CSIRO PUBLISHING

www.publish.csiro.au/journals/is *Invertebrate Systematics*, 2003, **17**, 361–386

Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae)

Philip S. Ward[A,C] *and Seán G. Brady*[A,B]

[A]Department of Entomology and Center for Population Biology, University of California, One Shields Avenue, Davis, CA 95616, USA.
[B]Current address: Department of Entomology, Comstock Hall, Cornell University, Ithaca, NY 14853, USA.
[C]To whom correspondence should be addressed. Email: psward@ucdavis.edu

Abstract. We investigated phylogenetic relationships among the 'primitive' Australian ant genera *Myrmecia* and *Nothomyrmecia* (stat. rev.) and the Baltic amber fossil genus *Prionomyrmex*, using a combination of morphological and molecular data. Outgroups for the analysis included representatives from a variety of potential sister-groups, including five extant subfamilies of ants and one extinct group (Sphecomyrminae). Parsimony analysis of the morphological data provides strong support (~95% bootstrap proportions) for the monophyly of (*1*) genus *Myrmecia*, (*2*) genus *Prionomyrmex*, and (*3*) a clade containing those two genera plus *Nothomyrmecia*. A group comprising *Nothomyrmecia* and *Prionomyrmex* is also upheld (85% bootstrap support). Molecular sequence data (~2200 base pairs from the 18S and 28S ribosomal RNA genes) corroborate these findings for extant taxa, with *Myrmecia* and *Nothomyrmecia* appearing as sister-groups with ~100% bootstrap support under parsimony, neighbour-joining and maximum-likelihood analyses. Neither the molecular nor the morphological data set allows us to identify unambiguously the sister-group of (*Myrmecia* + (*Nothomyrmecia* + *Prionomyrmex*)). Rather, *Myrmecia* and relatives are part of an unresolved polytomy that encompasses most of the ant subfamilies. Taken as a whole, our results support the contention that many of the major lineages of ants – including a clade that later came to contain *Myrmecia*, *Nothomyrmecia* and *Prionomyrmex* – arose at around the same time during a bout of diversification in the middle or late Cretaceous. On the basis of Bayesian dating analysis, the estimated age of the most recent common ancestor of *Myrmecia* and *Nothomyrmecia* is 74 million years (95% confidence limits, 53–101 million years), a result consistent with the origin of the myrmeciine stem lineage in the Cretaceous. The ant subfamily Myrmeciinae is redefined to contain two tribes, Myrmeciini (genus *Myrmecia*) and Prionomyrmecini (*Nothomyrmecia* and *Prionomyrmex*). Phylogenetic analysis of the enigmatic Argentine fossils *Ameghinoia* and *Polanskiella* demonstrates that they are also members of the Myrmeciinae, probably more closely related to Prionomyrmecini than to Myrmeciini. Thus, the myrmeciine ants appear to be a formerly widespread group that retained many ancestral formicid characteristics and that became extinct everywhere except in the Australian region.

Introduction

There is a widespread tendency – and perhaps a natural curiosity – to identify those taxa within a larger group that are especially 'primitive', that is, those species that have retained many characteristics thought to be ancestral for the group as a whole. Within the family Formicidae, the Australian genera *Myrmecia* Fabricius and *Nothomyrmecia* Clark have often been touted as exemplars of such primitiveness (Wheeler 1933; Wilson 1971; Taylor 1978; Hölldobler and Taylor 1984; Ogata 1991; Peeters 1997). Many aspects of the morphology and biology of these ants are relatively unspecialised in comparison to other formicids, and hence perhaps indicative of the conditions under which social behaviour arose in ants. Yet the phylogenetic relationship of these two ant genera to one another and to other ants, both living and fossil, has remained a topic of debate and uncertainty (Clark 1934, 1951; Brown 1954; Taylor 1978; Hölldobler and Wilson 1990; Ward 1990, 1994; Hashimoto 1991, 1996; Baroni Urbani *et al.* 1992; Grimaldi *et al.* 1997; Baroni Urbani 2000). In this paper, we investigate the relationship between *Myrmecia*, *Nothomyrmecia* and related taxa using morphological and molecular (DNA sequence) data. Clarification of the phylogenetic position of these groups within the family Formicidae can assist in evaluating their status as 'primitive' ants, and contribute to our understanding of the biogeographic history of ants.

The ant genus *Myrmecia* comprises 89 described species, all of which are confined to the Australian continent except for one endemic species in New Caledonia and one

 10.1071/IS02046 1445-5226/03/030361

introduced Australian species in New Zealand (Clark 1951; Taylor 1987, 1991; Ogata and Taylor 1991). Known by the vernacular name of 'bulldog ants' and – in those species with saltatory abilities – 'jumpers', these ants are easily recognised in the field by their relatively large body size, elongate mandibles, conspicuous protruding eyes and well-developed (and frequently employed) sting. The biology of certain *Myrmecia* species has been well studied and, as is the case with morphology, many features of ecology and behaviour appear to be primitive for ants as a whole (Wheeler 1933; Haskins and Haskins 1951; Freeland 1958; Wilson 1971; Crosland *et al.* 1988). Recent revisionary work (Ogata and Taylor 1991) has placed the species-level taxonomy on a firm footing. In addition, Ogata (1991) established nine species-groups in the genus *Myrmecia* and analysed phylogenetic relationships among them.

Nothomyrmecia consists of a single species, *N. macrops* Clark, which was known from only two workers apparently collected in Western Australia (Clark 1934) until rediscovered more than four decades later in South Australia (Taylor 1978). This was followed by a flurry of studies probing the behaviour, colony structure, ecology, genetics, internal anatomy and external morphology of this unusual ant (e.g. Taylor 1978; Kugler 1980; Wheeler *et al.* 1980; Ward and Taylor 1981; Hölldobler and Taylor 1984; Imai *et al.* 1991; Billen 1988, 1990*b*; Jaisson *et al.* 1992; Billen *et al.* 2000; Sanetra and Crozier 2001). *Nothomyrmecia* has been called a 'living fossil' (Taylor 1978) and 'arguably the most primitive living formicid' (Hölldobler and Taylor 1984). Similarities to the genus *Myrmecia* have often been noted (Brown 1954; Kugler 1980; Wheeler *et al.* 1980; Billen 1988; Billen *et al.* 1988, 2000), but whether these constitute synapomorphies or reflect plesiomorphic resemblance has not always been explicitly considered. In taxonomic treatments, *Nothomyrmecia* has been variously placed either in its own subfamily (Clark 1934, 1951; Taylor 1978; Hölldobler and Wilson 1990; Bolton 1994, 1995) or conjoined with *Myrmecia* in the subfamily Myrmeciinae (Brown 1954; Wilson *et al.* 1967; Brown and Taylor 1970; Wilson 1971). Recent phylogenetic analyses have tended to closely associate the two genera (Baroni Urbani *et al.* 1992; Ward 1994; Grimaldi *et al.* 1997; Baroni Urbani 2000), but the supporting evidence has been relatively weak, that is, based on few characters and not underpinned by high bootstrap or decay index values.

Although *Myrmecia* and *Nothomyrmecia* are endemic to the Australian region, there are morphologically similar fossil taxa from other parts of the world. These include the Baltic amber genus *Prionomyrmex* Mayr from the late Eocene or early Oligocene (Mayr 1868; Wheeler 1915; Dlussky 1997) and the South American genera *Ameghinoia* Viana & Haedo Rossi and *Polanskiella* Rossi de García, both from Argentine deposits of Eocene age (Viana and Haedo Rossi 1957; Brown and Taylor 1970; Rossi de García 1983; Petrulevicius 1999). A recent detailed study of *Prionomyrmex* by Baroni Urbani (2000) documented striking similarities between this genus and *Nothomyrmecia*, which led the author to synonymise the latter under *Prionomyrmex*. Although the results of our phylogenetic analyses support a close relationship between these two taxa, we treat *Nothomyrmecia* as a genus separate from *Prionomyrmex*. Justification for the resurrection of *Nothomyrmecia* is given later (see the section on 'Reclassification of the Myrmeciinae').

The Brazilian lower Cretaceous hymenopteran *Cariridris bipetiolata* Brandão & Martins-Neto was originally interpreted as a worker ant belonging to the subfamily Myrmeciinae (Brandão and Martins-Neto 1990), but Rasnitsyn (1994) and Verhaagh (1996) argued that *Cariridris* is a sphecid wasp. Grimaldi *et al.* (1997) similarly expressed doubt about its placement in Formicidae and noted that some features, such as the apparent postpetiole, could be preservation artefacts. Given these uncertainties, we have excluded *Cariridris* from consideration in this study.

Materials and methods

We used a combination of morphological and molecular (DNA sequence) data to examine phylogenetic relationships among *Myrmecia*, *Nothomyrmecia* and other ants. Four data sets were employed (Table 1): (*1*) MORPH1, the primary morphological data set; (*2*) MORPH2, a modification of MORPH1 such that the terminal taxa more closely match those for which sequence data were obtained (fossils removed and some higher taxa broken up into smaller units); (*3*) 18S+28S, a data set based on 18S and 28S ribosomal RNA (rRNA) sequences; and (*4*) COMBINED, a combination of the MORPH2 and 18S+28S data sets.

Table 1. Data sets used for phylogenetic analysis

Data set	No. of terminal taxa	Character type	No. of characters	No. of variable characters	No. of parsimony-informative characters
MORPH1	14 (extant + fossil)	Morphology	74	74	57
MORPH2	17 (extant)	Morphology	82	82	74
18S+28S	17 (extant)	DNA	2197[A]	353[B]	157[B]
COMBINED	17 (extant)	Morphology, DNA	2279[A]	435[B]	231[B]

[A]Excluding ambiguously aligned regions.

[B]Includes 60 sites with gaps treated as a fifth state (15 informative, 45 uninformative).

MORPH1 data set

For the basic morphological analysis, the ingroup was represented by four terminal taxa: genus *Myrmecia*, genus (and species) *Nothomyrmecia macrops*, species *Prionomyrmex longiceps* Mayr and species *Prionomyrmex janzeni* Baroni Urbani. The two species of *Prionomyrmex* were treated separately to test a claim (Baroni Urbani 2000) that the genus *Prionomyrmex* is paraphyletic if *Nothomyrmecia* is excluded from it.

We used multiple outgroups because the closest relatives of the ingroup have not been clearly identified. Our strategy was to examine a wide variety of potentially related ant taxa, focussing on morphologically generalised taxa. We excluded such derived groups as the doryline section (Bolton 1990*b*) and the subfamily Leptanillinae (Bolton 1990*a*) because no evidence links these specialised groups to myrmeciine ants. The subfamily Pseudomyrmecinae has been mentioned as a possible close relative of *Myrmecia* (Brown 1954; Hashimoto 1991; Ward 1994), so we treated the two principal genera of this subfamily as separate terminal taxa. The 'primitive' ant subfamily Ponerinae was represented by three genera (*Amblyopone* Erichson, *Paraponera* F. Smith and *Rhytidoponera* Mayr), also coded separately because of the likely paraphyly of this subfamily (Hashimoto 1991, 1996; Ward 1994; Grimaldi *et al.* 1997; Sullender and Johnson 1998). Other outgroups were the subfamilies Myrmicinae, Dolichoderinae and Formicinae, coded as terminal taxa at this level because their monophyly is well established (Baroni Urbani *et al.* 1992; Shattuck 1992*a*, 1995; Chiotis *et al.* 2000). The final ant outgroup was the Cretaceous fossil taxon Sphecomyrminae (Wilson *et al.* 1967; Dlussky 1975, 1983, 1987; Grimaldi *et al.* 1997). The aculeate wasp family Vespidae was treated as the 'outer outgroup', that is, it was used to root the tree, with no assumption being made that the ingroup taxa form a monophyletic group.

MORPH2 data set

A second morphological data set was established to correspond more closely to the 18S+28S molecular data set. As a result, this contained five ingroup taxa: *Nothomyrmecia macrops* and four different species-groups of *Myrmecia* (those for which sequence data were obtained). The two fossil species of *Prionomyrmex* were removed. Eleven morphological characters were added, to provide some resolution of relationships among the *Myrmecia* species-groups, and three morphological characters that became invariant were removed, giving a total of 82 characters. The set of outgroup taxa was modified such that the terminal taxa became the ant genera for which molecular data were available, namely *Pseudomyrmex* Lund, *Tetraponera* F. Smith, *Paraponera*, *Amblyopone*, *Rhytidoponera*, *Pogonomyrmex* Mayr, *Liometopum* Mayr, *Linepithema* Mayr, *Camponotus* Mayr and *Formica* Linnaeus. The morphological characters were coded for these taxa at the genus level. Vespidae remained coded at the family level, as in MORPH1. In addition, the bee genus *Apis* Linnaeus (Apidae) was added to MORPH2 and evaluated for all morphological characters, because this taxon was one of the outgroups in the molecular data set.

18S+28S data set

We generated sequence data (~2200 base pairs, bp, from the 18S and 28S rRNA genes) from single specimens of the following extant species: *Nothomyrmecia macrops*, *Myrmecia fulvipes* Roger, *Myrmecia picta* F. Smith, *Myrmecia pilosula* F. Smith, *Myrmecia pyriformis* F. Smith, *Pseudomyrmex gracilis* (Fabricius), *Tetraponera rufonigra* (Jerdon), *Paraponera clavata* (Fabricius), *Amblyopone pallipes* (Haldeman), *Rhytidoponera confusa* Ward, *Pogonomyrmex subdentatus* Mayr, *Linepithema humile* (Mayr), *Liometopum occidentale* Emery, *Formica moki* Wheeler, *Camponotus laevigatus* (F. Smith) and the vespid wasp *Mischocyttarus flavitarsis* (de Saussure). We chose species of *Myrmecia* from four divergent species-groups (*fulvipes* from the *mandibularis*-group, *picta* from the *picta*-group, *pyriformis* from the *gulosa*-group, and *pilosula* from the *pilosula*-group; see Ogata 1991) in order to broadly sample the diversity of this genus. 18S and 28S sequence data for *Apis mellifera* Linnaeus were taken from GenBank (accession numbers U89834, X89529 and AJ307465) and this species was used to root all molecular trees.

Material examined

For most of the taxa mentioned above we directly examined and dissected specimens in order to develop a comprehensive morphological character set, but we also extracted relevant information from the literature, especially for fossil taxa. Below we summarise the material examined and the literature sources used.

Nothomyrmecia macrops. Examined: small series of workers, two queens and one male. Information was also taken from Brown and Wilson (1959), Taylor (1978), Hölldobler and Engel (1979), Kugler (1980) and Billen (1988, 1990*b*).

Myrmecia. Examined: series of workers, queens and males from about 45 species, belonging to all nine species-groups. Additional information was taken from Clark (1951), Brown (1953*b*), Forbes (1967), Hölldobler and Engel (1979), Kugler (1980), Billen (1986, 1988, 1990*b*), Browning (1987), Ogata (1991) and Hashimoto (1991, 1996).

Prionomyrmex. Information on the worker caste was taken from descriptions and illustrations in Mayr (1868), Wheeler (1915) and Baroni Urbani (2000). A useful description of the male appears in Wheeler (1915).

Pseudomyrmex and *Tetraponera*. Examined: more than 100 species, all castes represented. See also information in Hölldobler and Engel (1979), Billen (1986), Attygalle *et al.* (1990), Ward (1990, 2001) and Hashimoto (1991, 1996).

Paraponera clavata. Examined: series of workers, queens and males.

Amblyopone. Examined: workers, queens and males, from about 10 species.

Rhytidoponera. Examined: workers and males from about 10 species; queens from two species.

Additional information on the last three ponerine genera was gleaned from Brown (1960), Hölldobler and Engel (1979), Ward (1980, 1994), Hashimoto (1991, 1996) and Kugler (1991).

Myrmicinae. Examined: representatives of the following genera (in most cases all castes), with the number of species indicated in parentheses: *Pogonomyrmex* (4), *Myrmica* Latreille (5), *Messor* Forel (1), *Aphaenogaster* Mayr (2), *Myrmicaria* Saunders (1) and *Megalomyrmex* Forel (1). Additional information was taken from Hölldobler and Engel (1979), Billen (1986), Hashimoto (1991, 1996) and Baroni Urbani *et al.* (1992).

Dolichoderinae. Examined: representatives of the following genera (all castes), with the number of species indicated in parentheses: *Dolichoderus* Lund (4), *Liometopum* (2), *Linepithema* (2), *Technomyrmex* Mayr (1) and *Leptomyrmex* Mayr (2). Additional information was taken from Pavan (1955), Miradoli Zatti and Pavan (1957), Hölldobler and Engel (1979), Attygale and Morgan (1984), Billen (1986, 1987), Hashimoto (1991, 1996), Baroni Urbani *et al.* (1992) and Shattuck (1992*a*, 1992*b*, 1995).

Formicinae. Examined: representatives of the following genera (all castes), with the number of species indicated in parentheses: *Oecophylla* F. Smith (2), *Camponotus* (4), *Formica* (4), *Cataglyphis* Foerster (1), *Melophorus* Lubbock (1), *Notostigma* Emery (1) and *Polyrhachis* F. Smith (1). Additional information was taken from Hölldobler and Engel (1979), Billen (1986), Agosti (1991), Hashimoto (1991, 1996) and Baroni Urbani *et al.* (1992).

Sphecomyrminae. Information on workers (*Sphecomyrma* Wilson & Brown, *Cretomyrma* Dlussky) and males (*Baikuris* Dlussky,

Dlusskyidris Bolton) was taken from Wilson *et al.* (1967), Wilson (1987), Dlussky (1975, 1983, 1987) and Grimaldi *et al.* (1997). In addition, in 1990 one of us (P.S.W.) examined the original holotype worker of *Sphecomyrma freyi* Wilson & Brown, before its disintegration and replacement with a neotype (see Grimaldi *et al.* 1997).

Vespidae and Apidae. The following taxa were examined: *Euparagia scutellaris* Cresson (male, female), *Mischocyttarus flavitarsis* (female), *Polistes* sp. (male), and *Apis mellifera* (worker). Information was also taken from Snodgrass (1956), Brothers (1975, 1999), Richards (1977), Baroni Urbani *et al.* (1992), Brothers and Finnamore (1993) and Grimaldi *et al.* (1997).

Most of the examined specimens reside as vouchers in the collection of the senior author at the University of California at Davis. Some material was borrowed from the Australian National Insect Collection, CSIRO Entomology, Canberra (ANIC).

Morphological characters

In developing a set of morphological characters we focused on features that varied among *Myrmecia*, *Nothomyrmecia* and *Prionomyrmex*, and between these ingroup taxa and the outgroups. In addition we sought traits that were potentially informative about outgroup relationships, even if they did not vary within the ingroup. In effect, we canvassed characters used in previous phylogenetic studies (Brothers 1975; Ward 1990, 1994; Bolton 1990*b*; Hashimoto 1991, 1996; Ogata 1991; Baroni Urbani *et al.* 1992; Brothers and Carpenter 1993; Grimaldi *et al.* 1997), evaluated these characters in the targeted taxa, and added new characters on the basis of direct examination of relevant material.

