THE ANT GENUS *LEPTANILLOIDES*: DISCOVERY OF THE MALE AND EVALUATION OF PHYLOGENETIC RELATIONSHIPS BASED ON DNA SEQUENCE DATA

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ABSTRACT

The male of the ant genus *Leptanilloides* is described, based on two specimens collected as isolated individuals in sweep samples from montane Costa Rica. Although not associated with ant workers or queens, these males are identified as *Leptanilloides mckennae* Longino on the basis of DNA sequence similarity to a reference worker of this species from Costa Rica. The male is slightly smaller than the worker, and has most traits expected of the dorylomorph group, including tergosternal fusion of abdominal segments 2 and 3 (but not 4); exposed spiracles on abdominal segments 5 and 6; a simplified volsella, lacking a cuspis; and absence of cerci. Additional attributes include: mandibles elongate-triangular and edentate; anterior clypeal lamella well developed; pronotum triangular in profile and much narrowed anteromedially; mesopleuron lacking posterior oblique sulcus; postpetiole absent; and wing venation reduced (forewing with pterostigma and one submarginal cell, but no discal cell). A molecular phylogenetic analysis, based on 5.1 kb of sequence data from five nuclear genes (18S rDNA, 28S rDNA, wingless, abdominal-A, and LW-rhodopsin), provides strong independent evidence that *Leptanilloides* is a member of the dorylomorph clade (parsimony bootstrap support of 100%; Bayesian posterior probability of 1.00), although relationships among taxa within that group remain poorly resolved. The dorylomorphs are nested within the formicoid clade, a very well supported group that contains the majority of extant ant subfamilies. Contrary to some previous suggestions *Leptanilloides* is not closely related to *Leptanilla*. The latter appears to stand well outside the formicoid clade, as sister taxon to all other extant ants sampled in this study (72% parsimony bootstrap, 1.00 posterior probability). The results highlight the repeated convergent evolution of similar morphology among subterranean ants.

Key words: Hymenoptera, Formicidae, phylogeny, morphology, dorylomorphs, doryline section, *Leptanilla*, subterranean ants, 18S rDNA, 28S rDNA, wingless, abdominal-A, LW-rhodopsin

INTRODUCTION

The study of ant assemblages living in subterranean habitats is one of the more exciting frontiers in myrmecology. Underground environments seem to harbor a disproportionate share of morphologically peculiar and phylogenetically interesting formicids (Brown *et al.*, 1971; Bolton, 1990b; López *et al.*, 1994; Ward, 1994). The ant subfamily Leptanilloidinae is one such group. It comprises two genera of small, blind ants, whose biology, distribution, and phylogenetic relationships are poorly understood (Bolton, 1990a, 2003; Brandão *et al.*, 1999; Brady & Ward, 2005). Confined to the New World, these ants are rarely collected and are assumed to be hypogaeic predators, possibly with legionary habits.

For many years the group was known only from a single species, *Leptanilloides biconstricta* Mann (1923), described from Bolivia. The genus came to be placed in the tribe Cerapachyini (Brown, 1975; Bolton, 1990a), and later in its own subfamily (Bolton, 1992). Our knowledge of the group was considerably expanded by Brandão *et al.* (1999) who revised the subfamily and described six new South American species, three of which were assigned to a new genus, *Asphinctanilloides*. Longino (2003) described another new species, *Leptanilloides mckennae*, from Costa Rica. This not only extended the known range of the subfamily from South America to Central America, but also blurred the morphological distinctions between the two genera.

The phylogenetic position of Leptanilloidinae has been a point of uncertainty. Mann (1923) and Borgmeier (1955) noted similarities to the Old World subfamily Leptanillinae, and to New World army ants (Ecitoninae). Brown (1975) considered *Leptanilloides* to be a ponerine ant in the tribe Cerapachyini, while Bolton (1990a, 1990c, 2003) provided compelling morphological evidence that it belongs with the dorylomorphs (previously known as the doryline section), a well defined monophyletic group that includes both cerapachyines and army ants, but not Leptanillinae or Ponerinae. A recent morphological phylogenetic analysis of the dorylomorphs (Brady & Ward, 2005) also concluded that Leptanilloidinae is part of this group, although a close association with Leptanillinae could not be confidently rejected in that study.

Until recently all collections of *Leptanilloides* and *Asphinctanilloides* have consisted of workers only, with males and queens being unknown. This paper provides the first description of leptanilloidine males (see also postscript at the end of this paper). Although the specimens were taken in sweep samples and were not associated with any specific ant colonies, their identity as *Leptanilloides* was confirmed through comparative analysis of DNA sequence data.

The males in question were brought to my attention by John T. ("Jack") Longino, who encountered them when processing ants from Costa Rican sweep samples collected by John Noyes. The males (two in number) were recognized as being unusual and it was hypothesized that they represented the hitherto unknown males of *Leptanilloides*. Since the samples were preserved in ethanol, and since workers of *L. mckennae* were also available in ethanol, the opportunity was presented to test this hypothesis through comparison of DNA sequences.

Once the identity of the males as *Leptanilloides* was resolved, a broader inquiry into the phylogenetic position of the genus was undertaken, using sequence data from five nuclear genes. This yielded a number of interesting results, not only concerning *Leptanilloides* but also regarding the phylogenetic relationships of other ant groups, including the enigmatic subfamily Leptanillinae.

