

A new ant genus from the Greater Antilles and Central America, *Zatania* (Hymenoptera: Formicidae), exemplifies the utility of male and molecular character systems

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Abstract. The ant genus *Prenolepis* (Hymenoptera: Formicidae) is the nominal member of the recently established *Prenolepis* genus-group within the subfamily Formicinae. Our molecular phylogenetic analyses using fragments from five nuclear genes (*arginine kinase*, *carbomoylphosphate synthase*, *elongation factor 1-alpha F1*, *elongation factor 1-alpha F2*, *wingless*) and one mitochondrial gene (*cytochrome oxidase I*) indicate that this genus is polyphyletic. Although the majority of *Prenolepis* species was found to belong to the same monophyletic group (*Prenolepis* sensu stricto), a smaller subset of *Prenolepis* species, all found in either Central America or the Greater Antilles, was robustly inferred to comprise a distinct lineage that is sister to the Old World genus *Paraparatrechina*. Here we describe this newly discovered lineage within the larger *Prenolepis* genus-group clade. The genus *Zatania*, **gen.n.** is composed of five extant species (*Zatania albimaculata*, *Zatania cisipa*, *Zatania gibberosa*, *Zatania gloriosa*, **sp.n.** and *Zatania karstica*) and one Dominican amber fossil species (*Zatania electra*[†], **sp.n.**). These are medium-sized ants (generally between 2.5 and 3 mm in total length) that are characterized by having long scapes and legs, and elongated mesosomata. A reliance on worker-based taxonomy has previously prevented the discovery of this new lineage because of worker convergence consisting of various combinations of elongated mesosomata, long scapes and legs, and a constriction immediately behind the pronotum, observed in several distinct lineages within the *Prenolepis* genus-group. However, we did find that male morphology complements our molecular results in revealing important diagnostic and potentially phylogenetically informative characters. Our study highlights the value for ant systematics to expand beyond its traditional foundation of worker-based morphology and embrace character systems from other castes and molecular data.

Introduction

The *Prenolepis* genus-group was defined by LaPolla *et al.* (2010a) to comprise the genera *Euprenolepis*, *Nylanderia*, *Paraparatrechina*, *Paratrechina*, *Prenolepis* and *Pseudolasius*

from the ant subfamily Formicinae. Evidence for the monophyly of this group is provided by morphological characters (LaPolla *et al.*, 2010a) and molecular phylogenetic analyses (Brady *et al.*, 2006; Moreau *et al.*, 2006; LaPolla *et al.*, 2010a). *Prenolepis* genus-group species are generally small-to-medium sized ants, possessing roughly triangular mandibles with between four and seven teeth, and 12-segmented antennae. Many species are generalist omnivores that inhabit the soil, leaf litter and rotten wood on the ground, where they form large

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nests. LaPolla *et al.* (2010a) provide a comprehensive review of the general morphology and biology of the group.

Efforts to understand the phylogenetic relationships within the *Prenolepis* genus-group led to the possibility that the genus *Prenolepis* might not be monophyletic. LaPolla *et al.* (2010a) found that Greater Antillean *Prenolepis* species may represent a distinct lineage that is sister to the Old World *Paraparatrechina* rather than to other species within *Prenolepis*, including the widespread North American species *Prenolepis imparis*. At the time of that study, there was only one representative species of this putative new lineage in the molecular phylogeny, so no taxonomic actions were taken.

To explore the identity of this Greater Antillean lineage, we generated molecular data from additional taxa, expanded our data to include several more gene fragments and conducted a detailed morphological examination. We conclude that not only does the Greater Antillean *Prenolepis* truly represent a separate lineage (the first suggestion that these species formed a monophyletic group came from Fontenla, 2000), but a species presently placed in *Nylanderia* also belongs to this lineage, as does a previously undescribed species from Central America. Additionally, a recently examined Dominican amber fossil species was also found to belong to this newly discovered lineage.

Here we describe this lineage as a new genus of ants found in the Greater Antilles and Central America (Fig. 1). We also argue that reliance on worker-based taxonomy has previously hindered the discovery of this new lineage because of repeating morphological forms in this caste shared by several distinct lineages within the *Prenolepis* genus-group. Our study thus highlights the value for ant systematics to expand beyond its traditional foundation of worker-based morphology and embrace character systems from other castes as well as molecular data.

Material and methods

Morphological work

Specimens examined for this study are deposited in the following institutions: AMNH, American Museum of Natural

History, New York, U.S.A.; IESC, Instituto de Ecología y Sistemática, Havana, Cuba; MCZC, Museum of Comparative Zoology, Cambridge, MA, U.S.A.; USNM, National Museum of Natural History, Washington, D.C., U.S.A.

All measurements were taken at various powers of magnification with a Nikon microscope using an orthogonal pair of micrometres, recorded to the nearest 0.001 mm and rounded to two decimal places for presentation. All measurements are given in millimetres. Digital colour images were created using a JVC KY-F75 digital camera (JVC, Yokohama, Japan) and AUTO-MONTAGE 5.0 (Syncroscopy, Frederick, MD, U.S.A.). Morphological terminology for measurements and indices employed throughout are defined following Bolton (1994) and Ward (2001), with modifications: CI, cephalic index = $100(\text{HW}/\text{HL})$; EL, eye length, maximum length of compound eye in full-face view; GL, gaster length, the length of the gaster in lateral view from the anteriormost point of the first gastral segment (third abdominal segment) to the posteriormost point of gaster; HL, head length, the length of the head proper, excluding the mandibles, measured in full-face view from the midpoint of the anterior clypeal margin to a line drawn across the posterior margin from its highest points (to accommodate species where the posterior margin is concave); HW, head width, the maximum width of the head in full-face view (in males, portion of the eyes that extends past the lateral margins of the head is included); PDH, propodeum height, height of the propodeum as measured in lateral view from the base of the metapleuron to the maximum height of the propodeum; PrFL, profemur length, the length of the profemur from its margin with the trochanter to its margin with the tibia; PrFW, profemur width, the maximum width of the profemur; PSC, pronotal setal count, the number of erect macrosetae on pronotum to one side of sagittal plane; PW, pronotal width, the maximum width of the pronotum in dorsal view; REL, relative eye length index = $100(\text{EL}/\text{HL})$; SI, scape index = $100(\text{SL}/\text{HW})$; SL, scape length, the maximum length of the antennal scape excluding the condylar bulb; TL, total length = $\text{HL} + \text{WL} + \text{GL}$; WL, Weber's length, in lateral view, the distance from the posteriormost border of the metapleural lobe to the anteriormost border of the pronotum, excluding the neck.



Fig. 1. Distribution of extant *Zatania* species.

Table 1. GenBank accession numbers and specimen codes.

