

Phylogeny and taxonomy of the *Prenolepis* genus-group of ants (Hymenoptera: Formicidae)

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Abstract. We investigated the phylogeny and taxonomy of the *Prenolepis* genus-group, a clade of ants we define within the subfamily Formicinae comprising the genera *Euprenolepis*, *Nylanderia*, **gen. rev.**, *Paraparatrechina*, **gen. rev. & stat. nov.**, *Paratrechina*, *Prenolepis* and *Pseudolasius*. We inferred a phylogeny of the *Prenolepis* genus-group using DNA sequence data from five genes (CAD, EF1 α F1, EF1 α F2, wingless and COI) sampled from 50 taxa. Based on the results of this phylogeny the taxonomy of the *Prenolepis* genus-group was re-examined. *Paratrechina* (broad sense) species segregated into three distinct, robust clades. *Paratrechina longicornis* represents a distinct lineage, a result consistent with morphological evidence; because this is the type species for the genus, *Paratrechina* is redefined as a monotypic genus. Two formerly synonymized subgenera, *Nylanderia* and *Paraparatrechina*, are raised to generic status in order to provide names for the other two clades. The majority of taxa formerly placed in *Paratrechina*, 133 species and subspecies, are transferred to *Nylanderia*, and 28 species and subspecies are transferred to *Paraparatrechina*. In addition, two species are transferred from *Pseudolasius* to *Paraparatrechina* and one species of *Pseudolasius* is transferred to *Nylanderia*. A morphological diagnosis for the worker caste of all six genera is provided, with a discussion of the morphological characters used to define each genus. Two genera, *Prenolepis* and *Pseudolasius*, were not recovered as monophyletic by the molecular data, and the implications of this result are discussed. A worker-based key to the genera of the *Prenolepis* genus-group is provided.

Introduction

During a recent study of the generic-level relationships among ants, Brady *et al.* (2006) found support for redefining the Lasiini to include the genera *Lasius*, *Myrmecocystus*, *Paratrechina*, *Prenolepis* and *Pseudolasius*. They did not include *Euprenolepis* in their analysis, but Moreau *et al.* (2006) did find *Euprenolepis* to belong to this clade as well (the species labeled *Plagiolepis* is actually *Euprenolepis*, and the species identified as *Euprenolepis* is actually *Plagiolepis*, according to vouchers examined by JSL). This represents a new phylogenetic arrangement from the ones outlined by both Agosti (1991) and Bolton (2003), although these genera have long

been thought to be closely related (Emery, 1906, 1925; Trager, 1984), as evidenced by the various tribal arrangements that have included these taxa (Bolton, 2003). Bolton (2003) acknowledged that his tribal arrangements of the Lasiini and Plagiolepidini would probably need to be re-evaluated in the future owing to the suspected homoplasious nature of the mesosomal and abdominal characters that have been used to separate these tribes. Overall, morphological characters among both lasiines and plagiolepidines have suffered from high character conflict and homoplasy levels (see Agosti, 1991), as indicated by low branch support values and low consistency and retention indices.

In this study we provide a taxonomic revision and phylogeny of the *Prenolepis* genus-group. We provide a morphological diagnosis for each genus and for the first time provide a comprehensive phylogeny of generic-level relationships. A re-evaluation of formicine tribes is outside of the scope of this

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study, and tribal-level relationships in this subfamily are currently being examined by SGB and colleagues. Instead, this study focuses on the clade containing *Euprenolepis*, *Paratrechina*, *Prenolepis*, *Pseudolasius*, and two genera revived in the present study, namely *Nylanderia* and *Paraparatrechina*. Most species in this group were at one time placed in the genus *Prenolepis* (broad sense) (see Taxonomic history section). We term these genera the *Prenolepis* genus-group as an informal name for this clade. We are not proposing the resurrection of the tribe Prenolepidini, which is currently in synonymy with Plagiolepidini, as this action would be best performed when the relationships across the entire subfamily are better understood.

The workers of the *Prenolepis* genus-group can be diagnosed by the following suite of morphological characters (modified and expanded from Bolton, 2003):

1. Mandibles roughly triangular, with four to seven teeth (*Pr. kohli* can possess a small eighth tooth on the inner mandibular margin);
2. Antennae 12-segmented (one species of *Pseudolasius* has 11-segmented antennae);
3. Torula close to the posterior clypeal margin, but not touching it;
4. Propodeal spiracle at or near the declivity of the propodeum;
5. Petiole scale in profile usually inclined forwards with a short anterior face and much longer posterior face.

Taxonomic history of the *Prenolepis* genus-group

Mayr described *Prenolepis* in 1861, and throughout the 19th century most species that later would be placed in *Paratrechina* were ascribed to *Prenolepis*. Motschoulsky (1863) described *Paratrechina* based on *Pa. currens* (which is now a synonym of *Pa. longicornis* Latreille, 1802), but the genus received little attention and was synonymized with *Prenolepis* by von Dalla Torre (1893). Emery (1925) removed *Paratrechina* from synonymy with *Prenolepis* as part of his world species catalogue, and it was at this time that many species were transferred to *Paratrechina* from *Prenolepis*. For a graphical summary of the taxonomic history of the *Prenolepis* genus-group see Fig. 1.

In 1906, Emery (1906) created three subgenera within *Prenolepis*, the nominal based primarily on the mesothoracic constriction immediately behind the pronotum, *Euprenolepis* based on worker polymorphism, and *Nylanderia* based on the

presence of thick, erect setae on the mesonotum and tibiae, with a layer of pubescence on the mesonotum. Later, Emery (1925) removed *Paratrechina* from synonymy with *Prenolepis* and transferred the subgenera *Euprenolepis* and *Nylanderia* to *Paratrechina*. The status of *Nylanderia* in particular has been uncertain since Emery's (1925) classification. Wheeler (1936) raised *Nylanderia* to full genus rank, but then Creighton (1950) returned it to subgeneric rank less than 15 years later. Chapman & Capco (1951) restored *Nylanderia* to full genus rank, only to have it challenged as a synonym of *Paratrechina* by Brown (1973). Smith (1979) treated *Nylanderia* as a subgenus of *Paratrechina*, but the last statement on the status of *Nylanderia* until this study was by Trager (1984), who proposed the synonymy of *Nylanderia* with *Paratrechina*, citing its lack of monophyly.

During this early period, the nominal subgenus *Paratrechina* contained a single species, *Paratrechina (Paratrechina) longicornis*. The subgenus was distinguished by an elongated, slender mesosoma compared to other subgenera of *Paratrechina*, together with a pair of erect setae on the pronotum and two pairs of erect setae on the mesonotum. Donisthorpe (1947) proposed the subgenus *Paraparatrechina* based on *Paratrechina (Paraparatrechina) pallida*. He distinguished his subgenus based on an elongated mesosoma with a pair of erect setae on the propodeum. Brown (1973) tentatively proposed the synonymy of *Paraparatrechina* with *Paratrechina*, and this was confirmed by Trager (1984), who cited the lack of monophyly of the subgenus as support for this treatment.

Euprenolepis was raised to full genus level by Brown (1953), and he also proposed the synonymy of *Chapmanella* with *Euprenolepis*. *Chapmanella* was established by Wheeler (1930), with small eyes and short palps the main morphological features separating it from other genera. Wheeler erroneously reported that the type species had six-segmented maxillary palps, but in fact they are four-segmented (Brown, 1953).

Emery (1887) established the genus *Pseudolasius*, based on a dimorphic worker caste with reduced maxillary and labial palp segmentation along with a few other minor morphological characters. Since that time the genus has received little taxonomic attention, but it has been suggested that it was a close relative of *Paratrechina* and *Prenolepis* (Trager, 1984).