MATERIALS AND METHODS

Critical material for this study was supplied by Jack Longino: an ethanol-preserved worker of *Leptanilloides mckennae* from Monteverde, Costa Rica (July 1995; leg. D. McKenna), and two unidentified male ants from Costa Rica, suspected to be *Leptanilloides*, and also held in ethanol. The males were collected at Estación Cacao, Prov. Guanacaste (22 February 2003; leg. J. S. Noyes), and Sendero Pilón, P. N. Arenal, Prov. Alajuela (26 February 2003; leg. J. S. Noyes),

respectively. A fresh, ethanol-preserved worker of a second species, *Leptanilloides nomada* Donoso, Vieira & Wild (2006) from Cotopaxi, Ecuador was provided by Alex Wild.

One of the two males, from Estación Cacao, was sacrificed for DNA extraction and sequencing, but the head, wings, and genitalia of this specimen were retained for morphological study and for comparison with the second male. Twenty-seven additional ant species were targeted for sequencing (Table 1). These were chosen to provide broad coverage of higher-level ant diversity, including nine other representatives of the dorylomorph group (belonging to the genera *Acanthostichus*, *Aenictus*, *Cerapachys*, *Cylindromyrmex*, *Dorylus*, *Neivamyrmex*, *Simopone*, and *Sphinctomyrmex*) and one representative of Leptanillinae (genus *Leptanilla*). Three non-formicid outgroups were also included: a vespid (*Mischocyttarus flavitarsis*), a bradynobaenid (*Chyphotes mellipes*), and an apid (*Apis mellifera*). All trees were rooted with *Apis mellifera*.

Molecular methods

DNA sequence data from five nuclear genes were utilized: the small subunit (18S) and large subunit (28S) ribosomal RNA genes, and the protein-encoding genes abdominal-A (*abd*-A), wingless (*wg*), and long wavelength rhodopsin (LW *Rh*). Seventy-eight new sequences were generated for this investigation, and the remainder were taken from Ward & Downie (2005). Methods of DNA extraction, amplification, purification, and sequencing followed Ward & Downie (2005). Sequences were collated with Sequencher (version 4.0.5), aligned with Clustal X version 1.8 (Thompson *et al.*, 1997), and manually edited with MacClade 4 (Maddison & Maddison, 2000). GenBank accession numbers for each sequence are given in Table 1.

Phylogenetic analysis

An intron in the LW *Rh* gene fragment and hypervariable regions of the 28S gene were removed prior to phylogenetic analyses, due to alignment difficulties. After exclusion of these sites the five-gene dataset consisted of 5135 characters (base pairs), of which 1336 were variable and 950 were parsimony-informative. Parsimony searches were carried out with PAUP* (version 4.0b10) (Swofford, 2003), using TBR branch swapping and 1000 random taxon additions. Gaps were treated as missing data. Bootstrap values were calculated using 1000 TBR replicates and 10 random taxon additions per replicate. Bayesian estimation of the phylogeny was conducted with Mr. Bayes v3b4 (Huelsenbeck & Ronquist, 2001), using a partitioned model as described in Ward & Downie (2005). The MCMC (Markov chain Monte Carlo) analysis was run for 10,000,000 generations, with sampling every 1000 generations, and a burn-in value of 1000 samples. The aligned data matrix used in phylogenetic analyses, with excluded sites identified, is available at TreeBase (M2144).

Metric measurements and indices

The following measurements (in mm) and indices were employed. Measurements were taken at 50× with a Wild M5A microscope and a Nikon dual-axis micrometer.

HL   Head length, measured in full-face (frontal) view, from the posterior margin to the midpoint of the anterior clypeal margin, excluding the clypeal apron.
HW   Maximum width of the head, including the eyes.
SL   Length of scape, excluding the basal condyle and neck.
EL   Length of compound eye, as seen in full-face view.
LHT   Length of the metatibia in dorsal view, excluding the medioproximal lobe.

Table 1. List of taxa included in the molecular phylogenetic analysis, with GenBank accession numbers.

