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PHYLOGENY OF SPECIES OF THE GENUS *LITOMOSOIDES* (NEMATATODA: ONCHOCERCIDAE): EVIDENCE OF RAMPANT HOST SWITCHING

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ABSTRACT: Filarioid nematodes of the genus *Litomosoides* occur in the abdominal and (or) thoracic cavities of marsupials, rodents, and bats of the Nearctic and Neotropical regions. In this study, the phylogenetic relationships among these nematodes were estimated with a parsimony analysis of morphological characters derived from species descriptions. This nonweighted analysis produced 20 shortest trees. The monophyly of the genus was not supported in that *Litomosoides thomomydis* and *Litomosoides westi* failed to group with the other members of the genus. When these 2 taxa (parasites of pocket gophers) were excluded, monophyly of *Litomosoides* was supported by 2 synapomorphies (structure of the walls and general shape of the stoma); however, ancestor–descendant relationships among the species in the genus were not well resolved. A posteriori reweighting of the characters produced a single tree, different from all 20 most parsimonious trees. Alternative host–parasite evolutionary models were tested against these results supporting the process of host switching as being most important in forming the patterns of mammal–nematode associations that have been detected in this group of nematodes.

Examining the patterns of occurrences of various kinds of parasites in hosts based on well corroborated phylogenies can lead to the investigation of questions that may be of interest to students of zoogeography, coevolution, and biological diversity (Brooks and McLennan, 1993). Phylogenetic trees derived separately of hosts and their parasites can be compared to one another, and this comparison can provide information on the extent of coevolution between parasites and their hosts (Brooks, 1985; Brooks and McLennan, 1993; Huelsenbeck and Rannala, 1997).

Species of *Litomosoides* Chandler, 1931 are filarioid nematodes in the family Onchocercidae occupying the thoracic and abdominal cavities of a wide range of small mammals. The range of mammalian hosts presently includes species representing 4 families of bats, 5 families of rodents, and 1 family of marsupial. These nematodes are reported exclusively from mammals in the Nearctic and Neotropical regions with most species occurring in mammals of South America. Each species of *Litomosoides* appears to be specific to 1 of 3 mammalian orders in that a species found in bats does not occur in marsupials or rodents (Table I). These nematodes have a wide distribution throughout the Neotropical and southern Nearctic regions and several species have been well studied from both experimental and morphological perspectives. As such, these nematodes offer an ideal model for continuing investigations into their history as parasites of New World mammals. The distribution of these nematodes through such a wide range of taxonomically distantly related mammals provides an opportunity to examine host–parasite associations from a phylogenetic perspective.

Filarioid nematodes of the genus *Litomosoides* usually are considered to have a reduced set of cephalic characters and cuticular ornamentation relative to corresponding characters found in free-living nematodes. These reductions are believed to be the result of adaptation to life within host tissue (Chitwood and Chitwood, 1974; Bain, 1981; Chabaud and Bain, 1994). Alternatively, these reductions could be a result of developmental constraints, an historical accident, or both (Brooks and McLennan, 1991). For example, the ancestor of the filarioid nematodes had reduced external cuticular ornamentation, so all of its descendants do also. This perceived paucity of morphological characters has hindered the development of phylogenetic hypotheses of the group (Chabaud and Bain, 1994), and up to this time no cladistic study has been performed among species of *Litomosoides.* Bain et al. (1989, 1991) posited that the ancestral hosts for members of the genus *Litomosoides* were microchiropteran bats of Central and South America and that as recently as 5 million years ago (mya), these parasites diversified via host switching into muroid rodents and marsupials. Interestingly, no hypothesis proposes an early origin in marsupials.

Understanding the difference between a host–parasite relationship that is produced as the result of a long-term historical association and one that exists due to an ecological event such as a host switch depends on the development and comparison of robust phylogenetic hypotheses for both the hosts and their parasites (Brooks, 1985). Complete phylogenetic tracking of a host lineage by a lineage of parasites is a clear example of cospeciation (Brooks and McLennan, 1993). The occurrence of sister taxa of parasites in hosts that are not sister taxa may be an example of host switching (Brooks, 1985; Brooks and McLennan, 1993).

Bain et al. (1989, 1991) proposed that host-switching events have been the primary mode of evolutionary diversification in *Litomosoides* (Fig. 1A). Alternatively, given that these nematodes are host specific, each to 1 of 3 mammalian orders (Rodentia, Chiroptera, or Marsupialia), it is possible that species of *Litomosoides* coevolved/cospeciated in synchrony with their host (Fig. 1B). The basic null hypothesis of this study is to assume complete cospeciation with their hosts. Any evidence of deviation would indicate host-switching phenomena.

Herein, we present a hypothesis of the phylogenetic relationships among species of the genus *Litomosoides* based on a cladistic analysis of morphological characters. We address the following questions: Do species of the genus *Litomosoides* form a monophyletic group? If the answer is yes, we ask what are the hosts of the taxa that occur at the base of the parasite tree? And, are there clades of *Litomosoides* that occur exclusively in bats, rodents, or marsupials. The trees that we constructed were based mostly on characters and features used commonly for species-level diagnoses.

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TABLE I. List of all the described species of *Litomosoides* with the host species and geographic distributions.

