

The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants

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Abstract. The ant subfamily Pseudomyrmecinae comprises three genera of hyperoptic, arboreal ants, widely distributed in tropical and subtropical regions: *Pseudomyrmex* (~200 species, New World), *Myrcidris* (two species, South America) and *Tetraponera* (~100 species, Palaeotropics). The phylogenetic relationships among these ants were investigated using DNA sequence data (~5.2 kb from 18S rDNA, 28S rDNA, wingless, abdominal-A, and long-wavelength rhodopsin genes) and 144 morphological characters, both separately and in combination. Data were gathered from a representative set of forty-nine pseudomyrmecine species, plus eighteen species from various outgroups. There was substantial agreement among the results obtained from different datasets, and from different methods of phylogenetic inference (parsimony, Bayesian inference). The monophyly of the following groups is strongly supported (100% bootstrap support and 1.00 posterior probability in the molecular dataset): Pseudomyrmecinae, *Pseudomyrmex*, and *Pseudomyrmex* + *Myrcidris*. The status of the genus *Tetraponera* is less clear: the DNA sequence data indicate that the genus is paraphyletic, but morphological features and a unique insertion in the 28S gene support the monophyly of this taxon. Seven of nine *Pseudomyrmex* species groups, established previously on the basis of morphology alone, are strongly upheld, but monophyly is rejected for the *P. pallens* group and the *P. viduus* group. In the latter case, molecular evidence indicates the existence of two independent clades, associated with the ant-plants *Triplaris* and *Tachigali*, respectively, whose convergent morphological features had caused them to be placed erroneously in the same species group. The present results confirm an earlier assertion that obligate associations with domatia-bearing plants have arisen at least twelve times in the subfamily. Molecular and morphological data support the hypothesis of a sister-group relationship between Pseudomyrmecinae and Myrmeciinae (84% parsimony bootstrap, combined dataset), which implies a Cretaceous origin of the stem-group pseudomyrmecines in the southern hemisphere. Pseudomyrmecines appear to have arisen in the Palaeotropics and later dispersed from Africa to South America, where they experienced a pronounced burst of diversification.

Introduction

Ants have undergone an impressive radiation since the Cretaceous, colonizing most terrestrial habitats and assuming keystone roles in many communities (Hölldobler & Wilson, 1990; Grimaldi & Agosti, 2000). The subfamily Pseudomyrmecinae is one of the more distinctive groups of ants inhabiting the arboreal stratum in tropical and subtropical regions. This subfamily comprises about 300

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species of gracile, fast-moving ants with large eyes, short antennal scapes, and a well-developed sting (Ward, 1990). Most pseudomyrmecine species have generalized twig-nesting habits, occupying dead stems and branches of many kinds of plants, usually in cavities previously excavated by coleopteran or lepidopteran larvae (Ward, 1991). More than forty species, however, are obligate inhabitants of specialized ant-plants (myrmecophytes). These ants colonize intrinsically hollow cavities (myrmecodomatia) in live plant tissue and in most instances appear to provide some degree of protection to the plant (Janzen, 1966, 1972; Benson, 1985; Ward, 1991; Davidson & McKey, 1993). It has been estimated that such mutualistic associations with domatia-bearing plants evolved at least twelve times within the subfamily (Ward, 1991).

Three genera of Pseudomyrmecinae are currently recognized: *Myrcondris* Ward (two species), *Pseudomyrmex* Lund (~200 species), and *Tetraoponera* F. Smith (~100 species) (numbers include undescribed taxa; Ward, unpublished). *Myrcondris* is known only from a few localities in Brazil and Guyana. *Pseudomyrmex* is widespread in the New World and reaches its greatest diversity in the South American tropics. Most species of *Pseudomyrmex* can be placed in one of nine morphologically defined species groups (Ward, 1989, 1993, 1999). *Tetraoponera* is restricted to the Palaetropics, with Eocene/Oligocene fossils known from Europe (Ward, 1990; Bolton, 1995). It has received less attention than *Pseudomyrmex*, but a taxonomic revision and phylogenetic study of the Indo-Australian species of *Tetraoponera* led to their partition into four species groups and to the inference that the genus originated in Africa and dispersed on several occasions into Asia (Ward, 2001).

Previous phylogenetic analyses – all based on morphology – also provided evidence for the monophyly of the three genera and suggested that *Myrcondris* is the sister group of (*Pseudomyrmex* + *Tetraoponera*) (Ward, 1990, 1991). Nevertheless, some of these results received only weak support and other questions remain unsettled, such as the sister group of Pseudomyrmecinae, the phylogenetic validity of the recognized species groups, and the relationships among them.

In this study, we used a combination of molecular (DNA sequence) data and morphology to investigate the phylogeny, biogeography and biological evolution of these ants.

Materials and methods

Taxa

Forty-nine species of pseudomyrmecines were selected for DNA sequencing (thirty-three *Pseudomyrmex*, fifteen *Tetraoponera*, and one *Myrcondris*), in such a way as to provide broad coverage of the species groups previously recognized within the subfamily on the basis of morphological features (Ward, 1989, 2001). Twelve of the selected pseudomyrmecine species are ant-plant specialists and two others are closely related to species that inhabit ant-plants. To this set of taxa we added, as outgroups, fifteen other ant species

from a variety of ant subfamilies and three non-ant Aculeata: a bradyobaenid, a vespid, and an apid (Appendix 1).

Morphological characters

The sixty-seven terminal taxa were assessed for 144 morphological characters (Appendix 2). These were drawn largely from features used in previous studies on pseudomyrmecine systematics (Ward, 1989, 1990, 1991, 1993, 1999, 2001). Additional traits that varied among ingroup or outgroup taxa were added to the data matrix. These included characters newly developed for this study and others taken from Hashimoto (1991, 1996), Baroni Urbani *et al.* (1992), Ward (1994), Ward & Brady (2003) and Bolton (2003). Of the 144 characters, twenty-two are based on the worker caste, three are queen based, and sixty-one are male based; the remainder are manifested similarly in workers and queens (forty-six), in queens and males (two), or in all three castes (ten). Characters involving the male genitalia and associated structures account for more than a third of the total (51/144).

One or (usually) more workers, queens and males were examined for each of the ant species treated as terminal taxa in this study. For *P. godmani*, no males were available, so this species was coded as unknown for male-only characters. Among outgroup taxa the following four taxon/caste combinations were unavailable: *Ectatomma opaciventre* male, *Proceratium stictum* queen, *Cerapachys larvatus* male, and *C. larvatus* queen. In these four instances, the scoring of male-only and queen-only characters was based on conditions observed in related congeners. The full data matrix is given in Appendix 3.

Molecular methods

Segments of 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*). The set of amplifying and sequencing primers used for most samples is given in Table 1. For some specimens, obtaining sequences of LW *Rh* and *abd-A* necessitated the use of alternative primer pairs that amplified shorter, overlapping, stretches of DNA. This applied mainly to older samples in which the DNA appeared to have been partially degraded. These alternative primer sets are documented in Table S1 of the supplementary material.

DNA was extracted from single individuals, usually adult worker ants but occasionally sexual forms or pupae, using the DNeasy Tissue Kit (Qiagen Inc., Valencia, California, U.S.A.) with a final wash performed with sterile water rather than the supplied buffer and at half the volume. In most instances, the same individual was sequenced for all five genes, but for five species (*P. gracilis*, *P. oki*, *P. termittarius*, *Myrcondris epicharis*, and *T. nigra*), a second individual from the same ant colony was used to complete the sequence dataset. Polymerase chain reaction (PCR) procedures for 18S and 28S were similar to those given in Ward &

Table 1. Primer sets.

Primer	Sequence (5' to 3')	Position	Source
18S-5F	TGGTTGATCCTGCCAGTAG	Drosophila 5–23	Wiegmann <i>et al.</i> (2000)
18S-847R	CACTCTAATTTKTTCAAAG	Drosophila 847–829	Wiegmann <i>et al.</i> (2000)
18S-629F	AAAGCTCGTAGTTGAATCTGTGT	Drosophila 629–651	This study
18S-1300R	CTGGTGAGGTTTCCCGTGTTG	Drosophila 1300–1280	This study
18S-1215F	GCTGAAACTTAAAGGAATTGACGGAAGGGCAC	Drosophila 1215–1246	Wiegmann <i>et al.</i> (2000)
18S-1975R	CACCTACGGAAACCTTGTTACGACTT	Drosophila 1975–1950	Wiegmann <i>et al.</i> (2000)
28S-3318F	CCCCCTGAATTTAAGCATAT	Drosophila 3318–3337	Schmitz & Moritz (1994)
28S-3706R	GGTTTACCCTGAACGGTT	Drosophila 3706–3688	This study
28S-3665F	AGAGAGAGTTCAAGAGTACGTG	Drosophila 3665–3686	Belshaw & Quicke (1997)
28S-4068R	TTGGTCCGTGTTTCAAGACGGG	Drosophila 4068–4047	Belshaw & Quicke (1997)
28S-4023F	CTACTGCTTTGGGTACTCT	Drosophila 4023–4041	This study
28S-4745R	ACACACTCCTTAGCGGA	Drosophila 4745–4729	Friedrich & Tautz (1997)
28S-4678F	GAAAGGCGTTGGTTGCTT	Drosophila 4678–4695	This study
28S-5015R	ACGGCTGTTACACGAA	Drosophila 5015–4999	This study
Wg578F	TGCACNGTGAARACYTGCTGGATGCG	Pheidole 578–603	This study
Wg1032R	ACYTCGCAGCACCARTGGAA	Pheidole 1032–1013	Abouheif & Wray (2002)
LR143F	GACAAAGTKCCACCRGARATGCT	Apis 143–165	This study
LR639ER	YTTACCGRITCCATCCRAACA	Apis ~639–624	This study
AA1182F	CCGGCGATATGAGTACGAAATTC	Myrmica 1182–1204	Modified from De Menten <i>et al.</i> (2003)
AA1824R	TAGAAYTGTGCCCGCTGCCAT	Myrmica 1824–1802	This study

Wg, wingless; LR, long-wavelength rhodopsin; AA, abdominal-A; F, forward primer; R, reverse primer.

Position numbers correspond to those in the following GenBank sequences: Drosophila = *D. melanogaster* (M21017); Pheidole = *P. morrissi* (AY101369.1); Apis = *A. mellifera* (U26026); and Myrmica = *M. rubra* (AF332515).

Brady (2003). For the protein-encoding genes, amplification typically consisted of forty cycles of 30 s at 95 °C, 30 s at 50–58 °C and 1 min 30 s at 72 °C, preceded by 1 min at 95 °C and followed by a final extension for 3 min at 72 °C. For most amplifications, PCR Master Mix (1.5 mM MgCl₂, 0.2 mM dNTPs, and 1 unit *Taq*) (Promega Biotech, Madison, WI), 0.4 µM each primer, and 3 µl of template, in a final reaction volume of 20 µl were used. The PCR products were purified by exonuclease I and shrimp acid phosphatase digestion of single-stranded DNA (primers) and dNTPs (ExoSAP-IT, USB Corporation, Cleveland, Ohio, U.S.A.), and sequenced in both directions on a Perkin-Elmer ABI 377 automated sequencer. GenBank accession numbers for individual sequences are given in Appendix 1.

Sequence alignment

Sequences were aligned with CLUSTAL X (1.8) (Thompson *et al.*, 1997), and then manually edited with MACCLADE 4 (Maddison & Maddison, 2000). Taking into account the inferred amino acid sequences, alignment was relatively straightforward for the three protein-encoding genes, and for the 18S ribosomal gene. These genes showed little variation in length, except for an intron in LW *Rh*, which always occurred at the same location and was removed from the dataset before analysis. Alignment of 28S sequences was more difficult, owing to the presence of hypervariable regions in the D1–D6 domains (*sensu* Hancock *et al.*, 1988). Multiple alignment was carried out first with the ingroup taxa

(subfamily Pseudomyrmecinae) using the program defaults of CLUSTAL X, and then the outgroup taxa were aligned with the pseudomyrmecines using the profile alignment option of CLUSTAL. From manual inspection we defined two sets of potentially excludable sites. The first set (339 sites in the CLUSTAL alignment) involved exclusion of all indel-rich hypervariable regions. In this case, all sites were excluded until there remained flanking nucleotides that were invariant, or nearly so, across all ant taxa. A second, less stringent, exclusion involved the removal of sites that were hypervariable within the ingroup (261 sites). Exploratory data analysis suggested that use of the less stringent exclusion set was a reasonable compromise between the loss of information within the ingroup (most pronounced with the more stringent exclusion) and incorrect homology assessment over the entire dataset (most pronounced with no exclusion of sites). The phylogenetic results reported here are based on the less stringent exclusion of 28S sequence sites. The concatenated, aligned, five-gene dataset, with 28S excluded sites identified, has been deposited with TREEBASE (M1940).

Phylogenetic analysis

Unweighted parsimony analysis of the morphological and molecular datasets, both separately and in combination, was conducted with PAUP* 4.0b10 (Swofford, 2003), using tree bisection reconnection (TBR) branch swapping and 100 random stepwise additions. Gaps in the sequence data were treated as missing data. Bootstrap values were

calculated using 1000 TBR replicates, with ten random taxon additions per bootstrap replicate.

For the DNA data, separate analyses were also performed for each of the five genes, and the degree of agreement among the different datasets was assessed by determining the number of instances in which well-supported clades (defined as those having bootstrap support > 80%) that were recovered from one dataset contradicted other well-supported clades from a different dataset. As an additional heuristic measure, the incongruence length difference (ILD) test (Farris *et al.*, 1995) was applied to all possible pairwise combinations of the five molecular datasets. ILD calculations were carried out with PAUP*, using 500 replicates and ten random taxon additions per replicate.

For model-based inference of the phylogeny, MODELTEST (3.06) (Posada & Crandall, 1998) in conjunction with PAUP* was employed to examine separately each of the five molecular datasets, applying likelihood ratio tests to choose a substitution model from among the fifty-six considered by the program. Three models were selected: TrNef + I + G (for 18S, 28S and *wg*), TVM + I + G (for LW *Rh*) and HKY + I + G (for *abd-A*). A Markov chain Monte Carlo (MCMC) analysis was then run with MRBAYES version 3b4 (Huelsenbeck & Ronquist, 2001), partitioning the dataset according to the five genes, and applying the closest available substitution models, namely the HKY + I + G model (nst = 2, rates = invgamma) to *abd-A* and the GTR + I + G model (nst = 6, rates = invgamma) to the other four genes. For each of the five partitions, MRBAYES estimated the proportion of invariant sites, the gamma distribution shape parameter, base frequencies, and the substitution rates (GTR model) or transition/transversion ratio (HKY model). The program default of four chains (three hot, one cold) was used and each MCMC run went for 10 000 000 generations, with sampling every 1000 generations. The burnin value (1000 samples) was determined by plotting the likelihood scores against the progress of the run and excluding preasymptotic values. Five independent runs were conducted, all of which gave similar output, suggesting that stationarity had been achieved. Separate Bayesian analyses were also run for each gene to examine potential conflict among genes.

For the concatenated (five-gene) dataset, the appropriateness of the partitioned Bayesian model relative to an unpartitioned (GTR + I + G) model was evaluated by a Bayes factor comparison (as in Nylander *et al.*, 2004). The Bayes factor, estimated from the ratio of the harmonic means of the marginal likelihoods of the partitioned and unpartitioned models, was approximately 480 log likelihood units, demonstrating a much better fit with the partitioned model and justifying the latter approach.