Taxon	Locality	18S	28S	wg	LW Rh	Abd-A
Formicidae						
Amblyoponinae						
Amblyopone pallipes	California, USA	AY703487	AY703554	AY703621	AY703755	AY703688
Dolichoderinae						
Leptomyrmex erythrocephalus	New South Wales, AS	AY703494	AY703561	AY703628	AY703762	AY703695
Liometopum occidentale	California, USA	AY867449	AY867465	AY867434	AY867496	AY867481
Dorylomorph group						
Acanthostichus kirbyi	Rondônia, BR	AY867438	AY867454	AY867423	AY867485	AY867470
Aenictus eugenii	Swaziland	AY867439	AY867455	AY867424	AY867486	AY867471
Cerapachys augustae	Arizona, USA	AY867440	AY867456	AY867425	AY867487	AY867472
Cerapachys larvatus	Aust. Cap. Terr., AS	AY703491	AY703558	AY703625	AY703759	AY703692
Cylindromyrmex striatus	Bolívar, VZ	AY867441	AY867457	AY867426	AY867488	AY867473
Dorylus helvolus	KwaZulu-Natal, SF	AY867442	AY867458	AY867427	AY867489	AY867474
Leptanilloides mckennae	Puntarenas, CR	AY867451	AY867467	--	AY867498	--
Leptanilloides male	Guanacaste, CR	AY867444	AY867460	AY867429	AY867491	AY867476
Leptanilloides nomada	Cotopaxi, EC	AY867443	AY867459	AY867428	AY867490	AY867475
Neivamyrmex nigrescens	Arizona, USA	AY867445	AY867461	AY867430	AY867492	AY867477
Simopone marleyi	KwaZulu-Natal, SF	AY867446	AY867462	AY867431	AY867493	AY867478
Sphinctomyrmex steinheili	Aust. Cap. Terr., AS	AY867447	AY867463	AY867432	AY867494	AY867479
Ectatomminae						
Ectatomma opaciventre	São Paulo, BR	AY703492	AY703559	AY703626	AY703760	AY703693
Typhlomyrmex rogenhoferi	Loreto, PE	AY703496	AY703563	AY703630	AY703764	AY703697
Formicinae						
Camponotus sp. nr. *vicinus*	California, USA	AY867448	AY867464	AY867433	AY867495	AY867480
Formica moki	California, USA	AY703493	AY703560	AY703627	AY703761	AY703694
Leptanillinae						
Leptanilla sp.	Eastern Cape, SF	AY867436	AY867452	AY867421	AY867483	AY867468
Myrmeciinae						
Myrmecia pyriformis	South Australia, AS	AY703500	AY703567	AY703634	AY703768	AY703701
Nothomyrmecia macrops	South Australia, AS	AY703501	AY703568	AY703635	AY703769	AY703702
Myrmicinae						
Aphaenogaster occidentalis	California, USA	AY867450	AY867466	AY867435	AY867497	AY867482
Myrmica tahoensis	California, USA	AY703495	AY703562	AY703629	AY703763	AY703696
Paraponerinae						
Paraponera clavata	Maranhão, BR	AY703489	AY703556	AY703623	AY703757	AY703690
Ponerinae						
Hypoponera opacior	California, USA	AY703488	AY703555	AY703622	AY703756	AY703689
Platythyrea mocquerysi	Madagascar	AY867437	AY867453	AY867422	AY867484	AY867469
Proceratiinae						
Proceratium stictum	Queensland, AS	AY703490	AY703557	AY703624	AY703758	AY703691
Pseudomyrmecinae						
Pseudomyrmex gracilis	Guanacaste, CR	AY703529	AY703596	AY703663	AY703797	AY703730
Tetraponera punctulata	Northern Terr., AS	AY703514	AY703581	AY703648	AY703782	AY703715
other Vespoidea						
Chyphotes mellipes	California, USA	AY703485	AY703552	AY703619	AY703753	AY703686
Mischocyttarus flavitarsis	California, USA	AY703486	AY703553	AY703620	AY703754	AY703687
Apis mellifera	California, USA	AY703484	AY703551	AY703618	AY703752	AY703685

CI   Cephalic index: HW/HL.
REL   Relative eye length: EL/HL.
REL2   Relative eye length, using HW: EL/HW.
SI   Scape index: SL/HW.

IDENTIFICATION OF *LEPTANILLOIDES* MALE FROM DNA SEQUENCE DATA

The *Leptanilloides mckennae* worker readily yielded 18S and 28S sequences but it proved difficult to obtain single-copy nuclear gene sequences from this specimen, possibly because of its age and preservation history. It is likely that the worker had been stored at an ethanol concentration of less than 95% (Longino, pers. comm.). Using primers that amplified short stretches of DNA, I was able to obtain sequence for the LW *Rh* gene, but not for *wg* or *abd*-A. This dictated use of a three-gene data set (18S, 28S, and LW *Rh*) to compare the putative *Leptanilloides* male with the *L. mckennae* worker and with other taxa.

The results were unambiguous: the male proved to be identical to *Leptanilloides mckennae* at the 18S and 28S genes, and almost identical (0.18% sequence divergence) at LW-rhodopsin. The only difference detected at the LW-rhodopsin gene was a single base substitution in an intron, which was 90 bp long in both the male and the *L. mckennae* worker. (The intron was 84 bp long in *Leptanilloides nomada* from Ecuador.) Over the concatenated three-gene data set the male exhibited 0.02% sequence divergence from *Leptanilloides mckennae*, 0.79% from *Leptanilloides nomada*, and moderate to large differences with all other sampled ants, with uncorrected sequence divergences ranging from 3.31% (with *Acanthostichus kirbyi*) to 7.76% (with *Leptanilla* sp.) (Table 2). Parsimony analysis of the sequence data placed the male as sister to *Leptanilloides mckennae*, to the exclusion of *Leptanilloides nomada*, with 100% bootstrap support (results not shown).

Table 2. Percent sequence divergence (uncorrected) between the putative *Leptanilloides* male and selected other taxa, individually and for three genes combined.