Nematode	Host	Distribution*
	Order Chiroptera (bats)	
Litomosoides sp.	*Artibeus jamaicensis*	M
Litomosoides artibei	*Artibeus cinerea*	C
Litomosoides braziliensis†	*Carollia perspicillata*	Bo, Br, C, CR, M, V
	Carollia brevicauda	Bo
	Phyllostomus sp.	V
	Glossophaga soricina	Br
	Myotis sp.	Br, V
Litomosoides caliensis	*Sturnira lilium*	C
Litomosoides chandleri†	*A. jamaicensis*	Br, C
	Sturnira lilium	Bo
	Sturnira oporaphilum	Bo
	Phyllonycteris poeyi	Br
	Tadarida laticaudata	Br
	Lasiurus ega	Bo
Litomosoides colombiensis	*Vampyrops dorsalis*	C
Litomosoides fosteri	*Glossophaga soricina*	P
Litomosoides guiterasi†	*Glossophaga soricina*	Bo, Br, C, M
	C. perspicillata	Br
	Pteronotus parnelli	Cb
	Eptesicus fuscus	AL, Cb
	Myotis lucifugus	AL
Litomosoides hamletti	*G. soricina*	Br
Litomosoides leonilavazqae	*Macrotus mexicanus*	M
Litomosoides molossi†	*Molossus molossus*	C
	Molossus major	Br
Litomosoides teshi†	*C. perspicillata*	C
	Order Rodentia (rodents)	
Litomosoides andersoni†	*Ctenomys opimus*	Bo
Litomosoides carinii†	*Rattus norvegicus*	V
	Sciurus sp.	Br
Litomosoides chagasfilhoi†	*Akodon cursor*	Br
Litomosoides circularis	*Calomys* sp.	Br
Litomosoides ctenomyos†	*Ctenomys opimus*	Bo
Litomosoides esslingeri†	*Oryzomys caliginosus*	C
	Oryzomys microtis	Bo
	Oecomys marmorae	Bo
	Eligmodontia typus	Bo
	Calomys lepidus	Bo
Litomosoides galizai†	*Oecomys trinitatis*	Br
Litomosoides hoplomyis†	*Hoplomys gymnurus*	C
	Proechimys semispinosus	C
Litomosoides kohnae†	*Nectomys squamipes*	Br
Litomosoides legerae†	*Oxymycterus quaestor*	Br
Litomosoides patersoni	*Holochilus vulpinus*	Br
Litomosoides scotti†	*Oryzomys palustris*	FL
Litomosoides sigmodontis†	*Sigmodon hispidus*	TX
Litomosoides silvai	*Akodon cursor*	Br
Litomosoides thomomydis†	*Thomomys talpoides*	CO
Litomosoides westi†	*Geomys bursarius*	CO
	Order Marsupialia	
Litomosoides barreti†	*Marmosa cinerea*	Br
Litomosoides petteri†	*M. cinerea*	Br

* AL = Alberta, Bo = Bolivia, Br = Brazil, C = Colombia, Cb = Cuba, CO = Colorado, CR = Costa Rica, FL = Florida, M = Mexico, P = Panama, TX = Texas, V = Venezuela.
† Designates species used in the present cladistic analysis.

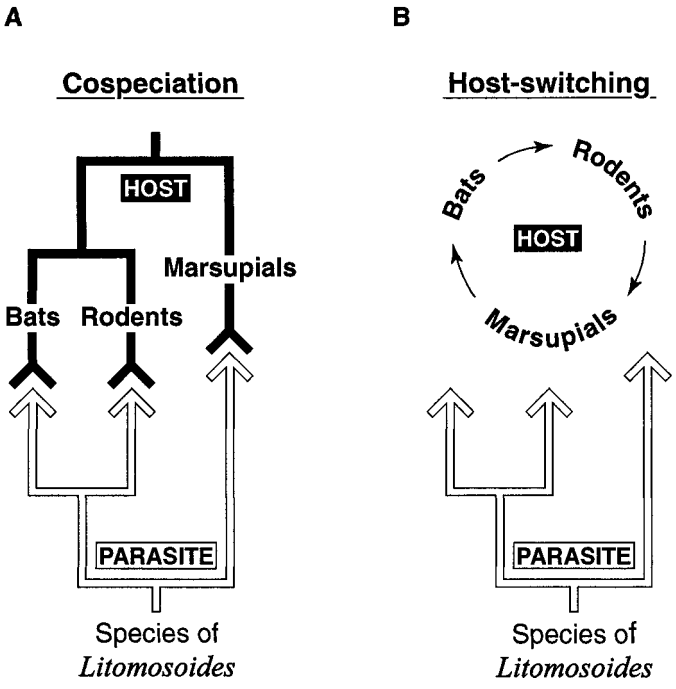

FIGURE 1. **A.** Possible model of strict cospeciation between *Litomosoides* species and their mammalian hosts in the Nearctic and Neotropical regions. This model implies that marsupials carried the ancestral lineage of *Litomosoides*, bats, and rodents following as more derived clades. As a consequence of this model of evolution, parasites of each mammalian order are predicted to form monophyletic groups (triangles). This model also implies the extinction of species of *Litomosoides* in all other mammalian orders. **B.** The alternative hypothesis would imply extensive host switching (arrows) between mammalian hosts, favored by close ecological associations. As a consequence, parasites of each mammalian order are not predicted to form monophyletic groups.

MATERIALS AND METHODS

Parasite collections

Parasites were isolated from mammals collected primarily in Bolivia (by S.L.G. or S.V.B.) from May through October 1984–1996. Rodents and marsupials were collected using Sherman™ live traps baited with oatmeal, bananas, sardines, and vanilla, or Macabee™ gopher traps. Bats were netted using mist nets set along or across streams, in flyways, and in fields of banana trees. Data on mammals are stored in either the Department of Mammalogy, American Museum of Natural History; the Division of Mammals, the Museum of Southwestern Biology, the University of New Mexico; or the National Museum of Natural History in La Paz, Bolivia.

In southeastern Nebraska, bats of the family Vespertilionidae were also examined for parasites, including *Eptesicus fuscus* (Beauvois), *Nycticeius humeralis* (Rafinesque), *Myotis keenii* (Merriam), *Myotis lucifugus* (Le Conte), *Lasionycteris noctivagans* (Le Conte), *Lasiurus borealis* (Müller), and *Lasiurus cinereus* (Beauvois). In Nebraska, bats were captured in mist nets set across streams and creeks from June through October 1995–1996.

Parasites were collected following the methods of Gardner (1996). From each host, all helminths found were collected, blood smears were made, samples of heart, liver, and kidney were preserved either in liquid nitrogen and transported back to permanent storage at −80 C or were frozen immediately at −80 C. Skins and skeletons were prepared using standard procedures (Yates et al., 1996).

