Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Molecular Phylogeny of the Ant Subfamily Formicinae (Hymenoptera, Formicidae) from China Based on Mitochondrial Genes

ZL Chen[1], SY Zhou[1], DD Ye[1], Y Chen[1], CW Lu[1]

1 - College of Life Sciences, Guangxi Normal University, Guilin, China

Article History
Edited by:
Gilberto M. M. Santos, UEFS - Brazil
Received 26 December 2012
Initial acceptance 19 February 2013
Final acceptance 08 April 2013

Keywords
Ant phylogeny; Formicidae; *Cyt b, COI, COII*

Corresponding author:
Shan-Yi Zhou
College of Life Sciences
Guangxi Normal University
Guilin, 541004, China.
E-Mail: syzhou5612@yahoo.com.cn

Abstract

To resolve long-standing discrepancies in the relationships among genera within the ant subfamily Formicinae, a phylogenetic study of Chinese Formicine ants based on three mitochondria genes (*Cyt b, COI, COII*) was conducted. Phylogenetic trees obtained in the current study are consistent with several previously reported trees based on morphology, and specifically confirm and reinforce the classifications made by Bolton (1994). The tribes Lasiini, Formicini, Plagiolepidini and Camponotini are strongly supported, while Oecophyllini has moderate support despite being consistent across all analyses. We have also established that the genus *Camponotus* and *Polyrhachis* are indeed not monophyletic. Additionally, we found strong evidence for *Polyrhachis paracamponota,* as described by Wu and Wang in 1991, to be corrected as *Camponotus* based on molecular, morphological and behavioral data.

Introduction

Ants are one of the most successful groups of eusocial insects. They act as an important part of the animal biomass in tropical rainforests and occupy key positions in many terrestrial environments (Wilson & Hölldobler 2005). Resolving the phylogeny of major ant lineages is vital for understanding the factors contributing to their success. Previous studies based on morphological (Baroni Urbani *et al*. 1992, Bolton 2003), fossil-based (Grimaldi *et al.* 1997, Dlussky 1999, Ward & Brady 2003, Bolton 2003), and molecular (Astruc *et al.* 2004, Saux *et al.* 2004, Ward & Brady 2003,Ward & Downie 2005, Ward *et al.* 2005, Brady *et al.* 2006, Moreau *et al.* 2006, Ouellette *et al.* 2006) data provided useful framework for understanding the relationships among ant subfamilies. However, relationships among genera within the subfamilies are not well understood. In addition, the genus-level phylogeny and classification of ant subfamilies remain controversial in many respects.

Formicinae is one of the most abundant ant subfamilies in the Holarctic (Wilson 1955). According to Bolton (2012), Formicinae includes 49 extant genera and over 3700 species and subspecies in the world. Although the subfamily includes a large number of abundant and ecologically important species that are often subjected to ecological and sociobiological studies, little is known about their phylogeny. Although there are several classifications based on a variety of morphological characteristics, such as sexual traits and larval morphology (Wheeler 1922, Emery 1925, Wheeler & Wheeler 1985, Agosti 1991, Bolton 1994, 2003), the tribes or genus-groups represent artificial assemblages and are used inconsistently by different myrmecologists or even by the same myrmecologist at different times. In particular, some aspects of worker morphology show a strong tendency towards convergence, making it challenging to infer phylogenetic relationships from morphological characteristics alone (Ward 2007). Indeed, Bolton has acknowledged that some tribes in his tribal arrangements would likely need to be re-evaluated (Bolton 2003).

No molecular phylogenetic study has been performed on the subfamily Formicinae in China to date. This study

aimed to establish molecular relationships among Formicinae members relative to previously established frameworks and to take a deeper look into species level relationships within more ambiguous assemblages. This was done by obtaining sequences of the mitochondrial genes cytochrome b (*Cyt b*), cytochrome oxidase subunit 1 (*COI*) and cytochrome oxidase subunit 2 (*COII*) and comparing them using Bayesian Inference (BI) (Nylander 2004), Maximum Parsimony (MP) and Neighbour Joining (NJ) (Swofford 2002).

Materials and Methods

Taxon sampling

In this study, a total of 47 species representing 14 genera from five tribes were selected to test the groups suggested by the tribal structure and dendrograms of Wheeler (1922), Emery (1925), Wheeler and Wheeler (1985), Agosti (1991), and Bolton (1994, 2003). *Cerapachys sulcinodis* from the subfamily Cerapachyinae and *Radoszkowskius oculata* from the family Mutillidae were added as outgroups. Apart from *R. oculata*, all other vouchers of Formicinae and *C. sulcinodis*, consisting of nestmate specimens from the same collection event have been deposited in the collection of Guangxi Normal University. Detailed information of the species studied is listed in Appendix 1.

DNA extraction, PCR, and sequencing alignment

Total genomic DNA was extracted from ground whole workers, of which the gasters were removed to minimize contamination from gut bacteria, using standard CTAB methods (slightly modified from Navarro *et al.* 1999). DNA sequence data from three protein-coding mitochondrial genes, namely *Cyt b*, *COI*, and *COII*, were obtained using conventional PCR methods (Villesen *et al.* 2004, Ward & Downie 2005). The sequences and positions on the mitochondrial DNA of the primers used for PCR and sequencing are shown in Table 1.

The primers J2791 and H3665 were used to amplify fragments of mitochondrial DNA that correspond to the 3' end of *COI*, *ITS*, and tRNA-leucine and the 5' end of *COII*. Fragments were sequenced in both directions, and the resulting chronograms were assembled and edited using DNAStar (Bioinformatics Pioneer DNAStar, Inc., WI). Sequence for each gene fragment was aligned using CLUSTALX v.1.83 (Thompson *et al.* 1997). Sites from the intergenic spacer (*ITS*) and tRNA-leucine were not used in the analyses. All new DNA sequences generated in this study were submitted to the NCBI GenBank database. Sequence data of the outgroup *R. oculata* was obtained via GenBank direct submission by Wei, S.J. and Chen, X.X. All GenBank accession numbers related to this study are listed in Appendix 1.

Phylogenetic analyses

Reconstruction of phylogenetic relationships among taxa was conducted using NJ, MP, and BI methods. NJ analysis was performed using PAUP* Version 4.0b10 (PPC) (Swofford 2002). Estimates of nodal support on distance trees were obtained using bootstrap analyses (1000 replications). MP analysis was also unweighted and performed using PAUP* Version 4.0b10 (PPC) (Swofford 2002). It involved the use of a heuristic search with random sequence addition (10 replicates each) and the TBR branch-swapping algorithm. Bayesian phylogenetics was used to estimate tree topology using MRBAYES v.3.1.2 (Ronquist & Huelsenbeck 2003). Data were partitioned by gene to yield a total of three data partitions, and the best-fitting model for each partition was selected using MRMODELTEST v. 2.2 (Nylander 2004) under Akaike information criteria (Posada & Buckley 2004).