The histories of change in nesting habits and geographical distribution among pseudomyrmecine ants were reconstructed on the phylogenies under parsimony (i.e. minimization of character state change), using the character tracing tools of MACCLADE 4 (Maddison & Maddison, 2000).

Results

Inferences from morphology

Analysis of the 144 character dataset produced 462 most-parsimonious trees (length = 658, consistency index = 0.278, retention index = 0.735), of which the strict consensus is depicted in Fig. 1. This retrieves the monophyly of the subfamily Pseudomyrmecinae (95% bootstrap support), the genera *Pseudomyrmex* (91%) and *Tetraponera* (61%), and the grouping of *Myrcidris* + *Pseudomyrmex* (77%). Within *Pseudomyrmex* and *Tetraponera*, most species cluster together in a manner consistent with the previously recognized species groups (Ward, 1989, 2001), with one exception: the four species representing the *P. pallens* group (*P. apache*, *P. elongatulus*, *P. pallens*, and *P. phyllophilus*) do not form a clade. This substantiates an earlier observation that the species in the *P. pallens* group are a somewhat heterogeneous assemblage and probably nonmonophyletic (Ward, 1989).

On the strict consensus tree (Fig. 1), the branch subtending the subfamily Pseudomyrmecinae has twelve changes (ACCTRAN optimization) or seventeen changes (DELTRAN optimization), emphasizing the distinctness of these ants. Most relationships among the outgroup taxa are not strongly supported (Fig. 1), with the exception of the monophyly of Myrmeciinae (*Myrmecia* and *Nothomyrmecia*). The analysis does not clearly identify a sister group of Pseudomyrmecinae.

DNA sequence characteristics

The aligned sequence data consist of 5191 bp (after excluding unalignable portions of 28S and an intron in LW *Rh*), of which 1316 sites are variable and 974 parsimony-informative. The characteristics of individual genes are reported in Table 2. The number of most-parsimonious trees is sharply reduced when the five genes are combined into a single dataset. Bases occur in approximately equal frequencies, but with a slight GC bias. For the concatenated dataset, the empirical base frequencies are: A 23.03%, C 26.34%, G 28.48% and T 22.15%. Base composition heterogeneity among taxa is not pronounced for the combined dataset (χ^2 87.45, d.f. = 198, P = 1.000) nor for four individual genes (χ^2 6.47–70.64, d.f. = 198, P = 1.000), but the *wg* gene is an exception (χ^2 237.21, d.f. = 198, P = 0.030). This last result is due largely to an unusually AT-rich *wg* sequence for *Mischocyttarus flavitarsis*; when this species is removed *wg* shows no significant departure from base frequency homogeneity (χ^2 221.86, d.f. = 195, P = 0.091). One should note that these χ^2 tests do not take into account phylogenetic structure, so the probability of type I error may be inflated.

Dataset conflict

The parsimony analyses of individual genes revealed almost no instances of strong conflict, i.e. no cases where

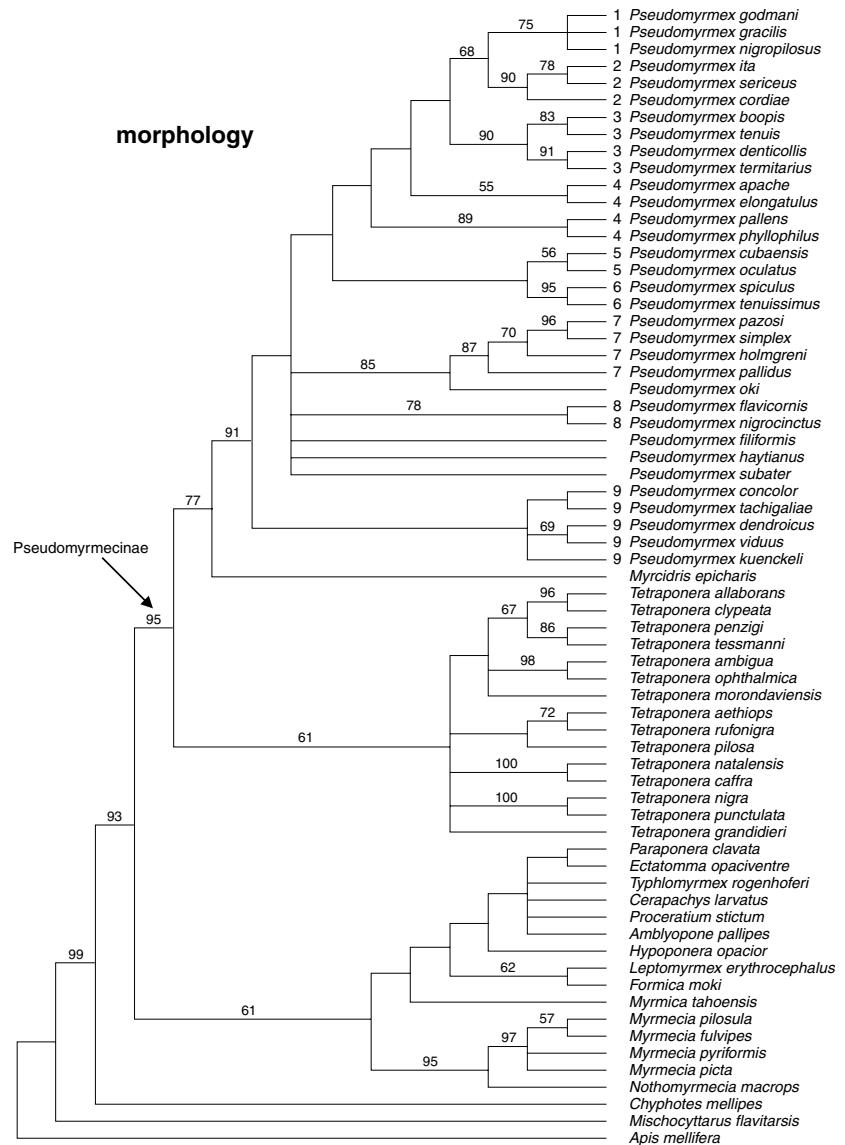


Fig. 1. Phylogeny of pseudomyrmecine ants and outgroups inferred from morphology. This is the strict consensus of 462 most-parsimonious trees. The numbers above the branches are bootstrap values (>50%) based on a separate analysis. The numbers next to the *Pseudomyrmex* species identify members of the same species group, defined previously on the basis of morphology (Ward, 1989): 1, *gracilis* group; 2, *sericeus* group; 3, *tenuis* group; 4, *pallens* group; 5, *oculatus* group; 6, *subtilissimus* group; 7, *pallidus* group; 8, *ferrugineus* group; 9, *viduus* group. *Pseudomyrmex* species without a number are unplaced to a species group (*incertae sedis*).

a clade of ants having strong support (bootstrap proportions > 80%) in one analysis is incompatible with a strongly supported clade in another analysis. The only exception is the 18S gene, which, because of the small number of parsimony-informative sites, has few well-supported clades, but does place *Myrcidris* as sister to a group of five *Tetraponera* species (bootstrap 92%), a result contradicted by *wg*, *LW Rh*, and 28S, all of which strongly support the hypothesis (bootstrap proportions > 85%) of a sister-group relationship between *Myrcidris* and *Pseudomyrmex*. Although not conforming to the definition of 'strong conflict' adopted above, the *wg* gene also strongly supports the paraphyly of *Tetraponera*, in contradiction of the 28S sequence data, which point to monophyly of the genus. The latter result has weak bootstrap support (62%), but includes an apparently unique insertion (see below). The results of ILD tests revealed significant

heterogeneity ($P < 0.05$) for seven of ten pairwise comparisons, but after application of the Bonferroni correction only three of these remained significant: 18S vs *wg* ($P \approx 0.04$), 18S vs *LW Rh* ($P < 0.02$) and *wg* vs 28S ($P < 0.02$).

Molecular phylogenies

The concatenated five-gene dataset yielded four most-parsimonious trees (length = 4891, consistency index = 0.420, retention index = 0.688), of which the strict consensus is well resolved (Fig. 2). Bayesian inference produced very similar results, such that the majority-rule consensus tree from the MCMC analysis (Fig. 3) is almost identical in topology to the strict consensus of the four most-parsimonious trees. Support levels for most clades are closely comparable

Table 2. Summary statistics for the datasets ($n = 67$ taxa in all cases).

Dataset	No. of characters	No. of variable characters	No. of parsimony-informative characters	No. of mpts	Length	CI	RI
18S	1868	169	90	10000+	336	0.607	0.756
28S	1835	467	308	8235	1421	0.507	0.715
wg	412	216	195	1344	1014	0.368	0.738
abd-A	618	220	174	1056	857	0.418	0.684
LW <i>Rh</i>	458	244	207	406	1143	0.351	0.638
Five genes	5191	1316	974	4	4891	0.420	0.688
Morphology	144	144	142	462	658	0.278	0.735
DNA + morphology	5335	1460	1116	10	5610	0.399	0.690

mpt, most-parsimonious tree; CI, consistency index; RI, retention index; wg, wingless; LW *Rh*, long-wavelength rhodopsin; *abd-A*, abdominal-A.

between phylogenetic methods (Fig. 2). Almost all groups having parsimony bootstraps (PB) > 80% have Bayesian posterior probabilities (PP) of 1.00, and for all groups with PP > 0.95 PB equals or exceeds 60%, with one exception: Bayesian analysis retrieved a clade (0.95 PP) consisting of all *Pseudomyrmex* species except *P. denticollis*, *P. termitarius*, *P. boopis*, *P. tenuis*, and *P. filiformis*. This result appears in only two of the four most-parsimonious trees and has PB < 50%.

Points of agreement with the morphology-based tree (and with traditional classification) include the monophyly of the subfamily Pseudomyrmecinae (100% PB, 1.00 PP) and the genus *Pseudomyrmex* (100% PB, 1.00 PP). A sister-group relationship between *Myrcidris* and *Pseudomyrmex* is also very strongly supported (100% PB, 1.00 PP). A notable departure from previous results is the inference that the Old World genus *Tetraponera* is paraphyletic, comprising five or six lineages that form a pectinate series, with the New World pseudomyrmecines at the tip. The most strongly supported elements of *Tetraponera* paraphyly are: (1) a sister-group relationship between three *Tetraponera* species (*T. aethiops*, *T. rufonigra* and *T. pilosa*; hereafter called the *rufonigra* group) and the New World species (*Myrcidris* + *Pseudomyrmex*), which has 91% bootstrap support (1.00 PP); and (2) the monophyly of a group containing all pseudomyrmecines except the *T. nigra* group (here represented by *T. nigra* and *T. punctulata*). The second pattern has 89% bootstrap support (1.00 PP).

The molecular data identify the subfamily Myrmeciinae as the group most closely related to Pseudomyrmecinae (69% PB, 1.00 PP), a result not obtained with the morphology-based tree (Fig. 1), although not strongly contradicted by it either. There is no indication of a close relationship between Myrmeciinae (represented by *Myrmica tahoensis*) and Pseudomyrmecinae.

Within *Pseudomyrmex*, most of the previously established species groups are recovered (Fig. 2), with two exceptions: the species belonging to the *P. pallens* group do not form a clade (a result also obtained with the morphological dataset), and the *P. viduus* group – an assemblage of myr-

mecophyte-inhabiting species, represented in this study by *P. concolor*, *P. dendroicus*, *P. kuenckeli*, *P. tachigaliae* and *P. viduus* – is not monophyletic. There is strong evidence (100% PB, 1.00 PP) that the two *Triplaris*-associated species (*P. dendroicus* and *P. viduus*) are more closely related to the *P. oculatus* group (represented by *P. oculatus* and *P. cubaensis*) than to the two *Tachigali* inhabitants, *P. concolor* and *P. tachigaliae*. These last two are sister taxa in this analysis (100% PB, 1.00 PP), but neither they nor the *Triplaris* ants are closely related to *P. kuenckeli*, a fifth member of the *P. viduus* group (and not an ant-plant specialist; Ward, 1999). If the *P. viduus* group is constrained to be monophyletic, there are four minimum-length trees, of length 4911, all of which are significantly more poorly supported by the data than the unconstrained trees (one-tailed Templeton test, $P < 0.01$). Thus, the *P. viduus* group, as defined by Ward (1999), is almost certainly not a clade.

Basally, the genus *Pseudomyrmex* comprises three apparent clades: (1) the *P. tenuis* group + *P. filiformis* (100% PB, 1.00 PP), (2) the *P. gracilis* group + *P. sericeus* group (100% PB, 1.00 PP), and (3) all other *Pseudomyrmex* (73% PB, 0.64 PP). The first two are also recovered by the morphology-based tree (Fig. 1), whereas the third group is not.

Among the outgroups there is very strong support (100% PB, 1.00 PP) for the monophyly of *Myrmecia*, Myrmeciinae, Ectatomminae (represented by *Typhlomyrmex* and *Ectatomma*), Formicidae, and for a group consisting of all sampled ant taxa except four poneromorph genera (*Amblyopone*, *Hypoconera*, *Paraponera* and *Proceratium*). The last group, here termed the 'formicoid clade' and comprising all extant ants except leptanillomorphs and some poneromorphs, is beginning to emerge from molecular analyses as one of the best-supported deep branches in ant phylogeny (Brady, 2003; Ward & Brady, 2003; Ohnishi *et al.*, 2004; Saux *et al.*, 2004). Our results also suggest that the bradynobaenid (*Chyphotes*) is more closely related to ants than is the vespidae (*Mischocyttarus*), but here there is a mismatch between strong support under parsimony (98% PB) and poor support under Bayesian inference (0.58 PP).

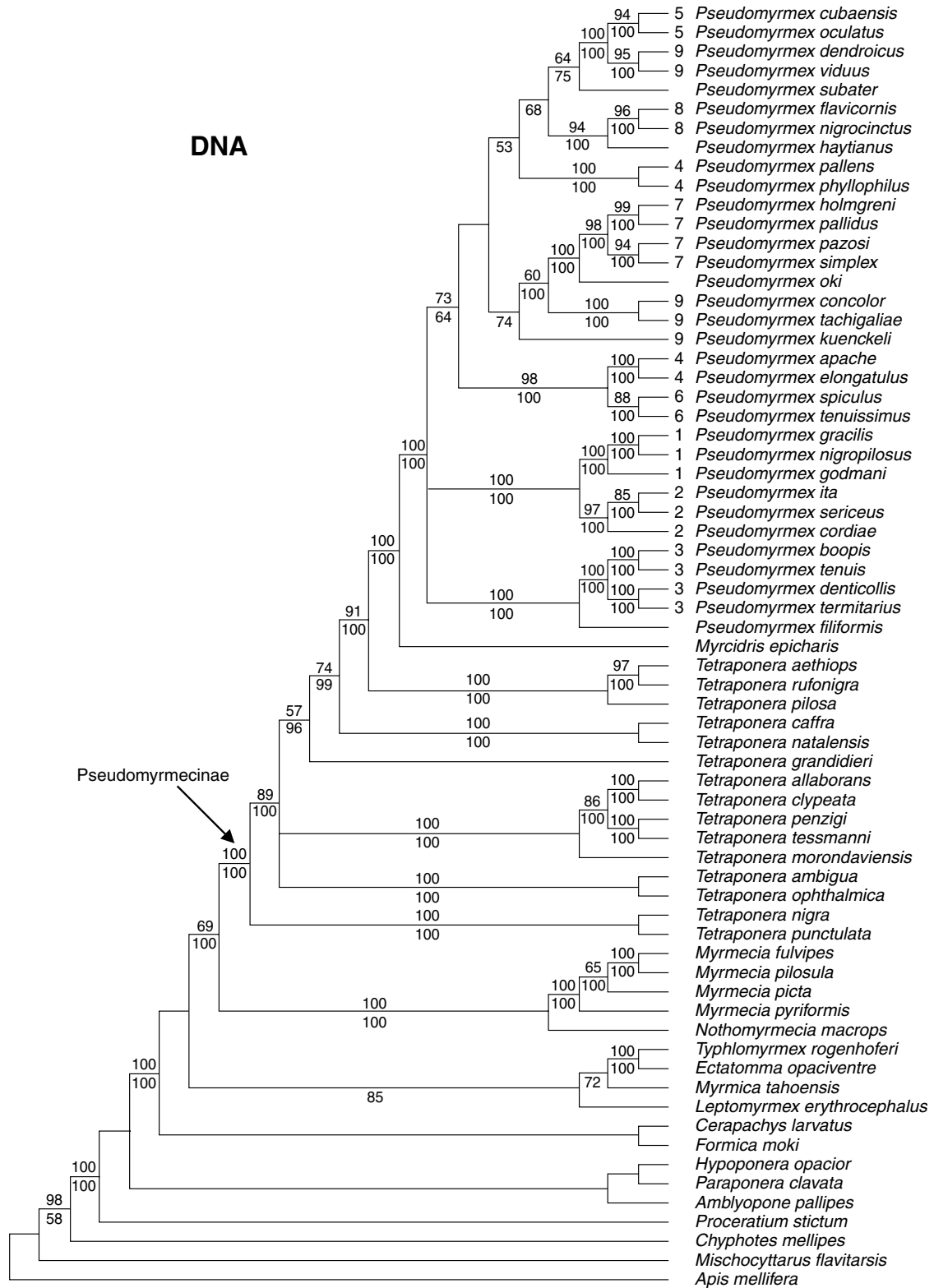


Fig. 2. Phylogenetic relationships inferred from DNA sequence data (five genes, 5.2 kb). This is the strict consensus of four most-parsimonious trees. The numbers on the branches are bootstrap values (above) and Bayesian posterior probabilities $\times 100$ (below). Species group numbers as in Fig. 1.