Adult filarioid nematodes isolated from the abdominal or thoracic regions of freshly killed hosts were fixed in glacial acetic acid and stored in 10% formalin or 70% ethanol. For study, nematodes were cleared gradually by evaporation of a 70% ethanol, 2% glycerol, and 2% lactic acid solution over a period of 5–7 days (Brant and Gardner, 1997).

To develop the character matrix for a phylogenetic analysis, quantitative and qualitative data were recorded using a calibrated ocular micrometer on a Zeiss Ultraphot microscope. Video images were captured and measured with a computer-aided image measurement system (Jandel JAVA™).

Phylogenetic analysis

We analyzed characters that traditionally are used to diagnose species of *Litomosoides*. Twenty-four taxa were included in the analysis (Tables I, II) of which the following 9 were identified from our field collection and used to verify the published descriptions: *Litomosoides brasiliensis* Lins de Almeida, 1936, *Litomosoides chandleri* Esslinger, 1973, *Litomosoides guiterasi* (Vigueras, 1934), *Litomosoides ctenomyos* Brant and Gardner, 1997, *Litomosoides esslingeri* (Esslinger, 1973), *Litomosoides andersoni* Brant and Gardner, 1997, *Litomosoides thomomydis* Gardner and Schmidt, 1986, *Litomosoides westi* Gardner and Schmidt, 1986. Character states for *Litomosa americana* were taken from specimens collected from *N. humeralis* in eastern Nebraska and character states for the remaining outgroups were taken from the literature (Boulenger, 1924; Vaz, 1934; Bain and Hocquet, 1968; Petit, 1980).

The morphological character states for the remaining 15 taxa were taken from the following references: Chandler, 1931; Sandground, 1934; Chitwood, 1938; Caballero and Caballero, 1939, 1944, 1947; Bain and Durette-Desset, 1973; Esslinger, 1973; Forrester and Kinsella, 1973; Padilha and de Faria, 1977; Bain et al., 1980, 1982, 1989; Muller, 1980; Gardner and Schmidt, 1986; Brant and Gardner, 1997; de Moraes Neto et al., 1997. *Litomosoides* sp. Chitwood, 1938, *Litomosoides circularis* Linstow, 1899, *Litomosoides fosteri* Caballero and Caballero, 1947, *Litomosoides leonilavazquezae* Caballero and Caballero, 1939, *Litomosoides hamletti* Sandground, 1934, *Litomosoides patersoni* Mazza, 1928, *Litomosoides silvai* Padilha and de Faria, 1977, and *Litomosoides artibei* Esslinger, 1973 were omitted from the phylogenetic analysis because of inadequate species descriptions. *Litomosoides caliensis* Esslinger, 1973 and *Litomosoides colombiensis* Esslinger, 1973 were omitted also as they were described only from microfilariae.

Lack of an explicit hypothesis of relationships for species of *Litomosoides* has left the choice of outgroups unclear; however, Bain et al. (1980, 1982, 1989, 1991) and Xie et al. (1994) suggested that the nearest relatives of the genus *Litomosoides* could be the genera *Acanthocheilonema* Cobbold, 1870 (parasites of insectivores, nearctic carnivores, pinnipeds, and some rodents), *Ackertia* (Chabaud and Anderson, 1959) (parasites of South American rodents), and *Litomosa* (parasites of World bats). For our analysis, 4 species from these 3 genera were used as outgroups, including: *Acanthocheilonema evansi* Boulenger, 1924, *Ackertia dorsti* Bain and Hocquet, 1968, *L. americana*, and *Litomosa hugoti* Petit, 1980.

A matrix representing 22 morphological characters (Table II) was used for the phylogenetic analysis. Characters were treated as unordered and were initially unweighted and coded as either binary or multistate. Character states were either scored 0 if the state occurred in the outgroup or scored as the alternatives 1 and 2. Character states found to be unclear in the literature were scored as ambiguous (?). The data matrix was analyzed using PAUP 3.1.1 (Swofford, 1993) and the character state distributions were investigated using MacClade version 3.01 (Maddison and Maddison, 1992). Heuristic searches in PAUP were performed using the following options: stepwise addition, addition sequence random with 1,000 repetitions, TBR, MULPARS holding 1 tree at each step, and character state optimization ACCTRAN.

RESULTS

Character analysis

From adult filarioid nematodes, 22 morphological characters were coded and are listed below in the order that they appear in the character matrix (Fig. 4; Table II):

1. Shape of head (0= blunt [Fig. 4A]; 1 = attenuated [Fig. 4B]).
2. Symmetry of arrangement of cephalic papillae (0 = sym-

TABLE II. Character states for adults of species of the genus *Litomosoides* and outgroups.*

Taxa	Characters																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Litomosoides baretti	1	0	1	0	1	1	1	1	1	0	0	1	1	1	0	0	1	0	1	1	1	1
Litomosoides brasiliensis	1	1	0	0	1	0	0	1	1	1	0	0	1	1	1	0	1	1	1	1	2	1
Litomosoides carinii	1	1	0	1	1	1	1	1	1	1	0	0	1	1	1	0	1	1	2	?	2	1
Litomosoides chandleri	1	0	1	0	1	0	0	1	1	1	1	1	1	1	0	1	1	0	0	1	2	1
Litomosoides esslingeri	1	0	0	0	1	1	0	1	1	1	0	1	1	1	0	0	1	0	1	1	1	1
Litomosoides guiterasi	1	0	0	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	2	1
Litomosoides hoplomyis	1	?	0	1	1	1	?	1	1	1	0	1	1	1	0	0	1	1	0	?	1	1
Litomosoides kohnae	1	1	0	1	1	0	1	1	1	0	0	1	1	1	0	0	1	0	1	0	1	1
Litomosoides legerae	1	0	0	0	1	0	0	1	1	0	0	1	1	1	0	0	1	0	1	0	1	1
Litomosoides molossi	1	?	0	?	1	1	?	1	1	1	0	1	1	1	0	1	1	1	2	?	2	0
Litomosoides petteri	1	0	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	0	0	0	2	0
Litomosoides scotti	1	0	1	0	1	0	0	1	1	0	0	1	1	1	?	0	1	1	0	?	2	1
Litomosoides sigmodontis	1	0	0	0	1	0	1	1	1	0	0	1	1	1	0	0	1	1	1	0	1	1
Litomosoides teshi	1	?	1	0	1	0	?	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1
Litomosoides thomomydis	0	0	1	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	2	?	0	1
Litomosoides westi	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	2	?	0	1
Litomosoides ctenomyos	1	0	1	1	1	0	1	1	1	0	0	1	1	1	0	0	1	1	1	1	1	1
Litomosoides andersoni	1	0	1	1	0	0	1	1	0	0	0	1	1	1	0	0	1	0	2	?	2	1
Litomosoides galizai	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	0	1	0	1	0	1	1
Litomosoides chagasfilhoi	1	1	0	0	1	1	1	1	1	0	0	1	1	1	0	0	1	0	1	1	1	1
Litomosa hugoti	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Acanthocheilonema evansi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Litomosa americana	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	2	?	0	1
Ackertia dorsti	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	?	0	0

