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Rediscovery of the ant genus *Amyrmex* Kusnezov (Hymenoptera: Formicidae) and its transfer from Dolichoderinae to Leptanilloidinae

PHILIP S. WARD[1] & SEÁN G. BRADY[2]

[1]*Department of Entomology, University of California, Davis, CA 95616, USA. E-mail: psward@ucdavis.edu*
[2]*Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington, DC 20013, USA. E-mail: bradys@si.edu*

Abstract

The ant genus *Amyrmex* Kusnezov (1953), previously known only from several males collected more than fifty years ago in Tucumán, Argentina, is redescribed on the basis of more recent material from Argentina and Brazil. Using DNA sequence data from seven nuclear genes we investigate the phylogenetic position of *Amyrmex* and demonstrate that it is a member of the subfamily Leptanilloidinae, rather than the Dolichoderinae to which it had been previously assigned. This placement is also supported by a reevaluation of morphological traits. *Amyrmex* is possibly a senior synonym of the worker-based genus *Asphinctanilloides* Brandão, Diniz, Agosti & Delabie (1999), but additional study is needed to establish generic limits within the Leptanilloidinae and to reliably associate male and worker castes.

Key words: ant taxonomy, molecular phylogenetics, dorylomorphs, *Leptanilloides, Asphinctanilloides*, Neotropical region

Introduction

The genus *Amyrmex* was established by Kusnezov (1953) for four small male ants collected in the Tucumán region of Argentina. Kusnezov recognized a single species, *Amyrmex golbachi*, which he placed in the subfamily Dolichoderinae, while noting peculiar features of the wing venation and abdominal morphology that introduced some uncertainty about its affinities. Since its original description *Amyrmex* has received scant attention from ant taxonomists. In his generic revision of the Dolichoderinae Shattuck (1992) synonymized *Amyrmex* under *Forelius*. Cuezzo (2000) resurrected *Amyrmex*, pointing out various distinctive features of morphology that do not agree with any known males of *Forelius*. No *Amyrmex* specimens have been reported besides the holotype, three paratypes, and one additional series of males from the Kusnezov collection (Cuezzo, 2000). All of this material is more than half a century old.

Recently one of us (PSW) discovered several males of *Amyrmex* among miscellaneous unidentified ant specimens in the Bohart Museum of Entomology, University of California at Davis (UCDC). Most of these males were collected in Malaise traps at a lowland rainforest site in Rondônia, Brazil in 1991. This newer material provided the opportunity to reexamine the identity of *Amyrmex* and to investigate its phylogenetic placement with DNA sequence data.

Materials and methods

The Bohart Museum of Entomology (UCDC) contains four males of an *Amyrmex* species from Fazenda Rancho Grande, Rondônia, Brazil (12–22 November 1991, leg. E. M. Fisher). These specimens were

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collected in lowland rainforest, as part of a series of Malaise trap samples (Eric Fisher, pers. comm.). The males are here referred to as *Amyrmex* BR01. They are morphologically uniform and differ only slightly from the type species, *A. golbachi* (see below). Morphological observations and metric measurements of these ants were made with a Wild M5 stereomicroscope, at 50×. Color images were taken with a Leica MZ16A stereomicroscope, JVC digital camera, and Automontage software. The sequence data reported here came from two specimens (CASENT0106161 and CASENT0106183) from which DNA was non-destructively extracted. Vouchers have been deposited in the Bohart Museum of Entomology (UCDC), the Museu de Zoologia, Universidade de São Paulo, Brazil (MZSP) and the California Academy of Sciences, San Francisco (CASC). In addition, we examined other miscellaneous leptanilloidine males in UCDC, including an *Amyrmex golbachi* male from Argentina. We carefully compared our material to the original description and illustrations of *Amyrmex golbachi* (Kusnezov, 1953) and to the images of topotypical specimens appearing on AntWeb (www.antweb.org). The AntWeb-imaged specimens are deposited in the Museum of Comparative Zoology, Harvard University (MCZC) (CASENT0172251, CASENT0172252, CASENT0172253) and apparently represent part of an old series from the Kusnezov collection.

