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Phylogeny and Biogeography of Dolichoderine Ants: Effects of Data Partitioning and Relict Taxa on Historical Inference

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Abstract.—Ants (Hymenoptera: Formicidae) are conspicuous organisms in most terrestrial ecosystems, often attaining high levels of abundance and diversity. In this study, we investigate the evolutionary history of a major clade of ants, the subfamily Dolichoderinae, whose species frequently achieve ecological dominance in ant communities. This group has also produced some of the world's most successful invasive ants. We use an extensive molecular data set (∼9 kb of sequence data from 10 nuclear genes, covering 48 dolichoderine species and 6 outgroup taxa) to infer the phylogenetic relationships, divergence dates, and biogeographic history of these ants. We evaluate the effects of data partitioning and outgroup composition on phylogenetic inference by estimating relationships under a series of increasingly partitioned data sets and by running analyses both with and without *Aneuretus simoni*, a rare and localized species that is the nearest living relative of Dolichoderinae. We also examine the effects of excluding 2 data partitions with significant base composition heterogeneity. Our results reveal 4 well-supported and mutually exclusive clades of dolichoderines, corresponding to 4 newly defined tribes: Bothriomyrmecini (B), Dolichoderini (D), Leptomyrmecini (L), and Tapinomini (T). All Bayesian and likelihood analyses yield the same unrooted (ingroup-only) topology, ((D,L),(B,T)), with the outgroups attaching either on the Dolichoderini branch or on the Tapinomini branch. Placement of the root is highly sensitive to choice of model partition and to inclusion/exclusion of *Aneuretus*. Bayes' factors strongly favor the more partitioned models, and in these Tapinomini is recovered as sister to the remaining dolichoderines, but only if *Aneuretus* is included. Exclusion of *Aneuretus* precludes recovery of this topology in all but the most highly partitioned Bayesian analyses and then only with nonsignificant support, underscoring the importance of relict, taxonomically isolated taxa for phylogenetic inference. Removal of 2 partitions with heterogeneous base composition also markedly increases support for placement of the root on the Tapinomini branch. Our divergence date estimates and biogeographic analyses indicate that crown-group dolichoderines arose about 65 million years ago (Ma), although this was preceded by a substantial period (30 million years) of stem group evolution. The 4 extant tribes are estimated to have crown-group origins in the late Paleocene or Eocene (40–60 Ma). Tapinomini and Bothriomyrmecini originated in the Paleotropics and subsequently dispersed to other biogeographic regions. Crown-group Leptomyrmecini arose and diversified in the Neotropics, but they also gave rise to one clade that colonized Australia about 30 Ma and subsequently experienced a massive radiation on that continent. This event occurred later than the diversification of dolichoderines in the northern hemisphere, so that by the time dolichoderines came to dominate the Australian fauna they had already declined in abundance in the Holarctic region. [Base composition; biogeography; data partitioning; Dolichoderinae; Formicidae; fossil record; outgroup; phylogenetic inference; relict taxa.]

Ants (Hymenoptera: Formicidae) are the world's most prominent social insects, numbering more than 12,000 described species (Bolton et al. 2007) and occupying nearly all major terrestrial habitats (Brown 2000). In their varied capacities as predators, scavengers, and herbivores, ants frequently exert strong effects on ecological communities (Hölldobler and Wilson 1990). Twenty-one extant subfamilies of ants are currently recognized, nearly all of which are monophyletic and hence mutually exclusive (Brady et al. 2006; Moreau et al. 2006; Ward 2007a; Rabeling et al. 2008). However, the species-level diversity of extant ants is unevenly distributed among these major clades, with 85% of all known species concentrated in just 4 of the subfamilies (Bolton et al. 2007).

This paper focuses on one of these species-rich clades, the subfamily Dolichoderinae, a cosmopolitan group with approximately 900 described species (Bolton 2007; Bolton et al. 2007) and many more awaiting formal description (Shattuck 1999; Andersen 2007). Dolichoderine ants are notable for their deployment of potent chemical defenses and their frequent assumption of numerical and behavioral dominance in ant communities (Andersen 1997; Holway 1999). They include some of the world's most successful invasive species, including the Argentine ant (*Linepithema humile*), the ghost ant (*Tapinoma melanocephalum*), and white-footed ants (*Technomyrmex* spp.) (Smith 1965; Deyrup 1991; Williams 1994; Holway et al. 2002).

The fossil record suggests that dolichoderine ants were even more dominant in the past. In fossil insect assemblages from the Green River Formation (Middle Eocene), Baltic amber (Late Eocene), and Florissant shales (Early Oligocene), dolichoderines constitute about two-thirds of all ant specimens (Dlussky and Rasnitsyn 2003). Since the Oligocene, however, these ants have declined in abundance in the Holarctic region, eclipsed by another highly diverse group, the Myrmicinae (Brown 1973; Dlussky and Rasnitsyn 2003). This pattern of retreat is also evident in the sister group of Dolichoderinae, the subfamily Aneuretinae. Although previously widespread across the northern hemisphere in the Tertiary (Dlussky and Rasnitsyn 2003), aneuretines are represented today by a single relict

species in Sri Lanka (Wilson et al. 1956). In the Neotropics and in the southern hemisphere—especially in Australia—dolichoderines have fared better, competing more successfully against myrmicines and ranking high in both abundance and diversity (Majer 1994; Andersen 1995). Whether this represents a recent radiation or whether it was contemporaneous with the diversification that occurred in the northern hemisphere during the Tertiary remains unclear because we lack a well-supported, time-dated phylogeny of the subfamily (cf. Shattuck 1995; Chiotis et al. 2000).

Here we reconstruct the evolutionary history of dolichoderine ants using a data set consisting of 10 nuclear genes obtained from a targeted subset of species. Our goal is to estimate relationships among the major lineages of this species-rich clade rather than to recover a detailed species-level tree. As such, we have employed the strategy of sequencing multiple genes from a set of exemplar taxa (∼50 species) drawn from a much larger species pool (900 + species). There are compelling arguments for inferring species phylogenies by integrating across estimated gene trees (Maddison and Knowles 2006; Edwards et al. 2007; Kubatko and Degnan 2007; Rosenberg and Tao 2008; Degnan and Rosenberg 2009). In this study, however, we adopt a concatenate-and-partition approach because we are concerned with deeper relationships in the tree and because our sampled species are taxonomically dispersed. Under these circumstances, lineage sorting and coalescent-associated branch length variation (Edwards 2009) are likely to be less important than in studies of closely related taxa.

We assess the effects of data partitioning and taxon discovery on phylogenetic inference by evaluating relationships under a series of increasingly partitioned data sets and by running analyses both with and without *Aneuretus simoni*, the rare relictual species that is the nearest living relative of Dolichoderinae. A growing body of evidence suggests that the outcome of phylogenetic analyses can be markedly influenced by choice of partition model (Brown and Lemmon 2007; McGuire et al. 2007; Li et al. 2008) and by outgroup composition (Philippe 1997; Holland et al. 2003; Hedtke et al. 2006; Rota-Stabelli and Telford 2008). We also examine the effects of excluding 2 data partitions in which there is significant base composition heterogeneity, another potentially confounding factor in phylogenetic estimation (Sanderson and Shaffer 2002). We demonstrate that data partitioning, outgroup membership, and base composition heterogeneity all have striking effects on our estimation of the dolichoderine phylogeny.

We evaluate the global biogeographic history of these ants using a combination of fossil-calibrated divergence dating methods and dispersal–vicariance analysis (DIVA). Our results reveal that diversification of crown-group dolichoderines postdates the K/T boundary and occurred later in the Australian region than in other parts of the world. Our findings also bring into question the identities ascribed to certain fossil ants from Baltic amber and suggest the need for taxonomic reassessment of this material.

Materials and Methods

Taxa

For molecular phylogenetic analyses, we chose a series of exemplar taxa to represent the diversity of the Dolichoderinae: 48 species belonging to 26 of 28 extant genera (Table 1). Nomenclature follows Bolton et al. (2007). Most genera were represented by multiple (2–5) species, chosen to capture the range of variation in the genus; those represented by a single species are either monotypic or species poor. Taxon sampling within a genus was guided by the results of previous taxonomic studies (e.g., Shattuck 1992; MacKay 1993; Bolton 2007; Longino 2007; Wild 2009), with species being chosen that would be expected to span the deeper nodes within the group. As outgroups we used *A. simoni*, the sole living species in the subfamily Aneuretinae (the sister group of the Dolichoderinae), and representatives from 5 more distantly related lineages of formicoid ants (Brady et al. 2006).

Sequencing and Sequence Annotation

We sequenced fragments from 10 nuclear genes—long-wavelength rhodopsin (LW Rh), elongation factor 1-alpha F1 copy (EF1aF1), elongation factor 1-alpha F2 copy (EF1aF2), abdominal-A (abdA), wingless (wg), arginine kinase (argK), enolase, rudimentary (CAD), 18S ribosomal DNA (rDNA), and 28S rDNA—for a total of about 9 kb of aligned sequence, excluding hypervariable regions of 28S and introns of the protein-coding genes. Our data matrix (54 taxa by 10 genes) contains no missing fragments (Table 1). Of the 540 sequences, 127 were previously published (Ward and Downie 2005; Brady et al. 2006; Ward 2007b); the remainder were generated for this study (GenBank accession numbers FJ939744–FJ940129). Sequencing procedures were similar to those described in Ward and Downie (2005), Brady et al. (2006), and Schultz and Brady (2008). Primers for 3 genes new to this study (argK, enolase, and CAD) are given in Table 2.

Sequences were collated in Sequencher v4.6 (Gene Codes Corporation, Ann Arbor, MI), aligned with ClustalX v1.81 (Thompson et al. 1997), and manually edited with MacClade v4.08 (Maddison D.R. and Maddison W.P. 2000). Alignment was straightforward for the exons of protein-coding genes and for 18S. In contrast, the introns of protein-coding genes and hypervariable regions of 28S proved difficult or impossible to align with confidence and were excluded from consideration in all analyses. Sequence characteristics are summarized in Table 3. The aligned data matrix has been deposited at TreeBase (matrix accession no. M4460).

Phylogenetic Analyses

Data partitions.—In both Bayesian and maximum likelihood (ML) analyses, we employed a series of data

