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Unraveling the evolutionary history of the hyperdiverse ant genus *Pheidole* (Hymenoptera: Formicidae)

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ABSTRACT

Pheidole is currently the most species rich genus of ants in the world, with many taxa still awaiting description. In this study, I reconstruct the phylogeny of *Pheidole* using molecular characters from three mitochondrial genes and two nuclear genes for ~140 species. The phylogenetic relationships of *Pheidole* are investigated with special interest in understanding factors that may have led to their remarkable diversity. The results presented here establish a framework for understanding the explosive radiation of this group by providing (1) a phylogenetic estimate, and (2) a comparative analysis of life history traits that are likely to have been important in the diversification of the group. In all analyses, *Pheidole* is recovered as a monophyletic lineage, and molecular clock estimates infer an age of 58.4–61.2 million years ago (Ma) for crown group members of the genus. Using an estimate of diversification rate, it appears that *Pheidole* has undergone 0.108–0.103 speciation events per million years. Previous hypotheses of species groups were largely not upheld in the analyses presented here. Workers of the genus *Pheidole* are dimorphic with a minor and major (soldier) subcaste. A third subcaste of super majors is known in eight species of *Pheidole* and this trait was found to have arisen multiple times throughout the phylogeny. Seed harvesting is common among species of the genus and is thought to be one of the factors leading to the diversification of the group, but additional data will be required to further test this hypothesis. To address biogeographic questions on the origin of the genus, both New and Old World species were included in these analyses, and the results suggest that *Pheidole* is New World in origin with a possible single introduction into the Old World.

1. Introduction

Ants are among the most ecologically and numerically dominant families of organisms in almost every terrestrial habitat throughout the world, although they include only about 1% of all described insect species, with approximately 11,500 extant species of ants in 288 genera (Bolton et al., 2006). The development of eusociality is thought to have been a driving force in the striking diversification and abundance of this group, and yet we are only recently beginning to resolve the evolution of the major lineages (Brady et al., 2006; Moreau et al., 2006) and factors (such as the rise of the angiosperms) that may have led to their diversification (Moreau et al., 2006). Although we now have a better understanding of the higher-level phylogenetic relationships within the ants, most species-level relationships and the factors that lead to their diversification are still poorly understood, including among them, the "hyperdiverse" genus *Pheidole*.

"Hyperdiversity" is a term used to describe a monophyletic group, as a genus or family, which exhibits an exceptionally large number of species compared to its sister group or other related group in the same higher taxon. The idea of adaptive radiations of species grew out of the observation that some clades appear to be unusually species rich compared with others (Darwin, 1859; Dobzhansky, 1951; Simpson, 1953; Hinton, 1976). Some well-known examples of hyperdiverse groups are the weevils (Farrell, 1998), marine gastropods of the genus *Conus* (Duda and Kohn, 2005), lycaenid butterflies in the tribe Eumaeini (Pierce et al., 2002), and fungal endophytes (Arnold et al., 2000). To explain unusual disparities in species number between clades, key innovation hypotheses (Hinton, 1976; Mitter et al., 1988; Sanderson and Donoghue, 1994) have been proposed. Hodges and Arnold (1995) defined key innovations as "biological traits that promote lineage diversification via mechanisms that increase the rate of speciation and/or decrease the rate of extinction". In order to identify potential key innovations in the genus, one must first infer the phylogeny.

With a worldwide distribution, the hyperdiverse myrmicine genus *Pheidole* is unsurpassed for number of species in a single ant genus (Wilson, 2003). *Pheidole* presently comprises more than

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9.5 percent of the entire known world ant fauna with over 1100 species described worldwide (Bolton et al., 2006). The only other ant genus that comes close to reaching this level of diversity is the formicine genus *Camponotus*, with about 1000 species currently described. However, recent findings suggest that *Camponotus* may not represent a monophyletic lineage (Brady et al., 2006). The biogeographic patterns of diversity between *Pheidole* and *Camponotus* are complementary, with *Pheidole* more species rich in the New World, and *Camponotus* more species rich in the Old World. The 600+ described species of *Pheidole* in the New World were recently the subject of a major revision by Wilson (2003) that included species descriptions and detailed morphological drawings of each species. Wilson (2003) proposed 19 species groups within the New World *Pheidole* based on overall similarity. Of these species groups, 17 are of New World origin and two are of Old World origin. The two Old World species groups are each represented in the New World by a single introduced species (*P. megacephala* and *P. teneriffana*). Of these New World species groups, the great majority of species fall into five assemblages (*diligens*, *fallax*, *flavens*, *pilifera*, and *tristis*). Wilson (2003) hypothesized that these five species groups are likely to be monophyletic lineages. He further considered the 12 remaining native New World groups to be monophyletic, with two exceptions. The *transversostriata* group was thought to be polyphyletic with two lines descended from within the *flavens* group, while the *granulata* group, with their rare and distinctive four-segmented antennal club were thought to be closely related to three species groups, *fallax*, *pilifera*, and *tristis*.

Ants in the genus *Pheidole* possess a dimorphic worker caste that is comprised of a minor worker subcaste and major worker subcaste, with these big-headed major workers sometimes referred to as soldiers. The earliest confirmed fossil specimens of *Pheidole* are found in the Florissant shales of Colorado, which is late Eocene, ~34 million years ago (Ma) in age (Carpenter, 1930). These compression fossils of two winged queens, although certainly members of the genus *Pheidole*, are not sufficiently well-preserved to be placed in an identifiable extant species group. Twenty-five additional fossils of *Pheidole* have been found in amber deposits from the Dominican Republic, dated early to middle Miocene (15–20 Ma). Eighteen of the specimens found in Dominican amber are thought to belong to the New World *flavens* group, with seven specimens belonging to the *sexspinosa* group, which is now restricted to the Old World (Wilson, 1985; Baroni Urbani, 1995).

The great diversity of *Pheidole* leads to the question: how long did it take to generate such remarkable diversity and what are the adaptations that promote ecological dominance in this group? We know from the fossil record that stem group *Pheidole* must be at least 34 Ma. During this time, ants in the genus *Pheidole* diversified quite extensively. What sets them apart from other New World ants with much less diversity? Several characters could be the "key innovation(s)" that enabled *Pheidole* to reach such ecological dominance. All known species of *Pheidole* are dimorphic (except six species of workerless social parasites), with minor workers performing most of the tasks within the nest and foraging, and large-headed majors specializing on colony defense and/or food processing. The evolution of worker polymorphism in ants has been hypothesized to be associated with a dietary change (Wilson, 1984; Hölldobler and Wilson, 1990; Ferster et al., 2006; Powell and Franks, 2006). Additionally, ants in *Pheidole* exhibit reduction of the sting in both the major and minor subcaste without an increase in defensive secretions. Defense of the colony and food sources are executed by cooperative fighting, instead of a "sting". Group retrieval of prey items is often accomplished by "spread-eagling" the prey or intruder.

Although the majority of species in the genus *Pheidole* possess a dimorphic worker caste, at least eight species (all belonging to the *pilifera* species group) possess an unusually large super major subcaste in addition to the typical minor and major subcastes (trimorphic worker caste). Did this super major subcaste evolve once or several times independently during the evolution of the genus, and is the appearance of super majors correlated with other life history characteristics that may have promoted diversification?

A large number of *Pheidole* major workers are also known to be involved in the milling of seeds harvested by the minor and major worker caste, and these seeds are often stored in granaries within the ant nest. Seed removal by ants may lead to dispersal or predation and Rodgerson (1998) has shown that relatively strong seeds are less likely to be removed by ants. Also, seed-removing by ants can have different effects on seed fate, with some ant species being more beneficial than others (Hughes and Westoby, 1992a,b). Not only are seeds gathered from their parent plant or the area near the parent plant, but several species of *Pheidole* have been observed to gather seeds from the feces of frugivorous birds (Byrne and Levey, 1993) and capuchin monkeys (Pizo and Oliveira, 1999). In addition, several seed harvesting ant species are often found in overlapping geographic ranges, thereby potentially exerting a strong predation pressure on many plants, but these broadly sympatric ant species are often segregated by microhabitat (Johnson, 2000) and/or regulated by rainfall (Kaspari and Valone, 2002). Not all seed harvesting by ants results in predation, and many seeds in granaries reach germination (Wheeler, 1910). Although other genera of ants are also know to harvest seeds (e.g., *Messor*, *Monomorium*, *Pogonomyrmex*), this behavioral innovation may have allowed *Pheidole* to radiate and take advantage of a food resource that many other ants cannot access. Did this "key innovation" evolve once and promote the proliferation of these ants, or has this behavior evolved multiple times throughout the history of *Pheidole* due to ecological factors? Both of these questions can be addressed once the evolutionary relationships in the genus have been inferred.

Here I reconstruct the phylogeny of over 140 species of *Pheidole* using molecular characters from three mitochondrial genes (*Cytochrome oxidase* I, *Cytochrome b*, 12S rDNA) and two nuclear genes (*Histone* H3 and *Long-Wavelength Rhodopsin*). A molecular based phylogeny will provide the beginning of a stable classification system for the group, as well as the framework to understand the explosive radiation of *Pheidole* through a comparative analysis of life history traits that may have been important in their diversification. Reconstructing the evolutionary relationships among *Pheidole* will also enable investigation in a central biogeographic question raised by Wilson (2003): did this dominant genus originate and proliferate in the New World before dispersing to other regions, or does it represent a more anciently derived, Gondwanan relict? Fossil evidence is inconclusive.

2. Methods and materials

2.1. Taxon sampling

The analysis presented here includes a total of 171 specimens. Included are 150 specimens representing ~140 species of *Pheidole*. Nine additional species from the tribe Pheidolini were included, as well as nine other Myrmicinae species. Finally, three genera outside of the myrmicines were included as outgroups (*Brachymyrmex* sp., *Lasius alienus*, and *Prenolepis imparis*). Table 1 contains a full list of all specimens, their taxonomic status (Bolton, 2003; Wilson, 2003), collection accession numbers, and GenBank accession numbers. Sequence for several of the outgroup taxa and *Pheidole rhea* were obtained from a previous study (Moreau et al., 2006) for the *Cytochrome Oxidase I* and *Long-wavelength Rhodopsin* genes. All sequences have been deposited in GenBank. The aligned data set for this study is available from TreeBASE (www.treebase.org,

Table 1
List of all specimens, taxonomic status (Old World *Pheidole* taxa include country of origin), collection accession numbers and GenBank accession numbers