Taxon	Specimen code	<i>argK</i>	<i>CAD</i>	<i>EF1αF1</i>	<i>EF1αF2</i>	<i>wingless</i>	<i>COI</i>
<i>Anoplolepis gracilipes</i>	CASENT0106057	JN562396	FJ982606	EF013223	—	EF013672	FJ982435
<i>Acropyga acutiventris</i>	USNM00756210	JN562399	FJ982607	FJ982521	FJ982564	FJ982522	FJ982436
<i>Lasius californicus</i>	CASENT0106045	JN562400	FJ982605	EF013268	EF013430	EF013706	FJ982437
<i>Myrmecocystus flaviceps</i>	CASENT0106055	JN562401	FJ982608	EF013294	EF013456	EF013724	FJ982438
<i>Euprenolepis procera</i>	USNM00756211	JN562402	—	FJ982485	FJ982565	FJ982523	FJ982439
<i>Euprenolepis wittei</i>	USNM00756212	JN562403	FJ982609	FJ982486	FJ982566	FJ982524	FJ982440
<i>Nylanderia amblyops</i>	USNM00756213	JN562404	FJ982610	FJ982487	FJ982567	FJ982525	FJ982441
<i>Nylanderia</i> GUY03	USNM00756214	JN562405	FJ982623	FJ982500	FJ982580	FJ982538	FJ982454
<i>Nylanderia</i> cf. <i>madagascarensis</i>	USNM00756215	JN562406	FJ982625	FJ982501	FJ982581	FJ982539	FJ982456
<i>Nylanderia</i> PER01	USNM00756216	JN562407	FJ982628	FJ982504	FJ982584	FJ982542	FJ982459
<i>Nylanderia phantasma</i>	USNM00756217	JN562408	FJ982630	FJ982506	FJ982586	FJ982544	FJ982461
<i>Nylanderia</i> cf. <i>vaga</i>	USNM00756218	JN562409	FJ982633	FJ982509	FJ982589	FJ982547	FJ982464
<i>Nylanderia vitiensis</i>	USNM00756219	JN562410	FJ982634	FJ982510	FJ982590	FJ982548	FJ982465
<i>Nylanderia vividula</i>	USNM00756220	JN562411	FJ982635	FJ982511	FJ982591	FJ982549	FJ982466
<i>Paraparatrechina glabra</i>	USNM00756221	JN562412	FJ982638	FJ982514	FJ982594	FJ982552	FJ982469
<i>Paraparatrechina</i> cf. <i>oceanica</i>	USNM00756222	JN562413	FJ982639	FJ982515	FJ982595	FJ982553	FJ982470
<i>Paraparatrechina</i> PHL01	USNM00756223	—	FJ982640	—	—	FJ982554	FJ982471
<i>Paraparatrechina</i> PHL02	USNM00756224	JN562414	FJ982641	JN562445	FJ982596	JN562463	FJ982472
<i>Paraparatrechina</i> PHL03	USNM00756225	JN562415	FJ982642	JN562446	FJ982597	FJ982555	FJ982473
<i>Paraparatrechina weissi</i>	USNM00756226	JN562416	FJ982643	FJ982516	FJ982598	FJ982556	FJ982474
<i>Paratrechina longicornis</i>	USNM00756227	JN562417	FJ982644	FJ982517	FJ982599	FJ982557	FJ982475
<i>Paratrechina longicornis</i>	USNM00756228	JN562418	FJ982645	JN562447	FJ982600	FJ982558	FJ982476
<i>Prenolepis imparis</i>	CASENT0106035	JN562419	FJ982647	EF013330	EF013492	EF013755	FJ982478
<i>Prenolepis imparis</i>	USNM00756229	JN562420	FJ982648	FJ982518	FJ982601	FJ982559	FJ982479
<i>Prenolepis</i> MAL01	USNM00756230	—	JN562431	JN562448	—	JN562464	JN562437
<i>Prenolepis melanogaster</i>	USNM00756231	JN562421	JN562432	JN562449	JN562458	JN562465	JN562438
<i>Prenolepis nitens</i>	USNM00756232	JN562422	FJ982649	FJ982519	FJ982602	FJ982560	FJ982480
<i>Prenolepis</i> PHL01	USNM00756233	—	FJ982650	FJ982520	—	FJ982561	FJ982481
<i>Prenolepis</i> PHL02	USNM00756234	JN562423	JN562433	JN562450	JN562459	JN562466	JN562439
<i>Prenolepis</i> VIE02	USNM00756235	JN562424	—	JN562451	—	—	JN562440
<i>Prenolepis</i> VIE05	USNM00756236	JN562425	JN562434	JN562452	—	JN562467	JN562441
<i>Pseudolasius australis</i>	CASENT0106005	JN562397	FJ982651	EF013339	EF013501	EF013763	FJ982482
<i>Pseudolasius</i> PHL01	USNM00756237	JN562398	FJ982652	JN562453	FJ982603	FJ982562	FJ982483
<i>Pseudolasius typhlops</i>	USNM00756238	JN562426	FJ982653	JN562454	FJ982604	FJ982563	FJ982484
<i>Zatania albimaculata</i>	CASENT0106052	JN562427	FJ982646	EF013329	EF013491	EF013754	FJ982477
<i>Zatania cisipa</i>	USNM00756239	JN562428	JN562435	JN562455	JN562460	JN562468	JN562442
<i>Zatania gibberosa</i>	USNM00756240	JN562429	—	JN562456	JN562461	JN562469	JN562443
<i>Zatania gloriosa</i>	USNM00756241	JN562430	JN562436	JN562457	JN562462	JN562470	JN562444

CASENT, California Academy of Sciences; USNM, National Museum of Natural History.

Molecular work

We tested the monophyly of *Zatania* using molecular phylogenetic data from 34 *Prenolepis* genus-group species and four outgroup genera. Multiple representatives from all *Prenolepis* genus-group genera were included. We sampled four of the five extant species of *Zatania* (only *Zatania karstica* is missing). We included two exemplars of the only known New World species of *Prenolepis* (*P. imparis*) sampled from opposite longitudinal ends of North America (Arizona and Maryland) and seven Old World species from this genus.

Sampled ants were preserved in ethanol or pinned dry prior to extraction. Genomic DNA was extracted from whole ants (except from *Zatania gibberosa*, which was sampled

nondestructively) using the Qiagen DNeasy Tissue Kit (Qiagen Inc., Valencia, CA, U.S.A.), following the manufacturer's standard protocol. Vouchers consisting of nestmate specimens from the same collection event are deposited in the National Museum of Natural History and the California Academy of Sciences (Table 1).

We generated DNA sequence data from five nuclear protein coding genes – *arginine kinase* (*ArgK*), *carbamoylphosphate synthase* (*CAD*), *elongation factor 1-alpha F1* (*EF1 α F1*), *elongation factor 1-alpha F2* (*EF1 α F2*) and *wingless* – and from the mitochondrial protein-coding gene *cytochrome oxidase I* (*COI*). Primers and PCR protocols follow Brady *et al.* (2006) and LaPolla *et al.* (2010a), except that the current study also includes an extended fragment from *CAD* and the new gene *ArgK*. Primers for these new fragments are from

Ward *et al.* (2010). Sequence chromatograms were assembled and edited using SEQUENCHER 4.10 and combined into a phylogenetic matrix using MACCLADE (Maddison & Maddison, 2000). All intron sequences were removed from all analyses; the resulting exon-only matrix was easily and unambiguously aligned in MACCLADE. The final data matrix contained a total of 4332 nucleotide characters (1011 of which were parsimony informative, PI) from the following gene fragments: *ArgK*, 673 characters (144 PI); *CAD*, 924 characters (183 PI); *EF1 α F1*, 776 characters (110 PI); *EF1 α F2*, 517 characters (77 PI); *wingless*, 400 characters (101 PI); and *COI*, 1042 characters (396 PI). GenBank accession numbers are reported in Table 1.

We used Bayesian inference (BI) to estimate tree topologies and branch lengths using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003) parallelized over eight Mac G5 processors. The data were partitioned by gene and, within each gene, by codon positions (1 + 2) and 3, for a total of 12 partitions. The best-fitting model for each partition was selected using JMODELTEST (Posada, 2008) under the corrected Akaike information criterion (AICc). All parameters were unlinked across partitions except branch lengths and topology. Partition-specific branch length rate multipliers (*m*) were unlinked and initially assigned a Dirichlet prior (*ratepr = variable*). Four independent runs were initiated using random topologies with branch length priors of *brlenspr = Unconstrained:Exponential(100)* to avoid becoming trapped in parameter space with distorted branch lengths and partition rate multipliers (Marshall, 2010). Each run was distributed across four chains with a heating parameter of 0.05 (compared with the default setting of 0.2); this was to increase mixing among chains. Runs were conducted for 50 million generations with a burn-in of 10 million generations. Convergence between independent Markov chain Monte Carlo (MCMC) runs was verified using potential scale reduction factor (PSRF) values (1.00 for all taxon bipartitions), the average standard deviation of split frequencies (0.001 by 10 million generations) and by plotting likelihood values across generations using TRACER 1.5 (Rambaut & Drummond, 2007). The resulting post burn-in trees from all four runs (a total of 160 million generations) were combined to infer posterior probabilities of clades and branch lengths.

We also conducted bootstrap analyses under maximum likelihood (ML) and maximum parsimony (MP) criteria. We used GARLI 2.0 (Zwickl, 2006) to infer 1000 partitioned ML bootstrap replicates using the same partitioning scheme and models as the BI analyses. MP bootstrap values were calculated using PAUP* 4.0a114 (Swofford, 2002). We performed 1000 bootstrap replicates, with each round consisting of 20 random-addition sequence replicates, with ten trees held at each step, with tree bisection reconnection (TBR) branch swapping and no limit to maxtrees.

We translated our nucleotide matrix using MACCLADE to create a concatenated amino acid matrix in order to identify potential synapomorphies for *Zatania* at the amino acid level. Both concatenated matrices (nucleotide and amino acid) are archived and publically available through the Dryad digital repository (<http://datadryad.org>) under doi:10.5061/dryad.2408n.

Results

Molecular phylogeny

The four *Zatania* species included in the molecular phylogeny (Fig. 2) together are monophyletic with strong support under all analytical methods: BI posterior probabilities (PP) = 1.0; ML bootstrap proportions (BP) = 100; MP BP = 100. Within this genus, *Zatania albimaculata* and *Z. gibberosa* are sister species with strong support (BI PP = 1.0; ML BP = 100; MP BP = 100); the species *Zatania cisipa* resolves as sister to (*Z. albimaculata* + *Z. gibberosa*) with strong support under BI (PP = 0.99), but with weaker bootstrap support (ML = 79 and MP = 59). Examination of the translated amino acid matrix identifies two unique amino acid residues found in all four *Zatania* species and no other taxa in our matrix: proline in *wingless* (position 82 in our concatenated amino acid matrix) and methionine in *EF1 α F1* (position 896). Furthermore, all *Zatania* species except *Zatania gloriosa* exclusively possess isoleucine in *ArgK* (position 1261).

The genus *Zatania* resolves as the sister group to *Parapatrechina* with strong support from BI (PP = 0.98), but with weaker bootstrap support (ML = 71 and MP = 61). *Prenolepis* is recovered as a monophyletic unit (after the taxonomic removal of *Z. albimaculata* and *Z. gibberosa*; see below) with strong support: BI PP = 1.0; ML BP = 100; MP BP = 100. The only known New World species, *P. imparis*, is nested well within a grade of Old World *Prenolepis* species. All other *Prenolepis*-group genera are also reconstructed as monophyletic with robust support (Fig. 2). The genus *Prenolepis* is sister to all other genera within the *Prenolepis* genus-group.