Results

DNA sequence composition

Table 2 shows the nucleotide content and substitution of three fragment sequences. The final data matrix contained 1830 characters (1049 variable sites, 897 parsimony-informative sites, 152 singleton sites) from the following gene fragments: *Cyt* b-447 characters (270 variable sites, 232 parsimony-informative sites, 38 singleton sites), COI-825 aligned characters (433 variable sites, 379 parsimony-informative sites, 54 singleton sites), and COII-558 characters (341 variable sites, 289 parsimony-informative sites, 52 singleton

Table 1. Sequences of primmer used in this study. Position refers to coordinates in the *Solenopsis invicta* mitochondrion complete genome, GenBank accession numbers: HQ215540. Primer combinations are as follows, with the forward primer listed Wrst for each pair: CB-11400–CB-11884, LCO1490–HCO2198, J2791–H3665, J2791–*COI*-R, CO-F–H3665.

Designation	Sequence (5'–3')	Position	Reference
CB-11400	TATGTACTACCHTGAGGDCAAATATC	9381-9406	Modified from Folmer *et al*. 1994
CB- 11884	ATTACACCNCCTAATTTATTAGGRAT	9840-9865	Modified from Folmer *et al*. 1994
LCO1490	GGTCAACAAATCATAAAGATATTGG	117-141	Modified from Folmer *et al*. 1994
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	700-726	Modified from Folmer *et al*. 1994
J2791	ATACCHCGDCGATAYTCAGA	1300-1319	Modified from Chiotis *et al*. 2000
CO-R	TCRTGRAAGAAGATTATTA	1650-1668	This study
CO-F	CTTTTATTAAAAATHAACAC	1586-1605	This study
H3665	CCACARATTTCWGAACATTG	2177-2196	Modified from Chiotis *et al*. 2000

sites). The base composition of these three fragments varied among the studied species. On average, the base composition was: T 40.8%, C 17.8%, A 31.9%, and G 9.5%, with a strong AT bias (72.7%) as is commonly found in other insect mitochondrial genomes (Vogler & Pearson 1996). The A+T contents of the third, second and first codon position from the three fragments were 84.2%, 66.2%, and 67.4%, respectively. The transitions of nucleotide substitution were more common than transversion with a transition. Numerically, the transversion between A and T was the highest among the four types of nucleotide transversions, whereas the transition between C and T was the highest of the two types of nucleotide transitions.

Amino acid composition and substitution saturation

The complete 1830 nucleotide sequence encoded 610 amino acids of 20 different types. Leucine (Leu) was the most frequent (13.53%) followed by isoleucine (Ile) (13.30%). Cysteine (Cys) was the least frequent, with a constant content of 0.29%. All three protein-coding genes were tested for saturation. These were achieved by plotting the numbers of observed substitutions versus the uncorrected p-distance estimates. The scattergrams (Fig. 1) show that TV increased along the uncorrected p-distance and TS reached saturation between certain pairs of taxa.

Phylogenetic trees

Phylogenetic analyses (Figs. 2 to 4) showed that the outgroups *C. sulcinodis* and *R. oculata* were well-resolved from the Formicinae taxa at the base of the trees with high confidence values (0.94 Bayesian posterior probability (PP), 100% NJ bootstrap, 99% MP bootstrap). As shown in Figure 5E (this Figure was synthesized from Figs. 2 to 4), all consensus trees strongly indicated that the 14 genera of Formicinae could be divided into five lineages, which we labeled as clades I-V, and consisted of genera from the tribes Lasiini, Formicini, Oecophyllini, Plagiolepidini and Camponotini, respectively. Our findings are consistent with morphological classifications of Bolton (1994) (Figs. 5E and 5F).

Clade I included four genera: *Lasius*, *Nylanderia*, *Prenolepis*, *Pseudolasius* (1.0 PP, 84% NJ bootstrap, 54% MP bootstrap). *Pseudolasius* appeared to be a sister group of (*Lasius* + (*Nylanderia* + *Prenolepis*)) in all three trees. These analyses showed that *Nylanderia* is a sister genus of *Prenolepis* with very strong support (1.0 PP, 90% NJ bootstrap, 89% MP bootstrap). A supported clade of ((*Formica* + *Polyergus*) + (*Proformica* + *Cataglyphis*)) (1.0 PP, 73% NJ bootstrap, 73% MP bootstrap) forms Clade II. Our analyses showed *Formica* as a sister genus of *Polyergus* (1.0 PP, 97% NJ bootstrap, 97% MP bootstrap), and *Proformica* as a sister genus of *Cataglyphis* with very strong support (1.0 PP, 97% NJ bootstrap, 91% MP bootstrap) in all trees. Clade III included only one species (*Oecophylla smaragdina*) and was placed as a sister group to Clade II. Although this species was not supported by strong bootstrap values (0.58 PP, 54% NJ bootstrap, 16% MP bootstrap), it was a consistent feature in all reconstructions. Clade IV comprised of three genera: *Anoplolepis*, a sister group to (*Plagiolepis* + *Lepisiota*). The genus *Plagiolepis* and *Lepisiota* also formed a sister group with good support in all trees. Clade V included *Camponotus* and *Polyrhachis* with very strong support (1.0 PP, 100% NJ bootstrap, 87% MP bootstrap). However the species-level phylogeny of the genera remains unresolved except for the distinct subclade of

Table 2. The content and substation of nucleotide sequences. Cs, conserved sites; V, variable sites; Pi, parsimony-informative sites; S, Singleton sites; ii, identical pairs; si, transitional pairs; sv, transversional pairs; R, Ts/Tv.

genes	Cs	V	Pi	S	Nuleotide content (%)					Nuleotide substitution			
					T	C	A	G	A+T	ii	si	sv	R
COI (825)	392	433	379	54	40.8	18.3	30.1	10.7	70.9	664	78	83	0.94
COII (558)	217	341	289	52	40.7	16.6	35.2	7.4	75.9	440	52	66	0.79
Cyt b (447)	177	270	232	38	40.7	18.4	31.2	9.7	71.9	349	45	52	0.88
Total (1830)	781	1049	879	152	40.8	17.8	31.9	9.5	72.7	1454	175	200	0.87

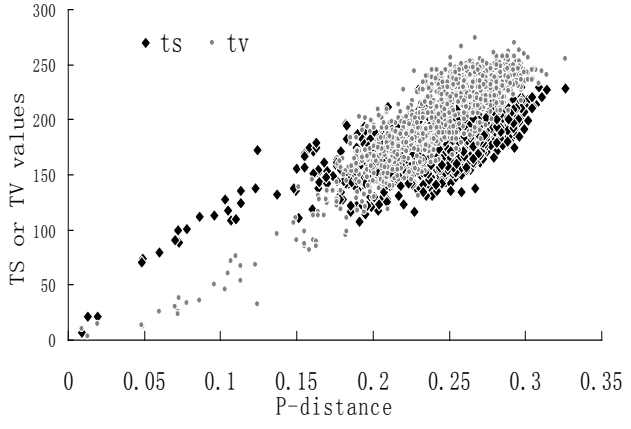

Fig.1. Scatterplots showing the number of substitutions (y-axes; TS, transitions; TV, transversions) versus uncorrected p-distance (x-axes) at each codon position.

