
Cladistic analysis of the fire ants of the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae)

JAMES P. PITTS, JOSEPH V. MCHUGH & KENNETH G. ROSS

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Results are presented from a phylogenetic study of the fire ants comprising the *Solenopsis saevissima* species-group (Hymenoptera: Formicidae). Six most-parsimonious trees were identified following a cladistic analysis utilizing 18 taxa and 36 morphological characters derived from three castes and two developmental stages. A strict consensus tree recovered the following relationships: (*S. daguerrei* ((*S. electra*, *S. pusilignis*) (*S. saevissima* (*S. pythia* (*S. interrupta*, *S. 'undescribed species'*, *S. weyrauchi* (*S. richteri*, *S. invicta* (*S. megergates* (*S. quinquecupis*, *S. macdonaghi*)))))))). This phylogenetic hypothesis implies trends in fire ant evolution towards both polygyny (multiple egg-laying queens per colony) and large major worker size. The phylogeny also provides a test of Emery's Rule, which is not supported in its strictest sense because the social parasite *S. daguerrei* is not the sister species to its host species. A modified version of Emery's Rule is supported, because the social parasite is the sister species to a larger clade containing its hosts, as well as nonhosts.

James P. Pitts, Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84326, USA. E-mail: jppitts@biology.usu.edu

Joseph V. McHugh and Kenneth G. Ross, Department of Entomology, University of Georgia, Athens, GA 30602, USA

Introduction

The *Solenopsis saevissima* species-group, formerly called the *S. saevissima* complex of the *S. geminata* species-group, is a primarily Neotropical assemblage whose members are commonly known as fire ants. Some species have become economically important pests in the United States where they invade lawns, pastures, and roadsides, attacking and stinging native animals, livestock, and humans when colonies are disturbed, and outcompeting native ants for food and nesting habitat (Lofgren *et al.* 1975; Jouvenaz 1990; Guillebeau *et al.* 2002). Annual economic losses attributable to the red imported fire ant, *Solenopsis invicta* Buren, in Georgia alone were recently estimated at over \$60 million (Guillebeau *et al.* 2002).

Although the pugnacious nature of these exotic ants makes them undesirable inhabitants of areas that they have colonized, they nonetheless have become premier objects of study for ecologists and evolutionary biologists. For instance, fire ants offer one of relatively few well-documented cases of the formation of a hybrid zone in historical times (Vander Meer *et al.* 1985; Ross *et al.* 1987a; Shoemaker *et al.* 1996), making them a rich source of information for genetic studies of speciation (Harrison 1990). Fire ants also show important variation in the social organization of their colonies, some of which appears to be under simple genetic control (Krieger & Ross 2002),

which makes them important model systems in the study of social evolution (Pamilo & Crozier 1995; Ross *et al.* 1987b, 1988, 2003; Ross & Trager 1990; Krieger & Ross 2002). In particular, the presence within single species of either a single egg-laying queen (monogyny) or multiple egg-laying queens (polygyny) in a colony has facilitated extensive comparative ecological and evolutionary studies of the causes and consequences of such variation (Vargo & Fletcher 1987; Ross & Keller 1995; Porter 1991; Porter *et al.* 1991). It is remarkable that in spite of the extensive research conducted on fire ants, no thorough phylogenetic analysis of the group has previously been conducted.

Currently there are approximately 185 species of fire ants known (Pitts *et al.* 2005). All are placed in the genus *Solenopsis* Westwood (Formicidae: Myrmicinae). Most of these species are characterized by small, monomorphic workers that form small colonies and display a lestoproct lifestyle, living in the nest walls of another ant species from which they steal food and brood (Thompson 1980, 1989). These habits have earned such *Solenopsis* species the name 'thief ants.' Three species of *Solenopsis* are considered to be inquiline social parasites of other *Solenopsis* species. The parasitic queen lives alongside the host queen, and these inquiline social parasites lack a worker caste, with the brood made up only of sexuals (Hölldobler &

Wilson 1990). Among the remaining *Solenopsis* are 20 New World species that differ greatly from the thief ants and social parasites in their biology. These species have polymorphic workers, form enormous colonies, and are highly aggressive in their foraging and defensive habits. The painful sting wielded by the workers has earned such *Solenopsis* species the name ‘fire ants.’ Four fire ant species are native to North America, while the remaining species are Neotropical; two of the latter species, *S. invicta* and *S. richteri*, were inadvertently introduced into the United States in the early 1900s.

Historically, *Solenopsis* has been a taxonomically difficult group (Creighton 1930, 1950; Wilson 1952; Buren 1972; Trager 1991). In South America, where species diversity is high, distinguishing *Solenopsis* species is exceedingly difficult due to a paucity of reliable diagnostic characters. This situation, which prompted Carlo Emery to call the group the *crux myrmecologorum* (Creighton 1930), led Wilson (1952) to reduce the number of fire ant species to three through several synonymies. Wilson apparently believed that the South American fire ants comprise little more than a large hybrid swarm of unstable variants. Further complicating matters is the fact that several of the species in North America (both native and introduced) actually do hybridize extensively where their ranges overlap (Shoemaker *et al.* 1996; Helms Cahan & Vinson 2003). The fire ants were revised by Trager (1991), although some unresolved taxonomic problems persisted. Pitts *et al.* (2005) address many of these concerns and propose modifications to Trager’s classification (see Table 1).

This phylogenetic study was undertaken with three main goals. The first objective was to examine the anatomy of queens, males, and larvae for phylogenetically informative characters. The adult form of the worker caste, the most readily collected and frequently studied form, is problematic for taxonomic and phylogenetic work because there is considerable morphological similarity across species due to the reduction in body size, loss of organs, and simplification of structures. This occurs even in species with polymorphic workers. Wheeler & Wheeler (1976, 1986, 1991) found that certain features of the larval morphology can be used to differentiate ant genera and published a key using these characters. Many species-level larval descriptions have been published, including descriptions of the larvae of several *Solenopsis* species (Wheeler & Wheeler 1955, 1977). Even so, in only two cases has ant larval morphology been used explicitly for phylogenetic purposes (Wheeler & Wheeler 1970; Schultz & Meier 1995), and larval characters of ants have yet to be used in species diagnoses. Also, Creighton (1930) noted that both adult males and alate (winged) queens appear to offer better characters for species determinations in *Solenopsis* than do the adult workers. Individuals of the sexual castes tend to be larger than workers, and the problem of loss of

Table 1 New classification of the *Solenopsis geminata* species-group.