The higher classification of the *Prenolepis* genus-group has been fraught with considerable uncertainty, with the genera being transferred back and forth among various tribal arrangements over the past century (see Bolton *et al.*, 2006

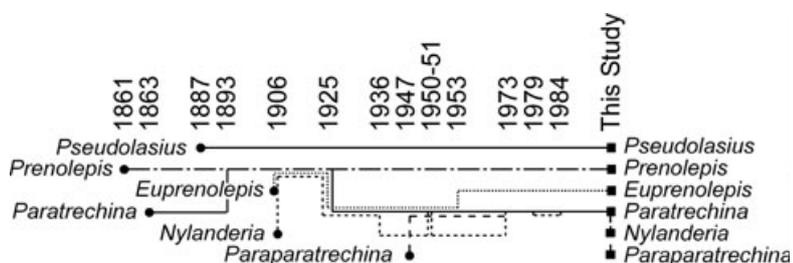


Fig. 1. Taxonomic changes at the generic level. Numerous genus-group names have been proposed for the species considered in this study. However, there has been little consistency in the treatment of many of these names, with various authors considering them full genera, subgenera or synonyms, often with little or no justification. Circles indicate establishment of genus; widely separated lines indicate status as a full genus, closely spaced lines indicate status as a subgenus, and joined lines indicate synonymy; squares indicate status proposed in this study.

for a review). For much of this time it was assumed that *Paratrechina* and *Prenolepis* were sister taxa (Emery, 1925; Wheeler & Wheeler, 1953; Trager, 1984), whereas their relationship to *Euprenolepis* and *Pseudolasius* was debated by various authors. More recently, Bolton (2003) placed *Euprenolepis*, *Paratrechina*, *Prenolepis* and *Pseudolasius* into the tribe Plagiolepidini along with several other genera, but no phylogenetic analysis was provided. The molecular phylogeny of Brady *et al.* (2006) found a clade of ((*Lasius* + *Myrmecocystus*) + (*Pseudolasius* + (*Prenolepis* + *Paratrechina*))), a grouping that various taxonomists have considered likely (Trager, 1984). The molecular phylogeny of Moreau *et al.* (2006) placed *Euprenolepis* within this clade as well (see above).

Overview of *Prenolepis* genus-group natural history

Prenolepis genus-group species are frequently collected when leaf litter extraction techniques such as Berlese funnels and Winkler bags are employed. Ward (2000) found that *Nylanderia* (listed as *Paratrechina*) was the fifth most frequently encountered ant genus in leaf-litter samples from around the world. Several species from different genera have become economically important invasive species, with *Paratrechina longicornis* (sometimes called the long-legged crazy ant) perhaps being the most notorious (see <http://www.issg.org/database/species/ecology.asp?si=958> for review).

Most species are small to medium-sized ants that generally inhabit soil, leaf litter, and rotten wood close to or on the ground. Many species form large, polydomous colonies consisting of hundreds to many thousands of individuals. Although nests are typically found under bark, in leaf litter, in soil, or under stones, most species are epigeic. However, many *Pseudolasius* species are putatively hypogaeic based on their morphology, and hypogaeic species are implicated among several other *Prenolepis* genus-group species. Most species appear to be generalist omnivores, with many species engaging in trophobiotic relationships with sternorrhynchan hemipterans when the opportunity to do so arises (Fig. 2A). There is suggested dietary specialization in some species, such as is observed in a few *Pseudolasius* species, which may enter into obligatory trophobiotic relationships with certain mealybug symbionts (Malsch *et al.*, 2001). The presence of corpulents (*sensu* Tschinkel, 1987) among the *Prenolepis* genus-group is best known among *Prenolepis* (sometimes called small or false honeypot ants) (Fig. 2B), but several genera express either the replete or the corpulent condition (research is needed to determine which condition they are expressing). For instance, swollen gasters have been observed in Australian *Nylanderia* workers (SOS, unpublished data) and in Malagasy *Paratrechina* workers (JSL, unpublished data). In *Euprenolepis*, the very unusual behaviour of nomadic fungivory has been observed in *E. procer* (Witte & Maschwitz, 2008), and it may be a genus-wide behaviour (LaPolla, 2009).

Prenolepis genus-group species can be found in a wide range of habitats, from deserts to tropical rain forests, and have achieved a virtual worldwide distribution. Two

species of *Prenolepis* and one species of *Pseudolasius* are described from mid-Eocene (*c.* 44 Ma) Baltic amber (Mayr, 1868; Wheeler, 1915). There are also several undescribed *Prenolepis* genus-group species known from mid-Miocene (*c.* 15–20 Ma) Dominican amber (Wilson, 1985; LaPolla, in preparation).

Phylogeny of the *Prenolepis* genus-group

Materials and methods

Phylogenetic relationships among *Prenolepis* genus-group lineages were inferred using molecular data from 46 *Prenolepis* genus-group species and four outgroup species that are putatively closely related based on the results of previous phylogenetic studies (Brady *et al.*, 2006; Moreau *et al.*, 2006). Sampled ants were preserved in ethanol (usually 95–100%) prior to extraction. Genomic DNA was extracted from whole ants using the Qiagen DNeasy Tissue Kit (Qiagen Inc., Valencia, CA) following the manufacturer's standard protocol. Vouchers for *Prenolepis* genus-group taxa, consisting of nestmate specimens from the same collection event, are deposited in the National Museum of Natural History.

DNA sequence data were obtained from four nuclear protein-coding genes—CAD, EF1 α F1, EF1 α F2 and wingless—and from the mitochondrial protein-coding gene COI. Primer sequences used for polymerase chain reaction (PCR) amplification are listed in Table S1. Products were sequenced on an ABI sequencer (ABI 377 or ABI 3100) using Big Dye Cycle Sequencing chemistry, employing the same primers as used for amplification. Fragments were sequenced in both directions, and the resulting chromatograms were assembled and edited using SEQUENCHER v.4.8. Sequences for each gene fragment were aligned using CLUSTALX v.1.83 (Thompson *et al.*, 1997). Unambiguous alignments could not be obtained for the two introns in the dataset (from EF1 α F1 and CAD), so these introns were excluded from all analyses. The final data matrix contained 3251 characters (785 parsimony-informative) from the following gene fragments: CAD—515 characters (107 parsimony-informative); EF1 α F1—776 characters (86 parsimony-informative); EF1 α F2—517 characters (78 parsimony-informative); wingless—400 characters (101 parsimony-informative); COI—1043 characters (410 parsimony-informative). GenBank accession numbers are reported in Table S2.

Bayesian phylogenetics was used to estimate tree topology and branch lengths, using MRBAYES v.3.1.2 (Ronquist & Huelsenbeck 2003) parallelized over eight Mac G5 processors. The data were partitioned by gene and by codon position, for a total of 15 data partitions. The best-fitting model for each partition was selected using MRMODELTEST v.2.2 (Nylander, 2004) under the Akaike information criterion (AIC) (Posada & Buckley, 2004). Two independent Markov chain Monte Carlo (MCMC) runs were conducted for 50 million generations, sampled every 1000 generations. Each run was distributed across four chains with a heating parameter of 0.01, in

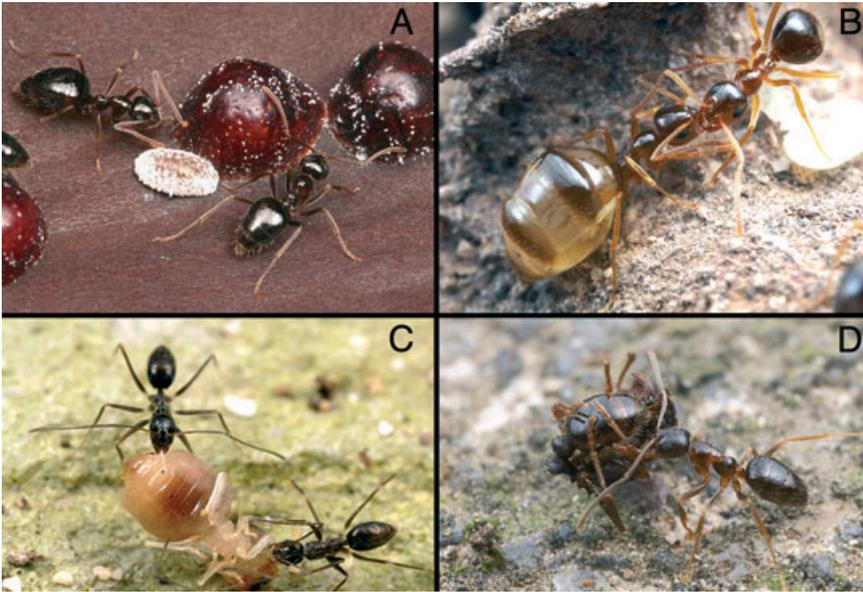


Fig. 2. (A) *Prenolepis imparis* workers with scales; (B) *Prenolepis imparis* workers. Note the corpulent worker on the left and the normal-sized worker on the right; (C) *Paratrechina longicornis* workers cooperating in carrying a termite carcass back to their nest; (D) *Nylanderia vividula* carries the carcass of *Tetramorium caespitum* back to its nest. Photos courtesy of Alex Wild.

order to increase mixing among chains compared to the default setting of 0.2. Convergence between runs was assessed using the average standard deviation of split frequencies (0.003 by 10 million generations) and by plotting likelihood values across generations using TRACER v.1.4 (Rambaut & Drummond, 2007). The two runs were combined after the removal of the first 10 million generations from each run as burn-in.