TABLE 1. Taxa sequenced in this study, with GenBank numbers

Subfamily	Species	Voucher specimen	GenBank accession numbers									
			18S	28S	wg	abdA	LW Rh	EF1aF1	EF1aF2	argK	Enolase	CAD
Dolichoderinae	*Anonychomyrma gilberti*	CASENT0106003	EF012835	EF012963	EF013671	EF013091	EF013543	EF013222	EF013384	FJ939841	FJ940077	FJ939895
Dolichoderinae	*Anonychomyrma itinerans*	CASENT0009959	FJ939744	FJ939776	FJ940012	FJ939808	FJ939979	FJ940044	FJ939947	FJ939842	FJ940078	FJ939896
Dolichoderinae	*Aptinoma antongil*	CASENT0489161	FJ939745	FJ939777	FJ940013	FJ939809	FJ939980	FJ940045	FJ939948	FJ939843	FJ940079	FJ939897
Dolichoderinae	*Aptinoma mangabe*	CASENT0106175	FJ939746	FJ939778	FJ940014	FJ939810	FJ939981	FJ940046	FJ939949	FJ939844	FJ940080	FJ939898
Dolichoderinae	*Arnoldius* AU01	CASENT0106155	FJ939747	FJ939779	FJ940015	FJ939811	FJ939982	FJ940047	FJ939950	FJ939845	FJ940081	FJ939899
Dolichoderinae	*Axinidris mlalu*	CASENT0090706	FJ939748	FJ939780	FJ940016	FJ939812	FJ939983	FJ940048	FJ939951	FJ939846	FJ940082	FJ939900
Dolichoderinae	*Axinidris murielae*	CASENT0093118	FJ939749	FJ939781	FJ940017	FJ939813	FJ939984	FJ940049	FJ939952	FJ939847	FJ940083	FJ939901
Dolichoderinae	*Azteca instabilis*	CASENT0106162	FJ939750	FJ939782	FJ940018	FJ939814	FJ939985	FJ940050	FJ939953	FJ939848	FJ940084	FJ939902
Dolichoderinae	*Azteca ovaticeps*	CASENT0106110	EF012842	EF012970	EF013678	EF013098	EF013550	EF013231	EF013393	FJ939849	FJ940085	FJ939903
Dolichoderinae	*Azteca schimperi*	CASENT0106172	FJ939751	FJ939783	FJ940019	FJ939815	FJ939986	FJ940051	FJ939954	FJ939850	FJ940086	FJ939904
Dolichoderinae	*Bothriomyrmex paradoxus*	JTLC000003511	FJ939752	FJ939784	FJ940020	FJ939816	FJ939987	FJ940052	FJ939955	FJ939851	FJ940087	FJ939905
Dolichoderinae	*Bothriomyrmex saundersi*	CASENT0172693	FJ939753	FJ939785	FJ940021	FJ939817	FJ939988	FJ940053	FJ939956	FJ939852	FJ940088	FJ939906
Dolichoderinae	*Chronoxenus javanus*	CASENT0106163	FJ939754	FJ939786	FJ940022	FJ939818	FJ939989	FJ940054	FJ939957	FJ939853	FJ940089	FJ939907
Dolichoderinae	*Doleromyrma darwiniana*	CASENT0009949	FJ939755	FJ939787	FJ940023	FJ939819	FJ939990	FJ940055	FJ939958	FJ939854	FJ940090	FJ939908
Dolichoderinae	*Dolichoderus debilis*	CASENT0106158	FJ939756	FJ939788	FJ940024	FJ939820	FJ939991	FJ940056	FJ939959	FJ939855	FJ940091	FJ939909
Dolichoderinae	*Dolichoderus decollatus*	CASENT0106165	FJ939757	FJ939789	FJ940025	FJ939821	FJ939992	FJ940057	FJ939960	FJ939856	FJ940092	FJ939910
Dolichoderinae	*Dolichoderus erectilobus*	CASENT0009956	FJ939758	FJ939790	FJ940026	FJ939822	FJ939993	FJ940058	FJ939961	FJ939857	FJ940093	FJ939911
Dolichoderinae	*Dolichoderus lamellosus*	CASENT0106159	FJ939759	FJ939791	FJ940027	FJ939823	FJ939994	FJ940059	FJ939962	FJ939858	FJ940094	FJ939912
Dolichoderinae	*Dolichoderus pustulatus*	CASENT0106164	FJ939760	FJ939792	FJ940028	FJ939824	FJ939995	FJ940060	FJ939963	FJ939859	FJ940095	FJ939913
Dolichoderinae	*Dolichoderus scabridus*	CASENT0106109	EF012860	EF012988	EF013696	EF013116	EF013568	EF013254	EF013416	FJ939860	FJ940096	FJ939914
Dolichoderinae	*Dorymyrmex bicolor*	CASENT0106031	EF012862	EF012990	EF013698	EF013118	EF013570	EF013257	EF013419	FJ939861	FJ940097	FJ939915
Dolichoderinae	*Dorymyrmex planidens*	CASENT0106156	FJ939761	FJ939793	FJ940029	FJ939825	FJ939996	FJ940061	FJ939964	FJ939862	FJ940098	FJ939916
Dolichoderinae	*Forelius chalybaeus*	CASENT0106157	FJ939762	FJ939794	FJ940030	FJ939826	FJ939997	FJ940062	FJ939965	FJ939863	FJ940099	FJ939917
Dolichoderinae	*Forelius pruinosus*	CASENT0106039	EF012866	EF012994	EF013702	EF013122	EF013574	EF013262	EF013424	FJ939864	FJ940100	FJ939918
Dolichoderinae	*Froggattella kirbii*	CASENT0009944	FJ939763	FJ939795	FJ940031	FJ939827	FJ939998	FJ940063	FJ939966	FJ939865	FJ940101	FJ939919
Dolichoderinae	*Gracilidris pombero*	CASENT0010797	FJ939764	FJ939796	FJ940032	FJ939828	FJ939999	FJ940064	FJ939967	FJ939866	FJ940102	FJ939920
Dolichoderinae	*Iridomyrmex* AU01	CASENT0106153	FJ939765	FJ939797	FJ940033	FJ939829	FJ940000	FJ940065	FJ939968	FJ939867	FJ940103	FJ939921
Dolichoderinae	*Iridomyrmex pallidus*	CASENT0106152	FJ939766	FJ939798	FJ940034	FJ939830	FJ940001	FJ940066	FJ939969	FJ939868	FJ940104	FJ939922
Dolichoderinae	*Iridomyrmex sanguineus*	CASENT0106154	FJ939767	FJ939799	FJ940035	FJ939831	FJ940002	FJ940067	FJ939970	FJ939869	FJ940105	FJ939923
Dolichoderinae	*Leptomyrmex burwelli*	CASENT0106001	EF012873	EF013001	EF013709	EF013129	EF013581	EF013274	EF013436	FJ939870	FJ940106	FJ939924
Dolichoderinae	*Leptomyrmex erythrocephalus*	CASENT0106077	AY703494	AY703561	AY703628	AY703695	AY703762	EF013275	EF013437	FJ939871	FJ940107	FJ939925
Dolichoderinae	*Linepithema humile*	CASENT0106119	EF012875	EF013003	EF013711	EF013131	EF013583	EF013277	EF013439	FJ939872	FJ940108	FJ939926
Dolichoderinae	*Linepithema keiteli*	CASENT0106160	FJ939768	FJ939800	FJ940036	FJ939832	FJ940003	FJ940068	FJ939971	FJ939873	FJ940109	FJ939927
Dolichoderinae	*Liometopum apiculatum*	CASENT0106033	EF012876	EF013004	EF013712	EF013132	EF013584	EF013278	EF013440	FJ939874	FJ940110	FJ939928
Dolichoderinae	*Liometopum occidentale*	CASENT0106078	AY867449	AY867465	AY867434	AY867481	AY867496	EF013279	EF013441	FJ939875	FJ940111	FJ939929
Dolichoderinae	*Loweriella boltoni*	CASENT0009998	FJ939769	FJ939801	FJ940037	FJ939833	FJ940004	FJ940069	FJ939972	FJ939876	FJ940112	FJ939930
Dolichoderinae	*Nebothriomyrmex majeri*	CASENT0106174	FJ939770	FJ939802	FJ940038	FJ939834	FJ940005	FJ940070	FJ939973	FJ939879	FJ940115	FJ939933
Dolichoderinae	*Ochetellus* cf *clarithorax*	CASENT0106166	FJ939771	FJ939803	FJ940039	FJ939835	FJ940006	FJ940071	FJ939974	FJ939881	FJ940117	FJ939935
Dolichoderinae	*Papyrius nitidus*	CASENT0106012	EF012905	EF013033	EF013741	EF013161	EF013613	EF013314	EF013476	FJ939882	FJ940118	FJ939936
Dolichoderinae	*Philidris cordatus*	CASENT0106011	EF012910	EF013038	EF013746	EF013166	EF013618	EF013320	EF013482	FJ939883	FJ940119	FJ939937
Dolichoderinae	*Ravavy miafina*	CASENT0080308	FJ939772	FJ939804	FJ940040	FJ939836	FJ940007	FJ940072	FJ939975	FJ939884	FJ940120	FJ939938
Dolichoderinae	*Tapinoma* MG03	CASENT0486546	FJ939773	FJ939805	FJ940041	FJ939837	FJ940008	FJ940073	FJ939976	FJ939886	FJ940122	FJ939940
Dolichoderinae	*Tapinoma melanocephalum*	CASENT0008659	FJ939774	FJ939806	FJ940042	FJ939838	FJ940009	FJ940074	FJ939977	FJ939887	FJ940123	FJ939941
Dolichoderinae	*Tapinoma sessile*	CASENT0106028	EF012938	EF013066	EF013774	EF013194	EF013646	EF013353	EF013515	FJ939888	FJ940124	FJ939942
Dolichoderinae	*Technomyrmex anterops*	CASENT0058901	FJ939775	FJ939807	FJ940043	FJ939839	FJ940010	FJ940075	FJ939978	FJ939889	FJ940125	FJ939943
Dolichoderinae	*Technomyrmex difficilis*	CASENT0106111	EF012940	EF013068	EF013776	EF013196	EF013648	EF013355	EF013517	FJ939890	FJ940126	FJ939944
Dolichoderinae	*Technomyrmex voeltzkowi*	CASENT0006839	EF012941	EF013069	EF013777	EF013197	EF013649	EF013356	EF013518	FJ939891	FJ940127	FJ939945
Dolichoderinae	*Turneria bidentata*	CASENT0106019	EF012948	EF013076	EF013784	EF013204	EF013656	EF013365	EF013527	FJ939893	FJ940129	FJ939946
Outgroups												
Aneuretinae	*Aneuretus simoni*	CASENT0007014	EF012833	EF012961	EF013669	EF013089	EF013541	EF013220	EF013382	FJ939840	FJ940076	FJ939894
Ectatomminae	*Rhytidoponera chalybaea*	CASENT0106000	EF012930	EF013058	EF013766	EF013186	EF013638	EF013343	EF013505	FJ939885	FJ940121	FJ939939
Myrmeciinae	*Myrmecia pyriformis*	CASENT0106088	AY703500	AY703567	AY703634	AY703701	AY703768	EF013292	EF013454	FJ939878	FJ940114	FJ939932
Myrmeciinae	*Nothomyrmecia macrops*	CASENT0106089	AY703501	AY703568	AY703635	AY703702	AY703769	EF013304	EF013466	FJ939880	FJ940116	FJ939934
Myrmicinae	*Manica bradleyi*	CASENT0106022	EF012878	EF013006	EF013714	EF013134	EF013586	EF013281	EF013443	FJ939877	FJ940113	FJ939931
Pseudomyrmecinae	*Tetraponera rufonigra*	CASENT0106099	AY703515	AY703582	AY703649	AY703716	AY703783	EF013362	EF013524	FJ939892	FJ940128	FJ940011

Note: Collection data for the voucher specimens are available on AntWeb (www.antweb.org).

TABLE 2. Primers used for sequencing argK (AK), enolase (EL), and CAD (CD) in ants

Primer	Sequence (5′ to 3′)	Coordinates	Source[a]
AK1F2	ATG GTT GAY GCY GCY GTT YTG GA	1–23	A
AK4F2	GTT GAY GCY GCY GTT YTG GAY AA	4–26	A
AK244F	GAY CCC ATY ATY GAC GAY TAY CA	244–266	A
AK346EF	AG GGT GAR TAC ATC GTR TCH ACT CG	~346–368	A
AK345ER	ACTYAC VGT VGG RTC RAG RTT	~345–331	A
AK392R	TC CAA RGA GCG RCC GCA TC	392–374	A
AK461R	GT GCT RGA YAC YTT CTC YTC CAT	461–439	A
AK720ER	AC CTG YCC RAG RTC ACC RCC CAT	~720–700	A
EL229F	GTA CCA TCA GGN GCN TCY ACY GG	229–251	B
EL427F	CCG AAT AAA TCS AAA CTT GGN GCR AAY GC	427–455	A
EL583F	ATY AAY GGW GGH TCH CAY GCT GG	583–605	A
EL488R	GC CTT GCA DAY WGC YAR RGA GAC ACC	488–463	A
EL644R	GT WGG HAR RAT CAT RAA YTC YTG CAT	644–619	A
EL794R	GC YTC YTT GTT CTC YAR AAT RTT YGG YGC	794–766	Modified from B
EL885R	CTT GTA GAA CTC NGA NGC NGC NAC RTC CAT	885–856	C
CD892F	GGY ACC GGR CGT TGY TAY ATG AC	892–914	A
CD1351F	ACG GTR CAG ACV TCV AAR GGH ATG GC	1351–1376	B
CD1423EF	AG GTR ATA CRA TCG GAR AGR CCD GA	~1423–1445	A
CD1491R	GCC GCA RTT NAG RGC RGT YTG YCC	1491–1468	B
CD1592R	GC RAA YAT YTT YCT RTC YTC RGT	1592–1570	A
CD1910R	CC GAG RGG RTC RAC RTT YTC CAT RTT RCA YAC	1910–1879	B
CD1955R	AG TGT YTG ACT CGG HGC DAC VAC RAT	1955–1930	A

Note: For a few samples containing poor-quality DNA or expanded introns, we employed additional primers to amplify shorter stretches of sequence (Table S1, available from http://www.sysbio.oxfordjournals.org/). Coordinates are based on GenBank sequences AF023619 (*Apis mellifera*, argK, numbering from the start of the coding sequence), XM_625053 (*A. mellifera*, enolase), and XM_393888 (*A. mellifera*, CAD), respectively.
[a]A = present study; B = Chris Schmidt (unpublished); C = Wild and Maddison 2008.

partitions of the 10-gene data set. The 5 main partition schemes were 1) "nuc1," in which the entire data set formed a single partition; 2) "nuc10," in which each of the 10 genes formed a separate partition; 3) "nuc18," in which each of the 8 protein-coding genes was divided into 2 partitions consisting of (i) codon positions 1 and 2 and (ii) codon position 3, and in which the 2 ribosomal genes formed the remaining 2 partitions; 4) "nuc22," in which each of 4 more conservative protein-coding genes (abdA, enolase, EF1aF1, EF1aF2) was divided into the same 2 partitions as in "nuc18," in which site-specific models were assigned to the other 4 protein-coding genes (i.e., each codon position formed a separate partition), and in which the 2 ribosomal genes formed the remaining 2 partitions; and 5) "nuc26," in which site-specific models were assigned to all 8 protein-coding genes and in which the 2 ribosomal genes formed the 2 remaining partitions. The choice of nucleotide substitution model for each partition (Table 3) was determined using the Akaike information criterion (Posada and Buckley 2004), as implemented in MrModeltest v2.2 (Nylander 2004). For each of the data partitions, we evaluated the homogeneity of base frequencies across taxa using PAUP* 4.0b10 (Swofford 2002). This revealed 2 partitions with heterogeneous base composition (wg third positions and EF1aF1 third positions), which in turn motivated 2 additional analyses: nuc1 and nuc26 (the latter renamed "nuc24") in which wg and EF1aF1 third positions were removed.