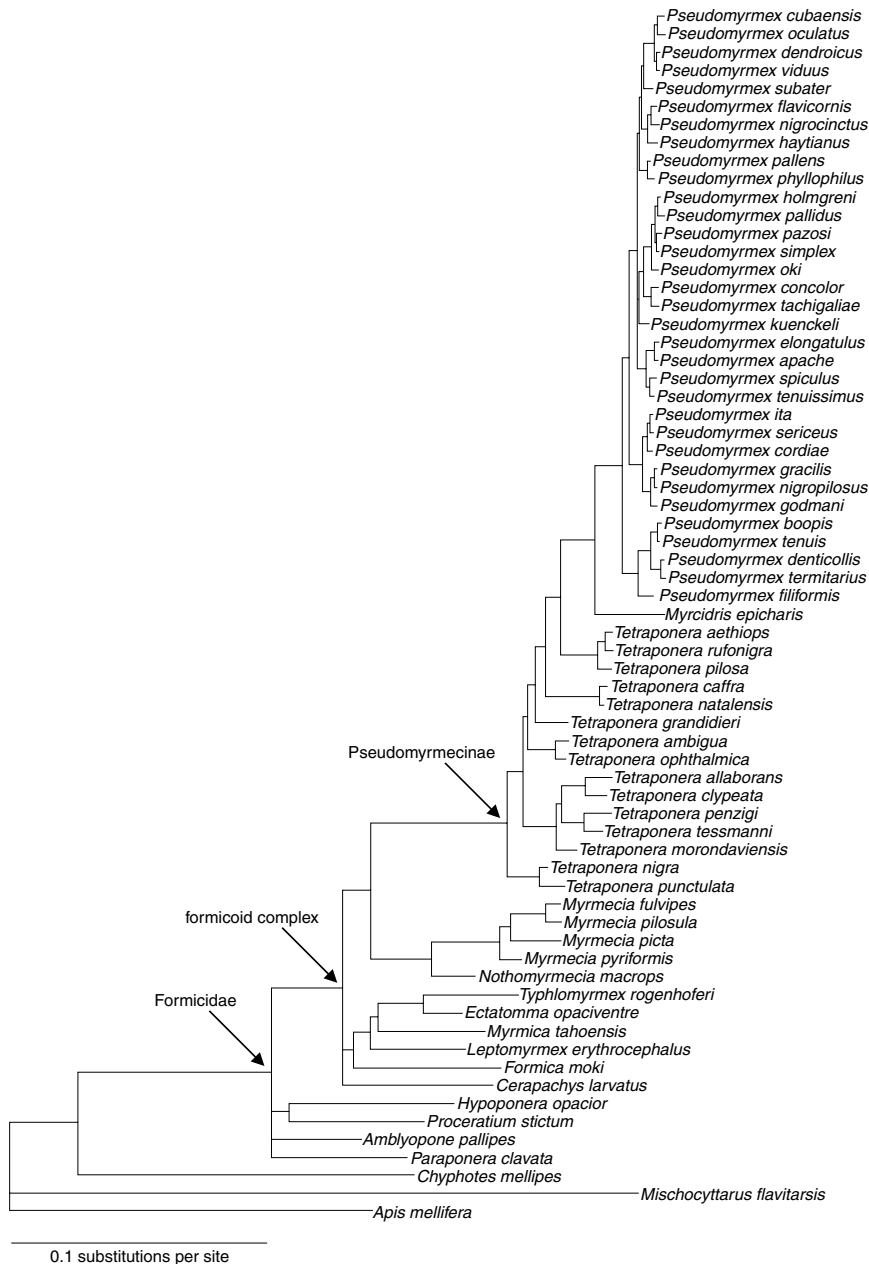


Fig. 3. Phylogram obtained with Bayesian estimate of phylogeny. This is the 50% majority-rule consensus tree of all sampled trees (after burnin) in the Markov chain Monte Carlo (MCMC) analysis. The branch lengths represent the means of the posterior probability distributions of branch lengths among all sampled trees. Note the long branch subtending Pseudomyrmecinae.

The branch length estimates from the Bayesian analysis (Fig. 3) highlight the long branch subtending the Pseudomyrmecinae, and the greater interspecific genetic divergences among the Old World species (*Tetraponera*) compared with those of the New World (*Myrcidris*, *Pseudomyrmex*).

Combined analysis of morphological and molecular data

Parsimony analysis of the combined dataset yielded results similar to the molecular tree, with most of the same

clades recovered except some basal groups within *Pseudomyrmex* (Fig. 4). This is not surprising, given the much larger number of characters contributed by the DNA sequence data (Table 2). Support for the paraphyly of *Tetraponera* drops, such that a clade consisting of (*T. rufonigra* group + (*Pseudomyrmex* + *Myrcidris*)) receives 79% PB (compared with 91% in the molecular dataset), and the monophyly of all pseudomyrmecines except the *T. nigra* group has 77% PB (compared with 89%). For most clades, bootstrap support remains about the same, but a sister-group relationship between Pseudomyrmecinae and Myrmeciinae is strengthened (from 69 to 84% PB).

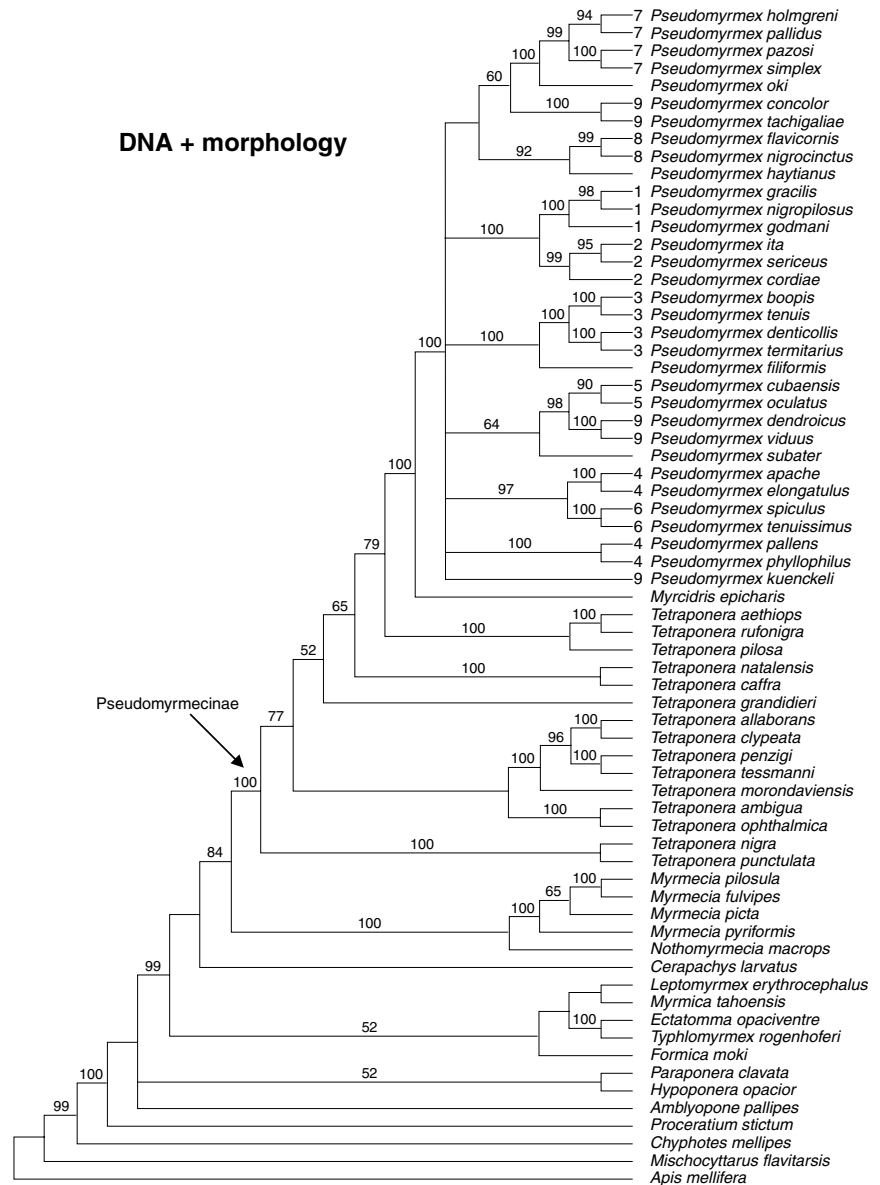


Fig. 4. Phylogenetic relationships inferred from parsimony analysis of combined morphological and molecular data. This is the strict consensus of ten most-parsimonious trees. The numbers above the branches are bootstrap values. Species group numbers as in Fig. 1.

Status of *Tetraponera*

Although the molecular data indicate that *Tetraponera* is paraphyletic, parsimony analysis under the constraint of monophyly resulted in trees of only slightly greater length (twelve trees of length 4904, compared with four trees of length 4891 in the unconstrained analysis). The unconstrained trees are only marginally better supported by the data (one-tailed Templeton tests, $P = 0.040\text{--}0.069$). Moreover, inspection of the parsimony and Bayesian trees produced by the analysis of individual genes shows that the paraphyly of *Tetraponera* is strongly supported (PB > 80%; PP > 0.95) only by the *wg* gene. The 28S gene and morphology indicate that *Tetraponera* is monophyletic, albeit with weak support (28S: 62% PB, 0.61 PP; morphology: 61%

PB). The three other genes (18S, *abd-A* and LW *Rh*) are individually inconclusive.

There are several morphological and molecular features that could be interpreted as synapomorphies of *Tetraponera*. These include (*w* = worker, *q* = queen) the angulate surface of the mandible above the trulleum (*w*), reduction in mandibular teeth (*w*, *q*), and the narrow notchlike cleft on the distal margin of the labrum (*w*, *q*). Among the 28S gene sequences there is a striking feature found in all species of *Tetraponera* examined to date: in a conserved region of the D2a subdomain there is a unique and apparently uncompensated single-base insertion of guanine. This insertion is absent from all other pseudomyrmecines and from all the outgroup taxa sampled here, including the three non-ant hymenopterans (*Apis mellifera*, *Mischocyttarus flavitarsis*,

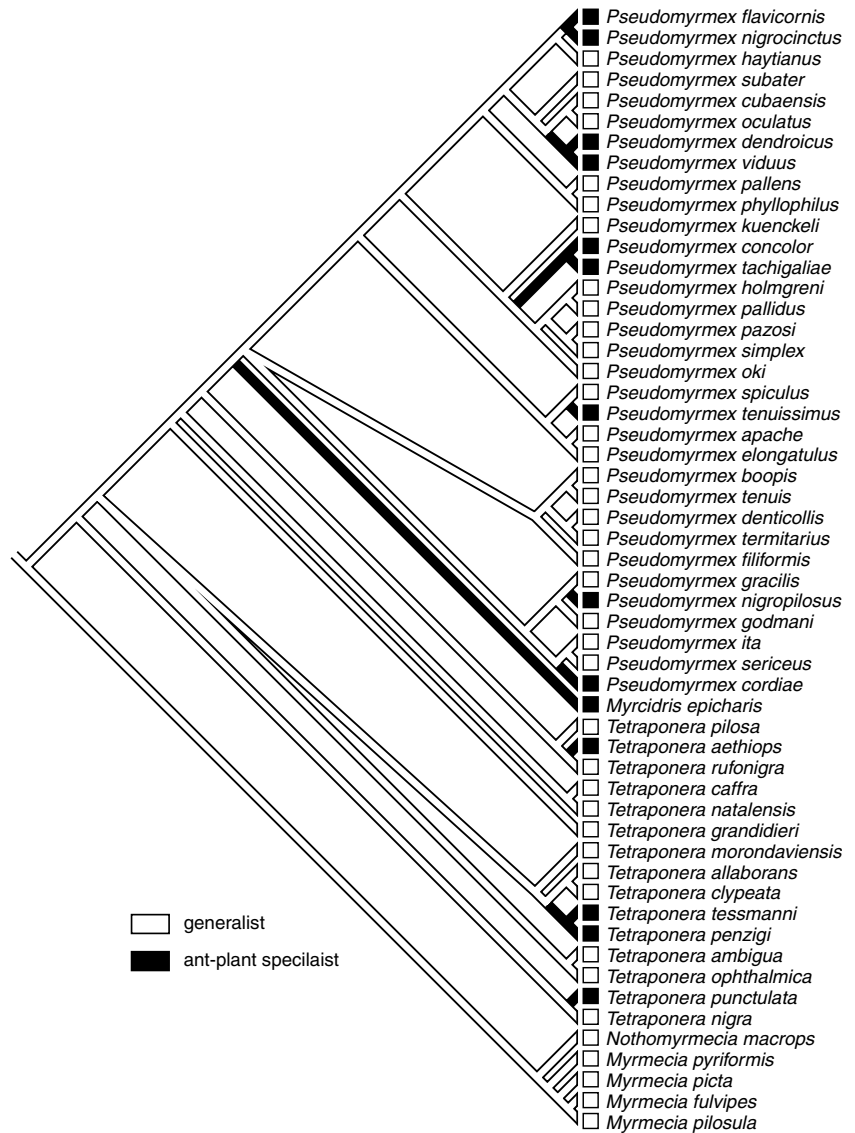


Fig. 5. Associations with ant-plants (myrmecophytes) traced on the phylogeny of Pseudomyrmecinae, as inferred from molecular data (Fig. 2). Two species, *Pseudomyrmex tenuissimus* and *Tetraponera punctulata*, are labelled as ant-plant specialists because very close relatives (*P. subtilissimus* and *T. tucuruua*), not sampled in this study, have this trait.