Bootstrap analyses under maximum likelihood (ML) and equally-weighted maximum parsimony (MP) criteria were also performed. Partitioned ML bootstrap analysis was conducted using MRFISHER (B.C. O'Meara, personal communication; see <http://www.brianomeeara.info/mrfisher>) under the same data partitions and models as used for MRBAYES. MRFISHER is a modified version of MRBAYES that searches for the optimal tree under a likelihood criterion using simulated annealing. A total of 300 bootstrap pseudoreplicates were conducted, with each pseudoreplicate lasting 2 million generations. MP bootstrap analysis was performed using PAUP* v.4.0b10 (Swofford, 2002). A total of 1000 pseudoreplicates were conducted, with each pseudoreplicate consisting of 100 random taxon-addition replicates, tree bisection–reconnection (TBR) heuristic searches, and no limit to MAXTREES.

Results and discussion

Our phylogeny (Fig. 3) strongly supports the splitting of *Paratrechina* (broad sense) into three separate genera: *Nylanderia* (1.0 Bayesian posterior probability (PP), 100% ML bootstrap, 99% MP bootstrap), *Paraparatrechina* (1.0 PP, 99% ML bootstrap, 93% MP bootstrap) and *Paratrechina* (strict sense) (1.0 PP, 100% ML, 100% MP bootstrap). The lack of monophyly of *Paratrechina* (broad sense) is not particularly surprising as, other than the presence of erect setae on the mesosoma, there are few morphological characters to support monophyly

of the group. *Paratrechina longicornis*, the type species for the genus, has long been considered morphologically unique compared to other species in the genus. *Paratrechina longicornis* possesses especially elongated scapes, five mandibular teeth, an elongated mesosoma with a unique pattern of erect setae, and an indistinct propodeum with a shallow declivity. This all stands in sharp contrast to the morphological characteristics of species within *Nylanderia* and *Paraparatrechina* (see Taxonomy section below). Our molecular phylogeny corroborates the separation of *Pa. longicornis* in placing this species as a distinct lineage that is sister to *Euprenolepis* (Fig. 3).

We also recover a well-supported clade of (*Pseudolasius* + (*Euprenolepis* + *Paratrechina*)) [1.0 PP, 99% ML bootstrap, 79% MP bootstrap]. Both *Euprenolepis* and *Pseudolasius* are characterized by the presence of major and minor castes (although this may not be the case for all *Euprenolepis* species, see below), whereas *Pa. longicornis* is monomorphic. Both *Euprenolepis* and *Pseudolasius* also possess reduced palpal formulas, whereas *Paratrechina* has the full complement of palpal segments and the segments are especially long compared to most *Prenolepis* genus-group species. However, *Euprenolepis* does share several morphological characters with *Paratrechina* (strict sense), including five mandibular teeth, an elongated mesosoma, and narrow and elongated procoxae. This clade also makes biogeographic sense, because with the transfer of the Afrotropical *Pseudolasius* to *Paraparatrechina* (see below) this group becomes a strictly southeast Asian/northern Australian clade.

Paraparatrechina is recovered as a monophyletic group (1.0 PP, 99% ML bootstrap, 93% MP bootstrap). *Paraparatrechina* is morphologically best characterized by the very uniform erect setal pattern on the mesosoma. All species possess erect setae on the mesosoma that are distinctly paired, with two pairs on the pronotum, one pair on the mesonotum and one pair on the propodeum. The presence of a pair of erect setae

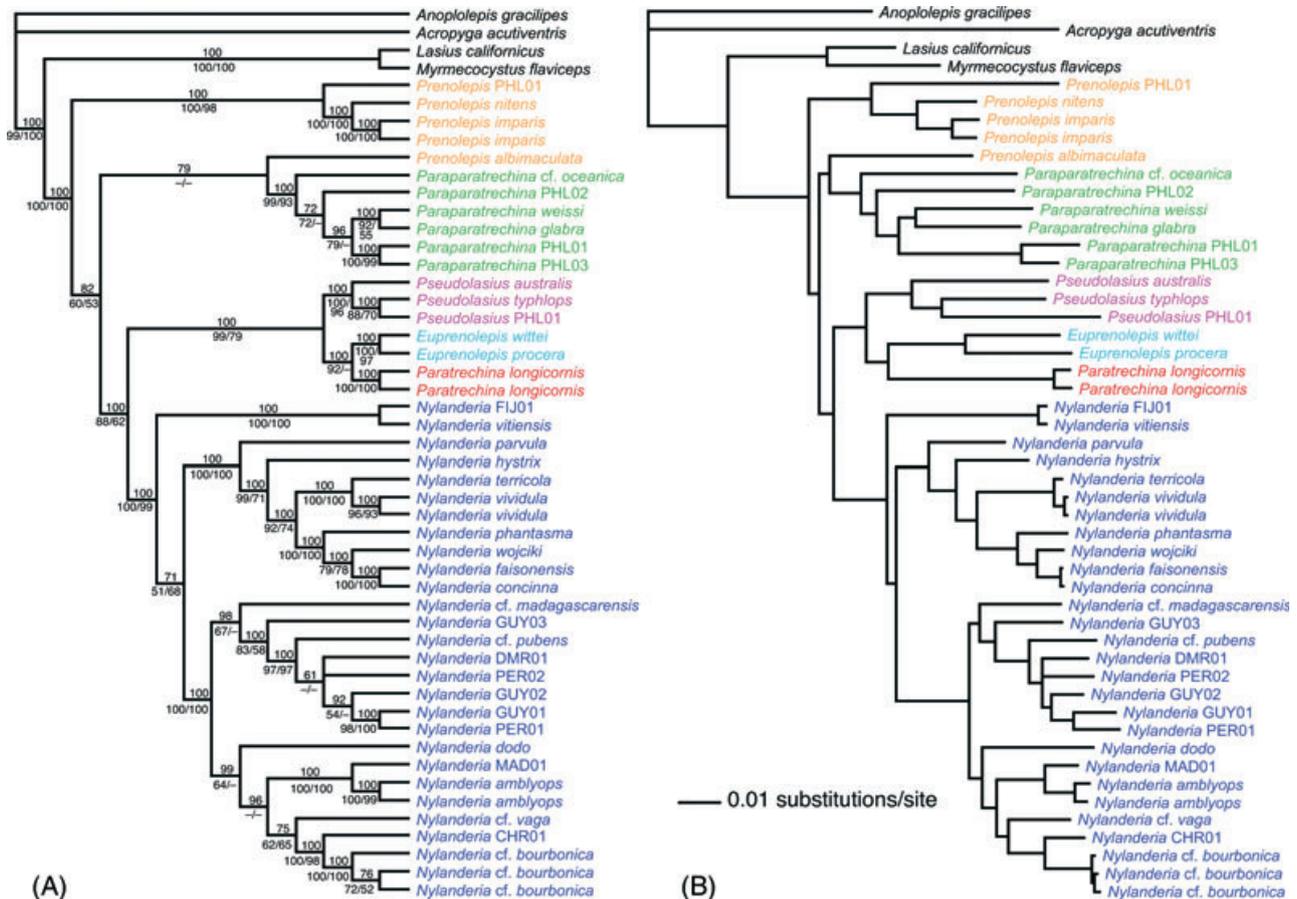


Fig. 3. Phylogeny of *Prenolepis* genus-group taxa. (A) Cladogram depicting the majority-rule consensus tree from Bayesian analysis. Numbers above branches are Bayesian posterior probabilities. Numbers below branches are bootstrap proportions from maximum likelihood and maximum parsimony analyses. (B) Phylogram of the Bayesian majority-rule consensus tree showing inferred branch lengths.

on the propodeum represents a morphological synapomorphy for the genus. Many species of *Paraparatrechina* also are characterized by the cuticular surface being iridescent. Such coloration is unusual in ants. For instance, many brown and dark brown species flash a metallic blue when observed under light microscopy. Upon closer inspection with electron microscopy, the cuticular surface of *Paraparatrechina albipes* is found to possess tiny striations that run along the surface and are responsible for the iridescent coloration (Fig. 4). Whether this phenomenon is widespread in the genus is unclear, but certainly warrants further investigation.