Taxon exclusion runs.—Among the 6 outgroup taxa, *A. simoni*, the sister taxon of Dolichoderinae, is of particular interest because of its rarity and taxonomic isolation. For reasons elaborated below (see Outgroup Sensitivity Experiments section), we conducted 2 sets of analyses for each partition model and for both Bayesian and ML bootstrap analyses, one in which *A. simoni* was included and one in which it was excluded.

Internal branch support.—With respect to the 4 major clades (tribes) of dolichoderines—here labeled B (Bothriomyrmecini), D (Dolichoderini), L (Leptomyrmecini), and T (Tapinomini)—all of our Bayesian and ML analyses generated the same unrooted (ingroup-only) tree, ((D,L),(B,T)), although the strength of support for the internal branch varied. With respect to the entire set of taxa, we always obtained 1 of 2 topological outcomes, as a result of the outgroups attaching to either the D branch or the T branch on the ingroup tree.

To isolate the effect of outgroup rooting on the strength of support for the ingroup branch separating (D,L) and (B,T), we examined the results of the 10 Bayesian and 10 likelihood analyses in which partitioning and outgroup composition were systematically varied. We did so by subjecting the post–burn-in trees (Bayesian analyses) and bootstrap trees (ML) from each analysis to a backbone constraint filter in PAUP* 4.0b10 (Swofford 2002) in which the ingroup branch of interest was the only resolved branch and from which the outgroup taxa had been excluded. The results of this filter indicate the strength of support for a key feature of ingroup topology independent of the influence of outgroup position.

Bayesian analyses.—We conducted Bayesian analyses using MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) with nucmodel = 4by4, nruns = 2, and samplefreq = 100, 200, 400, or 1000 depending on the number of generations. For partitioned analyses, all parameters were

TABLE 3. Sequence characteristics and models chosen for each data partition

Gene	No. sites	All taxa		Ingroup only		Dirichlet alpha	Model
		VS	PIS	VS	PIS		
18S	1858	66	35	44	25	1	GTR+I+G
28S	1809	260	130	168	91	10	GTR+I+G
abdA	600	163	127	137	108	10	HKY+I+G
abdA Pos 1	200	10	5	7	4	1	HKY+I
abdA Pos 2	200	7	1	6	1	1	F81
abdA Pos 1+2	400	17	6	13	5	1	HKY+I
abdA Pos 3	200	146	121	124	103	10	GTR+I+G
argK	672	258	207	220	175	10	SYM+I+G
argK Pos 1	224	40	27	30	21	2	SYM+I+G
argK Pos 2	224	19	11	16	9	1	GTR+I+G
argK Pos 1+2	448	59	38	46	30	2	GTR+I+G
argK Pos 3	224	199	169	174	145	10	GTR+G
CAD	1002	484	406	445	363	10	GTR+I+G
CAD Pos 1	334	104	74	89	67	2	HKY+I+G
CAD Pos 2	334	72	43	66	39	1	GTR+I+G
CAD Pos 1+2	668	176	117	155	106	2	GTR+I+G
CAD Pos 3	334	308	289	290	257	10	SYM+I+G
EF1a F1	1074	349	298	330	270	10	GTR+I+G
EF1a F1 Pos 1	358	24	17	24	17	1	GTR+I
EF1a F1 Pos 2	358	8	2	6	2	1	HKY+I
EF1a F1 Pos 1+2	716	32	19	30	19	1	GTR+I+G
EF1a F1 Pos 3	358	317	279	300	251	10	GTR+G
EF1a F2	516	175	162	147	117	10	K80+I+G
EF1a F2 Pos 1	172	12	11	6	5	1	GTR+I+G
EF1a F2 Pos 2	172	3	3	2	2	1	GTR+I+G
EF1a F2 Pos 1+2	344	15	14	8	7	1	GTR+I+G
EF1a F2 Pos 3	172	160	148	139	110	10	SYM+G
enolase	513	211	173	170	127	10	GTR+I+G
enolase Pos 1	171	41	30	33	22	1	GTR+I+G
enolase Pos 2	171	18	7	14	4	1	HKY+I+G
enolase Pos 1+2	342	59	37	47	26	1	GTR+G
enolase Pos 3	171	152	136	123	101	10	HKY+I+G
LW Rh	456	191	162	174	134	10	HKY+I+G
LW Rh Pos 1	152	47	40	42	34	2	SYM+I+G
LW Rh Pos 2	152	24	15	23	14	1	GTR+I+G
LW Rh Pos 1+2	304	71	55	65	48	2	GTR+I+G
LW Rh Pos 3	152	120	107	109	86	10	HKY+I+G
wg	414	207	153	162	113	10	GTR+I+G
wg Pos 1	138	45	28	29	20	2	GTR+I+G
wg Pos 2	138	38	24	27	17	1	GTR+G
wg Pos 1+2	276	83	52	56	37	2	GTR+I+G
wg Pos 3	138	124	101	106	76	10	GTR+G
All genes	8914	2364	1853	1997	1523		GTR+I+G

Note: VS = variable sites; PIS = parsimony-informative sites. Ingroup is Dolichoderinae. "Dirichlet alpha" is the Dirichlet distribution alpha parameter value assigned to that partition with the *prset* command in MrBayes.

unlinked across partitions except branch lengths and topology. Branch length rate multipliers were unlinked and initially assigned the default prior (*prset ratepr = variable*). All analyses were carried out using parallel processing (one chain per CPU) on networked Apple 8-core computers with Intel processors, in most cases with 8 chains per run. In a first round of analyses, runs employing the nuc1 and nuc10 models reached stationarity rapidly, within 1M to 4M generations, whereas the more highly partitioned analyses required many more burn-in generations and multiple paired runs. In this first round of partitioned analyses, we found that although most parameter values, including topology, appeared to have converged across runs (e.g., Potential Scale Reduction Factor convergence diagnostic ~1.0, average standard deviation [SD] of split frequencies <0.003), this was not true of total tree length (TL) nor of the partition rate multipliers (*m*). Estimates of TL were unreasonably high (TL 5.76–9.77 versus TL 1.31 with nuc1), and some of the *m* estimates were biologically implausible (e.g., much higher for second codon positions than third positions). Based on the findings of Marshall (Marshall et al. 2006; Marshall 2010) and the suggestions of an anonymous reviewer, we experimented with various modified settings in MrBayes to escape these suboptimal long-branch trees. The following strategy proved satisfactory in a second round of analyses: 1) Using the "*props*" command we increased the proposal rate from 1.000 to 10.000 and decreased the Dirichlet alpha parameter from 500 to 250 for the rate multipliers (proposal mechanism 26 in MrBayes). 2) We placed a shorter prior on the mean branch length, using the command *prset applyto = (all) brlenspr = unconstrained:exponential(100)*. This corresponds to a mean branch length prior of 0.01, in contrast to the MrBayes default of 0.1, and it more closely matches the mean branch length obtained in the

TABLE 4. Summary of Bayesian analyses

Model	Run	Generations	Burn-in	Marginal likelihood
nuc1	1	20M	1M	−54329.197 ± 0.072
nuc1	2	20M	1M	−54329.291 ± 0.075
nuc10	1	20M	3M	−53224.347 ± 0.133
nuc10	2	20M	3M	−53224.181 ± 0.138
nuc18	1	40M	10M	−51239.473 ± 0.192
nuc18	2	40M	10M	−51240.393 ± 0.176
nuc22	1	40M	20M	−51125.726 ± 0.222
nuc22	2	40M	20M	−51126.729 ± 0.243
nuc26	1	40M	27.5M	−50997.327 ± 0.359
nuc26	2	60M	40M	−50995.894 ± 0.288
nuc1/no *Aneuretus*	1	10M	1M	−52785.497 ± 0.101
nuc1/no *Aneuretus*	2	10M	1M	−52785.529 ± 0.103
nuc10/no *Aneuretus*	1	20M	5M	−51705.897 ± 0.132
nuc10/no *Aneuretus*	2	20M	5M	−51705.994 ± 0.143
nuc18/no *Aneuretus*	1	40M	5M	−49769.266 ± 0.156
nuc18/no *Aneuretus*	2	40M	5M	−49766.189 ± 0.159
nuc22/no *Aneuretus*	1	20M	10M	−49656.065 ± 0.239
nuc22/no *Aneuretus*	2	20M	10M	−49656.547 ± 0.224
nuc26/no *Aneuretus*	1	40M	20M	−49529.359 ± 0.197
nuc26/no *Aneuretus*	2	60M	20M	−49529.442 ± 0.247
nuc1/no wg&F1pos3	1	20M	10M	−45694.698 ± 0.354
nuc1/no wg&F1pos3	2	20M	10M	−45694.621 ± 0.355
nuc24/no wg&F1pos3	1	40M	20M	−43060.570 ± 0.234
nuc24/no wg&F1pos3	2	40M	20M	−43062.822 ± 0.297
nuc1/no wg&F1pos3/no *Aneuretus*	1	20M	10M	−44362.676 ± 0.354
nuc1/no wg&F1pos3/no *Aneuretus*	2	20M	10M	−44363.186 ± 0.365
nuc24/no wg&F1pos3/no *Aneuretus*	1	40M	20M	−41778.003 ± 0.233
nuc24/no wg&F1pos3/no *Aneuretus*	2	40M	20M	−41779.132 ± 0.282

unpartitioned (nuc1) analysis. 3) We applied a moderately informative Dirichlet prior to the rate multipliers, using alpha parameter values that reflected our prior expectation that 28S evolves more rapidly than 18S and that, for coding regions, third positions evolve faster than first positions, which evolve faster than second positions. These alterations markedly improved convergence between runs, decreased burn-in, increased the likelihoods, and yielded more plausible estimates of TL (1.2–1.3) and the rate multipliers (m). The results reported here are based on combining the post–burn-in data from the 2 runs that achieved the greatest marginal likelihoods, as summarized in Table 4. Marginal likelihoods were estimated from the harmonic means using Tracer v1.4 (Rambaut and Drummond 2007) by employing the weighted likelihood bootstrap estimator of Newton and Raftery (1994) as modified by Suchard et al. (2001), with standard error estimated using 1000 bootstrap pseudoreplicates.

Bayes' factor comparisons.—We used Bayes' factors (Kass and Raftery 1995) to evaluate the relative support for competing pairs of partition models. We calculated ln (Bayes' factor) as the difference in estimated marginal likelihoods for pairs of models, as described in Nylander et al. (2004).

Maximum likelihood.—We employed the program MrFisher (O'Meara 2008) to calculate ML bootstrap proportions under the same partition models analyzed with MrBayes. MrFisher is a modified version of MrBayes that retains the same nucleotide model specifications and the same facility for partitioned analyses but uses a simulated annealing algorithm to search for the optimal tree under a likelihood criterion. We carried out 200–800 ML bootstrap pseudoreplicates in MrFisher for all the models described above, running each pseudoreplicate for 2M generations. We compared the results generated by MrFisher with those obtained from GARLI v0.96 (Zwickl 2006) for 3 separate unpartitioned analyses. In all cases, tree topologies and bootstrap results were nearly identical.

Outgroup Sensitivity Experiments

Among the 6 outgroup taxa included in our data set, the closest relative to the Dolichoderinae is *Aneuretus simoni*, the sole surviving representative of a group (subfamily Aneuretinae) previously widespread in North America and Eurasia in the Tertiary (Dlussky and Rasnitsyn 2003). *Aneuretus simoni* is known from only a few rainforest sites in central Sri Lanka (Wilson et al. 1956). Were it not for the survival of this single relict species and—just as important—its discovery and recognition, our ability to reconstruct the phylogeny of the subfamily Dolichoderinae would depend on rooting with outgroups that are considerably more distantly related. Such "distant outgroup" problems affect other areas of ant phylogeny, including the root of the family Formicidae (Brady et al. 2006). New phylogenetically isolated ant species continue to be discovered, however, and they offer the possibility of resolving some of these problems (Rabeling et al. 2008). To explore the sensitivity of outgroup composition on phylogenetic estimation, we conducted Bayesian and likelihood analyses in

which *Aneuretus* was removed from the set of outgroup taxa, as described above.

Divergence Date Estimation

BEAST.—For divergence dating analyses using BEAST v1.4.8 (Drummond et al. 2006; Drummond and Rambaut 2007), lognormal prior age distributions were assigned to 6 internal nodes and to the root node, taking into account the entirety of the ant fossil record. The information we used to arrive at these decisions is given below. The 3 numbers listed for each calibration are our a priori ages (Ma) for these nodes, given as a 95% upper bound, median, and lower bound, respectively. Note that this last value coincides with the minimum age calibrations used for the r8s analyses (see below). Also provided in parentheses are the actual priors for the lognormal model parameters used by BEAST.