(*C. mitis* + (*C. vanispinus* + (*C. jianghuaensis* + *C. albosparsus*))). *C. singularis* is a sister species of other species of the genus *Camponotus* (including *Polyrhachis paracamponota*, excluding *C. yiningensis*) with very strong support (98% NJ bootstrap) in the NJ tree (Fig. 3) and modest support (67% MP bootstrap) in the MP tree (Fig. 2). However, in the BI tree (Fig. 4), *C. parius* first clustered with *C. wasmanni* with strong support (1.0 PP) and then as a sister group of *C. singularis* plus the rest of the species of *Camponotus* (including *P. paracamponota*, excluding *C. yiningensis*). *C. yiningensis* was tightly associated with *Polyrhachis* with very strong support (1.0 PP, 100% NJ bootstrap, 87% MP bootstrap), and further studies on its status are needed. The species *P. paracamponota* clustered with *Camponotus*, and was distinct from *Polyrhachis*.

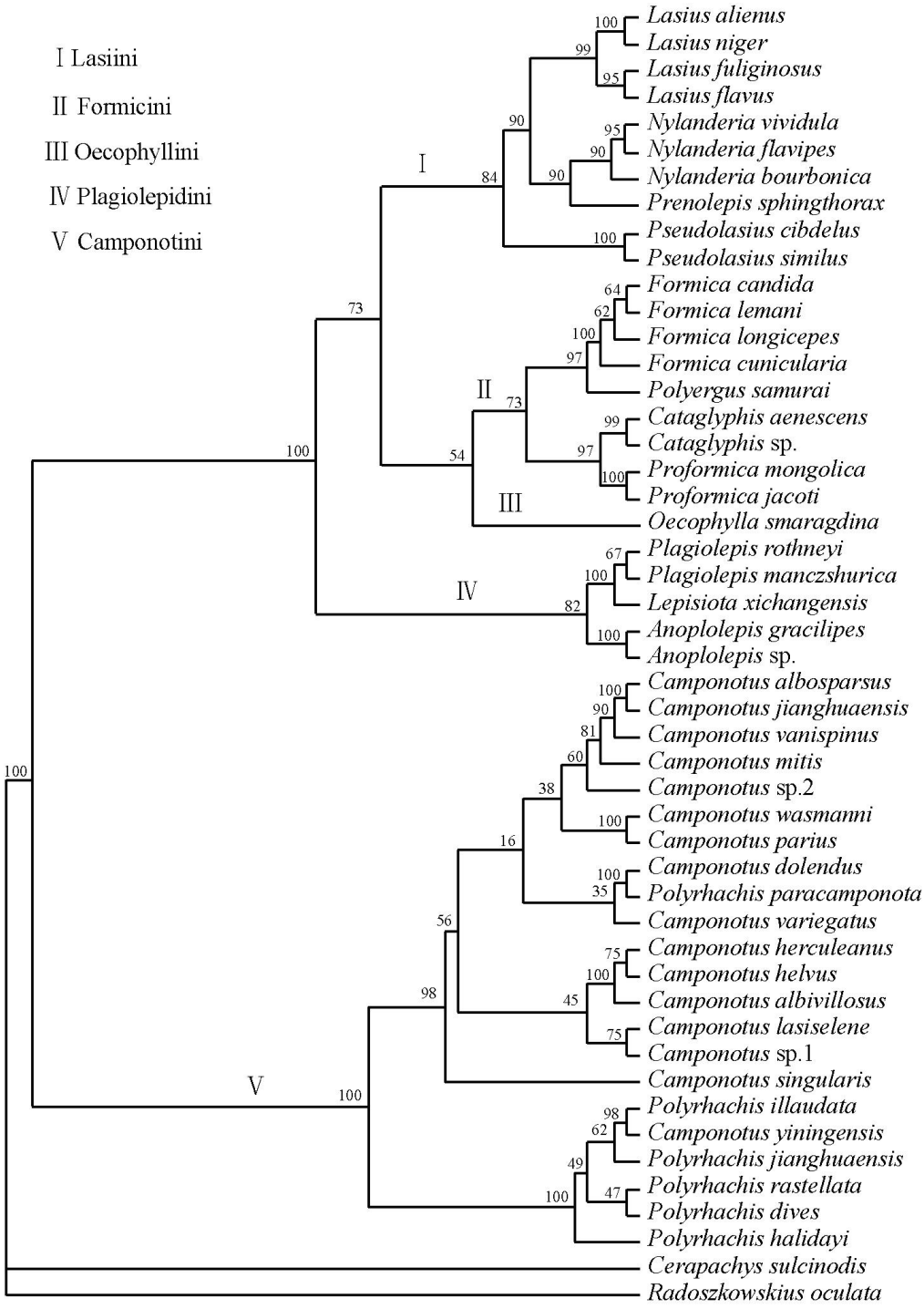

Fig. 2. Maximum-parsimony (MP) consensus tree from 1000 bootstrap replicates, obtained from 48 species of the concatenated sequences of the *Cytb* gene (447 bp), *COI* gene (825 bp) and *COII* gene (558 bp), with *Cerapachy sulcinodis* and *Radoszkowskius oculata* as the outgroups.