The genus *Prenolepis* is not recovered as monophyletic, with *Prenolepis albimaculata* found to be outside the genus; however, this result was only weakly supported by measures of clade support (Fig. 3A). The monophyly of *Prenolepis* is also questionable on morphological grounds (see under *Prenolepis* below for a discussion of morphological characters), with the Caribbean *Prenolepis* possibly representing a separate lineage within the *Prenolepis* genus-group. Given our limited sample size and uncertainty regarding the morphological limits of many species we do not feel justified in constructing a new

genus at this time to accommodate the Caribbean species, but this may be necessary in the future.

The genus *Pseudolasius* was also found not to be monophyletic, with *Ps. weissi* recovered within *Paraparatrechina*, and *Ps. dodo* falling within *Nylanderia*. Both of these results are well supported by posterior probability and bootstrap values (Fig. 3A). We have decided to transfer these species to *Paraparatrechina* and *Nylanderia*, respectively. *Pseudolasius weissi* possesses several morphological characteristics that suggest its placement within *Pseudolasius* rather than within *Paraparatrechina*, including the presence of a major worker caste and a reduced palpal formula (2, 3). However, the presence of worker polymorphism appears to have evolved independently several times within the *Prenolepis* genus-group (this study), and across the Formicinae the palpal count is known to be highly variable within genera (for an example within *Acropyga* see LaPolla, 2004a). *Pseudolasius weissi* possesses the following morphological characteristics that conform to *Paraparatrechina*: a 2:1:1 erect setal pattern on the mesosoma (observed in the minor caste—majors tend to display more setal variation), five mandibular teeth, scapes and femora/tibiae

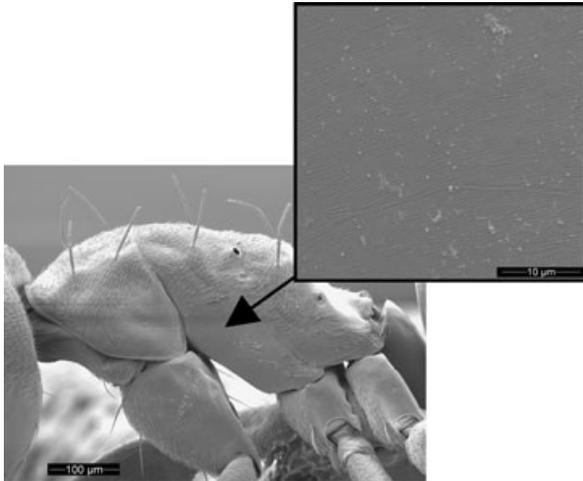


Fig. 4. *Paraparatrechina albipes* mesosoma. The arrow and insert indicate the area where the cuticle was examined under higher SEM magnification; note the many striations in the cuticle under higher magnification (2500 \times).

that lack erect setae, and a short, angular dorsal face of the propodeum. There are currently two recognized Afrotropical *Pseudolasius*, namely *Ps. bufonus* and *Ps. weissi* (LaPolla, 2004b). We transfer *Ps. bufonus* to *Paraparatrechina* as well, owing to its overall morphological similarity to *Ps. weissi*. One difference between the two is that *Ps. bufonus* has several erect setae on the mesosoma, a characteristic that differs from the 2:1:1 pattern of most *Paraparatrechina*. That being said, the males of both *Ps. weissi* and *Ps. bufonus* conform to what is observed for *Paraparatrechina* species, mainly that the parameres are elongated and thin, and have rounded apices.

The placement of *Ps. dodo* is less problematic. It appears that majors do not exist for this species, which would indicate that it does not belong in *Pseudolasius* (LaPolla, 2002). The species does have a reduced palpal formula (5:3), and although this has not been observed in any other *Nylanderia* species, palpal counts, as noted above, have been of limited utility in defining genera. The species has six mandibular teeth, as do all *Nylanderia*, and the molecular data strongly support its placement within this genus. Therefore, we have decided to transfer *Ps. dodo* to *Nylanderia*.

Worker polymorphism appears to have evolved several times within the *Prenolepis* genus-group. It is possible that *Pseudolasius*, as it is currently conceived, may be a 'wastebasket' group in which polymorphic *Prenolepis* genus-group species that possess reduced palps have been placed. A much larger sample of *Pseudolasius* will be required to define the limits of this group adequately. The larger clade of the *Prenolepis* genus-group and its sister taxon (*Lasius* + *Myrmecocystus*) may be instructive in examining worker polymorphism because polymorphism occurs in several independent lineages within these two genera.

Invasiveness is a further common characteristic among *Prenolepis* genus-group taxa. This group contains at least ten

known invasive species: *Nylanderia bourbonica*, *N. clandestina*, *N. flavipes*, *N. fulva*, *N. guatemalensis*, *N. pubens*, *N. vaga*, *N. vividula*, *Nylanderia* sp., *Paratrechina longicornis* (McGlynn, 1999; unpublished data). Several of these taxa, notably *N. bourbonica*, *N. vaga* and *P. longicornis*, are widespread tramp species frequently involved in human-mediated dispersal. However, even obtaining an exact species count is currently hampered by the taxonomic uncertainty surrounding most of these taxa (e.g. Trager, 1984). Our phylogenetic results indicate that invasiveness evolved many times within the *Prenolepis* genus-group, with at least four separate origins represented by *N. cf. pubens*, *N. vividula*, the *N. bourbonica/vaga* species complex, and *Paratrechina longicornis*. Increased phylogenetic taxon sampling and improved species-level taxonomy will be necessary to explore the issue of invasive origins in greater depth.

Synopsis of the *Prenolepis* genus-group

Euprenolepis Emery, 1906

= *Chapmanella*, Wheeler, W.M., 1930 (synonymy by Brown, 1953; here confirmed)

Nylanderia Emery, 1906, **gen. rev.**

= *Andragathus* Emery, 1922 (synonymy with *Paratrechina* by Agosti and Bolton, 1990; junior synonym of *Nylanderia*, **syn. nov.**, this study)

Paraparatrechina Donisthorpe, 1947, **gen. rev. & stat. nov.**

Paratrechina Motschoulsky, 1863

Prenolepis Mayr, 1861

Pseudolasius Emery, 1887

= *Nesolasius* Wheeler, W.M., 1935 (provisional synonymy by Brown, 1973; synonymy by Bolton, 1994; here confirmed)

Key to workers of the *Prenolepis* genus-group

This key can be modified to supplement many existing formicine keys such as Bolton (1994), Shattuck (1999) and Imai *et al.* (2003). In those keys, specimens that key to *Paratrechina* should be compared with couplets 3, 4 and 5 below to determine current generic placement. The relative eye length index (REL) is defined as $(EL/HL) \times 100$, where HL (head length) is the length of the head, excluding the mandibles, measured in full-face view, and EL (eye length) is the maximum length of the compound eye in full-face view.

1. Maxillary palps with 5 or fewer segments; species often strongly polymorphic, with a major and minor caste expressed 2
- Maxillary palps with 6 segments; species monomorphic to slightly polymorphic, with no discernable major or minor caste expressed 4

- 2. Polymorphic Afrotropical species *Paraparatrechina bufonus* and *weissi*
 - Monomorphic or polymorphic Australasian/Indoaustralian species 3
- 3. Eyes large, typically REL 20 or greater (one exception—*E. negrosensis*); labial palps typically with 4 segments; mesothorax constricted immediately behind pronotum; mandalus large and conspicuous (Fig. 7A), usually visible without dissection of mandible* *Euprenolepis*
 - Eyes small, REL less than 20, typically 15 or less; labial palps typically with 2 or 3 segments; mesothorax typically not constricted immediately behind pronotum; mandalus small and inconspicuous (Fig. 7F), usually not visible without dissection of mandible* *Pseudolasius*
- 4. Mandibles typically with 6 to 7 teeth, rarely 5 teeth present; if with 5 teeth, then mesothorax constricted immediately behind pronotum; scapes with or without erect setae ... 5
 - Mandibles always with 5 teeth; mesothorax not constricted immediately behind pronotum; scapes always lacking erect setae 6
- 5. Mesothorax constricted immediately behind pronotum; eyes largely above midlength of head (typically posterior of vertex); mandibles with distinct striations across anterior surface *Prenolepis*
 - Mesothorax not constricted immediately behind pronotum; eyes with about half their length above and half their length below midlength of head (typically at vertex); mandibles without striations across anterior surface *Nylanderia*
- 6. Erect setae (one pair) present on propodeum; erect setae on head form a pattern of 4 setae along posterior margin and 6 to 7 rows of setae from posterior margin

- to clypeal margin; dorsal face of propodeum typically short and angular; femora and tibiae lacking large erect setae *Paraparatrechina*
- Erect setae absent on propodeum; erect setae on head scattered across surface; dorsal face of propodeum broad and gently rounded; femora and tibiae with large erect setae *Paratrechina*

*This character can be difficult to determine unless the mandible is dissected. It is only required if specimens have 4-segmented labial palps and 5 mandibular teeth.