Subfamily/tribe	Genus	Species	Collection Accession Nos.	GenBank Accession No. for mtDNA COI	GenBank Accession No. for mt rDNA 12S	GenBank Accession No. for mtDNA cyt*b*	GenBank Accession No. for nDNA H3	GenBank Accession No. for nDNA LR
Myrmicinae/Pheidolini	*Pheidole*	*absurda*	RA0155	EF518305	EF518599	EF518453	EF518770	EF518934
Myrmicinae/Pheidolini	*Pheidole*	*adrianoi*	RA0332	EF518306	EF518600	EF518454	EF518771	EF518935
Myrmicinae/Pheidolini	*Pheidole*	*allarmata*	RA0109	EF518307	EF518601	X	EF518772	EF518936
Myrmicinae/Pheidolini	*Pheidole*	*amazonica*	CS0414	EF518308	EF518602	EF518455	EF518773	EF518937
Myrmicinae/Pheidolini	*Pheidole*	*ampla* gp. (Aust)	RA0358	EF518309	EF518603	EF518456	EF518774	EF518938
Myrmicinae/Pheidolini	*Pheidole*	*anastasii* (syn. of *bilimeki*)	RA0159	EF518310	EF518604	X	EF518775	X
Myrmicinae/Pheidolini	*Pheidole*	*artemisia* (syn. of *pilifera*)	RA0465	EF518311	EF518605	EF518457	EF518776	EF518939
Myrmicinae/Pheidolini	*Pheidole*	*astur*	CS0410	EF518312	EF518606	EF518458	EF518777	EF518940
Myrmicinae/Pheidolini	*Pheidole*	*barbata*	RA0141	EF518313	EF518607	EF518459	EF518778	X
Myrmicinae/Pheidolini	*Pheidole*	*bicarinata*	RA0197	EF518314	EF518608	EF518460	EF518779	EF518941
Myrmicinae/Pheidolini	*Pheidole*	*biconstricta*	RA0171	EF518315	EF518609	EF518461	EF518780	EF518942
Myrmicinae/Pheidolini	*Pheidole*	*bilimeki*	RA0162	EF518316	EF518610	X	EF518781	X
Myrmicinae/Pheidolini	*Pheidole*	n. sp. AZ-05	RA0571	EF518317	EF518611	EF518462	EF518782	EF518943
Myrmicinae/Pheidolini	*Pheidole*	*boltoni*	RA0176	EF518318	EF518612	EF518463	X	EF518944
Myrmicinae/Pheidolini	*Pheidole*	*boruca*	RA0153	EF518319	EF518613	X	EF518783	EF518945
Myrmicinae/Pheidolini	*Pheidole*	*browni*	RA0165	EF518320	EF518614	X	EF518784	EF518946
Myrmicinae/Pheidolini	*Pheidole*	*californica*	RA0146	EF518321	EF518615	X	EF518785	EF518947
Myrmicinae/Pheidolini	*Pheidole*	*caltrop*	RA0160	X	EF518616	EF518464	EF518786	X
Myrmicinae/Pheidolini	*Pheidole*	*carrolli*	RA0709	EF518322	EF518617	EF518465	EF518787	EF518948
Myrmicinae/Pheidolini	*Pheidole*	*casta*	RA0568	EF518323	EF518618	EF518466	EF518788	X
Myrmicinae/Pheidolini	*Pheidole*	*cavigenis*	RA0460	EF518324	EF518619	EF518467	EF518789	EF518949
Myrmicinae/Pheidolini	*Pheidole*	*cephalica*	CS0506	EF518325	EF518620	EF518468	EF518790	EF518950
Myrmicinae/Pheidolini	*Pheidole*	*cerebrosior*	RA0380	EF518326	EF518621	X	EF518791	EF518951
Myrmicinae/Pheidolini	*Pheidole*	*ceres*	CS0267	EF518327	EF518622	EF518469	EF518792	EF518952
Myrmicinae/Pheidolini	*Pheidole*	*clementensis*	RA0576	EF518328	EF518623	X	EF518793	EF518953
Myrmicinae/Pheidolini	*Pheidole*	*clydei*	RA0456	EF518329	EF518624	X	EF518794	EF518954
Myrmicinae/Pheidolini	*Pheidole*	*cocciphaga*	RA0174	EF518330	EF518625	EF518470	EF518795	EF518955
Myrmicinae/Pheidolini	*Pheidole*	*cockerelli*	RA0461	EF518331	EF518626	EF518471	EF518796	EF518956
Myrmicinae/Pheidolini	*Pheidole*	*coloradensis* A (syn. of *pilifera*)	RA0333	EF518332	EF518627	EF518472	EF518797	EF518957
Myrmicinae/Pheidolini	*Pheidole*	*coloradensis* B (syn. of *pilifera*)	RA0605	EF518333	EF518628	EF518473	EF518798	EF518958
Myrmicinae/Pheidolini	*Pheidole*	*comata* (Borneo)	RA0477	EF518334	EF518629	EF518474	EF518799	EF518959
Myrmicinae/Pheidolini	*Pheidole*	*constipata*	RA0570	EF518335	EF518630	EF518475	EF518800	EF518960
Myrmicinae/Pheidolini	*Pheidole*	*cramptoni*	CS0507	EF518336	EF518631	EF518476	EF518801	EF518961
Myrmicinae/Pheidolini	*Pheidole*	*crassicornis*	RA0614	X	EF518632	EF518477	EF518802	EF518962
Myrmicinae/Pheidolini	*Pheidole*	*davisi*	RA0497	EF518337	EF518633	EF518478	EF518803	EF518963
Myrmicinae/Pheidolini	*Pheidole*	*dentata*	CS0301	EF518338	EF518634	EF518479	EF518804	EF518964
Myrmicinae/Pheidolini	*Pheidole*	*desertorum*	CS0159	EF518339	EF518635	EF518480	EF518805	EF518965
Myrmicinae/Pheidolini	*Pheidole*	*diana*	RA0164	EF518340	EF518636	EF518481	EF518806	EF518966
Myrmicinae/Pheidolini	*Pheidole*	*diversipilosa*	RA0471	EF518341	EF518637	EF518482	EF518807	EF518967
Myrmicinae/Pheidolini	*Pheidole*	*dossena*	RA0156	EF518342	EF518638	EF518483	EF518808	EF518968
Myrmicinae/Pheidolini	*Pheidole*	*dugasi* (Thailand)	RA0318	EF518343	EF518639	EF518484	EF518809	EF518969
Myrmicinae/Pheidolini	*Pheidole*	*erratilis*	RA0163	EF518344	EF518640	EF518485	EF518810	EF518970
Myrmicinae/Pheidolini	*Pheidole*	*fimbriata*	RA0158	EF518345	EF518641	EF518486	EF518811	X
Myrmicinae/Pheidolini	*Pheidole*	*fiorii*	RA0182	EF518346	EF518642	EF518487	EF518812	EF518971
Myrmicinae/Pheidolini	*Pheidole*	*fissiceps*	RA0113	EF518347	EF518643	EF518488	EF518813	EF518972
Myrmicinae/Pheidolini	*Pheidole*	*flavens*	RA0180	EF518348	EF518644	EF518489	EF518814	EF518973
Myrmicinae/Pheidolini	*Pheidole*	*floridana*	RA0331	EF518349	EF518645	EF518490	EF518815	EF518974
Myrmicinae/Pheidolini	*Pheidole*	*furtiva*	RA0610	X	EF518646	EF518491	EF518816	EF518975
Myrmicinae/Pheidolini	*Pheidole*	*gatesi* (Vietnam)	RA0319	EF518350	EF518647	EF518492	EF518817	EF518976
Myrmicinae/Pheidolini	*Pheidole*	*gilvescens*	RA0139	EF518351	EF518648	EF518493	EF518818	X
Myrmicinae/Pheidolini	*Pheidole*	*granulata*	RA0572	X	EF518649	X	EF518819	X
Myrmicinae/Pheidolini	*Pheidole*	*harlequina*	RA0569	EF518352	EF518650	EF518494	EF518820	EF518977
Myrmicinae/Pheidolini	*Pheidole*	*hoplitica*	RA0528	EF518353	EF518651	EF518495	EF518821	EF518978
Myrmicinae/Pheidolini	*Pheidole*	*hyatti* (yellow form)	RA0450	EF518354	EF518652	EF518496	EF518822	EF518979
Myrmicinae/Pheidolini	*Pheidole*	*indagatrix*	RA0170	EF518355	EF518653	EF518497	EF518823	EF518980