* Character numbers correspond to those listed in results. Character states are as follows: 0 = plesiomorphic state; 1 and 2 = apomorphic state; ? = ambiguous.

TABLE III. List of each character and the corresponding consistency index (CI) after reweighting of characters from the original set of 20 most parsimonious trees.

	Character	CI
1.	Shape of the head	1.000
2.	Symmetry of cephalic papillae	0.333
3.	Lateral cephalic papillae	0.250
4.	Circumstomal papillae	0.143
5.	Shape of buccal capsule	1.000
6.	Cuticularization of buccal capsule	0.200
7.	Segments of buccal capsule	0.143
8.	Buccal capsule in esophagus	0.500
9.	Walls of buccal capsule	1.000
10.	Esophagus	0.250
11.	Position of vulva	0.250
12.	Shape of ovijector	0.333
13.	Shape of female tail	1.000
14.	Tail digits of female	1.000
15.	Ovaries in tail	0.200
16.	Cuticular tubercles on female tail	0.500
17.	Precloacal papillae on male	1.000
18.	Pericloacal papillae	0.200
19.	Number of postcloacal papillae	0.333
20.	Arrangement of postcloacal papillae	0.500
21.	Type of right and left spicule	1.000
22.	Total length ratio of right and left spicule	0.333

metrical [Fig. 4C]; 1 = asymmetrical [Fig. 4D]). Symmetrical arrangement of these papillae is considered ancestral (Anderson, 1968). The evolutionary trend suggested by many studies indicates a general reduction and fusing of cephalic papillae, with posterior migration of the remaining papillae, and asymmetrical arrangement, as noted in more derived forms (Anderson, 1968; Chitwood and Chitwood, 1974; Anderson and Bain, 1976; Willmott, 1981).

3. Cephalic papillae (0 = present [Fig. 4C]; 1 = reduced [Fig. 4E]). These papillae are well developed along the sides of the cephalic region in ancestral nematodes (Anderson, 1968).
4. Externolateral papillae (0 = 4 [Fig. 4E]; 1 = less than 4 [Fig. 4D]). These papillae occur in variable numbers forming a ring around the mouth.
5. Shape of stoma in longitudinal section (0 = triangular [Fig. 4A]; 1 = cylindrical [Fig. 4F]). A cylindrical stoma has long, cuticularized walls. A triangular stoma has its apex directed anteriad.
6. Relative thickness of stoma wall (0 = wall robust, thick [Fig. 4F]; 1 = wall thin [Fig. 4G]).
7. Segments of stoma (0 = present [Fig. 4H]; 1 = fused [Fig. 4F]). The segments of the stoma resemble the stoma segments of rhabditoid nematodes considered an ancestral group from which most of the present nematodes have diverged (Chitwood and Chitwood, 1974).
8. Stoma in esophagus (0 = embedded [Fig. 4A]; 1 = partially embedded [Fig. 4F]). In some of the taxa the stoma is enclosed completely by the esophagus.
9. Outer wall surface of the stoma (0 = outer walls smooth [Fig. 4B]; 1 = outer walls thickened irregularly [Fig. 4F]). In the ingroup, the stoma walls are smooth or have several constrictions, appearing bumpy.
10. Esophagus (0 = muscular; 1 = muscular/glandular). A fully muscular esophagus is considered ancestral (Bain et al., 1982). The derived condition exhibits a muscular portion of the esophagus that gradually changes to glandular toward the esophageal–intestinal junction.
11. Position of vulva (0 = posterior to esophageal–intestinal junction; 1 = in region of esophagus). The anteriad migration of the vulva toward the oral opening is considered a derived trait in the tissue-dwelling filarioid nematodes (Vaz, 1934; Bain and Durette-Desset, 1973).
12. Shape of ovijector (0 = with median constriction; 1 = rounded and muscular, no constriction) (Gardner and Schmidt, 1986; Brant and Gardner, 1997).
13. Shape of female tail (0 = blunt [Fig. 4I]; 1 = attenuated [Fig. 4J]).
14. Female caudal lappets (0 = present [Fig. 4I]; 1 = absent [Fig. 4J]). Tail digits are finger-like projections on the terminus of the tail.
15. Ovaries extending into the tail (0 = absent; 1 = present).
16. Cuticular tubercles on female tail (0 = absent; 1 = present). Minute tubercles found on cuticle of the distal portion of the female tail (Esslinger, 1973).
17. Precloacal papillae (0 = present; 1 = reduced [Fig. 4K]). These papillae occur anterior to the cloaca in males. The basic number of caudal papillae in secernentean nematodes is 10 pairs plus an unpaired precloacal papilla (Bain and Durette-Desset, 1973; Chabaud and Bain, 1994).
18. Pericloacal papillae (0 = present [Fig. 4K]; 1 = absent). These papillae occur in the immediate area of the cloaca in males. Possession of numerous papillae is considered ancestral (Bain and Durette-Desset, 1973; Chabaud and Bain, 1994).
19. Number of postcloacal papillae (0 = 8 [Fig. 4L]; 1 = 9–12; 2 = none [Fig. 4O]). The presence of 4 pairs of postcloacal papillae in males is considered ancestral (Bain and Durette-Desset, 1973; Chabaud and Bain, 1994).
20. Arrangement of postcloacal papillae on male tail (0 = asymmetrical [Fig. 4M]; 1 = symmetrical [Fig. 4L]; 2 = no papillae [Fig. 4O]). If the arrangement of these papillae on the ventral side of the male was symmetrical, then papillae were arranged in pairs rather than randomly. Taxa that do not possess papillae were scored as ambiguous.
21. Shape of right and left spicules (0 = outgroup state; 1 = *sigmodontis*-type [Fig. 4N]; 2 = *carinii*-type [Fig. 4O]). This character has been studied and described extensively (see Bain et al., 1989; Brant and Gardner, 1997). The outgroup state was any description of a spicule that could not be placed in either the *sigmodontis*- or the *carinii*-type. The *carinii*-type (e.g., *Litomosoides carinii* and *Litomosoides scotti*) is described by Bain et al. (1989: 285) translated as: ''The right spicule is sclerified to the distal extreme with a subterminal flange well marked on the dorsal side that may define a terminal hood (e.g., *L. carinii*); the talon is robust. The blade of the left spicule is composed of a simple, well sclerified portion; the handle is very membranous and is not well defined in *L. scotti.* The blade is shorter than the handle.'' The *sigmodontis*-type (e.g., *Litomosoides sigmodontis*) is described by Bain et al.