Taxonomy of the *Prenolepis* genus-group

Euprenolepis

Figures of worker. Head: Fig. 5A (major) and Fig. 5B (minor); mesosoma: Fig. 6A (major), Fig. 6B (minor); mandible: Fig. 7A (major).

Synopsis of taxonomic history (for full description see Bolton et al., 2006): *Euprenolepis* Emery, 1906: 134. As subgenus of *Prenolepis*. *Euprenolepis* as subgenus of *Paratrechina*, Emery, 1925: 223; *Euprenolepis* raised to genus and senior synonym of *Chapmanella*, Brown, 1953: 6. Type species: *Prenolepis (Euprenolepis) procera*, by original description.

Diagnosis. Mandible with 5 teeth; basal tooth with an obtuse angle on the inner mandibular margin (one known exception *E. negrosensis*, in which basal tooth is roughly quadriform relative to inner mandibular margin); mandalus

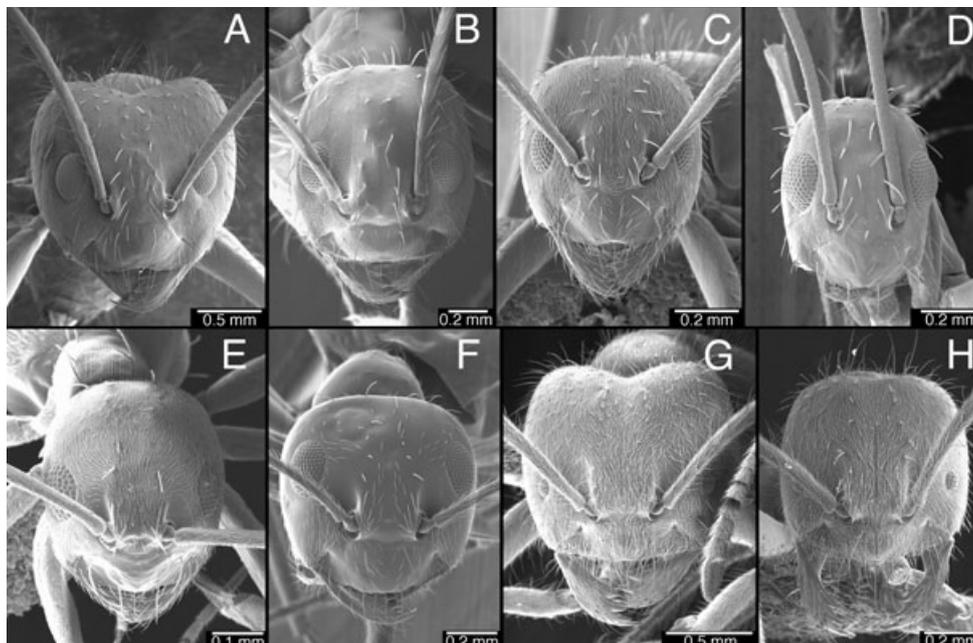


Fig. 5. Heads of *Prenolepis* genus-group workers in full frontal view. (A) *Euprenolepis* major; (B) *Euprenolepis* minor; (C) *Nylanderia*; (D) *Paratrechina*; (E) *Paraparatrechina*; (F) *Prenolepis*; (G) *Pseudolasius* major; (H) *Pseudolasius* minor.

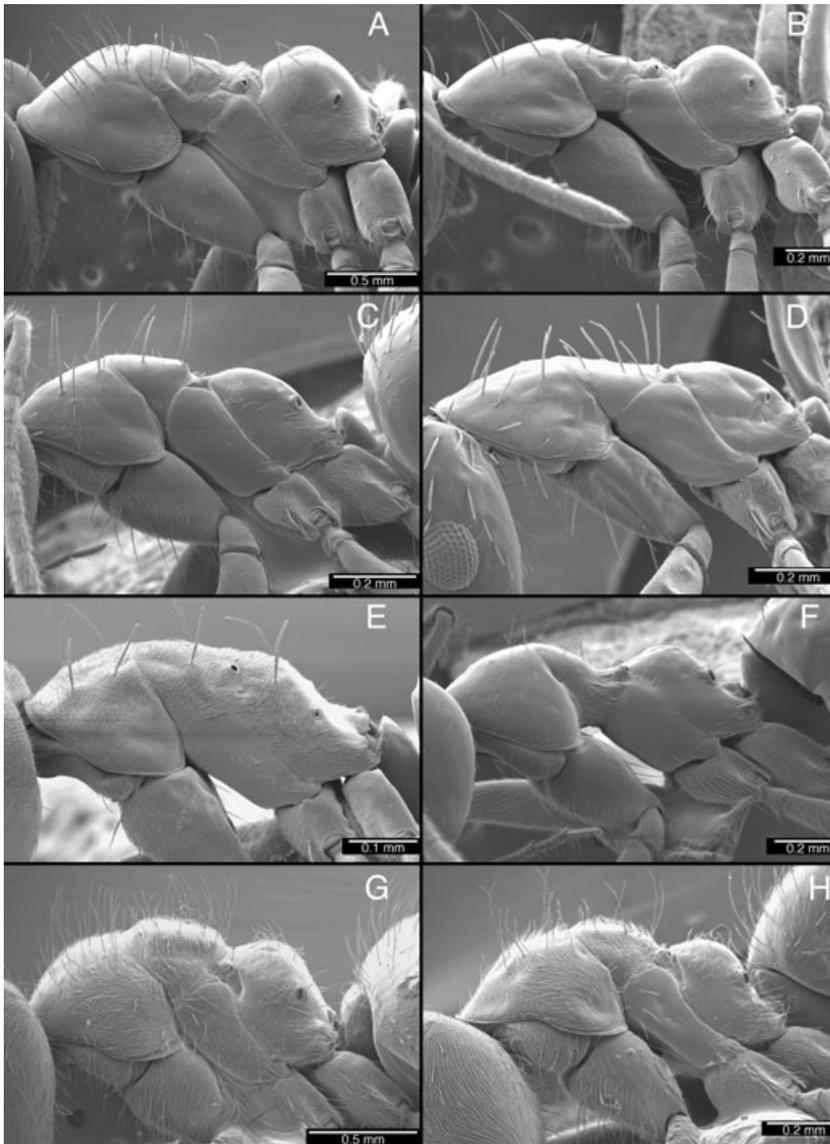


Fig. 6. Mesosoma of *Prenolepis* genus-group workers in lateral view. (A) *Euprenolepis* major; (B) *Euprenolepis* minor; (C) *Nylanderia*; (D) *Paratrechina*; (E) *Parapatrechina*; (F) *Prenolepis*; (G) *Pseudolasius* major; (H) *Pseudolasius* minor.

large and conspicuous; maxillary palps 3-segmented; labial palps 4-segmented. Eyes typically well developed and placed more anteriorly of midlength of head. Mesothorax constricted immediately behind pronotum. Erect setae on dorsum of head randomly placed; anterior portion of head slightly wider than preceding portion and flattened slightly; erect setae present on scapes, legs and dorsum of mesosoma, including propodeum; propodeum with a high-domed dorsal face; overall body shape long and slender.

Distribution. (Fig. 8A). This genus appears to be restricted to Southeastern Asia.

Notes. LaPolla (2009) recently revised the species-level taxonomy of this genus. In *E. procera*, workers are polymorphic with a major caste clearly expressed. In several

Euprenolepis species, however, the existence of worker polymorphism is unclear owing to the small number of available specimens (LaPolla, 2009). Even among *E. procera* specimens collected from a variety of localities, relatively few larger workers have been found.