Myrmicinae/Pheidolini	*Pheidole*	*indistincta*	RA0161	EF518356	EF518654	EF518498	EF518824	EF518981
Myrmicinae/Pheidolini	*Pheidole*	*innupta* (syn. of *alfaroi*)	RA0175	EF518357	EF518655	EF518499	EF518825	EF518982
Myrmicinae/Pheidolini	*Pheidole*	*inquilina*	RA0606	EF518358	EF518656	EF518500	EF518826	EF518983
Myrmicinae/Pheidolini	*Pheidole*	*jelskii*	RA0244	EF518359	EF518657	EF518501	X	EF518984
Myrmicinae/Pheidolini	*Pheidole*	*juniperae*	RA0527	EF518360	EF518658	EF518502	EF518827	EF518985
Myrmicinae/Pheidolini	*Pheidole*	*laselva*	RA0185	EF518361	EF518659	EF518503	EF518828	EF518986
Myrmicinae/Pheidolini	*Pheidole*	*laticornis*	RA0154	EF518362	EF518660	X	EF518829	X
Myrmicinae/Pheidolini	*Pheidole*	*littoralis*	RA0710	EF518363	EF518661	EF518504	EF518830	EF518987
Myrmicinae/Pheidolini	*Pheidole*	*macrops*	RA0578	EF518364	EF518662	EF518505	EF518831	EF518988
Myrmicinae/Pheidolini	*Pheidole*	*mamore*	RA0118	EF518365	EF518663	X	EF518832	EF518989
Myrmicinae/Pheidolini	*Pheidole*	*megacephala* (Aust)	RA0357	EF518366	EF518664	EF518506	EF518833	X
Myrmicinae/Pheidolini	*Pheidole*	*metallescens*	RA0524	EF518367	EF518665	EF518507	EF518834	EF518990
Myrmicinae/Pheidolini	*Pheidole*	*micula*	RA0467	EF518368	EF518666	EF518508	EF518835	EF518991
Myrmicinae/Pheidolini	*Pheidole*	*militicida*	RA0468	EF518369	EF518667	X	EF518836	EF518992
Myrmicinae/Pheidolini	*Pheidole*	*minutula*	RA0150	EF518370	EF518668	EF518509	EF518837	EF518993
Myrmicinae/Pheidolini	*Pheidole*	*moerens*	RA0128	EF518371	EF518669	X	EF518838	EF518994
Myrmicinae/Pheidolini	*Pheidole*	*morrisi*	RA0496	EF518372	EF518670	EF518510	EF518839	EF518995
Myrmicinae/Pheidolini	*Pheidole*	*nitella*	RA0179	EF518373	EF518671	EF518511	EF518840	EF518996
Myrmicinae/Pheidolini	*Pheidole*	*nitidicollis*	RA0183	EF518374	EF518672	EF518512	EF518841	EF518997
Myrmicinae/Pheidolini	*Pheidole*	*noda* (Vietnam)	RA0479	EF518375	EF518673	EF518513	EF518842	EF518998
Myrmicinae/Pheidolini	*Pheidole*	*obscurithorax*	RA0142	EF518376	EF518674	X	EF518843	EF518999
Myrmicinae/Pheidolini	*Pheidole*	*obtusospinosa* B	RA0641	EF518377	EF518675	EF518514	EF518844	EF519000
Myrmicinae/Pheidolini	*Pheidole*	*obtusospinosa* A	RA0218	EF518378	EF518676	EF518515	EF518845	EF519001
Myrmicinae/Pheidolini	*Pheidole*	*oceanica* (Palau)	RA0713	EF518379	EF518677	EF518516	X	EF519002
Myrmicinae/Pheidolini	*Pheidole*	*pacifica* A (syn. of *pilifera*)	RA0203	EF518380	EF518678	EF518517	EF518846	EF519003
Myrmicinae/Pheidolini	*Pheidole*	*pacifica* B (syn. of *pilifera*)	RA0575	X	EF518679	EF518518	EF518847	X
Myrmicinae/Pheidolini	*Pheidole*	*pallidula* (France)	RA0195	EF518381	EF518680	EF518519	EF518848	EF519004
Myrmicinae/Pheidolini	*Pheidole*	*pelor*	RA0525	EF518382	EF518681	EF518520	EF518849	EF519005
Myrmicinae/Pheidolini	*Pheidole*	*perpilosa*	RA0447	EF518383	EF518682	EF518521	EF518850	EF519006
Myrmicinae/Pheidolini	*Pheidole*	*pilifera*	RA0707	EF518384	EF518683	EF518522	EF518851	EF519007
Myrmicinae/Pheidolini	*Pheidole*	*plagiara* (Vietnam)	RA0482	EF518385	EF518684	EF518523	EF518852	EF519008
Myrmicinae/Pheidolini	*Pheidole*	*polymorpha* A	RA0564	EF518386	EF518685	EF518524	EF518853	EF519009
Myrmicinae/Pheidolini	*Pheidole*	*polymorpha* B	RA0565	EF518387	EF518686	EF518525	EF518854	EF519010
Myrmicinae/Pheidolini	*Pheidole*	*portalensis*	RA0577	EF518388	EF518687	EF518526	EF518855	EF519011
Myrmicinae/Pheidolini	*Pheidole*	*prostrata*	RA0184	EF518389	EF518688	EF518527	EF518856	EF519012
Myrmicinae/Pheidolini	*Pheidole*	sp. (eg-141) (Thailand)	RA0478	EF518390	EF518689	EF518528	EF518857	X
Myrmicinae/Pheidolini	*Pheidole*	*psammophila*	RA0573	EF518391	EF518690	X	EF518858	EF519013
Myrmicinae/Pheidolini	*Pheidole*	*quadrensis* (Borneo)	RA0320	EF518392	EF518691	EF518529	EF518859	EF519014
Myrmicinae/Pheidolini	*Pheidole*	*quadricuspis* (Borneo)	RA0480	EF518393	EF518692	EF518530	EF518860	EF519015
Myrmicinae/Pheidolini	*Pheidole*	*rhea* A	CS0161	DQ353372	EF518693	EF518531	EF518861	DQ353156
Myrmicinae/Pheidolini	*Pheidole*	*rhea* B	RA0533	EF518395	EF518694	EF518532	EF518862	EF519017
Myrmicinae/Pheidolini	*Pheidole*	*rhinoceros*	RA0181	EF518396	EF518695	EF518533	EF518863	EF519018
Myrmicinae/Pheidolini	*Pheidole*	*rufescens*	RA0607	EF518397	EF518696	EF518534	EF518864	EF519019
Myrmicinae/Pheidolini	*Pheidole*	*rugulosa*	RA0169	EF518398	EF518697	EF518535	EF518865	EF519020
Myrmicinae/Pheidolini	*Pheidole*	*sagittaria*	RA0157	X	EF518698	X	EF518866	X
Myrmicinae/Pheidolini	*Pheidole*	*sciara*	RA0580	EF518399	EF518699	EF518536	EF518867	EF519021
Myrmicinae/Pheidolini	*Pheidole*	*sciophila*	RA0204	EF518400	EF518700	EF518537	EF518868	EF519022
Myrmicinae/Pheidolini	*Pheidole*	*scrobifera*	RA0167	EF518401	EF518701	EF518538	EF518869	EF519023
Myrmicinae/Pheidolini	*Pheidole*	*senex*	RA0462	EF518402	EF518702	EF518539	EF518870	EF519024
Myrmicinae/Pheidolini	*Pheidole*	*sensitiva*	RA0172	EF518403	EF518703	EF518540	EF518871	EF519025
Myrmicinae/Pheidolini	*Pheidole*	*sexspinosa* gp. (Palau)	RA0712	EF518404	EF518704	X	EF518872	EF519026
Myrmicinae/Pheidolini	*Pheidole*	*sicaria*	RA0166	EF518405	EF518705	EF518541	EF518873	EF519027
Myrmicinae/Pheidolini	*Pheidole*	*sitiens*	RA0337	EF518406	EF518706	EF518542	EF518874	EF519028
Myrmicinae/Pheidolini	*Pheidole*	*soritis*	RA0466	EF518407	EF518707	EF518543	EF518875	EF519029
Myrmicinae/Pheidolini	*Pheidole*	*sospes*	RA0116	EF518408	EF518708	EF518544	EF518876	EF519030
Myrmicinae/Pheidolini	*Pheidole*	sp. (Aust)	RA0329	EF518409	EF518709	EF518545	EF518877	EF519031
Myrmicinae/Pheidolini	*Pheidole*	sp. (Sumatra)	RA0492	EF518410	EF518710	EF518546	EF518878	EF519032
Myrmicinae/Pheidolini	*Pheidole*	sp. 5 (Ivory Coast)	RA0536	EF518411	EF518711	EF518547	EF518879	EF519033
Myrmicinae/Pheidolini	*Pheidole*	sp. 8 (Ivory Coast)	RA0538	X	EF518712	EF518548	EF518880	X
Myrmicinae/Pheidolini	*Pheidole*	sp.1 (Madg)	CS0242	EF518412	EF518713	X	EF518881	EF519034

(*continued on next page*)

Table 1 (*continued*)

Subfamily/tribe	Genus	Species	Collection Accession Nos.	GenBank Accession No. for mtDNA COI	GenBank Accession No. for mt rDNA 12S	GenBank Accession No. for mtDNA cyt*b*	GenBank Accession No. for nDNA H3	GenBank Accession No. for nDNA LR
Myrmicinae/Pheidolini	*Pheidole*	sp.a (Ghana)	RA0557	EF518413	EF518714	EF518549	EF518882	EF519035
Myrmicinae/Pheidolini	*Pheidole*	sp.a (Indonesia)	RA0422	EF518414	EF518715	EF518550	EF518883	EF519036
Myrmicinae/Pheidolini	*Pheidole*	sp.a (Madg)	RA0314	EF518415	EF518716	EF518551	EF518884	EF519037
Myrmicinae/Pheidolini	*Pheidole*	sp.a (PNG)	RA0473	EF518416	EF518717	EF518552	EF518885	EF519038
Myrmicinae/Pheidolini	*Pheidole*	sp.b (Ghana)	RA0558	EF518417	EF518718	EF518553	EF518886	EF519039
Myrmicinae/Pheidolini	*Pheidole*	sp.b (Indonesia)	RA0423	EF518418	EF518719	EF518554	EF518887	EF519040
Myrmicinae/Pheidolini	*Pheidole*	sp.b (Madg)	RA0315	EF518419	EF518720	EF518555	EF518888	EF519041
Myrmicinae/Pheidolini	*Pheidole*	sp.b (PNG)	RA0516	EF518420	EF518721	EF518556	EF518889	EF519042
Myrmicinae/Pheidolini	*Pheidole*	sp.c (Ghana)	RA0559	EF518421	EF518722	X	EF518890	EF519043
Myrmicinae/Pheidolini	*Pheidole*	sp.c (Madg)	RA0316	EF518422	EF518723	EF518557	EF518891	EF519044
Myrmicinae/Pheidolini	*Pheidole*	sp.c (PNG)	RA0518	EF518423	EF518724	EF518558	EF518892	X
Myrmicinae/Pheidolini	*Pheidole*	sp.d (Ghana)	RA0563	EF518424	EF518725	EF518559	EF518893	EF519045
Myrmicinae/Pheidolini	*Pheidole*	sp.d (Madg)	RA0317	EF518425	EF518726	X	EF518894	EF519046
Myrmicinae/Pheidolini	*Pheidole*	sp.d (PNG)	RA0519	EF518426	EF518727	EF518560	EF518895	EF519047
Myrmicinae/Pheidolini	*Pheidole*	sp.e (PNG)	RA0520	EF518427	EF518728	EF518561	EF518896	EF519048
Myrmicinae/Pheidolini	*Pheidole*	*spadonia*	RA0379	EF518428	EF518729	EF518562	EF518897	EF519049
Myrmicinae/Pheidolini	*Pheidole*	*striaticeps*	RA0178	EF518429	EF518730	X	EF518898	X
Myrmicinae/Pheidolini	*Pheidole*	*subarmata*	RA0130	EF518430	EF518731	EF518563	EF518899	EF519050
Myrmicinae/Pheidolini	*Pheidole*	*tandjongensis* (Thailand)	RA0481	EF518431	EF518732	X	EF518900	EF519051
Myrmicinae/Pheidolini	*Pheidole*	*tepicana*	RA0451	EF518432	EF518733	EF518564	EF518901	EF519052
Myrmicinae/Pheidolini	*Pheidole*	*hyatti*	RA0567	EF518433	EF518734	EF518565	EF518902	EF519053
Myrmicinae/Pheidolini	*Pheidole*	*titanis*	RA0526	EF518434	EF518735	EF518566	EF518903	EF519054
Myrmicinae/Pheidolini	*Pheidole*	*tristicula*	CS0402	EF518435	EF518736	EF518567	EF518904	EF519055
Myrmicinae/Pheidolini	*Pheidole*	*truncula*	RA0168	EF518436	EF518737	EF518568	X	EF519056
Myrmicinae/Pheidolini	*Pheidole*	*tucsonica* (syn. of *gilvescens*)	CS0224	EF518437	EF518738	EF518569	EF518905	EF519057
Myrmicinae/Pheidolini	*Pheidole*	*tysoni*	RA0448	EF518438	EF518739	EF518570	EF518906	EF519058
Myrmicinae/Pheidolini	*Pheidole*	*umphreyi*	RA0177	EF518439	EF518740	EF518571	X	EF519059
Myrmicinae/Pheidolini	*Pheidole*	*vallicola*	RA0336	EF518440	EF518741	EF518572	EF518907	EF519060
Myrmicinae/Pheidolini	*Pheidole*	*variabilis* gp. (Aust)	RA0360	EF518441	EF518742	X	EF518908	EF519061
Myrmicinae/Pheidolini	*Pheidole*	*vinelandica* (syn. *bicarinata*)	RA0574	X	EF518743	EF518573	EF518909	EF519062
Myrmicinae/Pheidolini	*Pheidole*	*violacea*	RA0173	EF518442	EF518744	EF518574	EF518910	EF519063
Myrmicinae/Pheidolini	*Pheidole*	*vistana*	RA0208	EF518443	EF518745	EF518575	EF518911	EF519064
Myrmicinae/Pheidolini	*Pheidole*	*xanthogaster*	CS0399	EF518444	EF518746	EF518576	EF518912	EF519065
Myrmicinae/Pheidolini	*Pheidole*	*xerophila*	RA0494	EF518445	EF518747	EF518577	EF518913	EF519066
Myrmicinae/Pheidolini	*Pheidole*	*yaqui*	RA0579	EF518446	EF518748	EF518578	EF518914	EF519067
Myrmicinae/Pheidolini	*Aphaenogaster*	*texana*	RA0219	DQ353342	EF518749	EF518579	EF518915	DQ353198
Myrmicinae/Pheidolini	*Aphaenogaster*	*senilis*	RA0345	EF518447	EF518750	EF518580	EF518916	EF519068
Myrmicinae/Pheidolini	*Aphaenogaster*	sp. (Aust)	RA0356	EF518448	EF518751	X	EF518917	EF519069
Myrmicinae/Pheidolini	*Goniomma*	*hispanicum*	RA0341	DQ353300	EF518752	EF518581	EF518918	DQ353236
Myrmicinae/Pheidolini	*Messor*	*bouvieri*	RA0347	EF518449	EF518753	EF518582	EF518919	EF519070
Myrmicinae/Pheidolini	*Messor*	*julianus*	RA0348	DQ353349	EF518754	EF518583	EF518920	DQ353148
Myrmicinae/Pheidolini	*Messor*	*pergandei*	RA0349	EF518450	EF518755	EF518584	EF518921	EF519071
Myrmicinae/Pheidolini	*Ocymyrmex*	*picardi*	RA0254	DQ353328	EF518756	EF518585	EF518922	DQ353252
Myrmicinae/Pheidolini	*Oxyopomyrmex*	*insularis*	RA0346	EF518451	EF518757	EF518586	EF518923	DQ353147
Myrmicinae/Attini	*Atta*	sp.	CS0319	DQ353280	EF518758	EF518587	EF518924	DQ353250
Myrmicinae/Attini	*Cyphomyrmex*	sp.	CS0384	DQ353380	EF518759	EF518588	EF518925	DQ353251
Myrmicinae/Attini	*Trachymyrmex*	*jamaicensis*	RA0247	DQ353390	EF518760	EF518589	EF518926	DQ353224
Myrmicinae/Cephalotini	*Cephalotes*	sp.	CS0445	EF518452	EF518761	EF518590	X	EF519072
Myrmicinae/Cephalotini	*Cephalotes*	*unimaculatus*	RA0248	DQ353359	EF518762	EF518591	X	DQ353212
Myrmicinae/Cephalotini	*Procryptocerus*	*batesi*	CS0387	DQ353344	EF518763	EF518592	EF518927	DQ353190
Myrmicinae/Myrmicini	*Myrmica*	*incompleta*	RA0229	DQ353360	EF518764	EF518593	EF518928	DQ353225
Myrmicinae/Myrmicini	*Pogonomyrmex*	*maricopa*	CS0258	DQ353275	EF518765	EF518594	EF518929	DQ353178
Myrmicinae/Solenopsidini	*Tranopelta*	*subterranea*	CS0416	DQ353284	EF518766	EF518595	EF518930	DQ353284
Formicinae/Lasiini	*Lasius*	*alienus*	CS0268	DQ353288	EF518767	EF518596	EF518931	DQ353172
Formicinae/Plagiolepidini	*Brachymyrmex*	sp.	CS0108	DQ353294	EF518769	EF518598	EF518933	DQ353217
Formicinae/Plagiolepidini	*Prenolepis*	*imparis*	CS0297	DQ353397	EF518768	EF518597	EF518932	DQ353162