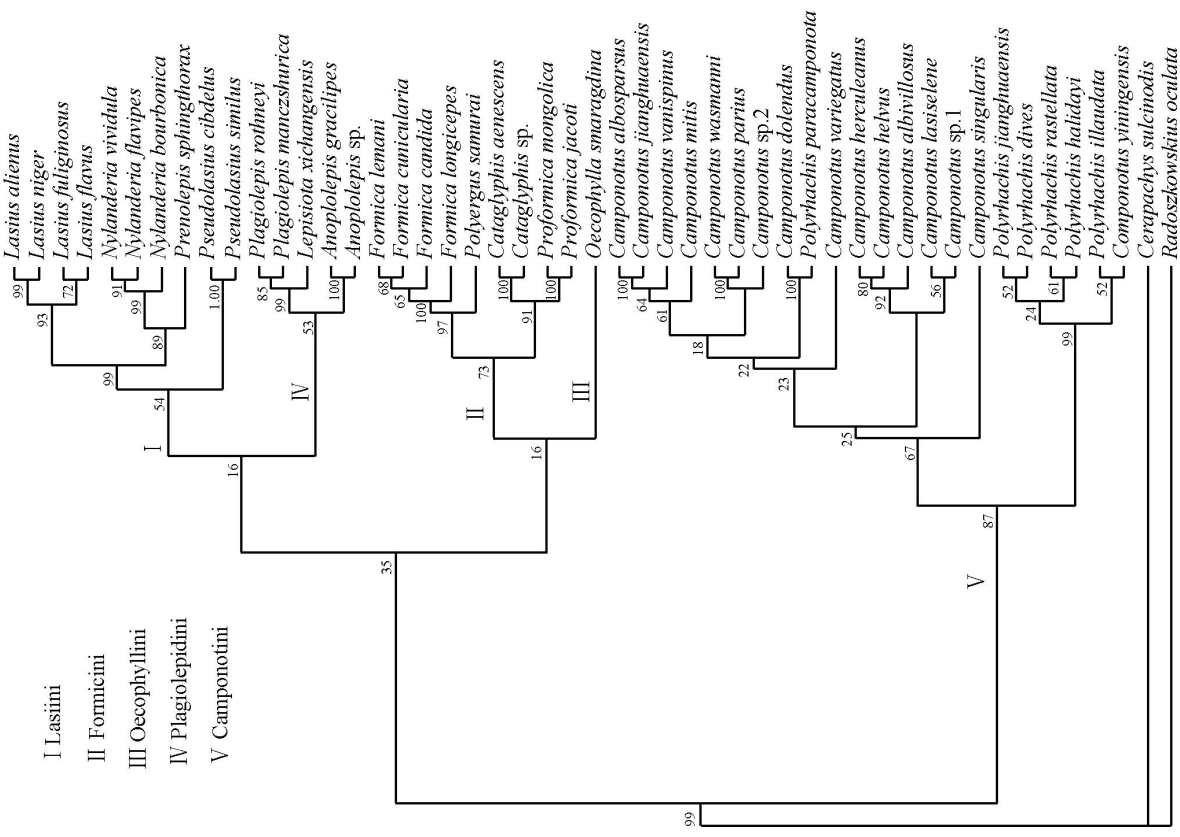

Fig. 4. Bayesian (BI) majority-rule consensus tree, obtained from 48 species of the concatenated sequences of the *Cyt b* gene (447 bp), *COI* gene (825 bp) and *COII* gene (558 bp) three partitions all under the same best-fit model (GTR+I+G) selecting by AIC in Modeltest, with *Cerapachy sulcinodis* and *Radoszkowskius oculata* as the outgroups.

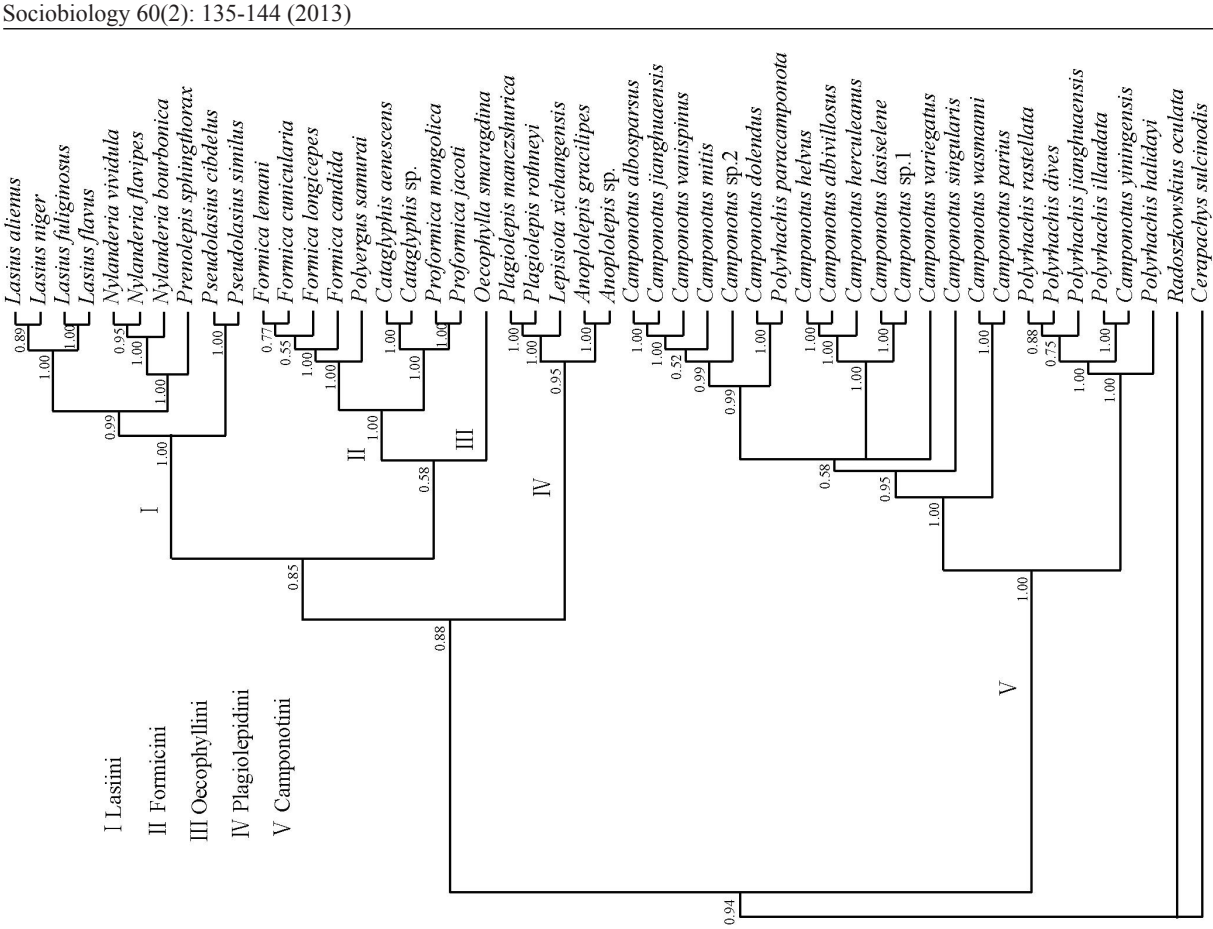

Fig. 3. Neighbor-joining (NJ) consensus tree from 1000 bootstrap replicates, obtained from 48 species of the concatenated sequences of the *Cyt b* gene (447 bp), *COI* gene (825 bp) and *COII* gene (558 bp), with *Cerapachy sulcinodis* and *Radoszkowskius oculata* as the outgroups.