Calibration 1. *Dolichoderus quadripunctatus* group stem. 65-54-42 (mean 2.5, SD 0.4, zero offset 42.0). Four species from the *D. quadripunctatus* group have been recorded in Baltic and Rovno ambers (ca. 42 Ma), along with a considerable diversity of *Dolichoderus* species from other species groups (Dlussky 2002). We therefore consider it likely that the *D. quadripunctatus* group arose at least 5–10 million years earlier. We use this information to calibrate the node representing the most recent common ancestor (MRCA) of *D. pustulatus*, a member of the *quadripunctatus* group (MacKay 1993), and *D. decollatus*. Based on the current state of *Dolichoderus* systematics, we believe that this is an appropriately conservative assignment that takes into account uncertainty about the relationship of Southeast Asian species such as *D. erectilobus* (Dill 2002) to those of other regions. This placement is also supported by our fossil cross-validation analysis (see below).

Calibration 2. *Liometopum* stem, *Tapinoma* stem. 70-54-42 (mean 2.5, SD 0.5, zero offset 42.0). These 2 genera are sister taxa, and both are recorded from the mid-Eocene: *Liometopum* in Baltic amber (Dlussky 1997) and *Tapinoma* in Rovno amber (Dlussky and Perkovsky 2002). Because this is an instance where taxa on both sides of the node are present in the fossil record, we place an older upper bound on this node than on node 1. See comments below about *Tapinoma* under node 6.

Calibration 3. *Azteca* stem, *Gracilidris* stem. 70-40-15 (mean 3.2, SD 0.5, zero offset 15.0). These 2 genera are also sister taxa. Both are present and well differentiated in Dominican amber (Wilson 1985; Wild and Cuezzo 2006), pointing to an origin well before the fossil date of 15 Ma. Multiple species of *Azteca* have been found in Dominican amber, and this group comprises more than 100 extant species (Bolton et al. 2007).

Calibration 4. *Technomyrmex* stem. 70-50-30 (mean 3.0, SD 0.4, zero offset 30.0). There is a definitive *Technomyrmex* specimen from Sicilian amber (30 Ma) (Bolton 2007) and an unconfirmed report from Hat Creek amber (55 Ma) (Poinar et al. 1999). Curiously, *Technomyrmex* has not been found in Baltic amber. The genus is tropicopolitan with about 90 extant species, mostly in the Old World. *Technomyrmex caritatus* from Dominican amber (Brandão et al. 1999) may belong to a different genus (Bolton 2007).

Calibration 5. *Leptomyrmex* stem. 70-45-15 (mean 3.4, SD 0.4, zero offset 15.0). *Leptomyrmex* is recorded from Dominican amber (15 Ma) (Baroni Urbani and Wilson 1987), and there is a *Leptomyrmex*-like male—now placed in the monotypic, extinct genus *Leptomyrmula*—in Sicilian amber (32 Ma) (Emery 1891). The relationship of *Leptomyrmula* to *Leptomyrmex* has not been clarified, and we consider *Leptomyrmula* to be *incertae sedis* in Dolichoderinae. Extant species of *Leptomyrmex* are confined to eastern Australia, New Guinea, and New Caledonia (Shattuck 1999). Most species have wingless ergatoid queens and hence limited dispersal capabilities. If the genus had a former distribution that encompassed both Australia and the Neotropics, this implies a considerably older age than that of the Dominican amber fossil.

Calibration 6. Tapinomini crown. 80-65-55 (mean 2.3, SD 0.6, zero offset 55.0). There are reports of both *Technomyrmex* (Poinar et al. 1999) and *Tapinoma* (S. B. Archibold, cited in Moreau et al. 2006) from Hat Creek amber (55 Ma), but detailed documentation is lacking in both cases. We take a conservative course and consider this evidence for the presence of the more inclusive clade (Tapinomini) to which these genera belong. The extinct genus *Eotapinoma* is recorded from Sakhalin amber (60 Ma) and Canadian Cretaceous amber (75 Ma) (Dlussky 1988, 1999), but there is uncertainty about the placement of this fossil within the Dolichoderinae.

Root node calibration: 120-110-100 (mean 2.3, SD 0.4, zero offset 100.0). We define the root node in our dating analyses as the MRCA of dolichoderomorphs (Dolichoderinae and Aneuretinae) and myrmeciomorphs (Myrmeciinae and Pseudomyrmecinae) (sensu Brady et al. 2006). The lower bound for this node is set by *Burmomyrma*, a putative aneuretine in Burmese amber (100 Ma; Dlussky 1996) and the oldest known fossil in the group. The median and upper bounds are based on previous ant-wide molecular divergence dating analyses that included information from the entire fossil record of aculeate Hymenoptera (Brady et al. 2006). Among other fossils that could be used as calibration points, we did not accept reports of *Anonychomyrma*, *Iridomyrmex*, and *Ochetellus* fossils from Baltic amber due to uncertainty over their taxonomic assignment (see also Dlussky and Rasnitsyn 2003).

The data were partitioned according to the nuc26 partition scheme described above. The nucleotide substitution model used for each partition was identical to that used for the nuc26 MrBayes analyses except that base frequencies were estimated for all partitions. An uncorrelated lognormal relaxed-clock model was implemented with a Yule process as the tree prior. Markov chain Monte Carlo searches were run for 20,000,000 generations with the first 2,000,000 generations discarded as burn-in. These searches achieved sufficient mixing as assessed by the high effective sample size values for

most parameters, plateaus for divergence time estimates over generations after burn-in, and repeatability of results over multiple independent runs. The results (after burn-in) from 2 independent runs were assessed using Tracer v1.4 (Rambaut and Drummond 2007) and then combined manually and visualized with FigTree v1.1.2. (Rambaut and Drummond 2008).

Penalized likelihood (r8s).—All divergence dating analyses with r8s v1.71 (Sanderson 2003) were conducted using the penalized likelihood method (Sanderson 2002), employing the truncated Newton algorithm and with the optimal smoothing parameter inferred using cross-validation. We used fossil-based minimum-age constraints on the 6 nodes discussed above. We evaluated 2 alternative nodal placements for one of these minimum-age constraints (Calibration 1) using fossil cross-calibration (Near and Sanderson 2004; Rutschmann et al. 2007). The *D. quadripunctatus* group is well represented in Baltic amber (Dlussky 2002). *Dolichoderus pustulatus* is an extant member of this group (MacKay 1993), whereas *D. erectilobus* is placed in the Southeast Asian *D. cuspidatus* group (Dill 2002). A strict taxonomic interpretation would place calibration at the MRCA of *D. pustulatus* and *D. erectilobus* (Calibration 1A in Table 5). However, given the lack of a comprehensive phylogenetic hypothesis for *Dolichoderus* species groups, we also considered a more conservative placement for this calibration, one node deeper in the tree at the MRCA of *D. erectilobus* and *D. decollatus* (Calibration 1B in Table 5). Fossil cross-validation involves fixing the age of each calibration separately and gauging its effect on the inferred ages for the other calibration nodes (Near and Sanderson 2004; Near et al. 2005). For this analysis, we used the topology and branch lengths of the nuc26 model tree obtained in our second round of Bayesian analyses. To check for multiple optima, analyses were run from multiple random starting points and with solutions randomly perturbed. The cross-validation results (Table 5) reveal that Calibration 1A is highly inconsistent with the other fossil calibrations, yielding estimates for these other nodes far older than the age of ants (115–135 Ma; Brady et al. 2006) and even Hymenoptera as a whole (∼230 Ma; Grimaldi and Engel 2005). Calibration 1B in contrast provides plausible age estimates that fall within the range of the other calibrations. We thus used Calibration 1B for all dating analyses.

The r8s program requires that at least one node be assigned either a maximum age or a fixed age. Using a maximum-age constraint for the root node proved unsatisfactory as the program simply inferred the age of that node to be identical to the chosen maximum age. As noted in other studies (e.g., Brady et al. 2006; Yang and Rannala 2006; Hugall et al. 2007), this practice can considerably inflate divergence dating estimates. Instead, we used a range of plausible fixed ages for the root node (the MRCA of dolichoderomorphs and myrmeciomorphs) in order to establish upper and lower bounds for our estimates. These fixed root ages were 100 and 120 Ma, as justified in the Root node calibration section above.

We obtained the mean and SD of node times using the profile command on the pool of all 6415 post–burn-in trees entirely congruent with the majority-rule consensus, including all compatible groups from the Bayesian nuc26 analyses. In this manner, we incorporated uncertainty in branch length estimation under our most favored partitioned model in the Bayesian analyses. Confidence intervals (CI) were calculated as ±1.96 SD.

Biogeographic Inferences

Dolichoderine ants include more than 900 described species, occurring in all of the world's major biogeographic regions (Shattuck 1992; Bolton et al. 2007). Our sample of 48 species from 26 genera broadly reflects the distribution of these ants, but it is not fully representative. For example, the 3 species of *Technomyrmex* that we sequenced—chosen to cover the range of phenotypic diversity in the genus (Bolton 2007)—are from 2 of the 5 biogeographic regions in which that genus occurs. We have no evidence for nonmonophyly of any of the genera. Hence, for the purposes of including more complete distributional information and inferring ancestral areas, we collapsed the species-level Bayesian tree to a genus-level cladogram, coded each terminal taxon for its known global distribution (Shattuck 1992, 1994), and employed a topology-based method of dispersal-vicariance analysis, as implemented in the program

TABLE 5. Fossil cross-validation

Calibration	Age	Node							∑dev	∑%dev
		1A	1B	2	3	4	5	6		
1A	42	Fixed	125	492	501	545	552	598	2616	62.3
1B	42	28	Fixed	72	74	79	81	87	251	6.0
2	42	43	68	Fixed	75	67	82	73	209	5.0
3	15	10	15	16	Fixed	18	17	19	135	9.0
4	30	19	30	28	33	Fixed	36	34	108	3.6
5	15	9	14	14	14	16	Fixed	17	143	9.5
6	55	33	51	46	57	51	62	Fixed	132	2.4

Note: Each row represents a separate analysis in which the calibration node ("Calibration") is fixed with the proposed age ("Age") and dates for the other nodes estimated using r8s. Calibrations are explained in the text. Nodes 1A and 1B are alternative calibration points using the same fossil evidence. ∑dev is the sum of the absolute values of differences between the estimated and proposed ages for each calibration node, and ∑%dev is this deviance expressed as a proportion of the proposed age for that calibration (Hugall et al. 2007). All ages are Ma.

DIVA 1.1 (Ronquist 1996). DIVA treats speciation by vicariance as the null model and attempts to minimize the number of implied dispersal and extinction events (Ronquist 1997). Importantly, it allows both terminal and ancestral nodes to occupy more than one area and is appropriate for complex biogeographic histories where there is no expectation of simple hierarchical relationships among areas (Sanmartín 2007).

Results

Phylogeny

In all analyses, across all character partitioning strategies, we find strong support (Bayesian posterior probability [PP] 1.00, ML bootstraps 92–100) for dolichoderine monophyly and for 4 mutually exclusive lineages within the subfamily: 1) genus *Dolichoderus*, 2) *Bothriomyrmex* and allies, 3) *Tapinoma*, *Technomyrmex*, and related genera, and 4) a large clade of Neotropical and Australian species (Fig. 1 and Table 6). These well-supported major clades provide the basis for a revised higher classification of the subfamily (Appendix) and are assigned the following oldest available tribal names: Dolichoderini, Bothriomyrmecini, Tapinomini, and Leptomyrmecini. There is good phylogenetic resolution among taxa within these 4 tribes, with most nodes moderately to very highly supported. In the few areas of the tree with very short branch lengths, however, there remains some uncertainty about intergeneric relationships (Figs. 1 and 2), most notably regarding the positions of *Doleromyrma* and *Nebothriomyrmex* within the Leptomyrmecini.

Relationships among the 4 major lineages (tribes) of Dolichoderinae are less clear, but all character partitions and modes of analysis yield the same *unrooted* (ingroup-only) topology: ((Dolichoderini, Leptomyrmecini), (Bothriomyrmecini, Tapinomini)), although the strength of support for the (DL-BT) bipartition varies (Figs. 1 and 3). Thus, estimating the phylogeny of the deeper branches of dolichoderines largely becomes an issue of the placement of the root.

We find that varying both partitioning and outgroup composition has striking effects on the position of the dolichoderine root (Figs. 1 and 3). In general, more highly partitioned analyses (nuc18, nuc22, and nuc26) and those with *Aneuretus* included in the set of outgroup taxa favor Tapinomini as sister to all other dolichoderines. When *Aneuretus* is excluded and/or when fewer partitions are employed (nuc1, nuc10), analyses yield Dolichoderini as sister to the other 3 tribes.

Bayes' factor comparisons strongly support the more partitioned models, with nuc26 favored over nuc22 (lnBF = 129.74), nuc22 favored over nuc18 (lnBF = 113.75), and nuc18 strongly favored over nuc10 (lnBF = 1984.30) (Fig. 4). Thus, the best-supported models and the analyses in which a crucial outgroup taxon (*Aneuretus*) is included both uphold the same result: placement of the dolichoderine root on the branch leading to Tapinomini. We therefore treat the nuc26 Bayesian consensus tree as our "preferred tree" (Figs. 1d and 2) and assume this topology for biogeographic analyses using DIVA and divergence dating estimates using r8s. For dating estimates using BEAST, the program infers the phylogeny as well as divergence times, and in fact, BEAST also recovered the same tree under the nuc26 model (see below).