The following metric measurements were used. HW: maximum width of head, including eyes; HL, head length, taken from the posterior margin of the head to the anterior clypeal margin; ML: chord length of mandible from the basal insertion to apex; SL: length of scape, excluding basal condyle and neck; LA2, LA3, LA4: length of second, third and fourth antennal segments, respectively; LA13: length of terminal (13th) antennal segment; EL: eye length, measured in full-face view; LHT, length of the metatibia in dorsal view, excluding the medioproximal lobe. The following indices are cited: CI (cephalic index) HW/HL, and SI (scape index) SL/HW.

We obtained fragments of seven nuclear genes from *Amyrmex* BR01: 18S rDNA, 28S rDNA, long wavelength rhodopsin (LW Rh), elongation factor 1-alpha F1 copy (EF1αF1), elongation factor 1-alpha F2 copy (EF1αF2), wingless (wg), and abdominal-A (abdA). Procedures for DNA extraction, amplification, and sequencing are given in Ward & Downie (2005) and Brady *et al.* (2006). The *Amyrmex* sequences (GenBank accession numbers FJ588487-FJ588493) were added to the 162-taxon data set of Brady *et al.* (2006), which comprises sequence data from the same seven genes for a wide array of ant taxa sampled throughout the formicid tree. The only subfamily excluded from our data matrix is the recently discovered Martialinae (Rabeling *et al.* 2008) for which the requisite sequence data are not yet available. As in Brady *et al.* (2006) we excluded introns of protein-coding genes and hypervariable regions of 28S, resulting in a data matrix of 5989 base pairs, with 1660 parsimony-informative sites and 2063 variable sites. The data set has no missing gene fragments.

Phylogenetic analyses of the new data matrix employed Bayesian, maximum parsimony (MP), and maximum likelihood (ML) methods. We conducted Bayesian analyses using MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) under the same data partition and model selection strategy as in Brady *et al.* (2006). This involved creating two partitions corresponding to codon positions 1+2 and 3 separately for each of the five protein-coding genes, and additional partitions for each of the two ribosomal genes, resulting in twelve total partitions. Each partition received the GTR+I+Γ model, determined using the AIC with Modeltest v3.06 (Posada & Crandall 1998). We conducted two independent MCMC runs for 50 million generations, distributed across four chains with a heating parameter of 0.01 (in order to increase mixing compared to the default setting of 0.2). Convergence between runs was assessed using the average standard deviation of split frequencies (0.017 upon completion) and by plotting likelihood values across generations using Tracer v1.4. A burn-in value of 20 million generations was established and only the post-burn-in generations from both runs were included in the results. We ran 1000 MP bootstrap replicates using PAUP v4.0b10 (Swofford 2002) with 10 random addition sequences per replicate, TBR branch swapping, no limit to MAXTREES, and gaps treated as missing data. We used GARLI v0.96 (Zwickl 2006) to conduct ML bootstrap analyses both for the combined 7-gene data set, and for each gene separately to assess concordance among genes in the placement of *Amyrmex*. For each ML analysis, we ran 500 bootstrap replicates using the GTR+I+Γ model, with each replicate consisting of 2 independent searches.

Results and discussion

Phylogeny

All phylogenetic analyses of the combined (7-gene) data set placed *Amyrmex* as sister to the two sampled *Leptanilloides* species, *L. nomada* Donoso, Vieira & Wild and *L. mckennae* Longino, with very high support: Bayesian posterior probability (PP) of 1.00 (Figure 1), ML bootstrap of 100, and MP bootstrap of 100. ML analyses of five individual genes likewise resulted in *Amyrmex* and *Leptanilloides* forming a clade (to the exclusion of all other taxa) with moderate to strong bootstrap support as follows: abdA = 98; EF1αF2 = 94; LW Rh = 98; wg = 98; 28S = 73. The other two genes, EF1αF1 and 18S, each placed these two genera within a larger, unresolved polytomy in the bootstrap consensus tree, and thus did not contradict this close relationship.