New classification (Pitts <i>et al.</i> 2005)	Trager (1991)
<i>S. virulens</i> species-group	<i>S. virulens</i> complex
<i>S. virulens</i>	<i>S. virulens</i>
<i>S. tridens</i> species-group	<i>S. tridens</i> complex
<i>S. substituta</i>	<i>S. substituta</i>
<i>S. tridens</i>	<i>S. tridens</i>
<i>S. geminata</i> species-group	<i>S. geminata</i> complex
<i>S. geminata</i>	<i>S. geminata</i> subcomplex
<i>S. amblychila</i>	<i>S. geminata</i>
<i>S. aurea</i>	<i>S. xyloni</i> subcomplex
<i>S. xyloni</i>	<i>S. amblychila</i>
<i>S. bruesi</i>	<i>S. aurea</i>
<i>S. gayi</i>	<i>S. xyloni</i>
<i>S. saevissima</i> species-group	<i>S. gayi</i> subcomplex
<i>S. interrupta</i>	<i>S. bruesi</i>
<i>S. invicta</i>	<i>S. gayi</i>
<i>S. macdonaghi</i>	<i>S. saevissima</i> complex
<i>S. megergates</i>	<i>S. saevissima</i> subcomplex
<i>S. pythia</i>	<i>S. interrupta</i>
<i>S. quinquecupis</i>	<i>S. invicta</i>
<i>S. richteri</i>	<i>S. macdonaghi</i>
<i>S. saevissima</i>	<i>S. megergates</i>
<i>S. weyrauchi</i>	<i>S. pythia</i>
<i>S. electra</i>	<i>S. quinquecupis</i>
<i>S. pusillignis</i>	<i>S. richteri</i>
<i>S. daguerrei</i>	<i>S. saevissima</i>
<i>S. hostilis</i>	<i>S. weyrauchi</i>
	<i>S. electra</i> subcomplex
	<i>S. electra</i>
	<i>S. pusillignis</i>
	Not assigned a subcomplex
	<i>S. daguerrei</i>
	<i>S. hostilis</i>

organs and simplification of associated structures generally is not as great a concern. Despite this potential, characters derived from the sexual castes have been used only by Creighton (1930) to distinguish *Solenopsis* subgenera (which are no longer in use), and by Trager (1991) and others to identify *S. pythia* Santschi. Worker larvae and adults of the sexual castes therefore remain under-utilized sources of information about fire ant relationships.

The second objective was to use data from these diverse morphological character systems to produce the first phylogenetic hypothesis for the *S. saevissima* species-group. A phylogenetic hypothesis for this group could be used to address several issues. It would indicate the closest relatives of *S. invicta*. It would permit evaluation of the monophyly of the supra-specific taxa proposed by Trager (1991). More generally, it would serve as an historical framework for assessing important trends in social evolution and for guiding comparative physiological, behavioural, and ecological studies of fire ants.

The final objective was to revise the classification of the species group in light of the phylogenetic study such that only natural, monophyletic groupings are recognized.

Materials and methods

Loaning institutions or depositories of specimens

The following acronyms are used for institutions that provided specimens for this study or serve as repositories for voucher material:

AEIC	American Entomological Institute, Gainesville, Florida.
AMNH	American Museum of Natural History, New York, USA.
BMNH	The Natural History Museum, London, UK.
CNCI	Canadian National Collections, Ottawa, Canada.
EMUS	Entomological Museum, Department of Biology, Utah State University, Logan, Utah, USA.
FSCA	Florida State Collection of Arthropods, Gainesville, Florida, USA.
ICIB	Museu de Entomologia, Instituto de Biologia, FEIS/UNESP, Ilha Solteira, São Paulo, Brazil.
IMLA	Fundacion e Instituto Miguel Lillo, Tucumán, Argentina.
JPPC	J. P. Pitts Personal Collection, Millville, Utah, USA.
LACM	Los Angeles County Museum of Natural History, Los Angeles, California, USA.
MACN	Museo Argentino de Ciencias Naturales, Buenos Aires, Argentina.
MCZ	Museum of Comparative Zoology, Cambridge, Massachusetts, USA.
MHNG	Muséum d'Histoire Naturelle, Geneva, Switzerland.
MZSP	Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil.
NHMB	Naturhistorisches Museum, Basel, Switzerland.
NMNH	National Museum of Natural History, Washington, D.C., USA.
SDPC	Sanford D. Porter Personal Collection, Gainesville, Florida, USA.
UCDC	University of California, Davis, California, USA.
UGCA	University of Georgia Collection of Arthropods, Athens, Georgia, USA.

Sample collection and specimens studied

Given the historically difficult taxonomy of the *S. saevissima* species-group, it was important to obtain specimens from localities throughout each species' range to infer the extent of geographically based intraspecific variation. Furthermore, it was necessary to have all relevant castes and life stages available to explore novel character systems, and these specimens needed to be associated with adult workers from the same

colony. Because such material is not readily available from museums, extensive sampling was conducted in South America by KGR, JPP, and others in 1988, 1992, 1998, and 2001 (see appendices A & B, Pitts *et al.* 2005). Voucher specimens from the 1998 and 2001 trips were deposited at the following institutions: EMUS, ICIB, JPPC, MCZ, MZSP, NMNH, and UGCA. Material from these collecting trips was identified by James C. Trager or JPP. These efforts resulted in the generation of new material from more than 1200 colonies.

Other large sources of new material include 118 colonies sampled in South America by Sanford D. Porter (USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL, USA) and more than 1500 undetermined *Solenopsis* colonies at the FSCA that were collected throughout South America primarily by William F. Buren.

Material examined

Although the main focus of this study is the *S. saevissima* species-group, specimens of many other *Solenopsis* species were studied in order to understand the normal levels of intra-specific variation within the genus and to look for characters of potential taxonomic and phylogenetic importance. The following list indicates the species and, in brackets, forms that were examined (M = adult males, Q = adult queens, W = adult workers, and L = larval workers): *S. abdita* Thompson [all], *S. amblychila* Wheeler [MQW], *S. aurea* Wheeler (MQW), *S. bruesi* Creighton [W], *S. carolinensis* (Forel) [QW], *S. chlytemnestra* Emery [W], *S. corticalis* Forel [QW], *S. daguerrei* Santschi [MQ], *S. electra* Forel [QW], *S. fugax* (Lat.) [MQW], *S. gayi* (Spinola) [MQW], *S. geminata* (Fabricius) [all], *S. globularia littoralis* (Smith) [all], *S. interrupta* Santschi [all], *S. invicta* Buren [all], *S. macdonaghi* Santschi [all], *S. megergates* Trager [all], *S. molesta* (Say) [all], *S. nigella* Emery [all], *S. nickersoni* Thompson [W], *S. pergandei* Forel [MQW], *S. picta* Emery [all], *S. pusillignis* Trager [all], *S. pythia* Santschi [QW], *S. quinquecuspis* Forel [all], *S. richteri* Forel [all], *S. saevissima* (Smith) [all], *S. substituta* Santschi [all], *S. succinea* Emery [QW], *S. tennesseensis* Smith [MW], *S. tenuis* Mayr [W], *S. texana* Emery [WL], *S. tonsa* Thompson [W], *S. tridens* Forel [all], *S. virulens* [W], *S. westwoodi* Forel [MQW], *S. weyrauchi* Trager [W], and *S. xyloni* McCook [all]. Pitts *et al.* (2005) describe the adult males, adult queens, and larval workers for all members of the *S. saevissima* species-group including the species referred to here as *S. 'undescribed species'*.