			Gene		
Taxon	18S	28S	LW *Rh* exon	LW *Rh* intron	all 3 genes (incl. intron)
Leptanilloides mckennae	0.00	0.00	0.00	1.11	0.02
Leptanilloides nomada	0.27	0.50	2.62	8.24	0.79
Acanthostichus kirbyi	0.54	1.50	16.38	33.00	3.31
Cylindormyrmex striatus	0.65	1.83	15.72	41.91	3.60
Cerapachys augustae	0.49	2.05	18.34	52.47	4.22
Cerapachys larvatus	0.43	2.94	18.12	50.57	4.34
Simopone marleyi	0.49	3.17	16.59	39.61	4.07
Sphinctomyrmex steinheili	0.70	1.74	18.78	52.73	4.21
Neivamyrmex nigrescens	2.32	4.05	19.00	38.03	5.54
Aenictus eugenii	1.62	5.74	17.47	41.79	5.91
Dorylus helvolus	1.14	4.84	19.65	44.20	5.60
Ectatomma opaciventre	0.97	4.41	16.16	53.24	5.10
Nothomyrmecia macrops	0.89	4.84	19.21	50.92	5.54
Amblyopone pallipes	1.40	4.27	17.90	55.13	5.54
Leptanilla sp.	1.78	8.06	22.05	56.38	7.76

The possibility exists that the *Leptanilloides* male, while genetically very close to *L. mckennnae*, belongs to a different (unknown) species. Without more information about the

diversity of *Leptanilloides* in Costa Rica and the degree of interspecific sequence divergence in the genus, this possibility cannot be entirely dismissed. The virtual identity of the male with *L. mckennae*, contrasted with its substantial divergence from *Leptanilloides nomada*, argues against this interpretation. *L. nomada* is clearly not conspecific with *L. mckennae* but it appears to be more closely related to the latter than to most other described species of the genus. Specifically, it has the combination of a reduced postpetiole (smaller than petiole) and enlarged abdominal segments 4-6, separated by distinct constrictions, that sets *L. mckennae* apart from most other described species of *Leptanilloides*.

Thus I conclude that the sequenced male (from Estación Cacao) and the morphologically similar specimen (from P. N. Arenal) represent the male caste of *Leptanilloides mckennae* Longino.

DESCRIPTION OF *LEPTANILLOIDES MCKENNAE* MALE

Head broader than long, with large eyes that occupy the anterior half of sides of head; mandibles slender, elongate-triangular, and overlapping at closure (Figure 1), masticatory margin edentate and rounding into unarmed basal margin; external margin of mandible weakly concave; maxillary palps 2-segmented; labial palps difficult to discern *in situ* but apparently similarly reduced; genal teeth and hypostomal teeth lacking; anterior margin of clypeus with a prominent, subtriangular, translucent lamella, bluntly pointed medially and with an anterior-posterior extension at this midpoint that is subequal to scape width; posterolateral margin of clypeus well marked, but posteromedial boundary obscure; antennal sockets horizontal and exposed, and located close to the anterior clypeal margin; antenna 13-segmented, each segment much longer than wide; scape and ultimate antennal segment subequal in length, each $0.12\times$ total length of antenna and $2.2\times$ length of the second antennal segment; front of head immediately posterior to antennal sockets slightly depressed, and furnished medially with a low, blunt, longitudinal ridge, probably homologous with the elevated frontal carinae of the worker; lateral ocelli separated from median ocellus by about their diameters.

Mesosoma with usual complement of sclerites (Figure 2); pronotum U-shaped in dorsal view and reduced anteromedially to a thin horizontal strip, set well below the level of the mesonotum; pronotum triangular in profile, with pointed posterior apex; mesonotum lacking notauli, but with a darkened, weakly impressed anteromedial suture; parapsidal sutures present; parascutal carina poorly developed and terminating before the transcutal cleft; axillae not meeting medially, connected by a narrow furrow; tegula very small and inconspicuous; mesopleuron lacking oblique transverse sulcus and hence not divided into anepisternum and katepisternum, but lower third of mesopleuron with a broad longitudinal furrow (possibly an artifact of cuticular collapse); boundary between metapleuron and propodeum effaced posteriorly; metapleural gland reduced and inconspicuous; propodeal spiracle small, circular, positioned at about midheight of propodeum and as far back as the posterior extremity of the metanotum. Legs slender (LHT/HL ~1.5); mesotibia and metatibia each with a single spur, barbulate and pectinate, respectively; tarsal claws lacking preapical tooth.

Wings somewhat infuscated and with reduced venation (Figure 3); pterostigma present; forewing with one submarginal cell; closed discal (medial) cell lacking, i.e., m-cu crossvein absent; veins M and Cu diverging distal to crossvein cu-a by a distance greater than the length of the crossvein; hindwing lacking closed cells; anterior margin of hindwing with 3-4 hamuli; jugal lobe absent.

Metasoma long and slender; abdominal segment 2 (petiole) subquadrate in profile (Figure 2), longer than high or wide, and only weakly constricted posteriorly (helcium thus apparently quite broad); spiracle on abdominal segment 2 located at anterodorsal extremity; abdominal segment 3 larger than petiole, and not developed as postpetiole nor separated from abdominal segment 4 by a marked constriction; abdominal spiracle 3 located on anterior third of tergite;

abdominal segments 2 and 3 with tergosternal fusion; abdominal segment 4 lacking tergosternal fusion; segment 4 with short but distinctly differentiated presclerites; spiracle present on anterior half of tergite 4; abdominal segments 5 and 6 apparently lacking differentiated presclerites, and not separated from succeeding segments by constrictions; abdominal spiracles 5 and 6 small and inconspicuous but exposed, i.e., visible at anterior margins of respective tergites, under normal distension; abdominal tergite 8 (pygidium) small and simple but visible dorsally, not wholly covered by abdominal tergite 7; cerci absent; subgenital plate (abdominal sternite 9) with posterior margin broadly concave but not bifurcate; anterior margin of subgenital plate with mesial apodeme better developed than lateral apodemes; basal ring not hypertrophied; paramere long and slender with upturned apex (Figure 2), about 1.5× petiole length; volsella simple, lobe-like, lacking differentiated cuspis.