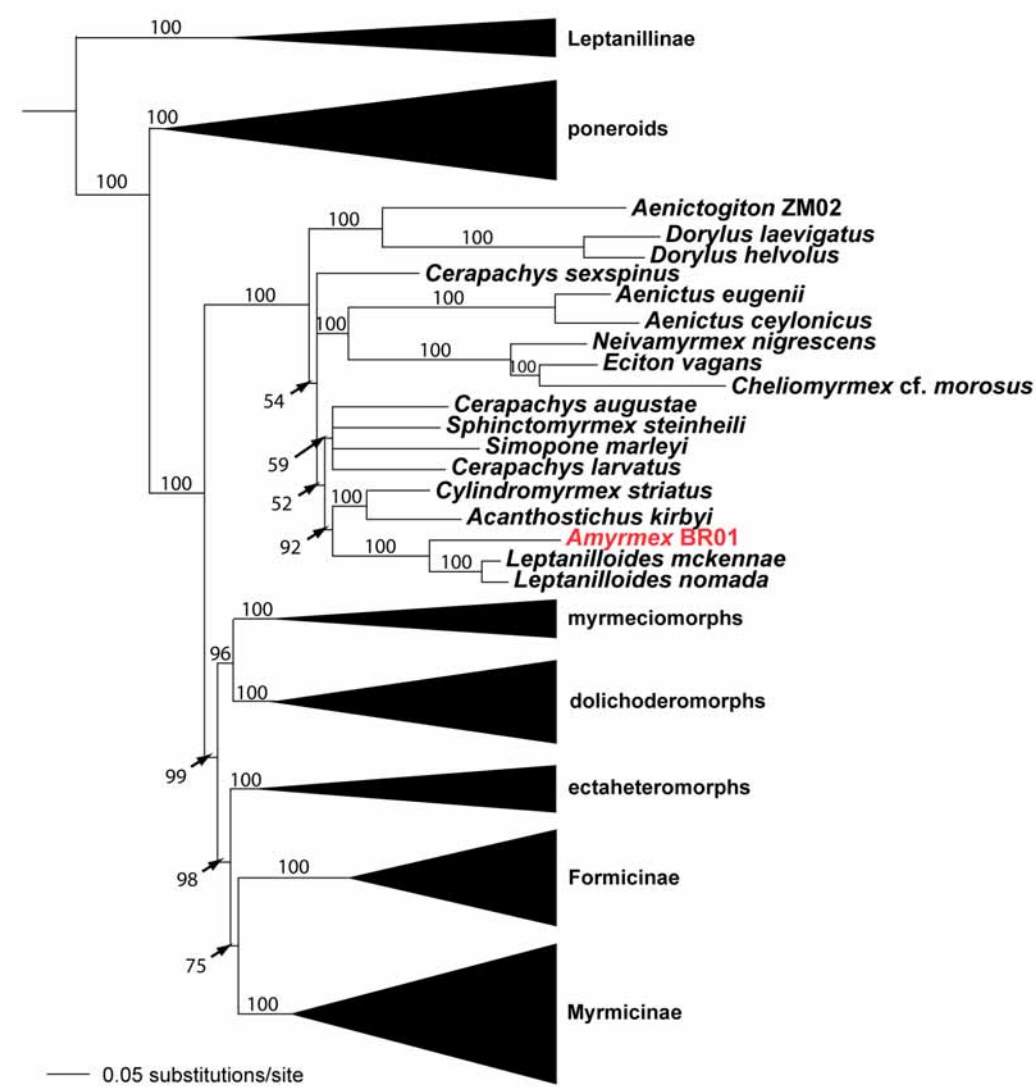


FIGURE 1. Bayesian phylogeny showing the placement of *Amyrmex* (in red font) as sister to *Leptanilloides*. Nodal support values (PP × 100) are indicated above branches. Only dorylomorph species are shown; see Brady *et al.* (2006) for details on relationships in other ant groups.

The phylogeny of the remaining dorylomorphs—the larger clade to which Leptanilloidinae belongs—was not changed substantially by the inclusion of *Amyrmex* in the data set, although *Amyrmex* broke the long branch connecting *Leptanilloides* to the other dorylomorphs. In our Bayesian analysis of the complete data set (Figure 1), Leptanilloidinae (including *Amyrmex*) remained sister to (*Acanthostichus* + *Cylindromyrmex*) with

PP of 0.92, a result similar to that obtained by Brady *et al.* (2006). However, our MP bootstrap analysis using the complete data set placed Leptanilloidinae as sister to all other dorylomorphs with moderate support (MP bootstrap of 73), while our ML bootstrap analysis was more agnostic by placing Leptanilloidinae as part of a large polytomy within the dorylomorphs. More intensive phylogenetic work will be required to infer with confidence the relationship of Leptanilloidinae to other taxa within the dorylomorphs.