Second, the DNA sequence data provide some support (69% PB, 1.00 PP) for a sister-group relationship between Pseudomyrmecinae and Myrmeciinae, a hypothesis also supported by several shared morphological features, such as the metabasitarsal sulcus, large eyes, and an elevated base of the sensilla basiconica on the antennae (Hashimoto, 1991; Ward, 1994). These traits are included in the morphological data matrix (Appendix 3), but the first two exhibit some homoplasy and their signal may also have been partly swamped by other characters. In this regard, it is instructive that the combined dataset (morphology + DNA sequence data) provides stronger bootstrap support for the hypothesis (84% PB). Given an estimated age of the most recent common ancestor of extant myrmeciines of 74 Mya (95% credibility interval of 53–101 Mya) (Ward & Brady, 2003), this implicates an origin of the stem-group pseudomyrmecines in the Cretaceous.

The molecular data do not support an earlier hypothesis (Ward, 1990; Baroni Urbani *et al.*, 1992; Grimaldi *et al.*, 1997) that the subfamily Myrmicinae is the sister group of Pseudomyrmecinae. The similarities between the two subfamilies are apparently due to convergence, especially of features associated with a postpetiole (e.g. a lengthening of pretergite IV relative to presternite IV, in those postpetiole taxa with a stridulitrum). It now appears that the postpetiole arose independently in the two groups, and separately in other ant lineages as well (Bolton, 2003). This is equally true of closed metacoxal cavities and naked pupae, two other features shared (but not uniquely) by the two subfamilies.

Third, the two New World genera of pseudomyrmecines (*Myrcidris* and *Pseudomyrmex*) together form a very well-supported clade (100% PB, 1.00 PP). This is contrary to the results of an earlier morphological analysis (Ward, 1990),

which inferred the following relationship: *Myrcidris* + (*Pseudomyrmex* + *Tetraponera*). In that study, however, an alternative arrangement in which *Myrcidris* and *Pseudomyrmex* were sister taxa was almost equally parsimonious, so that the molecular data can be said to have arbitrated among these alternatives and found much stronger evidence for the latter arrangement.

Fourth, in contrast to the situation with the New World pseudomyrmecines, it is unclear if the Old World species (genus *Tetraponera*) form a monophyletic group. In the five-gene analysis, *Tetraponera* is paraphyletic, with strong support, but the signal comes primarily from one gene (*wg*), with the 28S gene and morphology providing circumstantially strong evidence for monophyly. This evidence includes a unique insertion in the D2a domain of the 28S gene that is found in no other ants. If *Tetraponera* is monophyletic, then it is almost certainly subtended by a relatively short branch, which would militate against recovery of evidence for monophyly.

At this stage, the status of *Tetraponera* remains uncertain, and can only be resolved with additional data. For this reason, we refrain from making any changes in classification. It should be noted that if the genus proves to be paraphyletic – and therefore requires cleavage into multiple monophyletic subgroups – several genus-level names are already available: *Pachysima* Emery (type species *T. aethiops*) for the *rufonigra* group (here defined more inclusively than in Ward, 2001); *Tetraponera* (type species *T. nigra*) for the *T. nigra* group (represented in this study by *T. nigra* and *T. punctulata*); and *Sima* Roger (type species *T. allaborans*) for some fraction of the remaining species.

Species groups and ant–plant associations

Species groups within the genus *Pseudomyrmex*, established previously on the basis of morphology (Ward, 1989), hold up reasonably well under new scrutiny (Figs 2, 3). Seven of the nine species groups are recovered with high confidence: 88–100% PB in the molecular dataset, 90–100% PB in the combined dataset. The *P. pallens* group, whose artificiality was previously noted (Ward, 1989), is not monophyletic, however, nor is the *P. viduus* group. The latter is comprised mainly of species that inhabit specialized plants, in whose domatia the ants keep brood and scale insects and which they defend aggressively (Ward, 1991). The principal ant–plant associations are *Tachigali* (Fabaceae) and *Triplaris* (Polygonaceae). A previous phylogenetic analysis of these ants, based on morphology, found strong support for a sister-group relationship between two clades, composed of the *Triplaris*-associated and *Tachigali*-associated species, respectively (Ward, 1999). The molecular data strongly support the monophyly of these individual clades, but the data also strongly reject the hypothesis that they are sister taxa. Rather, the *Triplaris* ants and the *Tachigali* ants appear to have independently evolved from different groups of generalist *Pseudomyrmex* that inhabit dead twigs.

Morphology was evidently misleading in earlier phylogenetic analyses (Ward, 1991, 1999) – and even in the morphology-based tree in this study (see Fig. 1) – because the ants living in *Triplaris* and *Tachigali* have convergently evolved similar traits, such as shorter antennal scapes, reduced eyes, and more robust petioles. In the analysis by Ward (1999), all of the features supporting a sister-group relationship between the *Triplaris* ants and the *Tachigali* ants were worker and/or queen based, whereas the characters supporting the monophyly of each individual subgroup were based predominantly on male genitalia (see Ward, 1999: fig. 169). The monophyly of the individual subgroups is now strongly corroborated by the DNA sequence data. In retrospect, it seems that the male genital features provide a more reliable indication of relationships because they are not subject to the same ecologically driven convergence as worker and queen morphology.

An earlier morphological phylogenetic analysis concluded that obligate domatia-inhabiting ants evolved at least twelve times in the Pseudomyrmecinae (Ward, 1991) and the present results reinforce this conclusion. Among the species examined in this study, ten originations of ant–plant associations are implied (Fig. 5). There are additional myrmecophyte-associated species, in both *Pseudomyrmex* and *Tetraponera*, that are not closely related to those sampled here (Ward, 1991, 1999, 2001). Thus, it seems evident that twelve must be a minimum estimate of the number of times that this trait evolved. More exhaustive sampling of the 300+ species in the subfamily will be necessary to hone the details of this history.

Biogeography

Pseudomyrmecine ants are restricted largely to tropical and subtropical regions, with one large clade (*Pseudomyrmex* + *Myrcidris*) confined to the New World, and the remaining species (*Tetraponera*) endemic to the Old World. It would be of interest to know if the divergence between the two groups coincided with the separation of South America and Africa (~100 Mya), as was inferred recently for the Neotropical and Palaeotropical army ants (Brady, 2003). Using a dataset based on 18S and 28S sequence data and applying a Bayesian dating method, Ward & Brady (2003) estimated the date of divergence between *Pseudomyrmex* (represented by *P. gracilis*) and *Tetraponera* (represented by *T. rufonigra*) to be 54 Mya (95% credibility interval of 42–78 Mya), an age too young to be consistent with Gondwanan vicariance. The evidence from the present study that *Tetraponera* is paraphyletic does not alter this conclusion. In fact, as *T. rufonigra* is in the clade apparently most closely related to the New World pseudomyrmecines, the divergence date implies that there was a period of diversification of pseudomyrmecines in the Old World tropics in the Palaeocene, before dispersal to the New World. Baltic amber from the late Eocene or early Oligocene contains several morphologically disparate species of *Tetraponera* (Wheeler, 1915; Dlussky, 1997; Ward,

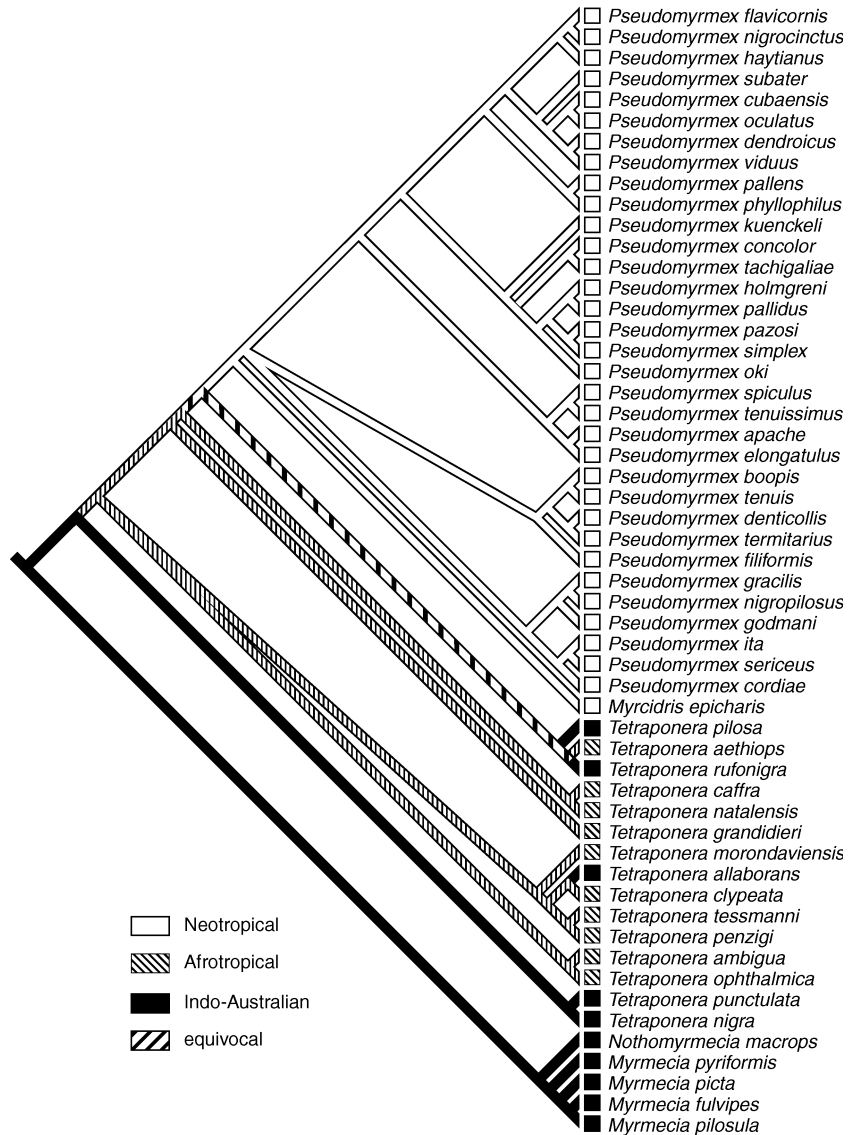


Fig. 6. Taxon distribution (Neotropical, Afrotropical, Indo-Australian) traced on the phylogeny of Pseudomyrmecinae, as inferred from molecular data (Fig. 2).

2001), consistent with the notion of an earlier period of diversification.

The hypothesis (Ward, 2001) that *Tetraponera* originated in Africa and dispersed to Asia (and then Australia) needs to be re-evaluated in light of the relationships inferred here, in particular the basal position of the *T. nigra* group, whose members are confined to the Indo-Australian region. If *Tetraponera* is paraphyletic in this manner, as inferred from the concatenated molecular dataset and the combined (molecular + morphological) dataset, then the ancestral area for *Tetraponera* is more likely to be in the Indo-Australian region rather than Africa, although much of the later history of the group appears to have involved the latter continent (Fig. 6).

There are currently about twice as many species of *Pseudomyrmex* (~200) as there are *Tetraponera*, with the latter genus having about twenty-five species in Africa, approxi-

mately forty in Madagascar and approximately thirty-five in the Indo-Australian region (Ward, 2001; unpublished). The difference in net diversification is even more striking if *Tetraponera* is paraphyletic, with the 200+ New World pseudomyrmecines being sister to a small clade (the *rufonigra* group) that contains two species in Africa and two species in Asia. Contrasts in species richness have been noted for other taxa inhabiting both Neotropical and Palaeotropical rainforests, with the Afrotropical region generally being the most impoverished (Amadon, 1973; Thorne, 1973; Robbins & Opler, 1997). Possible contributing factors include greater habitat heterogeneity in the Neotropics, associated with more active orogeny and other topographical peculiarities (McKey & Davidson, 1993), and higher extinction rates in the African forests, due to a smaller area and periods of climatic deterioration (Raven & Axelrod, 1974; Goldblatt, 1993; Livingston, 1993). Most

pseudomyrmecine species have 'generalized' twig-nesting habits and are not tied to particular plant species (unlike the minority of species that have become ant-plant inhabitants), but specialization is evident in preferences for different habitats, vegetation strata, and twig sizes. It seems reasonable to suppose that the more complex and heterogeneous vegetation in the Neotropics, coupled with less disruptive climatic change, afforded greater opportunities for diversification in the New World pseudomyrmecines.

Conclusions

Taken together, the molecular and morphological evidence presented here supports the hypothesis that the ant subfamilies Pseudomyrmecinae and Myrmecinae are sister taxa. Given their respective distributions, character traits and estimated divergence times (Ward & Brady, 2003), one can envisage an ancestral lineage of active, large-eyed, stinging ants – of moderately large body size – which ranged across some portion of Gondwana in the mid-Cretaceous, and which gave rise to these two groups. The pseudomyrmecines diversified in the course of adapting to arboreal conditions (unlike the predominantly ground-dwelling myrmecinae) and came to occupy and retain a much larger geographical range. The extant New World pseudomyrmecines, represented by the sister genera *Pseudomyrmex* and *Myrcidris*, are clearly a monophyletic group, but the status of the Palaeotropical species, currently placed in the genus *Tetraponera*, is unclear. Morphological features and a unique insertion in the 28S gene point to the monophyly of the Old World species, but DNA sequence data strongly suggest paraphyly – a conflict that can only be resolved with additional data. Maximum interspecific genetic divergences are greater in *Tetraponera* than *Pseudomyrmex*, probably reflecting the survival of older lineages in the Palaeotropics. The higher species richness and abundance of *Pseudomyrmex* in the New World may be attributed to a less disruptive climatic history, extensive habitat heterogeneity, and greater opportunities for specialization in the Neotropical forests.

Supplementary material

The following material is available at: <http://www.blackwellpublishing.com/products/journals/suppmat/SEN/SEN281/SEN281sm.htm>

Table S1. Alternative primers for LW *Rh* and *abd-A* genes, employed with a minority of samples. LR = LW *Rh*; AA = *abd-A*. F = forward primer; R = reverse primer. Position numbers correspond to those in the following GenBank sequences: *Apis* = *A. mellifera* (U26026); and *Myrmica* = *M. rubra* (AF332515).

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Appendix 1. List of sequenced taxa and GenBank accession numbers.