"X" denotes missing sequence information for taxon.

2005) or by request from the author. The exact collection data for each specimen can be obtained from the author. Voucher specimens have been deposited at Harvard University's Museum of Comparative Zoology, Cambridge, MA, USA.

2.2. DNA isolation

Field collections were made in 95% EtOH and kept in the laboratory until the time of DNA extraction. Total genomic DNA was isolated for one individual worker (except those species whose workers are very small, where DNA from two individuals was combined) in lysis buffer with a Teflon grinding implement, followed by purification using the DNeasy™ Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocols.

2.3. Polymerase chain reaction (PCR) amplification

For most specimens, five fragments were amplified via PCR (Mullis et al., 1987; Saiki et al., 1988) using specific primers for each gene region (Table 2): a fragment approximately 1000 base pairs (bp) in length containing the *Cytochrome Oxidase* I (COI) protein encoding mitochondrial molecular marker; a fragment approximately 450 bp in length of the *Cytochrome b* (cyt*b*) protein encoding mitochondrial molecular marker; a fragment approximately 360 bp in length of the small subunit (12S) ribosomal mitochondrial molecular marker; a fragment approximately 340 bp in length of the *Histone* H3 protein encoding nuclear marker and a fragment approximately 550 bp in length of the *Long-Wavelength Rhodopsin* (LR) protein encoding nuclear marker. Double-stranded DNA was amplified in 25 μL volume reactions: 16.15 μL ultra pure (HPLC quality) water, 2.5 μL 10× buffer, 1.5–2.5 μL 25 mM $MgCl_2$, 0.25 μL 100 mM dNTP, 1.2 μL of each primer (10 mM), 1 μL DMSO, and 0.2 μL Taq DNA Polymerase (Qiagen Inc., Valencia, CA). All reactions were initially denatured at 94 °C for 2 min in a MJ Dyad Thermal Cycler (MJ Research, Waltham, MA), then subjected to 35 cycles of 60 s at 94 °C denaturation, 60 s at 45–58 °C (annealing temperature depended on gene amplified) for annealing, and 2 min at 72 °C extension.

2.4. Sequencing

All sequencing was done using dye terminator cycle sequencing following the protocol specified by the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Revision B, August 1995, Perkin-Elmer, Norwalk, CT). Primers used for amplification served as sequencing primers. Additional internal primers were used for the COI protein encoding mitochondrial gene to provide overlapping sequence coverage for the entire region (Table 2). All samples were sequenced in both directions. Cycle sequencing reactions were performed in 12 μL reactions: 1.0 μL ABI Prism® BigDye™ v3.1 (Applied Biosystems Inc., Foster City, CA), 1.0 μL 5× buffer (buffer: 400 mM Tris at pH 9.0 and 10 mM $MgCl_2$), and 0.33 μL each (10 μM) primer. The remainder of the mixture was composed of ultra pure water and template to give 50–90 ng of template DNA in each reaction. Cycle sequence reaction parameters contained an initial denature step of 94 °C for 2 min, followed by 25 cycles of 10 s at 94 °C denaturation, 5 s at annealing 50 °C and 4 min at 60 °C (MJ Dyad Thermal Cycler, MJ Research, Waltham, MA).

2.5. Sequence alignment

After sequences were collected, they were analyzed and initially aligned using the computer programs Sequencing Analysis 3.7 (ABI Prism™ 2001) and Sequencher 4.5 (GeneCodes 2005), respectively. Conserved regions were identified and aligned, and gaps assigned to minimize changes using ClustalX 1.9a169 (Thompson et al., 1997). For all protein encoding genes, the inferred amino acid sequences were used, allowing for comparatively uncomplicated alignment. The aligned data set was finally viewed and further manually aligned using MacClade 4.06 (Maddison and Maddison, 2003).

2.6. Phylogenetic analysis

To infer relationships among the species of *Pheidole*, several phylogenetic analyses were performed using PAUP*4.0b10 (Swofford, 2001), GARLI v0.94 (Zwickl, 2006), and MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001). A variety of model based methods, in addition to maximum parsimony (MP), were employed to infer phylogenetic relationships. Parsimony searches were performed on the complete concatenated data set using the random stepwise addition option of the heuristic search for 500 replicates with tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, and equal weighting of all characters. If searches produced more than one tree, a strict consensus was performed to summarize data analyses. To measure the robustness of branching patterns of the parsimony trees, bootstrap analyses (bs) (Felsenstein, 1985; Hillis and Bull, 1993) were executed by using the closest stepwise addition of the heuristic search for 500 replicates.

In order to evaluate the fit of the data, likelihood analyses were conducted using the complete concatenated data set with GARLI v0.94 (Zwickl, 2006) and MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001). A series of nested hypotheses in which the null hypothesis (H_0) is a special case of the alternative hypothesis (H_1) were performed on various nucleotide substitution models using the likelihood ratio test (LRT) within Modeltest 3.06 (Posada and Crandall, 1998). A maximum likelihood search was implemented in GARLI v0.94 (Zwickl, 2006) with model parameters being estimated during the run, with genthreshfortopoterm = 10,000,000; scorethreshforterm = 0.05; significanttopochange = 0.05;

Table 2
Primer sequences for amplification and sequencing of the mitochondrial protein encoding *Cytochrome Oxidase* I (COI) mtDNA, mitochondrial protein encoding *Cytochrome b* (cyt*b*) mtDNA, mitochondrial ribosomal 12S rRNA, nuclear protein encoding *Histone* H3 nDNA, and nuclear protein encoding *Long-Wavelength Rhodopsin* (LR) nDNA

Gene	Primer	Sequence	Utility	Citation
COI	LCO1490	5′-GGTCAACAAATCATAAAGATATTGG-3′	Amplification/sequencing	Folmer et al. (1994)
COI	HCO2198	5′-TAAACTTCAGGGTGACCAAAAAATCA-3′	Sequencing	Folmer et al. (1994)
COI	Jerry	5′-CAACATTTATTTTGATTTTTTGG-3′	Sequencing	Simon et al. (1994)
COI	Ben	5′-GCTACTACATAATAKGTATCATG-3′	Amplification/sequencing	Simon et al. (1994)
cyt*b*	CB1	5′-TATGTACTACCATGAGGACAAATATC-3′	Amplification/sequencing	Chiotis et al. (2000)
cyt*b*	CB2	5′-ATTACACCTCCTAATTTATTAGGAAT-3′	Amplification/sequencing	Chiotis et al. (2000)
12S	12Sai	5′-AAACTAGGATTAGATACCCTATTA-3′	Amplification/Sequencing	Simon et al. (1994)
12S	12Sbi-f	5′-GAAAATGACGGGCAATTTGT-3′	Amplification/sequencing	Modified from Simon et al. (1994)
H3	H3F	5′-ATGGCTCGTACCAAGCAGACVGC-3	Amplification/sequencing	Colgan et al. (1998)
H3	H3R	5′-ATATCCTTRGGCATRATRGTGAC-3′	Amplification/sequencing	Colgan et al. (1998)
LR	LR143F	5′-GACAAAGTKCCACCRGARATGCT-3′	Amplification/sequencing	Ward and Downie (2005)
LR	LR639ER	5′-YTTACCGRTTCCATCCRAACA-3′	Amplification/sequencing	Ward and Downie (2005)

stopgen = 10,000,000; and stoptime = 10,000,000. This process was implemented several times to insure the topology converged on the same maximum likelihood tree. A single GTR+Γ+I model of sequence evolution was assumed to underlie all genes. To test the robustness of the final maximum likelihood (ML) tree, a bootstrap analysis was performed in GARLI v0.94 (Zwickl, 2006) for 500 pseudoreplicates.

The maximum likelihood model was used to determine whether the sequence among taxa was evolving at a constant rate and fit a molecular clock (Felsenstein, 1993). A procedure proposed by Felsenstein (1993) to test the H_0 of a molecular clock was used. This test uses a LRT to determine whether there are significant differences between the likelihood scores obtained from an analysis where the branch lengths are unconstrained compared to an analysis where the branch lengths are constrained so that terminal ends are contemporaneous. The likelihood test statistic is assumed to be approximately equal to a χ^2 distribution with $n-2$ degrees of freedom, where n equals the number of taxa sampled (Felsenstein, 1981).