The conclusion from DNA sequence data that *Amyrmex* is closely related to *Leptanilloides* is also consistent with a reconsideration of the morphological features of *Amyrmex* males and comparison with the recently described males of two *Leptanilloides* species (Donoso *et al*. 2006; Ward, 2007). Several morphological similarities are apparent between the males of these two genera including (1) edentate mandibles; (2) pronotum reduced anteromedially to a thin transverse strip set well below the level of the bulging mesonotum; (3) pronotum triangular in lateral profile, with apex directed toward the wing base, (4) absence of notauli; (5) absence of an oblique transverse suture on the mesopleuron; (6) nodiform and subquadrate petiole; (7) broad attachment between abdominal segments 2 and 3; (8) concave posterior margin of abdominal sternite 9; (9) absence of cerci; and (10) simplified, unbranched volsella. The wing venation is also similar in the two genera in that there is a single submarginal cell and the discal cell is absent, although venation also provides some features that may distinguish *Amyrmex* from other leptanilloidines, as discussed below.

Redescription of male of *Amyrmex* Kusnezov

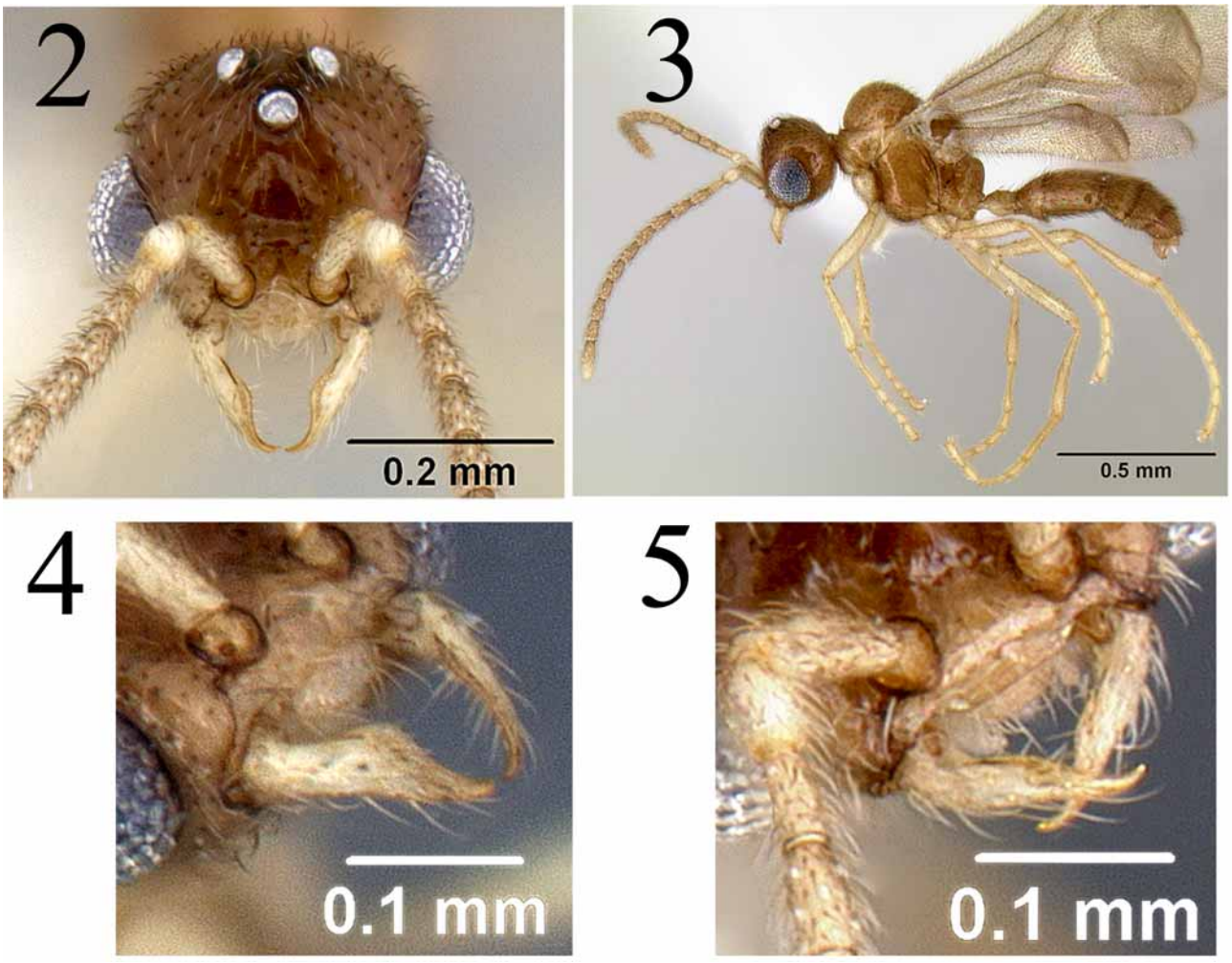
Head broader than long (CI 1.25–1.37), with large convex eyes that occupy the anterior two-fifths to one half of the sides of head (REL 0.43–0.57) (Figure 2); mandibles slender, elongate-triangular to sublinear, masticatory margin edentate and weakly differentiated from the unarmed basal margin; external margin of mandible curved basally, straight medially, and bent slightly mesad at apex (Figures 2, 4–5); mandible tips crossing at closure, mandible length subequal to eye length (ML/EL 0.88–1.10, ML/HW 0.35–0.41); genal teeth and hypostomal teeth lacking; clypeus short and transverse, bordered anteriorly by a thin clypeal lamella (apron); antennal sockets horizontal and exposed, and located close to the anterior clypeal margin; antenna 13-segmented, each segment longer than wide; scape of moderate length, SI 0.27–0.31; scape length subequal to or less than the length of ultimate antennal segment (SL/LA13 0.73–0.97), scape length 0.11–0.14× total length of antenna, less than twice the length of the second antennal segment (SL/LA2 1.50–1.78), and slightly more than half the combined length of the second, third and fourth antennal segments (SL/(LA2+LA3+LA4) 0.52–0.65); lateral ocelli separated from median ocellus by about their diameters.