Updated worker-based key to the genera of the *Prenolepis* genus-group

This key is modified from that found in LaPolla *et al.* (2010a) to include the new genus *Zatania*.

1. Maxillary palps with five or fewer segments; species often strongly polymorphic, with a major and minor caste expressed 2
 - Maxillary palps with six segments; species monomorphic to slightly polymorphic, with no discernable major or minor caste expressed 4
2. Polymorphic Afrotropical species.....*Parapatrechina* (*weissi* species-group)
 - Monomorphic or polymorphic Australasian/Indoaustralian species 3
3. Eyes large, typically REL ≥ 20 (one exception – *Euprenolepis negrosensis*); labial palps typically with four segments; mesothorax constricted immediately behind pronotum; mandalus large and conspicuous, usually visible without dissection of mandible (This character can be difficult to determine unless the mandible is dissected. It is only required if specimens have four-segmented labial palps AND five mandibular teeth.).....*Euprenolepis*

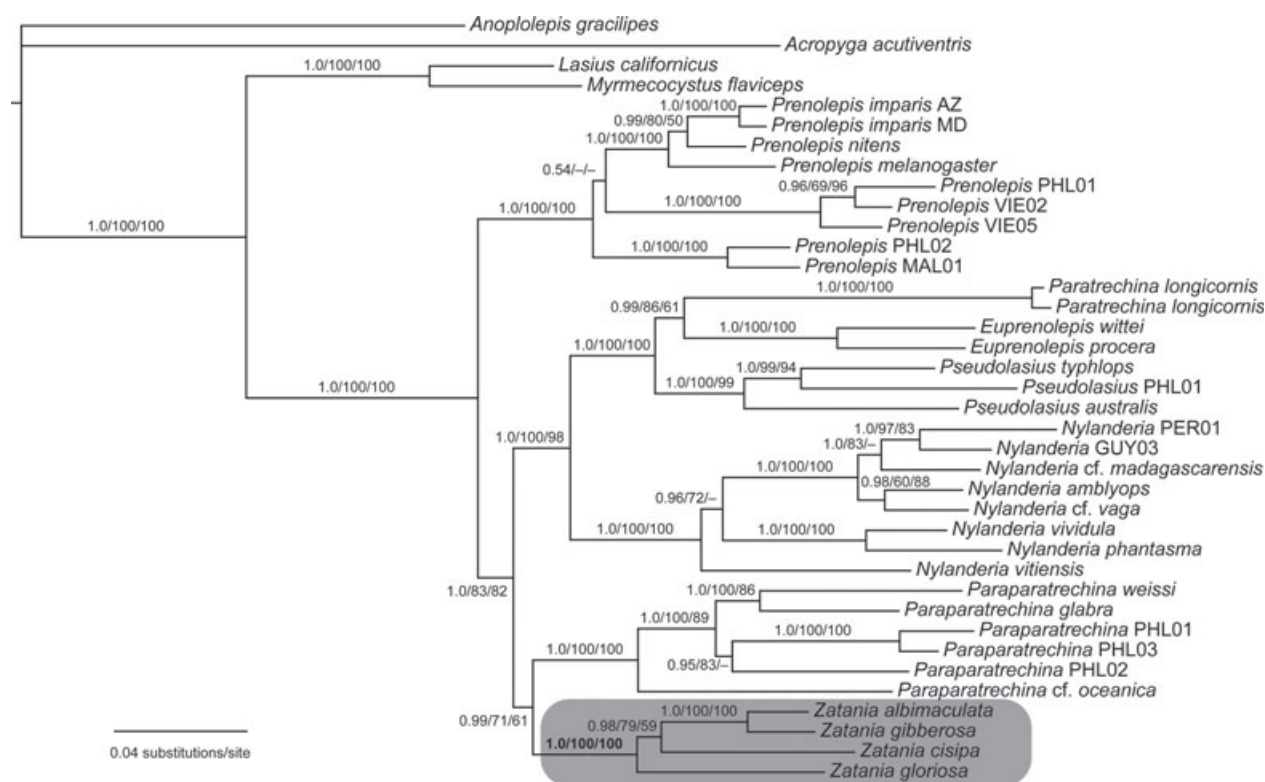


Fig. 2. Molecular phylogeny demonstrating the monophyly of *Zatania* and its phylogenetic position within the *Prenolepis* genus-group. The topology and branch lengths were inferred using Bayesian analyses. Support values are shown on branches in the following order: Bayesian posterior probabilities/maximum likelihood bootstrap proportions/maximum parsimony bootstrap proportions.

- Eyes small, REL < 20, typically 15 or less; labial palps typically with two or three segments; mesothorax typically not constricted immediately behind pronotum; mandibles small and inconspicuous, usually not visible without dissection of mandible *Pseudolasius*
- 4. Mandibles with between five and seven teeth; if five teeth, then mesothorax constricted immediately behind pronotum; scapes with or without erect setae 5
- Mandibles always with five teeth; mesothorax not constricted immediately behind pronotum; scapes always lacking erect setae 7
- 5. Eyes with about half their length above and half their length below midlength of head (typically at vertex) *Nylanderia*
- Eyes largely above midlength of head (typically posterior of vertex) 6
- 6. Head round in general appearance, with rounded, indistinct posterolateral corners; mesothorax always constricted immediately behind pronotum, typically with distinctly convex eyes (if eyes not distinctly convex, then head is distinctly round) *Prenolepis*
- Head rectangular in general appearance, with more angular, distinct posterolateral corners (one exception – *Zatania gloriosa*, which has a distinctly narrow, rectangular head with very rounded, indistinct posterolateral corners); if mesothorax

- constricted immediately behind pronotum then eyes relatively flat *Zatania*, **gen.n.**
- 7. Erect setae (one pair) present on propodeum; erect setae on head form a pattern of four setae along posterior margin and six or seven rows 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*

Description of *Zatania*, gen.n.

Type species of the genus: *Zatania cisipa* (Smith & Lavigne, 1973)

Etymology. The derivation of the genus name is a combination of the Greek prefix *za* (very) and *tany* (long), in reference to the long scapes, mesosomata and legs of species in this genus; it is a feminine noun.

Worker. Monomorphic, medium-sized (2–3 mm in total length) formicine ants, ranging in colour from brownish yellow to brown and reddish brown. Head with pubescence, and erect setae found along head margin; medially erect setae roughly

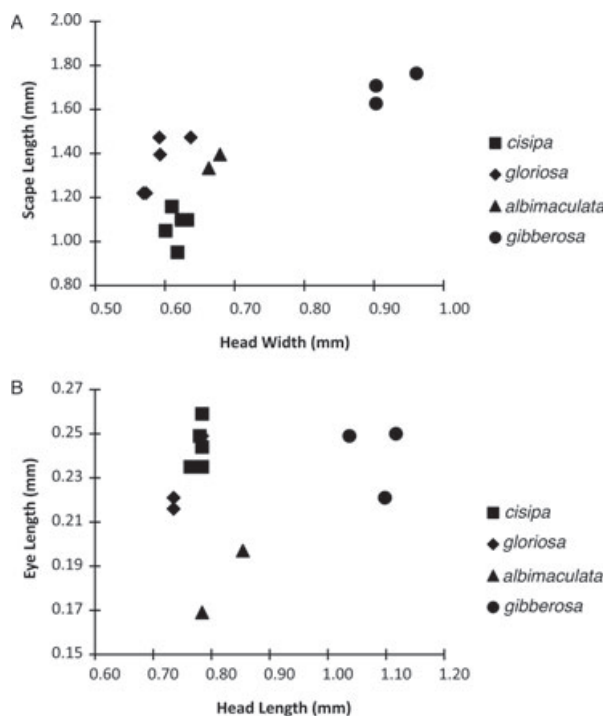


Fig. 3. Bivariate plots of morphological measurements.

paired, extended through medial portion of clypeus. Cuticle sculpturing variable; in some species opaque and slightly rugose, as in *Z. albimaculata*; in other species cuticle smooth and shining as in *Z. cisipa* and *Z. gloriosa*.

Antennae 12-segmented; scapes long (Fig. 3A), surpassing posterior margin by at least the length of the first four funicular segments. Scapes with either a dense layer of short erect setae and/or a layer of appressed setae that in some species is fairly scattered. Head longer than wide [cephalic index well below 100 (our recorded range, 75–87)].

Eyes medium to large, relative to head size; in *Z. cisipa* and *Z. gloriosa* eyes distinctly convex; three small, indistinct ocelli present.

Mandibles typical for a *Prenolepis* genus-group taxon, with five or six teeth: *Z. albimaculata*, *Z. gibberosa* and *Z. karstica* have five teeth; *Z. cisipa* and *Z. gloriosa* have six teeth; however, the tooth between the basal tooth and tooth 4 is much smaller than the other teeth, and is often missing, leaving a diastema between the basal tooth and tooth 4, effectively making the mandible five-toothed. Palps 6:4 segmented and long (at least as long as head).

Mesosoma distinctly elongated; in profile pronotum and mesonotum long; pronotum rises at less than 45° angle to mesonotum; in profile roughly triangular in shape at segment dividing pronotum and mesonotum; in some species mesonotum constricted immediately behind pronotum (*Z. albimaculata* and *Zatania electra*[†] possess the most extreme example of this character state); between 4–10 pronotal setae present.

