Survey of *Solenopsis* Fire Ants and Their Parasitoid Flies (Diptera: Phoridae: *Pseudacteon*) in Central Chile and Central Western Argentina

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ABSTRACT Twenty-two species of *Pseudacteon* flies (Diptera: Phoridae) are known to attack fire ants (*Solenopsis* spp.) in Argentina, Brazil, Bolivia, and Paraguay, but none are known in Chile. Surveys were conducted in central Chile and at similar latitudes in western Argentina to detect the presence of fire ants and parasitoid flies and to determine their relationship. Flies and fire ants were much more common and abundant in Argentina. In total, 100 colonies of four fire ant species were found at 63.6% of the sites surveyed. In contrast, only six colonies of one species, *Solenopsis gayi* (Spinola), were found at 4.7% of the sites surveyed in Chile. Our survey includes the first record of five parasitic fly species in central western Argentina and a new host, *Solenopsis quinquecuspis* Forel. The large form of *Pseudacteon obtusus* Borgmeier was found attacking *S. gayi* in Chile, which is the first record in that country, and the first record on this host species. The southern-most and western-most records were established for fire ant-decapitating flies. Mitochondrial DNA (mtDNA) indicates that *S. gayi* is genetically closer to the Argentine fire ants (*saevissima*-group) than the North American *S. geminata*-group. However, *S. gayi* venom alkaloid composition is similar to *S. geminata*-group, whereas cuticular hydrocarbon composition has characteristics of both groups. Analysis of mtDNA from the collected flies supports the monophyly of *P. obtusus* and suggests that the Chilean population is similar to populations in eastern Argentina. The presence of *P. obtusus* in Chile could be explained by immigrant parasitized fire ant species from Argentina or by an accidental introduction.

KEY WORDS *Solenopsis gayi*, *Pseudacteon obtusus*, geographical distribution, fire ants, biological control

Invasive species are widely recognized as significant agents of global change (Vitousek et al. 1996). In many cases, it is clear that invasive species benefit from the absence of coevolved parasites, predators, and competitors in their introduced range, which can lead to high population densities and concomitant ecological dominance (Keane and Crawley 2002). In some cases, the biology of invaders also is altered during introduction, leading to traits that allow introduced populations to become established and spread (Tsutsui et al. 2000, Tsutsui and Suarez 2003). Social insects, in particular, seem to often become invasive through the action of one or both of these mechanisms (Holway et al. 2002). Despite the important role a lack of natural enemies can play in the success of invasive species, there are few cases in which both invaders and their natural enemies have been examined in a broad ecological and geographic context. In particular, few studies have simultaneously examined the relationship among invasive species and their closest relatives and among parasites and their closest relatives. Such analyses are likely to be important for both understanding the mechanisms by which some species become damaging invaders, and for designing coherent strategies for controlling invasive species in their introduced ranges.

The genus *Solenopsis* (Hymenoptera: Formicidae) is a large, worldwide ant genus with 108 species in the New World (Bolton 1995), of which 21 are known as fire ants. Some *Solenopsis* are among the most damaging invasive species, including the red imported fire ant, *Solenopsis invicta* Buren; the black imported fire ant, *Solenopsis richteri* Forel; and the tropical fire ant *Solenopsis geminata* (F.). Attempts to control two of these species, *S. invicta* and *S. richteri,* in their introduced range have included the release of *Pseudacteon* phorid fly parasitoids as potential biological control agents (Porter and Gilbert 2004).

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All *Pseudacteon* phorid flies seem to be parasitoids of individual worker ants (Disney 1994, Feener and Brown 1997). The female fly inserts an egg into the thorax of a live worker ant. After hatching, the larva migrates into the head of the ant where it consumes all the tissue inside the head and ultimately decapitates the host. An adult fly emerges from the ant's mouth some weeks after the eggs were laid (Porter et al. 1995, Porter 1998).