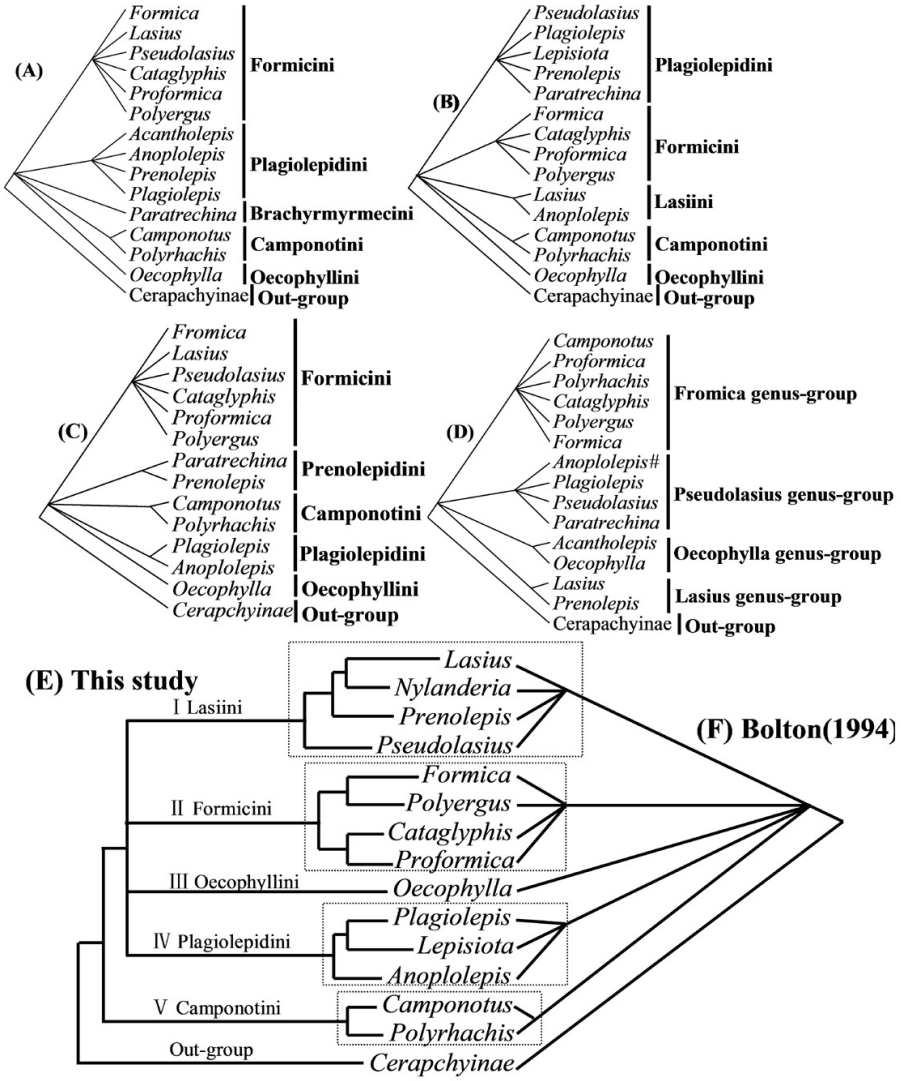

Fig. 5 Classifications of Formicine genera based on the schemes of: (A) Wheeler WM 1922; (B) Bolton 2003; (C) Wheeler, WM *et al*. 1985; (D) Agosti 1991; (E) This study; (F) Bolton 1994. {NB: only positions for species of interest in this phylogeny are noted; there are changes in classifications of other genera which are not being used in this study }.

Discussion

Results of the phylogenetic relationships of Formicinae in this study (Figs. 2 to 4, 5E) showed both similarities and differences compared with those of previous studies (Fig. 5A-5D, 5F). Surprisingly, results of our molecular phylogenetic trees have better fit with the morphological cladogram of Bolton (1994), with which they are congruent, than with that of Bolton (2003).

Clade I is best characterized morphologically with the worker alitrunk not conspicuously constricted or otherwise specialized and the mesonotum typically convex in profile view. The workers of *Lasius*, *Nylanderia* and *Prenolepis* shared the following morphological characters (Bolton 1994): mandibles roughly triangular with four to seven teeth, antennae 12-segmented, the torula close to but not touching the posterior clypeal margin. A propodeal spiracle present at or near the declivity of the propodeum, and the petiolar node in profile usually inclined forward, with a short anterior face and much longer posterior face. These data support the earlier hypothesis proposed by Bolton in 1994, into which *Pseudolasius*, *Prenolepis*, *Nylanderia* and *Lasius* were placed and formed the tribe Lasiini, but disagrees with that of Bolton (2003), in which the genera *Plagiolepis* and *Lepisiota* were added to form the tribe Plagiolepidini. In addition, these four genera formed a strongly supported group in all trees, especially in the case of the sister genus relationship between *Nylanderia* and *Prenolepis* (1.0PP, 90% NJ bootstrap, 99% MP bootstrap). These results are consistent with those of previous morphological (Emery 1925, Wheeler & Wheeler 1953, Trager 1984) and molecular studies (Brady *et al*. 2006), However, in the study of Moreau *et al*. (2006), the genus *Plagiolepis, Pseudolasius* and *Prenolepis* emerges first, followed by *Lasius* along with other two genera. Besides the study by LaPolla *et al.* (2010) in which *Prenolepis* was treated as being paraphyletic to the group. In addition, monophyly of the genus *Lasius* was strongly supported (0.99 PP, 90% NJ bootstrap, 99% MP bootstrap).

The results for clade II are consistent with those of previous studies (Bolton 1994, 2003) (Figs. 5E, 5F and 5B). Genera of the tribe Formicini share the following morphological features (Bolton 1994): 12-segmented antennae, antennal

sockets situated close to the posterior clypeal margin. Orifices of propodeal spiracle oval, elliptical, or as elongated slits and near-vertical or inclined from the vertical. All of these analyses provided strong support for the two sister-group relationships of (*Formica* + *Polyergus*) and (*Proformica* + *Cataglyphis*), which is consistent with the molecular studies of Moreau *et al*. (2006).