Specimen preparation and general methods

Larvae were prepared as outlined in Wheeler (1960). Only fourth instar worker larvae (or prepupae) were used in this study. The fourth instar larvae are discernible by their completely sclerotized mandibles (Petralia & Vinson 1979). Adult workers were air-dried after storage in 70%–85% ethanol in

preparation for stereoscopic light microscopy. This technique was unsatisfactory, however, for other castes because it often caused the head and mesosoma to collapse. To prevent such damage, all males and queens were saturated with amyl acetate and allowed to air-dry more gradually. This method also helped to maintain the natural coloration of specimens.

Adult *Solenopsis* were studied at 50× or 100× using a Wild M-5 or M-10 dissecting microscope. Larvae were studied at 400× or 1000× using a Leica DMRB compound microscope. For scanning electron microscopy, adults and larvae were dehydrated in ethanol, critical-point dried, and sputter-coated with gold. Initial attempts to dry specimens using hexamethyldisilazane (HMDS) following the procedure described by Nation (1983) caused an unacceptable amount of damage and were discontinued.

The morphological terminology used in this study follows that of Wheeler & Wheeler (1976) and Pitts *et al.* (2005).

Coding protocol

Thirty-six characters were analysed, including 19 characters derived from adult workers, eight from adult queens, five from adult males, and four from worker larvae (Table 2). Autapomorphies and characters with continuous, overlapping, or nondiscrete states were excluded. Taxa that were polymorphic for a particular character were scored as having all applicable states (e.g. 0&1, 0&2, etc.). Ten multistate characters were included in the analysis. Taxa that were missing data due to character inapplicability were scored as ‘-’; those that were missing data due to lack of material are scored as ‘?’.

Phylogenetic analysis can be sensitive to the choice and number of included taxa, making sampling strategy a serious concern (e.g. Lecointre *et al.* 1993; Sanderson 1996; Graybeal 1998; Hillis 1998). To address this concern, the ingroup includes 13 of the 14 described species that constitute the *S. saevissima* species-group, including all of the fire ant species and one of the three socially parasitic (inquiline) species. The social parasite *S. hostilis* was excluded from the phylogenetic analysis, as it has not been reported since it was first described (Borgmeier 1959) and specimens were not available for study. The third putative social parasite *Solenopsis solenopsidis* (Kusnezov), is not a member of the *S. saevissima* species-group and was not included in the study.

Adult character data were obtained from at least five individuals (if available) per species to account for interindividual and intercolony sources of variation. For common and wide-ranging species, we examined a minimum of 50 individuals from 50 nests collected from throughout the range. Larval character data were obtained from at least three individuals per species (if available). In common and wide-ranging species, at least 30 larvae from 30 different nests were examined.

Outgroup taxa

There are no formal phylogenetic hypotheses currently available to address either the position of *Solenopsis* within the

Table 2 Data matrix showing characters and character states used in a cladistic analysis of the *Solenopsis saevissima* species-group.

Species	workers																			queens					males					larvae								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36		
<i>Oxyopocus rastratus</i>	2	1	0	0	0	0	1	1	0	0	1	2	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	2	?	0	0	1	?	4	1	2	0
<i>S. molesta</i>	0	0	0	0	0	1	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3	1	2	0
<i>S. substituta</i>	1	2	1	1	0	0	1	1	2	0	1	2	0	1	1	1	0	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	3	2	1	1
<i>S. xyloni</i>	0	2	0	0	0	0	1	1	0&1	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	1	
<i>S. geminata</i>	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0&1	0	0	0	0	1	
<i>S. electra</i>	0	2	1	1	0	0	2	1	2	0	1	1	0	1	1	1	1	1	1	0	1	1	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	
<i>S. pusillignis</i>	0	2	1	1	0	0	1	1	2	0	1	1	0	1	1	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
<i>S. daguerrei</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	-	-	-	
<i>S. saevissima</i>	0	2	1	1	0	0	1	1	2	0	1	0	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	
<i>S. pythia</i>	0	2	1	1	0	0&1	1	1	2	0	1	0	0	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	?	?	?	?	?	?	?	?	?	
<i>S. interrupta</i>	0	2	1	1	0	0&1	1	1	2	0	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	
<i>S. 'undescribed species'</i>	0	2	1	1	1	1	1	1	2	0	1	0	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	?	?	?	?	?	?	?	?	
<i>S. weyrauchi</i>	0	2	1	1	1	1	1	1	2	0	1	0	0	1	1	1	1	1	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
<i>S. richteri</i>	0	2	1	1	0	1	1	1	1	1	1	0	0	1	1	0	1	1	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	2	2	1	1	
<i>S. invicta</i>	0	2	1	1	0	1	1	1	1	1	0	1	0	0	1	1	1	1	1	0&1	0	1	0	1	0	0	0	0	1	1	0&1	1	1	1	1	0	0	1
<i>S. megarctes</i>	0	2	1	1	0	0	0	1	0&2	0	1	0	0	0	0	0	1	1	1	0&1	0	1	0	1	0	1	1	1	0	1	1	1	1	1	1	2	1	1
<i>S. quinquecupis</i>	0	2	1	1	0	0&1	0	0	0&2	1	0	0	1	0	0	0	1	1	1	0&1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. macdonaghi</i>	0	2	1	1	0	0	0	0	0&2	0	0	0	1	1	0	0	1	1	1	0	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	1	1

Solenopsidini or the relationships of the *S. saevissima* species-group to other congeneric species-groups; therefore, outgroup selection was based on other criteria. Fire ants representing other species-groups within *Solenopsis* (*S. geminata*, *S. xyloni*, and *S. substituta*) and one thief ant species (*S. molesta*) were included as congeneric outgroups in the analyses. For a distant outgroup, *Oxyepoecus rastratus* Mayr was chosen because *Oxyepoecus* is thought to be closely related to *Solenopsis* (Bolton 1987; JPP unpublished data), and the worker larvae, adult males, and adult queens have been described (Kempf 1974; Wheeler & Wheeler 1980).

Morphological characters

The 36 characters used in the phylogenetic analysis are based on three different castes and two developmental stages (characters from adult workers [19], adult queens [8], adult males [5], and larval workers [4]; see Appendix 1). Many other character systems were examined but could not be included in the analysis. Although wing venation might provide useful phylogenetic data for determining the relationships of the tribe Solenopsidini, as proposed by Brown & Nutting (1950), the character system appears to be phylogenetically uninformative within the *S. saevissima* species-group. Similarly, the male genitalia within the *S. saevissima* species-group were found to be homogeneous and uninformative. The sting apparatus of queens and workers was a poor source of phylogenetic data because all potential character states that were observed were either continuously variable within species or were broadly overlapping among species.