In the list of characters below, the following abbreviations are used for metric measurements (in mm): HW, head width; SL, scape length; LF1, length of the first funicular segment; LF2, length of the second funicular segment; LF3, length of the third funicular segment; and WF2, width of the second funicular segment. The term 'poneroid group' is used in the sense of Bolton (1990*b*). We use the informal expression 'myrmeciine ants' to refer collectively to the genera *Myrmecia*, *Nothomyrmecia* and *Prionomyrmex*.

1. 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 essentially the morphological correlate of prognathy and is characteristic of all ants, including Sphecomyrminae.
2. Worker and queen. Broad anteromedial protrusion of labrum, proximally near its junction with clypeus: (0) absent; (1) present. Autapomorphy of *Myrmecia* (Ogata 1991).
3. Worker and queen. Clypeo-labral connection, in frontal view: (0) concealed by overhanging clypeus; (1) exposed. State (1) is an autapomorphy of *Myrmecia* (Ogata 1991).
4. Worker and queen. Anteromedial depression on anterior median lobe of clypeus: (0) absent; (1) present. As described by Ogata (1991), this appears to be an autapomorphy of *Myrmecia*, although it is somewhat variable in form.
5. Worker and queen. Anteromedial margin of clypeus: (0) not pointed; (1) pointed. A triangular clypeus with an acute point medially occurs in both species of *Prionomyrmex* (Wheeler 1915: 25; Baroni Urbani 2000: fig. 5). In *Nothomyrmecia* the anteromedial clypeal margin is more or less rounded. An acuminate clypeus shows up in scattered other taxa within subfamilies Myrmicinae, Formicinae and Ponerinae. The commonest condition by far in Myrmicinae and Formicinae is a non-acuminate clypeus, so these subfamilies are coded as '0'.
6. Worker and queen. Lateral clypeal carina: (0) absent; (1) present. From Baroni Urbani (2000), who discovered this feature shared by *Prionomyrmex* and *Nothomyrmecia*.
7. Worker and queen. Clypeus, posteromesial protrusion between frontal carinae and antennal sockets: (0) absent; (1) present. This feature is somewhat variably developed – possibly because of convergence – in Dolichoderinae, Myrmeciinae, Myrmicinae and Ponerinae.
8. Worker and queen. Mandible: (0) bidentate; (1) with >2 teeth. State (0) distinguishes *Sphecomyrma* from other ants.
9. Worker and queen. Mandible: (0) relatively short; (1) elongate and slender – mandible length about three-quarters or more of head length. By this definition, state (1) characterises *Myrmecia*, *Prionomyrmex* and *Nothomyrmecia*; *Amblyopone* is variable. Elongate-linear mandibles occur in a few other highly specialised ant taxa within other ant subfamilies (e.g. dacetine ants in the Myrmicinae, and *Myrmoteras* Forel in the Formicinae) but they are clearly secondarily derived and not part of the ground plan for those subfamilies.
10. Worker and queen. Closed mandibles: (0) broadly overlapping; (1) not broadly overlapping. In *Nothomyrmecia* and *Prionomyrmex* the masticatory margins of the closed mandibles oppose, but do not broadly overlap, along most of their length, and they form a tight closure with the anteromedially protruding clypeus. This condition does not occur in *Myrmecia*, or indeed most other ants. Non-overlapping mandibles occur as a derivative condition within the subfamily Myrmicinae (in *Lenomyrmex* Fernández & Palacio, *Tatuidris* Brown & Kempf, and the dacetine tribe group) but overlapping mandibles are almost certainly the plesiomorphic state for the subfamily (Bolton 1998, 2000).
11. Worker and queen. Stout, setiform cuticular projections on masticatory margin of mandible: (0) absent; (1) present. Such seta-like projections occur in *Prionomyrmex janzeni* (Baroni Urbani 2000: fig. 3) and in *Nothomyrmecia* (present study).
12. Worker and queen. Compound eyes: (0) separated from mandibular insertions by a well-developed malar area; (1) placed in anterior position on head, malar area much reduced. Forward-positioned compound eyes are characteristic of *Myrmecia*.
13. Worker and queen. Compound eyes: (0) not large, oval and strongly convex; (1) of such form. In *Myrmecia*, *Prionomyrmex* and *Nothomyrmecia* the eyes are not only large but also oval in shape and conspicuously protruding from the surface of the head. This condition is not seen in most other ants. For example, in the Pseudomyrmecinae the eyes are generally large but they are not as strongly convex when viewed in profile. Some large-eyed Formicinae approach this state and this subfamily is coded as polymorphic for this character.
14. Worker. Ocelli: (0) present, well developed; (1) absent or highly reduced. Workers of *Myrmecia* have well-developed ocelli, but they are absent or vestigial in *Nothomyrmecia* workers (Taylor 1978) and in most known specimens of *Prionomyrmex*. Of six *Prionomyrmex longiceps* workers with heads that Wheeler (1915) examined, all but one lacked ocelli. The ocellate individual was larger than the other workers as was Mayr's (1868) original worker, which also bore ocelli. Baroni Urbani (2000) does not mention the presence of ocelli in the two individuals of *P. janzeni* examined by him. We code *Prionomyrmex* as (1), in recognition of the loss or reduction of ocelli in this taxon.
15. Worker and queen. Scape: (0) relatively short, about 20% of the total length of the antenna or less; (1) elongate, ≥30% of antennal length. State (0) in Sphecomyrminae (Dlussky 1983), state (1) in most other ants.
16. Worker and queen. Relative length of second and third antennal segments, as expressed by the ratio LF1/LF2: (0) 0.50–1.15; (1) >1.20. The first funicular (second antennal) segment is less than, or approximately equal to, the second funicular (third

antennal) segment in *Prionomyrmex*, *Nothomyrmecia* and *Myrmecia*, as well as *Sphecomyrma* and some ponerines. In most other ants that have retained 12 antennal segments, the first funicular segment is conspicuously longer than the second.

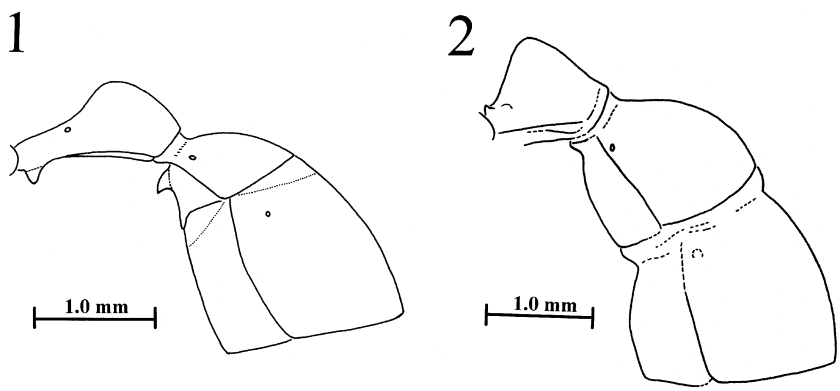
17. Worker and queen. Second antennal (first funicular) segment: (0) shorter than the fourth antennal (third funicular) segment; (1) as long as, or longer than, the fourth antennal segment. In *Nothomyrmecia*, *Myrmecia* and most other ants with 12 antennal segments, the length of the first segment of the funiculus equals or (more commonly) exceeds that of the third segment. *Prionomyrmex* is exceptional in having LF1/LF3 ~0.76 [based on illustrations in Wheeler (1915) and Baroni Urbani (2000)]. Such a low ratio is also seen in some ponerines and in *Sphecomyrma*.
18. Worker and queen. Third antennal (second funicular) segment: (0) not notably slender and elongate; (1) very slender (i.e. much longer than wide) and longer than the succeeding (third funicular) segment. An elongate second funicular segment has been noted as typical of *Sphecomyrma*, *Prionomyrmex*, *Nothomyrmecia* and *Myrmecia* (Wilson *et al.* 1967; Wheeler 1915; Ogata 1991; Grimaldi *et al.* 1997), but the feature has not been accurately characterised. The second funicular segment is not necessarily longer than all the others (the first funicular segment is as long as the second in *Nothomyrmecia* and in some *Myrmecia* species). Nor is it sufficient to state that it is longer than the third funicular segment: that is also true of many other ants, especially those with more compact antennae. The particular configuration of the second funicular segment that characterises workers and queens of the above ant taxa – and only a few others – is that the segment is both slender (more than twice as long as wide: LF2/WF2 > 2.00) and notably longer than the following (third funicular) segment (LF2/LF3 ≥ 1.10).
19. Male. Scape length: (0) one-quarter or more of the combined length of antennal segments 2–4; (1) about one-fifth or less of the combined length of antennal segments 2–4. Males of *Myrmecia*, *Nothomyrmecia* and – based on Wheeler's (1915) description – *Prionomyrmex* all have a very short, stocky scape, succeeded by much longer and more slender antennal segments 2–4, such that SL/(LF1 + LF2 + LF3) ranges from 0.16 to 0.22. This condition is not observed in most other ants. In males of Sphecomyrminae the ratio varies from about 0.23 to 0.38 and the scape is not notably thicker than the succeeding segments, based on descriptions and illustrations in Dlussky (1975, 1987) and Grimaldi *et al.* (1997). The ratio exceeds 0.30 in most males of Pseudomyrmecinae, but in some members of the *Pseudomyrmex gracilis*-group it is as low as 0.20, so the genus *Pseudomyrmex* has been coded as polymorphic.
20. Male. Second antennal segment (first funicular segment): (0) very short, less than one-third the length of third antennal segment; (1) longer, LF1/LF2 > 0.32. In most male ants the first funicular segment is one-third or more of the length of the succeeding segment, but it is shorter than this in *Prionomyrmex*, *Nothomyrmecia*, *Myrmecia* and some Ponerinae. *Tetraponera* is variable, as apparently are sphecomyrmine males, based on illustrations in Dlussky (1975, 1987) and Grimaldi *et al.* (1997).
21. 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).
22. Worker and queen. Antenna, peg of sensilla basiconica: (0) short (< 20 μm); (1) elongate (> 30 μm). Coded after Hashimoto (1991), who found elongate pegs in Amblyoponini (*Amblyopone*), Ectatommini (*Gnamptogenys* Roger, *Rhytidoponera*) and Myrmicinae (16 genera sampled), and short pegs in all other taxa examined, including *Myrmecia*. The short condition is also seen in *Nothomyrmecia* (present study).
23. Worker and queen. Antenna, aperture of sensilla trichoidea curvata: (0) extending beyond the base of the hair; (1) not extending beyond the base. This also follows Hashimoto (1991), who noted that a small aperture occurred only in Ectatommini (represented in his study by *Gnamptogenys* and *Rhytidoponera*) and Myrmicinae. Assessment of this feature requires cross-sectioning of the antenna. The condition is unknown in *Nothomyrmecia*, *Paraponera* and the fossil taxa.
24. Worker and queen. Conspicuous standing hairs on antennal scape: (0) absent or confined to a few hairs near the apex of the scape; (1) common. Antennal pilosity varies widely in ants, and would not normally be considered a useful character for a higher-level phylogenetic analysis. It is included here because it is one of the few differences between the two described species of *Prionomyrmex* (Baroni Urbani 2000), and these two taxa have been coded separately in order to assess the strength of support for monophyly of the genus. The scape is densely hairy in *Nothomyrmecia*, variable in *Myrmecia*.
25. Worker. Promesonotal suture: (0) mobile; (1) fused. The pronotum freely articulates with the mesonotum in most taxa considered here (Bolton 1990*b*; Baroni Urbani *et al.* 1992; Ward 1994). It is very probable that this was the condition in the fossil taxa *Prionomyrmex* and *Sphecomyrma* because their promesonotal suture is well marked and their morphology is similar to that of extant ants that show mobility of the suture. For this reason they have been coded '0'.
26. Worker. Mesonotum: (0) of normal length; (1) short and transverse, much wider than long and much shorter than the dorsal face of the propodeum. A very reduced mesonotum is characteristic of the Amblyoponini (Ward 1994) and *Paraponera*.
27. Male. Notauli ('Mayrian furrows') on mesoscutum: (0) present, strongly impressed; (1) absent or weakly developed. Notauli are well developed in the males of *Prionomyrmex* (Wheeler 1915) and *Myrmecia* but absent in *Nothomyrmecia* (Taylor 1978). Notauli occur elsewhere in male Formicidae having rather 'generalised' morphology (e.g. *Paraponera*, *Rhytidoponera*, *Amblyopone*, *Myrmica*) and in other aculeate Hymenoptera (Richards 1977), but they appear to have been repeatedly lost or reduced.
28. Male. Distinct posterior oblique sulcus on mesepisternum: (0) present; (1) absent. Such a sulcus, which divides the mesepisternum into an upper anepisternum and a lower katepisternum (Tulloch 1935), is evident in males of most ants, but it is essentially absent – or represented at most by a weak broad furrow – in males of *Myrmecia* and *Nothomyrmecia*. Wheeler's (1915: 27) description of a *Prionomyrmex* male makes no mention of a mesopleural sulcus, suggesting that it might be absent, but because Wheeler does not explicitly describe the mesepisternum, the condition in *Prionomyrmex* remains uncertain.
29. Worker and queen. Metapleural gland: (0) absent; (1) present. This gland is a synapomorphy of all ants, including Sphecomyrminae (Dlussky 1975; Grimaldi *et al.* 1997). Secondary loss has occurred in a few genera of Formicinae (Hölldobler and Engel-Siegel 1985).
30. Worker and queen. Metapleural gland opening: (0) not flanked above by carina-like flange that is directed anterodorsally; (1) with such a flange. State (1) is seen in *Nothomyrmecia*, *Myrmecia* and Pseudomyrmecinae. Unfortunately, the descriptions and illustrations of *Prionomyrmex* (Mayr 1868; Wheeler 1915; Baroni Urbani 2000) are not sufficiently detailed to reveal the condition in this taxon. A possibly homologous cuticular flange

that lies above the metapleural gland orifice occurs in the doryline section and in some Leptanillinae (Bolton 1990*a*; Baroni Urbani *et al.* 1992).

31. Worker and queen. Metapleural gland opening: (0) not located immediately above the posteroventral margin of the metapleuron (separated by a distance greater than the diameter of the opening); (1) located immediately above the lower margin of the metapleuron. State (1) is an autapomorphy of Pseudomyrmecinae (Ward 1990).
32. Worker, queen and male. Metacoxal cavities: (0) open; (1) closed. Coded in part after Ward (1990, 1994) and Baroni Urbani *et al.* (1992). *Amblyopone* is considered 'open' (contra Ward 1990), because the endpoints of the cuticle surrounding the metacoxal cavity are overlapping but not fused.
33. Worker, queen and male. Paired propodeal spines or teeth: (0) absent; (1) present. One of the more notable features of *Prionomyrmex* is the presence of a pair of short, blunt teeth on the propodeal dorsum, posterior to the spiracles (Mayr 1868; Wheeler 1915; Baroni Urbani 2000). These are absent in *Nothomyrmecia*, *Myrmecia* and Pseudomyrmecinae. Propodeal spines or teeth occur in other ant taxa, especially within the subfamily Myrmicinae.
34. Male. Propodeal spiracle: (0) slit-shaped; (1) round to elliptical. By 'slit-shaped' we mean that the aperture of the spiracle is very elongate (length four or more times the width), with parallel sides. Among the taxa considered in this study, a slit-shaped propodeal spiracle is seen in males of *Nothomyrmecia*, *Myrmecia*, *Paraponera* and Sphecomyrminae. The condition in *Prionomyrmex* is unknown. Pseudomyrmecinae and Formicinae are polymorphic. These two subfamilies were miscoded as '1' in Baroni Urbani *et al.* (1992) and Grimaldi *et al.* (1997); Pseudomyrmecinae was similarly miscoded in Baroni Urbani (2000).
35. Worker, queen and male. Foretibial spur (calcar) with conspicuous velum: (0) present; (1) absent. The base of the foretibial spur has a distinct translucent lamella (velum), unobstructed by cuticular teeth, in *Myrmecia* (Schönitzer and Lawitsky 1987) and *Nothomyrmecia* (present study). The condition in *Prionomyrmex* cannot be determined from the original descriptions or illustrations. A foretibial spur with a proximal velum was observed in a Cretaceous male ant provisionally identified as belonging to the subfamily Sphecomyrminae (Grimaldi *et al.* 1997: 18). A distinct velum also occurs in some Ponerini (Schönitzer and Lawitsky 1987) and in *Amblyopone*, but not in most other ant taxa considered here. In *Paraponera* males, the foretibial spur has an apparent velum, that is, a transparent lamella, most readily discerned with back-lighting, but it is overgrown by fine cuticular teeth except at the distal extremity. In *Paraponera* workers and queens, the entire structure is obscured by cuticula. To reflect this ambiguity *Paraponera* is coded as '?' (unknown). Vespidae have a velum with a serrated rim (Schönitzer and Lawitsky 1987).
36. Worker, queen and male. Number of apical hind tibial spurs: (0) 2; (1) 1. Presence of two hind tibial spurs is the condition in the three myrmeciine genera (Ogata 1991; Baroni Urbani 2000): Pseudomyrmecinae (Ward 1990), Sphecomyrminae (Wilson *et al.* 1967; Dlussky 1975) and Vespidae (Brothers 1975). Reduction to a single spur (or none) has occurred in Formicinae, Dolichoderinae (Shattuck 1992*a*), Myrmicinae and in many members of the poneroid group.
37. Worker and queen. Basitarsal sulcus on anterior surface of mid and hind tarsi: (0) absent; (1) present. This trait is seen in *Myrmecia*, *Nothomyrmecia* and *Prionomyrmex*. A basitarsal sulcus is also present in two of the three genera of Pseudomyrmecinae (Ward 1990) and is here considered part of the ground plan of that subfamily. A similar sulcus also occurs in *Paraponera*, suggesting either convergence or an early origin of the feature in ant evolution, followed by multiple losses. *Sphecomyrma freyi* lacks the sulcus (P. S. Ward, personal observation).
38. Worker and queen. Tarsal claws: (0) bifurcate, with submedian tooth in addition to apical tooth; (1) simple (lacking submedian tooth). Bifurcate tarsal claws occur in *Myrmecia*, *Nothomyrmecia*, *Prionomyrmex*, Sphecomyrminae, Pseudomyrmecinae (incorrectly coded as simple in Baroni Urbani 2000), and widely but not universally within the poneroid group. Vespidae are variable but bifurcate claws are considered part of the ground plan (Brothers 1975).
39. 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. Divergence of M and Cu near the cu-a crossvein occurs in *Myrmecia*, *Nothomyrmecia*, Sphecomyrminae and some Ponerinae, based on material examined in this study and on information and illustrations in Brown and Nutting (1950), Taylor (1978) and Grimaldi *et al.* (1997). Given Wheeler's (1915) statement that the wing venation of *Prionomyrmex* is 'almost exactly' like that of *Myrmecia*, we assume that the same condition applies to *Prionomyrmex*. In most other taxa considered in this study, the divergence of M and Cu is notably distad of the cu-a crossvein (*Amblyopone* and *Rhytidoponera* are polymorphic).
40. Queen (if winged) and male. Forewing, distal section of cubital vein [Cu-A1 of Brown and Nutting (1950) or Cu1 of Gauld and Hanson (1995)]: (0) present, at least as a fold; (1) absent. Loss of the distal portion of the cubital vein is an apparent autapomorphy of Sphecomyrminae (Grimaldi *et al.* 1997). Convergent secondary loss occurs within the various groups of ants (e.g. in some species of Myrmicinae and Dolichoderinae with much reduced wing venation), but a distally developed cubital vein is evidently part of the ground plan for those groups and for other taxa considered here (Brown and Nutting 1950).
41. Queen (if winged) and male. Hind wing jugal lobe: (0) present; (1) absent. Coded on the basis of information in Wheeler (1915), Dlussky (1975), Grimaldi *et al.* (1997) and Baroni Urbani *et al.* (1992), except that the latter paper incorrectly states that the lobe is absent in *Paraponera* (it is actually present and well developed in that genus). Although this character is variable in Vespidae, the presence of a jugal lobe is considered the ancestral condition in that family.
42. Worker and queen. Tergosternal fusion of abdominal segment 2 (the petiole): (0) absent or incomplete, such that there remains mobility between the two sclerites; (1) complete, such that there is no independent mobility of the two plates. There is free movement between the petiolar tergum and sternum in Pseudomyrmecinae, *Nothomyrmecia*, *Myrmecia*, *Rhytidoponera* and *Amblyopone*. Interestingly, there is partial tergosternal fusion anteriorly in *Amblyopone* (Ward 1994) and in *Nothomyrmecia* (present study), restricting mobility of the plates to the posterior half. Petiolar fusion cannot be directly assessed in the fossil taxa *Prionomyrmex* and *Sphecomyrma*, but the well-marked boundaries between the terga and sterna (Baroni Urbani 2000: fig. 6; Grimaldi *et al.* 1997: fig. 3) indicate lack of complete tergosternal fusion. The remaining ant taxa considered here (Appendix 1) show complete tergosternal fusion of the petiole. Following Grimaldi *et al.* (1997), we consider the ground plan for Vespidae to be lack of fusion (state '0').
43. Worker and queen. Abdominal segment 2 with well-differentiated and slender anterior peduncle: (0) absent; (1) present (Fig. 1). Coded in part after Ogata (1991). A well-