Propodeum variable: in *Z. albimaculata*, *Z. electra*[†], *Z. gibberosa* and *Z. karstica*, propodeum dorsal face dome-like

and higher than remainder of mesosoma; in *Z. cisipa* and *Z. gloriosa* dorsal face gently rounded and not higher than remainder of mesosoma. In *Z. gibberosa* dorsal face with abundant short erect setae; in other species, dorsal face with only scattered or no erect setae. Petiole cuneate, broadly rounded dorsally and not surpassing height of propodeum.

Legs notably long; in darker coloured species, tarsi lighter in colour than remainder of leg.

Gaster robust, densely covered with erect setae and in some species with layer of pubescence.

Queen. Queens generally have the same coloration (although they are typically darker), setation and pubescence patterns as seen in workers.

Male. (males only examined in *Z. cisipa*, *Z. electra*[†], *Z. gibberosa* and *Z. gloriosa*)

Coloration similar to workers, but generally lighter overall. Cuticle surface, pubescence and setation patterns generally follow worker morphology.

Antennae 13-segmented; scapes long, as in workers, with similar pubescence and setation patterns.

Head shape variable; in *Z. cisipa* and *Z. gloriosa* compound eyes very large and convex, taking up most of lateral margin, with three very large ocelli; in *Z. gibberosa* compound eyes and ocelli considerably smaller, and head more similar to worker in general shape.

Mandibles in *Z. cisipa* and *Z. gloriosa* with prominent, pointed apical tooth; remainder of masticatory margin smooth, with indistinct, rounded basal angle that seamlessly blends into inner mandibular margin. In *Z. gibberosa* mandible also with prominent, pointed apical tooth, but masticatory margin serrated into generally indistinct denticles, most prominent and tooth-like immediately behind apical tooth; basal angle rounded, but more distinct than observed in *Z. cisipa* and *Z. gloriosa*; as in workers, palps 6:4 segmented and long.

Mesosoma robust, with small collar-like pronotum, overarched by rounded and prominent mesonotum; dorsal and declivitous faces of propodeum difficult to distinguish; propodeum linear in profile, at approximately a 45° angle.

Legs long as in workers.

Gaster generally similar to workers. Genitalia variable by species, but with some generally similar characteristics: parameres elongate and narrow; digiti and cuspi meet dorsally; digiti and cuspi shorter than aedeagal valves; digiti and cuspi very similar in overall shape and conformation (see Figs 28, 38).

Synopsis of *Zatania* species

Zatania albimaculata (Santschi, 1930), **comb.n.** Cuba
Zatania cisipa (Smith & Lavigne, 1973), **comb.n.** Puerto Rico
Zatania electra[†], **sp.n.** Extinct, Dominican Amber fossil
Zatania gibberosa (Roger, 1863), **comb.n.** Cuba and Haiti
 = *Zatania gibberosa rogeri* (Mann, 1920)
Zatania gloriosa, **sp.n.** Honduras and Mexico (Chiapas)
Zatania karstica (Fontenla, 2000), **comb.n.** Cuba

Key to workers of extant *Zatania* species

1. Yellow species; scapes with a dense layer of short, decumbent setae (Fig. 11) *cisipa*
 – Brown to reddish brown species (some species strongly bicoloured); scapes with either appressed (Fig. 19) or decumbent pubescence (if decumbent pubescence present then species is dark-brown to reddish brown) (Fig. 7) 2
2. Dorsal face of propodeum only slightly convex, below level of mesonotum; cuticle shiny (Fig. 18) *gloriosa*
 – Dorsal face of propodeum strongly convex, dome-like, well above level of mesonotum; cuticle opaque (Figs 6, 14, 21) 3
3. Body uniformly dark brown *karstica*
 – Body distinctly bicoloured, with reddish brown head and mesosoma, and darker brown gaster 4
4. Larger species (SL > 1.6 mm; HL > 1.0 mm; ML > 1.7 mm); head with a layer of pubescence; mesosoma with abundant erect setae *gibberosa*
 – Smaller species (SL < 1.5 mm; HL < 1.0 mm; ML < 1.5 mm); head without a layer of pubescence, although sometimes scattered pubescence present; mesosoma with scattered erect setae *albimaculata*

Species accounts

Zatania albimaculata (Santschi, 1930), **comb.n.**
 [Figs 4–7 (worker)]

Prenolepis gibberosa albimaculata Santschi, 1930: 81 (worker described). Type locality: Cuba: Piedra Gorda, Baracoa (depository of type specimens unknown). Fontenla, 2000: 81, raised to species (here confirmed).

Worker diagnosis. Head and mesosoma reddish brown, with darker gaster; head with scattered erect setae and only sparse pubescence; scapes with dense layer of short, erect setae; head cuticle slightly rugose; mesosoma with scattered short, erect setae and sparse pubescence; strong constriction immediately behind pronotum; dorsal face of propodeum convex with only very scattered erect setae and pubescence.

Measurements ($n = 2$): TL 2.20–2.40 mm; HW 0.66–0.68 mm; HL 0.78–0.85 mm; EL 0.17–0.2 mm; SL 1.30–1.40 mm; PW 0.42–0.47 mm; WL 1.43–1.47 mm; PrFL 0.98–1.10 mm; PrFW 0.14 mm; GL 0.98–1.1 mm.
 Indices: CI 80–85; REL 22–23; SI 201–205.

Notes. Workers of this species most closely resemble *Z. gibberosa* with regards to coloration, but *Z. albimaculata* is smaller, less robust and possesses much less pilosity. We have not seen males of *Z. albimaculata*, but based on Fontenla (2000) there appears to be significant differences in male genitalic characters that also separate these two species. Fontenla (2000) reported that this species mostly forages in trees.

Zatania cisipa (Smith & Lavigne, 1973), **comb.n.**
 [Figs 8–11 (worker), 22–23 (queen), 24–29 (male)]

Paratrechina (*Nylanderia*) *cisipa*, Smith D.R. & Lavigne, 1973: 184 (worker, queen and male described). Holotype worker, Puerto Rico: Luquillo Experimental Forest, El Verde Field Station, 1500 ft, 1 July 1970 (R.J. Lavigne) (USNM) [examined]. LaPolla *et al.*, 2010a: 127, combination in *Nylanderia*.

Worker diagnosis. Overall brownish yellow, with darker gaster; head with layer of pubescence and distinctly convex eyes; scapes with dense layer of short, slender, decumbent setae; mesosoma with scattered erect setae on pronotum and mesonotum; dorsal face of propodeum gently rounded; gaster with dense layer of pubescence.

Measurements ($n = 6$): TL 2.50–2.70 mm; HW 0.6–0.63 mm; HL 0.76–0.78 mm; EL 0.24–0.26 mm; SL 1.00–1.20 mm; PW 0.46–0.50 mm; WL 1.10–1.33 mm; PrFL 0.83–0.88 mm; PrFW 0.16–0.18 mm; GL 1.22–1.40 mm.

Indices: CI 78–81; REL 30–33; SI 154–176.

Notes. This is the only yellow species known in the genus, and is therefore easy to identify. The yellow coloration is probably an adaptation, reflecting the fact that workers are nocturnal foragers (J.S. LaPolla, personal observation). At El Verde Field Station in Puerto Rico, during the day no workers were ever seen foraging, but at night it was one of the most commonly encountered ant species, often observed in large foraging trails with hundreds of individual workers moving on the forest floor. This appears to be a tree hollow-nesting species, as nests were observed by both Smith & Lavigne (1973) and J.S. LaPolla in living tree hollows at a height of about 1–1.5 m from the ground.