Results of cladistic analysis

The data matrix (Table 2) was generated using WinClada (BETA) version 0.9.9 (Nixon 1999). Parsimony analyses were performed using NONA version 2.0 (Goloboff 1997) with all characters analysed as unordered. A heuristic search was performed in NONA using the following command sequence: 'hold 1000; hold/100; mult*100'; (hold 1000 trees in memory; hold 100 starting trees in memory; perform tree bisection-reconnection branch swapping on 100 random addition replicates). The parsimony ratchet (Nixon 1999) was implemented using WinClada, with 200 iterations performed per run and one tree held per iteration. The parsimony ratchet was performed three times each for character samplings of 10% (four characters), 15% (five characters), and 20% (six characters), for a total of nine executions. Bremer support values (Bremer 1994) were calculated for each tree using NONA (command sequence: 'hold 1000; sub 1; find *; <enter>, hold 8000; sub 3; find *; <enter>, hold 10 000; sub 5; find *; <enter>, bsupport'). Successive approximations weighting (SAW; Farris 1969) was implemented using the squared consistency index as a weighting function in NONA (command sequence: 'run swt.run mult*10;') to determine which topologies from an

initial set of most-parsimonious trees obtained under equal weight might be preferred based on the relative support they received from the most consistent characters (Carpenter 1988, 1994).

Phylogenetic analyses

The total evidence parsimony analysis using all 36 characters found six most-parsimonious trees (length 80; CI 0.61; RI 0.69). No additional minimum-length trees were found by the parsimony ratchet. A strict consensus tree (Fig. 1A) was calculated from the original six shortest trees. Successive approximations weighting (SAW) yielded a single most-parsimonious tree with the same topology as one of the original six trees (Fig. 1B).

In order to ascertain whether the large proportion of missing data for *S. weyrauchi* and *S. daguerrei* significantly affected the results, an analysis was performed excluding these two species. It is notable that 47% of the data were coded as missing for *S. weyrauchi* because only workers were available for study, whereas 53% of the data were coded as missing for *S. daguerrei* because this inquiline species lacks a worker caste. The parsimony analysis resulted in two most-parsimonious trees (length 77; CI 0.63; RI 0.72), the strict consensus of which is presented as Fig. 1C. Successive approximations weighting of these two trees yielded a single most-parsimonious tree that is concordant with the SAW-preferred tree from the full data analysis.

Discussion

Evolutionary implications of the phylogenetic analyses

Analyses of numerous characters from both traditional and novel morphological character systems has produced the first rigorous phylogenetic hypothesis of the *S. saevissima* species-group, a taxon of ecologically and economically important ants. Although the number of most parsimonious trees recovered was relatively small and the CI value falls within the normal range for phylogenetic studies of this size (see Sanderson & Donoghue 1989), the Bremer Support values are low. Such low support values may be attributable to the taxonomic scope of the study (i.e. congeneric species). In such groups of closely related taxa, few informative morphological characters are expected due to the relatively recent divergence of the taxa. One might also expect the phylogenetic signal from those characters to be confused by hybridization events or the absence of lineage sorting of ancestral polymorphisms.

Despite the large amount of missing data for *S. weyrauchi* and *S. daguerrei*, inclusion of these taxa did not significantly impact the results of the full analysis. The strict consensus trees and SAW-preferred shortest trees were concordant between the analyses with the two taxa included and the analyses with them removed.