developed and attenuate petiolar peduncle is present in *Nothomyrmecia*, absent in *Prionomyrmex* and variable in *Myrmecia*. It is also a common (although not universal) condition in Pseudomyrmecinae. In other taxa considered here the anterior peduncle tends to be short or absent.

44. Worker, queen, male. Junction of abdominal segments 2 and 3: (0) lacking a distinct constriction; (1) marked by a distinct constriction, at least ventrally and usually on all sides, resulting in the formation of an isolated node-like or scale-like petiole. Some degree of isolation of abdominal segment 2 (the petiole) from the rest of the gaster occurs in all ants.
45. Worker, queen. Dorsal portion of abdominal segment 2: (0) consisting of a flat surface extending uninterrupted to the posterior margin, without descending into a posterior face; (1) with a well-developed posterior face, resulting in a marked dorsal constriction between abdominal segments 2 and 3. State (1) is characteristic of most ants, but in Amblyoponini the petiole lacks a posterior face (Brown 1960; Ward 1994).
46. Worker and queen. Presclerites of abdominal segment 3 (i.e. the helcium): (0) unfused; (1) fused. Coded on the basis of information in Ward (1990, 1994), Bolton (1990*b*), Baroni Urbani *et al.* (1992) and Grimaldi *et al.* (1997). The helcium is unfused in *Nothomyrmecia*, *Myrmecia*, Pseudomyrmecinae, some Dolichoderinae and most Myrmicinae, and fused in Ponerinae and in all Formicinae examined. Lack of fusion is here considered the ground plan for Myrmicinae and Dolichoderinae, as well as Vespidae. This character cannot be assessed in the fossil taxa.
47. Worker, queen and male. Helcial sternite (presternite of abdominal segment 3): (0) overlapped by helcial tergite; (1) not overlapped. State (1) is an autapomorphy of Myrmicinae.
48. Worker and queen. Helcial tergite (i.e. pretergite of abdominal segment 3) with internal anteromedial lobe for attachment of tergal muscles: (0) absent; (1) present. Hashimoto (1996) discovered this interesting feature: a cuticular extension on the internal anteromedial border of the helcial tergite, directed posteriorly and functioning as a point of origin for a specific bundle of tergal muscles ('no. 8') that serve to elevate the gaster. In a wide-ranging survey of various aculeate Hymenoptera, Hashimoto (1996) found the internal helcial lobe present in Ectatommini (*Rhytidoponera*) and Myrmicinae (five genera sampled), and absent from other taxa examined including *Myrmecia*, *Pseudomyrmex*, *Tetraponera*, Amblyoponini, Ponerini and Proceratiini. In the present study, we found the lobe to be also lacking in *Nothomyrmecia* and *Paraponera*, and present in four additional ectatommine genera (*Ectatomma* F. Smith, *Gnamptogenys*, *Heteroponera* Mayr and *Acanthoponera* Mayr).
49. Worker and queen. Postsclerites of abdominal segment 3: (0) not completely fused; (1) completely fused. Complete tergosternal fusion of abdominal segment 3, posterior to the helcium, occurs in all poneroid group females except *Adetomyrma* Ward (Ward 1994).
50. Worker and queen. Diaphanous longitudinal keel on poststernite of abdominal segment 3, near its anterior margin: (0) absent; (1) present (Fig. 1). This is an autapomorphy of *Nothomyrmecia* (present study).
51. Worker, queen and male. Sinuous medial protrusion on posterior margin of abdominal sternite 3: (0) absent; (1) present (Fig. 1). This is another morphological novelty of *Nothomyrmecia*. The protruding posterior margin of the poststernite of abdominal segment 3 is slightly thickened and forms part of the ventral stridulatory organ of *Nothomyrmecia* (see Taylor 1978), serving as a plectrum (scraper) that moves against the file on the presternite of abdominal segment 4. A posteromedial protrusion of abdominal sternite 3 is not seen in *Myrmecia* or *Prionomyrmex* (Fig. 2), consistent with the confirmed absence of a ventral stridulitrum in the former genus and suggesting the absence of such an organ in *Prionomyrmex* (contrary to the inference of Baroni Urbani 2000).
52. Worker, queen and male. Dorsal stridulatory organ, with stridulitrum (file) on abdominal pretergite 4 and posterior margin of preceding segment serving as plectrum: (0) absent; (1) present. Such a structure is absent in *Myrmecia* and *Nothomyrmecia*, but present in Pseudomyrmecinae, Myrmicinae and some Ponerinae (Markl 1973; Baroni Urbani *et al.* 1992). It is coded as unknown in *Sphecomyrma* and *Prionomyrmex*.
53. Worker, queen and male. Abdominal segment 3 in dorsal view: (0) not forming a postpetiole; (1) forming a node-like postpetiole: strongly constricted from abdominal segment 4 and distinctly smaller. A well-differentiated postpetiole occurs in *Myrmecia*, *Prionomyrmex*, Pseudomyrmecinae and Myrmicinae, but not *Nothomyrmecia* (although abdominal segment 3 is distinctly narrower than segment 4). Among ants of the poneroid group, it is sometimes difficult to code this character because of intermediate conditions. By the preceding criteria, a well-developed postpetiole can be considered present in *Paraponera*, absent in *Amblyopone* and *Rhytidoponera*. The feature has evidently evolved more than once in ants.
54. Worker and queen. Dorsal midline length of third abdominal segment excluding the helcium (i.e. length of post-tergite): (0) markedly less than that of fourth abdominal post-tergite ($\leq 0.80\times$); (1) subequal to, or greater than, the length of abdominal post-tergite 4 ($> 0.80\times$). Condition (0) is seen in all



Figs 1–2. Lateral view of worker abdominal segments 2–4. *1*, *Nothomyrmecia macrops*. *2*, *Prionomyrmex janzeni* (modified from Baroni Urbani 2000).

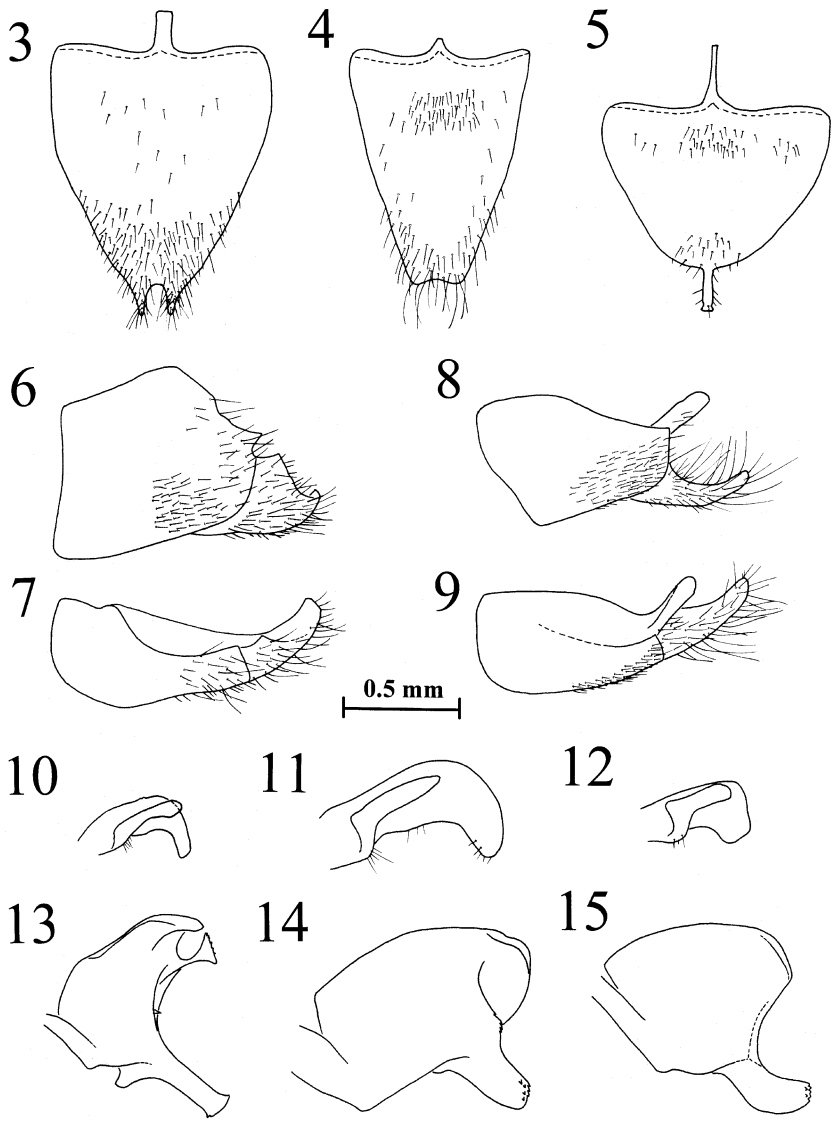
three myrmeciine genera, as well as Pseudomyrmecinae, Myrmicinae, *Sphecomyrma* and *Euparagia* Cresson (Vespidae). A long third abdominal segment, relative to the fourth, occurs in Formicinae and in most Dolichoderinae and Ponerinae.

55. Worker and queen. Height of third abdominal segment, as seen in profile under normal distension of the gaster: (0) subequal to that of fourth abdominal segment (~0.90–1.05×); (1) distinctly less than that of the fourth abdominal segment (≤ 0.80×). This feature shows a similar but not identical distribution of states as character no. 53 (presence/absence of postpetiole). The discrepancy occurs because *Nothomyrmecia*, although not possessing a postpetiole, nevertheless has a third abdominal segment markedly smaller than the following segment. This is otherwise rare in ants without a postpetiole, occurring in *Aneuretus* Emery (Wilson *et al.* 1956) and some taxa in the doryline section.
56. Worker and queen. Abdominal segment 4 with differentiated presclerites, separated from the postsclerites by distinctive girdling: (0) absent; (1) present. State (1) occurs in *Myrmecia*, *Prionomyrmex* (Baroni Urbani 2000: fig. 6), Pseudomyrmecinae, Myrmicinae, and the poneroid group. The ground plan for the Vespidae is considered to be absence of differentiated presclerites (Grimaldi *et al.* 1997).
57. Worker and queen. Presclerites of abdominal segment 4: (0) not fused laterally; (1) fused laterally. Fusion occurs in *Myrmecia* and all Ponerinae (except *Adetomyrma*). Myrmicinae is polymorphic, but unfused in genera such as *Myrmica*, *Pogonomyrmex* and *Aphaenogaster*; where fusion occurs (e.g. *Myrmicaria*) it is evidently secondarily. This character cannot be assessed in *Prionomyrmex*.
58. Worker and queen. Pretergite of abdominal segment 4: (0) subequal to or shorter than presternite; (1) notably longer than presternite. State (1) is characteristic of Pseudomyrmecinae and most Myrmicinae (Ward 1990). Contrary to the statement in Hashimoto (1996: 354), the pretergite is always longer than the presternite in pseudomyrmecines. Where pretergite 4 is short in Myrmicinae (e.g. *Myrmicaria*), it is apparently associated with loss of the dorsal stridulitrum.
59. Worker and queen. Tergosternal fusion of postsclerites of abdominal segment 4: (0) absent; (1) present. Such fusion occurs only in the Ponerinae, although it is lacking in the enigmatic *Adetomyrma* (Ward 1994).
60. Worker and queen. Posterior margin of abdominal sternite 6: (0) simple; (1) with a median cariniform protuberance, as seen in lateral or ventral view (Pavan 1955: fig. 1). A beak-like median point or protuberance on the posterior margin of sternite 6 was noted by Pavan (1955) and Miradoli Zatti and Pavan (1957) as being characteristic of Dolichoderinae and Aneuretinae, occurring even in taxa (*Liometopum*, *Tapinoma* Foerster) in which Pavan's gland (see below, character 66) is apparently lost. The protuberance is most evident in those dolichoderines that have a well-developed longitudinal median carina on abdominal sternite 6, because the protuberance marks the endpoint of this carina. In taxa lacking the carina (e.g. *Iridomyrmex* Mayr, *Technomyrmex*), the protuberance may be reduced or absent.
61. Worker and queen. Acidopore: (0) absent; (1) present. This is an autapomorphy of Formicinae.
62. Worker and queen. Furcula of sting apparatus: (0) present and well developed; (1) very reduced or absent. The furcula is well developed in *Myrmecia*, *Nothomyrmecia* and most other ants considered here, but there has been extreme reduction (fusion to sting base) or loss in Dolichoderinae, Formicinae and some taxa in the poneroid group (Kugler 1980, 1991, 1992; Hermann and Blum 1981).
63. Worker and queen. Pygidial gland: (0) absent; (1) present. This gland, opening between abdominal tergite 6 and tergite 7 (the pygidium), is present in all major ant groups, including *Myrmecia* and *Nothomyrmecia*, but is generally lacking in Formicinae (Hölldobler and Engel 1979; Billen 1987). A 'pygidial gland', differing in structural detail, occurs in the formicine genus *Polyergus* and is considered to be a non-homologous feature (Hölldobler 1985).
64. Worker and queen. Production of monoterpene iridoids (by the pygidial gland): (0) absent; (1) present. To our knowledge, Dolichoderinae are unique among ants in producing a group of cyclopentanoid monoterpenes known as iridoids (Attygalle and Morgan 1984; Shattuck 1992*a*).
65. Worker and queen. Postpygidial gland: (0) absent; (1) present. Coded after Hölldobler and Engel (1979), who found this gland present in *Myrmecia*, *Nothomyrmecia*, Pseudomyrmecinae and most poneroid group taxa, but absent in *Amblyopone*, Myrmicinae, Formicinae, Aneuretinae and Dolichoderinae.
66. Worker and queen. Pavan's gland: (0) absent; (1) present. This gland occurs in Dolichoderinae and Aneuretinae only (Billen 1987). It is absent, presumably secondarily, in at least some species of *Liometopum* and *Tapinoma* (Pavan 1955). The gland opens between and abdominal sternites 6 and 7, and it appears to be a source of trail pheromone.
67. Worker and queen. Sting bulb gland: (0) absent; (1) present. Among extant ants this feature is apparently uniquely shared by *Myrmecia* and *Nothomyrmecia* (Billen 1990*b*). The condition in *Prionomyrmex* cannot be determined.
68. Worker and queen. Apical cell membrane of Dufour's gland with numerous, robust microvilli: (0) absent; (1) present. Such apical microvilli occur in the Dufour's gland of *Myrmecia* and *Nothomyrmecia* (Billen 1986, 1988) and not in other taxa examined (Attygalle *et al.* 1990; Billen 1986).
69. Male. Paramere, when viewed laterally and ventrally: (0) not divided by a suture into distinct apical/ventromesial and proximal/dorsolateral sections; (1) so divided. In males of *Myrmecia* and *Nothomyrmecia*, there is a distinct suture between an apical and ventromesial portion of the paramere and a proximal/dorsolateral portion. In a ventral view of the paramere the suture has an oblique longitudinal orientation, then becomes transverse as it crosses the outer face of the paramere (Figs 6, 8). Most outgroup taxa lack this feature. The paramere is divided in Dolichoderinae (Krafchick 1959) and Vespidae (Richards 1977), but of a different configuration.
70. Male. Volsella: (0) not reduced; (1) reduced to a small setose lobe closely appressed to (or fused with) the inner wall of the paramere. State (1) is an autapomorphy of Pseudomyrmecinae (Ward 1990). The volsella is well developed, with differentiated cuspis and digitus, in *Myrmecia* (Forbes 1967), *Nothomyrmecia* (Fig. 10) and other taxa considered here.
71. Male. Aedeagus, posteroventral projection, armed with stout teeth or spines: (0) absent; (1) present. The aedeagus has a lobe-like posteroventral projection, adorned apically with spines or teeth, in males of *Myrmecia* (Figs 14, 15) and *Nothomyrmecia* (Fig. 13). Despite considerable variation in the shape of this spiny lobe, it appears to be present in almost all *Myrmecia* species, with the exception of one species in which the apical spines have been lost and a second species in which the lobe has been reduced to a dentate but non-projecting posteroventral corner (Browning 1987). This trait was not observed in the outgroup taxa examined.
72. Worker, queen and male. Larva with ventral food pocket (trophothylax): (0) absent; (1) present. This is an autapomorphy of Pseudomyrmecinae (Wheeler and Wheeler 1976; Ward 1990).