Analyses were also performed with MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001), with model parameters being estimated during the run, and using the default value of four Markov chains. Multiple chains can assist in navigating tree-space more easily and help avoid entrapment in local topological optima. A "temperature" parameter of 0.2 was implemented to produce incremental heating of each chain, with higher temperature values producing greater differences between chains, since they are less constrained by likelihood scores in moving through tree-space (Wilcox et al., 2002). The Markov chain Monte Carlo (MCMC) length was 30,000,000 generations, with the chain sampled every 100 generations after the initial burn-in period of 100,000 generations. A second independent run was implemented for 10,000,000 generations to compare to the results of the previous run. Bayesian posterior probabilities (bpp) were estimated as the proportion of trees sampled after burn-in that contained each of the observed bipartitions (Rannala and Yang, 1996; Larget and Simon, 1999). A single GTR+Γ+I model of sequence evolution was assumed to underlie all gene regions. Convergence of chains was confirmed in all Bayesian analyses by examination of the average standard deviation of split frequencies.

In addition, an analysis was conducted using "mixed models" or partitioned analysis of molecular sequence evolution for 10,000,000 generations with MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001), with model parameters being estimated during the run, and using the default value of four Markov chains. In these analyses, each separate gene region was assigned its own GTR+Γ+I model.

2.7. Fossil constraints

The use of fossils in concert with molecular data can take two forms: (1) fossils can serve as fixed "calibration" points used to calculate absolute branching times, or (2) they can serve as maximum or minimum age "constraints" (Sanderson, 1997). For this study, I selected three fossils that can be confidently placed in the tree to use as separate minimum age constraints. I used only the oldest confirmed member of each extant species group, genus, or subfamily to calibrate divergence times of modern crown-group *Pheidole*. The fossil calibration points applied to the stem group used in this study as minimum age constraints are as follows: *Pheidole sexspinosa* group, 15–20 million years ago (Ma) (Baroni Urbani, 1995); the genus *Pheidole*, 34 Ma (Carpenter, 1930) (i.e., *Pheidole* + *Cephalotes*); and the genus *Cephalotes*, 15–20 Ma (De Andrade and Baroni Urbani, 1999). Following the results of Moreau et al. (2006) for the oldest age estimate for the Myrmicinae, the root node was given a fixed age of 114 Ma. To account for fossils with unsure stratigraphic ages from dated formations, such as Dominican Republic amber (15–20 Ma), two separate molecular clock analyses were performed with the minimum and maximum age for those formations, plus all other fossils resulting in an age range for the genus.

To insure that the use of the above fixed age of the root node was not resulting in a misleading age for *Pheidole*, the analyses were repeated again with the following three fossils: *Pheidole sexspinosa* group, 15–20 million years ago (Ma) (Baroni Urbani, 1995); the genus *Pheidole*, 34 Ma (Carpenter, 1930) applied to the stem group; and the genus *Cephalotes*, 15–20 Ma (De Andrade and Baroni Urbani, 1999). In this second analysis, the root node was given a maximum (fixed) age of 92 Ma following the sister group relationship between Myrmicinae and Formicinae from the oldest known fossil of the subfamily Formicinae (Grimaldi and Agosti, 2000).

2.8. Molecular clock analyses

Penalized likelihood (Sanderson, 2002) (PL) is a semi-parametric smoothing method. Penalized likelihood assumes that there is an autocorrelation of substitution rates and attempts to minimize rate changes between ancestral/descendant branches on a tree (i.e., at the nodes). PL attempts to combine the statistical power of parametric methods (models of molecular evolution) with the robustness of non-parametric methods. A smoothing parameter can vary from very small, in which case each branch of the phylogeny has a different substitution rate (saturated model), to very large, in which parameters are essentially clock-like. The core of the penalized likelihood method is determining the optimal smoothing level. The program r8s v1.7 (Sanderson, 2003) implements a data driven cross-validation procedure that systematically prunes terminals from the tree, then estimates parameters from the submatrix and a given smoothing value. It then tries to predict the data for pruned taxa using the estimated parameters. Finally, it calculates a chi-squared error associated with the difference between the predicted and observed data. The optimal smoothing level is chosen as the one that minimizes the chi-squared error (Iturralde-Vinent and MacPhee, 1996; Sanderson, 2003). Standard deviations on age estimates where calculated via non-parametric bootstrapping for 100 pseudoreplicates with branch lengths and divergence times re-estimated in each replicate.

2.9. Estimating diversification rates

To assess the diversification rate of the genus *Pheidole*, the method of Magallón and Sanderson (2001) was implemented using the known extant species numbers and estimated ages for the genus crown group. To estimate the number of speciation events per million years in the absence of extinction the following formula was used: $[\ln(n) - \ln(2)]/t$, where n = extant species numbers and t = estimated age of the genus (Magallón and Sanderson, 2001).

2.10. Hypothesis testing and character mapping

To test alternative hypotheses for the evolution of the species based on previous taxonomic definitions of the species groups and biogeographic hypotheses (Wilson, 2003) constraint tree searches were implemented in GARLI v0.94 (Zwickl, 2006) and the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) was executed to investigate significant differences in tree lengths. This test was performed using RELL with 10,000 bootstrap replicates, and the results evaluated as a one-tailed test.

To study the evolution of polymorphism and seed harvesting in *Pheidole*, these traits were mapped onto the maximum likelihood chronogram. Information regarding presence or absence of seed harvesting follows: S.P. Cover (pers. comm.), Eguchi (2001), Hölldobler and Wilson (1990), Johnson (2000), Shattuck and Barnett (2001) and Wilson (2003). Possible patterns in the evolution of seed harvesting were investigated using analyses of directionality and

models of evolution with the software package BayesTraits (Pagel and Meade, 2006) using the BayesMultiState method (Pagel et al., 2004). These analyses were implemented in a likelihood framework with presence or absence of seed harvesting included for all species of *Pheidole*, when known. Models of gradual ($\kappa = 1$) versus punctuated ($\kappa = 0$) models of evolution were tested (Pagel, 1994), as well as estimated from the data. These tests also allowed for investigating the potential directionality of the evolution of this trait.

3. Results

3.1. Simple sequence statistics

This study produced a final aligned 2738 bp fragment with most taxa sequenced for the following five genes regions: a fragment spanning the mitochondrial *Cytochrome Oxidase* I (COI) (1066 bp) gene, a portion of the mitochondrial gene *Cytochrome b* (cyt*b*) (446 bp), a portion of the mitochondrial small subunit 12S rDNA (346 bp), a fragment of the nuclear protein encoding gene *Histone* H3 (324 bp), and a fragment of the nuclear protein encoding gene *Long-Wavelength Rhodopsin* (LR) (556 bp, including 114 bp of an intron). The aligned fragment contained 1105 sites that were constant (40.4%), 321 sites that were variable (11.7%) and 1312 sites that were parsimoniously informative (47.9%). Examinations of base composition of the entire data set resulted in the following: A: 0.29628; C: 0.19646; G: 0.16248; T: 0.34477.

3.2. Parsimony phylogenetic analyses

The maximum parsimony (MP) analysis of all characters resulted in two most parsimonious trees ($L = 23632$) with a CI of 0.122 and a RI of 0.427. The bootstrap values recovered with the maximum parsimony criterion (MP bs) are included in Fig. 1.

3.3. Maximum likelihood phylogenetic analyses

The best fit maximum likelihood (ML) model determined using the LRT, as well as, Modeltest 3.06 (Posada and Crandall, 1998) was the GTR+Γ+I. A maximum likelihood search in GARLI v0.94 (Zwickl, 2006) using this model resulted in one maximum likelihood tree with a $-\ln L = 96672.97392$ (Fig. 1). The parameter values as estimated in PAUP*4.0b10 (Swofford, 2001) from this tree were: A ⇔ C: 0.22749, A ⇔ G: 5.13765, A ⇔ T: 0.35715, C ⇔ G: 0.89657, C ⇔ T: 3.69217, G ⇔ T: 1.0 for the GTR model, estimated base composition was A = 0.374361, C = 0.169986, G = 0.044111, T = 0.411542, $\alpha = 0.473495$ for the Γ distribution, and $I = 0.367282$ for the proportion of invariable sites. Maximum likelihood was also used to test for a clock-like evolution. The molecular clock tree produced with the same parameter estimates above gave a likelihood score of $-\ln L = 97006.95437$, indicating that the molecular clock should be rejected ($\chi^2 = 667.96$, df = 169, $P < 0.0001$). The bootstrap values recovered with the maximum likelihood criterion (ML bs) are included in Fig. 1.

3.4. Bayesian inference phylogenetic analyses

The likelihood analysis of all characters in MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001) using the GTR+Γ+I (INVGAMMA) model of sequence evolution run for 30,000,000 generations resulted in a sample of trees with a mean likelihood score of $-\ln L = 96684.91439$. The mean parameter values as estimated were: A ⇔ C: 0.20560, A ⇔ G: 5.11948, A ⇔ T: 0.33930, C ⇔ G: 0.86535, C ⇔ T: 3.24169, G ⇔ T: 1.0 for the GTR model estimated base composition was A = 0.368033, C = 0.175376, G = 0.042793, T = 0.413799, $\alpha = 0.464854$ for the Γ distribution, and I (PINVAR) = 0.363214 for the proportion of invariable sites. The average standard split frequencies of the chains after 30,000,000 generations was 0.017, suggesting that the chains had reached convergence. The Bayesian posterior probabilities (bpp) are included in Fig. 1. The results of the second Bayesian run of 10,000,000 generations resulted in a nearly identical topology, with a sample of trees with a mean likelihood score of $-\ln L = 96689.76994$, but that differed slightly in some of the posterior probabilities, which is to be expected. The average standard split frequencies of the chains after the second run with 10,000,000 generations was 0.0095, suggesting that the chains had reached convergence.

The likelihood analysis in MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001) using the mixed model of sequence of evolution, in which each gene region was assigned its own GTR+Γ+I model of sequence evolution, resulted in a sample of trees with a mean likelihood score of $-\ln L = 96830.83337$, although after 10,000,000 generations the chains still had not reached convergence. Overall topology and posterior probabilities recovered for the mixed model analysis tended to agree with those of the single common model Bayesian analysis (results not shown).

3.5. Molecular dating

Age estimation using the maximum likelihood topology with estimated branch lengths using the both the maximum and minimum ages for the Dominican Amber fossil specimens within the penalized likelihood framework with ±1.96 times the standard deviation (SD) of the bootstrapped samples resulted in an age estimation of 58.4 ± 6.76–61.2 ± 3.04 Ma for the modern crown-group *Pheidole*. Even when the analyses were completed with the more conservative criterion for root node age, the modern crown-group *Pheidole* were still recovered with an age range of 60.21–63.2 Ma using the penalized likelihood method.

3.6. Phylogenetic relationships of Pheidole

All parsimony, maximum likelihood, and Bayesian Inference tree topologies show strong support (98% ML bs; 87% MP bs; 100% bpp) for the monophyly of the genus *Pheidole* (Fig. 1). Of the species included in these analyses, 10 of Wilson's (2003) 17 species groups are represented. Only the crassicornis species group of the 10 species groups included was recovered as a monophyletic lineage (but see below for results of hypothesis testing). Interestingly, all analyses recovered *Pheidole fimbriata* as sister to all the *Pheidole* species included. To assure that this was not an artifact of missing sequence data for *P. fimbriata* for the *Long-wavelength Rhodopsin* (LR) gene, the LR gene was eliminated for all samples and a Bayesian Inference analysis for 10,000,000 generations was implemented resulted in a sample of trees with a mean likelihood score of $-\ln L = 88648.12883$. Again, the same topology was recovered, with *P. fimbriata* as the earliest extant *Pheidole* species (results not shown), suggesting this result is not due to missing information for this single sample.