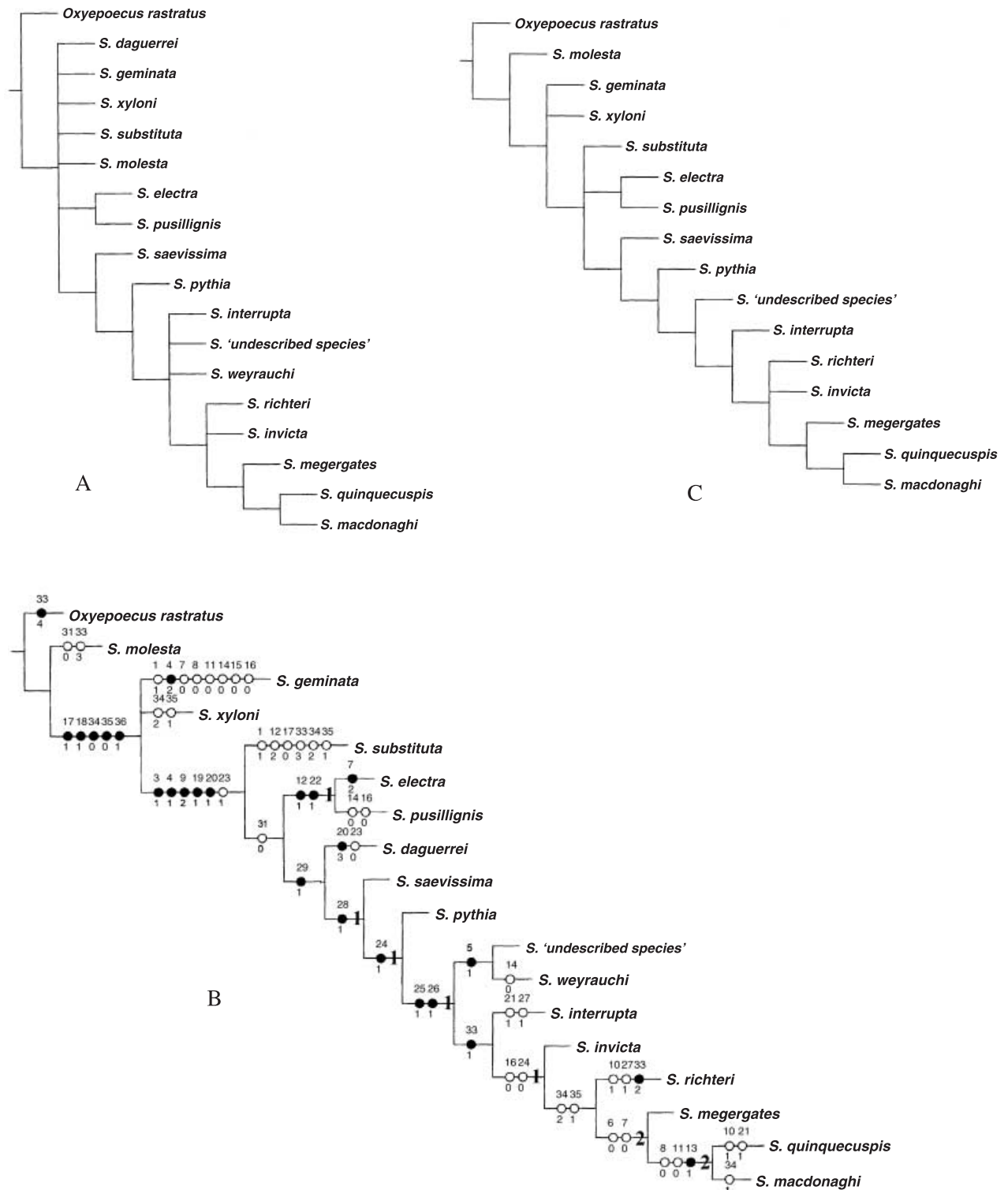


Fig. 1 A–C. —A. Strict consensus of six minimum-length cladograms from parsimony analysis of full data set. —B. One of six shortest cladograms (length 80; CI 61; RI 69) obtained by parsimony analysis of full data set, illustrating SAW-preferred topology (Bremer support values are given near representative nodes; filled circles represent apomorphic characters and open circles represent homoplastic characters). —C. Strict consensus of two minimum-length cladograms from parsimony analysis of data set with *Solenopsis daguerrei* and *S. weyrauchi* removed.

The *S. saevissima* species-group is characterized by having adult workers that are strongly polymorphic and have a long scape and long first flagellomere, and adult workers and queens that have weak sculpturing, a strongly developed median clypeal tooth, and a small or absent ventral petiolar process (Pitts *et al.* 2005). Although the species-group is distinct in having this combination of features, our phylogenetic study is equivocal about its monophyly. The group was recovered in three of the six most parsimonious trees from the full analysis, including the SAW-preferred tree (Fig. 1B).

Our phylogenetic analyses reveal several interesting evolutionary trends and relationships within the *S. saevissima* species-group. First, there seems to be a trend towards increased size of the major workers, a feature that is evident from both the SAW tree (Fig. 1B) and the consensus tree (Fig. 1A). The strongest evidence for this trend comes from the fact that the most recently derived clade consists of the three species with the largest major workers, *S. megergates*, *S. macdonaghi*, and *S. quinquecupis*. While it is tempting to assume that the character states defining this apical clade are simply features correlated with large size, this is not the case. The major workers of species such as *S. electra*, *S. 'undescribed species'*, and *S. interrupta* have some of the same character states that define the large-major-worker clade (e.g. presence of a median ocellular pit), yet they are not placed within this clade. Further evidence for the proposed trend toward larger majors is the fact that the fire ant species with the smallest workers, *S. electra* and *S. pusillignis*, are basal to the remaining species of fire ants with polymorphic workers.

The phylogenetic analyses reveal a second trend that has profound implications for social evolutionary theory. This concerns the issue of whether monogyny (single-queen colonies) or polygyny (multiple-queen colonies) represents the ancestral form of colony social organization in various clades of social Hymenoptera. Monogyny generally has been assumed to be the primitive condition in most groups (e.g. Hölldobler & Wilson 1977; Fletcher & Ross 1985; Hölldobler & Wilson 1990; Bourke & Franks 1991; cf. Nonacs 1988; Ward 1989a; Ross & Carpenter 1991), and detailed genetic analyses have supported this view of the polarity of social organization in the *S. saevissima* species-group, as well as in the fire ant *S. geminata* (Krieger & Ross 2002; Ross *et al.* 2003). Our morphology-based, species-level phylogeny of the *S. saevissima* species-group lends strong additional support to the view that polygyny is a secondary form of social organization in fire ants. Both the SAW tree (Fig. 1B) and the consensus tree (Fig. 1A) reveal that all of the species known or believed to exhibit polygyny (*S. invicta*, *S. macdonaghi*, *S. megergates*, *S. quinquecupis*, and *S. richteri*; Krieger & Ross 2002, 2005) form the most derived clade in the species-group. As polygyny is not known in the more basal species, except for an unsubstantiated report in *S. pythia* (see Pitts

et al. 2005), this form of social behaviour may represent a synapomorphy for the derived clade referred to above.

A third important conclusion of the phylogenetic analyses is the biogeographical implication that most fire ant sister taxa have distributions that are either parapatric or allopatric (see Pitts *et al.* 2005 for range maps). The most obvious case involves the sister species *S. weyrauchi* and *S. 'undescribed species'*, whose ranges are separated by the vast distance between the Andes and the mountains of south-eastern Brazil. Also, the ranges of the sister species *S. electra* and *S. pusillignis* do not overlap, although *S. pusillignis* has been collected so infrequently that the true extent of its range remains unknown. The range of *S. saevissima* apparently overlaps that of *S. invicta* only marginally at its extreme southern limits, as does the range of *S. invicta* with respect to that of *S. richteri*. Finally, the ranges of the sister species *S. quinquecupis* and *S. macdonaghi* apparently overlap only minimally in northern Uruguay, while the range of *S. megergates* overlaps little with that of *S. quinquecupis* in the same area and not at all with that of *S. macdonaghi*. This biogeographical pattern supports the idea that speciation within the *S. saevissima* species-group occurs principally through processes involving the geographical isolation of incipient sister species (Barraclough & Vogler 2000), an idea proposed more generally for ants by Ward (1989a). The only known instances of broad overlapping of native fire ant ranges involve *S. richteri*, the range of which completely encompasses those of *S. quinquecupis* and *S. megergates* as well as much of the range of *S. macdonaghi*. These unusual patterns presumably can be explained by allopatric speciation followed by subsequent range expansion of one or both incipient sister species.

A final important conclusion from our analyses concerns the relationship of the inquiline social parasite *S. daguerrei* to its hosts and the relevance that this holds as a test of Emery's Rule (Emery 1909), a general hypothesis of the relationships between social parasites and their hosts in the Hymenoptera (Ward 1989b, 1996; Carpenter *et al.* 1993; Agosti 1994; Choudary *et al.* 1994; Taber 1998; Fanelli *et al.* 2001). *Solenopsis daguerrei* does not have a sister-group relationship with any of its known hosts, *S. invicta*, *S. richteri*, and *S. macdonaghi* (e.g. Fig. 1). Therefore, Emery's Rule is unjustified in its strictest sense in the case of fire ants, and we can discount the possibility that *S. daguerrei* arose via sympatric speciation directly from its current host(s) or an immediate ancestor of these hosts. A modified version of the Rule is applicable, however, because *S. daguerrei* is sister species to a larger clade containing its current hosts as well as nonhosts. Thus, we cannot rule out a scenario in which the social parasite speciated sympatrically from a free-living ancestor that subsequently evolved into several species, including the current hosts, with *S. daguerrei* losing the ability to parasitize many of these descendent species.