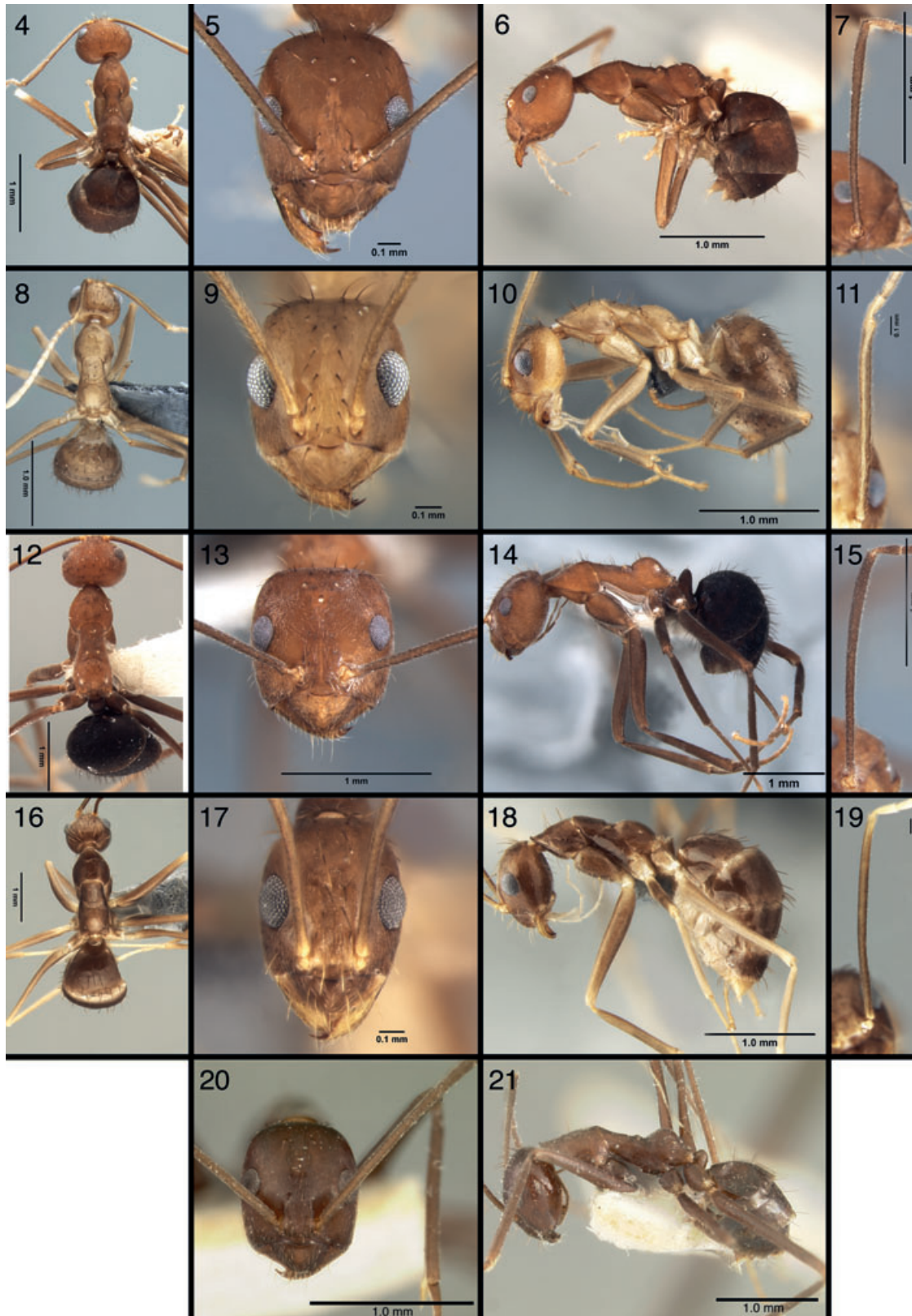
Zatania electra[†], **sp.n.**
 [Figs 30a, b and 31b (worker), 31a (male)]

Holotype worker, Dominican Amber fossil, AMNH DR10-159; one paratype worker, Dominican Amber fossil (MCZC); and one paratype male, AMNH PB-118-25.

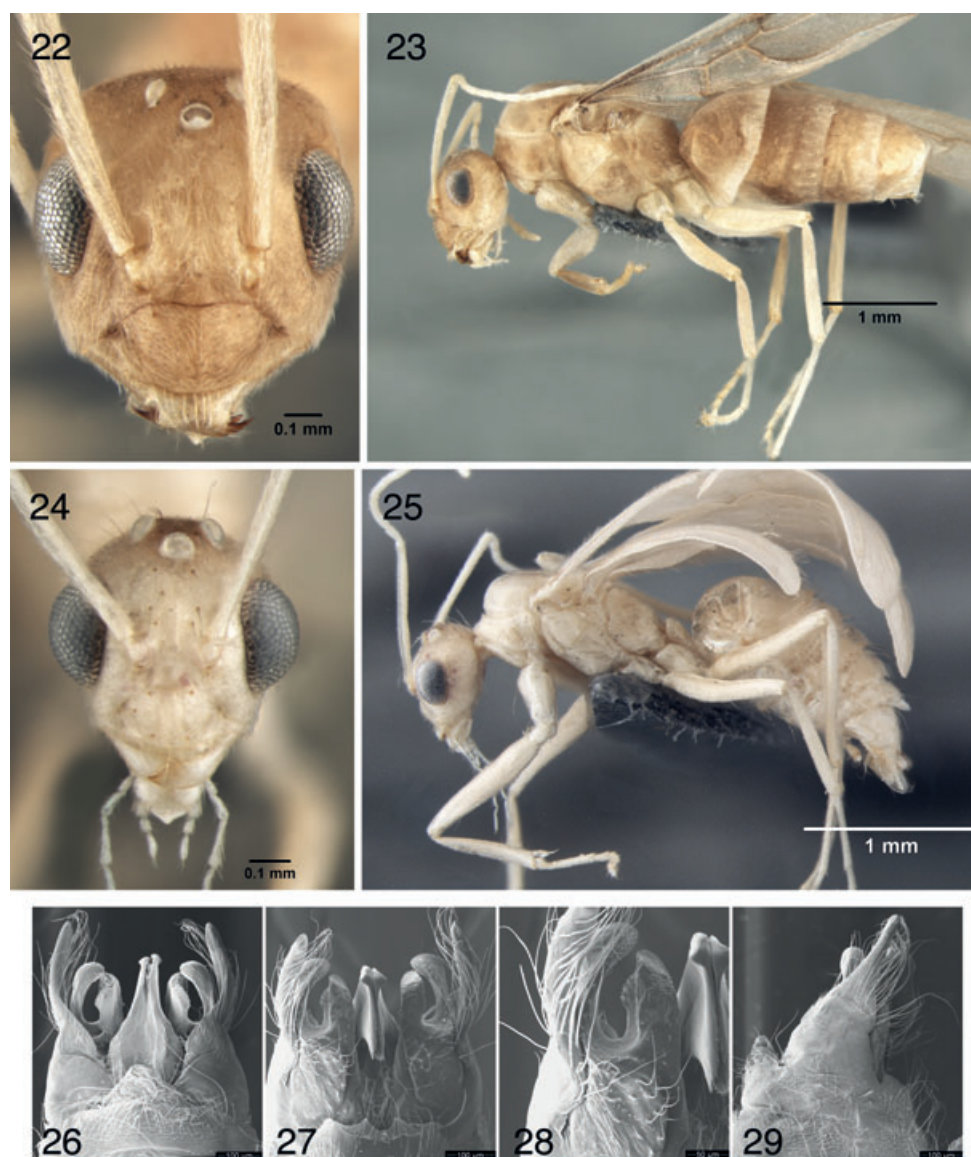
Worker diagnosis. Dominican amber fossil species.
 Measurements ($n = 1$): TL 2.80 mm; HW 0.57 mm; HL 0.73 mm; EL 0.17 mm; SL 1.18 mm; PW 0.41 mm; WL 1.14 mm; PrFL 0.84 mm; PrFW 0.16 mm; GL 0.94 mm.
 Indices: CI 78; REL 23; SI 207.

Species description

Worker. Coloration difficult to ascertain in amber matrix, but appears to be either uniformly dark or reddish brown. Head distinctly longer than wide (CI 78) and strongly oval in shape; mandibles seemingly with five teeth; posterolateral corners of head indistinct and rounded; posterior margin short; scattered macrosetae on head; difficult to ascertain if pubescence is present. Scapes surpass posterior margin by about length of first six or seven funicular segments; scapes with thin, short erect setae, difficult to ascertain if pubescence present.



Figs 4–21. *Zatania* workers in various views (left to right: dorsal, full frontal, lateral and scape): 4–7, *Zatania albimaculata*; 8–11, *Zatania cisipa*; 12–15, *Zatania gibberosa*; 16–19, *Zatania gloriosa*; 20–21, *Zatania karstica*.



Figs 22–29. *Zatania cisipa* reproductives: 22, queen in full-frontal view; 23, queen in lateral view; 24, male in full-frontal view; 25, male in lateral view; 26–29, male genitalia (dorsal, ventral, ventral close-up of digitus and cuspis, and lateral parameres).

Eyes relatively small, but distinctly convex. Pronotum with scattered erect setae; erect setae on notum; propodeal erect setae shorter than other notal setae; distinct constriction present immediately behind pronotum; metanotal area long; dorsal face of propodeum dome-like, higher than remainder of notum; gaster with scattered erect macrosetae.

Queen. Unknown.

Male. Coloration difficult to ascertain in amber matrix but appears to be either uniformly dark or reddish brown. Head slightly wider than long, dominated by large, convex compound eyes that take up most of lateral margin of head; mandibles, with prominent, pointed apical tooth; masticatory margin partly obscured, but seemingly with indistinct denticles,

similar to what is observed in *Z. gibberosa* males; head with scattered erect macrosetae; difficult to ascertain if pubescence present. Scapes surpass posterior margin by about length of first five or six funicular segments; scapes with short, thin erect setae. Mesosoma modified for presence of wing; erect setae scattered across mesonotum and metanotum; propodeum indistinct with very short dorsal face and long declivitous face. Gaster with scattered erect setae; genitalia with long, narrow parameres, similar to what is observed in *Z. cisipa* and *Z. gloriosa*.

Measurements ($n = 1$): TL 2.85 mm; HW 0.68 mm; HL 0.62 mm; EL 0.29 mm; SL 0.98 mm; PW 0.51 mm; WL 1.28 mm; PrFL 1.01 mm; PrFW 0.15 mm; GL 0.95 mm.