Species of *Pseudolasius* that possess 5 mandibular teeth, 4-segmented labial palps and have a constriction of the mesothorax immediately behind the pronotum can be difficult to separate from *Euprenolepis* species. Fortunately, few *Pseudolasius* species have this suite of morphological features. The most reliable morphological character to separate these two genera is the presence of larger eyes in *Euprenolepis*. All *Pseudolasius* have some reduction in eye size relative to most other *Prenolepis* genus-group species, and many *Pseudolasius* species display extreme eye reduction. All *Euprenolepis*, with the exception of *E. negrosensis*, have large eyes. In the

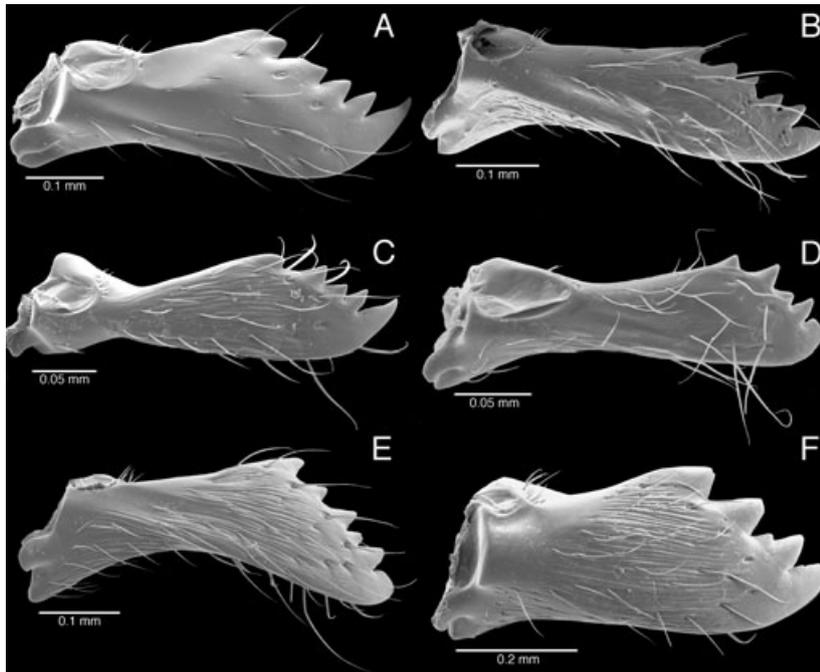


Fig. 7. Mandibles of *Prenolepis* genus-group workers in full frontal view. (A) *Euprenolepis* major; (B) *Nylanderia*; (C) *Paratrechina*; (D) *Parapatrechina*; (E) *Prenolepis*; (F) *Pseudolasius* major.

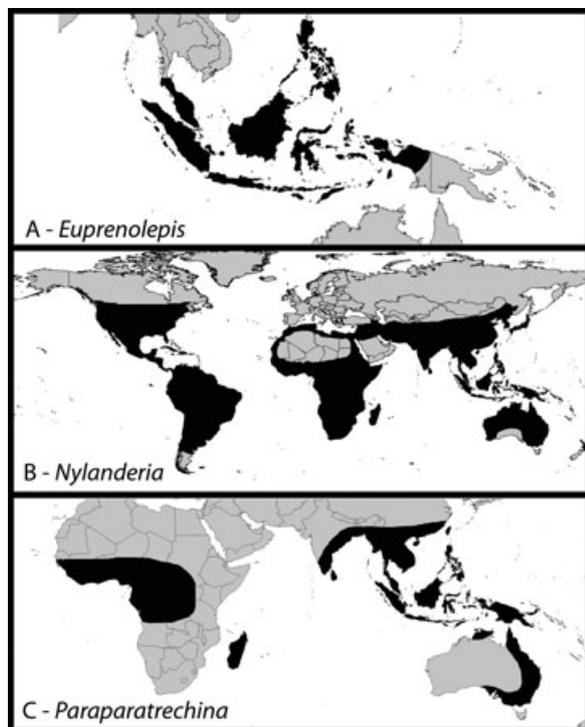


Fig. 8. *Prenolepis* genus-group distributions based on LaPolla (2009) for *Euprenolepis*, and material examined for all genera.

case of *E. negrosensis*, all other morphological characters suggest its placement within the genus. Another character that separates the two genera is the condition of the mandalus. In *Euprenolepis* the mandalus (Fig. 7A) is large and conspicuous

(often visible without dissection), usually at least 2× the size of the mandalus found in *Pseudolasius*.

Nylanderia, gen. rev.

Figures of worker. Head: Fig. 5C; mesosoma: Fig. 6C; mandible: Fig. 7B.

Synopsis of taxonomic history (for full description see Bolton *et al.*, 2006): *Nylanderia* Emery, 1906: 133. As subgenus of *Prenolepis*. As subgenus of *Paratrechina*, Emery, 1925: 217. *Nylanderia* raised to genus, Wheeler, 1936: 210; as subgenus of *Paratrechina*, Creighton, 1950: 405; *Nylanderia* revived as genus, Chapman & Capco, 1951: 214; provisional junior synonym of *Paratrechina*, Brown, 1973: 183; as subgenus of *Paratrechina*, Smith, D.R., 1979: 1443; *Nylanderia* as junior synonym of *Paratrechina*, Trager, 1984: 51. Type species: *Formica vividula*, by original description.

= *Andraganthus* Emery, 1922: 111. *Andraganthus* as junior synonym of *Paratrechina*, Agosti & Bolton, 1990; this study in synonymy with *Nylanderia*, **syn. nov.** Type species: *Andraganthus hubrechtii*, by monotypy.

Diagnosis. Mandible with 6 to 7 teeth; maxillary palps 6-segmented; labial palps 4-segmented; erect setae on dorsum of head randomly placed; typically with abundant erect setae on head, scapes, legs and dorsum of mesosoma, although a few species lack erect setae on head, scapes and legs. Eyes typically well developed and placed midlength and laterally on head. Dorsal mesosomal setae arranged loosely in pairs; propodeum lacking erect setae (with one exception); propodeum with a low- to high-domed dorsal face; generally overall mesosoma shape compact.

Distribution. (Fig. 8B). This genus is essentially world-wide in distribution, but its exact northern limits are uncertain, and it is curious that apparently no native species are found in Europe. The genus reaches its highest diversity in the tropics, but in many temperate areas *Nylanderia* form important components of the fauna (Ward, 2000). Humans have dispersed a few species around the globe, and there are several economically important invasive species in this genus.

Notes. This is by far the most diverse of the *Prenolepis* genus-group genera. This genus is currently under revision by the authors and is certain to increase tremendously in recognized species as studies are completed.

Although most species of this genus are easily recognizable, there are several *Nylanderia* species (*N. darlingtoni*, *N. otome* and *N. yamburu*) that superficially resemble *Paratrechina* in that they lack erect setae on the legs and/or scapes and have paired erect setae on the pronotum and mesonotum. Scapes without erect setae or with very few setae appear more commonly in New World *Nylanderia* (particularly among the North American species), with the condition only spottily observed for species outside this region. However, essentially all species can be placed into *Nylanderia* based on the lack of erect setae on the propodeum and the presence of six mandibular teeth. The single known exception is an undescribed species from Papua New Guinea, which has numerous elongate setae on the propodeum. However, in this case the setae differ in number and structure from those found in other genera considered here. Undoubtedly, as taxonomic revision of *Nylanderia* progresses there will be some adjustments regarding to which genus a few species are ascribed, so the generic species list reported below will probably change in the future.

Combinations in *Nylanderia*. The following names are **comb. rev.** from *Paratrechina*: *consuta*, *darlingtoni*, *pearsei*.