Body size small; total length, excluding appendages, approximately 2.7 mm; *integument* mostly smooth and shiny, with scattered piligerous punctures; *pilosity* common on most of body, suberect to decumbent. *Color*: yellowish-brown, dorsum of head a contrasting dark brown; abdominal segments 4-8 medium brown.

Comparison of the two males

The two males were closely comparable in terms of external morphology, the chief difference between them being larger eye size in the Arenal male (see measurements below). Head shape, antennal proportions, integument sculpture, color, and overall habitus were otherwise very similar. The preceding description is a composite for the head, wings and genitalia, and is based on the Arenal male alone for other details.

Measurements (in mm) and indices:

	HW	HL	SL	LHT	CI	SI	REL	REL2
P.N. Arenal	0.639	0.472	0.242	0.724	1.35	0.38	0.54	0.40
Estación Cacao	0.592	0.452	0.221	—	1.31	0.37	0.47	0.36

Comments

To the extent that the *Leptanilloides mckennae* male is representative it suggests the following provisional diagnosis for males of the subfamily Leptanilloidinae: mandibles elongate-triangular and edentate; palp formula 2,2 or less; antennae 13-segmented; anterior clypeal lamella present; pronotum triangular in profile, U-shaped in dorsal view, and narrow and strap-like anteromedially; notauli absent; mesopleuron lacking oblique transverse suture; petiole nodiform; postpetiole absent; abdominal spiracles 5 and 6 visible under normal distension; posterior margin of subgenital plate emarginate; cerci absent; mesotibia and metatibia each with single apical spur; forewing with one submarginal cell and no discal (medial) cell.

Several features of the *Leptanilloides mckennae* male match those of the worker caste of the same species, such as the well developed, medially pointed clypeal lamella; the form of the tibial spurs; the location of the abdominal spiracles; and overall body size (2.7 mm long versus ~3.1 mm in the worker). General traits of the *Leptanilloides* male that provide support for placement of the genus in the dorylomorph group include tergosternal fusion of abdominal segments 2 and 3 (but not 4); exposure of abdominal spiracles 5 and 6 under normal distension; absence of cerci; and simplified volsella. Interestingly the *Leptanilloides* male also shares some specific attributes with army ant males including a triangular pronotum (in profile); reduced parascutal carina; absence of an oblique transverse sulcus on the mesopleuron; and relatively small tegula. In other respects, however, *Leptanilloides* lacks some of the key characteristics of male army ants such as

large size (relative to workers), elongate and sickle-shaped mandibles, robust metasoma, and modified genitalia.

RELATIONSHIP OF *LEPTANILLOIDES* TO OTHER ANTS: MOLECULAR EVIDENCE

Following identification of the sequenced male as *Leptanilloides mckennae*, it was possible to use this specimen as the representative for this species in a more detailed (five-gene) molecular phylogenetic analysis. This overcame the problems associated with the single *L. mckennae* worker whose DNA was less well preserved.

Under both parsimony and Bayesian approaches the closeness of the two *Leptanilloides* species is confirmed, and there is unequivocal support (parsimony bootstrap 100%, Bayesian posterior probability 1.00) for placement of *Leptanilloides* in the dorylomorph group (Figure 4). The parsimony analysis indicates that *Leptanilloides* is sister to all other dorylomorphs (bootstrap support of 72%), but Bayesian inference places *Leptanilloides* as sister to (*Acanthostichus* + *Cylindromyrmex*) with a posterior probability of 0.89. In general, relationships among the dorylomorph taxa are not well resolved, except for the pairing of *Acanthostichus* and *Cylindromyrmex*. Both parsimony and Bayesian trees recover army ant monophyly, but without strong support (cf. Brady, 2003). The dorylomorphs are placed firmly in the formicoid clade (parsimony bootstrap 100%, Bayesian posterior probability 1.00), a group that is increasingly well supported by molecular data (Ward & Brady, 2003; Saux *et al.*, 2004; Ward & Downie, 2005).

Within the formicoid clade, the remaining taxa (representatives from Ectatomminae, Myrmicinae, Formicinae, Dolichoderinae, Myrmeciinae, and Pseudomyrmecinae) appear to form a clade that is sister to the dorylomorphs, but low support levels (parsimony bootstrap 57%, Bayesian posterior probability 0.68) undermine the reliability of this hypothesis. Among the remaining formicoids, two groups are well supported, however, at least under Bayesian inference (posterior probability of 1.00): (Ectatomminae + Myrmicinae + Formicinae) and (Pseudomyrmecinae + Myrmeciinae).