Insights into the evolutionary route by which *S. daguerrei* acquired its habits as an inquiline can be further gleaned by considering the biology of non-fire ant species of *Solenopsis* and of species in its possible sister genera, *Carebarella* and *Oxyepoecus* (Bolton 1987). Most of the smaller *Solenopsis* species are lestoproctid ants that hide in the nest of a host ant and surreptitiously steal food and brood, and at least one *Carebarella* species (*C. bicolor* Emery) seems to display similar lestoproctid habits (Eidmann 1936; as *C. punctatorugosa*). Moreover, some *Oxyepoecus* species apparently behave as inquilines of *Pheidole* and *Solenopsis*, spending their entire life-cycle in the host nest where they are dependent on the hosts for food and brood care (Ettershank 1966; Kempf 1974; Hölldobler & Wilson 1990). Such behaviours suggest that *Solenopsis* species have inherited a predisposition for symbioses with other ants. Although presumed intermediate stages between lestoproctidism and inquilinism (e.g. parabiosis, xenobiosis) have not been reported in *Solenopsis*, it is reasonable to speculate that *S. daguerrei* evolved its most extreme form of ant social parasitism from a previous lestoproctid relationship with its hosts (Wilson 1971; Hölldobler & Wilson 1990; cf. Buschinger 1986). Definitive evidence addressing this hypothesis will be gained only when the closest relatives of the *S. saevissima* species-group are determined and the natural history of these species becomes better understood.

Addition of new data sources

Taxonomic revisions and phylogenetic analyses of ants historically have relied almost exclusively on characters pertaining to adult workers. In the *S. saevissima* species-group, morphological data derived from adult workers when augmented with data from less accessible castes and life stages produced a more robust and well-resolved phylogenetic hypothesis than was previously possible. Thus, as has been shown in studies of other taxa (e.g. Ward 1990, 1993; Eernisse & Kluge 1993; Wheeler *et al.* 1993; Taber 1998; Heterick 2001; Schulmeister 2003), utilization of multiple character systems can play an integral part in resolving difficult phylogenetic relationships.

Previous studies addressing fire ant phylogeny

Ross & Trager (1990) produced an early study of the relationships of several *S. saevissima*-group species based on allozyme data. They proposed that *S. richteri* and *S. quinquecupis* are sister species, in contrast to the results of the current study. They also reported an undescribed cryptic species from central Argentina (designated 'species x'). Although diagnosable genetically, this species is morphologically indistinguishable from *S. quinquecupis* (only adult workers of this putative cryptic species were available for the present study). Given its morphological identity, it was surprising that this cryptic species was not inferred to be the sister taxon of

S. quinquecupis in the allozyme study, but instead tended to group with *S. macdonaghi* or *S. invicta* (Ross & Trager 1990).

Some shortcomings of the allozyme analyses may explain the discrepancies between this earlier study and the morphological study presented here. For instance, several *S. saevissima*-group species were not included in the allozyme study and, among those that were included, only limited parts of the known ranges were sampled. Relatively few loci were studied. In addition, allozyme data present special challenges for phylogeny reconstruction (e.g. Buth 1984). Nevertheless, ongoing analyses of allozymes, as well as mitochondrial DNA sequences, from numerous diverse samples continue to suggest the possibility of additional cryptic species within nominal *S. invicta*, *S. richteri*, and *S. saevissima* (Shoemaker *et al.* 2003; KGR and D. D. Shoemaker, unpublished data). The issue of cryptic species and their relationships to the nominal species of the *S. saevissima* species-group, however, must await evaluation of additional DNA sequences from several nuclear genes.

Krieger & Ross (2005) produced a phylogeny of the alleles of the nuclear gene *Gp-9* based on nucleotide sequence data from virtually all described *S. saevissima* group species. This gene is unusual in that it seems to be directly involved in regulating social organization and, thus, has been subject to occasional directional selection over its evolutionary history. Complete *Gp-9* allele phylogenies support some of the relationships proposed here, including a sister-group relationship between *S. electra* and *S. pusillignis* and the presence of an apical clade consisting of *S. invicta*, *S. richteri*, *S. megergates*, *S. macdonaghi*, and *S. quinquecupis*. Interestingly, monophyly of the *S. saevissima*-group species was not supported by the sequence analysis, reflecting the equivocal support for the group from our morphological analyses. There are also many points of disagreement concerning species relationships between the *Gp-9* and morphological data, presumably stemming in some measure from the modest divergence among, and thus limited phylogenetic information content of, *Gp-9* sequences from most species in the group. In an effort to resolve this disagreement, a simultaneous analysis of morphological, *Gp-9*, and mtDNA sequence data is intended in the future, but is not presented here.

Our phylogenetic hypotheses provide consistent support for a sister-group relationship between *S. electra* and *S. pusillignis*, as Trager (1991) formalized by terming this clade the *S. electra* subcomplex (see Table 1). We feel that it is unwarranted at this juncture to divide the *S. saevissima* species-group into smaller divisions and thus we do not offer formal names for this clade or for any other presumed monophyletic subgroup.

A final notable feature of our phylogenetic hypothesis is that *S. saevissima* and *S. pythia* do not appear to be sister species, despite the similar appearance of the workers. In many cases it is impossible to distinguish between the two species

using worker characters alone. In light of this resemblance, coupled with the sporadic distribution of *S. pythia*, Trager (1991) suggested that this species might be an inquiline social parasite of *S. saevissima*. If this is the case, *S. pythia* may not belong to the species-group and our placement of it in the phylogeny would be in error due to our combining data from two different species, workers of *S. saevissima* and queens of *S. pythia*. Further lending strength to the conclusion that *S. pythia* may not belong to the species-group is the fact that the queens lack a bidentate process on the metasternum, a structure that is found in the rest of the species in this group but is lacking in many of the thief ants.

However, the idea that *S. pythia* is a social parasite of *S. saevissima* can be rejected for two reasons. First, *S. pythia* queens lack the typical reductions in sculpture, wing venation, and other features typically seen in inquiline queens, such as *S. daguerrei* (Hölldobler & Wilson 1990). Second, *S. pythia* queens and associated workers have been collected from sites far outside the known range of *S. saevissima* (see Pitts et al. 2005). The only reasonable conclusions that can be drawn are that *S. pythia* is not a parasite of *S. saevissima* and that the workers are those of *S. pythia*. The absence of the bidentate process on the metasternum of *S. pythia* thus would seem to represent an independent loss of this feature. Given that *S. saevissima* appears from our analyses to be the sister taxon of a larger clade in which *S. pythia* is the most basal species, the close resemblance of the workers of these two species may simply reflect their joint retention of plesiomorphic traits of the entire clade.