Yet another line of evidence in favor of rooting the dolichoderine tree on the Tapinomini branch comes from analyses in which 2 character partitions were removed. Examination of base frequencies revealed homogeneity across taxa for all but 2 partitions: third codon positions in wg and third codon positions in EF1aF1. When those positions were excluded, support for rooting on the Tapinomini branch increases under both Bayesian and likelihood methods, although this support continues to be adversely affected by the removal of *Aneuretus* (Fig. 5).

From the perspective that better supported groups are more likely to be diagnosable, we examined whether our data set contained any obvious "molecular synapomorphies" for either BLT (Bothriomyrmecini + Leptomyrmecini + Tapinomini) or BLD (Bothriomyrmecini + Leptomyrmecini + Dolichoderini), the clades resulting from rooting on the Dolichoderini branch and the Tapinomini branch, respectively. We did this by examining apomorphy lists for the Bayesian nuc1 and nuc26 trees (Fig. 1a,d, respectively), as generated by PAUP* under the parsimony criterion. Most sites reconstructed by PAUP* as changing on the branches subtending BLT and BLD are quite homoplastic (consistency index < 0.500), and there are no diagnostic or near-diagnostic apomorphies for BLT. On the other hand, there are 3 near-diagnostic sites for BLD, involving 28S (position 5226: G), abdA (position 1374: A) and enolase (position 8699: T). In fact, the last 2 sites in combination uniquely diagnose the group, at least for the set of exemplar taxa sequenced in this study.

Divergence Dates

Analysis of the data with BEAST using the nuc26 partition strategy resulted in a chronogram whose topology was identical with the MrBayes results under the same partitioning scheme. Notably, the BEAST analysis placed the root of the dolichoderine tree on the branch leading to Tapinomini with solid support (Fig. 6).

The BEAST chronogram estimates the crown-group origin of Dolichoderinae at 67 Ma (CI = 61–74; Fig. 6 and Table 7). The origin of the crown dolichoderomorphs (Dolichoderinae + Aneuretinae) occurred considerably earlier, approximately 98 Ma (CI = 82–114). The mean crown-group ages of the 4 dolichoderine tribes fall between 57 Ma (Tapinomini) and 42 Ma (Bothriomyrmecini). A large, well-supported clade consisting of almost exclusively Australian genera ("DNAPPTOFI") originated between approximately 23 Ma (crown-group age; CI = 17–30) and 33 Ma (stem-group age; CI = 24–43).

Divergence date estimates from r8s were generally similar to those generated using BEAST (Table 7). Some

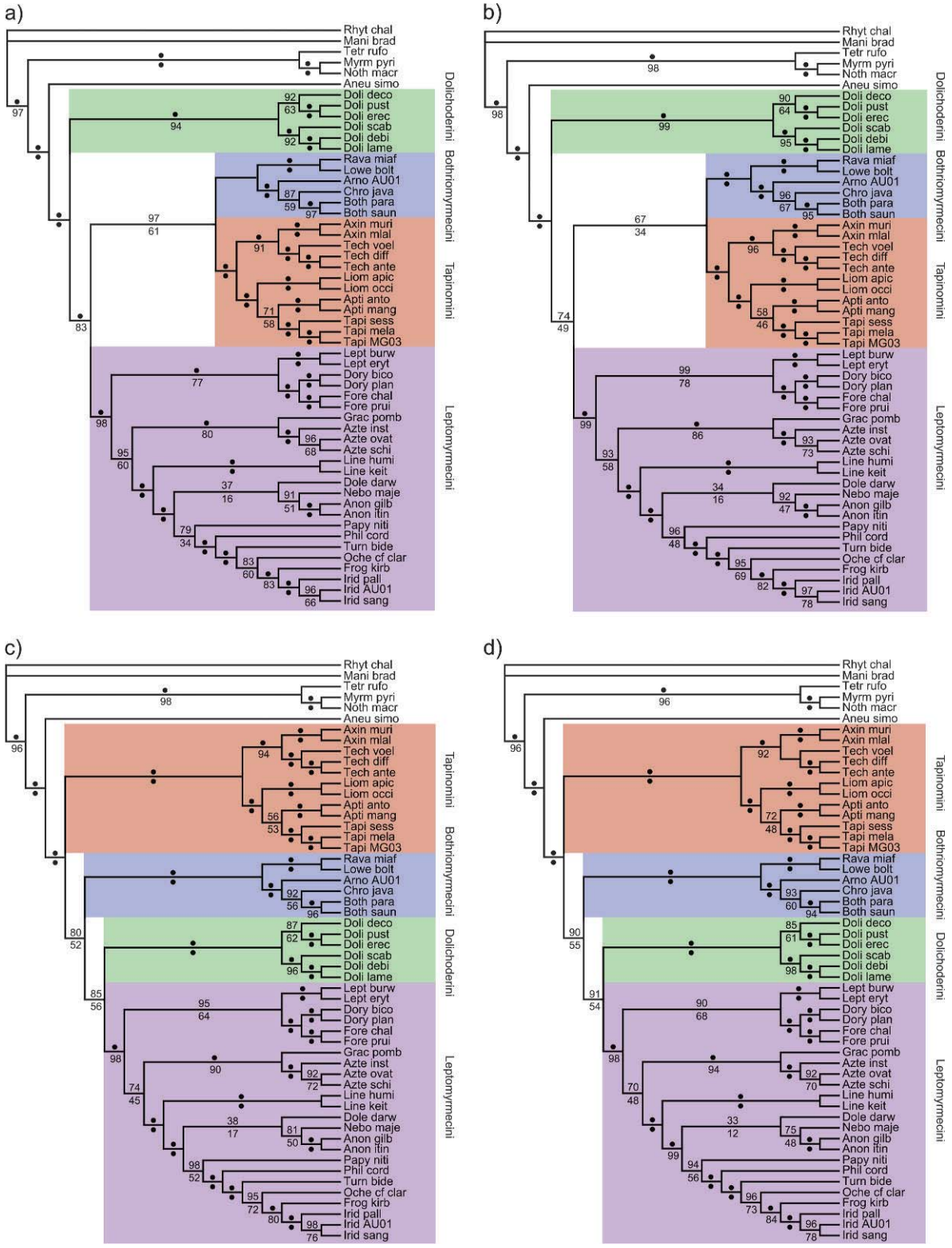

FIGURE 1. Results of analyses under 4 models of data partitioning: (a) nuc1, in which the entire data set formed a single partition; (b) nuc10, in which each of the 10 genes formed a separate partition; (c) nuc18, in which each of the 8 protein-coding genes was divided into 2 partitions consisting of (i) codon positions 1 and 2 and (ii) codon position 3, and in which the 2 ribosomal genes formed the remaining 2 partitions; and (d) nuc26, in which site-specific models were assigned to each protein-coding gene, that is, in which each codon position formed a separate partition for a total of 24 partitions and in which the 2 ribosomal genes formed the 2 remaining partitions. Numbers above branches are Bayesian PPs (×100) from MrBayes; numbers below branches are ML bootstrap proportions (×100) from MrFisher. Nodes with a dot received a support value of 100. Taxon names are represented by the first 4 characters of the genus name and species name; for full species names refer to Figure 2.

TABLE 6. Support values for tribes and other major clades, under different model conditions, based on Bayesian PPs and ML bootstraps

Clade	Bayesian				ML			
	nuc1	nuc10	nuc18	nuc26	nuc1	nuc10	nuc18	nuc26
Dolichoderomorphs	1.00	1.00	1.00	1.00	100	100	100	100
Dolichoderinae	1.00	1.00	1.00	1.00	100	100	100	100
Tapinomini	1.00	1.00	1.00	1.00	100	100	100	100
Bothriomyrmecini	1.00	1.00	1.00	1.00	100	100	100	100
Dolichoderini	1.00	1.00	1.00	1.00	94	99	100	100
Leptomyrmecini	1.00	1.00	1.00	1.00	98	99	98	98
LFD[a]	1.00	0.99	0.95	0.90	77	78	64	68
GA[b]	1.00	1.00	1.00	1.00	80	86	90	94
DNAPPTOFI[c]	1.00	1.00	1.00	1.00	100	100	100	99
L-DNAPPTOFI[d]	1.00	1.00	1.00	1.00	100	100	100	100

Note: [a]LFD = *Leptomyrmex* + *Forelius* + *Dorymyrmex*.
[b]GA = *Gracilidris* + *Azteca*.
[c]DNAPPTOFI = Australian clade: MRCA of *Anonychomyrma* and *Iridomyrmex*.
[d]L-DNAPPTOFI = *Linepithema* + Australian clade.

deeper nodes such as Dolichoderinae and Dolichoderini were estimated by r8s to be 4–7 million years younger compared with the BEAST values, whereas some shallower nodes showed slightly older dates in r8s, including the Australian "DNAPPTOFI" clade which is 6 million years older in the r8s analysis. The age estimate for the dolichoderomorph node was sensitive to alternative root ages in the r8s analysis, varying by 13 million years between assigned root ages of 100 and 120 Ma. The BEAST estimate for this node was 98 Ma, with a credibility interval (82–114 Ma) that essentially encompasses the range of values generated by r8s with the 2 assigned root ages: 84 (81–88) Ma for root age = 100 Ma and 97 (92–102) Ma for root age = 120 Ma. The age estimates of nodes within Dolichoderinae, by contrast, were not very sensitive to alternative root ages in r8s, varying by 0–3 million years. The confidence intervals generated in the r8s analyses were considerably narrower compared with BEAST, presumably at least in part because r8s does not incorporate topological uncertainty. These r8s results are based on our final Bayesian nuc26 estimate with a TL of 1.30. A preliminary r8s analysis based on a phylogram with a suboptimal TL of 5.76 yielded estimates that differed at most by less than 1 million years (results not shown), indicating that the inflated TL produced by the default MrBayes settings did not adversely affect divergence date estimates.

Biogeography

DIVA suggests the following biogeographic scenario for the Dolichoderinae. The ancestral dolichoderine is inferred to have been widespread, occupying a composite of all 6 biogeographic regions (Fig. 7), although this may be partly an artifact of the way DIVA optimizes toward the base of the tree (Sanmartín 2007). The early branching lineages Tapinomini and Bothriomyrmecini are estimated to have their crown-group origins in the Afrotropical and Oriental regions, respectively. Both clades have remained concentrated in the Paleotropics, but a few representatives have colonized the Nearctic and Neotropical regions (examples include species in the genera *Bothriomyrmex*, *Technomyrmex*, *Liometopum*, and *Tapinoma*). Because we treat the genus *Dolichoderus* (= tribe Dolichoderini) as a single entity, we cannot make a statement about crown-group distribution, but this clade currently has a widespread distribution, being absent only from the Afrotropics. The remaining lineage, tribe Leptomyrmecini, originated and diversified in the Neotropics, where it is now represented by hundreds of species, but leptomyrmecines also invaded North America (*Forelius*, *Dorymyrmex*) and Australia. One of the 2 implied dispersal events to Australia, involving the common ancestor of *Linepithema* and *Iridomyrmex*, led to a spectacular radiation that has produced some of that continent's most dominant ants (Andersen 1995). Our divergence dating estimates place this Australian radiation in the Miocene.

DISCUSSION

Comparison with Previous Studies

The genus-level classification of Dolichoderinae is relatively stable, thanks to a comprehensive morphological revision (Shattuck 1992) that carefully evaluated the status of all genus group taxa. In the present study, we found no evidence for nonmonophyly of any of the currently recognized extant genera. In contrast, we find limited support for previous hypotheses about relationships among genera. Earlier studies of phylogenetic relationships among dolichoderine genera were based on morphology (Shattuck 1995; Brandão et al. 1999) and the mitochondrial gene cytochrome b (Chiotis et al. 2000). These studies produced trees with weak support at most nodes. In general, our results do not agree with the topologies recovered in these earlier analyses, although there are some elements in common: a clade comprising *Dorymyrmex* and *Forelius*, for example, and subsets of other genera from the tribes Leptomyrmecini and Tapinomini. The morphological studies suggested that *Leptomyrmex* is sister to the rest of the Dolichoderinae, an arrangement not supported by our results.