In clade III, the genus *Oecophylla* was separated as a distinct lineage. This result is well supported by previous morphological studies (Wheeler 1922, Wheeler & Wheeler 1985, Bolton 1994, 2003) (Fig. 5), which showed *Oecophylla* as the tribe Oecophllini. In our molecular phylogeny, *Oecophylla* appears to be a sister of Formicini but with low bootstrap support (0.58 PP, 0.54% NJ bootstrap, 16% MP bootstrap). However, this topology is in agreement with that of Moreau *et al*. (2006). Wilson and Taylor (1964) also suggested that *Oecophylla* and clade II cannot be given much credence considering the separate placement in morphologically and parsimony-based phylogenies, as well as its current geographical separation. However, fossil evidence indicate that *Oecophylla* previously occurred in Europe, suggesting that these genera may have shared a common ancestor.

Clade IV is a well supported clade consisting of members from the tribe Plagiolepidini (*Anoplolepis* + (*Plagiolepis* + *Lepisiota*)) (0.95 PP, 82% NJ bootstrap, 53% MP bootstrap). Bolton (1994) had previously placed the three genera into the tribe Plagiolepidini based on a morphological study (Fig. 5F) and the current study is the first to arrive at the same placement based on molecular phylogenetics. This tribe is distinguished by the following features: worker with 11-segmented antennae, antennal sockets fused with the posterior clypeal margin, and palp formula of 6,4. Surprisingly, Bolton (2003) proposed the genus *Plagiolepis* and *Lepisiota* to be included in the tribe Plagiolepidini (Fig. 5B). Although Bolton (2003) represents a more comprehensive summary of ant morphological characters assembled to date than his previous treatment (Bolton 1994), it is likely that this reflects a genuine conflict between morphology and molecular data.

Clade V is strongly supported in all trees (1.0 PP, 100% NJ bootstrap, 87% MP bootstrap) and consists of *Camponotus* and *Polyrhachis*. This result is in agreement with previous morphological (Wheeler 1922, Emery 1925b, Wheeler & Wheeler 1985, Bolton 1994, 2003) (Figs. 5) and molecular studies (Astruc *et al.* 2004, Brady *et al.* 2006, Moreau *et al.* 2006). The tribe Camponotini can be characterized by its 12-segmented antennae, with antennal sockets situated far behind the posterior clypeal margin, and a palp formula of 6,4. *Camponotus* is however a paraphyletic group, as is noted in other studies (Brady *et al.* 1999, Astruc *et al.* 2004, Brady *et al.* 2006). *Camponotus yiningensis* has been placed outside of the genus *Camponotus*, which has been confirmed not to be monophyletic (Brady *et al.* 1999, 2000; Astruc *et al.* 2004, Brady *et al.* 2006). Morphological characters also reflected close, and sometimes overlapping, relationships between *Camponotus* and *Polyrhachis.* For instance, many species of *Camponotus* acquired distinctive spines, and many species of *Polyrhachis* have camber-shaped alitrunks. The species *Polyrhachis paracamponota* was first described by Wang and Wu in 1991 based on a single holotype worker which possesses pronotal spines, and was placed in the genus *Polyrhachis*. But having pronotal spines is very common in *Camponotus* and *Polyrhachis,* this morphological character could not be used for distinguishing between the two genera. The original descriptions exact match with the morphological character of the genus *Camponotus*. In our opinion, the authorships also had the same idea, so this species be named "*paracamponota*". Besides, this species has polymorphic workers, and they have been observed to tunnel into the soil for subterranean nesting. In contrast, the workers of *Polyrhachis* are exclusively monomorphic, and can only use existing cavities in the soil or under stones for nesting, but never excavate tunnels themselves. Our phylogenetic reconstruction indicated that this species is associated with *Camponotus*, and is clearly separated from *Polyrhachis*. As such, there is strong evidence from morphological, behavioristic and molecular data that *Polyrhachis paracamponota* should be placed as a member of *Camponotus*.

Conclusion

In conclusion, our study of the phylogenetic relationship of Formicinae from China based on sequences from three protein-coding mitochondrial genes (*Cyt b, COI, COII*) confirms and reinforces the findings of previous morphological studies (Bolton 1994). The tribes Lasiini (*Pseudolasius*, *Prenolepis*, *Paratrechina*, *Lasius*), Formicini (*Formica*, *Cataglyphis*, *Proformica*, *Polyergus*), Plagiolepidini (*Lepisiota*, *Plagiolepis*, *Anoplolepis*), and Camponotini (*Camponotus*, *Polyrhachis*) are strongly supported, while Oecophyllini has moderate support despite being consistent across all analyses. We have also established that the genus *Camponotus* and *Polyrhachis* are indeed not monophyletic. Additionally, evidence from molecular, morphological and behavioral data indicates that *Polyhachis paracamponota* should be corrected as *Camponotus*.

Acknowledgments

We sincerely thank Professor Yu-Feng Xu (National Taiwan Normal University, Taiwan), Dr. Jun-Hao Tang (National University of Singapore) and Dr. John R. Fellowes (Kadoorie Farm and Botanic Garden, Hong Kong) for reviewing the English text. We thank two anonymous reviewers for helpful comments on the manuscript. Thanks also to Chao-Tai Wei (Guangxi Normal University) for providing us with some ant materials, De-Long Zeng (Guangxi Normal University) for helpful assistance and comments on phylogeny analysis. This study was supported by the National Natural Science

Foundation of China (Project Nos. 30770258 and 31071971), Foundation of the Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection, Ministry of Education, Guangxi Normal University.

References

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

Astruc, C., Julien, J. F., Errard, C. & Lenoir, A. (2004). Phylogeny of ants (Formicidae) based on morphology and DNA sequence data. Mol. Phylog. Evol., 31: 880-893. doi: 10.1016/j.ympev.2003.10.024

Baroni Urbani, C., Bolton, B. & Ward, P. S. (1992). The internal phylogeny of ants (Hymenoptera: Formicidae). Syst. Entomol., 17: 301-329.

Bolton, B. (1994). Identification guide to the ant genera of the world. *Harvard University Press, Cambridge, Massachusetts*, 222 pp.

Bolton, B. (2011). Catalogue of species-group taxa. http://gap.entclub.org/contact.html. *(accessed date: 1 March, 2011)*.

Bolton, B. (2003). Synopsis and classification of Formicidae. Mem. Am. Entomol. Inst., 71: 1-370.