Mesosoma with distinctive pronotum (Figure 3): U-shaped in dorsal view and reduced anteromedially to a thin horizontal strip, set well below the level of the dorsally protruding mesonotum; pronotum triangular in profile, with pointed posterior apex directed towards the wing base; mesonotum lacking notauli; parapsidal sutures very weakly impressed, barely discernable; 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; mesoscutellum prominently bulging, as seen in lateral view; metapleural gland reduced and inconspicuous; propodeal spiracle small, circular, positioned at about midheight of propodeum and slightly posterior to the metanotum. Legs slender (LHT/HL ~1.2); mesotibia and metatibia each with a single short spur; tarsal claws lacking preapical tooth.

Wings with reduced venation (Figure 6); pterostigma present; forewing with elongate submarginal cell, four times longer than wide, and longer than basal cell; base of Rs weak, and absent in one species (*Amyrmex* BR02), resulting in confluence of the basal and submarginal cells; submarginal cell extending distad of stigma and terminating in an acute point; no free M vein after Rs+M; discal (medial) cell lacking, i.e., m-cu crossvein absent; A merging into cu-a, which curves anteriorly to join M+Cu at the point where veins M and Cu diverge, hence no free A vein distal to cu-a (Figure 6); hindwing lacking closed cells; anterior margin of hindwing with 1–4 hamuli; jugal lobe absent.

Metasoma slender in profile, obovate in dorsal view, widest at abdominal segment 5; abdominal segment 2 (petiole) subquadrate in profile (Figure 3), longer than high or wide, and only weakly constricted posteriorly, the helcium thus apparently quite broad; spiracle on abdominal segment 2 located on anterior third, near 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 lacking well differentiated presclerites, and not separated from succeeding segments by constrictions; abdominal spiracles 5 and 6 not discernable in specimens examined but possibly present at anterior margins of respective tergites; 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; basal ring not hypertrophied; paramere small and slender with rounded apex, paramere about 0.8× petiole length; volsella a simple, elongate-triangular lobe, lacking differentiated cuspis.