Family (subfamily)	Taxon	Locality (date)	GenBank accession numbers			LW Rh	abt-A
			18S	28S	wg		
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex apache</i>	Arizona, U.S.A. (2001)	AY703518	AY703585	AY703652	AY703786	AY703719
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex boopis</i>	Bolívar, Colombia (1992)	AY703519	AY703586	AY703653	AY703787	AY703720
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex concolor</i>	Amazonas, Brazil (1999)	AY703520	AY703587	AY703654	AY703788	AY703721
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex cordata</i>	Santa Cruz, Bolivia (1993)	AY703521	AY703588	AY703655	AY703789	AY703722
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex cubaensis</i>	Pinar del Río, Cuba (2001)	AY703522	AY703589	AY703656	AY703790	AY703723
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex dendroicus</i>	Santa Cruz, Bolivia (1993)	AY703523	AY703590	AY703657	AY703791	AY703724
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex denticollis</i>	Formosa, Argentina (2002)	AY703524	AY703591	AY703658	AY703792	AY703725
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex elongatulus</i>	Veracruz, Mexico (1985)	AY703525	AY703592	AY703659	AY703793	AY703726
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex filiformis</i>	MG do Sul, Brazil (1996)	AY703526	AY703593	AY703660	AY703794	AY703727
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex flavicornis</i>	Guanacaste, CR (2000)	AY703527	AY703594	AY703661	AY703795	AY703728
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex godnani</i>	Santa Cruz, Bolivia (1993)	AY703528	AY703595	AY703662	AY703796	AY703729
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex gracilis</i>	Guanacaste, CR (2000)	AY703529	AY703596	AY703663	AY703797	AY703730
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex haytianus</i>	Distrito Nacional, DR (1992)	AY703530	AY703597	AY703664	AY703798	AY703731
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex holmgreni</i>	Santa Cruz, Bolivia (1993)	AY703531	AY703598	AY703665	AY703799	AY703732
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex ita</i>	Puntarenas, CR (1990)	AY703532	AY703599	AY703666	AY703800	AY703733
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex kuenekei</i>	Matto Grosso, Brazil (1996)	AY703533	AY703600	AY703667	AY703801	AY703734
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex nigrocinctus</i>	Guanacaste, CR (2000)	AY703534	AY703601	AY703668	AY703802	AY703735
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex nigropilosus</i>	Guanacaste, CR (1989)	AY703535	AY703602	AY703669	AY703803	AY703736
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex oculatus</i>	Santa Cruz, Bolivia (1993)	AY703536	AY703603	AY703670	AY703804	AY703737
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex oki</i>	Puntarenas, CR (1990)	AY703537	AY703604	AY703671	AY703805	AY703738
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex pallens</i>	Santa Cruz, Bolivia (1993)	AY703539	AY703606	AY703673	AY703807	AY703740
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex pallidus</i>	Heredia, CR (2000)	AY703538	AY703605	AY703672	AY703806	AY703739
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex pazosi</i>	Villa Clara, Cuba (2001)	AY703540	AY703607	AY703674	AY703808	AY703741
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex phyllophilus</i>	Minas Gerais, Brazil (1996)	AY703541	AY703608	AY703675	AY703809	AY703742
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex sericeus</i>	Santa Cruz, Bolivia (1993)	AY703542	AY703609	AY703676	AY703810	AY703743
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex simplex</i>	Guanacaste, CR (2000)	AY703543	AY703610	AY703677	AY703811	AY703744
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex spiculatus</i>	Limón, CR (1988)	AY703544	AY703611	AY703678	AY703812	AY703745
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex subater</i>	Villa Clara, Cuba (2001)	AY703545	AY703612	AY703679	AY703813	AY703746
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex tachigaliae</i>	Loreto, Peru (1996)	AY703546	AY703613	AY703680	AY703814	AY703747
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex tenuis</i>	Maranhão, Brazil (1999)	AY703547	AY703614	AY703681	AY703815	AY703748
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex tenuissimus</i>	Santa Cruz, Bolivia (1993)	AY703548	AY703615	AY703682	AY703816	AY703749
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex termitarius</i>	Santa Cruz, Bolivia (1993)	AY703549	AY703616	AY703683	AY703817	AY703750
Formicidae (Pseudomyrmecinae)	<i>Pseudomyrmex viduus</i>	Guanacaste, CR (2000)	AY703550	AY703617	AY703684	AY703818	AY703751
Formicidae (Pseudomyrmecinae)	<i>Myrcidris epiccharis</i>	Amazonas, Brazil (1987)	AY703517	AY703584	AY703651	AY703785	AY703718
Formicidae (Pseudomyrmecinae)	<i>Tetraponera aethiops</i>	Centr. Afr. Republic (2001)	AY703502	AY703569	AY703636	AY703770	AY703703
Formicidae (Pseudomyrmecinae)	<i>Tetraponera allaborans</i>	Karnataka, India (1999)	AY703503	AY703570	AY703637	AY703771	AY703704
Formicidae (Pseudomyrmecinae)	<i>Tetraponera ambigua</i>	West Cape, RSA (1999)	AY703504	AY703571	AY703638	AY703772	AY703705

Appendix 1. Continued

Family (subfamily)	Taxon	Locality (date)	GenBank accession numbers				LW Rh	abd-A
			18S	28S	wg			
Formicidae (Pseudomyrmecinae)	<i>Tetraponera caffra</i>	KwaZulu Natal, RSA (1999)	AY703505	AY703572	AY703639	AY703773	AY703706	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera clypeata</i>	West Cape, RSA (1999)	AY703506	AY703573	AY703640	AY703774	AY703707	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera grandidieri</i>	Fianarantsoa, MA (1997)	AY703507	AY703574	AY703641	AY703775	AY703708	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera morondaviensis</i>	Toliara, MA (1993)	AY703508	AY703575	AY703642	AY703776	AY703709	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera natalensis</i>	KwaZulu Natal, RSA (1999)	AY703509	AY703576	AY703643	AY703777	AY703710	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera nigra</i>	Kerala, India (1999)	AY703510	AY703577	AY703644	AY703778	AY703711	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera ophthalmica</i>	Coast, Kenya (1990)	AY703511	AY703578	AY703645	AY703779	AY703712	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera penzigi</i>	Rift Valley, Kenya (2002)	AY703512	AY703579	AY703646	AY703780	AY703713	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera pilosa</i>	Singapore (1988)	AY703513	AY703580	AY703647	AY703781	AY703714	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera punctulata</i>	Northern Territory, AS (1999)	AY703514	AY703581	AY703648	AY703782	AY703715	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera rufonigra</i>	Karnataka, India (1999)	AY703515	AY703582	AY703649	AY703783	AY703716	
Formicidae (Pseudomyrmecinae)	<i>Tetraponera tessmanni</i>	Centr. Afr. Republic (2001)	AY703516	AY703583	AY703650	AY703784	AY703717	
Formicidae (Amblyoponinae)	<i>Amblyopone pallipes</i>	California, U.S.A. (2002)	AY703487	AY703554	AY703621	AY703755	AY703688	
Formicidae (Cerapachyinae)	<i>Cerapachys larvatus</i>	Aust. Cap. Terr. AS (1999)	AY703491	AY703558	AY703625	AY703759	AY703692	
Formicidae (Dolichoderinae)	<i>Leptomyrmex erythrocephalus</i>	New South Wales, AS (1999)	AY703494	AY703561	AY703628	AY703762	AY703695	
Formicidae (Ectatomminae)	<i>Ectatomma opaciventre</i>	São Paulo, Brazil (1998)	AY703492	AY703559	AY703626	AY703760	AY703693	
Formicidae (Ectatomminae)	<i>Typhlomyrmex rogenhoferi</i>	Loreto, Peru (2002)	AY703496	AY703563	AY703630	AY703764	AY703697	
Formicidae (Formicinae)	<i>Formica moki</i>	California, U.S.A. (2001)	AY703493	AY703560	AY703627	AY703761	AY703694	
Formicidae (Myrmecinae)	<i>Myrmecia fulvipes</i>	New South Wales, AS (1988)	AY703497	AY703564	AY703631	AY703765	AY703698	
Formicidae (Myrmecinae)	<i>Myrmecia picta</i>	South Australia, AS (1999)	AY703498	AY703565	AY703632	AY703766	AY703699	
Formicidae (Myrmecinae)	<i>Myrmecia pilosula</i>	Aust. Cap. Terr. AS (1999)	AY703499	AY703566	AY703633	AY703767	AY703700	
Formicidae (Myrmecinae)	<i>Myrmecia pyriformis</i>	South Australia, AS (1998)	AY703500	AY703567	AY703634	AY703768	AY703701	
Formicidae (Myrmecinae)	<i>Nothomyrmecia macrops</i>	South Australia, AS (1999)	AY703501	AY703568	AY703635	AY703769	AY703702	
Formicidae (Myrmecinae)	<i>Myrmica tahoenis</i>	California, U.S.A. (2002)	AY703495	AY703562	AY703629	AY703763	AY703696	
Formicidae (Paraponerinae)	<i>Paraponera clavata</i>	Maranhão, Brazil (1999)	AY703489	AY703556	AY703623	AY703757	AY703690	
Formicidae (Ponerinae)	<i>Hypoponera opacior</i>	California, U.S.A. (2002)	AY703488	AY703555	AY703622	AY703756	AY703689	
Formicidae (Proceratiinae)	<i>Proceratium stictum</i>	Queensland, AS (1989)	AY703490	AY703557	AY703624	AY703758	AY703691	
Bradynobaenidae (Chyphotinae)	<i>Chyphotus mellipes</i>	California, U.S.A. (2002)	AY703485	AY703552	AY703619	AY703753	AY703686	
Vespidae (Polistinae)	<i>Mischocyttarus flavitarsis</i>	California, U.S.A. (2002)	AY703486	AY703553	AY703620	AY703754	AY703687	
Apidae (Apinae)	<i>Apis mellifera</i>	California, U.S.A. (2003)	AY703484	AY703551	AY703618	AY703752	AY703685	

AS, Australia; CR, Costa Rica; DR, Dominican Republic; MA, Madagascar; RSA, Republic of South Africa; wg, wingless; LW Rh, long-wavelength rhodopsin; abd-A, abdominal-A.

Appendix 2

List of morphological characters

The following linear measurements and indices are utilized in the character descriptions below: HW, head width, including eyes; HL, head length, excluding mandibles; N4, midline distance from the posterior margin of the head to a line drawn across the anterior margin of the compound eyes; SL, scape length; MD1–MD9, a series of mandibular measurements taken with the mandible removed from the head capsule (see Ward, 1989: 454); MFC, minimum distance between the frontal carinae; ASD, distance between the median lobes of the antennal sclerites; ASO, distance between the lateral margins of the antennal sclerites; EW, maximum eye width; EL, maximum eye length, in full-face view; LF1–LF3, length of first, second and third funicular segments, respectively (second, third and fourth antennal segments); FL, profemur length; FW, profemur width; DPW, dorsal petiole width; PH, petiole height; PL, petiole length; PPW, dorsal width of postpetiole; CI = HW/HL; SI = SL/HW; ASI = ASD/ASO; FCI = MFC/HW; REL = EL/HL; OI = EW/EL; FI = FW/FL; PLI = PH/PL; and PWI2 = DPW/PPW. For further explanation and illustration of these measurements and indices, see Ward (1989, 1999).

1. *Worker, mandible, basal margin*: (0) diverging from external margin distally ($MD1/MD2 < 0.95$); (1) sub-parallel to external margin or converging slightly towards it distally ($MD1/MD2 = 0.96–1.16$).
2. *Worker and queen, mandible*: (0) much longer than wide, maximum width about one-quarter or less of length ($MD2/MD3 = 0.15–0.25$); (1) width about one-third of length ($MD2/MD3 = 0.30–0.40$); (2) relatively broad, width more than two-fifths of length ($MD2/MD3 = 0.41–0.52$).
3. *Worker, mandible, basal margin*: (0) much shorter than masticatory margin ($MD5/MD9 < 0.80$); (1) subequal to, or slightly less than, masticatory margin ($MD5/MD9 = 0.82–1.05$); (2) much longer than masticatory margin ($MD5/MD9 > 1.15$).
4. *Worker, mandible, distalmost mesial basal tooth, if present*: (0) located midway to two-thirds of distance along basal margin ($MD4/MD5 = 0.50–0.65$); (1) located more distal ($MD4/MD5 = 0.68–0.82$).
5. *Worker, mandible, third tooth on masticatory margin (counting back from the apical tooth)*: (0) located closer to apical tooth than to apicobasal tooth ($MD7/MD9 < 0.44$); (1) located about midway between apical tooth and apicobasal tooth ($MD7/MD9 = 0.45–0.62$); (2) located notably closer to apicobasal tooth ($MD7/MD9 > 0.62$).
6. *Worker, mandible, fourth tooth on masticatory margin (counting back from the apical tooth)*: (0) located closer to apical tooth than to apicobasal tooth ($MD8/MD9 < 0.40$); (1) located at about midpoint of masticatory margin ($MD8/MD9 = 0.40–0.60$); (2)

located closer to (or corresponding to) apicobasal tooth ($MD8/MD9 \geq 0.62$).

7. *Worker and queen, mandible, proximal basal tooth*: (0) absent; (1) present.
8. *Worker and queen, mandible, median number of teeth on masticatory margin*: (0) three; (1) four; (2) five; (3) six to seven; (4) eight to ten; (5) more than ten.
9. *Worker and queen, mandible, venter*: (0) not sharply bounded at the masticatory margin by a trenchant ridge, which terminates at the apicobasal tooth; (1) with such a ridge.
10. *Worker, mandible, in lateral view such that the face of the external margin is perpendicular to the plane of view*: (0) rounded, or at most obtusely angled, above the trulleum; (1) sharply angulate above the trulleum.
11. *Worker and queen, mandible*: (0) relatively short ($MD3/HL < 0.80$); (1) elongate and slender, length of mandible (when dissected) more than four-fifths head length ($MD3/HL > 0.80$).
12. *Queen, mandible*: (0) not broadened apicobasally ($MD2/MD3 < 0.55$); (1) much broadened by a mesial expansion of the apicobasal area (area at the junction of the basal and masticatory margins) ($MD2/MD3 > 0.70$).
13. *Queen, mandible*: (0) basal face (i.e. region above basal margin) rounding obtusely into the anterodorsal face along most of its length, the anterodorsal face flat or convex over most of its surface; (1) basal face rounding sharply into the anterodorsal face, the latter with an obliquely transverse concavity or impression; (2) basal face rounding sharply into the anterodorsal face, the latter with a broad, longitudinal concavity or depression below the juncture of the two faces; (3) juncture of basal and anterodorsal faces marked proximally by a line of margination, which begins above the trulleum and continues obliquely across the anterodorsal face towards the middle of the masticatory margin, such margination being flanked laterally by a similarly oblique concavity on the anterodorsal face; (4) basal face rounding obtusely into anterodorsal face except basally above the trulleum where there is a sharp carina, flanked laterally by a marked concavity.
14. *Queen, mandible, external margin*: (0) not incised basally; (1) sharply incised basally such that the dorsal abductor swelling and immediately distal section of the mandible form an angle of 100° or less, in a frontal view of the head.
15. *Male, mandible, basal margin*: (0) one-half or less the length of the masticatory margin ($MD5/MD9 = 0.30–0.52$); (1) three-fifths or more the length of the masticatory margin ($MD5/MD9 \geq 0.58$).
16. *Male, median number of teeth or denticles on masticatory margin, including apical tooth and apicobasal tooth or angle*: (0) one to five; (1) six to twelve; (2) more than twelve.
17. *Worker and queen, number of maxillary palp segments*: (0) six; (1) five; (2) four; (3) three or less.

18. *Worker and queen, number of labial palp segments*: (0) four; (1) three or less.
19. *Worker and queen, labrum, distal margin*: (0) with a broad, V-shaped cleft or emargination; (1) with a narrower notchlike cleft.
20. *Worker, labrum*: (0) lacking prominent teeth or tubercles; (1) with a single median tubercle, near the proximal margin; (2) with a median tubercle near the proximal margin, widely flanked by a lateral pair (situated closer to the margin); (3) with a pair of tubercles, closely flanking the midline near the proximal margin (no median tubercle); (4) with a widely flanking lateral pair of tubercles near the proximal margin; median tubercle present on distal third of labrum above the cleft.
21. *Worker and queen, labrum*: (0) lacking a transverse protruding ridge; (1) with such a ridge, protruding anteriorly, near junction with clypeus.

State 1 is an autapomorphy of *Myrmecia* (Ogata, 1991).

22. *Worker and queen, clypeolabral connection, in frontal view*: (0) concealed by overhanging clypeus or frontoclypeal complex; (1) exposed.

State 1 is an autapomorphy of *Myrmecia* (Ogata, 1991).