Brady, S.G., Gadau, J. & Ward, P.S. (1999). Is the ant genus *Camponotus* paraphyletic? *4th International Hymenopterists Conference,* Glen Osmond, South Australia, Canberra, Australia.

Brady, S.G., Gadau, J. & Ward, P.S. 2000. Systematics of the ant genus *Camponotus* (Hymenoptera: Formicidae): a preliminary analysis using data from the mitochondrial gene cytochrome oxidase I. *In: Austin, A.D., Dowton, M. (Eds.), Hymenoptera. Evolution, Biodiversity and Biological Control.* CSIRO Publishing, Collingwood, Victoria, pp. 131-139, xi+ 468 pp.

Brady, S.G., Fisher, B.L., Schultz, T.R. & Ward, P.S. (2006). Evaluating alternative hypotheses for the early evolution and diversification of ants. Proc. Nat. Acad. Sci. USA., 103: 18172-18177. doi: 10.1073/pnas.0605858103

Chiotis, M., Jermiin, L.S. & Crozier, R.H. (2000). A Molecular Framework for the Phylogeny of the Ant Subfamily Dolichoderinae. Mol. Phylog. Evol., 17(1): 108-116. doi: 10.1006/mpev.2000.0821

Dlussky, G.M. (1999). The first find of the Formicoidea (Hymenoptera) in the lower Cretaceous of the northern hemisphere. [In Russian.] Paleontol. Zhurnal, 3: 62-66.

Emery, C. (1925). Hymenoptera. Fam. Formicidae. Subfam. Formicinae. Gen. Insectorum, 183: 1-302.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. and Biotech., 3(5): 294-299.

Grimaldi, D., Agosti, D. & Carpenter, J.M. (1997). New and rediscovered primitive ants (Hymenoptera: Formicidae) in Cretaceous amber from New Jersey, and their phylogenetic relationships. Am. Mus. Nov., 3208: 1-43.

Huelsenbeck, J.P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. Bioinformatics, 17: 754-755.

Johnson, R.N., Agapow, P.M. & Crozier, R.H. (2003). A tree island approach to inferring phylogeny in the ant subfamily Formicinae, with especial reference to the evolution of weaving. Mol. Phylog. Evol., 29: 317-330. doi: 10.1016/S1055-7903(803)00114-3

LaPolla, J.S, Brady, S.G. & Shattuck, S.O. (2010). Phylogeny and taxonomy of the *Prenolepis* genus-group of ants (Hymenoptera: Formicidae). Syst. Entomol., 35: 118-131. doi: 10.1111/j.1365-3113.2009.00492.x

Moreau, C.S.., Bell, C.D., Vila, R., Archibald, S.B. & Pierce, N.E. (2006). Phylogeny of the ants: diversification in the age of angiosperms. Science, 312: 101-104. doi: 10.1126/science.1124891

Navarro E., Jaffre T., Gauthier D., Gourbiere F., Rinaudo G., Simonet P. & Normand P. (1999). Distribution of Gymnostoma spp. microsymbiotic Frankia strains in New Caledonia is related to soil type and to host-plant species. Mol. Ecol., 8: 1781-1788.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. (2004). Bayesian phylogenetic analysis of combined data. Syst. Biol., 53: 47-67 doi: 10.1080/10635150490264699

Nylander, J.A.A. (2004). MrModeltest v2, Program distributed by author. *Evolutionary Biology Centre, Uppsala University, Uppsala*.

Ouellette, G.D., Fisher, B.L. & Girman, D.J. (2006). Molecular systematics of basal subfamilies of ants using 28S rRNA (Hymenoptera: Formicidae). Mol. Phylog. Evol., 40: 359-369. doi: 10.1016/j.ympev.2006.03.017

Posada, D. & Buckley, T.R. (2004). Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol., 53: 793-808. doi: 10.1080/10635150490522304

Ronquist, F. & Huelsenbeck, J.P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19: 1572-1574.

Saux, C., Fisher, B.L. & Spicer, G.S. (2004). Dracula ant phylogeny as inferred by nuclear 28S rDNA sequence and implications for ant systematics (Hymenoptera: Formi-

cidae). Mol. Phylog. Evol., 33: 457-468. doi: 10.1016/j.ympev.2004.06.017

Swofford, D.L. (2002). PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Vol. 4. *Sinauer Associates, Sunderland, MA*.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc. Acids Res., 25: 4876-4882.

Trager, J.C. (1984). A revision of the genus *Nylanderia* (Hymenoptera: Formicidae) of the continental United States. Sociobiology, 9: 49-162.

Villesen, P., Mueller, U.G., Schultz, T.R., Adams, R.M.M. & Bouck, A.C. (2004). Evolution of ant-cultivar specialization and cultivar switching in *Apterostigma* fungus-growing ants. Evolution, 58: 2252-2265. doi: 10.1111/j.0014-3820.2004.tb01601.x

Vogler, A.P. & Pearson, D.L. (1996). A molecular phylogeny of the tiger beetles (Cicindelidae): congruence of mitochondrial and nuclear rDNA data sets. Mol. Phylog. Evol., 6: 321-338.

Wang, C.L. & Wu, J. (1991). Taxonomic Studies on the Genus *Polyrhachis* Mayr of China (Hymenoptera: Formicinae). For. Res., 4(6): 596-601.

Ward, P.S. & Brady, S.G. (2003). Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). Invert. Syst., 17, 361-386. doi: 10.1071/IS02046

Ward, P.S. & Downie, D.A. (2005). The ant subfamily Pseudomyrmecinae (Hymenoptera: Formicidae): phylogeny and evolution of big-eyed arboreal ants. Syst. Entom., 30: 310-335. doi: 10.1111/j.1365-3113.2004.00281.x

Ward, P.S., Brady, S.G., Fisher, B.L. & Schultz, T.R. (2005). Assembling the ant "Tree of Life" (Hymenoptera: Formicidae). Myrmecol. Nachrichten, 7: 87-90.

Ward, P.S. (2007). Phylogeny, classification, and species-level taxonomy of ants. (Hymenoptera: Formicidae). Zootaxa, 1668: 549-563.

Wheeler, W.M. (1922). Ants of the American Museum Congo expedition. A contribution to the myrmecology of Africa. VII. Keys to the genera and subgenera of ants. Bul. Am.. Mus. Nat. Hist., 45: 631-710.

Wheeler, G.C. & Wheeler, J. (1953). The ant larvae of the subfamily Formicinae. Ann. Entom. Soc. Am., 46: 126-171.

Wheeler, G.C. & Wheeler, J. 1985. A simplified conspectus of the Formicidae. *T*rans. Am. Entom. Soc.*,* 111: 255-264.