Body size very small; total length, excluding appendages, approximately 1.1–1.7 mm; HW 0.32–0.41, LHT 0.29–0.39; *integument* mostly smooth and shiny, with scattered piligerous punctures; *pilosity* common on most of body, suberect to decumbent. *Color*: body yellowish-brown to medium-brown, head and posterior margins of abdominal segments 4–7 darker, appendages (antennae, mandibles, legs) lighter.



FIGURES 2–5. Automontage images of *Amyrmex* males. 2. *Amyrmex* BR01 (CASENT0106184), dorsal (full-face) view of head; 3. *Amyrmex* BR01 (CASENT0106184), lateral view of body; 4. *Amyrmex* BR01 (CASENT0106184), close-up of right mandible; 5. *Amyrmex golbachi* (CASENT0106195), close-up of right mandible.

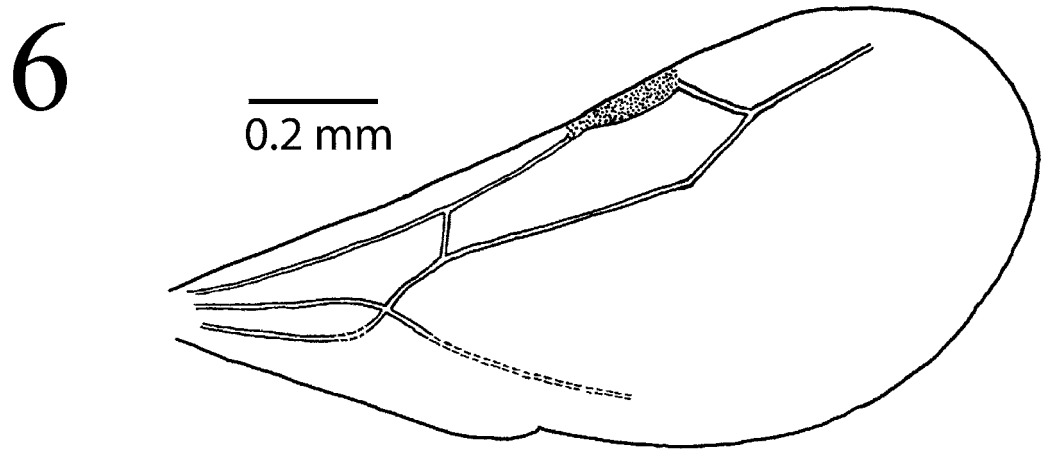


FIGURE 6. Right forewing, male *Amyrmex* BR01 (CASENT0106185).

The above description is a composite, based on the following material:

Amyrmex golbachi, 1 male, ARGENTINA Formosa: Estancia Guaycolec, 25km N Formosa, 185m, 25°59′S 58°12′W, 17–20.xii.1998, Malaise trap, S. L. Heydon (UCDC) (CASENT0106195).

Amyrmex BR01, 4 males, BRAZIL Rondônia: Fazenda Rancho Grande, 62km S Ariquemes, 165m, 12–22 November 1991, 10°18' S, 62°53'W, E. M. Fisher (CASC, MZSP, UCDC) (CASENT0106161, CASENT0106183, CASENT0106184, CASENT0106185).

Amyrmex BR02, 1 male, BRAZIL Rondônia: Fazenda Rancho Grande, 62km S Ariquemes, 165m, 12–22 November 1991, 10°18' S, 62°53'W, E. M. Fisher (UCDC) (CASENT0106186).

The male of *Amyrmex golbachi* from Argentina (Formosa) matches the original description (Kusnezov 1953) and the images of *A. golbachi* males from Tucumán on AntWeb (www.antweb.org). Differences between the three taxa are summarized in Table 1.

TABLE 1. Differences among males of three species of *Amyrmex*. Contrasts in mandible shape are depicted in Figures 4–5. "B" and "SM" refer to the basal cell and submarginal cell, respectively.