23. *Worker, median portion of clypeus, upper (anterodorsal) surface*: (0) continuous, broadly convex, nontruncate, with the insertions of the lowermost clypeal setae visible in a full-face frontal view; below such insertions the upper surface forms a distinct (usually sharp) juncture with the lower, anteroventral surface; in an anterior view of the clypeus the setae are clearly confined to the area above this juncture; (1) discontinuous, truncate (at least laterally), the insertions of the lowermost clypeal setae occurring below the truncation and often hidden in full-face view of the head; in anterior view of the clypeus (mandibles removed) the setae thus occur below the apparent anterodorsal margin; surface below the truncation often merging inconspicuously into the true ventral surface without a sharp juncture.

Further discussion (and illustration) of the two states is given in Ward (1990).

24. *Worker, median portion of clypeus*: (0) laterally rounded; (1) laterally angulate.
25. *Worker, median portion of clypeus, ventral surface*: (0) without transverse carina; (1) with transverse carina, anterior to the posteroventral border.
26. *Worker, median portion of clypeus, insertions of lowermost clypeal setae*: (0) visible in full-face frontal view of head; (1) not visible in full-face frontal view.
27. *Worker, anterolateral extremity of clypeus*: (0) not fully concealing the dorsal abductor swelling of the mandible, when head is observed in full-face view; (1) covering the dorsal abductor swelling of the mandible, in full-face view.
28. *Worker and queen, clypeus, lamellate clypeal apron on anterior margin*: (0) absent; (1) present.

This feature is characteristic of Ectatomminae and Heteroponerinae (Bolton, 2003).

29. *Worker and queen, clypeus, posteromesial protrusion between frontal carinae and antennal sockets*: (0) absent; (1) present.

In state 0 the posteromedial margin of the clypeus terminates approximately in line with the anterior margins of the antennal sclerites, or only slightly posterior to this.

30. *Worker and queen, frontal carinae*: (0) fusing with antennal sclerites anteriorly; (1) not fusing with antennal sclerites anteriorly, but continuing forward onto the median clypeal lobe.
31. *Worker and queen, frontal carinae*: (0) closely adjacent (worker FCI = 0.009–0.090); (1) moderately well separated (worker FCI = 0.100–0.180); (2) widely separated (worker FCI = 0.190–0.380).
32. *Worker, median lobe of antennal sclerite*: (0) little expanded laterally (ASI = 0.40–0.60); (1) moderately expanded laterally (ASI = 0.62–0.74); (2) strongly expanded laterally (ASI = 0.75–1.00).
33. *Worker and queen, number of antennal segments*: (0) twelve; (1) eleven.
34. *Male, number of antennal segments*: (0) thirteen; (1) twelve.
35. *Worker and queen, scape length relative to head width*: (0) short (worker SI = 0.36–0.54); (1) medium (worker SI = 0.55–0.72); (2) long (worker SI > 0.75).
36. *Male, scape length relative to head width*: (0) relatively short, about one-fifth or less of head width (SI = 0.15–0.22); (1) longer (SI > 0.22).
37. *Male, scape*: (0) less than 0.4 times the combined length of antennal segments 2–4; (1) \geq 0.4 times the combined length of antennal segments 2–4.
38. *Worker, antenna*: (0) moderately expanded apically, last antennal segment less than 1.7 times width of second antennal segment; (1) strongly enlarged apically, maximum width of last antennal segment 1.7–2.2 times width of second antennal segment. Character 16 of Ward (1999).
39. *Worker and queen, antenna, socket of sensilla basiconica*: (0) even with the cuticular surface; (1) elevated above the cuticular surface. An elevated socket has been recorded in *Myrmecia*, *Nothomyrmecia* and *Pseudomyrmecinae* (Hashimoto, 1991; Ward, 1994).
40. *Worker and queen, compound eye*: (0) notably elongate, more than 1.5 times as long as wide (worker OI = 0.48–0.66); (1) oval, \leq 1.5 times as long as wide (worker OI = 0.67–0.88).
41. *Worker and queen, compound eye in relation to HL*: (0) short (worker REL < 0.24); (1) of moderate length (worker REL = 0.25–0.33); (2) long (worker REL = 0.34–0.47); (3) very long (worker REL = 0.48–0.88).
42. *Male, eye size*: (0) relatively small (male REL = 0.34–0.48); (1) larger (male REL > 0.48).
43. *Worker and queen, anterior margin of compound eye, as seen in full-face view of head*: (0) located on upper half of head (worker N4/HL = 0.30–0.48); (1) located on lower 50–70% of head length (worker N4/HL = 0.50–0.71); (2) located more anteriorly (worker N4/HL = 0.72–0.90).

44. *Worker, compound eye with long axis directed*: (0) anteriorly or anterolaterally; (1) anteromesially.
45. *Worker, typical number of ocelli*: (0) three; (1) two; (2) none.
46. *Worker and queen, foramen magnum*: (0) situated at about centre of underside of head, not distant from the buccal cavity; (1) situated at posterior end of head, well separated from the buccal cavity by a much expanded genal area.

Character state 1 is the morphological correlate of prognathy and is characteristic of all ants (Ward & Brady, 2003), and a few other vespoids.

47. *Worker and queen, head*: (0) much longer than wide (worker CI = 0.55–0.62); (1) moderately elongate (worker CI = 0.65–0.92); (2) about as wide as, or wider than, long (worker CI = 0.93–1.25).
48. *Worker and queen size*: (0) small (median worker HW = 0.45–0.65); (1) medium (median worker HW = 0.68–1.14); (2) large (median worker HW = 1.15–2.10, or greater).
49. *Worker and queen, predominant sculpture on upper third of head*: (0) densely imbricate-punctulate or rugulose-punctulate, and opaque; (1) punctulate, imbricate-punctulate, or coriarious-punctulate, sublucid; (2) scattered fine punctures (<0.010 mm diameter) on a mostly smooth, shiny background; (3) coarser punctures (at least some with diameter >0.010 mm), occurring in medium to high density, on a mostly smooth, shiny background.
50. *Worker and queen, predominant colour of mesosoma*: (0) black or dark brownish-black; (1) bicoloured, orange and brown-black; (2) medium brown; (3) orange-brown.
51. *Worker, promesonotal suture*: (0) mobile; (1) inflexible.
52. *Male, distinct posterior oblique sulcus on mesepisternum*: (0) absent; (1) present.

A distinctly impressed sulcus is seen in most male ants, but it is absent or much reduced in Myrmeciinae (Ward & Brady, 2003) and a few other taxa.

53. *Worker, convex, platelike metanotum*: (0) absent; (1) present.
54. *Worker and queen, metapleural gland*: (0) absent; (1) present.
55. *Worker and queen, metapleural gland opening*: (0) not flanked above by carinalike flange that is directed anterodorsally; (1) with such a flange.
56. *Worker and queen, metapleural gland opening*: (0) separated from the posteroventral margin of the metapleuron by a distance greater than the diameter of the opening; (1) located immediately above the lower margin of the metapleuron.

State 1 is an apparent synapomorphy of the Pseudomyrmecinae, although a similar development occurs in some taxa in the doryline section.

57. *Worker and queen, metapleural gland opening*: (0) not in the form of a curved slit, directed dorsally to posterodorsally and subtended below by a convex rim of cuticle; (1) of such a configuration.

This feature, characteristic of Ectatomminae and Myrmicinae, is described by Bolton (2003: 45) and illustrated in Ward (1994: 166).

58. *Worker, queen and male, metacoxal cavities*: (0) open; (1) closed.

In the 'closed' condition, the metacoxal cavity is completely encircled by a fused sclerotized ring (e.g. Ward, 1990: fig. 5).

59. *Worker, standing pilosity, visible in outline on mesosoma dorsum*: (0) common, ≥ 12 standing hairs; (1) sparse, ≤ 10 standing hairs.
60. *Worker, standing pilosity, visible in outline on the external faces of the mesotibia and metatibia*: (0) absent or almost so (none to two standing hairs in total, on both faces); (1) sparse (four to eight standing hairs in total); (2) common (ten or more standing hairs in total).

This count excludes apical tufts of setae.

61. *Worker, appressed pubescence on mesosternum*: (0) absent on most of surface; (1) present on most of surface.

Character 26 in Ward (2001). In worker ants, the mesosternum is usually predominantly smooth and shiny, lacking both standing pilosity and pubescence, except at the margins. A conspicuous mat of appressed pubescence covers most of the mesosternum in some African and Oriental species of *Tetraponera*.

62. *Worker, profemur*: (0) slender (FI = 0.15–0.35); (1) moderately broad (FI = 0.36–0.46); (2) very broad (FI = 0.47–0.55).
63. *Worker, queen and male, number of apical metatibial spurs*: (0) two; (1) one or none.
64. *Worker and queen, metabasitarsal sulcus*: (0) absent; (1) present.
65. *Worker and queen, metabasitarsal sulcus*: (0) absent or simple; (1) subtended by prominent raised ridge.
66. *Queen (if winged) and male, forewing*: (0) with three submarginal cells (*sensu* Gauld & Bolton, 1988); (1) typically with two submarginal cells; (2) typically with one submarginal cell (or lost altogether).

These submarginal cells correspond to cells 1R1, 1Rs and 2Rs of Goulet & Huber (1993), and to the incorrectly named 'cubital cells' of Smith (1943) and Ward (1990, 2001). *Myrmica* is coded as '1' because an interrupted Rs vein partially divides the single submarginal cell into two.

67. *Queen (if winged) and male, forewing veins M and Cu diverging*: (0) opposite, or close to, the cu-a crossvein; (1) distad of the cu-a crossvein by more than the length of the crossvein.
68. *Worker and queen, petiole in profile*: (0) slender, height less than 0.75 times length (worker PLI = 0.25–0.74); (1) more robust, height more than 0.75 times length (worker PLI = 0.76–1.28).
69. *Worker and queen, petiole*: (0) not strongly laterally compressed, petiole width more than 0.70 times petiole height; (1) markedly compressed from side to side, such that petiole width little more than 0.5 times height (worker DPW/PH = 0.60–0.68).

70. *Worker and queen, petiole width in relation to postpetiole*: (0) ≤ 0.75 times postpetiole width (worker PWI2 = 0.45–0.76); (1) more than 0.75 times postpetiole width (worker PWI2 = 0.78–0.90).
71. *Worker and queen, tergo-sternal fusion of abdominal segment 2 (petiole)*: (0) absent or incomplete, such that some free movement is possible between the tergum and sternum; (1) complete.
72. *Worker, queen and male, anteroventral extremity of helcial tergite*: (0) embraced laterally by the petiolar tergite only; (1) embraced laterally, at least in part, by flanges or posterolateral arms that originate on the petiolar sternite.

In generalized ants lacking tergo-sternal fusion of the petiole, the petiolar sternite has short internal posterolateral projections, developed near but anterior to the posterior margin, that provide an articular surface for the anteroventral margin of the helcium. Laterally the helcial tergite is embraced solely by the petiolar tergite (either the tergite proper or the laterotergite, in those ants having a differentiated laterotergite). The development of posterolateral arms on the petiolar sternite that arch back to about the level of the posteromedial margin and partly embrace the helcial tergite is a trait that is apparently shared uniquely by Pseudomyrmecinae and Myrmeciinae, although some Ponerini (e.g. *Harpegnathos*) begin to approach this condition. In ants where the petiolar tergite and sternite have completely fused, leaving no trace of a suture (among outgroups used in this study: *Formica* and *Myrmica*), assessment of this character is ambiguous.

73. *Worker, queen and male, articular posteromedial margin of petiolar sternite (i.e. the part of the margin that articulates with the helcial sternite)*: (0) not subtended below by a strong ridge that connects to the lateral flanges that surround the anteroventral extremity of the helcial tergite; (1) with such a connecting ridge.

The ridge can be sinuous and close to the point of sternal articulation (*Pseudomyrmex*) or evenly arched and more distant (*Tetraoponera*; see next character).

74. *Worker, queen and male, articulation between petiolar sternite and helcial sternite*: (0) at or close to the posteroventral margin of the petiole; (1) strongly displaced dorsomesially, and attended below by a hoodlike extension of the petiolar sternite that forms the posteroventral extremity of the petiolar sternite.

Character 28 of Ward (2001).

75. *Worker, queen and male, hoodlike posteroventral extension of petiolar sternite with narrow medial notch*: (0) absent; (1) present.

This feature appears to be unique to *Tetraoponera natalensis* and its closest relatives.

76. *Worker and queen, presclerites of abdominal segment 3 (i.e. the sclerites of the helcium)*: (0) not fused; (1) fused.
77. *Worker and queen, helcial tergite (pretergite of abdominal segment 3) with internal anteromedian lobe for attachment of tergal muscles*: (0) absent; (1) present.

From Hashimoto (1996) and Ward & Brady (2003).

78. *Worker and queen, postsclerites of abdominal segment 3*: (0) not completely fused; (1) completely fused.

Complete tergo-sternal fusion of abdominal segment 3, posterior to the helcium, is characteristic of the doryline section and most poneromorphs.

79. *Worker, queen and male, abdominal segment 3 in dorsal view*: (0) not forming a postpetiole; (1) forming a nodelike postpetiole: strongly constricted from abdominal segment 4 and distinctly smaller in size.

A distinct postpetiole is present in Pseudomyrmecinae, *Myrmecia*, *Myrmica*, and *Paraponera*, among the taxa considered in this study. *Cerapachys larvatus* is coded as ambiguous (?) because of its intermediate condition.

80. *Worker and queen, dorsal midline length of third abdominal segment excluding the helcium (i.e. length of post-tergite 3)*: (0) subequal to, or greater than, the length of fourth abdominal post-tergite ($> 0.80\times$); (1) markedly less than the length of abdominal post-tergite 4 ($< 0.80\times$).

From Ward & Brady (2003).

81. *Worker, queen and male, dorsal stridulatory organ, with stridulitrum (file) on abdominal pretergite 4 and with posterior margin of post-tergite 3 serving as plectrum*: (0) absent; (1) present.

Such a structure occurs in Pseudomyrmecinae, Myrmicinae and some poneromorphs.

82. *Worker and queen, abdominal segment 4 with differentiated presclerites, separated from the postsclerites by distinctive girdling*: (0) absent; (1) present.

83. *Worker and queen, pretergite of abdominal segment 4, if present*: (0) subequal to or shorter than presternite; (1) notably longer than presternite.

State 1 is a feature of Pseudomyrmecinae and most Myrmicinae (Ward, 1990; Ward & Brady, 2003). In pseudomyrmecines, the pretergite is typically 1.5–2 times the length of the presternite, although the ratio is as low as 1.2 in a few species of *Tetraoponera*.

84. *Worker and queen, tergo-sternal fusion of postsclerites of abdominal segment 4*: (0) absent; (1) present.

85. *Worker and queen, abdominal tergite 4, pubescence consisting of*: (0) relatively dense mat of fine, appressed hairs, separated by less than their lengths; (1) scattered, relative sparse, appressed hairs separated by their lengths or more.

86. *Worker and queen, furcula of sting apparatus*: (0) present and well developed; (1) very reduced/absent.

87. *Worker and queen, sting apparatus, median connection of spiracular plates*: (0) sclerotized; (1) membranous.

In *Tetraoponera* and in most Myrmicinae, the connection between the spiracular plates is essentially membranous (Kugler, 1978; Ward, 1990). *Pseudomyrmex* species generally show a distinctly sclerotized median connection but in the *tenuis* group the connection is weakly sclerotized and approaches state 1.