The future

Our phylogenetic hypotheses set the stage to begin addressing additional fundamental questions about the evolutionary history of fire ants and their close relatives. For example, resolution of the monophyletic status of the *S. saevissima* species-group and its phylogenetic placement within the genus *Solenopsis*, especially with respect to the fire ants of North America, is pertinent to the issue of how many times the fire ant life-history syndrome has arisen. Another issue of importance is whether the remaining species-groups within *Solenopsis* are monophyletic. In particular, it has been suggested that the *S. geminata* species-group, which comprises the North American fire ant species, is characterized only by symplesiomorphies (Pitts et al. 2005) and thus may be paraphyletic (but see Krieger & Ross 2005). The nature and placement of this group assumes special significance with regard to the issue of the number of origins of the fire ant syndrome. A final important task for the future is to learn about the relationships within the Solenopsidini. Because the Solenopsidini includes several other genera such as *Carebarella*, *Monomorium*, *Megalomyrmex*, and *Oxyepoecus*, which vary both in queen number and in the propensity for symbioses with other ants, this group may provide essential information for testing theories of social evolution.

Resolution of the systematic issues outlined here will benefit from the addition of new information derived from multiple sources, including molecular data and anatomical data derived from various life stages and castes. Robust analyses considering large and diverse data sets will be able to address basic evolutionary questions about the remarkable natural history of these ants.

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Appendix 1

Morphological characters for in the analyses of the *S. saevissima* species-group.

Adult major worker characters

1. *Lateral margin of propodeum*: rounded [0], ridge-like [1].
2. *Size of compound eye*: small, less than 15 facets [0], medium, between 40 and 60 [1], large, 70 or greater [2] (Fig. 2A).
3. *Median clypeal tooth*: absent [0], present [1] (Fig. 2A).
4. *Ventral petiolar process*: present [0], absent [1] (Fig. 2B).
5. *Mesonotal pilosity*: with ≤ 26 setae [0] (Fig. 2B), ≥ 30 setae [1].
6. *Median frontal streak*: absent [0], present [1] (e.g. Fig. 3C). (*Solenopsis pythia*, *S. interrupta*, and *S. quinquecupis* are polymorphic for this character, with various levels of development found even within single colonies.)
7. *Median ocellus*: present (with or without obvious lens) in largest major workers [0], absent [1] (Fig. 2A,C).
8. *Piligerous foveolae*: on head ovate to elongate [0], round [1].
9. *Mandibular costulae*: absent [0], absent mesally [1] (Fig. 2A,C), present throughout [2] (Fig. 2D).
10. *Pronotum*: rounded anterolaterally, bosses absent [0], quadrate anterolaterally, distinct bosses present [1].
11. *Promesonotal suture*: angulate medially, sometimes projecting upward [0], gently curved medially, never projecting upward [1]. (This character is present in the largest workers of *S. macdonaghi* and *S. quinquecupis* but is not present in workers of every colony. Similarly sized specimens of *S. megergates* lack this character.)
12. *Propodeum sculpture*: glabrous posteroventrad to spiracle [0] (Fig. 2B), granulate posteroventrad to spiracle [1].
13. *Propodeum*: in largest major workers curves upward from metanotal groove to flattened posterior portion [0], produced upward from metanotal groove above and curves downward to flattened posterior portion (in lateral view, propodeum appears to have an anterior lobe projecting dorsally) [1]. (This character is present in the largest workers of *S. macdonaghi* and *S. quinquecupis* but is not present in workers of every colony. Similarly sized specimens of *S. megergates* lack this character.)
14. *Postpetiole striae of posterior face*: present on lower 0.75 or more [0], present on lower 0.50 or less [1] (Fig. 2E).
15. *Postpetiole shape*: much broader than high [0], as high or higher than broad [1] (Fig. 2E).
16. *Postpetiole sculpture of posterior face*: with upper 0.50 weakly to distinctly granulate [0], glabrous [1].
17. *Colony worker size distribution*: monomorphic [0], polymorphic [1].
18. *Flagellomere*: as broad or broader than long [0], longer than broad [1].
19. *Scape length*: short, not reaching occiput [0], long, bypassing occiput [1] (Fig. 2F).

Adult queen characters

20. *Clypeus*: bidentate [0], tridentate [1] (Fig. 3A), rounded, lobate [2].
21. *Median frontal streak*: absent [0], present [1] (Fig. 3C). (This character is polymorphic for *S. invicta* and *S. megergates*. This character was coded as absent for *S. richteri* because the surrounding integument is as darkly coloured as a normal frontal streak, thus precluding the presence of a streak.)
22. *Ocello-ocular index (OOI)*: > 0.80 , usually much greater [0] (Fig. 3A), < 0.75 [1] (Fig. 3B). (OOI = OOD/OD where ocello-ocular distance (OOD) is the distance from the middle of the median ocellus to a line drawn across the posterior margins of the compound eyes, and ocellar distance (OD) is the distance from the middle of the median ocellus to the midpoint of a line drawn between the lateral ocelli measured with the head in full-face, dorsal view.)
23. *Eye placement*: low on head, upper 0.33 of eye at midpoint of head, as seen in frontal (view [0], midpoint of eye at midpoint of head, as seen in frontal view [1] (Fig. 3A,B).
24. *Head sculpture*: with small piligerous foveolae with interfoveolar space glabrous and < 0.005 mm [0], large piligerous foveolae with interfoveolar space striate and > 0.01 mm [1].
25. *Integument around parapsidal lines*: with maculations absent or not distinctly margined [0] (Fig. 3D), with maculations present, distinct, edges clearly indicated by abrupt colour change [1] (Fig. 3E).
26. *Mesonotal anteromedian maculation*: absent or not distinctly margined [0] (Fig. 3E), with maculations present, distinct, edges clearly indicated by abrupt colour change [1] (Fig. 3D).
27. *Median maculation of T1*: always absent or with indistinct posterior margin (colour gradually changing) [0], tergal maculation with distinct, clearly indicated posterior margin [1]. (*Solenopsis richteri* and *S. interrupta* only occasionally exhibit a tergal maculation with a distinct posterior margin.)

Adult male characters

28. *Head*: with area posterior to ocellar triangle glabrous [0], weakly to strongly granulate [1].
29. *Head striae anterior to occipital carina*: present [0], absent [1].
30. *Mandibular colour*: yellow [0], brown [1]. (*Solenopsis invicta* and *S. macdonaghi* were coded as polymorphic for this character. Some specimens have mandibles of either colour, while others have mandibles that are yellow basally and brown apically.)
31. *Color of male*: predominantly yellow brown [0], dark brown to black [1].
32. *Pubescence on mesonotum* sparse: [0] (Fig. 3F), dense [1] (Fig. 3G).