(1989: 285) translated as: ''The right spicule is poorly sclerified as the distal region is slender and tapering sustained by two fine cuticular borders ending in one short membranous partition that has generally a dorsal coil upon the spicule; frequently, the talon does not form a well defined flange or sclerified apical hood, and is shorter and narrower than the carinii type. The blade of the left spicule has a sclerified axis that is longer than the handle; the anterior half of the handle is bordered by large, membranous, longitudinally pleating alae that are visible without dissection.''

22. Ratio of Length of right spicule to left spicule (0 = 1.0–2.5; 1 = 3.0–7.0). In the specimens examined, as well as in the literature, there was a consistent gap between ratios of 2.5 and 3.0.

PHYLOGENETIC ANALYSIS

The cladistic analysis produced 20 most parsimonious trees, length = 67, consistency index (CI) = 0.3582 (Kluge and Farris, 1969). The strict consensus tree is shown in Figure 2. As defined, species assigned to *Litomosoides* do not form a monophyletic group because 2 of the ingroup taxa (*L. thomomydis* and *L. westi*) are grouped with the outgroup. Characters 1 (shape of the head), 5 (shape of the stoma), 9 (walls of the stoma), and 17 (precloacal papillae on male) are uniquely derived and unreversed (CI = 1 in all 20 trees). Branch A, supporting the ingroup (except *L. thomomydis* and *L. westi*) is defined by 5 characters of which only character 1 (shape of the head) is uniquely derived and unreversed. Characters 5 (shape of the stoma) and 9 (walls of stoma) support branch B that separates *L. andersoni* from the rest of the species in the genus.

Because of the amount of homoplasy present in the data set, characters were reweighted a posteriori using the maximum value of the rescaled CI for each character (Farris, 1969). Reweighting of the characters enabled us to assess the strength of each character and compare the resulting tree to the original 20 most parsimonious trees. This procedure resulted in a single tree with length = 15.34, CI = 0.67 that was different from the