Indices: CI 101; REL 47; SI 145.

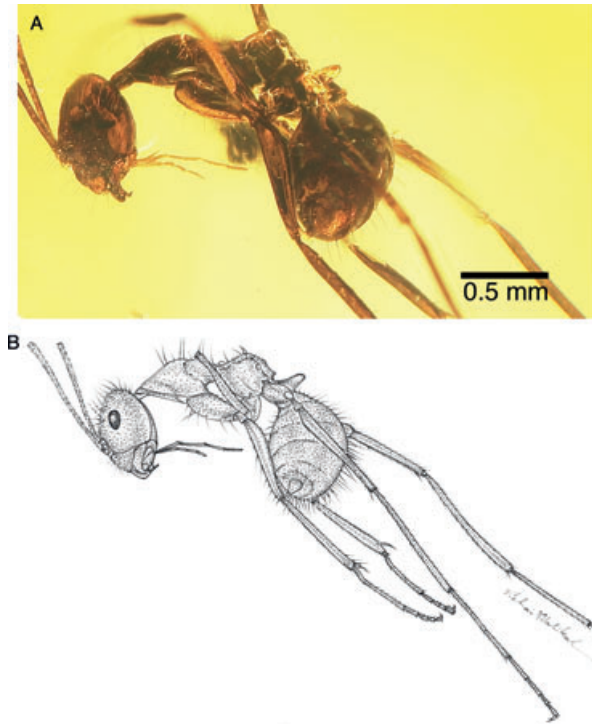


Fig. 30. *Zatania electra*[†]: A, photograph of holotype worker; B, illustration of holotype worker.

Etymology. The species epithet is in reference to the fact that this is a fossil species found in amber (*electra* is Greek for amber).

Notes. This is the only fossil species known in the genus. The workers, with respect to some morphological features, resemble the workers of *Z. albimaculata* and *Z. karstica*, especially with regards to the relatively small size of the compound eyes and the constriction of the mesosoma immediately behind the pronotum. The dome-like propodeum is most similar to *Z. gibberosa* and *Z. karstica*. The apparently uniform body colour of the fossil species is only shared with *Z. karstica* among extant species. Interestingly, however, despite the similarities of the workers to the three mentioned extant species, the narrow male parameres most closely resemble those of *Z. cisipa* and *Z. gloriosa*. The narrow head of the *Z. electra* worker is most similar to what is observed in *Z. gloriosa*[†]. It therefore appears that *Z. electra*[†] has an interesting combination of morphological features observed among all of the known extant species.

Zatania gibberosa (Roger, 1863), **comb.n.**
[Figs 12–15 (worker)]

Prenolepis gibberosa, Roger, 1863: 161 (worker described). Type locality: Cuba (depository of type specimens unknown). *Prenolepis* (*Prenolepis*) *gibberosa* var. *rogeri* Mann, 1920: 434 (worker described). Holotype worker, Cuba: Yateras District, Guantanamo, San Blas (NMNH) (examined). Fontenla,

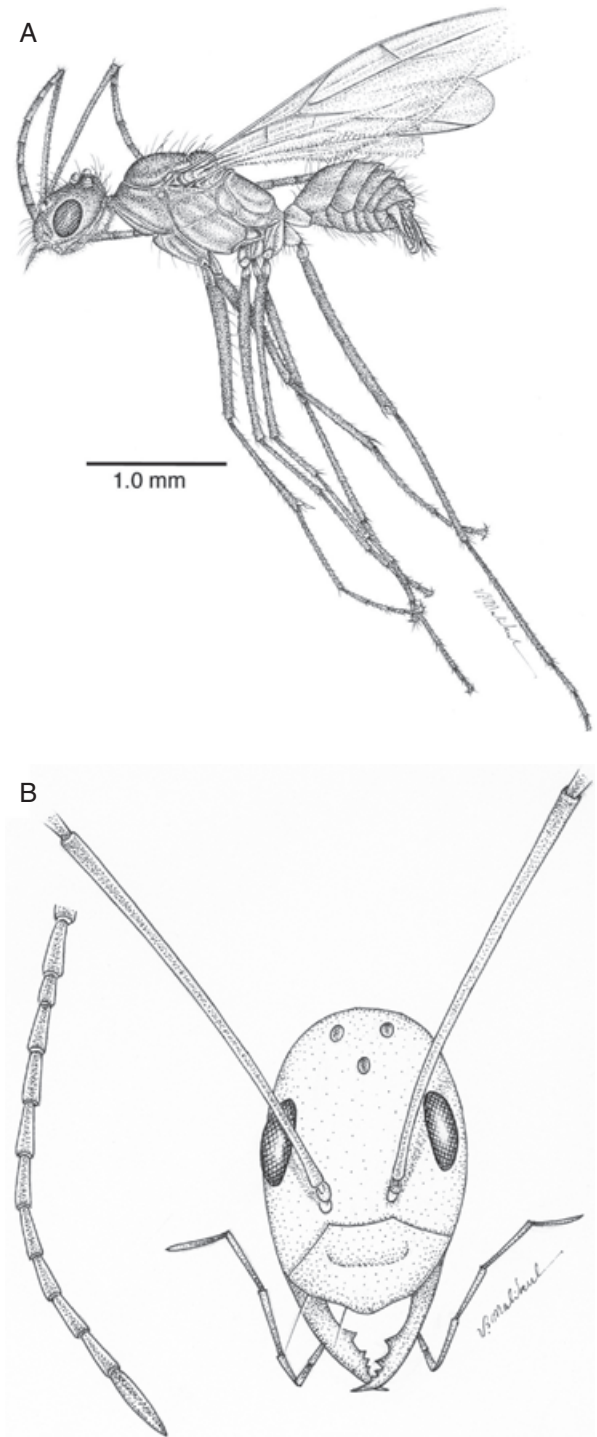


Fig. 31. *Zatania electra*[†]: A, male in lateral view; B, worker in full-frontal view.

2000: 82, junior synonym of *Prenolepis gibberosa* (here confirmed, but within *Zatania*), **comb.n.**

Worker diagnosis. Large, robust species; head and mesosoma reddish brown, with darker gaster; entire body distinctly

opaque (although there is some variation in gaster opacity, see below) with abundant erect setae and pubescence; scapes with very dense layer of suberect pubescence; dorsal face of propodeum distinctly dome-like and with abundant erect setae. Measurements ($n = 3$): TL 3.0–3.1 mm; HW 0.9–0.96 mm; HL 1.0–1.1 mm; EL 0.22–0.25 mm; SL 1.63–1.8 mm; PW 0.6–0.65 mm; WL 1.73–1.85 mm; PrFL 1.32–1.35 mm; PrFW 0.17–0.24 mm; GL 1.2–1.3 mm.

Indices: CI 82–87; REL 20–24; SI 180–189.

Notes. This species has the largest and most robust workers within the genus; the large, pilose workers of this species effectively separate it from other species. It possesses abundant erect setae on the head and mesosoma, including the propodeum, which is in contrast to *Z. albimaculata*, the species most likely to be confused with *Z. gibberosa*. Mann (1920) described a variety of *Z. gibberosa* (var. *rogeri*, then in *Prenolepis*) based on the gaster being shiny as opposed to opaque. Fontenla (2000) stated that this character was variable and that the morphological division between opaque and shiny gasters was unclear. In fact we have seen specimens in which the gaster is both shiny in some places and opaque in other places. There is also some variation in the size of workers (suggesting workers might be slightly polymorphic), worker coloration and mesosomal setation (Fontenla, 2000), but with the fairly low number of specimens available for this study we cannot correlate these morphological characters in such a way that suggests there is more than one species present (as opposed to geographical variation). However, this species is reported from across Cuba and into Haiti (although interestingly it has not been collected from the Dominican Republic), and a more detailed study is needed to determine if cryptic species are present. For now we maintain the synonymy established by Fontenla (2000), until additional data suggest otherwise. Both Mann (1920) and Fontenla (2000) report *Z. gibberosa* from forests; Mann observed nests in decayed logs. Where it occurs, it is apparently an active (workers move about quickly) and abundant species.

Zatania gloriosa, sp.n.

[Figs 16–19 (worker), 32–33 (queen), 34–39 (male)]

Holotype worker, MEXICO: Chiapas: 2 km south of Playón de la Gloria, 16.1385°N, 90.90147°W, 22 July 2007 (R.S. Anderson) (USNM). Six paratype workers from the same locality as the holotype (USNM, MCZC).

Worker diagnosis. A slender species, with a distinctly narrow head; overall coloration brown with areas of brownish yellow on head (mandibles and antennae, particularly around torulae and proximal end of scapes and funiculus), mesosoma along segmental margins, legs at joints (mesoleg and metaleg almost entirely brownish yellow), and gaster venter and along segmental margins; propodeum with low, gently rounded dorsal face. Measurements ($n = 5$): TL 2.49–2.69 mm; HW 0.57–0.64 mm; HL 0.74–0.78 mm; EL 0.22–0.25 mm; SL

1.22–1.47 mm; PW 0.44–0.50 mm; WL 1.27–1.44 mm; PrFL 0.98–1.10 mm; PrFW 0.16–0.19 mm; GL 1.31–1.44 mm.