73. Worker, queen and male. Pupa: (0) enclosed in cocoon; (1) naked. Among the taxa considered here, the pupae are always naked in Pseudomyrmecinae, Myrmicinae and Dolichoderinae. Pupae may be enclosed in cocoons or naked in formicine and ponerine ants (Baroni Urbani *et al.* 1992), but a cocooned pupa is much more common and is considered the ground plan of Formicinae and the genus *Amblyopone*.
74. Worker, queen. Apterous worker caste: (0) absent; (1) present. Synapomorphy of Formicidae.

The following supplementary characters were used to discriminate among the four *Myrmecia* species-groups represented in the MORPH2 data set.

75. Worker and queen. Well-developed occipital carina: (0) absent; (1) present. Treatment follows Ogata (1991), except that a well-developed carina is considered lacking in *Nothomyrmecia* because there is no distinctly elevated ridge. In the three *Myrmecia* species-groups with an occipital carina there is an elevated ridge, sharply separating an area of dense sculpture and pilosity from a much smoother occiput. The condition in *Prionomyrmex* cannot be determined unambiguously from the original descriptions and illustrations. An occipital carina is variably present in the outgroups.
76. Worker and queen. Antennal scape: (0) short; (1) long, SL > (0.5HW + 1.20). This is the formula used by Ogata (1991) to distinguish *Myrmecia* species-groups with short and long scapes (see his fig. 29). By this criterion, the scape is long in *Prionomyrmex*, and short in *Nothomyrmecia* and Pseudomyrmecinae. Other outgroups tend to be variable.
77. Worker and queen. Subapical portion of mandible with supplementary ventral tooth, below the main series (Ogata 1991: fig. 33): (0) absent; (1) present.
78. Worker. Mesonotum: (0) relatively elongate; (1) broader, such that mesonotal length/width <0.90. Character state distinctions are from Ogata (1991), but note that his 'mesonotal index' is



Figs 3–15. Male terminalia: (*3–5*) ventral view of sternite 9, (*6–9*) paired lateral and dorsal views of left paramere, (*10–12*) lateral view of left volsella, and (*13–15*) lateral view of aedeagus. *3*, *6*, *7*, *10*, *13*, *Nothomyrmecia macrops*; *4*, *8*, *9*, *Myrmecia picta*; *5*, *M. varians* Mayr; *11*, *M. tarsata* F. Smith; *12*, *15*, *M. pilosula*; *14*, *M. nobilis* (Clark).

misstated to be 'width/length' when it is actually 'length/width'. Also, in contrast to Ogata (1991), *Nothomyrmecia* is here considered to have a relatively elongate mesonotum (length/width ~1.0), as in *Prionomyrmex* (Baroni Urbani 2000: fig. 5).

79. Worker. Posteromedial margin of mesonotum: (0) rounded; (1) straight. Coded after Ogata (1991).
80. Worker and queen. Postpetiole: (0) large, hemispherical; (1) smaller, subconical. Coded after Ogata (1991). In most *Myrmecia* species the postpetiole is rather broad (ratio of postpetiole width to gaster width in workers ≥0.60), but in the *M. gulosa*-group and *M. nigrocincta*-group it is more slender (Ogata 1991: 371). The postpetiole is broad in *Prionomyrmex* (postpetiole width/gaster width ~0.78) and *Paraponera* (0.84), variable in Pseudomyrmecinae (0.50–0.78), and slender in Myrmicinae (usually <0.50). Other outgroups are non-postpetiolate and hence coded as 'inapplicable'.
81. Male. Sternite VIII, conspicuous setae on posterior margin: (0) present; (1) absent. The posterior margin of sternite VIII is hairy in males of the *Myrmecia gulosa*-group, *M. nigrocincta*-group, *M. urens*-group, and most outgroups, and devoid of pilosity in other *Myrmecia* taxa examined and in *Nothomyrmecia*.
82. Male. Hypopygium (sternite IX), anteromedial region with setae: (0) absent or sparse (Fig. 3); (1) moderately common (Figs 4, 5). Isolated anteromedial patches of appressed setae – separated from the pilosity on the posterior half of sternite IX – appear to be present in males of most *Myrmecia* species-groups but are generally absent in the *M. gulosa*-group, *M. nigrocincta*-group and *M. aberrans*-group. Anteromedial patches of setae are absent in *Nothomyrmecia* (a few scattered setae are present: Fig. 3) and outgroups.
83. Male. Hypopygium (sternite IX), with thin digitiform posteromedial protrusion: (0) absent; (1) present (Fig. 5). A very thin, elongate posteromedial process on sternite IX, about as narrow as the anteromedial apodeme of the sternite and often bent dorsad at the apex, is characteristic of most *Myrmecia* species-groups. It is lacking in the *M. picta*-group, *M. urens*-group, *M. nigrocincta*-group and in most species of the *M. gulosa*-group. This feature was not observed in *Nothomyrmecia* (Fig. 3) or in the outgroups.
84. Male. Paramere with dorsomesial process ('dorsal median projection' of Forbes 1967): (0) absent; (1) present (Figs 8, 9). A lobe-like dorsal projection, emerging from the mesial wall of the paramere, occurs in males of the *Myrmecia gulosa*-group, *M. nigrocincta*-group and *M. picta*-group. It is absent in the other taxa, including *Nothomyrmecia* (Figs 6, 7).
85. Male. Digitus of volsella, in lateral or mesial view: (0) of approximately constant or narrowing width distally (Figs 10, 11); (1) enlarged distally, in form of a hammer or anvil (Fig. 12). A distally enlarged, anvil-shaped digitus appears to characterise males of all *Myrmecia* species-groups except the *M. gulosa*-group and *M. nigrocincta*-group. Two exceptional species in the *M. gulosa*-group that have an anvil-shaped digitus (*M. mjobergi* Forel and *M. regularis* Crawley; see Browning 1987) are considered to represent secondary modifications within the group. A distally enlarged digitus is not seen in *Nothomyrmecia* (Fig. 10) or most outgroups.

The morphological data matrices (MORPH1 and MORPH2) are given in Appendix 1.

DNA sequence data

All sequence data reported in this study are new except those for *Apis mellifera*. Genomic DNA was isolated from ethanol-preserved adult females (minus the gaster) or, in a few instances, worker pupae. Specimens were dried, frozen at –80°C, and ground in lysis buffer. Extraction was performed by standard protocols from the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) or a CTAB-phenol extraction method (Hunt and Page 1994).

We obtained sequences from the nuclear ribosomal genes 18S and 28S using the primer combinations reported in Table 2. These nuclear genes encode the large subunit (28S) and small subunit (18S) of the ribosome. We sequenced a portion of 28S that encompasses the D1 and D2 expansion regions (Hancock *et al.* 1988), which have proven useful for inferring deep splits within other hymenopteran groups (e.g. Belshaw *et al.* 1998, 2000; Cameron and Mardulyn 2001). Likewise, fragments from 18S appear to robustly resolve Mesozoic-level divergences in taxa such as Lepidoptera (Wiegmann *et al.* 2000).

Amplification by polymerase chain reaction (PCR) typically consisted of 35–40 cycles of 1 min at 95°C, 1 min at 47–54°C, and 1 min 30 s at 72°C, with an initial denaturation step of 2 min at 95°C and a final extension step of 7 min at 72°C. A concentration of 1.5 mM of $MgCl_2$ was used in a final reaction volume of 25 μL. PCR product was purified either with Microcon 100 microconcentrators (Amicon, Danvers, MA, USA) or by the enzymatic method ExoSap-IT (USB, Cleveland, OH, USA). Automated fluorescent dye sequencing reactions were conducted on an ABI Prism 377 DNA Sequencer (Perkin Elmer, Wellesley, MA, USA). Both strands were sequenced for all taxa.

Table 2. Primers used for both PCR amplification and DNA sequencing of 18S and 28S gene fragments

Position denotes coordinates in the *Drosophila melanogaster* 18S or 28S gene, using the numbering of Tautz *et al.* (1988), as corrected by Linares *et al.* (1991). Primer combinations are as follows, with the forward primer listed first for each pair: rc18A–18N′, rc18H′–18 L, 28SA–28SC, Bel28S–revBel28S

Locus, primer	Sequence 5′ → 3′	Position	Reference
18S			
rc18A	TGGTTGATCCTGCCAGTAG	5–23	Wiegmann *et al.* (2000)
18N′	CACTCTAATTTKTTCAAAG	847–829	Wiegmann *et al.* (2000)
rc18H′	GCTGAAACTTAAAGGAATTGACGGAAGGGCAC	1215–1246	Wiegmann *et al.* (2000)
18L	CACCTACGGAAACCTTGTTACGACTT	1975–1950	Wiegmann *et al.* (2000)
28S			
28SA	CCCCCTGAATTTAAGCATAT	3318–3337	B. Sullender (personal communication)
28SC	CGGTTTCACGTACTCTTGAA	3692–3673	B. Sullender (personal communication)
Bel28S	AGAGAGAGTTCAAGAGTACGTG	3665–3686	Belshaw and Quicke (1997)
revBel28S	TTGGTCCGTGTTTCAAGACGGG	4068–4047	Belshaw and Quicke (1997)

Sequence chromatograms were assembled with Sequencher version 4.0.5 (GeneCodes Corporation, Ann Arbor, MI, USA). Gene sequences of the ants and vespid wasp were aligned with ClustalW version 1.74 (Thompson *et al.* 1994) with the default settings of a gap-opening penalty 10, and a gap-extension penalty 0.1 in pairwise and 0.05 in multiple alignments. The resulting alignment was modified by hand to correct a few obvious alignment errors. The 18S and 28S sequence data from *Apis mellifera* were subsequently aligned with the above ant and wasp sequences with the 'Profile Alignment Mode' of ClustalX version 1.8 (Thompson *et al.* 1997).

Nine ambiguously aligned regions were identified by visual inspection and removed from the analysis. We excluded any region where it was plausible – without considering taxon identities – to change the alignment in any way because of gaps. In these cases we eliminated all sequences on either side until a 'stable' region was achieved, almost always (17 out of 18 cases) in the form of a completely homogeneous nucleotide position. Most of these excluded regions were hypervariable, meaning that they contained a high proportion of indels (insertions or deletions). Although some useful information is undoubtedly discarded by this exclusion procedure (Lee 2001*a*), such a conservative approach reduces the chances of spurious homology assignment due to alignment errors in hypervariable regions (Lutzoni 1997; Lutzoni *et al.* 2000). Gaps in the non-excluded sequence were coded both as a 'fifth state' (Maddison 1993) and as 'missing'. Parsimony analyses were conducted under both procedures; results reported here are based on treatment of gaps as a 'fifth state' unless otherwise indicated. When considering the ant data alone, all gaps in the non-excluded sequence were single-base insertion or deletion events.

Uncorrected pairwise sequence divergence values were calculated by PAUP* version 4.0b8 (Swofford 2002). This program was also used to conduct a χ^2-test of base composition homogeneity across taxa. The DNA sequences have been deposited in GenBank under accession numbers AY218290–AY218353. A matrix containing the sequence alignment is available online from TreeBase (http://www.treebase.org; matrix accession number M1367).

Phylogenetic analyses

PAUP* version 4.0b8 (Swofford 2002) was used for all phylogenetic analyses unless otherwise stated. For morphological, molecular and combined data sets, we obtained the most parsimonious (MP) tree(s) using the branch-and-bound algorithm. For molecular data, we also employed maximum-likelihood (ML) and neighbour-joining (NJ) methods. We used MODELTEST version 3.06 (Posada and Crandell 1998) to initially estimate maximum-likelihood values under 56 different substitution models, which were then subjected to hierarchical likelihood ratio tests to determine the most appropriate model to be used in ML analysis (Posada and Crandell 2001). The selected model was TrNef+I+G, which employs two transition rates and one transversion rate, allows for both invariant sites and site-to-site rate variation, and assumes equal base frequencies. We used this model to estimate the ML tree by a successive iteration strategy (after Swofford *et al.* 1996; Maddison *et al.* 1999). An initial heuristic tree bisection–reconnection (TBR) search simultaneously estimated parameter values from the data and inferred an ML tree. These parameter values were fixed and additional TBR searches with multiple random-addition replicates were conducted in order to search for trees of higher likelihood. If a more likely tree resulted, parameter values were reoptimised and fixed on the new ML tree, and more searches were conducted. This procedure was repeated until the likelihood scores stabilised to arrive at a final ML tree. The NJ tree was computed with Kimura two-parameter distances (Kimura 1980) under the minimum evolution criterion.

Branch support was assessed using the nonparametric bootstrap (Felsenstein 1985) under the same search conditions described for MP and NJ above. For ML bootstrap runs, parameter values from the final ML tree were fixed for all ML bootstrap runs, and each replicate consisted of five random-addition TBR heuristic searches. In all cases, 1000 bootstrap replicates were conducted.

We used the incongruence length difference (ILD) (Mickevich and Farris 1981; Farris *et al.* 1995) to measure levels of heterogeneity among the 18S, 28S and MORPH2 data sets. However, we did not use this test to evaluate combinability of data, because of several arguments against this procedure (Baker *et al.* 2001; Yoder *et al.* 2001; Dowton and Austin 2002). Invariant and autapomorphic characters were removed before ILD analysis because of differences in the proportions of parsimony-informative characters among our data sets (Cunningham 1997; Lee 2001*b*). For each analysis we ran 1000 randomisation replicates using branch-and-bound searches.

We tested for the presence of rate consistency among lineages (i.e. a molecular clock) with a log-likelihood ratio test (Felsenstein 1988; Huelsenbeck and Rannala 1997). We then used a program provided by J. Thorne that implements the Bayesian method of Thorne *et al.* (1998) and Kishino *et al.* (2001) for estimating divergence times. This technique does not require a molecular clock, but instead employs a model that allows evolutionary rates between ancestor and descendant branches to change in an autocorrelated fashion. We used 120 million years ago (Mya) (± 20 million years s.d.) as the *a priori* date for the root node, and also constrained seven internal nodes with minimum ages based upon the fossil record (Table 3). The ML tree was the input tree for this dating analysis. The program uses a Markov chain Monte Carlo (MCMC) technique to evaluate the posterior distribution for divergence times of each node in the tree.

Phylogenetic relationships

Inferences from morphology

Analysis of the MORPH1 data set yielded seven MP trees (length 122, consistency index 0.61, retention index 0.67). The strict consensus of these trees is similar in topology to the bootstrap majority-rule consensus tree (Fig. 16) but slightly less resolved: Dolichoderinae and Formicinae do not form a clade and *Paraponera* is part of the basal polytomy. There is strong support for monophyly of the ingroup, that is, for a clade comprising *Myrmecia*, *Nothomyrmecia* and *Prionomyrmex* (97% bootstrap proportions), and for the genus *Prionomyrmex* itself (94%). A clade consisting of *Prionomyrmex* and *Nothomyrmecia* is also upheld (85%). Among the outgroups we obtain much less confident resolution of relationships. There are two well-supported clades, neither particularly novel: *Pseudomyrmex* + *Tetraponera* (99%) and a group comprising all ants except Sphecomyrminae (94%). The subfamilies Dolichoderinae and Formicinae appear together with less certainty (66%). We are unable to identify with confidence (high bootstrap support) the sister-group of the myrmeciine ants. On the basis of the bootstrap tree (Fig. 16), *Myrmecia* and relatives are part of a large polytomy that encompasses most of the outgroup taxa.

A similar picture emerges from the analysis of the MORPH2 data set, which excludes fossil taxa and subdivides other taxa to be more compatible with the molecular data set. Analysis of this second data set produced two MP trees (length 148, consistency index 0.55, retention

index 0.74). The strict consensus of these two trees shows greater resolution than was obtained with the MORPH1, but the bootstrap tree (Fig. 17) shows strong support for only a few groups: the extant myrmeciine genera (97% bootstrap proportions), genus *Myrmecia* (93%) and pairs of genera representing subfamilies whose monophyly has never been seriously questioned: Pseudomyrmecinae (represented by *Pseudomyrmex* and *Tetraponera*, at 98% bootstrap proportions), Dolichoderinae (*Liometopum* and *Linepithema*, 91%) and Formicinae (*Formica* and *Camponotus*, 89%). Beyond this, outgroup relationships are poorly resolved and the sister-group of the myrmeciines remains unclear.

DNA sequence characteristics

We obtained molecular data consisting of 1440 aligned nucleotide positions from the 18S gene and 951 positions from 28S. From this total we identified 194 ambiguously aligned positions (17 from 18S and 177 from 28S) and eliminated these from all analyses. Data from the two genes were combined to yield an overall data set of 2197 nucleotide positions, and this was used for all molecular phylogenetic analyses. Results from the ILD test did not reveal significant amounts of heterogeneity ($P = 0.07$) between the 18S and 28S data sets. The ILD test did indicate more-pronounced heterogeneity ($P = 0.01$) between the combined (18S+28S) data set and the morphological data set (MORPH2), although a standard 5% level of significance may be quite conservative for this test (Darlu and Lecointre 2002). When gaps in the sequence data were treated as a 'fifth state', there were 45 sites with parsimony-uninformative gap characters and 15 with parsimony-informative gap characters (Table 1).

Uncorrected pairwise sequence divergence values from the 28S data ranged from 0 to 2% between species of *Myrmecia*, 3 to 4% between *Myrmecia* species and *Nothomyrmecia*, 3 to 8% between ant subfamilies, and 11 to 16% between ants and the aculeate outgroups. Values from the 18S data were lower (as expected) and ranged from 0 to 2% within ants and 3 to 4% between ants and the other Aculeata. Observed mean base composition for the combined 18S+28S data set was distributed fairly evenly among the four bases (A, 23%; C, 26%; G, 29%; T, 22%). Between-species comparisons of base frequency showed that this base composition did not vary significantly among taxa ($\chi^2 = 10.634$, d.f. = 48, $P = 1.00$).