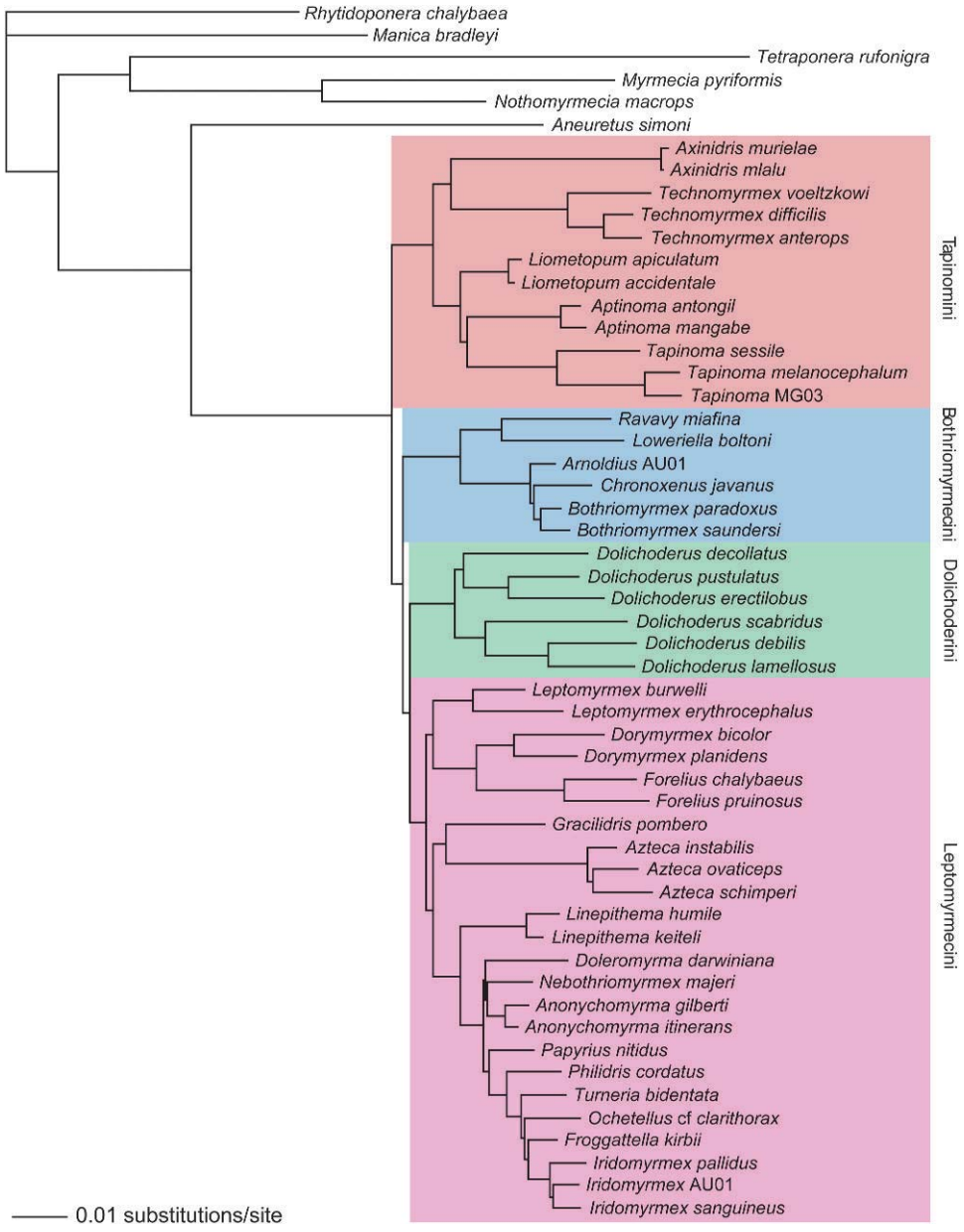

FIGURE 2. Phylogram of Dolichoderinae based on Bayesian analysis of the most partitioned model (nuc26). This corresponds to the cladogram in Figure 1d.

Two multi-gene molecular phylogenetic analyses of ants as a whole (Brady et al. 2006; Moreau et al. 2006) each included modest representation of dolichoderine ants and yielded results partially concordant with those reported here. Brady et al. (2006) found *Dolichoderus* to be sister to the other Dolichoderinae (PP=1.00), whereas we now conclude, based on multiple lines of evidence, that this result is less well supported than placement of the root on the Tapinomini branch (Fig. 3). Moreau et al. (2006) obtained Tapinomini as sister to the remaining dolichoderines (PP = 0.71), but they also recovered a *Dolichoderus* + *Bothriomyrmex* clade (PP = 0.71), so that their unrooted topology does not correspond to the one that we consistently obtain in this study.

Dolichoderine Evolution

The position of *Dolichoderus* within Dolichoderinae has implications for the evolution of body form in the subfamily. Workers of *Dolichoderus* have thick integuments with well-developed cuticular sculpture, and they frequently have strong body armament (spines) (Shattuck 1992), features essentially absent from the remaining dolichoderines but present in many other ants.

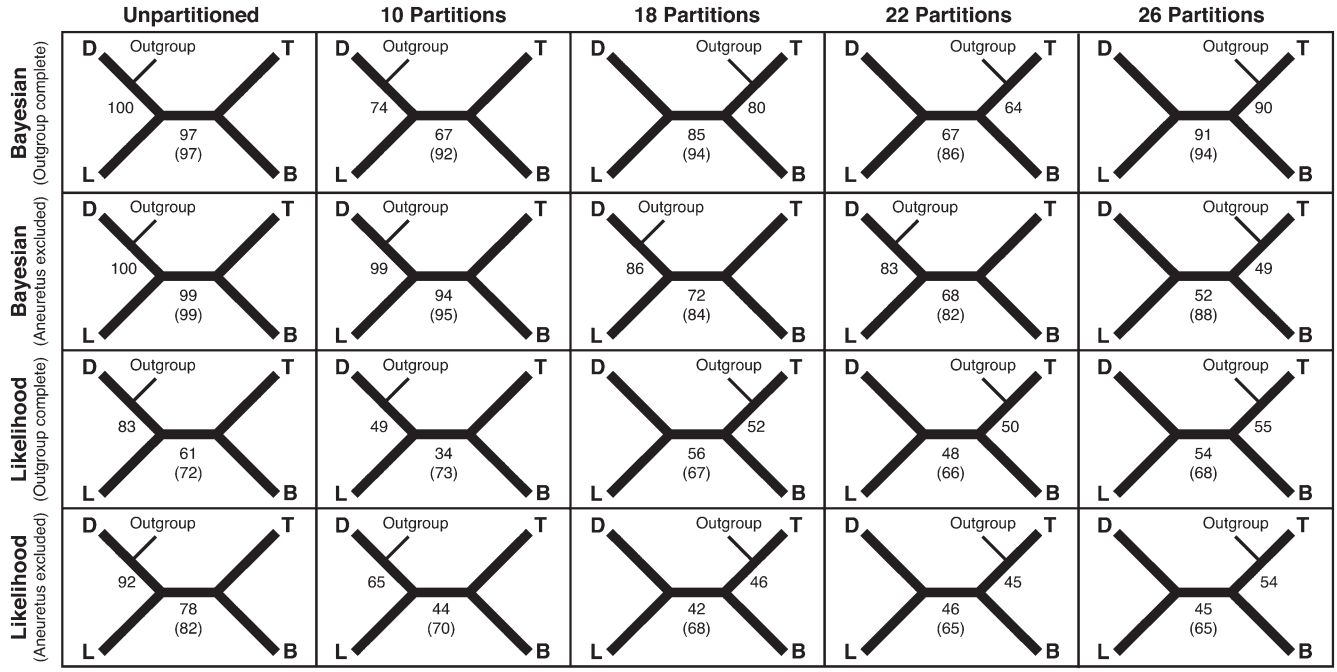

FIGURE 3. The effects of data partitioning and of including/excluding from the outgroup the relict, monotypic genus *Aneuretus*, the sister group of the Dolichoderinae. D = Dolichoderini, L = Leptomyrmecini, T = Tapinomini, and B = Bothriomyrmecini. In all outcomes, support for the monophyly of the Dolichoderinae has a PP of 1.00 (Bayesian) and a bootstrap proportion of 1.00 (ML) and the unrooted (ingroup only) topology remains stable. Outcomes vary, however, in the position of the root and in support for the branch separating (D,L) and (T,B), with a general trend toward rooting at D when data are divided into fewer partitions and/or when *Aneuretus* is excluded versus rooting at T when data are more highly partitioned and/or when *Aneuretus* is included. Bayesian analyses were conducted using MrBayes and ML analyses using MrFisher. Topologies and support values are based on majority-rule consensus trees, including all compatible groups for the post–burn-in trees (Bayesian analyses) or bootstrap trees (ML analyses). Numbers in parentheses indicate support for the ingroup branch separating (D,L) and (T,B) when considering ingroup taxa only, that is, when erosion of support solely due to the repositioning of the outgroup is ignored.

Given that *Dolichoderus* is nested within the dolichoderine tree, the thin flexible cuticle and reduced body sculpture typical of most dolichoderines—as well as their sister group, the Aneuretinae—is most parsimoniously interpreted as the ancestral condition for the subfamily. The thick cuticular sculpture and armament of *Dolichoderus* is therefore likely to represent a convergence with well-armored ants in other subfamilies.

Tapinoma, *Aptinoma,* and *Technomyrmex* are unique among ants in having a highly reduced petiole (Shattuck 1992; Bolton 2007). In our analyses, they are closely related but they do not form a monophyletic group (Fig. 1). Our phylogenetic results indicate that the highly reduced petiole evolved at least twice or that there was reversal in *Axinidris* and *Liometopum*.

Historical Biogeography

Both divergence dating methods (BEAST and r8s) yield estimates for the origin of crown-group Dolichoderinae in the Paleocene (~60–67 Ma) and indicate that this was preceded by a long period (~30 million years) of stem lineage evolution. Early dolichoderine-like fossils such as *Eotapinoma* (Cretaceous-Paleocene) and *Zherichinius* (Paleocene), whose placement in the subfamily has been a matter of uncertainty (Dlussky and Rasnitsyn 2003), likely represent members of dolichoderine stem lineages. Thus, the relatively long branch inferred for stem group Dolichoderinae in our chronograms can be attributed to a series of extinctions of these early dolichoderine lineages. These fossils and the first fossil crown-group representatives of the subfamily are

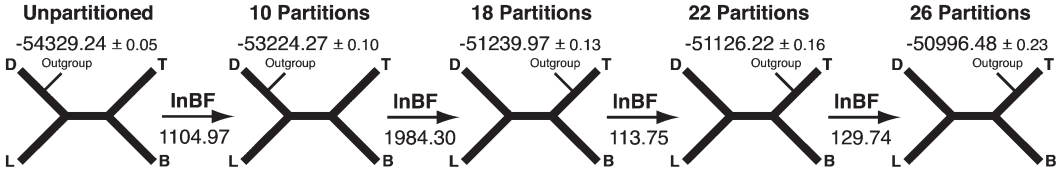

FIGURE 4. Bayes' factor (BF) comparisons of post–burn-in marginal likelihoods, calculated in Tracer using the method of Newton and Raftery (1994) as modified by Suchard et al. (2001), for Bayesian analyses in which data partitioning was varied. As judged by BFs, more highly partitioned models confer significantly higher likelihoods upon the data than do the less partitioned models. BFs are given as lnBF. Taxon abbreviations as in Figure 3.

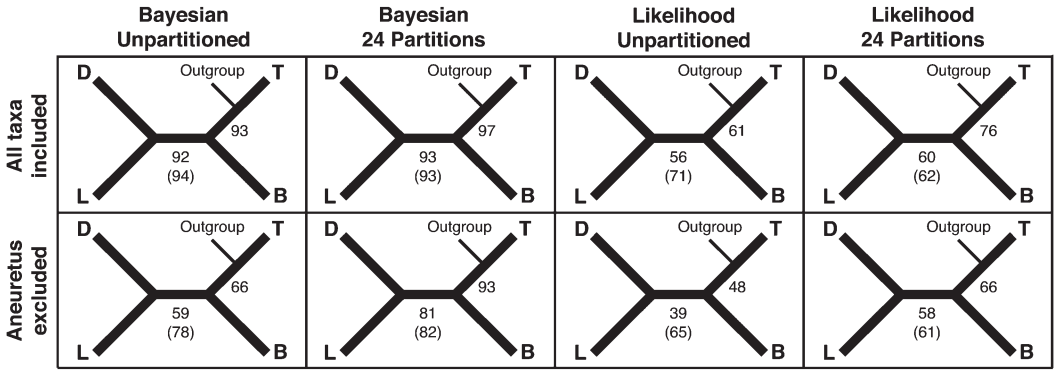

FIGURE 5. Results of analyses from which 2 data partitions with heterogeneous base composition (wg and EF1aF1 third positions) were excluded. In analyses that include all taxa, support for the topology rooted at the Tapinomini (T) is much improved compared with analyses in which the heterogeneous partitions are included (cf. Fig. 3). Support for this rooting is eroded when *Aneuretus*, the relict, monotypic sister group of the Dolichoderinae, is excluded (Row 2), although less so than in analyses in which the heterogeneous positions are included (cf. Fig. 3). Numbers in parentheses indicate support for the ingroup branch separating (D,L) and (T,B) when considering ingroup taxa only, that is, when erosion of support solely due to the repositioning of the outgroup is ignored. Taxon abbreviations as in Figure 3.

from North America and Eurasia (Dlussky 1997, 1999; Poinar et al. 1999), implicating a northern hemisphere origin for Dolichoderinae.