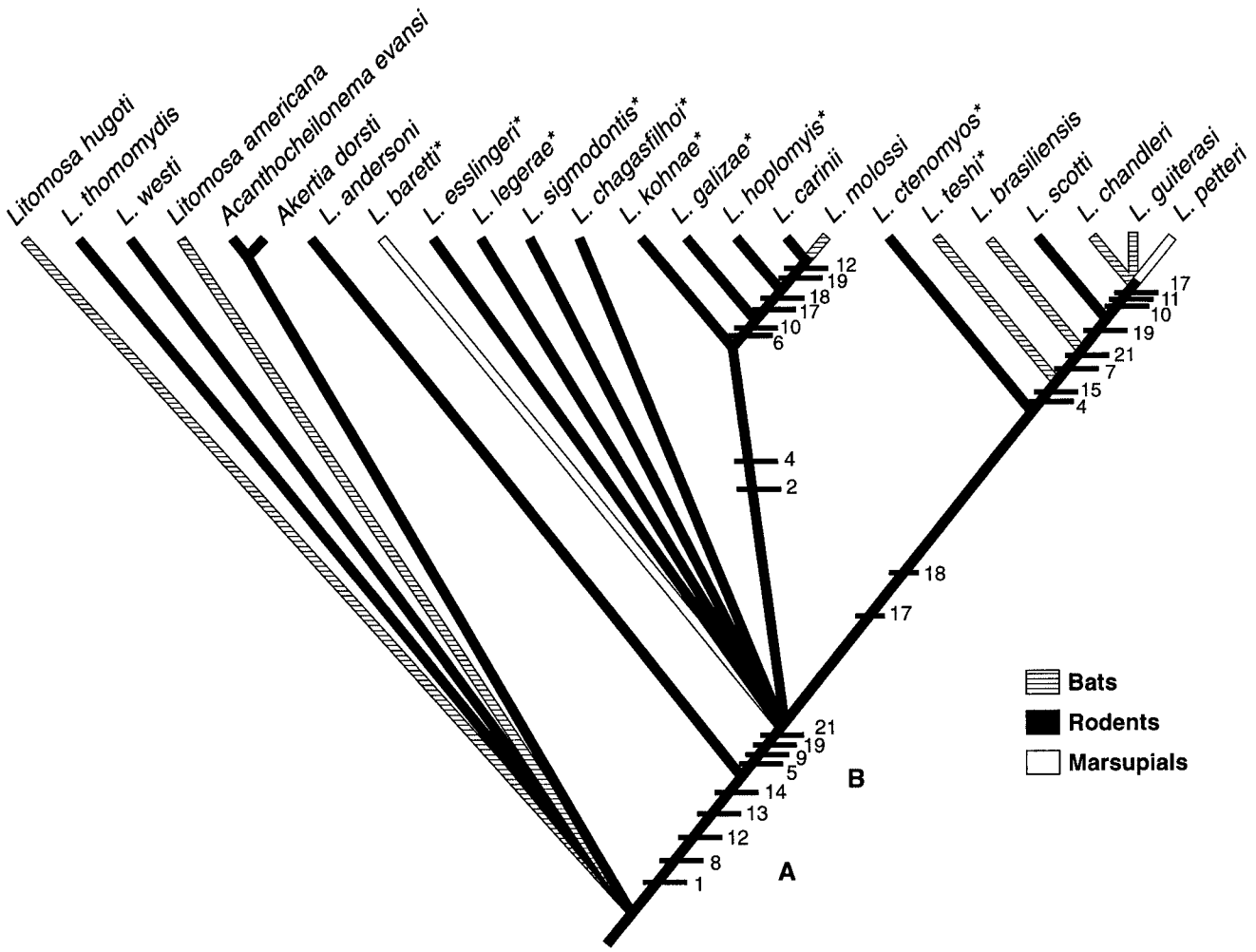

FIGURE 2. The strict consensus tree of the 20 most parsimonious trees, length = 67, generated from the cladistic analysis of the adult character data matrix presented in Table II. Species of *Litomosoides* are preceded by L. Characters are indicated following a dash and bolded number and unique shared derived characters are outlined. Letters correspond to clades discussed in the text. An asterisk denotes species of *Litomosoides* with the *sigmodontis*-type of spicule. Indicated by branch shading, the host group is mapped onto the cladogram for each species of *Litomosoides*. See Table I for the host group and distribution for each species of *Litomosoides*.

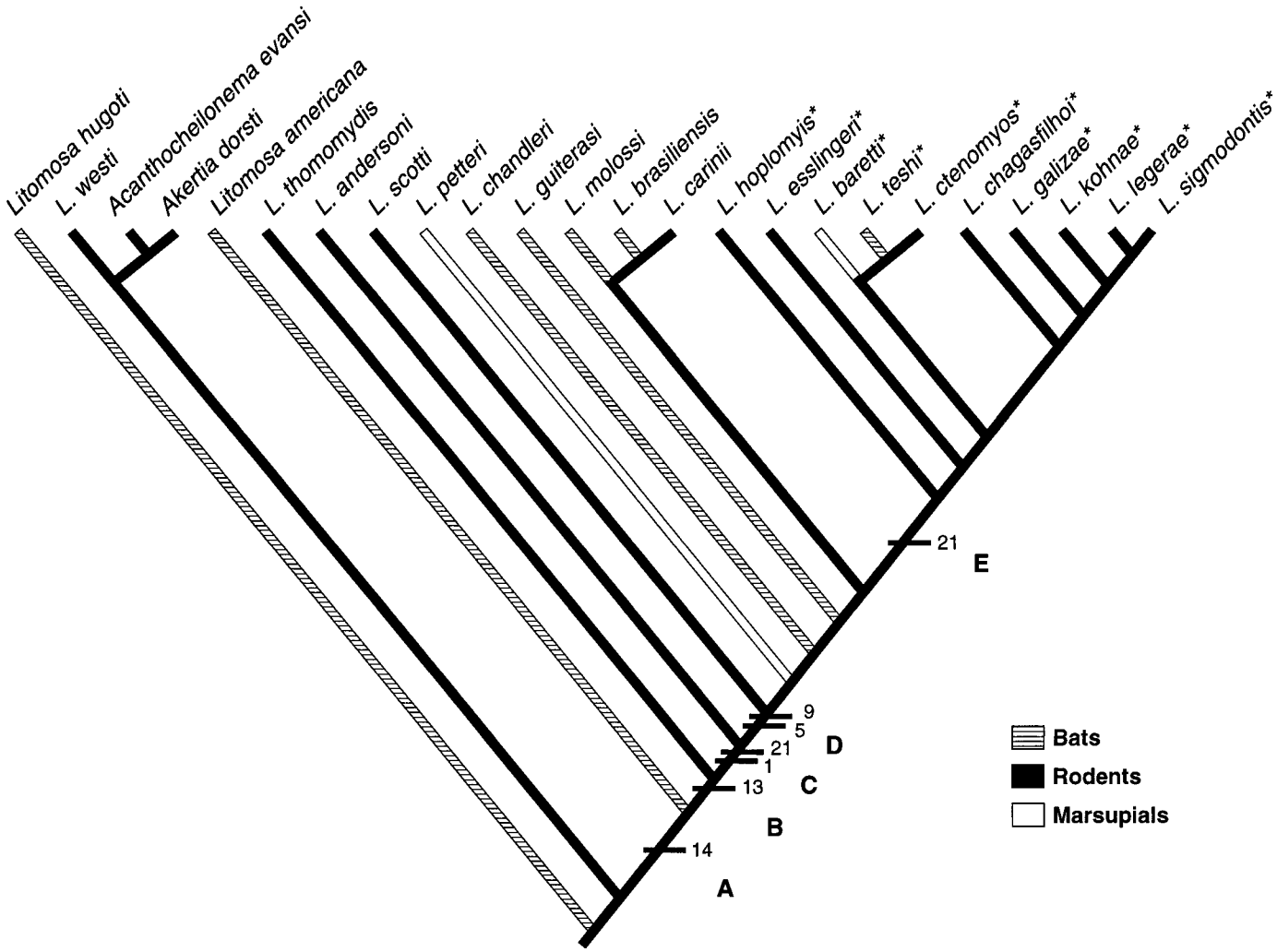

FIGURE 3. The reweighted tree obtained by successive approximations, starting with the 20 parsimonious trees (length = 15.4), generated from analysis of the adult characters listed in Tables II and III. Characters are indicated following a dash; numbers and letters correspond to the characters in the results section. An asterisk denotes species of *Litomosoides* with the *sigmodontis*-type of spicule. Indicated by branch shading, the host group is mapped onto the cladogram for each species of *Litomosoides.*

original set of 20 trees (Fig. 3). However, in agreement with the set of 20 most parsimonious trees, the reweighted tree also fails to support *Litomosoides* and *Litomosa* as monophyletic groups.