The ant genus *Leptanilla*—and by implication other Leptanillinae such as *Protanilla* and *Anomalomyrma*—is not closely related to *Leptanilloides* and other dorylomorphs. It lies well outside the formicoid clade, as the sister group of all other ants sampled in this study (parsimony bootstrap 71%, Bayesian posterior probability 1.00). An earlier study, based on 28S data only, also suggested a basal position for *Leptanilla* among the Formicidae (Saux *et al.*, 2004). This result should be viewed with caution, however, since *Leptanilla* shows divergent sequence evolution and its placement adjacent to the aculeate outgroups might be an artifact of long-branch attraction (see Bergsten, 2005).

DISCUSSION AND CONCLUSIONS

Three subterranean ant taxa, Leptanillinae, Leptanilloidinae, and *Apomyrma*, have workers of similar appearance that share a suite of characteristics, including small size, pale integument, absence of eyes, absence of frontal lobes, very narrow clypeus, exposed antennal sockets, short scapes, reduced abdominal segment 3 (compared with segment 4), flexible promesonotal suture, and well developed sting. The last two features are probably ancestral for ants as a whole, but the others appear to be traits associated with adaptation to underground habitats. Saux *et al.* (2004) demonstrated with 28S sequence data that *Apomyrma*, formerly associated with the Leptanillinae (Bolton, 1990b, 2003), is actually a member of the subfamily Amblyoponinae, in accordance with its original suggested placement (Brown *et al.*, 1971). The present study confirms that the superficial similarity between *Leptanilloides* and *Leptanilla* is no more than that, and that both groups arise far apart on the ant tree (Figure 4).

Recent molecular studies (Ward & Brady, 2003; Ohnishi *et al*., 2004, Saux *et al*., 2004; Ward & Downie, 2005; present work) have produced results consistent with the hypothesis that the evolutionary history of ants involved an early diversification of poneromorph-like taxa, within which a second stronger radiation occurred that generated most of modern ant diversity (Figure 5). Highly specialized subterranean ants occur in both the early ("basal") lineages and in various groups within the younger formicoid clade. This indicates that colonization of, and restriction to, the underground environment, with attendant convergence in morphology and behavior, has occurred on multiple occasions during ant evolution. Given the frequency of specialized hypogaeic species in lineages at the base of the ant phylogeny, the possibility could be entertained that these taxa reflect the ancestral condition for Formicidae, but this implies one or more transitions from subterranean to above-ground habits, which seems improbable. Moreover, epigaeic taxa are prevalent throughout the fossil record of ants, beginning in the Cretaceous (Grimaldi & Agosti, 2000; Nel *et al*., 2004). Nevertheless semi-cryptic nesting habits (e.g., living in rotten logs) could have provided the ancestral ants with opportunities for specialization in both directions.

POSTSCRIPT

As this manuscript was being prepared I learned of the discovery of additional new *Leptanilloides* species in Cotopaxi, Ecuador (J. M. Vieira and D. A. Donoso, pers. comm.), with ergatogyne queens and males. Descriptions of these taxa and castes appear elsewhere (Donoso *et al.*, 2006).