Pheidole is a member of the tribe Pheidolini. Exemplars from five additional genera from this tribe of the 10 currently recognized were included in this analysis [*Aphaenogaster* (three species); *Goniomma* (one species); *Messor* (three species); *Ocymyrmex* (one species); and *Oxyopomyrmex* (one species)], but none of the analyses recovered Pheidolini as a monophyletic lineage. In fact, *Pheidole* was not recovered as closely related to any of the other Pheidolini tribal members included in the analyses.

3.7. Estimating diversification rates

Using the method of Magallón and Sanderson (2001) diversification rates were estimated using the known extant species

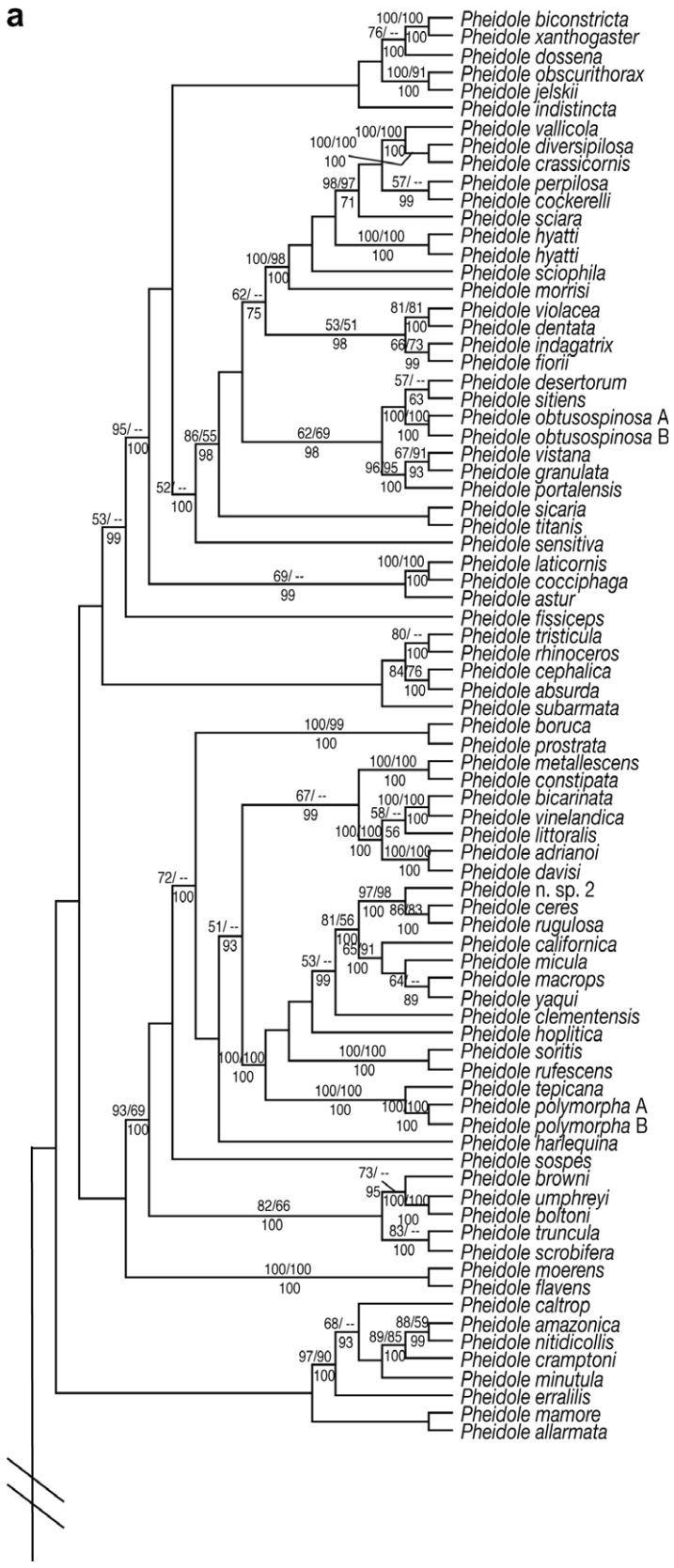

Fig. 1. Single tree inferred with GARLI under maximum likelihood search with a GTR+Γ+I model of sequence evolution from the analysis of the complete data set. Values above the branches represent GARLI maximum likelihood bootstrap percentages greater than 50% (ML bs) and PAUP* parsimony bootstrap percentages greater than 50% (MP bs). Values below the branches represent posterior probability values from Bayesian analysis greater than 50% (bpp). (a) Top portion of tree; (b) bottom portion of tree.

numbers and estimated ages for *Pheidole* crown group. To estimate the number of speciation events per million years in the absence of extinction the following formula was used: $[\ln(n) - \ln(2)]/t$, where $n = 1100$ and $t = 58.4$ or 61.2 Ma (using the minimum and maxi-

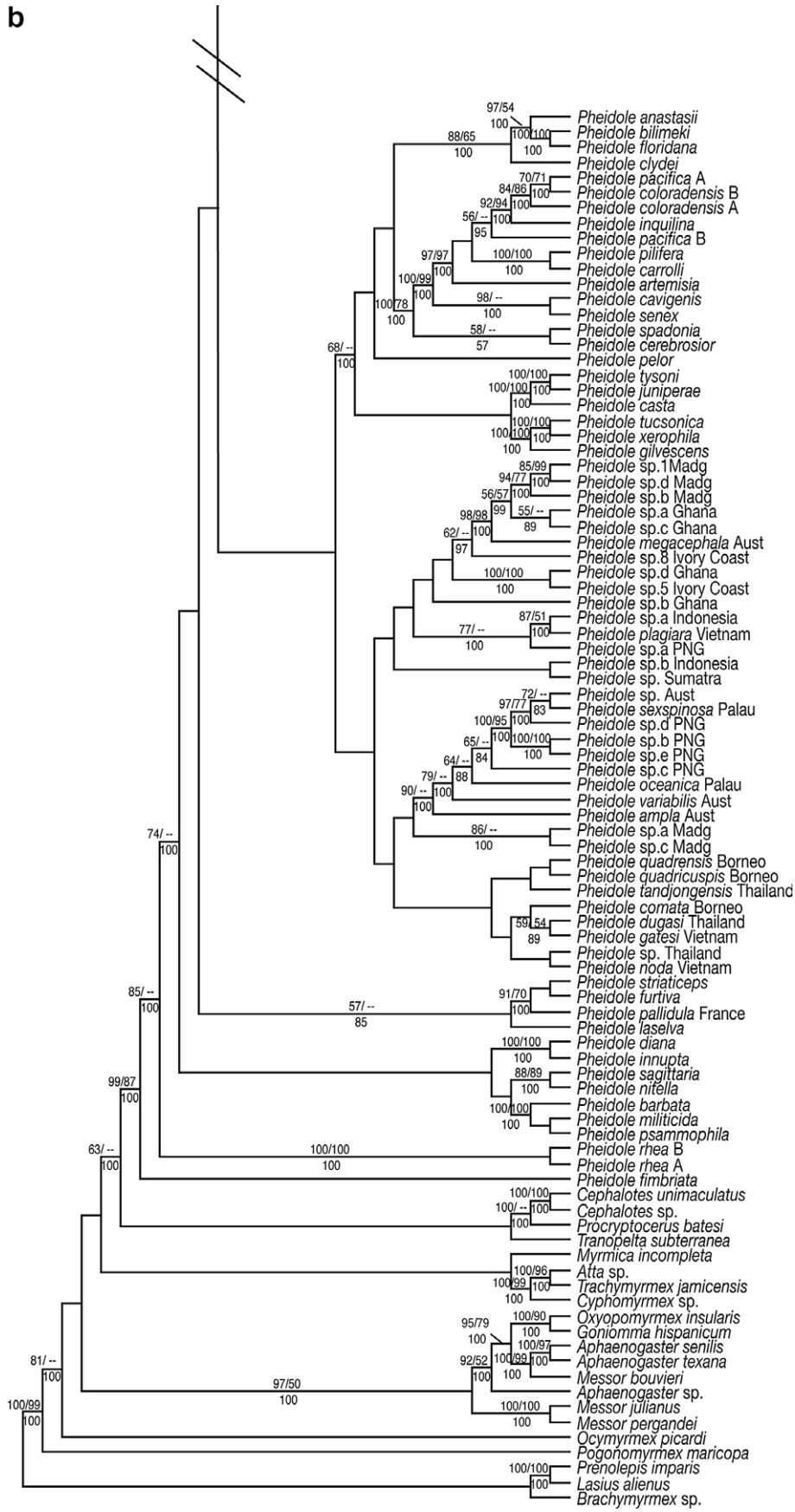

Fig. 1 (*continued*)

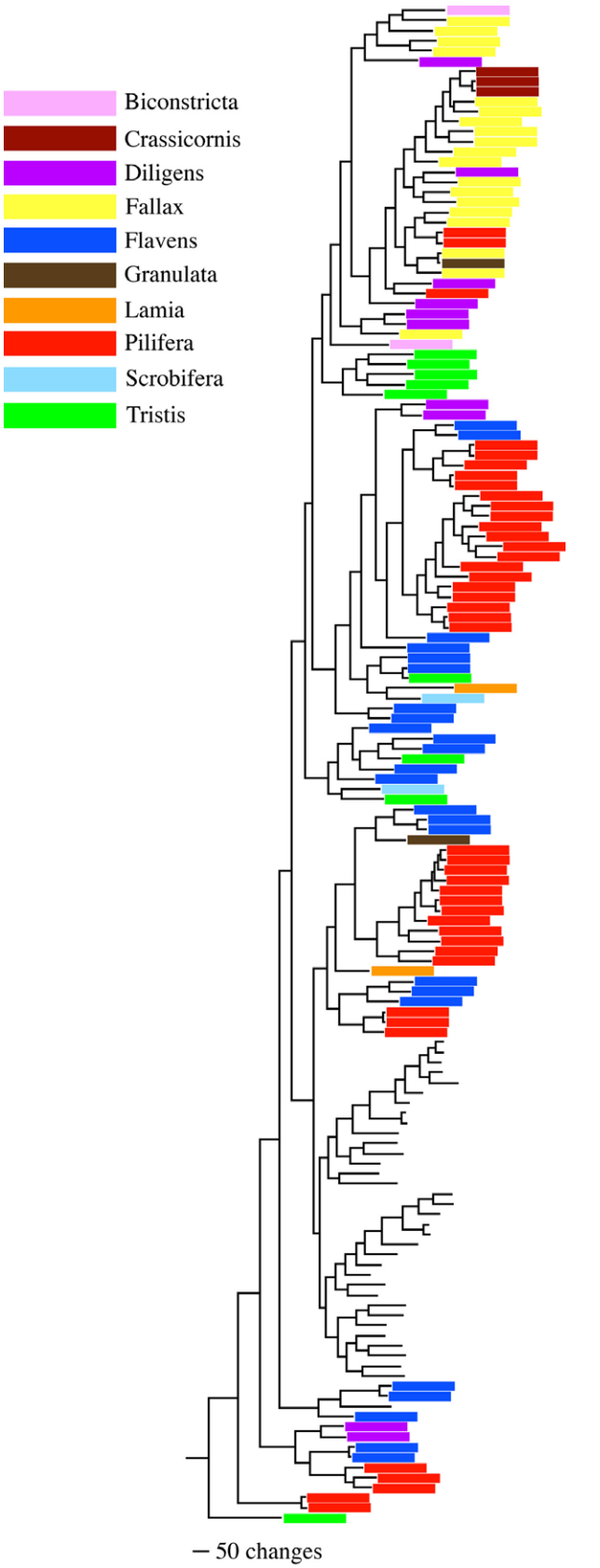

Fig. 2. Species groups identified by Wilson (2003) replace taxon names for New World species on single tree inferred shown with branch lengths proportional to estimated divergence with GARLI under maximum likelihood search with a GTR+Γ+I model of sequence evolution from the analysis of the complete data set. Outgroup taxa have been removed.

mum estimated ages from this study). From this analysis it appears that *Pheidole* has undergone 0.108–0.103 speciation events per million years.