88. *Male, abdominal sterna VI, VII and VIII, posterolateral corners*: (0) rounded, not produced ventrally; (1) angulate and produced ventrally.

89. *Male, abdominal sternum VIII, posterior margin*: (0) concave; (1) straight or weakly convex.

90. *Male, sternum IX (hypopygium), anterolateral extremities, position in relation to anteromedial apodeme*: (0) posterior to, or even with, the apodeme; (1) anterior to the apodeme.

Character 43 in Ward (2001).

91. *Male, sternum IX (hypopygium), anterolateral arms*: (0) simple; (1) subtended by a thin, lamellate anteromesial extension.

Character 44 in Ward (2001).

92. *Male, sternum IX (hypopygium)*: (0) without carinae preceding the posterior margin; (1) with paired, lateral transverse carinae, preceding the posterior margin.

93. *Male, sternum IX (hypopygium), posterior margin*: (0) without a rounded, protruding, medial lobe; (1) with such a lobe.

94. *Male, sternum IX (hypopygium), posteromedial margin*: (0) lacking a concavity or indentation; (1) with a shallow to moderate concavity or indentation, wider than long; (2) with a deep, semicircular or notchlike concavity, as long as or longer than wide.

95. *Male, sternum IX (hypopygium), with thin, digitiform, posteromedial protrusion*: (0) absent; (1) present.

Character 83 in Ward & Brady (2003).

96. *Male, sternum IX (hypopygium), posterior margin with posterolateral shoulders*: (0) absent; (1) present.

97. *Male, tergum VIII (pygidium), posteromedial margin*: (0) directed posteriorly or posteroventrally; (1) strongly recurved and directed anteroventrally.

98. *Male, pygostyles*: (0) distinctly differentiated from the remnants of tergites IX and X, as a result of a weakly sclerotized or membranous basal connection; (1) fused with the remnants of tergites IX and X through an uninterrupted, evenly sclerotized connection.

99. *Male, paramere, inner proximal dorsal margin, as seen in dorsal view*: (0) diverging gradually from midline; (1) diverging suddenly from midline at a sharply rounded angle.

Character 51 of Ward (2001).

100. *Male, paramere, inner proximal dorsal margin*: (0) not suddenly directed lateroventrally and passing below inner distal dorsal margin; (1) of such a form.

Character 52 of Ward (2001).

101. *Male, paramere, inner proximal dorsal margin, with posteriorly directed lobe*: (0) absent; (1) present.

The proximal portion of the inner dorsal margin of the paramere terminates in a ligulate lobe in *Tetraponera natalensis* and related species.

102. *Male, paramere, mesial dorsoventral lobe*: (0) absent; (1) present.

This structure (illustrated in Ward, 1990: 466) is a dorsoventral, mesially projecting lobe or lamellate ridge on the inner (mesial) surface of the paramere near its distal end. It is found in almost all pseudomyrmecines, although the orientation of the lobe, as seen in mesial view, varies from vertical to somewhat oblique (e.g. Ward, 1999: 468). In some *Tetraponera* species, the lobe has apparently been lost or modified beyond recognition.

103. *Male, paramere, mesial dorsoventral lobe, as seen in posterior view*: (0) not dorsally and ventrally truncate, and subrectangular, with a straight inner edge; (1) of such a form.

104. *Male, paramere, mesial dorsoventral lobe, as seen in posterior view*: (0) not subtriangular and protruding mesially; (1) of such a form.

105. *Male, paramere, mesial dorsoventral lobe, as seen in mesial view*: (0) without a pair of keel-like ridges extending about two-thirds of the distance down the inner surface of the paramere; (1) of such a form.

106. *Male, paramere, mesial dorsoventral lobe, as seen in mesial view*: (0) more or less vertical; (1) with a notably oblique orientation, from anterodorsal to posteroventral.

107. *Male, paramere, mesial dorsoventral lobe in the form of an isolated, digitiform process, attached ventrally and directed dorsally*: (0) absent; (1) present.

This modification of the mesial dorsoventral lobe is uniquely characteristic of the *Pseudomyrmex pallidus* group.

108. *Male, paramere, posteromesial surface*: (0) without a large, mesially directed, saucer- or cup-shaped concavity; (1) with a large, saucer-shaped concavity, partly carinate and directed mesially; (2) with a large, smooth, cup-shaped concavity (subcircular or elongate), continuously carinate and directed mesially.

109. *Male, paramere, posteromesial concavity (or equivalent region in taxa lacking the concavity)*: (0) with at least some standing pilosity; (1) lacking standing pilosity.

Character 52 of Ward (1999).

110. *Male, paramere, subterminal posterodorsal surface*: (0) without a smooth, saucer-shaped concavity, directed dorsomesially; (1) with such a concavity.

111. *Male, paramere, distal end, in lateral view*: (0) posterodorsal extremity not sharply angled; (1) posterodorsal extremity sharply angled.

112. *Male, paramere, distal end, in lateral and mesial views*: (0) not in the form of a long, cone-shaped process extending from 'shield wall' of the mesial dorsoventral lobe; (1) of such a form.

113. *Male, paramere, distal end, in lateral and mesial views*: (0) not truncate, subquadrate and directed posteroventrally; (1) of such a form.

114. *Male, paramere, distal end, with slender digitiform apex*: (0) absent; (1) present.

A paramere with a very long, fingerlike tip, extending from the mesial dorsoventral lobe, characterizes the *Pseudomyrmex subtilissimus* group.

115. *Male, paramere, that part of distal end beyond the mediodorsal impression (or, where latter is obscure, the region just distad of the volsella)*: (0) not highly reduced in size relative to remainder of paramere, - more than one-quarter length of remainder; (1) much reduced in size, \leq one-quarter length of remainder.

In most pseudomyrmecines, the mediodorsal impression is a useful landmark, visible as an impression on the dorsal margin of the paramere, when the latter is viewed in profile (illustrated in Ward, 1990: 466). Reduction of

the portion of the paramere distad of this (or distad of the apex of the volsella, when the mediodorsal impression is not evident) is characteristic of some *Tetraponera* species. This character is inapplicable or ambiguous in the outgroup taxa.

116. *Male, paramere, distal end*: (0) without a large, thin posterodorsal lobe, preceding apex; (1) with such a lobe.
117. *Male, paramere, distal end*: (0) without a mediodorsal lobe or ridge, connected with, and proximal to, the upper extremity of the mesial dorsoventral lobe; (1) with a small lobe or ridge in such a position; (2) with a large, fingerlike lobe in such a position.
118. *Male, paramere, distal end*: (0) without a mediodorsal lobe, separated from, and proximal to, the upper extremity of the mesial dorsoventral lobe; (1) with a small rounded lobe in such a position; (2) with a slender fingerlike process (longer than wide) in such a position.
119. *Male, paramere, distal end, inner (mesial) face*: (0) without an expanded, horizontal keel-like lobe or ridge (continuous with the mesial dorsoventral ridge) on the lower margin of the mediodorsal impression, above the volsella; (1) with such a lobe or ridge.
120. *Male, paramere, distal end, in posterolateral or dorsal view, with very deep, obliquely transverse impression*: (0) absent; (1) present.

Character 56 of Ward (2001).

121. *Male, paramere, distal end, portion distad of the mesial dorsoventral lobe, as seen in mesial view*: (0) not dorsoventrally truncate, subquadrate, and much higher than long; (1) of such a form.

In state 1, the part of the paramere distad of the mesial dorsoventral lobe is markedly truncate, elongate-subrectangular in shape, and three to six times higher than long. This condition is unique to the *Pseudomyrmex tenuis* group and *P. filiformis*.

122. *Male, paramere, with process ('dorsal median projection' of Forbes, 1967) emerging from the dorsomesial surface of the paramere*: (0) absent; (1) present.

Character 84 of Ward & Brady (2003).

123. *Male, paramere, when viewed laterally and ventrally*: (0) not divided by a suture into distinct distal/ventromesial and proximal/dorsolateral sections; (1) so divided.

Character 69 of Ward & Brady (2003).

124. *Male, volsella*: (0) moderately well developed, usually with a differentiated digitus and cuspis; (1) reduced to a small, setose fingerlike lobe; (2) fused to the lower, inner (mesial) wall of the paramere.
125. *Male, volsella, principle lobe (digitus), as seen in lateral or mesial view*: (0) of approximately constant or narrowing width distally; (1) enlarged distally in the form of a hammer or anvil.

Character 85 of Ward & Brady (2003).

126. *Male, aedeagus, inner face*: (0) without a flat, platelike surface separated from the dorsal margin by a

membranous strip or groove, such that a subdorsal margin is evident; (1) of such a form.

127. *Male, aedeagus, lateral apodeme*: (0) markedly shorter in length than the anterior apodeme; (1) about as long as, or longer than, the anterior apodeme.
128. *Male, aedeagus, external face, with >-shaped carina whose tip extends just beyond the posterior margin*: (0) absent; (1) present.
129. *Male, aedeagus, external face, with J-shaped carina whose long straight section is directed dorsally, and remote from the posterior margin of the aedeagus, and whose short curved section originates anteromedially*: (0) absent; (1) present.
130. *Male, aedeagus, external face, with broadly curved carina originating anteromedially and directed posterodorsally*: (0) absent; (1) present.
131. *Male, aedeagus, external face, with horizontal carina, originating anteromedially and directed distad towards posterior margin of aedeagus*: (0) absent; (1) present.
132. *Male, aedeagus, external face, arched carina originating anteroventrally and terminating at or near posterodorsal tooth*: (0) absent; (1) present.

Character 68 of Ward (2001).

133. *Male, aedeagus, external face*: (0) without cornuti; (1) with six to eight cornuti.

State 1 is an autapomorphy of *Myrcidris* (Ward, 1990).

134. *Male, aedeagus, posterior or posteroventral margin*: (0) lacking a row of fine teeth or denticles; (1) with a row of denticles.

When the aedeagus is viewed in profile these denticles may not be readily visible if the posterior margin of the aedeagus is bent laterad (see character 139).

135. *Male, aedeagus, posteroventral extremity in lateral view*: (0) without a ventrally directed tooth or sharp angle; (1) with a single ventrally directed tooth or sharp angle; (2) with a pair of ventrally directed teeth.
136. *Male, aedeagus, thin translucent lamella protruding from anterodorsal margin*: (0) absent; (1) present.

Character 71 of Ward (1999).

137. *Male, aedeagus, with large, rounded, lamellate, posterodorsal protrusion*: (0) absent; (1) present.
138. *Male, aedeagus, prominent posteroventral projection, armed with stout teeth or spines*: (0) absent; (1) present.

Synapomorphy of Myrmecinae. Character 71 of Ward & Brady (2003).

139. *Male, aedeagus, posterior margin*: (0) directed predominantly posteriorly or posterolaterally; (1) bent laterad at right angles to the sagittal plane, along most of its length.
140. *Male, aedeagus, prominent digitiform lobe projecting laterally from dorsal margin*: (0) absent; (1) present.
141. *Worker, queen and male, larva with ventral food pocket (trophothylax)*: (0) absent; (1) present.

This structure is unique to the subfamily Pseudomyrmecinae (Wheeler & Wheeler, 1976; Ward, 1990).

142. *Worker, queen and male, pupa*: (0) enclosed in cocoon; (1) naked.

143. *Male, pupa, antennae*: (0) passing laterally on either side of mandibles; (1) passing ventrally below the mandibles.

Positioning of the proximal segments of the antennae of the male pupa below the mandibles – as opposed to either side of them – appears to be unique to the genus *Pseudomyrmex*. The description of the male pupa of *Myrcidris*

(Ward, 1990: 465) is in error: the antennae actually pass laterally around the mandibles in this genus.

144. *Female, apterous worker caste*: (0) absent; (1) present. Synapomorphy of Formicidae.

Most characters were treated as ordered, in the sequence given above. The following were considered to be unordered: 13, 20, 49, 50.

Appendix 3. Morphological data matrix. ?, missing, unknown or ambiguous; p, polymorphic; n, not applicable. For the purposes of the phylogenetic analysis, the last two categories were treated as missing/unknown.

	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000	0000000000
	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
<i>Pseudomyrmex apache</i>	0200121310	0000010000	0010010000	0001010010	2p20011113	0101110110	0100011000
<i>Pseudomyrmex boopis</i>	0201121310	0000010000	0010010000	0001010010	312001220p	0101110110	0100011110
<i>Pseudomyrmex concolor</i>	1200121210	0000012100	0010000000	11010p1110	2p1001p133	0101110102	0100011100
<i>Pseudomyrmex cordiae</i>	0201011410	0000021100	0010010000	0001000010	3120011100	0101110110	0100011100
<i>Pseudomyrmex cubaensis</i>	1200121210	0000010100	0010010000	0001010?10	3120011112	0101110100	0p00011p00
<i>Pseudomyrmex dendroicus</i>	1200121310	0010010000	0010000000	1101011010	2110012232	0101110102	0100011p00
<i>Pseudomyrmex denticollis</i>	0201121310	0000010000	0011010000	0001011010	312001p203	0101110110	0100011000
<i>Pseudomyrmex elongatulus</i>	0201121210	0000010000	0010010000	0001011010	3120011113	0101110110	0100011000
<i>Pseudomyrmex filiformis</i>	1200121310	0000011000	0010010000	0101000010	312001112p	0101110100	0100011000
<i>Pseudomyrmex flavicornis</i>	0200121310	0000011100	00100p0000	0p01011010	2120011100	0101110100	0100011000
<i>Pseudomyrmex godmani</i>	0201011410	0000??0000	0010010000	000?0??010	3?20012200	0?01110102	01000??100
<i>Pseudomyrmex gracilis</i>	0201011410	0000010000	0010010000	0001000010	312001221p	0101110102	0100011000
<i>Pseudomyrmex haytianus</i>	1200121310	0000010100	0011010000	0p01010010	3120011111	0101110110	0100011000
<i>Pseudomyrmex holmgreni</i>	1200121210	0000011100	0011010000	0001011110	312001111p	0101110110	0p00011000
<i>Pseudomyrmex ita</i>	0201011410	0000020000	0010010000	0001000010	3120011100	0101110110	0200011100
<i>Pseudomyrmex kuenckeli</i>	0201121310	0000010000	0010000000	1101011010	2110012222	0101110102	0000011100
<i>Pseudomyrmex nigrocinctus</i>	0201121310	0000011100	00100p0000	0p010p0010	2120011113	0101110100	0100011000
<i>Pseudomyrmex nigropilosus</i>	0201011410	0000010000	0010010000	0001000010	312001121p	0101110102	0100011000
<i>Pseudomyrmex oculatus</i>	0200121210	0000010100	0010010000	0001010010	3120011102	0101110101	0100011100
<i>Pseudomyrmex oki</i>	1200121210	0000011100	0011010000	0001010110	3120011112	0101110100	0200011000
<i>Pseudomyrmex pallens</i>	0201121210	0000010000	0010010000	0001010010	3120011113	0101110100	0100011000
<i>Pseudomyrmex pallidus</i>	1200121210	0000011100	0011010000	0001011110	3120011113	0101110110	0100011000
<i>Pseudomyrmex pazosi</i>	1200121210	0000011100	0011010000	0001011110	3120011123	0101110110	0200011000
<i>Pseudomyrmex phyllophilus</i>	0201121210	0000010000	0010010000	0001010010	3120011102	0101110100	0100011000
<i>Pseudomyrmex sericeus</i>	0201011410	0000020000	0010010000	0001000010	3120011100	0101110110	0200011100
<i>Pseudomyrmex simplex</i>	1200121210	0000011100	0011010000	0001011110	3120011123	0101110110	0200011000
<i>Pseudomyrmex spiculus</i>	0200121310	0000010100	0010010001	0001001010	3120011002	0101110110	0200011000
<i>Pseudomyrmex subater</i>	1200121210	0000011100	0010000000	0001010010	p120011130	0101110100	0100011p00
<i>Pseudomyrmex tachigaliae</i>	1211021310	0041011100	0011000000	0001011110	2010011112	0101110100	0100011p00
<i>Pseudomyrmex tenuis</i>	0201121310	0000010000	0010010000	0001010010	312001220p	0101110110	0100011110
<i>Pseudomyrmex tenuissimus</i>	0200121210	0000010100	001001?001	000101p010	3120010002	0101110110	0200011000
<i>Pseudomyrmex termitarius</i>	0201121310	0000010000	0011010000	0001011010	3120012203	0101110110	0000011000
<i>Pseudomyrmex viduus</i>	1201121310	0000011100	0010010000	0101011010	2110011132	0101110102	0p00011100
<i>Myrcidris epicharis</i>	110n221110	0000101100	0000000000	1210011111	2110011023	0101110100	0201011p00
<i>Tetraponera aethiops</i>	1110220101	0020100011	0000100000	2201011011	1010012210	0101110110	0001011001
<i>Tetraponera allaborans</i>	11212n0001	0000100012	0000001000	1201111111	2010211120	0101110110	1101021000
<i>Tetraponera ambigua</i>	121n120101	0000100010	0000000000	2201111111	2111111113	0101110100	p100011000
<i>Tetraponera caffra</i>	11202n0001	0000100014	0000000000	1201010?11	2110111113	0101110110	0201011p00
<i>Tetraponera clypeata</i>	11212n0001	0000100010	0000001000	1201111111	1010211010	0111110110	1101021000
<i>Tetraponera grandidieri</i>	011n220101	0000100013	0000000000	1201210011	1010211213	0101110110	0001011000
<i>Tetraponera morondaviensis</i>	120n120101	0100100000	0000000000	1201111111	1110211112	0111110100	1101011000
<i>Tetraponera natalensis</i>	11202n0001	00001p0014	0000000000	1201010011	2p10111213	0101110110	0101011001
<i>Tetraponera nigra</i>	1110220101	0031100011	0000000000	1201110111	1110211210	0101110102	0101111000
<i>Tetraponera ophthalmica</i>	020n120101	0000100010	0000000000	1201111111	2111211013	0101110110	0100011000
<i>Tetraponera penzigi</i>	110n220101	0001100010	0000000000	2201010111	1010211110	0111110110	1101021101
<i>Tetraponera pilosa</i>	1110220101	00001p0010	0000100000	1201110011	2110011210	0101110110	0p01011000
<i>Tetraponera punctulata</i>	110n220101	0031100011	0000000000	1201p1p111	p110211130	0101110100	0p01111000