In Table III, characters 1, 5, 9, 13, 14, 17, and 21 have a reweighted CI = 1 that support the following groups in Figure 3: Branch A, character 14 (tail digits of female) supports the ingroup taxa excluding *L. westi* but including *L. americana.* Branch B, supported by character 13 (shape of female tail), excludes *L. americana*, grouping the remaining ingroup taxa. Branch C, supported by characters 1 (shape of the head) and 21 (type of right and left spicule), placed *L. thomomydis* as the sister group to the rest of species of *Litomosoides*. *Litomosoides thomomydis* is basal in both the reweighted tree and the strict consensus tree. Supported by characters 5 (shape of the stoma) and 9 (walls of stoma), branch D is the single branch uniting the same taxa as the strict consensus tree (Fig. 2, branch B). Finally, branch E, supported by character 21 (type of right and left spicules), defines a clade that corresponds to the *sigmodontis*-type of spicules. The *carinii*-type of spicules is ancestral in both the strict consensus and the reweighted trees. The reweighted tree partially resolves the polytomy in the outgroup present in the strict consensus tree; *L. westi* is united by character 8 (stoma in esophagus) with *A. evansi* and *A. dorsti*; and both species of *Litomosa* fail to form a monophyletic group in this tree and in the strict consensus tree (Figs. 2, 3).

To test the null hypothesis of a host–parasite coevolution, a constraint tree was created that forced the ingroup taxa to group according to their host affiliation such that all nematodes parasitizing each group (order) of mammals form a monophyletic group (Fig. 1A). Enforcing this constraint on the original data set resulted in 102 equally parsimonious trees, length = 80, CI = 0.30 (trees not shown). Using the Templeton test (Templeton, 1983), parsimony scores of these 102 trees were compared to the original 20 trees, length = 67 (13 steps shorter). All constraint trees were significantly worse ($P < 0.05$) than the 20 most parsimonious trees. Enforcing the host constraint on the reweighted data-set resulted in 150 equally parsimonious trees,

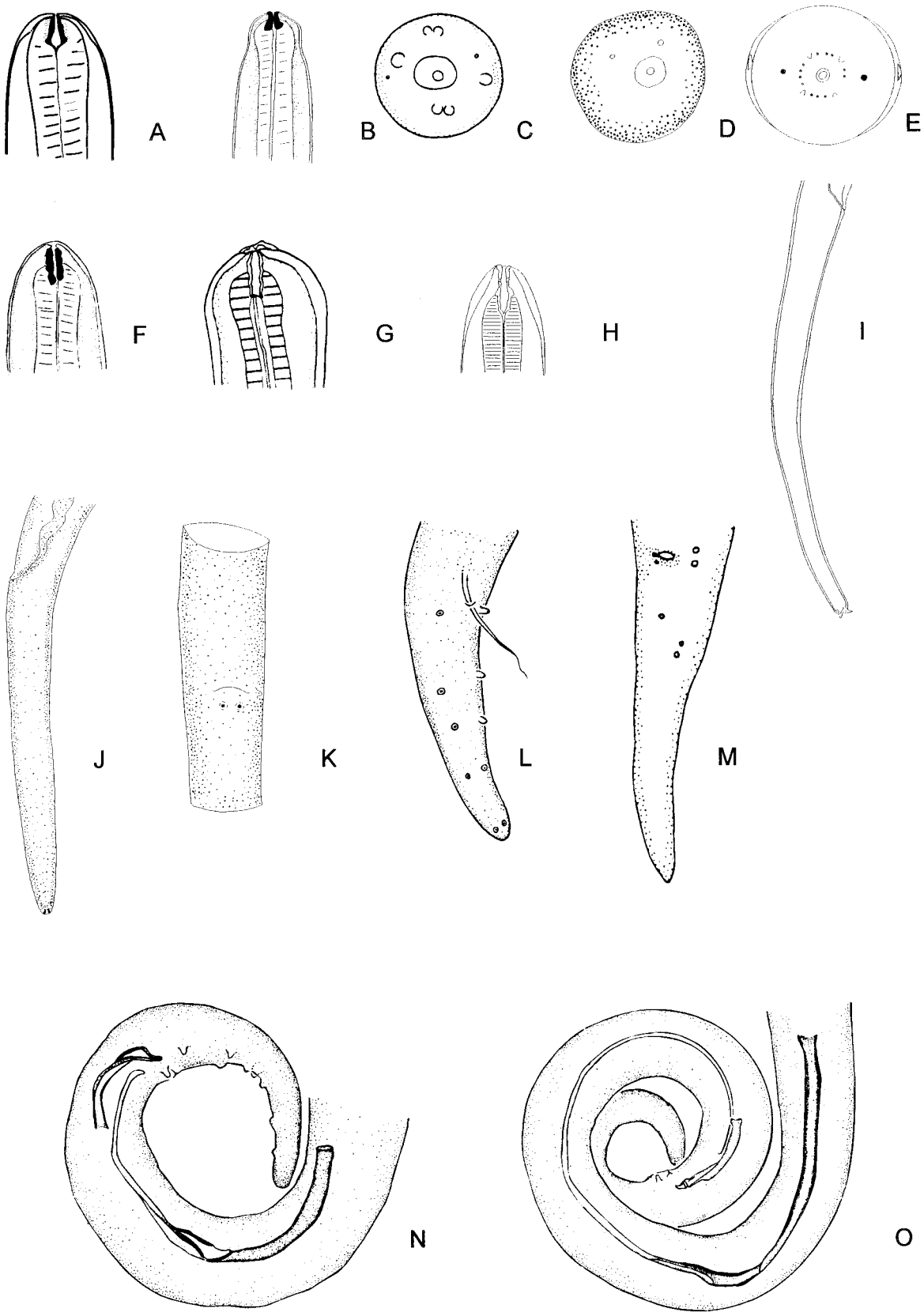

FIGURE 4. Diagrams of some of the characters used in the phylogenetic analysis. Figures B, D, F, J, K, L, N, and O modified from Brant and Gardner (1997). Figure C modified from Forrester and Kinsella (1973). Figure M modified from Bain et al. (1980). Figures A, E, and I adapted from Gardner and Schmidt (1986). Figure H modified from Bain et al. (1980).

length = 23.4 (trees not shown). Using the Templeton test, these 150 trees were compared to the reweighted tree (length = 15.36) and all constraint trees were significantly worse $P < 0.02$ (trees not shown).