3.8. Hypothesis testing and character mapping in Pheidole

3.8.1. Monophyly of Wilson's (2003) species groups

As mentioned above, only one of Wilson's (2003) species groups included in this analysis was recovered as a monophyletic lineage (Fig. 2). To test the remaining nine species groups these competing tree topologies were further compared using the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) to test for significant differences in tree lengths. In this analysis, tree topologies obtained when each of the nine species groups was constrained as a monophyletic lineage were compared with the maximum likelihood tree. Only the *scrobifera* species group hypothesis was uncovered by this analysis as not significantly different at the $\geqslant 0.05$ level (Table 3), and all other species groups differed significantly from the maximum likelihood topology.

3.8.2. Evolution of polymorphism

All of species of *Pheidole* are dimorphic, possessing both minor and major workers (soldiers), with the exception of at least eight New World species, which also possess an unusually large super major subcaste in addition to the typical minor and major subcastes (i.e., a trimorphic worker caste). Nine specimens from five trimorphic species (*P. obtusospinosa*, *P. polymorpha*, *P. rhea*, *P. tepicana*, and *P.* n. sp. 2) were included in this analysis. These taxa were not recovered as a monophyletic lineage (Fig. 3) and the results of this study support at least four independent origins of this super major subcaste.

3.8.3. Seed harvesting in Pheidole

The species included in this study known to harvest seeds were mapped on the maximum likelihood tree (Fig. 3). It is clear that seed harvesting is a widespread trait that has probably evolved multiple times and in some cases appears to have been lost in entire lineages (Fig. 3). The results of the BayesMultiState (Pagel et al., 2004) analyses suggest that a gradual model ($\kappa = 1$: $-\ln L = 30.657406$) rather than a punctuated model ($\kappa = 1$: $-\ln L = 35.046291$) of evolution of this behavior is a better explanation of the data ($\chi^2 = 8.77777$, df = 1, $P < 0.003$) (Pagel, 1994). When the value of κ is estimated from the data, a gradual model of the evolution of this trait is again preferred ($\kappa = 0.986280$). In addition, the results of both BayesMultiState (Pagel et al., 2004) analyses suggest that the transition from seed harvesting to lack of seed harvesting is a better model of the evolution of this trait ($\kappa = 1$: presence to absence 1.475037; absence to presence 0.000000) ($\kappa = 0$: presence to absence 0.093282; absence to presence 0.000000). Nevertheless, due to the limited information available regarding the presence or absence of seed harvesting in many species and the vast diversity of *Pheidole* species that have not been included in this study, these results can only be viewed as a first attempt to understand the evolution of this behavior.

3.8.4. Biogeographic History of the Genus Pheidole

Although the maximum likelihood analysis recovered the Old World species of *Pheidole* as a monophyletic lineage (with the exception of *P. pallidula* from France) nested well within the New World taxa, this clade lacked support and was recovered as a polytomy for the Old World taxa plus the one additional New World clade in the maximum parsimony and Bayesian inference analyses. A search was implemented in GARLI v0.94 (Zwickl, 2006) in which the Old World taxa were constrained to be non-monophyletic. This competing tree topology was compared to the maximum likelihood topology, which recovered the Old World taxa as a monophy-

Table 3
Results of Shimodaira–Hasegawa test evaluated using RELL bootstrap (one-tailed test) with 10,000 replicates

	−ln*L*	Difference −ln*L*	*P*-value
Maximum likelihood topology	96672.97392	(Best)	
biconstrica species group + (2/7 species included)	96806.71242	133.73848	0.0004[a]
diligens species group + (10/90 species included)	96963.37392	290.39998	0.0000[a]
fallax species group + (19/103 species included)	97614.66235	941.68841	0.0000[a]
flavens species group + (23/165 species included)	97587.19506	914.22112	0.0000[a]
granulata species group + (2/5 species included)	96791.12270	118.14875	0.0004[a]
lamia species group + (2/4 species included)	96764.00738	91.03344	0.0000[a]
pilifera species group + (41/48 species included)	97410.65061	737.67667	0.0000[a]
scrobifera species group + (2/12 species included)	96710.40449	37.43055	**0.0840**
tristis species group + (9/132 species included)	97128.31058	455.33663	0.0000[a]
Old World taxa ≠ (35 species included)	96791.73190	118.75796	0.0004[a]

"+" denotes constrained as monophyletic. "≠"denotes constrained as non-monophyletic. Species groups follow Wilson (2003) with number of species included in this study from the total described. Bold values indicate hypotheses that cannot be rejected based on the Shimodiara–Hasegawa test.
[a] Denotes hypotheses that differ at the 0.05 level.

letic lineage, using the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) to test for significant differences in tree lengths and was found to be a significantly worse fit to the data (Table 3).

4. Discussion

The "hyperdiverse" ant genus *Pheidole* was recovered as a monophyletic lineage in all phylogenetic analyses with high support (98% ML bs; 87% MP bs; 100% bpp). *Pheidole* is a member the tribe Pheidolini, as currently defined, and several Pheidolini species were included in this study. Of the five other Pheidolini genera included in this study (*Aphaenogaster*, *Goniomma*, *Messor*, *Ocymyrmex*, and *Oxyopomyrmex*), none were found to be sister to *Pheidole*, suggesting the tribe is not monophyletic. Although they do not possess a dimorphic worker caste, and therefore differ from all extant species of *Pheidole*, species of the genus *Aphaenogaster* have long been thought to be closely related to and possibly nested within *Pheidole* or vice-versa (Emery, 1914; Brown, 1949). Although only three species of *Aphaenogaster* were included here, this hypothesized close relationship between *Pheidole* and *Aphaenogaster* was never observed. This result was also found in two previous molecular studies (Brady et al., 2006; Moreau et al., 2006) suggesting that that *Pheidole* and *Aphaenogaster* are not closely related, and that Pheidolini as it is currently defined is not a true monophyletic unit and should be reassessed. This study also corroborates the findings of Brady et al. (2006) that both the genus *Aphaenogaster* and *Messor* are probably not monophyletic lineages (Fig. 1). More interestingly, all analyses recovered ((*Cephalotes* + *Procryptocerus*) + *Tranopelta*) as sister to *Pheidole*, although these taxa are not even members of the Pheidolini tribe (Fig. 1), but belong to two separate tribes (Cephalotini and Solenopsidini). These results argue for a more careful evaluation of myrmicine tribal boundaries.

Results of the penalized likelihood molecular clock analysis suggest the genus is 58.4–61.2 MY old. The results of these analyses of the most recent common ancestor of *Pheidole* are in the same range as findings from a previous study of ant divergence times (Moreau et al., 2006). It should be noted that this age for *Pheidole* is considerably older than the oldest known fossils (34 Ma) and suggests that either the molecular clock method is overestimating the age of the genus, or the sparseness of the fossil record has not yet unveiled older fossils of the genus. This lack of a fossil record has led several authors to conclude that *Pheidole* is a rather recent lineage, and that this remarkable diversity arose in a rather narrow window of time (Brown, 1973; Naves, 1985; Wilson, 2003). The results presented here suggest that *Pheidole* has had substantially more time to diversify, and this could help explain the large number of species. But time alone is not sufficient to explain *Pheidole*'s hyperdiversity. Other ant genera, such as *Myrmica*, *Pogonomyrmex*, and *Proceratium* are as old or older than *Pheidole* and only *Camponotus* comes near to being described as hyperdiverse. Understanding the factors that have allowed *Pheidole* to become so diverse and ecologically dominant will be required to explain the hyperdiversity of this group.

To estimate the diversification rate of *Pheidole* the method of Magallón and Sanderson (2001) was implemented using the known extant species numbers and estimated ages for *Pheidole* crown group. From this analysis, it appears *Pheidole* has undergone 0.108–0.103 speciation events per million years in the absence of extinction. Although many angiosperm clades have been shown to have a much higher rate than *Pheidole* (Magallón and Sanderson, 2001), this rate is higher than that of beetles (0.048–0.068 MY: Hunt et al., 2007) and other animal groups in which this has been tested (0.066 MY: McPeek and Brown, 2007).

Of the 10 species groups included in this study proposed by Wilson (2003) based on overall morphological similarity, only *crassicornis* was recovered as monophyletic and *scrobifera* was not found to be significantly different at the ⩾0.05 level from the maximum likelihood topology (Table 3). Unfortunately only two of the 12 species of the *scrobifera* species group were included in this study, so further data are needed to confirm the potential monophyly of this species group.

Interestingly, the earliest diverging species of *Pheidole* recovered in all analyses is *P. fimbriata* (Fig. 1). This species is nocturnal, nests in the ground, and has a wide distribution from Mexico to Argentina found mainly in tropical forests. The foraging behavior of this species is unusual for a member of *Pheidole*, with an almost column-raiding-like behavior (similar to behaviors observed in army ants) and this species has even been observed to tend lycaenid butterfly larvae. Since only a small number of the extant species could be included in this phylogenetic study (∼140 of the over 1100 species), it will be interesting to see if *P. frimbriata* will continue to be recovered as sister to the remaining species with the inclusion of additional taxa. Also noteworthy is the next most early lineage within *Pheidole*. All analyses recovered *P. rhea*, one of the trimorphic, seed harvesting species, as the next earliest diverging lineage.