Molecular phylogenies

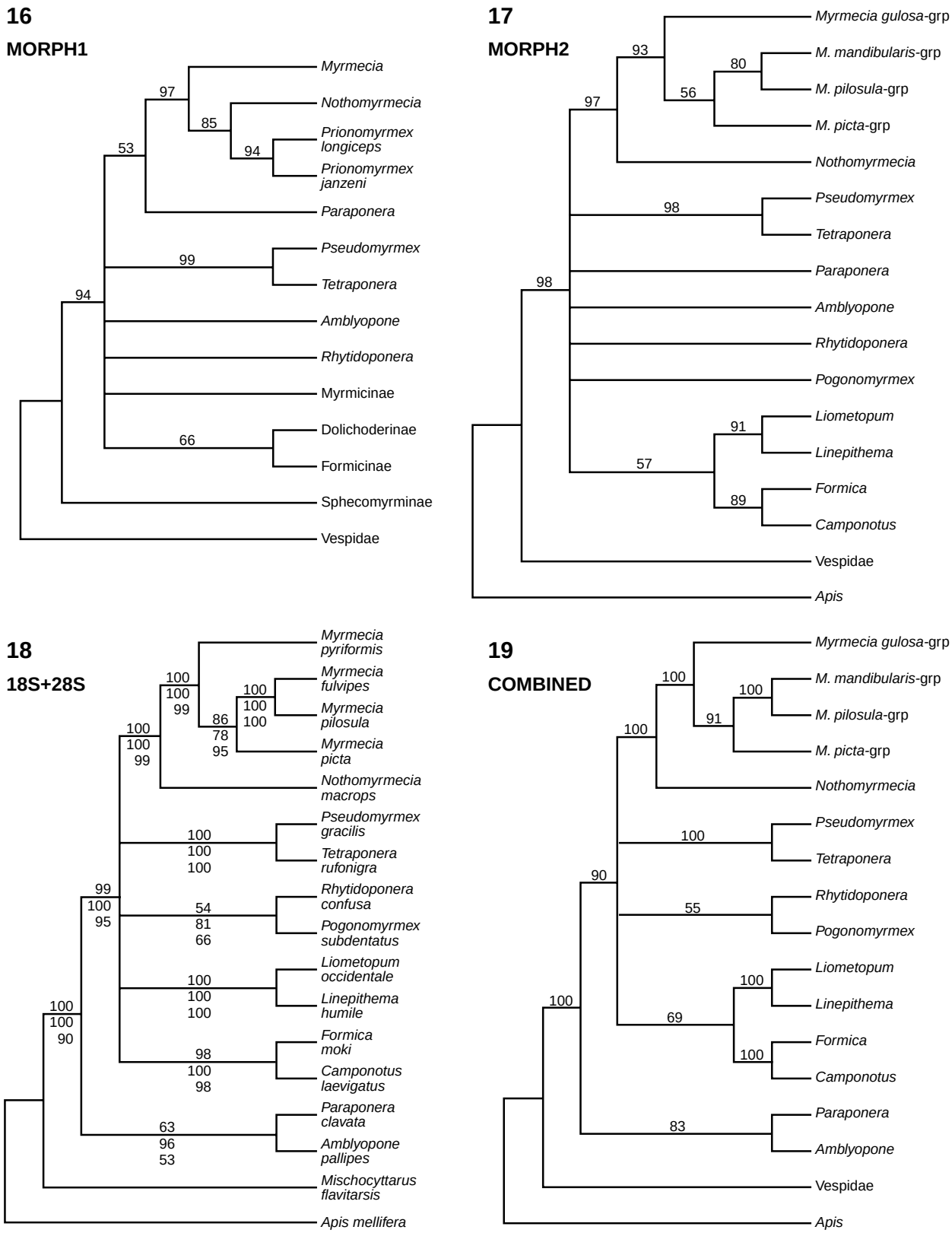
Analysis of the 18S+28S data set yielded a single MP tree (length 562, consistency index 0.77, retention index 0.68). The bootstrap majority-rule consensus tree (Fig. 18) is less resolved, however, and is similar to the result obtained with morphology (Fig. 18). There is 100% bootstrap support for monophyly of a group containing the extant myrmeciines (i.e. *Myrmecia* + *Nothomyrmecia*) and for the genus *Myrmecia* (represented by four species, each from a different species-group). There is also strong support for clusterings of outgroup taxa that belong to the same subfamily, with the exception of Ponerinae (*Pseudomyrmex* + *Tetraponera*, 100%; *Liometopum* + *Linepithema*, 100%; *Formica* + *Camponotus*, 98%). Two of the ponerine genera, *Amblyopone* and *Paraponera*, form a more weakly supported clade (63%), as do *Pogonomyrmex* and *Rhytidoponera* (54%). Most interesting of all is a well-supported group (99%) consisting of all ant taxa except *Amblyopone* and

Table 3. Estimated divergence times of the nodes in Fig. 20, based on Bayesian analysis
Also indicated are the minimum age constraints applied to seven nodes before the analysis, and the justification for these constraints

Node	Estimated age (Mya)	95% CL (Mya)	Minimum age (Mya)	Justification for minimum age constraint
1	99	82–126	52	*Leptothorax* (Myrmicinae) in Hat Ck amber (Poinar *et al.* 1999)
2	76	49–105	42	*Camponotus* and *Formica* in Baltic amber
3	54	42–78	42	*Tetraponera* in Baltic amber
4	65	44–93	42	*Liometopum* in Baltic amber
5	8	0–29		
6	32	10–64		
7	44	19–77		
8[A]	74	53–101	50	*Prionomyrmex* in Baltic amber
9	99	84–124	65	Dolichoderinae diverse in Sakhalin amber; possibly in Medicine Hat amber (Dlussky 1999*b*)
10	103	89–129		
11	106	93–134	92	*Kyromyrma* (Formicinae) in New Jersey amber (Grimaldi and Agosti 2000)
12	108	94–136		
13	112	84–145		
14[B]	130	107–166		

[A]Node 8 represents the myrmeciine ants.

[B]The root node (14) was assigned a prior date of 120 Mya (± 20 million years s.d.), after Grimaldi and Agosti (2000).



Figs 16–19. Bootstrap majority-rule consensus trees obtained by parsimony analysis of different data sets (MORPH1, MORPH2, 18S+28S and COMBINED). The three bootstrap values given for the 18S+28S tree are the results obtained with parsimony (above branch) and neighbour-joining and maximum-likelihood analyses (below branch).

Paraponera. Strong evidence for this clade did not emerge from analysis of the morphological data sets, although it does not conflict with any well-supported groups appearing in the morphological trees (Figs 16, 17). As for morphology, the sequence data do not identify unambiguously the sister-group of *Myrmecia* + *Nothomyrmecia*. The foregoing results were obtained with gaps coded as 'fifth state'. Coding gaps as 'missing data' yielded the same tree topology with similar bootstrap proportions.

The ML and NJ bootstrap results are virtually identical to those obtained with parsimony (Fig. 18). In particular, support remains high for *Myrmecia* + *Nothomyrmecia*, for most subfamily pairings, and for a clade containing all examined ants except *Amblyopone* and *Paraponera*. Similar to the case with parsimony, neither the ML nor the NJ trees resolve the sister-group of (*Myrmecia* + *Nothomyrmecia*) with high bootstrap support.

The optimal ML tree (Fig. 20) was achieved after the first round of searches; additional random-addition replicates with fixed parameter values did not result in a lower likelihood score [–ln(L) = 5837.58]. This tree does not contradict any clades in the parsimony bootstrap tree (Fig. 18). Some internal branches on the ML tree are very short, especially those connecting exemplars from different subfamilies. Using a log-likelihood ratio (LR) test, we rejected the hypothesis of constant rates among lineages (–2ln(LR) = 46.21, d.f. = 15, $P < 0.005$), indicating the absence of a tree-wide molecular clock.

We used Bayesian dating analysis (Thorne *et al.* 1998; Kishino *et al.* 2001), which does not assume rate constancy, to infer divergence dates for nodes on the ML tree (Table 3). The estimated age of the most recent common ancestor of the extant myrmeciines is 74 million years (95% confidence limits 53–101 million years). Inferred dates for other nodes are reported in Table 3. These dates remained stable after multiple MCMC searches, each starting from different random states. In addition, changes to prior values of rate parameters resulted in only minor fluctuations (results not shown). We also explored the sensitivity of these results to changes in the prior probability distribution assigned to the age of the root node. Varying the prior age between 110 (± 20) and 140 (± 40) Mya changed the estimated age of the myrmeciine lineage by only 1–7 million years (Table 4). Not surprisingly, the posterior probability of the root node (i.e. the estimated age of the most recent common ancestor of all ants) is more strongly affected by changes in the prior.

Combined analysis

A comparison of the bootstrap consensus trees obtained from analyses of the two data sets (Figs 17, 18) does not reveal strong disagreement. Although there are a few clades that appear in only one of the two consensus trees, there are no instances in which such clades conflict with groups appearing in the other tree. Thus, any disagreement between the two data sets involves groups that have very little support (bootstrap proportions <50%). Consequently a combined treatment of the data seems merited.

Analysis of the COMBINED (morphological and molecular) data set produced two MP trees (length 719, consistency index 0.72, retention index 0.69). The bootstrap tree (Fig. 19) is consistent with those derived from the individual data sets (Figs 17, 18). There continues to be strong support for the monophyly of (*1*) the genus *Myrmecia*, (*2*) a clade containing *Myrmecia* and *Nothomyrmecia*, (*3*) each of the outgroup subfamilies Pseudomyrmecinae, Formicinae and Dolichoderinae, and (*4*) the family Formicidae (100% bootstrap support in all instances). Paraphyly of the Ponerinae is also strongly upheld (90% bootstrap support), such that a

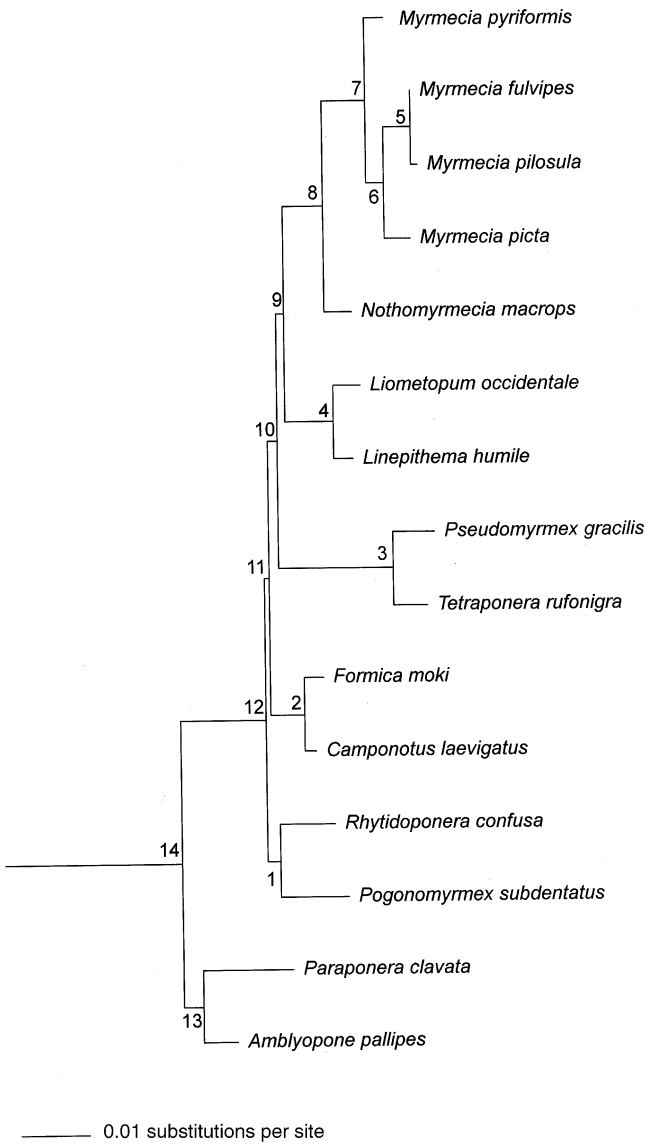


Fig. 20. Maximum-likelihood tree from 18S+28S sequence data, with vespoid outgroups removed. Numbers refer to the nodes, whose estimated divergence times are given in Table 3.

Table 4. Effects of assigning different prior probability distributions to the age of the root node on the estimated divergence times of the myrmeciine node and the root node

Prior on root node (mean ± s.d.) (Mya)	Estimated age of myrmeciine node (Mya)	95% CL (Mya)	Estimated age of root node (Mya)	95% CL (Mya)
110 ± 20	73	53–98	126	105–158
120 ± 20	74	53–101	130	107–166
120 ± 40	78	54–114	143	107–207
140 ± 40	80	54–119	149	109–215

clade consisting of *Paraponera* and *Amblyopone* (with 83% bootstrap support) is sister to the other extant ant taxa in this study. The sister-group of the myrmeciines remains uncertain: the combined morphological and molecular data do not confidently resolve the polytomy consisting of myrmeciines, Pseudomyrmecinae, Dolichoderinae, Formicinae, Myrmicinae and at least one ponerine genus (*Rhytidoponera*). There is some support for a clade consisting of Dolichoderinae and Formicinae (69% bootstrap support), and a weak indication of a sister-group relationship between *Rhytidoponera* (Ponerinae : Ectatommini) and *Pogonomyrmex* (Myrmicinae) (55%).

Although not a focus of our study, relationships within the genus *Myrmecia* are similarly resolved by both the molecular and morphological data sets, and by the combined data. Among the four species-groups represented in our data, all analyses yield the following arrangement, with moderate to high bootstrap support for all clades: (*gulosa*-group + (*picta*-group + (*pilosula*-group + *mandibularis*-group))). Ogata (1991) delimited nine species-groups in *Myrmecia* and analysed relationships among them, using worker morphology. Our findings agree with the results that he obtained (his fig. 53) when he used both *Nothomyrmecia* and Pseudomyrmecinae as outgroups.

Reclassification of the Myrmeciinae

Subfamily classification

The results given here establish with high certainty the monophyly of a group containing *Myrmecia*, *Nothomyrmecia* and *Prionomyrmex*. In the interests of informativeness and utility, we suggest that all three genera should be placed in a single, expanded subfamily Myrmeciinae. To reflect current phylogenetic knowledge, we propose the recognition of two tribes within the subfamily: Prionomyrmecini, containing *Prionomyrmex* and *Nothomyrmecia*; and a monotypic Myrmeciini (comprising *Myrmecia* alone). The Argentine fossils *Ameghinoia* and *Polanskiella* are treated as *incertae sedis* in Myrmeciinae, for reasons given below (under 'Status of South American fossil taxa'). The proposed new classification of the subfamily is as follows:

Subfamily Myrmeciinae Emery, 1877
- Tribe Myrmeciini Emery, 1877
  - Genus *Myrmecia* Fabricius, 1804
    - = *Promyrmecia* Emery, 1911 (synonymy by Brown 1953*a*)
    - = *Pristomyrmecia* Emery, 1911 (synonymy, under *Promyrmecia*, by Clark 1943)
    - = *Halmamyrmecia* Wheeler, 1922 (synonymy, under *Promyrmecia*, by Clark 1943)
- Tribe Prionomyrmecini Wheeler, 1915
  - = Nothomyrmecii Clark, 1934 (synonymy by Baroni Urbani 2000)
  - Genus *Prionomyrmex* Mayr, 1868
  - Genus *Nothomyrmecia* Clark, 1934 **stat. rev.**
- Genus *Ameghinoia* Viana & Haedo Rossi, 1957 *incertae sedis*
- Genus *Polanskiella* Rossi de Garcia, 1983 *incertae sedis*

Resurrection of the genus Nothomyrmecia

Nothomyrmecia was recently synonymised under *Prionomyrmex* (Baroni Urbani 2000), an action justified by two arguments. First, it was claimed that *Prionomyrmex* is paraphyletic relative to *Nothomyrmecia*. Second, it was argued that the two taxa are so similar that their placement in the same genus would be uncontested were it not for their disjunct spatiotemporal distribution. Neither argument withstands scrutiny.

In the current study we provide strong evidence for the monophyly of *Prionomyrmex* (94% bootstrap support), even stronger than that indicating a sister-group relationship between *Prionomyrmex* and *Nothomyrmecia* (85%) (Fig. 16). In fact, the 'paraphyly argument' can be turned on its head: there is a possibility that a taxon formed by the union of *Prionomyrmex* and *Nothomyrmecia* would itself be paraphyletic since there are South American fossil taxa to which *Nothomyrmecia* may be more closely related (see below).

Also, substantial morphological differences exist between *Nothomyrmecia* and *Prionomyrmex*, providing adequate justification for keeping them as separate genera by the standards typically applied in ant taxonomy. Perhaps the most striking difference between the two taxa involves the presence (*Prionomyrmex*; Fig. 2) and absence (*Nothomyrmecia*; Fig. 1) of a postpetiole. In *Nothomyrmecia*, there is not even an obvious constriction between abdominal segments 3 and 4, as is the case in those few ant genera (e.g. *Cerapachys* F. Smith, *Proceratium* Roger) that could be considered polymorphic for the presence/absence of a postpetiole.

Other notable differences between *Nothomyrmecia* and *Prionomyrmex* include features of the head, mesosoma, petiole and abdominal sternite 3 (Table 5). Differences extend to both sexes. Moreover, for some characters the

apparent apomorphic state occurs in *Prionomyrmex* (e.g. presence of postpetiole, propodeal teeth, and acuminate clypeal margin), whereas in other instances *Nothomyrmecia* has the derived condition (e.g. loss of notauli in the male; presence of longitudinal keel on sternite 3).

Diagnosis of subfamily Myrmeciinae

This diagnosis is based on features observed in *Myrmecia*, *Nothomyrmecia* and *Prionomyrmex*. Putative apomorphic conditions are indicated with an asterisk (*).

1. *Worker, queen. Mandibles multidentate and elongate, such that mandible length is three-quarters or more of head length.
2. Worker, queen, male. Palp formula 6, 4 (possibly reduced in *Prionomyrmex janzeni*).
3. Worker and queen. Clypeus with posteromesial protrusion between frontal carinae and antennal sockets.
4. *Worker, queen. Eyes large, oval and conspicuously protruding from the surface of the head.
5. *Male. First antennal segment (scape) very short and stocky, such that SL/(LF1 + LF2 + LF3) ~ 0.20.
6. Worker and queen. First funicular segment less than, or approximately equal to, second funicular segment in length (LF1/LF2 = 0.65–1.15).
7. Worker and queen. Second funicular segment slender (more than twice as long as wide; LF2/WF2 > 2.00) and notably longer than the third funicular segment (LF2/LF3 ≥ 1.10).
8. Worker. Promesonotal suture flexible.
9. *Male. Mesepisternum lacking distinct posterior oblique sulcus, at most a weak furrow present (condition in *Prionomyrmex* unclear).
10. Worker, queen, male. Metacoxal cavities open (condition in *Prionomyrmex* unknown).
11. *Worker, queen. Metapleural gland opening flanked above by carina-like flange and separated from ventral margin of the metapleuron by a distance greater than the diameter of the opening (condition in *Prionomyrmex* unknown).
12. Worker, queen, male. Hind tibia with two apical spurs, the posterior one usually pectinate.
13. Worker, queen. Basitarsi of mid and hind legs with longitudinal sulcus.
14. Worker, queen, male. Tarsal claws bifurcate, with submedian tooth in addition to apical tooth.
15. Queen, male. Forewing Cu and M veins diverging at, or near, the cu-a crossvein.
16. Queen, male. Forewing crossvein m-cu joining M distad of the divergence between M and Rs.
17. Queen, male. Forewing with two submarginal cells (cells 1R1 and 1Rs, in the terminology of Goulet and Huber 1993).
18. Queen, male. Hind wing jugal lobe present.
19. Worker, queen. Petiole, postpetiole and abdominal segment 4 lacking tergosternal fusion (some fusion occurs anteriorly in the petiole of *Nothomyrmecia*).
20. *Worker, queen. Third abdominal segment substantially smaller than fourth abdominal segment such that, when observed in profile under normal gastral distension, the height of third abdominal segment distinctly less than that of the fourth abdominal segment (≤ 0.80×).
21. Worker, queen. Sting well developed (there is some reduction in *Nothomyrmecia* compared with *Myrmecia*: see Kugler 1980).
22. *Male. Paramere divided by an oblique longitudinal suture into (*i*) an apical and ventromesial portion, to which the volsella is attached, and (*ii*) a proximal and dorsolateral portion (condition in *Prionomyrmex* unknown).