Wilson, E.O. (1955). A monographic revision of the ant genus *Lasius*. Bul. Mus. Compar. Zool., 113: 1-201.

Wilson, E.O, & Taylor, R.W. (1964). A fossil ant colony: new evidence of social antiquity. Psyche, 71: 93-103.

Wilson, E.O. & Holldobler, B. (2005). The rise of the ants: a phylogenetic and ecological explanation. Proc. Nat. Acad. Sci. USA, 102: 7411-7414.

Appendix 1

Species	Collection locality	Voucher specimen	GenBank accession numbers		
			Cyt b	*COI*	*COI & COII*
Lepisiota xichangensis	Jingxi, Guangxi	GXJX0006	JQ681097	JQ681046	JQ680992
Plagiolepis manczshurica	Helan Mt, Inner Mongolia	NMHL0422	JQ681098	JQ681047	JQ680993
Plagiolepis rothneyi	Xiangtou Mt, Guangdong	GDXT0122	JQ681099	JQ681048	JQ680994
Anoplolepis gracilipes	Beiliu, Guangxi	GXBL0001	JQ681100	JQ681049	JQ680995
Anoplolepis sp.	Bohai, Yunnan	YNBH0003	JQ681101	JQ681050	JQ680996
Pseudolasius cibdelus	Jingxi, Guangxi	GXJX0031	JQ681102	JQ681051	JQ680997
Pseudolasius similus	Jingxi, Guangxi	NMHL0269	JQ681103	JQ681052	JQ680998
Prenolepis sphingthorax	Jingxi, Guangxi	GXJX0144	JQ681104	JQ681053	JQ680999
Cataglyphis aenescens	Heze, Shandong	Shandong_70	JQ681105	HQ619705	JQ681000
Cataglyphis sp.	Yangling, Shanxi	SXYL0007	JQ681106	JQ681054	JQ681001
Formica candida	Xiaowutai Mt, Hebei	Hebei_50	JQ681107	HQ619704	JQ681002
Formica longicepes	Helan Mt, Inner Mongolia	NMHL0227	JQ681108	JQ681055	JQ681003
Formica cunicularia	Xiaowutai Mt, Hebei	Hebei_307	JQ681109	HQ619714	JQ681004
Formica lemani	Xiaowutai Mt, Hebei	Hebei_251	JQ681110	HQ619712	JQ681005
Proformica mongolica	Helan Mt, Inner Mongolia	NMHL0045	JQ681111	JQ681056	JQ681006
Proformica jacoti	Xiaowutai Mt, Hebei	HBXW0039	JQ681112	JQ681057	JQ681007
Nylanderia flavipes	Heze, Shandong	SDHZ0104	JQ681113	JQ681058	JQ681008
Nylanderia vividula	Guilin, Guangxi	GXGL0111	JQ681149	JQ681093	JQ681044
Nylanderia bourbonica	Jingxi, Guangxi	GXJX0022	JQ681114	JQ681059	JQ681009
Lasius niger	Xiaowutai Mt, Hebei	HBXW0263	JQ681115	JQ681060	JQ681010
Lasius flavus	Helan Mt, Inner Mongolia	NMHL0320	JQ681116	JQ681061	JQ681011
Lasius fuliginosus	Xiaowutai Mt, Hebei	HBXW0266	JQ681117	JQ681062	JQ681012
Lasius alienus	Helan Mt, Inner Mongolia	NMHL0316	JQ681118	JQ681063	JQ681013
Oecophylla smaragdina	Xiangtou Mt, Guangdong	GDXT0104	JQ681119	JQ681064	JQ681014
Polyrhachis illaudata	Jingxi, Guangxi	GXJX0141	JQ681120	JQ681065	JQ681015
Polyrhachis halidayi	Jingxi, Guangxi	GDJX0024	JQ681121	JQ681066	JQ681016
Polyrhachis rastellata	Rong'an, Guangxi	GXRA0045	JQ681122	JQ681067	JQ681017
Polyrhachis dives	Beiliu, Guangxi	GXGL0099	JQ681123	JQ681068	JQ681018
Polyrhachis jianghuaensis	Beiliu, Guangxi	GXBL0006	JQ681124	JQ681069	JQ681019
Polyrhachis paracampponota	Jingxi, Guangxi	GXJX0009	JQ681125	JQ681070	JQ681020
Camponotus variegatus	Jingxi, Guangxi	GXJX0155	JQ681126	JQ681071	JQ681021
Camponotus herculeanus	Helan Mt, Inner Mongolia	NMHL0273	JQ681127	JQ681072	JQ681022
Camponotus albosparsus	Jingxi, Guangxi	GXJX0130	JQ681128	JQ681073	JQ681023
Camponotus vanispinus	Jingxi, Guangxi	GXJX0007	JQ681129	JQ681074	JQ681024
Camponotus wasmanni	Xiangtou Mt, Guangdong	GDXT0102	JQ681130	JQ681075	JQ681025
Camponotus dolendus	Jingxi, Guangxi	GXJX0036	JQ681131	JQ681076	JQ681026
Camponotus jianghuaensis	Rong'an, Guangxi	GXRA0010	JQ681132	JQ681077	JQ681027
Camponotus mitis	Bohai, Yunnan	YNBH0111	JQ681133	JQ681078	JQ681028
Camponotus helvus	Jingxi, Guangxi	GXJX0015	JQ681134	JQ681079	JQ681029
Camponotus yiningensis	Jingxi, Guangxi	GXJX0013	JQ681135	JQ681080	JQ681030
Camponotus albivillosus	Helan Mt, Inner Mongolia	NMHL2122	JQ681136	JQ681081	JQ681031
Camponotus lasiselene	Jingxi, Guangxi	GXJX0012	JQ681137	JQ681082	JQ681032
Camponotus parius	Beiliu, Guangxi	GXBL0009	JQ681138	JQ681083	JQ681033
Camponotus singularis	Beiliu, Guangxi	GXBL0008	JQ681139	JQ681084	JQ681034
Camponotus sp. 1	Jingxi, Guangxi	GXJX0017	JQ681140	JQ681085	JQ681035
Camponotus sp. 2	Jingxi, Guangxi	GXJX0123	JQ681141	JQ681086	JQ681036
Polyergus samurai	Beiliu, Guangxi	GXBL0212	JQ681142	JQ681087	JQ681037
Out-group					
Cerapachys sulcinodis	Beiliu, Guangxi	GXBL0095	JQ681145	JQ681090	JQ681040
Radoszkowskius oculata	From GenBank		NC_014485	NC_014485	NC_014485