Fourth instar larval worker characters

33. *Occipital setal row*: bifid [0] (Fig. 4A), simple mesally, bifid laterally [1] (Fig. 4B), simple [2] (Fig. 4C), bifid mesally, simple to denticulate laterally [3], brachyplumose [4].
34. *First setal row on vertex*: bifid [0] (Fig. 4A), brachyplumose [1], simple [2] (Fig. 4C).
35. *Second setal row on vertex*: bifid laterally [0] (Fig. 4A), simple [1], brachyplumose [2].
36. *Setae below antennae*: denticulate [0], simple [1] (Fig. 4A,C).

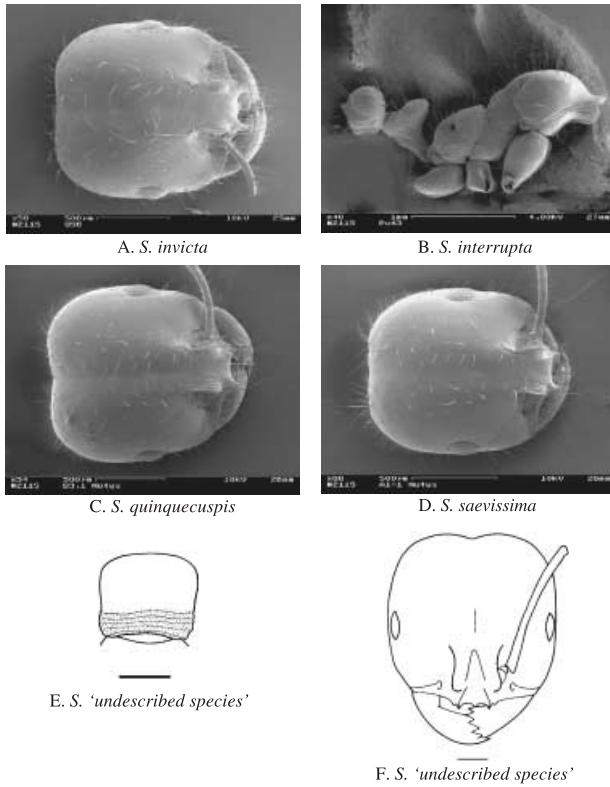


Fig. 2 A–F. —A. Head of major worker of *Solenopsis invicta*, frontal view. —B. Mesosoma of major worker of *S. interrupta*, lateral view. —C. Head of major worker of *S. quinquecupis*, frontal view. —D. Head of major worker of *S. saevissima*, frontal view. —E. Postpetiole of major worker of *S. 'undescribed species'*, posterior view, scale = 0.22 mm —F. Head of major worker of *S. 'undescribed species'*, frontal view, scale bars = 0.22 mm.

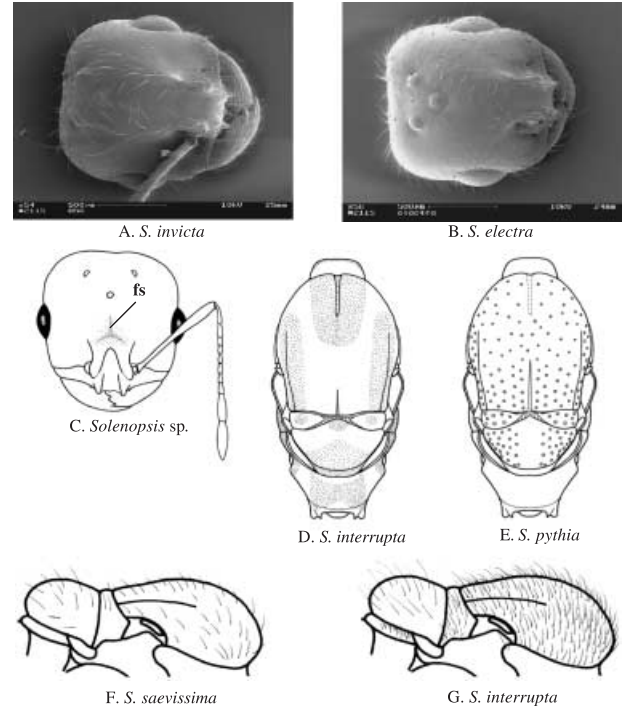


Fig. 3 A–G. —A. Head of queen of *Solenopsis invicta*, frontal view. —B. Head of queen of *S. electra*, frontal view. —C. Head of queen of *S. solenopsis* sp. with frontal view. —D. Mesosoma of queen of *S. interrupta*, dorsal view. —E. Mesosoma of queen of *S. pythia*, dorsal view. —F. Mesosomal dorsum of male of *S. saevissima*, dark form, lateral view. —G. Mesosomal dorsum of male of *S. interrupta*, lateral view. *Abbreviations:* fs, median frontal streak.

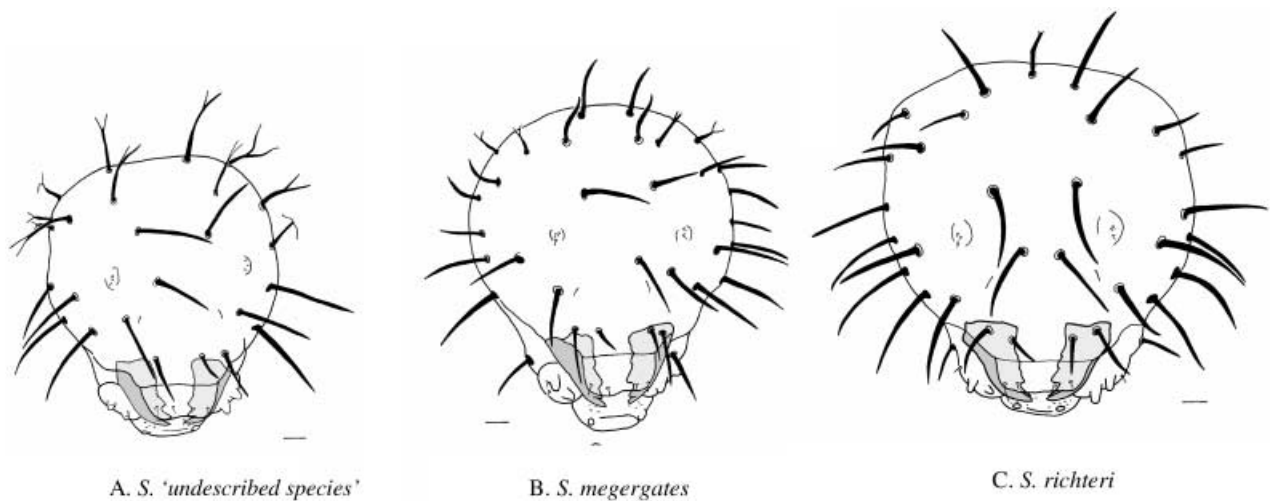


Fig. 4 A–C. —A. Larval head capsule of *Solenopsis 'undescribed species'*, frontal view. —B. Larval head capsule of *S. megergates*, frontal view. —C. Larval head capsule of *S. richteri*, frontal view; scale bar for all = 46 µm.