Table 5. Major differences between *Nothomyrmecia* and *Prionomyrmex*

The hypothesised apomorphic state is italicised. w, worker; m, male. All the worker characters listed here for *Nothomyrmecia* are similarly expressed in the queens, but the queen caste is unknown for *Prionomyrmex*

Character	*Nothomyrmecia*	*Prionomyrmex*
Postpetiole (w, m)	Absent	*Present*
Propodeal spines (w, m)	Absent	*Present*
Anterior petiolar peduncle (w, m)	*Long*	Short
Notauli (m)	*Absent*	Present
Longitudinal keel on sternite 3 (w)	*Present*	Absent
Posterior margin of sternite 3 (w, m)	*Sinuous*, *protruding*	Simple
Anterior clypeal margin (w)	Rounded	*Acuminate*
Scape length (w)	Short (SL/HW ~ 1.10)[A]	*Long* (*SL/HW ~ 1.25*)[A]
Relative lengths of first and second funicular segments (w)	*LF1/LF2 ~ 1.04*	LF1/LF2 ~ 0.67
Relative lengths of first and third funicular segments (w)	*LF1/LF3 ~ 1.15*	LF1/LF3 ~ 0.76

[A]This difference is more apparent when using an index [SL/(0.5HW + 1.2)] designed to separate groups of *Myrmecia* species with long and short scapes (see Ogata 1991: 364). For *Nothomyrmecia* workers, this index is ~0.76, for *Prionomyrmex* ~1.12.

23. *Male. Aedeagus with posteroventral projection, armed at the apex with stout teeth or spines (condition in *Prionomyrmex* unknown).

In addition, the workers of *Nothomyrmecia* and *Myrmecia* possess a unique gland, the sting bulb gland (Billen 1990*b*); they exhibit similar ultrastructure of the Dufour's gland (Billen 1988); and the immature stages of the two genera are very similar (Wheeler *et al.* 1980). The glandular characters are evidently apomorphic, but the larval similarities may be plesiomorphic.

The 18S and 28S sequences contain six sites at which specific nucleotides appear to be uniquely shared by *Myrmecia* and *Nothomyrmecia*. All other examined taxa, including *Mischocyttarus* and *Apis*, are fixed for an alternative base (at positions 549, 1642, 1646, 1651 and 2244) or a gap (position 2202). Position numbers are those of our aligned sequence data (TreeBase accession number M1367).

Diagnosis of tribe Prionomyrmecini

The newly defined tribe Prionomyrmecini can be distinguished from tribe Myrmeciini (and hence genus *Myrmecia*) by the following worker- and queen-based features.

1. *Masticatory margins of the closed mandibles meeting along most of their length but not broadly overlapping, and forming a tight closure with the anteromedially protruding clypeus.
2. *Stout setiform cuticular projections on masticatory margin of mandible.
3. *Lateral clypeal carina present.
4. Compound eye separated from base of mandible by an extensive malar area.
5. Clypeo-labral connection, in frontal view, concealed by overhanging clypeus.
6. *Worker ocelli reduced or absent.

Status of South American fossil taxa

Ameghinoia piatnitzkyi Viana & Haedo Rossi was described as a fossil formicid on the basis of three alate females from Argentine deposits now assigned to the Ventana formation (Eocene/early Oligocene) (Viana and Haedo Rossi 1957; Petrulevicius 1999). *Ameghinoia* was originally placed in the subfamily Ponerinae and later transferred to Myrmeciinae (Brown and Taylor 1970). Still later, Rossi de García (1983) described another similar formicid, *Polanskiella smekali*, from 11 female specimens from the same locality and geological formation as *Ameghinoia*. *Polanskiella* has been almost entirely overlooked in the myrmecological literature. We have not examined the fossils but we find enough information in the original descriptions and illustrations to support placement of these taxa in Myrmeciinae, as follows. (*1*) The mandibles are large, elongate and multidentate. (*2*) The compound eye appears to be large and convex (Viana and Haedo Rossi 1957: plate figs 1–2; Rossi de García 1983: fig. 1). (*3*) The third abdominal segment is markedly smaller than the fourth. The ratio of (height of third abdominal segment)/(height of fourth abdominal segment) is about 0.5 and 0.6 in *Polanskiella* and *Ameghinoia*, respectively, while the corresponding width ratio in *Ameghinoia* is about 0.5. (*4*) A postpetiole is developed, that is, the third segment is not only much smaller than the fourth but also separated from it by a marked constriction (see especially Viana and Haedo Rossi 1957: plate fig. 3). (*5*) The overall habitus matches that of other myrmeciines, including the rather large size (2–3 cm in length), long appendages, and evenly rounded profile of the propodeum.

Various features point to the exclusion of *Ameghinoia* and *Polanskiella* from other postpetiolate ant groups such as Myrmicinae and Pseudomyrmecinae. For example, veins M and Cu diverge opposite or near the cu-a crossvein (as in *Myrmecia* and *Nothomyrmecia*), not markedly distal to the crossvein as in the aforementioned subfamilies (Brown and Nutting 1950). Moreover, in *Ameghinoia* and *Polanskiella* the m-cu crossvein joins M distad of its divergence with Rs, giving the discal (first medial) cell five sides, a feature almost never seen in Myrmicinae (Brown and Nutting 1950). That *Ameghinoia* and *Polanskiella* are not members of the subfamily Pseudomyrmecinae is indicated not only by the proximal divergence of the median and cubital veins but also by the elongate mandibles and elongate scapes, with the latter surpassing the posterior margin of the head (Viana and Haedo Rossi 1957: plate figs 1–2; Rossi de García 1983: fig. 2).

In the original description of *Polanskiella*, it is distinguished from *Ameghinoia* principally by differences in forewing venation, specifically the shape of the two submarginal cells and the discal cell (Rossi de García 1983). Yet a figure of *Polanskiella* (Rossi de García 1983: fig. 5) appears to belie these distinctions: it shows venation more like that of *Ameghinoia*. For example, the discal cell is elongate, not short and pentagonal as claimed for *Polanskiella*. Some additional reported characteristics of the two taxa require confirmation, including the apparent lack of tibial spurs in *Ameghinoia* (Viana and Haedo Rossi 1957) and the putative 8-segmented antenna of *Polanskiella* (Rossi de García 1983). It seems quite likely that *Polanskiella* will prove to be a junior synonym of *Ameghinoia*, although this issue cannot be definitively resolved without additional study.

To test the hypothesis that these two fossil taxa belong in the Myrmeciinae, we treated *Ameghinoia* and *Polanskiella* as a composite taxon and evaluated '*Ameghinoia*/*Polanskiella*' for as many characters as possible from data set MORPH1. We were able to score 17 of 74 characters, with the remaining characters being coded as unknown (Appendix 1). Despite the potential for instability introduced by the addition of a taxon with so many unknown states (Huelsenbeck 1991; Nixon

1996), the results of a parsimony analysis place '*Ameghinoia*/*Polanskiella*' firmly in Myrmeciinae, with 92% bootstrap support (Fig. 21). The South American fossils are further nested within Myrmeciinae as part of a weakly supported clade (67%) that also contains *Nothomyrmecia* and *Prionomyrmex*. In all seven of the MP trees (length 122, consistency index 0.61, retention index 0.68), *Nothomyrmecia* is the sister-group of '*Ameghinoia*/ *Polanskiella*', although this receives little bootstrap support (54%).

Most of the diagnostic characters of the Prionomyrmecini (listed above) cannot be assessed in the South American fossils. Given this state of uncertainty, it seems preferable to classify *Ameghinoia* and *Polanskiella* as *incertae sedis* in Myrmeciinae until additional material can be studied. The preceding analysis suggests the likelihood, however, that the South American taxa are more closely related to *Nothomyrmecia* than to *Myrmecia*.

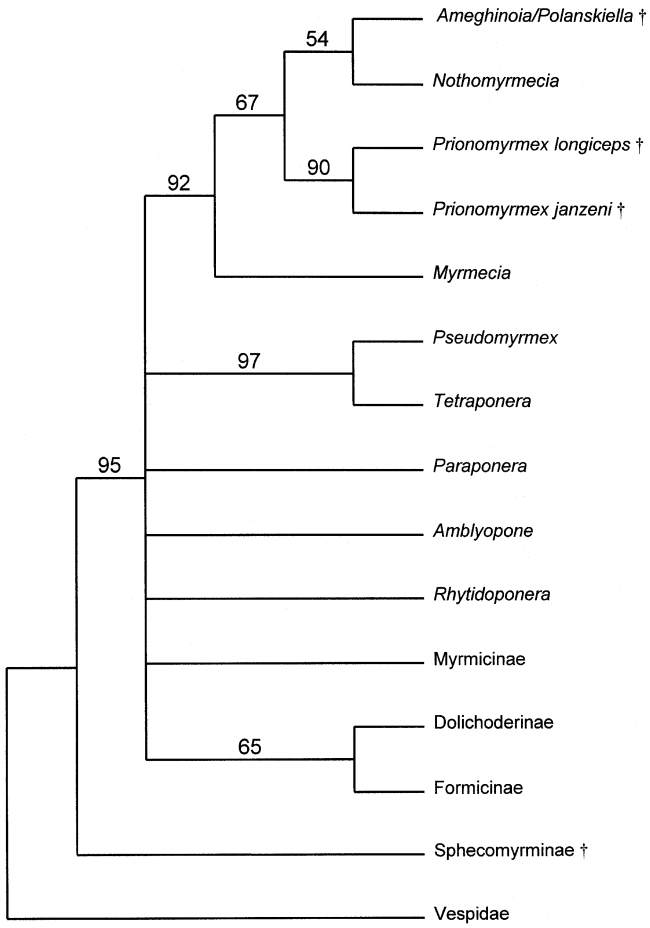


Fig. 21. Estimated phylogeny of myrmeciine ants, including the South American fossil taxa *Ameghinoia* and *Polanskiella*, based on morphology (MORPH1 data set). This is the bootstrap consensus tree; it is identical in topology to the strict consensus of the seven MP trees except that (Dolichoderinae + Formicinae) collapses in the latter. † = Extinct taxon.

Discussion

Biogeographic implications

These results highlight the fact that myrmeciine ants were previously more widespread than their current distribution would indicate, being represented by fossil taxa in South America and Europe and by extant species in the Australian region. Given the restriction of most records to the Southern Hemisphere, this suggests the hypothesis that the Myrmeciinae arose in Gondwana in the late Mesozoic, were isolated on different southern continents by plate tectonics, and subsequently dispersed across the Tethys Sea to Europe in the early Tertiary. Our estimate of the age of the most recent common ancestor of the group (between 53 and 101 million years, on the basis of molecular sequence data) is consistent with this scenario, as is the apparent absence of fossil myrmeciines from other parts of the Northern Hemisphere, despite a reasonably extensive fossil record (e.g. Carpenter 1930; Wilson 1985, 1988; Dlussky 1983, 1987, 1988, 1996, 1999*b*). Plotting the phylogeny of the Myrmeciinae – including *Ameghinoia*/*Polanskiella* – on a map of the world (Fig. 22) further emphasises a southern origin and indicates that myrmeciines probably also had a foothold in Africa in the late Cretaceous or early Tertiary. In this context, the absence of fossil myrmeciines from the Afrotropical region has little significance, given that there are almost no records of any fossil ants from this part of the world.

Biological studies of *Myrmecia* and *Nothomyrmecia* indicate that the workers forage solitarily, do not recruit nestmates to food sources, do not use trail pheromones, and (with some exceptions in *Myrmecia*) seldom engage in trophallaxis (Haskins and Haskins 1951; Freeland 1958; Hölldobler and Taylor 1984; Jaisson *et al.* 1992). These ants rely largely on visual and tactile cues for prey capture, and their chemical communication systems are notably less sophisticated than those of most other ants (Hölldobler and Wilson 1990). In light of these features it is perhaps not surprising that myrmeciine ants have gone extinct everywhere except on the relatively isolated landmasses of Australia and New Caledonia. Even within Australia these ants show several hallmarks of 'relict' taxa (Brown 1953*b*).

For example, both species richness and abundance of *Myrmecia* are greatest in the southern third of the Australian continent (Ogata and Taylor 1991; Shattuck 1999), and even within this southern heartland many species are rare and localised (Clark 1951; Brown 1953*b*). The factors responsible for the sharp attenuation of diversity and abundance in northern Australia are unclear. One possibility is that *Myrmecia* ants are intrinsically ill-adapted to tropical climates, but another contributing factor could be the presence of competitively aggressive ants that entered northern Australia relatively recently (*c.* 20 Mya) as the Australian plate came into close proximity with South-East Asia (Hall 1998). One such probable latecomer is the weaver

ant genus *Oecophylla*, and Brown (1953*b*: 11) provides circumstantial evidence that some of the *Myrmecia* species in north Queensland rainforest are adversely affected by the presence of *Oecophylla*. *Nothomyrmecia macrops* has an even more restricted distribution, being confined to a particular woodland habitat that occurs as a thin fringe along the southern margin of the Australian continent (Taylor 1978; Watts *et al.* 1998).

Relationship of Myrmeciinae to other ants

Most ant species fall into a few well-defined clades, corresponding largely to currently recognised subfamilies (Ward 1990; Baroni Urbani *et al.* 1992; Shattuck 1992*a*; Grimaldi *et al.* 1997), with the notable exception of the Ponerinae (see below). Our study contained exemplars from most of these clades, in order to include all potential close relatives of Myrmeciinae. Notwithstanding this broad coverage and the use of large morphological and molecular data sets (Table 1), we have been unable to identify confidently the sister-group of these ants. Components of the unresolved polytomy include Pseudomyrmecinae, Dolichoderinae, Formicinae, Myrmicinae and the ponerine tribe Ectatommini, in addition to the Myrmeciinae (Fig. 19). The Bayesian estimates of the divergence times of these groups (i.e. the estimated ages of nodes 1 and 9–12 in Fig. 20) are all quite close (99–108 Mya), and have broadly overlapping confidence limits (Table 3). Thus, resolving the relationships among these taxa with high certitude may require substantial additional data.

In contrast, our molecular and combined data sets place two ponerine taxa (*Amblyopone*, *Paraponera*) outside this polytomous grouping, with strong (90–100%) bootstrap support. This result is consistent with previous work suggesting ponerine paraphyly (Hashimoto 1991, 1996; Ward 1994; Grimaldi *et al.* 1997; Sullender and Johnson 1998). It will be interesting to see how our understanding of ponerine relationships develops under the scrutiny of additional characters and taxa.

The findings of the current study with respect to the higher phylogeny of the Formicidae are summarised in Fig. 23. This tree is based on those clades that received more than 50% bootstrap support in either the morphological or molecular analyses. We feel that this schema more faithfully represents the current state of knowledge than the more 'resolved' ant phylogenies appearing in recent papers, in which basal relationships are very poorly supported (Baroni Urbani *et al.* 1992; Grimaldi *et al.* 1997; Grimaldi and Agosti 2000). In conjunction with estimated divergence times (Table 3), Fig. 23 highlights the hypothesis that the immediate ancestors of the Myrmeciinae and most other ant subfamilies arose rather suddenly about 100 Mya. In addition, if the tree has been properly rooted, it implicates an early Cretaceous origin of ants (cf. Crozier *et al.* 1997; Rust and Andersen 1999; Lattke in press). The fossil record has not yielded definitive ants (Formicidae) from this period (Grimaldi and Agosti 2000), but the existence of two genera of stem-group formicoids (Armaniidae) in the lower Cretaceous (Dlussky 1999*a*) adds to the plausibility of crown-group origin at that time. These Cretaceous origins can be contrasted with the apparent delay until the early Tertiary of the attainment of high species diversity and behavioural dominance by ants (Grimaldi and Agosti 2000).

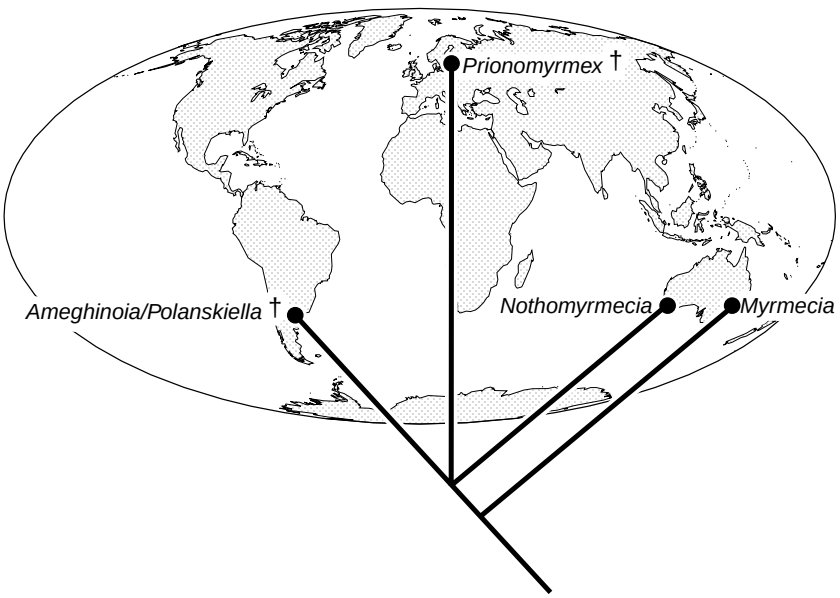


Fig. 22. Phylogeny of the Myrmeciinae plotted on a map of the world. The relationship among *Nothomyrmecia*, *Prionomyrmex* and *Ameghinoia*/*Polanskiella* has been depicted as a trichotomy because there is not strong evidence to resolve this (see Fig. 21). † = Extinct taxon.