The following names are all **comb. nov.** in *Nylanderia*. They are listed under their previous combinations as catalogued in Bolton *et al.* (2006). **From *Paratrechina*:** *amblyops*, *amblyops rubescens*, *amia*, *anthracina*, *arenivaga*, *arlesi*, *aseta*, *assimilis*, *austrorocidua*, *birmana*, *birmana hodgsoni*, *bourbonica*, *bourbonica farquharensis*, *bourbonica ngasiyana*, *brasiliensis*, *braueri*, *braueri donisthorpei*, *braueri glabrior*, *bruesii*, *burgesi*, *caeciliae*, *caeciliae elevata*, *cisipa*, *clandestina*, *colchica*, *comorensis*, *concinna*, *dispar*, *dugasi*, *faiso-nensis*, *flavipes*, *formosae*, *fulva*, *fulva biolleyi*, *fulva cubana*, *fulva fumata*, *fulva fumatipennis*, *fulva incisa*, *fulva longis-capa*, *fulva nesiotis*, *goeldii*, *gracilis*, *grisoni*, *grisoni fuscata*, *guatemalensis*, *guatemalensis cocoensis*, *guatemalensis itinerans*, *gulinensis*, *helleri*, *hubrechtii*, *humbloti*, *hystrix*, *incallida*, *indica*, *integra*, *jaegerskioeldii*, *johannae*, *johannae dibullana*, *kraepelini*, *laevigata*, *lepida*, *lietzi*, *madagascarensis*, *madagascarensis ellisii*, *madagascarensis rufescens*, *madagascarensis sechellensis*, *manni*, *mendica*, *mexicana*, *microps*, *mixta*, *myops*, *nodifera*, *nubatama*, *nuggeti*, *obscura*, *obscura bismarckensis*, *obscura celebensis*, *obscura minor*, *obscura papuana*,

ogasawarensis, *opisophthalmia*, *otome*, *parvula*, *phantasma*, *picta*, *pieli*, *pubens*, *rosae*, *ryukyuensis*, *sakurae*, *sharpii*, *sikora*, *silvestrii*, *silvestrii kuenzleria*, *simpliciuscula*, *sindbadi*, *smythiesii*, *staudingeri*, *steeli*, *steinheili*, *steinheili minuta*, *stigmatica*, *tasmaniensis*, *taylori*, *taylori levis*, *teranishii*, *terricola*, *tjibodana*, *tococae*, *traegaordhi*, *traegaordhi natalensis*, *vaga*, *vagabunda*, *vitiensis*, *vididula*, *vididula antillana*, *vididula australis*, *vididula docilis*, *vididula kuenzleri*, *vididula mjobergi*, *waelbroeckii*, *weissi*, *weissi nimba*, *wojciki*, *yaeyamensis*, *yambaru*, *yerburyi*, *zelotypa*. **From *Pseudolasius*:** *dodo*.

Paratrechina

Figures of worker. Head: Fig. 5D; mesosoma: Fig. 6D; mandible: Fig. 7C.

Synopsis of taxonomic history (for full description see Bolton *et al.*, 2006): *Paratrechina* Motschoulsky 1863: 13. As junior synonym of *Prenolepis*, Dalla Torre, 1893: 177; revived as genus, Emery, 1925: 216. Type species: *Paratrechina currens* (junior synonym of *Paratrechina longicornis*); by subsequent designation of Wheeler (1911).

Diagnosis. Mandible with 5 teeth; maxillary palps 6-segmented; labial palps 4-segmented; erect setae on dorsum of head randomly placed; scapes lacking erect hairs; abundant erect setae on legs and dorsum of mesosoma. Eyes well developed and convex, surpassing outline of head in full frontal view, placed midlength and laterally on head. Dorsal mesosomal setae arranged loosely in pairs; propodeum lacking erect setae; propodeum with a low-domed dorsal face; overall mesosoma shape long and slender.

Distribution. (Fig. 9A). *Paratrechina longicornis* is a well-known invasive species that now has a pantropical distribution. It has also become established in more temperate localities by living in buildings and other man-made structures. Greenhouses in North America are sometimes infested with *Pa. longicornis* (Wetterer *et al.*, 1999).

Notes. Taxonomists have long commented on the unusual morphology of *Pa. longicornis*, such as its elongated scapes and tibiae and unusual mesosomal setal pattern compared to other '*Paratrechina*, s.l.' (Trager, 1984). Our results here confirm that it is in fact a separate, distinct lineage from other *Prenolepis* genus-group members. In his reorganization of the genus, Emery (1925) recognized *Pa. longicornis* as distinct from the rest of the genus by placing it in its own monotypic subgenus. Here we recognize *Paratrechina* as a monotypic genus.

This is a notoriously invasive species that has spread worldwide around the tropics and subtropics. It is interesting to note that our analysis places it within a clade containing *Pseudolasius* and *Euprenolepis*, two genera that are restricted to southeastern Asia. Therefore, it may be reasonable to assume that *Pa. longicornis* is native to this region, and not to Africa as has been previously suggested. It is also interesting to note that to date no *Euprenolepis* or *Pseudolasius* are known

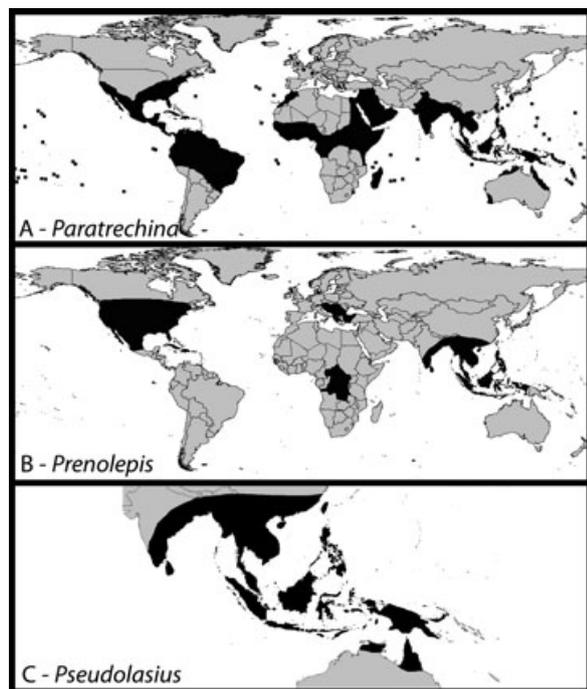


Fig. 9. *Prenolepis* genus-group distributions based on Wetterer (2008) for *Paratrechina*, and material examined for all genera. Note that *Paratrechina* is highly invasive and that the distribution reported here represents both natural and introduced populations, and that many northern records represent occurrences restricted to indoor sites.

to have been introduced by human activities, so whatever characteristics of *Paratrechina* that make it a good invader seem restricted to this single lineage.

Paratrechina longicornis currently contains the subspecies *Pa. l. hagemanni* (Forel, 1901), described from worker(s) collected at Boma, Democratic Republic of Congo. Wheeler (1922) treated this form as a junior synonym of *longicornis* (without comment), whereas Emery (1925) removed it from synonymy as a variety of *longicornis* (again without comment). In his original description, Forel (1901) states that this taxon is very similar to *longicornis* and differs in being 'perhaps' slightly smaller and with the metanotum slightly more convex. All other characters mentioned match *longicornis*, and, upon examination of type material, *Pa. l. hagemanni* (Forel) is here returned to synonymy with *Pa. longicornis* (**syn. nov.**), leaving *Paratrechina* with a single valid species.

***Parapatrechina*, gen. rev. & stat. nov.**

Figures of worker. Head: Fig. 5E; mesosoma: Fig. 6E; mandible: Fig. 7D.

Synopsis of taxonomic history (for full description see Bolton *et al.*, 2006): *Parapatrechina* Donisthorpe, 1947: 192. Described as a subgenus of *Paratrechina*. As provisional junior synonym of *Paratrechina*, Brown, 1973: 183; as junior

synonym of *Paratrechina*, Trager, 1984. Type species: *Paratrechina* (*Parapatrechina*) *pallida*, by monotypy.

Diagnosis. Mandible with 5 teeth; maxillary palps 6-segmented; labial palps 4-segmented; erect setae on dorsum of head distinctly paired; erect setae on head form a pattern of four setae along posterior margin and six to seven rows of paired setae from posterior margin to clypeal margin; scapes and legs lack erect setae; head (excluding clypeal surface) and mesonotal dorsum covered in dense pubescence. Eyes typically well developed and placed midlength towards midline and laterally on head. Erect mesosomal setae distinctly paired, with two pairs on pronotum, one pair on mesonotum and one pair on propodeum; propodeum typically with short dorsal face compared to longer posterior face; generally overall mesosoma shape compact, although in a few species the mesosoma is elongated.

Distribution. (Fig. 8C). This genus appears to be restricted to the Palearctic of Africa, Asia and Australia.