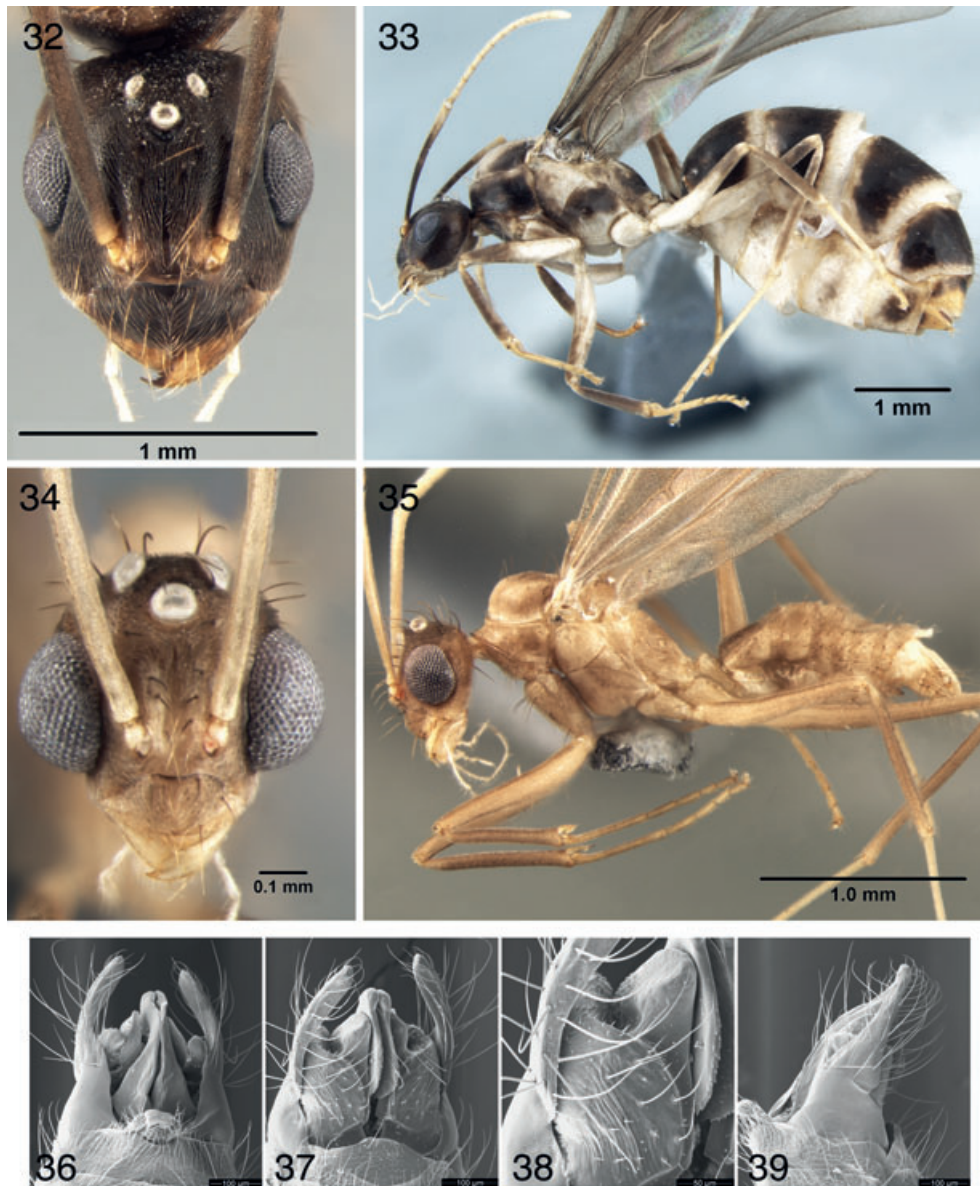
Indices: CI 76–78; REL 29–32; SI 212–235.

Species description

Worker. Overall coloration brown, with areas of brownish yellow across body; cuticle smooth and shining. Head distinctly longer than wide (CI 76–78) and strongly oval in shape; mandibles brownish yellow, typically with six teeth (although in some specimens fifth tooth between basal tooth and fourth tooth small and sometimes abraded away or otherwise missing); posterolateral corners of head indistinct and rounded; posterior margin short; erect macrosetae concentrated medially, where they are roughly paired and along head margins; head with layer of pubescence. Scapes surpass posterior margin by length of first five funicular segments; scapes lacking erect setae, but layer of pubescence present; antennae lighter than head, becoming brownish yellow to yellow around torulae and proximal end of scapes and funiculus. Eyes large and convex taking up about one-third of lateral margin of head. Pronotum with scattered erect setae; notum with sparse pubescence; metanotal area distinct, but short; dorsal face of propodeum not higher than remainder of notum, gently rounded; legs lighter than body; procoxae brown, with mesocoxae and metacoxae yellowish brown; prolegs yellowish brown, with mesoleg femur brownish becoming brownish yellow to white; metaleg entirely brownish yellow to white; gaster with scattered erect setae and layer of pubescence; dorsally gaster brown except along segmental margins, which are brownish yellow to white; ventrally gaster brownish yellow to white.

Queen. Generally matches worker description, with modifications expected for caste. Coloration pattern generally similar to workers, but darker brown and more white found on funiculus, mesosoma, legs and gaster than in workers.

Male. Overall coloration with yellowish brown head, yellow antennae and remainder of body golden brown. Head longer than broad, dominated by large, convex compound eyes that take up most of lateral margin of head; mandibles yellow, with prominent, pointed apical tooth; remainder of masticatory margin smooth, with indistinct, rounded basal angle that seamlessly blends into inner mandibular margin; erect macrosetae concentrated medially, where they are roughly paired, and along head margins; head with layer of pubescence. Scapes surpass posterior margin by length of first three funicular segments; scapes lacking erect setae, but layer of pubescence present. Mesosoma modified for presence of wing; erect setae scattered across mesonotum and metanotum; propodeum indistinct, with very short dorsal face and long declivitous face. Gaster with scattered erect setae and layer of pubescence; whitish in colour in area immediately preceding genitalia (see Figs 36–39). Measurements ($n = 2$): TL 2.68–2.86 mm; HW 0.64–0.65 mm; HL 0.56–0.59 mm; EL 0.29–0.31 mm; SL 1.02–1.03 mm; PW 0.57–0.58 mm; WL 1.05–1.16 mm; PrFL 0.94–0.99 mm; PrFW 0.15–0.16 mm; GL 1.04–1.14 mm. Indices: CI 110–116; REL 52–53; SI 145.



Figs 32–39. *Zatania gloriosa* reproductives: 32, queen in full-frontal view; 33, queen in lateral view; 34, male in full-frontal view; 35, male in lateral view; 36–39, male genitalia (dorsal, ventral, ventral close-up of digitus and cuspis, and lateral parameres).

Etymology. The species epithet is in reference to the type locality and also the interesting coloration pattern of the workers of this species.

Non-type material. HONDURAS: Atlantida: 10 km SSW Tela, 15.69714°N, 87.47451°W, 610 m a.s.l., 17 June 2010, LLAMA#Wm-C-08-2-02, tropical wet forest, ex. sifted leaf litter (two workers).

Notes. The coloration pattern of *Z. gloriosa* workers and queens is particularly striking, and is unlike any other species in the genus. The worker has a distinctly narrow, oval-shaped head, also unlike any other extant species in the genus (it is

similar to the fossil species *Z. electra*[†]). The type series nest was collected from a rainforest in sifted leaf litter, as were the two workers collected from Honduras.

Zatania karstica (Fontenla, 2000), **comb.n.**

[Figs 20, 21 (worker)]

Prenolepis karstica Fontenla, 2000: 84 (worker described). Holotype worker, Cuba: Pinar del Río Province, near Ceja de Francisco, Sierra de Mesa (IESC) (examined).

Worker diagnosis. Overall coloration uniformly dark brown; scattered erect setae on head, pronotum and gaster; no erect

setae on scapes, mesonotum or propodeum; whitish pubescence scattered across body; dorsal face of propodeum distinctly dome-like.

Notes. The workers of this species are the only extant species that is uniformly coloured, making them relatively easy to identify. Fontenla (2000) discussed the interesting behaviour of *Z. karstica* workers. In other *Zatania* species workers are fast-moving individuals (not surprising given the long legs observed in workers), but in *Z. karstica* the workers are slower (although Fontenla did not qualify this), and they often display what he termed a frozen pose as they moved along. Fontenla (2000) suggested that this behaviour was related to the fact that the species is putatively restricted to living on limestone mogotes, and that the coloration of *Z. karstica* mimics *Temnothorax* ants (*Temnothorax gundlachi* and *T. poeyi*), with which it lives sympatrically. It has been suggested that the coloration of the *Temnothorax* ants is aposematic (Wilson, 1988; Fontenla, 1995), and therefore Fontenla (2000) speculated that *Z. karstica* is engaged in some kind of convergent mimicry to protect them against *Anolis* lizards, presumably one of their main predators. We have not personally observed this species in the field, but *Z. karstica* may be an interesting candidate for the study of adaptations (morphological and behavioural) of ants to living on limestone mogotes.