Our divergence dating and DIVA analyses suggest that the crown-group Tapinomini, the sister group of all other extant dolichoderines, arose in the Afrotropics

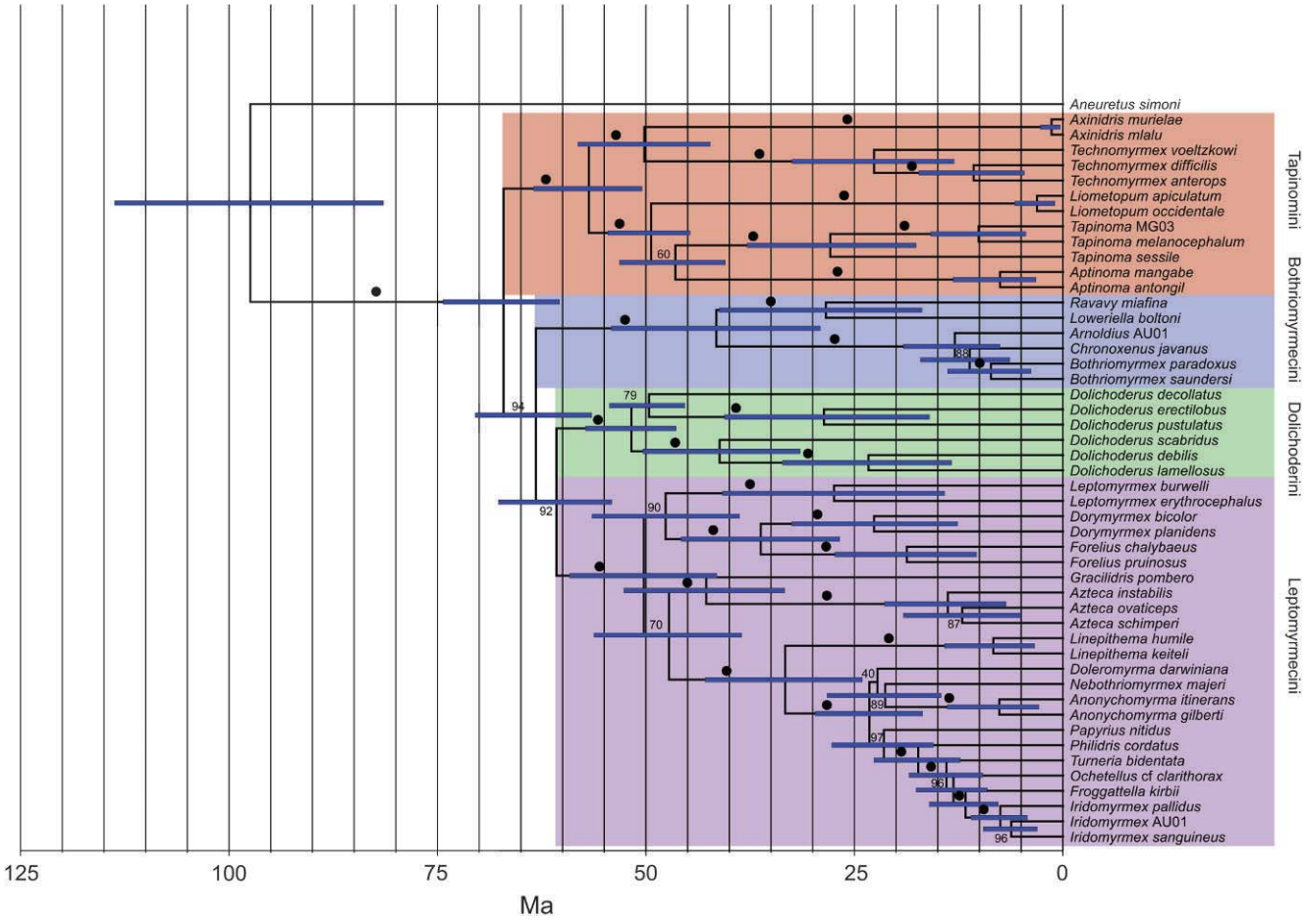

FIGURE 6. Topology and chronogram for the Dolichoderinae based on analyses using a 26-partition strategy with BEAST. Numbers above branches are Bayesian PPs (×100), with a dot indicating a support value of 100. Horizontal blue bars represent the 95% credibility limits for node ages.

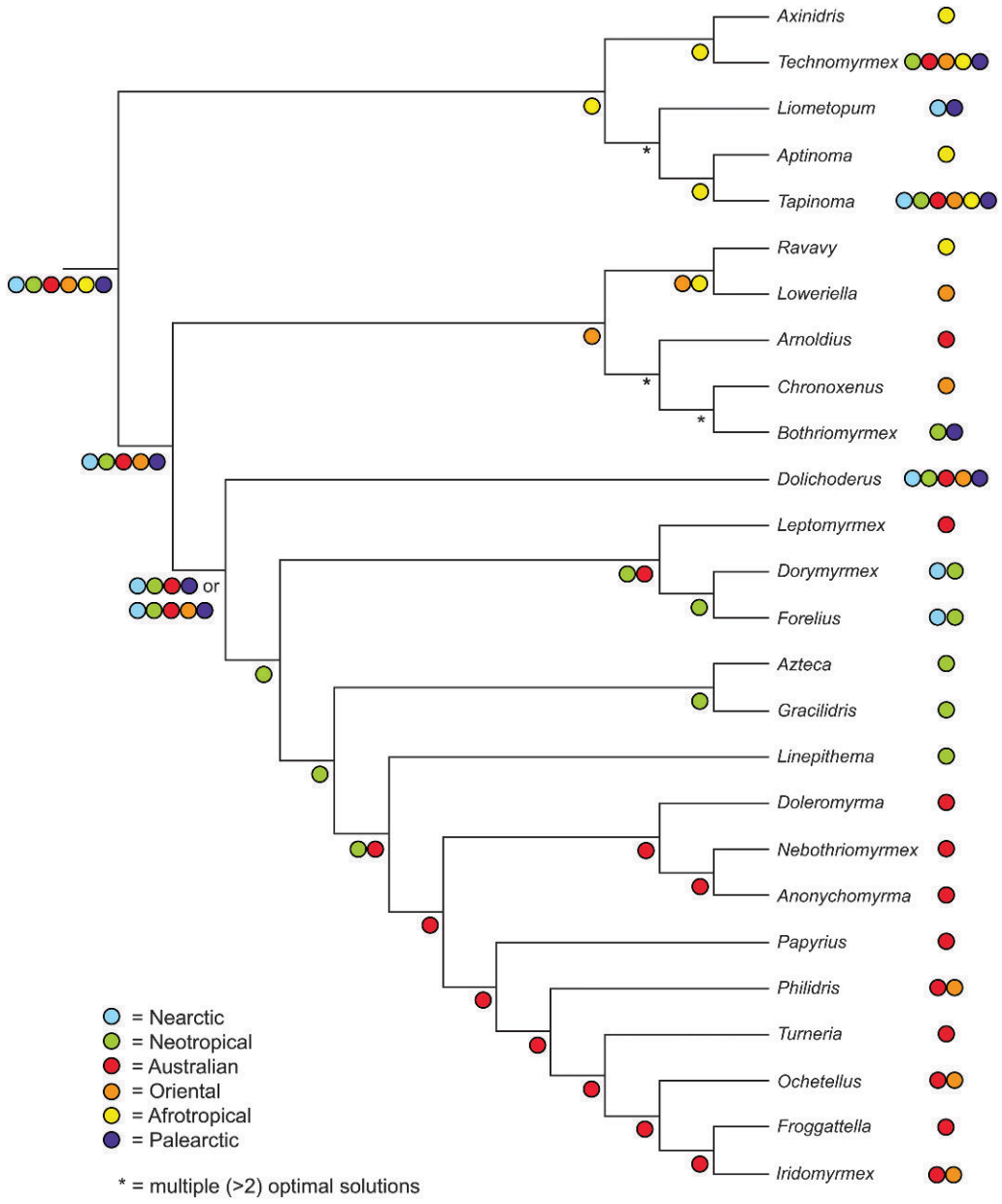

FIGURE 7. Biogeographic history of dolichoderine ant genera as reconstructed by DIVA. In this analysis, the tree based on 48 exemplar species (Fig. 2) was collapsed to a genus-level cladogram. This allowed the incorporation of more complete distributional information about these ants beyond that contributed by the limited species sample.

<60 Ma. The presence of this group in Canadian Hat Creek amber (55 Ma) and Baltic amber (~42 Ma) indicates that it must have rapidly radiated and dispersed to Europe and North America, if this scenario is correct. Dolichoderine ants are numerically dominant in Baltic amber, constituting about two-thirds of all ant inclusions, and most of these appear to be tapinomines (Wheeler 1915; Dlussky and Rasnitsyn 2003). In younger fossil deposits from the Holarctic region, the relative abundance of dolichoderines is greatly reduced (e.g., 4% of all ants in a Middle Miocene deposit from Stavropol, Russia—Dlussky and Rasnitsyn 2003). The tribe Bothriomyrmecini, sister group of the remaining dolichoderines, also appears to have originated in the Paleotropics (more specifically in the Oriental region, under DIVA optimization), and this clade has remained almost entirely confined to the Old World. We made no attempt to estimate the geographic origin of Dolichoderini because we treated the sole genus, *Dolichoderus*, as a single unit in DIVA. Future studies of this widespread genus, found in all biogeographic regions except the Afrotropics, would be illuminating.

The fourth major lineage of dolichoderines, tribe Leptomyrmecini, arose in the Neotropics about 50 Ma and has subsequently become a prominent and species-rich group in this region. The genus *Azteca*, for example,

TABLE 7. Divergence time estimates (Ma) and 95% credibility/confidence intervals (CI) for selected crown-group clades using BEAST and r8s

Clade	BEAST		r8s (root = 100)		r8s (root = 120)	
	Mean	CI	Mean	CI	Mean	CI
Dolichoderomorphs	98	82–114	84	81–88	97	92–102
Dolichoderinae	67	61–74	60	59–62	63	60–65
Tapinomini	57	51–64	55	55–55	55	55–55
Bothriomyrmecini	42	29–54	40	36–44	42	37–46
Dolichoderini	52	47–57	45	42–47	46	43–49
Leptomyrmecini	50	42–59	51	48–55	53	49–57
LFD	48	39–57	50	46–53	51	47–55
GA	43	34–53	45	41–49	47	42–51
DNAPPTOFI	23	17–30	29	25–33	30	25–34
L-DNAPPTOFI	33	24–43	39	34–43	40	35–45
Azteca	14	7–22	12	10–14	13	11–15
Iridomyrmex	8	4–11	10	8–12	10	8–12
Linepithema	8	4–14	10	8–13	11	8–13

Note: Clade acronyms are defined in Table 6.

contains more than 100 described species, and these ants frequently dominate arboreal ant communities in the New World tropics (Longino 2007). In Dominican amber (15–20 Ma), dolichoderines are the most numerically abundant subfamily of ants, and most specimens are *Azteca* species (Wilson 1985). Two subgroups of leptomyrmecines have colonized Australia. The older of these 2, *Leptomyrmex*, is sister to the New World clade of (*Forelius* + *Dorymyrmex*) and is currently confined to mesic forest in eastern Australia and adjacent Melanesia (Shattuck 1999). Curiously, *Leptomyrmex* has been recorded from Dominican amber (Baroni Urbani and Wilson 1987), although our results suggest a reinterpretation: that the New World fossil "*Leptomyrmex*" represents an extinct stem lineage of (*Forelius* + *Dorymyrmex*).

The second Australian subgroup, here labeled with the acronym "DNAPPTOFI" (after the first letter of each contemporary genus), is sister to the Neotropical genus *Linepithema*. Our estimate for the age of stem DNAPPTOFI—about 33 Ma—corresponds to the time when Australia and southern South America last shared a land connection via Antarctica, approximately 30–28 Ma according to most biogeographical reconstructions (Sanmartín and Ronquist 2004). Recent phylogenetic and biogeographical work within the genus *Linepithema* indicates that this clade likely originated in southern South America (Wild 2009). This combined information is consistent with dispersal (range expansion) of the MRCA of *Linepithema* and DNAPPTOFI from southern South America to Australia in the late Eocene or early Oligocene, followed by isolation of the Australian lineage through severance of the connection between the 2 continents. The DNAPPTOFI leptomyrmecines evidently underwent a burst of diversification after their isolation in Australia and are now dominant ants in most Australian ecosystems (Andersen 1995). This radiation took place in the Miocene, which contrasts with the situation in the northern hemisphere where the fossil record suggests that dolichoderines attained dominance and diversity in the Eocene and had declined in abundance by the Middle Miocene (Dlussky and Rasnitsyn 2003).