	Amyrmex golbachi	*Amyrmex* BR01	*Amyrmex* BR02
Body size	larger: HW 0.38	larger: HW 0.35–0.41	smaller: HW 0.32
Leg length	longer: LHT 0.33	longer: LHT 0.32–0.39	shorter: LHT 0.29
Mandibles	sublinear	elongate-triangular	sublinear
Eye size	smaller: REL 0.43	larger: REL 0.52–0.57	larger: REL 0.51
B cell and SM cell	not confluent	not confluent	confluent

Relationship to other Leptanilloidinae

From the foregoing description the following differences emerge between the known males of *Leptanilloides* (Donoso *et al.* 2006; Ward 2007) and *Amyrmex*:

Amyrmex

Small body size, HW 0.32–0.41, LHT 0.29–0.39

Scape shorter: SI 0.27–0.31; scape less than twice the length of the second antennal segment (SL/LA2 1.5–1.8)

Legs shorter, LHT/HL ~1.2

Paramere small, ~0.8× petiole length

Veins M and Cu diverging at crossvein cu-a

Submarginal cell elongate: about four times longer than wide, longer than the basal cell, extending distad of stigma and terminating in an acute point

No free M vein after Rs+M

Leptanilloides

Variable body size, HW ~0.40–0.64, LHT 0.40–0.72

Scape disproportionately longer: SI 0.37–0.41; scape more than twice the length of second antennal segment (SL/LA2 ~2.2)

Legs disproportionately longer, LHT/HL ~1.5

Paramere large, ~1.5× petiole length

Veins M and Cu diverging distal to crossvein cu-a by a distance greater than the length of the crossvein

Submarginal cell less elongate: no more than three times longer than wide, shorter than the basal cell, and terminating at level of stigma

Free M vein after Rs+M (may be weak)

However, in the UCDC collection there are several other male specimens that weaken these distinctions. First, there are seven additional leptanilloidine males—apparently representing two species—collected at Fazenda Rancho Grande, Rondônia, Brazil from the same series as *Amyrmex* BR01 and *Amyrmex* BR02 (12–22 November 1991, leg. E. M. Fisher) (CASENT0106187 to CASENT0106193). These are small (HW 0.22–0.38) and similar to *Amyrmex* except that (1) the forewing submarginal cell is less elongate, shorter in length than the basal cell, and does not exceed the stigma; (2) the mandibles are elongate-linear and bowed (i.e., falcate); and (3) the parameres are broad and paddle-shaped, subequal in length to the petiole. Thus, from a single collection from this one rainforest site in Rondônia there are males representing at least four species of Leptanilloidinae, two of them conforming to the strict *Amyrmex* diagnosis (above) and two other *Amyrmex*-like males. Second, a single male from Barro Colorado Island, Panama (12.viii.1978, leg. R. B. & L. S. Kimsey) (CASENT0106194) matches the *Amyrmex* description except that it is larger (HW 0.48, LHT 0.43) with disproportionately longer, falcate mandibles (ML/HW 0.52, ML/EL 1.33), and a short submarginal cell, not exceeding the stigma and approximately equal in length to the basal cell.

It is important to note that our concept of the male caste of *Leptanilloides* is based on only two species, *L. mckennae* Longino (Ward 2007) and *L. nubecula* Donoso, Vieira & Wild (Donoso *et al.* 2006), both with relatively large workers (HW 0.54–0.64). The *L. mckennae* males are also large (HW 0.59–0.64). The measurements given for the male of *L. nubecula* suggest a rather small male (HW 0.32, HL 0.32; see Donoso *et al.* 2006) but these may be in error. Based on the scale bar in the illustration of the male (Donoso *et al.* 2006, figure 25) HW should be about 0.37 and HL 0.29, and both of these values are unusually low in relation to the size of the workers (HW 0.54–0.56) and gyne (HW 0.74). In any event these two species do not adequately represent the spectrum of diversity within the genus. No males have been associated with workers of the smaller species of *Leptanilloides* such as *L. biconstricta* Mann and *L. sculpturata* Brandão, Diniz, Agosti & Delabie. The leptanilloidine males from Rondônia and Panama with short submarginal cells and falcate mandibles might belong here.