Discussion

Fontenla (2000) provided an initial attempt at a morphological phylogeny for *Prenolepis*. This phylogeny contained relatively few taxa (12 species included), and was inferred using solely worker-based morphology. It uncovered a monophyletic group containing *Z. albimaculata*, *Z. gibberosa* and *Z. karstica* (all three were then classified as *Prenolepis*), and he was the first to suggest that *Prenolepis* might not be monophyletic. He cites oval pronotal shape and nesting behaviour (arboreal or limestone nesting) as the two synapomorphies for these three species. We are not certain as to what he meant by the oval pronotum character, but the pronotal shape of *Zatania* workers is different from many *Prenolepis* species (see below), especially the North American *P. imparis*. *Zatania gloriosa* seems to nest at least occasionally in the ground, so the genus is not exclusively restricted to arboreal or limestone nest sites (but the other four extant species are putatively not ground nesting). However, based on our phylogeny, arboreal and limestone nesting species do form a monophyletic group within the genus, and interestingly these are all from the Greater Antilles.

Our molecular phylogeny confirms that *Zatania* represents a lineage of ants distinct from *Prenolepis* and all other genera within the genus-group. The three new *Zatania* species we added to our phylogenetic analyses cluster together with *Z. albimaculata* from LaPolla et al. (2010a) with high posterior probabilities (1.0) and bootstrap values (100), strongly supporting the monophyly of this genus. Inspection of our molecular matrix also identifies several amino acid changes that are unique within *Zatania*. Although our sampling within the *Prenolepis* genus-group is by no means comprehensive at

the species level, these amino acid characters may serve as synapomorphies for *Zatania* within the group.

The molecular data also suggest a sister-group relationship between *Zatania* and *Paraparatrechina*, although not all forms of phylogenetic analyses provide high support for this conclusion. Compared with LaPolla et al. (2010a), support for this relationship is considerably stronger in the current study, which includes additional taxa and molecular characters. Support values changed from BI PP = 0.79, ML BP < 50 and MP BP < 50 in LaPolla et al. (2010a) to BI PP = 0.99, ML BP = 71 and MP BP = 61 in the current study. Even given this trend, however, the current molecular data must still be viewed as reflecting some uncertainty concerning this relationship. We can also identify two morphological features of males that perhaps unite these two genera: narrow parameres and a similar-shaped aedeagal valve (LaPolla et al., 2010b). The aedeagal valves in the two genera share similarities with respect to a distinctly rounded ventral margin, with teeth present and a dorsal margin with a triangular section extending past the apodeme. Interestingly, a similarly shaped aedeagal valve is also observed in most species of *Myrmecocystus* (Snelling, 1976), which may indicate that this condition is pleisomorphic, or that it has evolved at least two times within the group.

In keys to the workers of New World formicine genera (Bolton, 1994; Fisher & Cover, 2007; LaPolla et al., 2010a), the species placed here in *Zatania* would key to the genus *Prenolepis*, in which three *Zatania* species were previously classified. There are several morphological features that provide a means to distinguish between these two genera. In the New World the only known *Prenolepis* species is *P. imparis*. *Zatania* species possess very long scapes, heads that are distinctly longer than wide (*P. imparis* has a head that is nearly as wide as it is long), an elongated, linear pronotum in profile (in *P. imparis* the pronotum is short and distinctly rounded in profile) and distinctly long legs (especially the femur and tibia).

We provide a modified key that will allow *Zatania* workers to be distinguished from other genera within the *Prenolepis* genus-group. *Zatania* workers are most likely to be confused with Old World *Prenolepis* workers. At present, *Zatania* is only known from the Greater Antilles and Central America, and *Prenolepis* is absent from these areas, so *Zatania* workers could seemingly be separated from *Prenolepis* workers based on geography. As other ant genera, however, have been found to have a disjunct distribution between Asia and Central America (e.g. Longino & Hartley, 1994), it is possible that *Zatania* does in fact occur in Asia. The Asian tropics, where *Prenolepis* reaches highest species diversity, have been poorly collected, and as new collections are made, the discovery of *Zatania* in Asia would not be surprising. The main diagnostic features that separate *Zatania* and *Prenolepis* workers are found in the head and mesothorax. *Prenolepis* workers generally have a round head, with very indistinct posterolateral corners, whereas *Zatania* workers have a rectangular head, with more distinct posterolateral corners. The one exception is found in *Z. gloriosa*, which has a rectangular head, but also has very indistinct posterolateral corners. *Prenolepis* also always

have a mesothorax that is constricted immediately behind the pronotum. This condition is observed among the extant members of *Zatania* in *Z. albimaculata*, *Z. gibberosa* and *Z. karstica* (the fossil species *Z. electra*[†] also has constricted mesothorax). In these *Zatania* species, however, the eyes are small relative to the size of the head, and they are relatively flat, whereas in most *Prenolepis* the eyes are larger relative to the size of the head and distinctly convex. In species such as *P. imparis*, where the eyes are not distinctly convex, their head is still distinctly rounded, thereby placing it within *Prenolepis* (with other features noted in the previous paragraph).

The main difficulty in distinguishing between the various lineages within the *Prenolepis* genus-group at the morphological level arises from the fact that, in several groups, a similar morphological form is observed among workers, confounding the use of worker morphology as a basis for generic level recognition. An elongated worker mesosoma is present in *Euprenolepis*, *Paratrechina* and *Prenolepis*, as well as in the new genus described here. Accompanying this elongated mesosoma are elongated scapes and legs, and typically a distinct constriction of the mesosoma behind the pronotum. To a lesser degree, this suite of morphological traits is seen in a few species of *Pseudolasius* and *Nylanderia* as well. The reliance on worker-based taxonomy has hindered the discovery of this new lineage, and it remains a possibility that other lineages are currently undiscovered within the *Prenolepis* genus-group, based on the level of worker morphological convergence observed. Upon closer inspection, differences in worker morphology can usually be observed, but it is often difficult to ascertain their relative phylogenetic importance a priori. An example can be seen in separating *Pseudolasius* and *Euprenolepis*, as both are distinct lineages based on molecular data. With closer examination it was found that the mandalus of the mandible in each genus was different (LaPolla, 2009), but without the molecular phylogenetic results it would have been difficult to assess if this morphological difference represented a true synapomorphy for *Euprenolepis*.

The males of *Zatania*, however, do provide a series of diagnostic morphological features for the genus. A notable difference between males of *P. imparis* and *Zatania* is observed in the antennal scapes. *Zatania* males possess long scapes compared with the short scapes of *P. imparis*. *Zatania* males possess long, narrow parameres, digiti and cuspi that meet dorsally, digiti that are shorter than the aedeagal valves (in *P. imparis* the digiti are much longer than the aedeagal valves), and robust, distinctly shaped digiti (see Figs 28 and 38). These genitalic characters are putatively morphological synapomorphies for the genus. It is encouraging that male-based morphology provides a means to indentify some clades within the *Prenolepis* genus-group that are highly supported by our molecular data. The problem of course remains that for many species males are unknown. *Zatania* males are distinctive in several ways, the most obvious being their elongated scapes and legs, and the structure of the genitalia. It would be prudent for future morphologically based research of this genus-group to include males whenever possible.

Other studies have also illustrated the value of male characters to ant systematics. Wild (2007) gives an excellent example in which male characters provide critical information towards delimiting species-groups within *Linepithema*. Male morphology can also provide robust phylogenetic information in cases where taxa are known only from males (e.g. *Aenictogiton*; Brady & Ward, 2005). Recent efforts to construct comprehensive diagnoses (Bolton, 2003) and identification keys (see the summary in Yoshimura & Fisher, 2011) for males will also serve to increase the applicability of this character system. Unfortunately, in some ant groups, males are either too rare in collections (e.g. African ponerines; Bolton & Fisher, 2008) or are even undiscovered as yet (e.g. *Lachnomymex*; Feitosa & Brandão, 2008), and thus cannot yet provide significant systematic information. An increase in targeted collecting activities and male-worker association via molecular diagnostics may increase the utility of male morphology in these cases (e.g. Fisher & Smith, 2008).

Our results support the notion that continued reliance on worker-based morphology may impede progress in higher-level ant systematics. It is true that some groups previously established using worker morphology have maintained their integrity with the addition of new character systems, especially molecular data; the most notable example is the strong molecular support for the vast majority of ant subfamilies established using morphology (Brady *et al.*, 2006; Moreau *et al.*, 2006). However, molecular data are rapidly uncovering robust clades that worker-based characters have failed to identify. For example, molecular data (Ward & Brady, 2003; Brady *et al.*, 2006; Moreau *et al.*, 2006) consistently and robustly indicate a formicoid clade that includes several taxa previously identified as belonging to a separate group (the ponerines) by using worker characters. Ward (2011) summarizes examples of long-established ant genera that molecular data have revealed to be non-monophyletic. He outlines a new strategy in ant systematics consisting of the following steps: (i) the generation of a molecular phylogenetic hypothesis, with taxon sampling guided by existing taxonomic knowledge; (ii) the re-examination of morphology in light of any novel molecular results; (iii) the production of new morphology-based diagnoses. The current study, along with LaPolla *et al.* (2010a), provides two examples of how this newly emerging paradigm promises to increase our understanding of the systematics of ants and other insect groups.

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