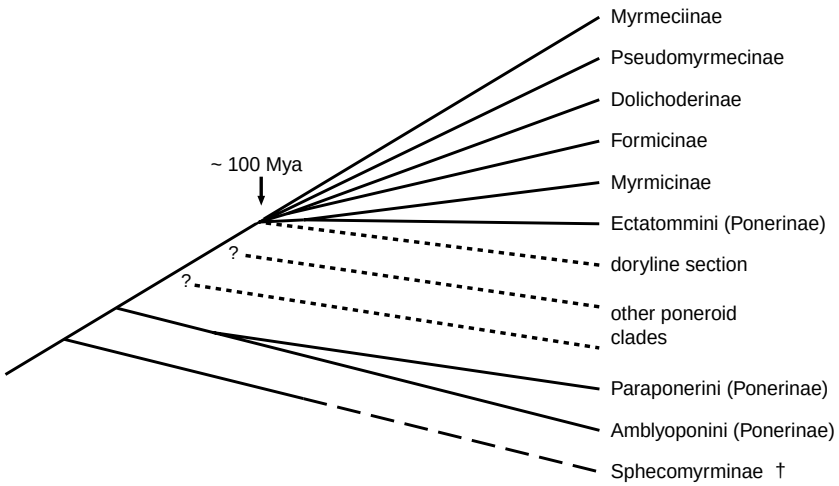


Fig. 23. Hypothesis of relationships and relative ages of the major lineages of ants, based on morphological and molecular evidence. Groups subtended by dotted lines were not directly considered in this study. 'Other poneroid clades' include Apomyrminae, Leptanillinae and the remaining tribes of Ponerinae. Excluded from the figure are the subfamilies Aneuretinae (the probable sister-group of Dolichoderinae; see Billen 1990*a*; Baroni Urbani *et al.* 1992) and the Formiciinae (Lutz, 1986), an extinct group of uncertain phylogenetic affinity. † = Extinct taxon.

Notwithstanding the 'bushiness' of parts of the ant tree, our data provide strong evidence that *Myrmecia* and *Nothomyrmecia* form a clade to the exclusion of all other extant ants. It is notable, however, that the myrmeciines are not the sister-group of all other ants, despite their apparent retention of many archaic biological traits. This points to the likely convergent evolution of many derived features of social life in ants.

Acknowledgments

For provision of specimens we thank Steve Shattuck (CSIRO Entomology, Canberra, Australia) and Jürgen Gadau (Universität Würzburg, Germany). We are also grateful to Bryan Danforth, John Huelsenbeck and Jeff Thorne for providing computer resources and software assistance, and to Ross Crozier, Bryan Danforth, Brian Fisher, John Lattke, Ted Schultz, Steve Shattuck and two anonymous reviewers for helpful comments on the manuscript. This research was supported in part by NSF grants DEB-9903650 to Philip Ward and INT-9904233 to Seán Brady.

References

Agosti, D. (1991). Revision of the oriental ant genus *Cladomyrma*, with an outline of the higher classification of the Formicinae (Hymenoptera: Formicidae). *Systematic Entomology* **16**, 293–310.

Attygalle, A. B., and Morgan, E. D. (1984). Chemicals from the glands of ants. *Chemical Society Reviews* **13**, 245–278.

Attygalle, A. B., Billen, J. P. J., Jackson, B. D., and Morgan, E. D. (1990). Morphology and chemical contents of Dufour glands of *Pseudomyrmex* ants (Hymenoptera: Formicidae). *Zeitschrift für Naturforschung C*, *Journal of Biosciences* **45**, 691–697.

Baker, R. H., Wilkinson, G. S., and DeSalle, R. (2001). Phylogenetic utility of different types of molecular data used to infer evolutionary relationships among stalk-eyed flies (Diopsidae). *Systematic Biology* **50**, 87–105.

Baroni Urbani, C. (2000). Rediscovery of the Baltic amber ant genus *Prionomyrmex* (Hymenoptera, Formicidae) and its taxonomic consequences. *Ecologae Geologicae Helvetiae* **93**, 471–480.

Baroni Urbani, C., Bolton, B., and Ward, P. S. (1992). The internal phylogeny of ants (Hymenoptera: Formicidae). *Systematic Entomology* **17**, 301–329.

Belshaw, R., and Quicke, D. L. J. (1997). A molecular phylogeny of the Aphidiinae (Hymenoptera: Braconidae). *Molecular Phylogenetics and Evolution* **7**, 281–293.

Belshaw, R., Fitton, M., Herniou, E., Gimeno, C., and Quicke, D. L. J. (1998). A phylogenetic reconstruction of the Ichneumonoidea (Hymenoptera) based on the D2 variable region of 28S ribosomal RNA. *Systematic Entomology* **23**, 109–123.

Belshaw, R., Dowton, M., Quicke, D. L. J., and Austin, A. D. (2000). Estimating ancestral geographical distributions: a Gondwanan origin for aphid parasitoids? *Proceedings of the Royal Society of London, Series B* **267**, 491–496.

Billen, J. P. J. (1986). Comparative morphology and ultrastructure of the Dufour gland in ants (Hymenoptera: Formicidae). *Entomologia Generalis* **11**, 165–181.

Billen, J. (1987). Morphology and ultrastructure of the abdominal glands in dolichoderine ants (Hymenoptera, Formicidae). *Insectes Sociaux* **33**, 278–295.

Billen, J. (1988). Comparaison entre les fourmis australiennes des genres *Myrmecia* et *Nothomyrmecia*. *Actes des Colloques Insectes Sociaux* **4**, 27–33.

Billen, J. (1990*a*). Phylogenetic aspects of exocrine gland development in the Formicidae. In 'Social Insects and the Environment. Proceedings of the 11th International Congress of IUSSI, 1990'. (Eds G. K. Veeresh, B. Mallik and C. A. Viraktamath.) pp. 317–318. (Oxford and IBH: New Delhi, India.)

Billen, J. (1990*b*). The sting bulb gland in *Myrmecia* and *Nothomyrmecia* (Hymenoptera: Formicidae): a new exocrine gland in ants. *International Journal of Insect Morphology and Embryology* **19**, 133–139.

Billen, J. P. J., Jackson, B. D., and Morgan, E. D. (1988). Secretion of the Dufour gland of the ant *Nothomyrmecia macrops* (Hymenoptera: Formicidae). *Experientia (Basel)* **44**, 715–719.

Billen, J., Ito, F., and Peeters, C. (2000). Novel exocrine glands in the hindleg tarsi of the ant *Nothomyrmecia macrops*. *Australian Journal of Zoology* **48**, 661–667.

Bolton, B. (1990*a*). The higher classification of the ant subfamily Leptanillinae (Hymenoptera: Formicidae). *Systematic Entomology* **15**, 267–282.

Bolton, B. (1990*b*). Army ants reassessed: the phylogeny and classification of the doryline section (Hymenoptera, Formicidae). *Journal of Natural History* **24**, 1339–1364.

Bolton, B. (1994). 'Identification Guide to the Ant Genera of the World.' (Harvard University Press: Cambridge, MA, USA.)

Bolton, B. (1995). 'A New General Catalogue of the Ants of the World.' (Harvard University Press: Cambridge, MA, USA.)

Bolton, B. (1998). Monophyly of the dacetonine tribe-group and its component tribes (Hymenoptera: Formicidae). *Bulletin of the Natural History Museum, Entomology Series* **67**, 65–78.

Bolton, B. (2000). The ant tribe Dacetini. *Memoirs of the American Entomological Institute* **65**, 1–1028.

Brandão, C. R. F., and Martins-Neto, R. G. (1990). [Untitled. *Cariridris* Brandão & Martins-Neto, new genus. *Cariridris bipetiolata* Brandão & Martins-Neto, new species.] pp. 201–202. In Brandão, C. R. F., Martins-Neto, R. G., and Vulcano, M. A. 'The earliest known fossil ant (first southern hemisphere Mesozoic record) (Hymenoptera: Formicidae: Myrmeciinae)'. *Psyche (Cambridge)* **96**, 195–208.

Brothers, D. J. (1975). Phylogeny and classification of the aculeate Hymenoptera, with special reference to Mutillidae. *University of Kansas Science Bulletin* **50**, 483–648.

Brothers, D. J. (1999). Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). *Zoologica Scripta* **28**, 233–249.

Brothers, D. J., and Carpenter, J. M. (1993). Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera). *Journal of Hymenoptera Research* **2**, 227–304.

Brothers, D. J., and Finnamore, A. T. (1993). Superfamily Vespoidea. In 'Hymenoptera of the World: An Identification Guide to Families'. (Eds H. Goulet and J. T. Huber.) pp. 161–278. (Research Branch, Agriculture Canada and Canada Communications Group: Ottawa, Canada.)

Brown, W. L., Jr (1953*a*). Characters and synonymies among the genera of ants. Part I. *Breviora* **11**, 1–13.

Brown, W. L., Jr (1953*b*). Revisionary notes on the ant genus *Myrmecia* of Australia. *Bulletin of the Museum of Comparative Zoology* **111**, 1–35.

Brown, W. L., Jr (1954). Remarks on the internal phylogeny and subfamily classification of the family Formicidae. *Insectes Sociaux* **1**, 21–31.

Brown, W. L., Jr (1960). Contributions toward a reclassification of the Formicidae. III. Tribe Amblyoponini (Hymenoptera). *Bulletin of the Museum of Comparative Zoology* **122**, 143–230.

Brown, W. L., Jr, and Nutting, W. L. (1950). Wing venation and the phylogeny of the Formicidae. *Transactions of the American Entomological Society* **75**, 113–132.

Brown, W. L., Jr, and Taylor, R. W. (1970). Superfamily Formicoidea. In 'The Insects of Australia'. (Ed. CSIRO.) pp. 951–959. (Melbourne University Press: Melbourne, Australia.)

Brown, W. L., Jr, and Wilson, E. O. (1959). The search for *Nothomyrmecia*. *Western Australian Naturalist* **7**, 25–30.

Browning, G. P. (1987). 'Taxonomy of *Myrmecia* Fabricius (Hymenoptera: Formicidae).' PhD Thesis. (University of Adelaide: Australia.)

Cameron, S. A., and Mardulyn, P. (2001). Multiple molecular data sets suggest independent origins of highly eusocial behavior in bees (Hymenoptera: Apinae). *Systematic Biology* **50**, 194–214.

Carpenter, F. M. (1930). The fossil ants of North America. *Bulletin of the Museum of Comparative Zoology* **70**, 1–66.

Chiotis, M., Jermiin, L. S., and Crozier, R. H. (2000). A molecular framework for the phylogeny of the ant subfamily Dolichoderinae. *Molecular Phylogenetics and Evolution* **17**, 108–116.

Clark, J. (1934). Notes on Australian ants, with descriptions of new species and a new genus. *Memoirs of the National Museum of Victoria* **8**, 5–20.

Clark, J. (1943). A revision of the genus *Promyrmecia* Emery (Formicidae). *Memoirs of the National Museum of Victoria* **13**, 83–149.

Clark, J. (1951). 'The Formicidae of Australia. Vol. 1. Subfamily Myrmeciinae.' (CSIRO: Melbourne, Australia.)

Crosland, M. W. J., Crozier, R. H., and Jefferson, E. (1988). Aspects of the biology of the primitive ant genus *Myrmecia* F. (Hymenoptera: Formicidae). *Journal of the Australian Entomological Society* **27**, 305–309.

Crozier, R. H., Jermiin, L. S., and Chiotis, M. (1997). Molecular evidence for a Jurassic origin of ants. *Naturwissenschaften* **84**, 22–23.

Cunningham, C. W. (1997). Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* **14**, 733–740.

Darlu, P., and Lecointre, G. (2002). When does the incongruence length difference test fail? *Molecular Biology and Evolution* **19**, 432–437.

Dlussky, G. M. (1975). Superfamily Formicoidea Latreille, 1802. Family Formicidae Latreille, 1802. In 'Hymenoptera Apocrita of the Mesozoic.' (Ed. A. P. Rasnitsyn.) pp. 114–122. *Trudy Paleontologicheskogo Instituta, Akademiya Nauk SSSR* **147**, 1–134. [In Russian.]

Dlussky, G. M. (1983). A new family of Upper Cretaceous Hymenoptera: an "intermediate link" between the ants and the scolioids. *Paleontologicheskii Zhurnal* **1983**(3), 65–78. [In Russian.]

Dlussky, G. M. (1987). New Formicoidea (Hymenoptera) of the Upper Cretaceous. *Paleontologicheskii Zhurnal* **1987**(1), 131–135. [In Russian.]

Dlussky, G. M. (1988). Ants of Sakhalin amber (Paleocene?). *Paleontologicheskii Zhurnal* **1988**(1), 50–61. [In Russian.]

Dlussky, G. M. (1996). Ants (Hymenoptera: Formicidae) of Burmese amber. *Paleontologicheskii Zhurnal* **1996**(3), 83–89. [In Russian.]

Dlussky, G. M. (1997). Genera of ants (Hymenoptera: Formicidae) from Baltic amber. *Paleontologicheskii Zhurnal* **1997**(6), 50–62. [In Russian.]

Dlussky, G. M. (1999*a*). The first find of Formicoidea (Hymenoptera) in the lower Cretaceous of the Northern Hemisphere. *Paleontologicheskii Zhurnal* **1999**(3), 62–66. [In Russian.]

Dlussky, G. M. (1999*b*). New ants (Hymenoptera, Formicidae) from Canadian amber. *Paleontologicheskii Zhurnal* **1999**(4), 73–76. [In Russian.]

Dowton, M., and Austin, A. D. (2002). Increased congruence does not necessarily indicate increased phylogenetic accuracy—the behavior of the incongruence length difference test in mixed-model analyses. *Systematic Biology* **51**, 19–31.

Emery, C. (1877). Saggio di un ordinamento naturale dei Mirmicidei, e considerazioni sulla filogenesi delle formiche. *Bullettino della Società Entomologica Italiana* **9**, 67–83.

Emery, C. (1911). Hymenoptera. Fam. Formicidae. Subfam. Ponerinae. *Genera Insectorum* **118**, 1–125.

Fabricius, J. C. (1804). 'Systema Piezatorum secundum ordines, genera, species, adjectis synonymis, locis, observationibus, descriptionibus.' (C. Reichard: Brunswick.)

Farris, J. S., Källersjö, M., Kluge, A. G., and Bult, C. (1995). Testing significance of incongruence. *Cladistics* **10**, 315–319.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1988). Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics* **22**, 521–565.

Forbes, J. (1967). The male genitalia and terminal gastral segments of two species of the primitive ant genus *Myrmecia* (Hymenoptera: Formicidae). *Journal of the New York Entomological Society* **75**, 35–42.

Freeland, J. (1958). Biological and social patterns in the Australian bulldog ants of the genus *Myrmecia*. *Australian Journal of Zoology* **6**, 1–18.

Gauld, I. D., and Hanson, P. E. (1995). The structure of the Hymenoptera. In 'The Hymenoptera of Costa Rica'. (Eds P. E. Hanson and I. D. Gauld.) pp. 102–137. (Oxford University Press: Oxford, UK.)

Goulet, H., and Huber, J. T. (Eds) (1993). 'Hymenoptera of the World: An Identification Guide to Families.' (Research Branch, Agriculture Canada and Canada Communications Group: Ottawa, Canada.)

Grimaldi, D., and Agosti, D. (2000). A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants. *Proceedings of the National Academy of Sciences USA* **97**, 13678–13683.

Grimaldi, D., Agosti, D., and Carpenter, J. M. (1997). New and rediscovered primitive ants (Hymenoptera: Formicidae) in Cretaceous amber from New Jersey, and their phylogenetic relationships. *American Museum Novitates* **3208**, 1–43.

Hall, R. (1998). The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In 'Biogeography and Geological Evolution of SE Asia'. (Eds R. Hall and J. D. Holloway.) pp. 99–131. (Backhuys: Leiden, The Netherlands.)

Hancock, J. M., Tautz, D., and Dover, G. A. (1988). Evolution of the secondary structure and compensatory mutations of the ribosomal RNAs of *Drosophila melanogaster*. *Molecular Biology and Evolution* **5**, 393–414.

Hashimoto, Y. (1991). Phylogenetic study of the family Formicidae based on the sensillum structures on the antennae and labial palpi (Hymenoptera, Aculeata). *Japanese Journal of Entomology* **59**, 125–140.

Hashimoto, Y. (1996). Skeletomuscular modifications associated with the formation of an additional petiole on the anterior abdominal segments in aculeate Hymenoptera. *Japanese Journal of Entomology* **64**, 340–356.

Haskins, C. P., and Haskins, E. F. (1951). Notes on the biology and social behavior of the archaic ponerine ants of the genera *Myrmecia* and *Promyrmecia*. *Annals of the Entomological Society of America* **43**, 461–491.

Hermann, H. R., and Blum, M. S. (1981). Defensive mechanisms in the social Hymenoptera. In 'Social Insects. Vol. 2'. (Ed. H. R. Hermann.) pp. 77–197. (Academic Press: New York, USA.)

Hölldobler, B. (1985). A new exocrine gland in the slave raiding ant genus *Polyergus*. *Psyche (Cambridge)* **91**, 225–235.

Hölldobler, B., and Engel, H. (1979). Tergal and sternal glands in ants. *Psyche (Cambridge)* **85**, 285–330.

Hölldobler, B., and Engel-Siegel, H. (1985). On the metapleural gland of ants. *Psyche (Cambridge)* **91**, 201–224.

Hölldobler, B., and Taylor, R. W. (1984). A behavioral study of the primitive ant *Nothomyrmecia macrops* Clark. *Insectes Sociaux* **30**, 384–401.

Hölldobler, B., and Wilson, E. O. (1990). 'The Ants.' (Harvard University Press: Cambridge, MA, USA.)

Huelsenbeck, J. P. (1991). When are fossils better than extant taxa in phylogenetic analysis? *Systematic Zoology* **40**, 458–469.

Huelsenbeck, J. P., and Rannala, B. (1997). Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science* **276**, 227–232.

Hunt, G. J., and Page, R. E. J. (1994). Linkage analysis of sex determination in the honey bee (*Apis mellifera*). *Molecular Genetics* **244**, 512–518.

Imai, H. T., Taylor, R. W., Kubota, M., Ogata, K., and Wada, M. Y. (1991). Notes on the remarkable karyology of the primitive ant *Nothomyrmecia macrops*, and of the related genus *Myrmecia* (Hymenoptera: Formicidae). *Psyche (Cambridge)* **97**, 133–140.

Jaisson, P., Fresneau, D., Taylor, R. W., and Lenoir, A. (1992). Social organization in some primitive Australian ants. I. *Nothomyrmecia macrops* Clark. *Insectes Sociaux* **39**, 425–438.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**, 111–120.

Kishino, H., Thorne, J. L., and Bruno, W. J. (2001). Performance of a divergence time estimation method under a probabilistic model of rate evolution. *Molecular Biology and Evolution* **18**, 352–361.

Krafchick, B. (1959). 'A Comparative Study of the Male Genitalia of North American Ants (Formicidae) with emphasis on Generic Differences.' PhD Thesis. (University of Maryland: College Park, MD, USA.)