A further complication is that neither males nor DNA sequence data are available for the other worker-based leptanilloidine genus *Asphinctanilloides* Brandão, Diniz, Agosti & Delabie (1999), which was recovered as sister to *Leptanilloides* in morphological phylogenetic analyses (Brandão *et al.* 1999; Brady & Ward 2005). The three known species of *Asphinctanilloides* are associated with lowland Amazon and Atlantic coastal rainforest, which contrasts with the predominantly Andean and Central American distribution of *Leptanilloides*. Thus it seems quite possible that *Amyrmex*, known currently from the Amazon basin of Brazil and from northern Argentina, represents a senior synonym of *Asphinctanilloides*. For this reason we refrain

from describing any of the *Amyrmex*-like males as new species since names may already be available for them (i.e., *Asphinctanilloides anae* Brandão, Diniz, Agosti & Delabie, *A. amazona* Brandão, Diniz, Agosti & Delabie and *A. manauara* Brandão, Diniz, Agosti & Delabie). Confirming this will require more extensive study. Since it may prove difficult to find worker-associated males of leptanilloidines in the field, DNA sequencing offers a reliable way of associating these disparate castes, if the material is sufficiently well preserved.

In the original description of *Asphinctanilloides* the workers were said to be distinguished from those of *Leptanilloides* by several features including (1) presence of a metanotal groove, (2) reduced postpetiole, smaller than the petiole as seen in profile, and (3) abdominal segments 5 and 6 lacking differentiated presclerites and hence without constrictions between abdominal segments 4 and 5, and 5 and 6 (Brandão *et al.* 1999). With the subsequent discovery of *Leptanilloides* species whose workers have a metanotal groove and a short postpetiole the first two characters have lost their diagnostic value (Longino 2003; Donoso *et al.* 2006). Brandão *et al.* (1999) also documented differences in the sting apparatus between *Leptanilloides* and *Asphinctanilloides*, however, and these have not been evaluated in the newly described species of *Leptanilloides*.

We examined two workers of *Asphinctanilloides amazona* (BRAZIL Amazonas: 28km N Manaus, 1.xii.1998, Berlese soil sample, leg. M. Verhaagh; CASENT0006016, CASENT0006815) (CASC) and found that in both workers abdominal segment 5 has a differentiated presclerite, and is separated from abdominal segment 4 by a weak constriction. No such constriction occurs between abdominal segments 5 and 6. Further assessment of the morphological differences between the two genera is warranted. (Attempts to extract DNA from *Asphinctanilloides* workers belonging to this series were unsuccessful, apparently due to their initial collection into low concentration ethanol.)

Nevertheless, if a reduced worker postpetiole and an undifferentiated presclerite on abdominal segment 6 are derived features within Leptanilloidinae then it might still be possible to define *Asphinctanilloides* (or *Amyrmex*, if the two prove to be synonyms) in such a way that it is monophyletic, but this could also render *Leptanilloides* paraphyletic. These considerations, together with the discovery of a variable assortment of leptanilloidine males from scattered Neotropical localities, make the delimitation of genera in this group an ongoing challenge.

Conclusions

The ant genus *Amyrmex* Kusnezov was described over half a century ago from several males collected in Tucumán, Argentina, and it has been in a state of taxonomic limbo since then. Placed awkwardly in the subfamily Dolichoderinae—and even synonymized under the dolichoderine genus *Forelius* for a period of time—it is here shown to be a member of the subfamily Leptanilloidinae, within the dorylomorph clade. The association was established with DNA sequence data, but it is also supported by a reevaluation of morphological features. The relationship of *Amyrmex*, still known only from males, to the two worker-based leptanilloidine genera, *Leptanilloides* and *Asphinctanilloides*, remains uncertain, although the geographical distribution of *Amyrmex* suggests that it might be a senior synonym of *Asphinctanilloides*. Establishing generic limits within the Leptanilloidinae will require additional assessment of worker- and male-based material. It is becoming increasingly apparent that DNA sequences, in addition to resolving phylogenetic relationships among taxa, also offer a powerful source of evidence for correctly associating the male and female castes of ants such as leptanilloidines (Ward 2007) and dorylines (Schöning *et al.* 2008), where the workers tend to be subterranean and the males are collected separately at lights or in Malaise traps. This emphasizes the importance of collecting specimens into media, such as 95% ethanol, that provide adequate long-term preservation of DNA.

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