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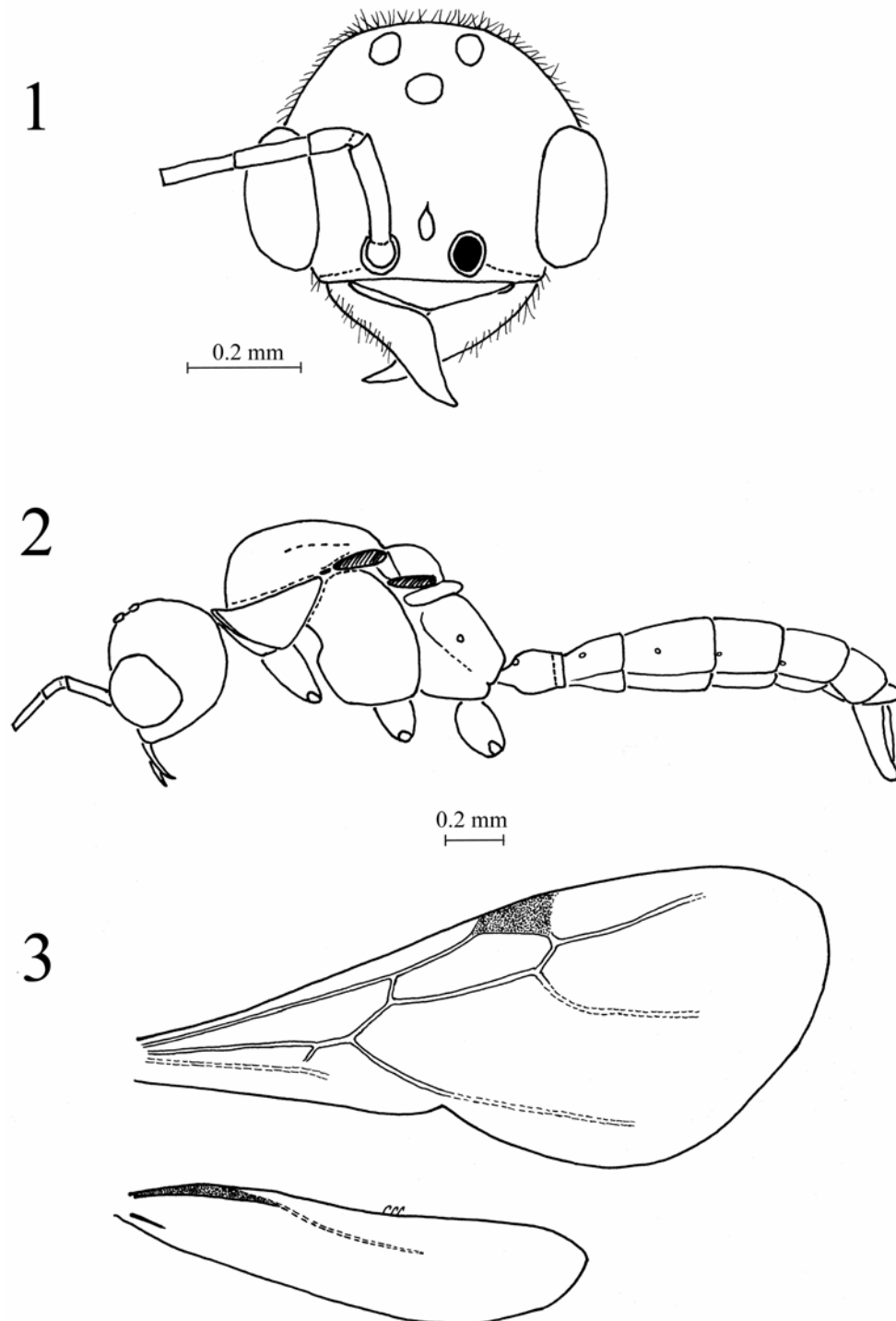
Figures 1-3. *Leptanilloides mckennae* male. 1, Frontal (full-face) view of head; 2, lateral view of body; and 3, wing venation. Figures 2 and 3 are drawn to the same scale.
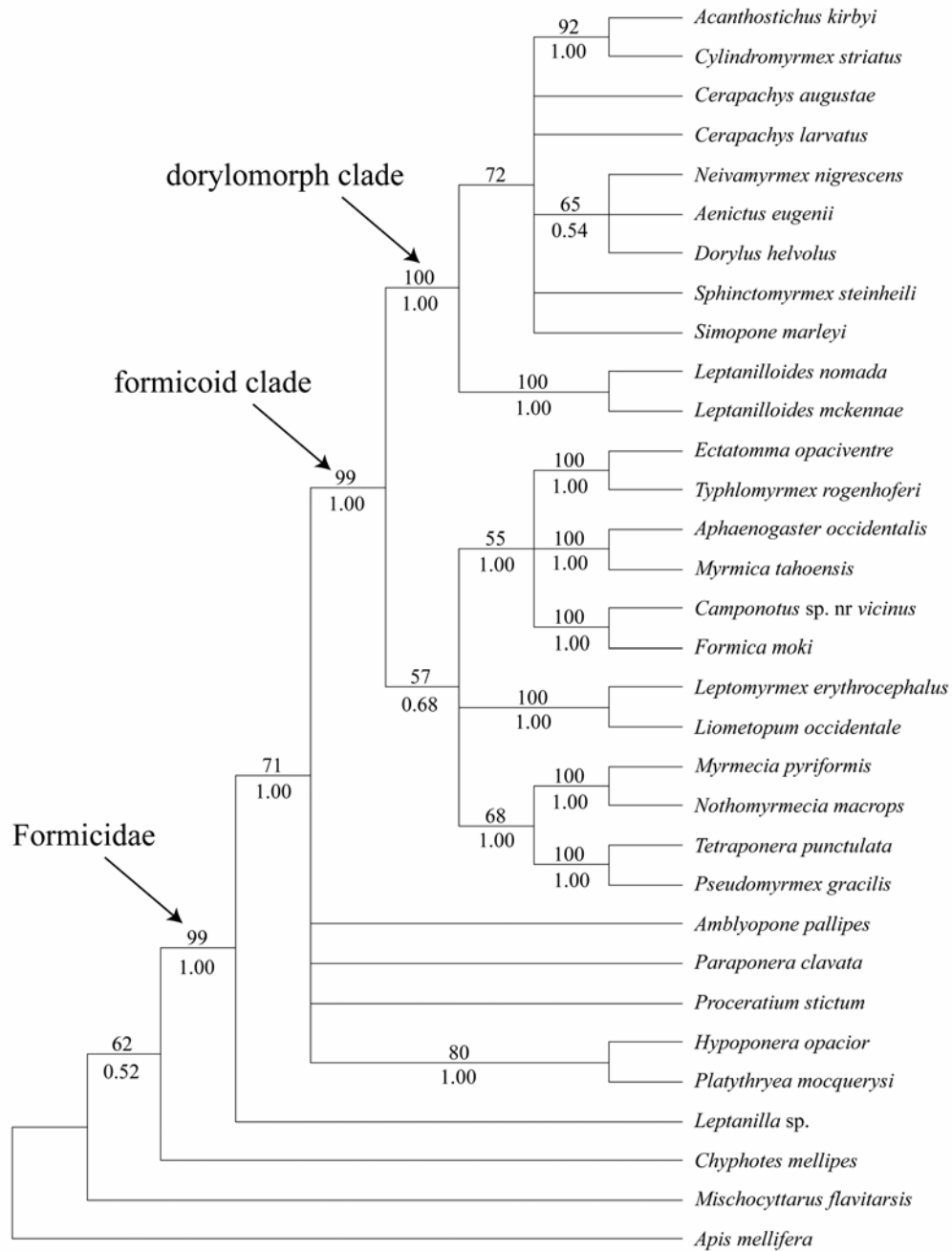
Figure 4. Phylogenetic relationships of *Leptanilloides* and other ant taxa, based on analysis of five nuclear genes (18S, 28S, *wg*, *abd-A*, and LW *Rh*; total: 5.1 kb). Numbers above each node are parsimony bootstrap values; those below are Bayesian posterior probabilities. The tree depicted is the 50% majority-rule consensus tree obtained from parsimony bootstrap analysis. The Bayesian tree (majority-rule consensus of all sampled trees after burn-in) is almost identical in topology except that *Leptanilloides* appears as sister to (*Acanthostichus* + *Cylindromyrmex*) with posterior probability of 0.89.
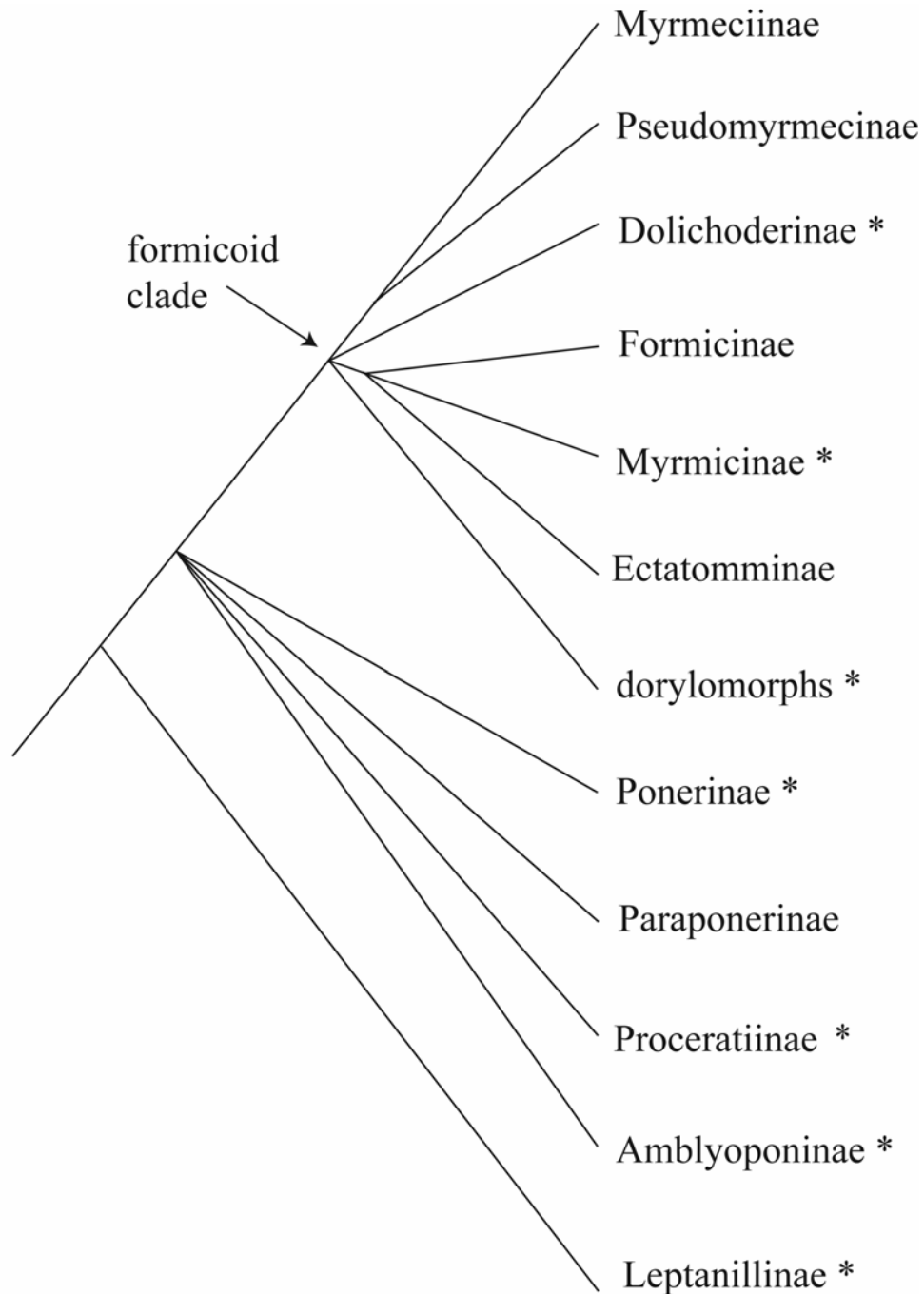
Figure 5. Relationships among major groups of extant ants, as inferred from DNA sequence data. Based on the findings of Ward & Brady (2003), Ohnishi *et al.* (2004), Saux *et al.* (2004), Ward & Downie (2005), and present study. The subfamilies Aneuretinae and Heteroponerinae (see Bolton, 2003) are here treated as part of Dolichoderinae and Ectatomminae, respectively. Asterisks signify groups containing genera whose workers are entirely hypogaeic and blind.