Notes. The mesosomal setal pattern of these ants (two pairs of pronotal erect setae; one pair of mesonotal erect setae and one pair of propodeal erect setae) is remarkably uniform across the genus. These are generally small ants (the majority of species are just over 1 mm to just under 2 mm in total length) that, except for a few known species, have a compact mesosoma. In at least three species a more elongated, slender mesosoma is noted: *P. pallida* and two undescribed Afrotropical species (LaPolla *et al.*, in preparation). In these species the general form of the mesosoma roughly resembles that of *Pa. longicornis*: elongate and slender with a propodeum not angular with a short dorsal face, but instead with a dorsal face that is rounded and broad. In every other diagnostic morphological character, however, the species conform to the morphology listed above for *Parapatrechina*.

Two polymorphic species from the Afrotropics, *weissi* and *bufonus*, superficially resemble *Pseudolasius* (until this study they were in that genus). A major worker caste is clearly expressed in these species and they possess many morphological features that suggest a hypogaecic lifestyle. Presently, they represent the only known cases of worker polymorphism within *Parapatrechina*.

New combinations in Parapatrechina. The following names are all **comb. nov.** in *Parapatrechina*. They are listed under their previous combinations as catalogued in Bolton *et al.* (2006). **From *Paratrechina*:** *albipes*, *butteli*, *butteli bryanti*, *butteli malaccana*, *caledonica*, *dichroa*, *emarginata*, *foreli*, *foreli nigriventris*, *glabra*, *iridescens*, *koningsberg-eri*, *lecamopteridis*, *minutula*, *minutula buxtoni*, *nana*, *net-tae*, *oceanica*, *opaca*, *opaca metallescens*, *pallida*, *pusillima*, *sauteri*, *subtilis*, *subtilis termitophila*, *tapinomoides*. **From *Pseudolasius*:** *bufonus*, *weissi*.

Prenolepis

Figures of worker. Head: Fig. 5F; mesosoma: Fig. 6F; mandible: Fig. 7E.

Synopsis of taxonomic history (for full description see Bolton *et al.*, 2006): *Prenolepis* Mayr, 1861: 52. Type species: *Tapinoma nitens*, by subsequent designation of Bingham, 1903: 325.

Diagnosis. Mandible with 5 to 7 teeth (in *Pr. kohli* up to 8 teeth observed in some specimens); maxillary palps 6-segmented; labial palps 4-segmented; erect setae on dorsum of head randomly placed; with erect setae on scapes, legs and dorsum of mesosoma, including propodeum. Eyes well developed, often strongly convex, and placed posteriorly on the head. Mesothorax constricted immediately behind pronotum; propodeum with a high-domed dorsal face, with entire propodeum often distinctly rounded in overall shape; overall mesosoma shape long and slender.

Distribution. (Fig. 9B). *Prenolepis* has a curious distribution, with a wide-ranging species found in North America, one species known from southern Europe and Anatolia and three species from Cuba (although see below), but the genus reaches its highest species diversity levels in southeastern Asia and southern China. One species is found in the rainforests of West Africa.

Notes. Recent descriptive work (Xu, 1995; Zhou & Zheng, 1998; Fontenla, 2000; Zhou, 2001) has expanded this genus to 35 species and subspecies. However, this genus is in need of global taxonomic revision and caution is advised before further species are described within it. In fact, the monophyly of the genus may be in question.

The Antillean *Prenolepis* (*Pr. albimaculata* (Cuba only), *Pr. gibberosa* (Cuba and Haiti) and *Pr. karstica* (Cuba only)) possess 5 teeth, and although we retain them provisionally within *Prenolepis* our phylogenetic results suggest that they may represent a separate lineage within the *Prenolepis* genus-group (Fig. 3). This result was also suggested by Fontenla (2000) in his morphologically based phylogenetic analysis of the genus. The three Antillean species possess ocelli, whereas all other species (with the exception of the Asian *Pr. naorojii*) do not. This character may be of phylogenetic importance. It is also interesting to note that an undescribed fossil of an Antillean *Prenolepis* species from Dominican amber possesses five teeth and ocelli (LaPolla, in preparation). Given our limited taxon sampling in this group, however, any taxonomic decisions regarding the generic status of the Antillean species requires further investigation.

Another interesting species is *Pr. kohli*, which we are transferring to *Prenolepis* from *Paratrechina*. This is an African species described from the Democratic Republic of the Congo, and it has a number of morphological traits that suggest that it belongs in *Prenolepis*. Among these characters are: (i) a mesothoracic constriction immediately behind the

pronotum (although not as pronounced as in other species); (ii) mandibles with more than six teeth (up to eight in some specimens, although the eighth tooth is usually very small and found on the inner mandibular margin); (iii) striations across the mandibles as seen in most *Prenolepis* species; (iv) very long scapes that surpass the posterior margin of the head by more than half their length; (v) eyes convex, placed posterior to the midline of the head; and (vi) the propodeum with very high-domed shaped dorsal face. This is the only *Prenolepis* known from Africa. It is to be hoped that in the future molecular data can be gathered from this species to examine whether or not this placement is correct.

The following name is a **comb. rev.** in *Prenolepis* from *Paratrechina*: *kohli*.

Pseudolasius

Figures of worker. Head: Fig. 5G (major), Fig. 5H (minor); mesosoma: Fig. 6G (major), 6H (minor); mandible: Fig. 7F.

Synopsis of taxonomic history (for full description see Bolton *et al.*, 2006): *Pseudolasius* Emery, 1887: 244. Type species: *Formica familiaris*, by subsequent designation of Bingham (1903: 337),: 244.

= *Nesolasius* Wheeler, W.M., 1935: 50. As subgenus of *Pseudolasius*. As provisional junior synonym of *Pseudolasius*, Brown, 1973: 183; as junior synonym of *Pseudolasius*, Bolton, 1994: 50 (here confirmed). Type species: *Pseudolasius* (*Nesolasius*) *typhlops*, by original description.

Diagnosis. Mandible with 4 to 7 teeth; maxillary palps 2- to 5-segmented; labial palps 2- to 4-segmented; mandalus small and inconspicuous. Setae on dorsum of head randomly placed; with erect setae on scapes, legs and dorsum of mesosoma, including propodeum; body often covered in dense pubescence. Eyes often poorly developed, typically placed below midlength. Workers strongly polymorphic, with a major caste present; posterior margin of head in majors typically emarginate. Propodeum with a low- to high-domed dorsal face; overall mesosoma shape compact.

Distribution. (Fig. 9C). This genus is restricted to southern Asia, from India to China, stretching southwards to northern Australia. It appears to be restricted to tropical localities.

Notes. The polymorphic nature of this genus is well known, but overall this is a fairly heterogeneous group that is in need of taxonomic revision. There are clearly some lineages that have taken a hypogaecic evolutionary route and look quite different from the larger, presumably epigaecic species. A polymorphic species placed in *Pseudolasius* until the current study (*Paraparatrechina weissi*) did not group with the few other polymorphic *Pseudolasius* sampled in our molecular phylogeny. Additional study is required to determine

if other species currently placed within *Pseudolasius* need to be transferred to other genera.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI 10.1111/j.1365-3113.2009.00492.x

Table S1. Primers used for PCR amplification and sequencing. For EF1 α F1, initial PCR amplifications were conducted using F1-383F and F1-1887R. This PCR product was used for nested reamplifications using the following primer pairs: F1-792F and F1-1189R; F1-1109F and F1-1551R; F1-1424F and F1-1829R. For wingless, in some cases an initial amplification was conducted using Wg503F and Wg1032, followed by semi-nested reamplification using Wg578F and Wg1032F.

Table S2. GenBank accession numbers and voucher codes.

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Acknowledgements

This project would not have been possible without the kind support of the many biologists who sent us specimens for both molecular and morphological analysis. For specimens we sincerely thank: Gary Alpert, Daniel Burckhardt, Stefan Cover, Lloyd Davis, Brian Fisher, Milan Janda, Fabio Penati, Martin Pfeiffer, Phil Ward, Jim Wetterer, Volker Witte and Seiki Yamane. We thank Alex Wild for the use of his photographs. Katrina Pagenkopp and Nor Dahlan provided valuable laboratory support. Scott Whittaker provided assistance with the SEM work. Helpful comments and suggestions about the manuscript were provided by three anonymous reviewers. This research was supported by the National Science Foundation under grants DEB-0743542, EF-0431330, and the Australian Commonwealth Environment Research Facilities' Taxonomy Research and Information Network.

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Accepted 20 May 2009