis for relationships of *L. chavezii* based on approaches from comparative morphology and suggested placement of the genus within the Molineinae, but not as a transitional form.

The present analyses provided the first explicit test of monophyly for Nematodirinae and of the range of alternative concepts for *Lamanema*. Powerful jackknife and Bremer support are apparent for recognition of the Nematodirinae (Fig. 1). *Lamanema* is consistently excluded from the Nematodirinae irrespective of the conventions used for character coding and whether or not Hennig’s Auxiliary Principle is enforced (Table II); suspension of the Auxiliary Principle in the context of coding for this monotypic genus appears warranted (Brooks and McLennan, 2002). There is no apparent phylogenetic basis for *Lamanema* as a nematodirine, but the current analyses have failed to reveal an unequivocal linkage to other taxa within the *Trichostrongylina*. Durette-Desset (1985) and Rickard and Hoberg (2000) would both suggest affinities to the Molineinae, and a series of more extensive phylogenetic studies designed explicitly to examine the relationships for *Lamanema* are requisite but are beyond the scope of the current analyses.

Monophyly for nematodirines is linked to 7 unequivocal synapomorphies that constitute phylogenetic diagnosis for the subfamily (Fig. 1). Analysis corroborated exclusion of the monotypic *Lamanema*, as *L. chavezii* lacks 6 of the 7 unequivocal synapomorphies that diagnose the Nematodirinae (Rickard and Hoberg, 2000). Additionally, autapomorphies including the divergence of bursal rays 2 and 3 (character 21), structure of the accessory bursal membrane (character 20), and a unique pattern of enterohepatic development (character 19) further define the distinct nature of *Lamanema*. In this regard, hypotheses for independent derivation of several characters may warrant further examination.

We assume here that large eggs (character 10) have been independently derived in the Molineinae (*Lamanema*), *Trichostrongyloidea* (species of *Marshallagia* Orloff, 1933), and in genera and species of the Nematodirinae. Extended development and molting of late larval stages also appears to be associated with those species in which large eggs are typical. In *Lamanema* and probably all Nematodirinae (data are not available for *Murielus* and *Nematodrioides*), development to the infective third larval stage occurs within the egg (character 22). Among species of *Marshallagia*, eggs are up to 200 µm in length and hatching is delayed until the second larval stage (Skjabin et al., 1954). Large eggs, in conjunction with delayed hatching, may represent specific adaptations for enhancing survival and transmission in extreme environments (see below). We postulate that this pattern of ontogeny is convergent where it occurs across the *Trichostrongylina*, particularly when considered within the context of a suite of other characters.

The neodont (character 1) is also regarded as homoplasious within the *Trichostrongylina*, consistent with hypotheses out-
lined by Durette-Desset (1985) and Durette-Desset et al. (1994). The esophageal structure, found among the Haemonchinae (Trichostrongyloidea), Nematodirinae (excluding Murielus), and Lamanema (Molineinae) is not considered an indicator of common ancestry.

The structure of the bursa and, specifically, the highly divergent disposition of rays 2 and 3 (character 21), is not often observed among the Trichostrongylina. In specimens of Lamanema, rays 2 and 3 are relatively robust and curved anteriorly, with the tips sharply separated. Among some Trichostrongyloidea, including the Haemonchinae, some Cooperininae, Pseudostertagia bullosa (Ransom and Hall, 1912), and Parostertagia heterospiculum Schwartz and Alicata, 1933, a similar pattern for rays 2 and 3 has been noted (Gibbons and Khalil, 1982). In contrast, rays 2 and 3 are parallel and narrow among nematodirines and most Molineinae, which represents the pleisiomorphic condition (Durette-Desset et al., 1999). Additionally, an accessory bursal membrane (character 20) associated with dorsal papillae (rays 7) in Lamanema is apomorphic and similar to that observed in Trichostrongyloidea (see Hoberg and Lichtenfels, 1994).

Life history patterns are relatively uniform among trichostrongylines and progression of infections and ontogeny of parasitic larvae are usually limited to the lumen or mucosa of the gastrointestinal system. Among trichostrongylid parasites in artiodactyls, an enterohemepic cycle is only seen in Cooperioides hepaticae, where development of parasitic stages occurs in the lumen of the small intestine, but immature adults migrate into the bili ducts prior to patency (Anderson, 1992). The enterohemepic cycle in L. chavezi (character 19) differs considerably as third-stage larvae migrate via the circulation to the liver, molt to the fourth stage in the hepatic parenchyma, and then return to the small intestine to complete development (Guerrero et al., 1973).

Durette-Desset (1978) and Sutton and Durette-Desset (1985) concluded that Lamanema represented a transitional form between the Molineinae and Nematodirinae. This contention of a relationship for Lamanema and basal nematodirines appears to be refuted by the present analyses (Fig. 1). Consequently, the historical relationships for the nematodirines require further examination, as the former conclusion figured prominently in hypotheses about evolution of these nematodes, a putative ancestral association with camelds, and a reticulate status for Nematodirus lamae in the Neotropical region (e.g., Rossi, 1983; Durette-Desset, 1985; Durette-Desset et al., 1994).

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