DISCUSSION

Based on this analysis, we conclude that species of the genus *Litomosoides,* as defined at present, do not form a monophyletic group. Moreover, reweighting of the characters resulted in a single tree that was significantly worse relative to each of the original 20 most parsimonious trees, indicating character homoplasy. On examination of the characters in Table II and of the character CIs in Table III, these incongruences become evident because there are few synapomorphies (characters 1, 5, 9, and 17) and no single character supports the monophyly of all ingroup taxa.

Six characters contained phylogenetic information and had been considered previously stable by Bain et al. (1989) as diagnostic for species of *Litomosoides*: shape of spicules (character 21), form of the stoma (characters 5, 9), and presence and disposition of the caudal papillae of the male (characters 17, 19, 20) (Sandground, 1934; Bain et al., 1989). Chitwood and Chitwood (1974) considered the stoma too variable to use as a character for differentiation among filarioid nematodes. Bain et al. (1989) defined 2 groups of *Litomosoides* that correspond to the structure of both the right and left spicule; *sigmodontis*-type and *carinii*-type (character 21). Both of these types of spicules are found in nematodes occurring in marsupials, rodents, and bats with geographic distributions in both North and South America. The strict consensus tree did not show distinct monophyletic groups of either spicule type and did not correspond to host monophyly or to the geographic distributions of the parasites (Fig. 2; Table I). In the tree constructed with character reweighting, all taxa with the *sigmodontis*-type of spicule were placed in a derived monophyletic group, suggesting that the *carinii*-type of spicule may be the ancestral condition (Fig. 3, clade E).

Caudal papillae in the males have also been regarded as significant and stable in the evolution and classification of filarioid nematodes. As these nematodes evolved, there was evidently a migration of the anterior precloacal and pericloacal papillae to the postcloacal position (Sandground, 1934; Bain and Durette-Desset, 1973; Esslinger, 1973; Bain, 1981; Bain et al., 1989; Chabaud and Bain, 1994). Our results show that these 2 characters, the arrangement and number of postcloacal papillae (characters 19 and 20), contain little or no phylogenetic information in hypothesizing relationships among *Litomosoides.*

Bain et al. (1989) considered the structure of the stoma to be the most significant morphological character that can be used to differentiate members of this genus from other members of Onchocercidae and within *Litomosoides.* In both the unweighted strict consensus tree and the reweighted tree characters 5 and 9 (shape and walls of stoma, respectively) grouped a majority of the ingroup taxa, excluding 3 taxa that are morphologically distinct from other members of *Litomosoides* (*L. andersoni, L. thomomydis,* and *L. westi*). Our analysis supports the assertion of Bain et al. (1989). The incompatibility of the set of 20 most parsimonious trees and the reweighted tree suggests that most of the characters presently used for species diagnosis lack much phylogenetic information. Not all characters are of equal value in elucidating relationships among taxa, and the decision of what characters to emphasize and the weight given to these characters is subject to debate (Goloboff, 1993).

Litomosoides thomomydis and *L. westi* (both parasites of rodents of the family Geomyidae in Central North America) share morphological similarities (spicules and stoma) with the genus *Litomosa* (Bain et al., 1989), but they did not group with either *L. hugoti* (Old World distribution in bats) or *L. americana* (Nearctic distribution in 1 species of bat). Our results indicate (as suggested by Bain et al. [1989]) that *L. thomomydis* and *L. westi* do not belong to the genus *Litomosoides.* In addition, parasite taxa found in marsupials were not basal clades, indicating they were not the ancestral hosts of *Litomosoides.* Furthermore, *Litomosoides baretti* and *Litomosoides petteri,* both found in the marsupial *Marmosa cinerea* in Brazil, were not sister species and were placed in distant positions on the consensus tree (Figs. 2 and 3). The analysis enforcing a topological host constraint, supporting the monophyly of parasites of marsupials, rodents, and bats, yielded significantly longer trees indicating that host switching was the primary mode of evolution in species of the genus *Litomosoides* (Fig. 1B).

Bain et al. (1991) postulated that *Litomosoides* were fundamentally parasites of South American bats that switched subsequently into rodents. On the strict consensus tree, the host of the basal taxon, *L. andersoni,* is an hystricognath rodent, suggesting that these nematodes may have originated at an early stage in the evolution of this host group and then diversified subsequently in bats (Figs. 2, 3). Additionally, our results show the most basal taxa on the tree are found in rodents and a marsupial and not in bats.

Species included presently in *Litomosoides* do not comprise a monophyletic group, and our analysis does not support either of the above hypotheses. However, within a phylogenetic framework, our results agree with the assertions of Bain et al. (1989) indicating that host switching is the primary process that shaped the current patterns of distribution of species of the genus *Litomosoides* among New World mammals (Fig. 1B) but disagree with the host of origin as a bat. Knowledge of the appropriate intermediate host species for each species of *Litomosoides* is probably critical to evaluate further the mode of evolution shaping this host–parasite association.

Our results are the first attempt at reconstructing the phylogenetic relationships among the species of *Litomosoides.* The paucity of morphological characters in this group and the extent of homoplasy evident from the analysis indicates that robust phylogenetic analyses depending only on morphology will be difficult. Future efforts in the direction of developing biological databases of DNA sequences should improve our resolution and ability to provide a more accurate history of the relationships between these filarioid nematodes and their hosts.

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