Several other interesting species-level relationships were recovered in this study. All New World species of *Pheidole* possess a three-segmented antennal club, with the exception of four species (*granulata* species group), which possess a four-segmented club. Two of these four-segmented club species were included in this study: *P. clydei* and *P. granulata*. As these two species were never recovered as a monophyletic group, and the results of the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) demonstrated significant differences in tree lengths when they were constrained as a monophyletic lineage (Table 3), it seems likely that this similarity in antennal club number is due to morphological convergence. Both S.P. Cover and P.S. Ward (pers. comm.) have

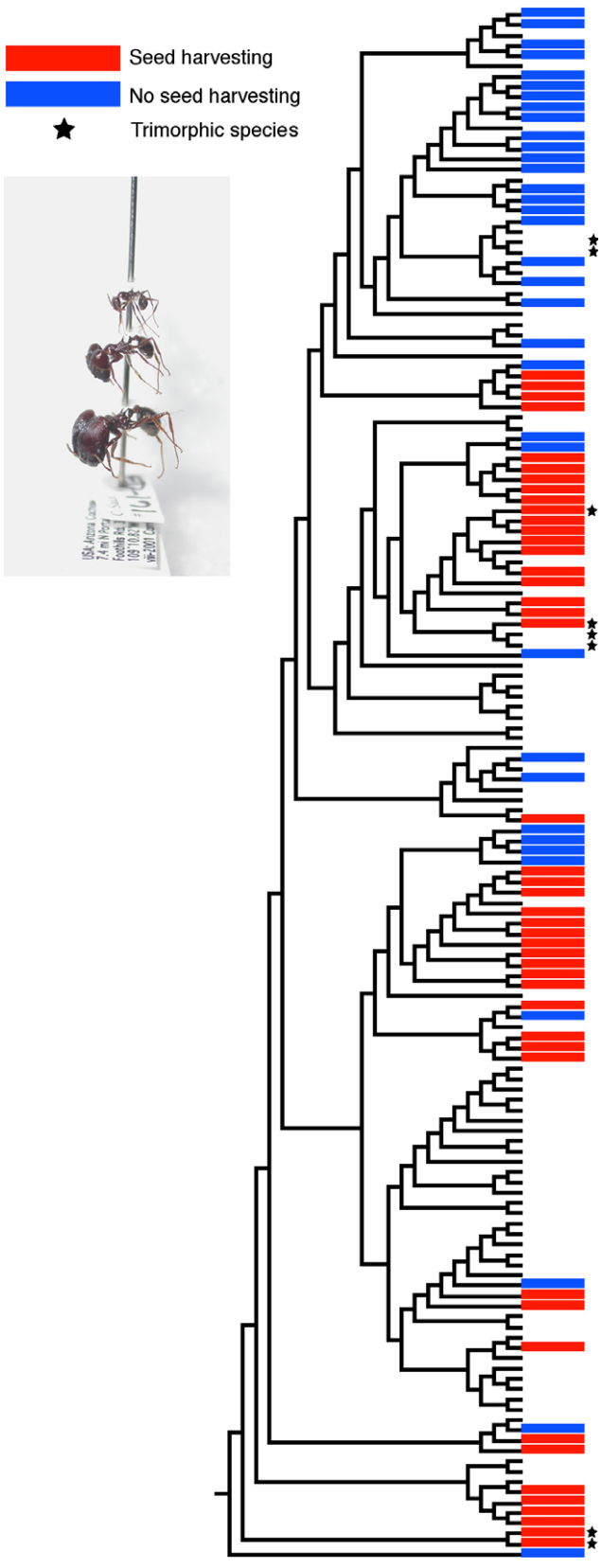

Fig. 3. Seed harvesting, when known, designated by red bars for presence or blue bars for absence on the maximum likelihood tree. Trimorphic species are denoted with a "star". Outgroup taxa have been removed. Image of *Pheidole rhea*, a trimorphic species (image by CSM).

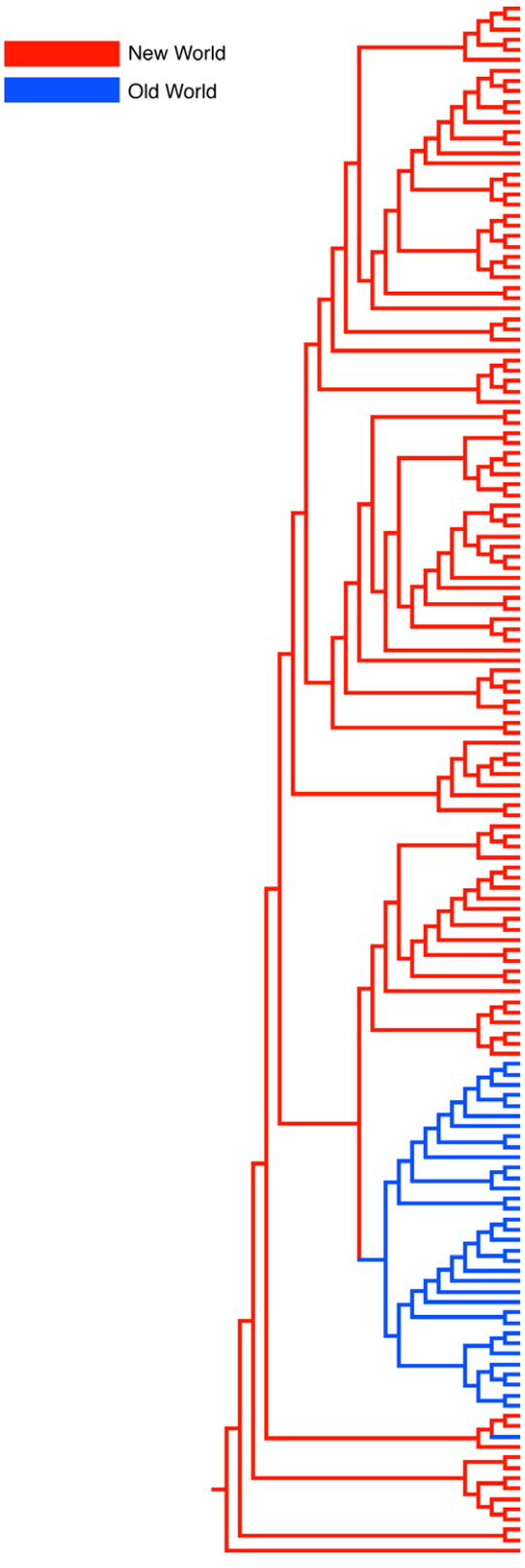

Fig. 4. Biogeographic origin of species mapped on the maximum likelihood tree: New World (red bars) and Old World (blue bars). Outgroup taxa have been removed.

hypothesized, based on overall morphological similarity, that *P. granulata* might be more closely related to *P. vistana*, although the latter lacks the four-segmented antennal club. A close relationship between *P. granulata* and *P. vistana* was recovered in this study (Fig. 1).

Also included in this analysis were several taxa that have been synonomized within another species (see Table 1). Of those where both the valid species and the synonomized taxa were included, only *P. vinelandica* + *P. bicarinata* could not be ruled out as a valid synomony. In order for *P. anastasii* to be a valid member of *P. bilimeki*, *P. floridana* would also have to be accepted as a synonym of *P. bilimeki*, but this and similar cases should be considered carefully as gene trees can remain paraphyletic after recent speciation events. Due to the following inferred relationship ((*P. tucsonica* + *P. xerophila*) + *P. gilvescens*), this is also the case for *P. tucsonica*, which is currently a synomized within *P. gilvescens* where *P. xerophila* would also have to be synonomized within *P. gilvescens* or all three species would have to be included under *P. xerophila*, as both names where first used by Wheeler (1908). The matter is more complicated for the *P. pilifera* species complex (Fig. 1), and suggests that further research into the species-level relationships among these closely related taxa are needed. One interesting point is the position of *P. inquilina*, considered a valid species and a workerless social parasite of *P. coloradensis*. (The latter is a synonomized species now considered a member of *P. pilifera* and nested well within this clade; Wilson, 2003.) *Pheidole inquilina* was recovered in all analyses as sister to the species (and the nest from which it was collected) that it is known to parasitize and one of the included species of *P. pacifica*.

Eight known New World species of *Pheidole* are trimorphic, possessing a super major worker subcaste in addition to the typical minor and major subcastes (Fig. 3) found from the southwestern United States into Mexico. The limited geographic range and number of species that possess this super major subcaste has begged the question of a single origin of this trimorphic worker subcaste. Included in these study were nine specimens from five of these trimorphic species (*P. obtusospinosa*, *P. polymorpha*, *P. rhea*, *P. tepicana*, and *P.* n. sp. 2) and the results presented here indicates that not only are they not monophyletic, but there been at least four independent origins of the trait (Fig. 3). These trimorphic species often co-occur with other species of *Pheidole* in dry, arid habitats. All trimorphic species of *Pheidole* are considered to be seed harvesters with the exception of *P. tepicana*, which is thought to be a more general scavenger, although seed caches have been collected in its nests. The possession of the super major subcaste is thought to allow these species to take advantage of food resources unavailable to other co-occurring species of *Pheidole*, such as larger seeds. The results of this study indicate that the similar ecology of these species may have selected for convergence in the formation of the super major subcaste, rather than shared evolutionary history.

Seed milling and harvesting is common among species of *Pheidole*, but unfortunately there are many species for which life history information is not yet available. The available data are mapped on the maximum likelihood tree (Fig. 3). It is clear that seed harvesting is widespread and in some cases appears to have been absent in entire lineages (Fig. 3), which are more general scavenger and predators. Unfortunately due to the lack of information for presence or absence of seed harvesting for many of the species, conclusions cannot be drawn regarding the potential for this character to be a possible key innovation for this hyperdiverse group. Not only do we need diet preference information for many of the species included here, but many additional taxa are needed to investigate the role this behavior may have played in the evolution of this genus. Also needed to make any meaningful conclusions regarding the potential role of this behavior as a key innovation is information regarding the number of species per clade in which seed harvesting is known. The results of the BayesTraits (Pagel and Meade, 2006) BayesMultiState (Pagel et al., 2004) analyses suggest that the polarity of this trait was more likely to have been from the presence of seed harvesting to the loss of this behavior, and that a gradual model of evolution is the best explanation of the data, but again with such limited knowledge of the behavior of many species, even these conclusions should be taken with caution. We are only now beginning to understand the immense diversity of this group and the addition of further behavioral data and a larger sampling of the species within *Pheidole* are needed. One conclusion that can be drawn from this analysis with reference to seed harvesting, although we do not know the condition in the ancestor to *Pheidole*, is that this behavior does appear to have evolved or been lost multiple times throughout the genus.

Although species of *Pheidole* are found worldwide, Wilson (2003) suggested that the New World may be the cradle for this hyperdiverse genus. The division of New and Old World taxa is not thought to be a natural separation, and in the words of Creighton (1950) in describing the partition of species of the genus *Pheidole*:

> "No plan which rules out the possibility of relationship between Old and New World species is likely to find many champions however useful it may be." p. 161

Interestingly, the results of this phylogenetic analysis suggest that not only is *Pheidole* New World in origin, but there may have been a single introduction into the Old World (with a secondary introduction of a single taxon in Mediterranean Europe). Although lacking bootstrap or posterior probability support, the maximum likelihood topology recovered the Old World taxa, with the exception of the French species, as a monophyletic lineage (Fig. 4). If this topology reflects the true biogeographic history of the genus, then *Pheidole* is New World in origin with a single introduction into the Old World. Since the statistical support for this monophyletic Old World clade is lacking and the maximum parsimony and Bayesian inference found this clade to be a polytomy (suggesting at least one to three independent origins into the Old World), a maximum likelihood search was performed in which the Old World taxa were constrained to be non-monophyletic. Using the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) to test for significant differences in tree lengths, this competing topology was found to be significantly different at the $\geqslant 0.05$ level from the maximum likelihood topology (Table 3). Although another topology where the Old World taxa were allowed to be non-monophyletic was found to be a significantly worse fit to the data, this does not necessarily support the hypothesis of a single introduction into the Old World of *Pheidole* from a New World stock (although it is suggestive). However, both of these analyses support the hypothesis of Wilson (2003) of a New World origin for the genus.

So why then is *Pheidole* so diverse? Unfortunately much more data and natural history information are needed to solve this puzzling question. As Pie and Traniello (2007) showed in their examination of morphological variation among 231 species of *Pheidole*, morphological evolution in the genus has been greatly conserved despite substantial ecological diversification. The results of this study are not able to identify unambiguously those characters or behaviors that may have been the key innovations that promoted the diversification of *Pheidole*, but we now have a better understanding of the timing and evolution of this genus. This study highlights the need for more studies on the phylogeny, morphological evolution, behavioral ecology, and natural history within this ecologically dominant group.

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