Kugler, C. (1980). The sting apparatus in the primitive ants *Nothomyrmecia* and *Myrmecia*. *Journal of the Australian Entomological Society* **19**, 263–267.

Kugler, C. (1991). Stings of ants of the tribe Ectatommini (Formicidae: Ponerinae). *Insecta Mundi* **5**, 153–166.

Kugler, C. (1992). Stings of ants of the Leptanillinae (Hymenoptera: Formicidae). *Psyche (Cambridge)* **99**, 103–115.

Lattke, J. (In press). Biogeografía de las hormigas neotropicales. In 'Hormigas de la Región Neotropical'. (Ed. F. Fernández.) (Instituto Humboldt: Bogotá, Colombia.)

Lee, M. S. Y. (2001*a*). Unalignable sequences and molecular evolution. *Trends in Ecology and Evolution* **16**, 681–685.

Lee, M. S. Y. (2001*b*). Uninformative characters and apparent conflict between molecules and morphology. *Molecular Biology and Evolution* **18**, 676–680.

Linares, A. R., Hancock, J. M., and Dover, G. A. (1991). Secondary structure constraints on the evolution of *Drosophila* 28 S ribosomal RNA expansion segments. *Journal of Molecular Biology* **219**, 381–390.

Lutz, H. (1986). Eine neue Unterfamilie der Formicidae (Insecta: Hymenoptera) aus dem mittel-eozänen Olschiefer der "Grube Messel" bei Darmstadt (Deutschland, S-Hessen). *Senckenbergiana Lethaea* **67**, 177–218.

Lutzoni, F. M. (1997). Phylogeny of lichen- and non-lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. *Systematic Biology* **46**, 373–406.

Lutzoni, F. M., Wagner, P., Reeb, V., and Zoller, S. (2000). Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. *Systematic Biology* **49**, 628–651.

Maddison, D. R., Baker, M. D., and Ober, K. A. (1999). Phylogeny of carabid beetles as inferred from 18S ribosomal DNA (Coleoptera: Carabidae). *Systematic Entomology* **24**, 103–138.

Maddison, W. P. (1993). Missing data versus missing characters in phylogenetic analysis. *Systematic Biology* **42**, 576–581.

Markl, H. (1973). The evolution of stridulatory communication in ants. In 'Proceedings of the VIIth IUSSI International Congress, London, 10–15 September 1973.' pp. 258–265. (University of Southampton: UK.)

Mayr, G. (1868). Die Ameisen des baltischen Bernsteins. *Beiträge zur Naturkunde Preussens* **1**, 1–102.

Mickevich, M. F., and Farris, J. S. (1981). The implications of congruence in *Menidia*. *Systematic Zoology* **30**, 351–370.

Miradoli Zatti, M. A., and Pavan, M. (1957). Studi sui Formicidae. III. Nuovi reperti dell'organo ventrale nei Dolichoderinae. *Bollettino della Società Entomologica Italiana* **87**, 84–87.

Nixon, K. C. (1996). Paleobotany in cladistics and cladistics in paleobotany: enlightenment and uncertainty. *Review of Palaeobotany and Palynology* **90**, 361–373.

Ogata, K. (1991). Ants of the genus *Myrmecia* Fabricius: a review of the species groups and their phylogenetic relationships (Hymenoptera: Formicidae: Myrmeciinae). *Systematic Entomology* **16**, 353–381.

Ogata, K., and Taylor, R. W. (1991). Ants of the genus *Myrmecia* Fabricius: a preliminary review and key to the named species (Hymenoptera: Formicidae: Myrmeciinae). *Journal of Natural History* **25**, 1623–1673.

Pavan, M. (1955). Studi sui Formicidae. I. Contributo alla conoscenza degli organi gastrali dei Dolichoderinae. *Natura (Milan)* **46**, 135–145.

Peeters, C. (1997). Morphologically "primitive" ants: comparative review of social characters, and the importance of queen–worker dimorphism. In 'The Evolution of Social Behavior in Insects and Arachnids'. (Eds J. C. Choe and B. J. Crespi.) pp. 372–391. (Cambridge University Press: Cambridge, UK.)

Petrulevicius, J. F. (1999). Insectos del Cenozoico de la Argentina. *Revista de la Sociedad Entomológica Argentina* **58**, 95–103.

Poinar, G., Archibald, B., and Brown, A. (1999). New amber deposit provides evidence of early Paleogene extinctions, paleoclimates, and past distributions. *Canadian Entomologist* **131**, 171–177.

Posada, D., and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Posada, D., and Crandall, K. A. (2001). Selecting the best-fit model of nucleotide substitution. *Systematic Biology* **50**, 580–601.

Rasnitysn, A. P. (1994). Concerning Michael Prentice's observations on aculeate wasps in Lebanese amber. *Sphecos* **27**, 11–12.

Richards, O. W. (1977). 'Hymenoptera: Introduction and Key to Families. Handbooks for the Identification of British Insects. Vol. 6, No. 1 (2nd Edn)'. (Ed. A. Watson.) (Royal Entomological Society: London, UK.)

Rossi de Garcia, E. (1983). Insectos fósiles en la Formación Ventana (Eoceno). Provincia de Neuquén. *Revista Asociación Geológica Argentina* **38**, 17–23.

Rust, J., and Andersen, N. M. (1999). Giant ants from the Paleogene of Denmark with a discussion of the fossil history and early evolution of ants (Hymenoptera: Formicidae). *Zoological Journal of the Linnean Society* **125**, 331–348.

Sanetra, M., and Crozier, R. H. (2001). Polyandry and colony genetic structure in the primitive ant *Nothomyrmecia macrops*. *Journal of Evolutionary Biology* **14**, 368–378.

Schönitzer, K., and Lawitzky, G. (1987). A phylogenetic study of the antenna cleaner in Formicidae, Mutillidae, and Tiphiidae (Insecta, Hymenoptera). *Zoomorphology (Berlin)* **107**, 273–285.

Shattuck, S. O. (1992*a*). Higher classification of the ant subfamilies Aneuretinae, Dolichoderinae and Formicinae (Hymenoptera: Formicidae). *Systematic Entomology* **17**, 199–206.

Shattuck, S. O. (1992*b*). Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). *Sociobiology* **21**, 1–181.

Shattuck, S. O. (1995). Generic-level relationships within the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). *Systematic Entomology* **20**, 217–228.

Shattuck, S. O. (1999). 'Australian Ants: Their Biology and Identification.' (CSIRO Publishing: Melbourne.)

Snodgrass, R. E. (1956). 'The Anatomy of the Honey Bee.' (Cornell University Press: Ithaca, NY, USA.)

Sullender, B. W., and Johnson, M. J. (1998). A preliminary molecular phylogeny for the Formicidae. [Abstract.] In 'Social Insects at the Turn of the Millennium. Proceedings of the XIII International Congress of IUSSI, Adelaide, Australia, 29 December 1998 to 3 January 1999'. (Eds M. P. Schwarz and K. Hogendoorn.) p. 460. (XIII Congress of IUSSI: Adelaide, Australia.)

Swofford, D. L. (2002). 'PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), version 4.0.' (Sinauer Associates: Sunderland, MA, USA.)

Swofford, D. L., Olsen, G. J., Waddell, P. J., and Hillis, D. M. (1996). Phylogenetic inference. In 'Molecular Systematics. 2nd Edn'. (Eds D. M. Hillis, C. Moritz and B. K. Mable.) pp. 407–514. (Sinauer Associates: Sunderland, MA, USA.)

Tautz, D., Hancock, J. M., Webb, D. A., Tautz, C., and Dover, G. A. (1988). Complete sequence of the rRNA genes in *Drosophila melanogaster*. *Molecular Biology and Evolution* **5**, 366–376.

Taylor, R. W. (1978). *Nothomyrmecia macrops*: a living-fossil ant rediscovered. *Science* **201**, 979–985.

Taylor, R. W. (1987). A checklist of the ants of Australia, New Caledonia and New Zealand (Hymenoptera: Formicidae). Report No. 41. (CSIRO Division of Entomology: Canberra, Australia.)

Taylor, R. W. (1991). *Myrmecia croslandi* sp. n., a karyologically remarkable new Australian jack-jumper ant (Hymenoptera: Formicidae: Myrmeciinae). *Journal of the Australian Entomological Society* **30**, 288.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–4680.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**, 4876–4882.

Thorne, J. L., Kishino, H., and Painter, I. S. (1998). Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution* **15**, 1647–1657.

Tulloch, G. S. (1935). Morphological studies of the thorax of the ant. *Entomologica Americana* **15**, 93–131.

Verhaagh, M. (1996). Warum die älteste bekannte Ameise keine ist (Hymenoptera). *Beiträge der Hymenopterologen-Tagung in Stuttgart* **1996**, 11.

Viana, M. J., and Haedo Rossi, J. A. (1957). Primer hallazgo en el hemisferio sur de Formicidae extinguidos y catálogo mundial de los Formicidae fósiles. Primera parte. *Ameghiniana* **1**, 108–113.

Ward, P. S. (1980). A systematic revision of the *Rhytidoponera impressa* group (Hymenoptera: Formicidae) in Australia and New Guinea. *Australian Journal of Zoology* **28**, 475–498.

Ward, P. S. (1990). The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): generic revision and relationship to other formicids. *Systematic Entomology* **15**, 449–489.

Ward, P. S. (1994). *Adetomyrma*, an enigmatic new ant genus from Madagascar (Hymenoptera: Formicidae), and its implications for ant phylogeny. *Systematic Entomology* **19**, 159–175.

Ward, P. S. (2001). Taxonomy, phylogeny and biogeography of the ant genus *Tetraponera* (Hymenoptera: Formicidae) in the Oriental and Australian regions. *Invertebrate Taxonomy* **15**, 589–665.

Ward, P. S., and Taylor, R. W. (1981). Allozyme variation, colony structure and genetic relatedness in the primitive ant *Nothomyrmecia macrops* Clark (Hymenoptera: Formicidae). *Journal of the Australian Entomological Society* **20**, 177–183.

Watts, C. H. S., McArthur, A. J., and Foster, R. (1998). Notes on the distribution of the dinosaur ant *Nothomyrmecia macrops* Clark (Hymenoptera: Formicidae) in South Australia. *Australian Entomologist* **25**, 29–31.

Wheeler, G. C., and Wheeler, J. (1976). Ant larvae: review and synthesis. *Memoirs of the Entomological Society of Washington* **7**, 1–108.

Wheeler, G. C., Wheeler, J., and Taylor, R. W. (1980). The larval and egg stages of the primitive ant *Nothomyrmecia macrops* Clark (Hymenoptera: Formicidae). *Journal of the Australian Entomological Society* **19**, 131–137.

Wheeler, W. M. (1915). The ants of the Baltic Amber. *Schriften der Physikalisch-Ökonomischen Gesellschaft zu Königsberg* **55**, 1–142.

Wheeler, W. M. (1922). Observations on *Gigantiops destructor* Fabricius and other leaping ants. *Biological Bulletin* **42**, 185–201.

Wheeler, W. M. (1933). 'Colony Founding among Ants, with an Account of Some Primitive Australian Species.' (Harvard University Press: Cambridge, MA, USA.)

Wiegmann, B. M., Mitter, C., Regier, J. C., Friedlander, T. P., Wagner, D. M., and Nielsen, E. S. (2000). Nuclear genes resolve Mesozoic-aged divergences in the insect order Lepidoptera. *Molecular Phylogenetics and Evolution* **15**, 242–259.

Wilson, E. O. (1971). 'The Insect Societies.' (Harvard University Press: Cambridge, MA, USA.)

Wilson, E. O. (1985). Ants from the Cretaceous and Eocene amber of North America. *Psyche (Cambridge)* **92**, 205–216.

Wilson, E. O. (1987). The earliest known ants: an analysis of the Cretaceous species and an inference concerning their social organization. *Paleobiology* **13**, 44–53.

Wilson, E. O. (1988). The biogeography of the West Indian ants (Hymenoptera: Formicidae). In 'Zoogeography of Caribbean Insects'. (Ed. J. K. Liebherr.) pp. 214–230. (Cornell University Press: Ithaca, NY, USA.)

Wilson, E. O., Eisner, T., Wheeler, G. C., and Wheeler, J. (1956). *Aneuretus simoni* Emery, a major link in ant evolution. *Bulletin of the Museum of Comparative Zoology* **115**, 81–99.

Wilson, E. O., Carpenter, F. M., and Brown, W. L., Jr (1967). The first Mesozoic ants, with the description of a new subfamily. *Psyche (Cambridge)* **74**, 1–19.

Yoder, A. D., Irwin, J. A., and Payseur, B. A. (2001). Failure of the ILD to determine data combinability for slow loris phylogeny. *Systematic Biology* **50**, 408–424.

Manuscript received 29 October 2002; revised and accepted 21 February 2003.

Appendix 1. Morphological data sets, MORPH1 (74 characters) and MORPH2 (85 characters)

The composite taxon '*Ameghinoia*/*Polanskiella*' was added to MORPH1 to test the placement of the South American fossils (see text). Three constant characters in MORPH2 (nos 5, 33 and 40) were excluded before analysis. ?, Missing or unknown; p, polymorphic; –, inapplicable.

Taxon	1 10 20 30 40 50 60 70 80
MORPH1	
Myrmecia	11110011100110101110100p000111000000100000p11000000010111000001010111010 01
Nothomyrmecia	10000111111011101110 10? 10011110000001000001110000110001000000001010111010 01
Prionomyrmex longiceps	1? 00111111? 011100110??? 1000? 1??? 1?? 0? 0000? 011???? 00? 1011???? 0? ??????????? 1
Prionomyrmex janzeni	1? 0011111110111001????? 000?? 1? 0? 1?? 010??? 0011???? 00? 1011??? 00? ??????????? 1
Pseudomyrmex	10000001000000111 0p1100p001011110p10001010p11000000110110100001010000101 11
Tetraponera	10000001000000111 00p100p001011110p10101010111000000110110100001010 0? 010111
Paraponera	10000011000001100010000? 11100100000? 01000010111001001111111010 00? 0? 00? 000001
Amblyopone	10000011p000011110010101010010000010001p01001010010000010110100010000? 000001
Rhytidoponera	10000011000001 1pp0pp011110001000011100p01001110110010p01101000101000000001
Myrmicinae	10000011000001111001011p10p01001p111011011p11011000110110100001000000000011
Dolichoderinae	100000p100000p111p01000p00101001p11101110110100000000p00000101110100000011
Formicinae	100000010000pp111001000p001010010p11011011011100000001000000110000000000001
Sphecomyrminae	1? 00000000000000010p??? 000p0100? 000000010001 1??? 000? 00000? 000? ??????????? 1
Vespidae	0000000p000p000000010000--110--00000000000p00000000000000000000000000000p0
Ameghinoia/*Polanskiella*	1?????? 11?? 01? 1?????????????????? 0?? 0?? 0??? 111???? 00? 101??????????????????
MORPH2	
Myrmecia gulosa-group	11110011100110101110100p000111000000100000111000000010111000000 10? 01110100111100100010
M. mandibularis-group	1111001110011010111010? 10001110000001000000111000000010111000000 10???? 10100100111011101
M. picta-group	1111001110011010111010? 00001110000001000000111000000010111000000 10???? 10100100000011011
M. pilosula-group	1111001110011010111010? 00001110000001000000111000000010111000000 10101? 10100100111011101
Nothomyrmecia	10000111111011101110 10? 10011110000001000001110000110001000000001010111010 01 00000-10000
Pseudomyrmex	10000001000000111 0p1100p001011110p10001010p11000000110110100001010000101 11000ppp00000
Tetraponera	10000001000000111 00p100p001011110p10101010111000000110110100001010 0? 010111000ppp00000
Paraponera	10000011000001100010000? 11100100000? 01000010111001001111111010 00? 0? 00? 00000101011010001
Amblyopone	10000011p000011110010101010010000010001p01001010010000010110100010000? 00000100p11-00001
Rhytidoponera	10000011000001 1pp0pp011110001000011100p01001110110010p01101000101000000001 1p0---00000
Pogonomyrmex	10000011000001111001? 11p10p01001p111011011p11011000110110100001000000000011000--100000
Liometopum	10000011000 00p111101? 0? 10010100101110110110111000000010000010111000? 00001100000-00000
Linepithema	10000011000001111p01? 0? 00010100101110 1p01101110000000100000101110100000001100000-00000
Formica	10000001000000111001000p001010010p11011011011100000001000000110000000000p100000-00000
Camponotus	10000001000 00p111001000p0010p0010p11011011011100000001000000110000000000 1pp0pp-00000
Vespidae	0000000p000p000000010000--110--00000000000p00000000000000000000000000000p0100---00000
Apis	00100000010000101001000 1--110--00001000000000000000001000000000000? 0? 0010000---0? 00-

Appendix 2. Keys to the genera of Myrmeciinae

Workers and queens

1. Postpetiole present and slender, such that the height of abdominal segment 3 about 0.50–0.65× height of abdominal segment 4; eyes apparently in a relatively posterior position on the head, well separated from the mandibular base (Argentina, Eocene/early Oligocene, two extinct species described, one from each genus) . . . . . *Ameghinoia* and *Polanskiella*

   Without the above combination of features: either eyes situated far forward close to the base of the mandibles, and/or postpetiole much broader (height ratio of abdominal segments 3 and 4 about 0.80), or a postpetiole absent 2

2. Compound eye well separated from the mandibular base, by a distance greater than the eye diameter; lateral clypeal carina present; closed mandibles with the masticatory margins confluent, not overlapping; base of the mandibles fitting tightly against the protruding anterior clypeal margin 3

   Compound eye situated far forward on side of head, separated from the mandibular base by a distance much less than the eye diameter; lateral clypeal carina absent; closed mandibles broadly overlapping, with the masticatory margins not confluent; base of the mandibles not fitting tightly against the anterior clypeal margin (Australia, *c.* 90 extant species) . . . . . *Myrmecia*

3. Postpetiole present, i.e. third abdominal segment notably smaller than fourth abdominal segment *and* separated from the latter by a marked constriction (Fig. 2); short paired teeth on posterodorsal surface of propodeum; abdominal sternite 3 lacking longitudinal keel (Baltic amber, two extinct species) . . . . . *Prionomyrmex*

   Postpetiole absent (third abdominal segment much smaller than fourth abdominal segment but not separated from the latter by a marked constriction) (Fig. 1); propodeum lacking teeth; abdominal sternite 3 with a median longitudinal keel (Australia, one extant species) . . . . . *Nothomyrmecia*

Males (unknown in Ameghinoia *and* Polanskiella*)*

1. Notauli present; postpetiole present; posterior margin of sternite 3 simple 2

   Notauli absent; postpetiole absent; posterior margin of sternite 3 sinuous, protruding medially (as in Fig. 1) . . . *Nothomyrmecia*

2. Propodeum with short paired teeth . . . . . *Prionomyrmex*

   Propodeum lacking teeth . . . . . *Myrmecia*