The New World genus *Linepithema* has recently spawned one of the world's most successful invasive ants, the Argentine ant, *L. humile*. Inadvertently introduced by humans within the last 150 years to parts of North America, Hawaii, Mediterranean Europe, South Africa, and Australia, *L. humile* has caused widespread elimination or decline of native ant populations in most of these areas (Holway et al. 2002). The region where it has been least successful in usurping the native ant fauna is Australia (Majer 1994), the only place where it encounters species from the sister group of *Linepithema*, that is, DNAPPTOFI. Thus, the invasion success of *L. humile* appears to be inversely related to its phylogenetic relatedness to potential competitors, a pattern recently noted for invasive grasses in California (Strauss et al. 2006). *Linepithema humile* provides only a single putative example among the ants, however, and further studies of invasive ant success and phylogenetic relatedness are warranted.

If our phylogeny, age estimates, and biogeographic reconstructions are accurate, then reports of 3 genera within the DNAPPTOFI clade, *Anonychomyrma*, *Iridomyrmex* and *Ochetellus*, from Baltic amber (42 Ma) (Dlussky 1997) must be in error. Workers of most dolichoderine ants have a rather uniform habitus (Shattuck 1992), and placement of fossil specimens in extant genera can pose difficulties (Dlussky and Rasnitsyn 2003). Even the distinctive *Leptomyrmex*-like fossils from Dominican amber were placed in a completely different subfamily for a period of time (Wilson 1985). We thus suggest that these apparent leptomyrmecines from Baltic amber may actually represent convergently similar species in the tribe Tapinomini, a conclusion that should be evaluated with additional morphological work.

General Implications for Phylogenetic Inference

Our results add to the growing body of evidence that data partitioning can have substantial effects on phylogenetic reconstruction (Brown and Lemmon 2007). Empirical studies across a range of taxa have reported significant changes in Bayesian PPs for some clades based solely on different partitioning strategies (e.g., Castoe et al. 2004; Mueller et al. 2004; Nylander et al. 2004; Brandley et al. 2005; Castoe and Parkinson 2006; McGuire et al. 2007; Li et al. 2008). In our case, the influence of data partitioning manifests itself most dramatically as alternate rootings of the ingroup (Dolichoderinae). The simplest partitioning scheme in our Bayesian analyses produced a 1.00 PP for a root position on the Dolichoderini branch, but support for this rooting erodes under more complex partitioning schemes, with the most complex model yielding 0.90 PP for an alternative rooting on the Tapinomini branch (0.97 when heterogeneous third positions of wg and EF1aF1 are

removed). If we conclude, based on multiple lines of evidence (statistical comparisons of partition models, inclusion/exclusion of *Aneuretus*, inclusion/exclusion of heterogeneous third positions, independent BEAST analysis), that the proper rooting of the Dolichoderinae is indeed on the Tapinomini branch, then we find that commonly employed forms of partitioning produce incorrect results, in some cases with strong support. Unpartitioned analyses support the incorrect placement of the root with 1.00 PP (Bayesian) and 0.83 bootstrap proportion (ML) (Fig. 3). Partitioning by gene, another common strategy, also supports the incorrect result. Only partitioning the data both by gene and by codon position within gene reverses this effect. Support for the correct rooting is weaker, however, when first- and second-codon positions are grouped together in a single partition (nuc 18) compared with the nuc26 model, in which all codon positions receive a separate partition, that is, when site-specific rates are modeled in all 8 protein-coding genes (PP increase from 0.80 to 0.90). Curiously, in the intermediate case (nuc22), in which each of the codon positions receives a separate partition in 4 more rapidly evolving protein-coding genes but in which first and second positions remain grouped together in 4 more slowly evolving protein-coding genes, support for the correct rooting is worse than in the nuc18 partition model (0.64 vs. 0.80). This may be related to an interaction effect between partitioning and the influence of the 2 partitions with heterogeneous base frequencies (Fig. 5). Based on the paucity of variable sites within some of the first and second positions (Table 3), the nuc26 partitioning scheme possibly introduces some degree of overparameterization (Sullivan and Joyce 2005). However, noticeable biases due to overparameterization have rarely been demonstrated in empirical data sets (Kelchner and Thomas 2006). In our study, the fully partitioned Bayesian analyses generate results that are in significantly better agreement with analyses that consider other potential sources of error, including taxon representation and base composition heterogeneity.

Partitioning has similar corrective effects on the likelihood results, but support for the correct phylogeny is much weaker, rising only to a best case of 0.55 bootstrap support under site-specific rates (0.76 when heterogeneous third positions of wg and EF1aF1 are removed). In fact, under the ML bootstrap criterion, the difference in support for the outgroup rooting in the nuc18, nuc22, and nuc26 analyses is, statistically speaking, equivalent. A recent study of ray-finned fishes (Li et al. 2008) also reported greater sensitivity of Bayesian methods to the effect of partitioning schemes on tree topology compared with ML. This difference between Bayesian and likelihood analyses with regard to partitioning deserves further investigation.

Our results also demonstrate the far-reaching effects of taxon representation, another factor with potentially strong impacts on phylogenetic inference (reviewed by Heath et al. 2008). When the sister group of the Dolichoderinae, the relict, monotypic *A. simoni*, is excluded from our analyses, most partitioning schemes are unable to reconstruct the correct rooting in Bayesian analyses. Presumably, this is because *Aneuretus* breaks the long branch separating Dolichoderinae from the next closest outgroups (Fig. 2). In likelihood analyses, partitioning by codon position (18, 22, and 26 partitions) does correct for the absence of *Aneuretus*, but with only poor support. This outcome underscores the importance of taxon discovery for phylogenetics, especially in hyperdiverse and incompletely known groups such as insects. Without the information provided by *A. simoni*, at least for the 10 genes analyzed here, the phylogeny of the Dolichoderinae would be reconstructed incorrectly under most Bayesian analyses, with high support under some partitioning schemes. The species-level taxonomy of ants is far from complete, with the actual number of species likely more than twice the ~12,000 currently described taxa (Ward 2007a). Although many of these new species are easily assigned to existing genera, the discovery of phylogenetically isolated species continues unabated. For example, our study included 3 dolichoderine genera discovered only within the past several years: *Gracilidris* (Wild and Cuezzo 2006), *Ravavy*, and *Aptinoma* (Fisher 2009). Two of these (*Gracilidris, Ravavy*) are monotypic, and one (*Ravavy*) is known only from males. More strikingly, *Martialis heureka*, an ant species described from a single specimen collected recently in the Amazon rainforest, may be the sister group of all extant Formicidae (Rabeling et al. 2008). The continued discovery of such relict taxa and their strong influence on phylogenetic inference argues that the exploration of biodiversity and the search for taxonomically unusual species should remain a high priority in systematics.

Supplementary Material

Supplementary material can be found at http://www.sysbio.oxfordjournals.org/.

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APPENDIX

Revised Classification of the Dolichoderinae

The last comprehensive taxonomic revision of the ant subfamily Dolichderinae was that of Shattuck (1992, 1994, 1995). His treatment recognized 22 extant genera and 15 extinct fossil genera and showed that previous tribe-level classifications were poorly supported. In the absence of clear subdivisions, Shattuck placed all genera in a single tribe, Dolichoderini, congruent with the subfamily. Bolton (2003) followed the same arrangement. Since then, one genus has been transferred from Formicinae to Dolichoderinae (LaPolla and Longino 2006) and subsequently synonymized under an existing dolichoderine genus (Fisher and Bolton 2007); one genus originally placed in Dolichoderinae, *Amyrmex*, has been transferred to Leptanilloidinae (Ward and Brady 2009); and several new dolichoderine genera have been described (Dubovikov 2004; Wild and Cuezzo 2006; Fisher 2009). In addition, Dubovikov (2005) erected a new tribe, Iridomyrmecini, to which he assigned 2 groups of taxa: *Iridomyrmex* and several other Australian genera (in subtribe Iridomyrmecina) and *Bothriomyrmex* and related genera (in new subtribe Bothriomyrmecina). The tribe Tapinomini was resurrected to contain all remaining dolichoderine genera. Most of these groupings, as defined by Dubovikov, are not justified by the results of our study, but we recognize a tribe Bothriomyrmecini (**n. stat.**) whose composition is similar to Dubovikov's (2005) subtribe Bothriomyrmecina except that *Nebothriomyrmex* and *Ctenobethylus* are excluded.

In our proposed higher level classification of the ant subfamily Dolichoderinae, we recognize 4 tribes corresponding to the 4 major clades that emerged as very strongly supported groups in all of our molecular phylogenetic analyses (Table 6). There are 2 extant genera, *Anillidris* and *Ecphorella*, of which we were unable to obtain specimens for sequencing. These 2 genera, along with the extinct fossil genus *Ctenobethylus*, are provisionally placed in the new classification on the basis of morphological similarities to other taxa. The remaining extinct genera are too poorly characterized to allow confident placement, and they are treated as *incertae sedis* within Dolichoderinae.

The diagnoses below are based on extant taxa. The following abbreviations are used: w = worker; q = queen (gyne); m = male. Molecular synapomorphies are referenced by their position in our data matrix (TreeBase matrix accession no. M4460). † = extinct fossil taxon.

Tribe Dolichoderini Forel 1878

Diagnosis. Hypostoma with anterolateral tooth (w, q, m); mesosternum with convex anteromedial margin (w, q); mandibular dentition well developed (w, q, m); declivitous face of propodeum concave (w, q, m); integument thick and often strongly sculptured (w, q). 28S, C→A (position 5912); 28S, G→T (position 5921); 28S, →T (position 5940; insertion); 28S, G→A (position 5941); 28S, C→T (position 5950).

Genus *Dolichoderus* Lund 1831

Tribe Tapinomini Emery 1913

Diagnosis. Petiolar scale usually reduced or absent (w); if not, then either propodeal spiracle in mediodorsal position (*Axinidris*) or metanotal groove weakly developed such that mesonotum and propodeum form a continuous convex surface (*Liometopum*). CAD, G→A (position 3024); CAD, T→A (position 3039); 28S, C→T (position 4680); 28S, G→A (position 4695); 28S, C→T (position 4855).

Genus *Aptinoma* Fisher 2009
Genus *Axinidris* Weber 1941
†Genus *Ctenobethylus* Brues 1939
Genus *Ecphorella* Fisher 2009
Genus *Liometopum* Mayr 1861
Genus *Tapinoma* Foerster 1850
Genus *Technomyrmex* Mayr 1872

Tribe Bothriomyrmecini Dubovikov 2005 ***n. stat.***

Diagnosis. Compound eyes small, composed of 10–40 ommatidia, and placed in anterior position on head (w); posteromedial extension of clypeus absent (w); medial hypostoma reduced or absent (w, q, m). LW Rh, G→T (position 291); ArgK, C→T (position 2145); CAD, C→T (position 2468, nonsynonymous substitution).

Genus *Arnoldius* Dubovikov 2005
Genus *Bothriomyrmex* Emery 1869
Genus *Chronoxenus* Santschi 1919
Genus *Loweriella* Shattuck 1992
Genus *Ravavy* Fisher 2009

Tribe Leptomyrmecini Emery 1913 ***rev. stat.***

Diagnosis. Morphologically heterogeneous assemblage, recognized primarily by disagreement with the 3 preceding diagnoses. Hypostoma lacking anterolateral tooth (w, q, m); mesosternum with straight anteromedial margin (w, q); petiolar scale present (w); propodeal spiracle in lateral or posterodorsal position (w); metanotal groove well developed (w). ArgK, G→A (position 1848); 18S, C→G (position 3527); 18S, C→T (position 3536).

Genus *Anillidris* Santschi 1936
Genus *Anonychomyrma* Donisthorpe 1947
Genus *Azteca* Forel 1878
Genus *Doleromyrma* Forel 1907
Genus *Dorymyrmex* Mayr 1866
Genus *Forelius* Emery 1888

Genus *Froggattella* Forel 1902
Genus *Gracilidris* Wild and Cuezzo 2006
Genus *Iridomyrmex* Mayr 1862
Genus *Leptomyrmex* Mayr 1862
Genus *Linepithema* Mayr 1866
Genus *Nebothriomyrmex* Dubovikov 2004
Genus *Ochetellus* Shattuck 1992
Genus *Papyrius* Shattuck 1992
Genus *Philidris* Shattuck 1992
Genus *Turneria* Forel 1895

Incertae sedis *in Dolichoderinae*

†Genus *Alloiomma* Zhang 1989
†Genus *Asymphylomyrmex* Wheeler 1915
†Genus *Elaeomyrmex* Carpenter 1930
†Genus *Elaphrodites* Zhang 1989
†Genus *Emplastus* Donisthorpe 1920
†Genus *Eotapinoma* Dlussky 1988
†Genus *Eurymyrmex* Zhang, Sun & Zhang 1994
†Genus *Kotshkorkia* Dlussky 1981
†Genus *Leptomyrmula* Emery 1913
†Genus *Miomyrmex* Carpenter 1930
†Genus *Petraeomyrmex* Carpenter 1930
†Genus *Pityomyrmex* Wheeler 1915
†Genus *Protazteca* Carpenter 1930
†Genus *Proiridomyrmex* Dlussky and Rasnitsyn 2003
†Genus *Zherichinius* Dlussky 1988