<i>Tetraponera rufonigra</i>	020n120201	0021110010	0000100000	1201111011	101001p21p	0101110102	0001011001
<i>Tetraponera tessmanni</i>	1100120201	0001103100	0000000000	2201011111	0010211023	0111110110	1101021101
<i>Amblyopone pallipes</i>	n0nnnnnn00	1n00nn1100	0001001010	2200111001	010n211102	0001000002	000001010n
<i>Cerapachys larvatus</i>	020n000500	00000?3100	00?0n0001?	1000111100	1110212130	1101110102	0010020101
<i>Ectatomma opaciventre</i>	020n000500	0000113100	0000000110	2200200001	1000212202	1101001002	001001010n
<i>Formica moki</i>	020n000400	0000100000	00?0?10000	2100211001	2100011202	0101000110	001002110n
<i>Hypoponera opacior</i>	010n010400	0000nn3100	00000010?0	1200201101	0020211012	0101000110	001001110n
<i>Leptomyrmex erythrocephalus</i>	010n000500	0000020010	0000001000	2100211001	0010210200	0111000110	001002110n
<i>Myrmecia fulvipes</i>	n0nnnn1500	1n00100000	11?1n00010	2200100011	2120012210	0001100002	0001010100
<i>Myrmecia picta</i>	000n001500	1000100000	11?1n00010	2200100010	2020012201	0001100002	1001010000
<i>Myrmecia pilosula</i>	n0nnnn1500	1n00100000	11?1n00010	2200200011	2020012200	0001100002	0001010100
<i>Myrmecia pyriformis</i>	0001001500	1000100000	11?1n00010	1200200011	2020012212	0001100002	1001010000
<i>Myrmica tahoensis</i>	020n010400	0000100000	0000000010	2200211001	00102121?2	1101001100	0010011100
<i>Nothomyrmecia macrops</i>	000n000500	1000010000	0000000010	1200200011	2110212232	0001100002	000101000n
<i>Paraponera clavata</i>	0201011500	0000nn1100	0011010010	2200200001	01102122?0	1101000002	0001010000
<i>Proceratium stictum</i>	000n220100	0000102100	00?0010010	1000211001	011n211102	1101000102	001002100n
<i>Typhlomyrmex rogenhoferi</i>	020n010500	0000013100	0000000110	2200111101	0010211102	0101001002	011002010n
<i>Chyphotes mellipes</i>	n0nnnn0n00	0n00nn00n0	0000?0000n	nn00011n?1	212n212133	n0n0nnn0nn	nn0001000n
<i>Mischocyttarus flavitarsis</i>	120n220100	00001000n0	000000000n	1000011n00	312n002200	n0n0nnn0nn	n00000000n
<i>Apis mellifera</i>	01nnnn0n00	00001000n0	010000000n	n000011n00	312n002210	n0n0nnn0nn	n010000nnn

0000000000 0000000000 0000000001 1111111111 1111111111 1111111111 1111111111 1111111111 1111
 7777777778 8888888889 9999999999 0000000001 1111111112 2222222223 3333333334 4444
 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234

<i>Pseudomyrmex apache</i>	0100000011	1110000000	0001000000	0100010110	0000001000	0001010000	0000100000	1111
<i>Pseudomyrmex boopis</i>	0110000011	1110000000	0010000000	0100000000	0000001000	1001010100	0000000000	1111
<i>Pseudomyrmex concolor</i>	0100000011	1110000000	0002000000	0100100100	1000001000	0001010000	0001010000	1111
<i>Pseudomyrmex cordiae</i>	0110000011	1110000000	0001000000	0110000000	0000001000	0001010010	0000100000	1111
<i>Pseudomyrmex cubaensis</i>	0100000011	1110000000	0000000000	0100100001	0000001000	0001010000	0000100000	1111
<i>Pseudomyrmex dendroicus</i>	0100000011	1110000000	0001000000	0100000210	0000000000	0001010001	0000100010	1111
<i>Pseudomyrmex denticollis</i>	0110000011	1110000000	0010000000	0100000100	0000001000	1001010100	0000000000	11?1
<i>Pseudomyrmex elongatulus</i>	0100000011	1110000000	0000000000	0100010110	0000001000	0001010000	0000100000	1111
<i>Pseudomyrmex filiformis</i>	0100000011	1110000000	0001000000	0100000000	0000001000	1001010000	0000000000	1111
<i>Pseudomyrmex flavicornis</i>	0100000011	1110000000	0002000000	0100000000	0000002000	0001010001	0001001000	1111
<i>Pseudomyrmex godmani</i>	0110000011	11100000??	??????????	??????????	??????????	??????????	??????????	11?1
<i>Pseudomyrmex gracilis</i>	0110000011	1110000000	0101000000	0100000000	0100001000	0001010000	0001100000	1111
<i>Pseudomyrmex haytianus</i>	0100000011	1110000000	0001000000	0100000100	1000002000	0001010000	0001000000	11?1
<i>Pseudomyrmex holmgreni</i>	0100000011	1110000100	0001001000	0100001000	0000010100	0001010000	0001000000	1111
<i>Pseudomyrmex ita</i>	0110000011	1110000000	0000000000	0110000000	0000001000	0001010010	0000100000	1111
<i>Pseudomyrmex kuenckeli</i>	0110000011	1110000000	0001000000	0100000100	0000001100	0001010000	0000100000	1111
<i>Pseudomyrmex nigrocinctus</i>	0100000011	1110000000	0001000000	0100000000	0000002000	0001010001	0001001000	1111
<i>Pseudomyrmex nigropilosus</i>	0110000011	1110000000	0101000000	0100000000	0100001000	0001010000	0001100000	1111
<i>Pseudomyrmex oculus</i>	0100000011	1110000000	0001000000	0100100001	0000001000	0001010001	0000100000	1111
<i>Pseudomyrmex oki</i>	0100000011	1110000100	0001000000	0100001000	0000000000	0001010000	0001000000	1111
<i>Pseudomyrmex pallens</i>	0100000011	1110000000	0000000000	0101000000	0000001100	0001010010	0001100000	1111
<i>Pseudomyrmex pallidus</i>	0100000011	1110000100	0001001000	0100001000	0000010000	0001010000	0001000000	1111
<i>Pseudomyrmex pazosi</i>	0100000011	1110100100	0001001000	0100001000	0000010200	0001010000	0001000000	1111
<i>Pseudomyrmex phyllophilus</i>	0100000011	1110000000	0000000000	0101000000	0000001100	0001010010	0001100000	11?1
<i>Pseudomyrmex sericeus</i>	0110000011	1110000000	0000000000	0110000000	0000001000	0001010010	0000100000	1111
<i>Pseudomyrmex simplex</i>	0100000011	1110100100	0001001000	0100001000	0000010200	0001010000	0001000000	1111
<i>Pseudomyrmex spiculus</i>	0100000011	1110000000	0000000000	0100000000	0001001000	0001010000	0000100000	1111
<i>Pseudomyrmex subater</i>	0100000011	1110000000	0001000000	0100000100	0000001000	0001010000	0000100000	1111
<i>Pseudomyrmex tachigaliae</i>	0100000011	1110000001	1001000000	0100100100	1000001000	0001010000	0001010010	1111
<i>Pseudomyrmex tenuis</i>	0110000011	1110000000	0010000000	0100000000	0000001000	1001010100	0000000000	1111
<i>Pseudomyrmex tenuissimus</i>	0100000011	1110000000	0000000000	0100000000	0001001000	0001010000	0000100000	11?1
<i>Pseudomyrmex termitarius</i>	0110000011	1110000000	0010000000	0100000100	0000001000	1001010100	0000000000	11?1
<i>Pseudomyrmex viduus</i>	0100000011	1110000000	0001000000	0101010210	0000000000	0001010001	0000100010	1111
<i>Myrcidris epicharis</i>	0100000011	1110000010	0000000100	0101000000	0000000000	0001010000	0010000000	1101
<i>Tetraponera aethiops</i>	0100000011	1110001001	0001000000	0100000000	0000100000	0001000000	0001000000	1101
<i>Tetraponera allaborans</i>	0111000011	1110101000	0000000000	0100000000	0000000001	000100p000	10010000?0	1101

Appendix 3. Continued

	0000000000	0000000000	0000000001	1111111111	1111111111	1111111111	1111111111	1111111111	1111
	7777777778	8888888889	9999999990	0000000001	1111111112	2222222223	3333333334	4444	
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234	
<i>Tetraoponera ambigua</i>	0111000011	1110001000	0000000000	0100000000	0000100010	0002000000	0001010001	1101	
<i>Tetraoponera caffra</i>	0111100011	1110001000	0001011010	1100000000	0000000000	0001000000	0001200000	1101	
<i>Tetraoponera clypeata</i>	0111000011	1110101000	0000000000	0100000100	0000?00001	0001001000	1001000010	1101	
<i>Tetraoponera grandidieri</i>	0100000011	1110101000	0000000000	0100000000	0000000000	0001000000	1000000010	1101	
<i>Tetraoponera morondaviensis</i>	0111000011	1110001001	1000000010	0100000000	0000000000	0002000000	1001000000	1101	
<i>Tetraoponera natalensis</i>	0111100011	1110001000	0001011010	1100000000	0000000000	0001000000	0001200000	1101	
<i>Tetraoponera nigra</i>	0100000011	1110001001	1001000011	0100000110	0010000000	0002001000	0100100000	11?1	
<i>Tetraoponera ophthalmica</i>	0111000011	1110001000	0000000000	0100000000	0000100010	0002000000	0001010001	1101	
<i>Tetraoponera penzigi</i>	0111000011	1110001001	1000000000	0100000000	0000100000	0002001000	1001000010	1101	
<i>Tetraoponera pilosa</i>	0100000011	1110001000	0000000000	00000n0000	0001000000	0001000000	0001000000	11?1	
<i>Tetraoponera punctulata</i>	0100000011	1110001001	1001000011	0100000110	0010000000	0002001000	0100100000	1101	
<i>Tetraoponera rufonigra</i>	0100000011	1110001000	0001000010	0100000000	0000100000	0001001000	0001000000	1101	
<i>Tetraoponera tessmanni</i>	0111000011	1110001000	0000000000	00000n0000	0001100000	0001001000	1000000010	1101	
<i>Amblyopone pallipes</i>	0000010100	0101000000	0000000000	00000n0010	0000n00000	0000100000	0001000000	0001	
<i>Cerapachys larvatus</i>	00000101?0	0100110000	0001000n00	00000n0000	0000n00000	0000000000	0001000000	0001	
<i>Ectatomma opaciventre</i>	1000011100	1101100000	0000000000	00000n0000	0000n00000	0000100000	1001000000	00?1	
<i>Formica moki</i>	1?00010000	00n001?000	0000010000	00000n0000	0000n00000	0000000000	1000100000	0001	
<i>Hypoponera opacior</i>	1000010100	1101000000	0000000000	00000n0010	0000n00000	0000100000	0001000000	0001	
<i>Leptomyrmex erythrocephalus</i>	1000010000	00n0010001	0000000100	00000n0000	0000n00000	0000100000	0001000000	0101	
<i>Myrmecia fulvipes</i>	0100000011	0100000000	0000100000	00000n0010	1000n00000	0010100000	0010000100	0001	
<i>Myrmecia picta</i>	0100000011	0100000000	0001000000	00000n0000	0001n00000	0110100000	0000000100	0001	
<i>Myrmecia pilosula</i>	0100000011	0100000000	0000100000	00000n0010	0000n00000	0010100000	0000000100	0001	
<i>Myrmecia pyriformis</i>	0100000011	0100000000	0000100000	0100000000	0000n00000	0110000000	0000000100	0001	
<i>Myrmica tahoensis</i>	1?00001011	1110101000	0000000000	00000n0010	0000n00000	0000000000	0001000000	0101	
<i>Nothomyrmecia macrops</i>	0100000001	00n0000000	0002000000	00000n0010	0000n00000	0010000000	0000000101	00?1	
<i>Paraponera clavata</i>	1000010110	1101100000	0000000000	00000n0000	0001n00000	0000100000	0001000000	00?1	
<i>Proceratium stictum</i>	1000010100	0101000000	0000000n00	00000n0000	1000n00000	0000100000	1001000000	0001	
<i>Typhlomyrmex rogenhoferi</i>	1000011100	0101000000	0000100000	00000n0000	0000n00000	0000100000	0001000000	00?1	
<i>Chyphotes mellipes</i>	1000000000	00n010?01?	0000000000	00000n0000	0000n00000	0000000000	0000000000	???0	
<i>Mischocyttarus flavitarsis</i>	0000000000	00n000?010	0000000n00	00000n0000	0001n00000	0000000000	0000000000	00?0	
<i>Apis mellifera mellifera</i>	0nnnnnn000	00n000001?	0000000nnn	nnnnnnnnnn	nnnnnnnnnn	nnnnnn0?000	0000000000	0000	