Pseudacteon species have been collected in South America, North America, Europe, Asia, Australia, and Indonesia (Disney 1994, Brown and Feener 1998, Disney and Michailovskaya 2000). Twenty-two species of *Pseudacteon* flies have been found attacking fire ants in the *S. saevissima* species-group in South America (Porter and Pesquero 2001, Calcaterra et al. 2005, Folgarait et al. 2005). Three of them have been released as biocontrol agents against the imported fire ants *S. invicta* and *S. richteri* in the United States (Porter and Gilbert 2004). Previous surveys for fire ants and phorid flies have concentrated on east central to northeastern Argentina and southeastern Brazil, the major areas of occurrence of *S. invicta* and *S. richteri* (Ross and Trager 1990, Trager 1991, Pitts 2002, Ross and Shoemaker 2005, Shoemaker et al. 2006). Information on fire ants occurring in western Argentina and central Chile is scarce (Snelling and Hunt 1975, Calcaterra et al. 2005), because intra- and interspecific variation in many important morphological characters impedes their identification (Pitts 2002). Thus, the systematic of fire ants remains confused and the best keys available are difficult to use in the laboratory and essentially impossible to use in the field.

South American fire ant parasitoid flies have been reported from Argentina, Brazil, Bolivia, and Paraguay (Williams et al. 1973; Williams 1980; Fowler et al. 1995; Pesquero et al. 1996; Orr et al. 1997; Calcaterra et al. 2005; Folgarait et al. 2003, 2005). However, to our knowledge, they have never been reported from Chile. South American fire ants occur in almost all habitats from the Amazon Basin of Brazil to ≈42° S latitude in the Río Negro province, Argentina (Hays 1958, Kusnezov 1978, Trager 1991). However, only one fire ant species, *Solenopsis gayi* (Spinola), has been reported from Chile (Snelling and Hunt 1975, Trager 1991). The scarcity of *Solenopsis* in Chile is likely due to the dispersal barrier of the Andes Mountains, which prevents the westward expansion of Argentinean fire ants (Goetsch 1931, Hays 1958).

Although *S. gayi* occurs only in South America (Trager 1991), it was tentatively placed within the North American *S. geminata* species-group (Trager 1991, Pitts et al. 2005) based on a single morphological character. *S. gayi* is the smallest polymorphic South American fire ant species. In southern Perú, *S. gayi* is sympatric with *Solenopsis bruesi* Creighton, but it is apparently limited to low elevations (up to 500 m above sea level). *S. gayi* also has been reported as introduced in Colombia (Trager 1991). Although distribution information is scarce, *S. gayi* seems to be limited to central Chile, in the Chilena phytogeographical province (Cabrera and Willink 1980) or the Espinal region (30–37° S), one of the Chilean regions with the highest number of ant species (Snelling and Hunt 1975, Trager 1991, Medel and Fuentes 1995).

One objective of this work was to determine whether fire ants in central Chile are also attacked by *Pseudacteon* flies. Additionally, surveys were conducted in similar latitudes in central western Argentina to detect the presence of fire ants and their fly parasitoids and to determine the degree of relationship between parasitic flies and their fire ant hosts on both sides of the Andes.

Materials and Methods

Surveys. The exploration was conducted at sites where *S. gayi* had been reported previously (Snelling and Hunt 1975), mainly in central Chile (Coquimbo, Valparaíso, Metropolitana, Libertador General O'Higgins, Maule, Bio Bio, and Araucaria regions). Sites in central western Argentina were selected based on latitudes similar to those for the sites in Chile (La Pampa, Mendoza, San Juan, San Luis, Río Negro, Neuquén, and Chubut provinces). Collections and observations were made in October 2004 (Argentina), January 2005 (Chile and Argentina), and January 2006 (Argentina).

Roadsides, pastures, camping areas, riversides, and parks were surveyed for ant mounds and/or foragers during daylight hours. Stones were removed to search for fire ant nests when there were no visible mounds. Any nests that were discovered were opened to disturb the colony and thus attract phorid flies. All flies were collected using hand aspirators, tentatively identified in the field with 10× hand lens, and preserved in 96% ethanol for further lab identification (Porter and Pesquero 2001) and to conduct genetic studies. Workers from all fire ant colonies were collected and preserved in 96% ethanol for morphological determination and genetic studies. Worker samples also were taken from the same fire ant nests for analysis of venom alkaloids and cuticular hydrocarbons, which are also good biochemical taxonomic characters (Ross et al. 1987, Vander Meer and Lofgren 1988).

Weather variables were measured on the top of the nests by using a Kestrel 3000 portable weather station, and altitude, latitude, and longitude were recorded using a Garmin III GPS unit. Voucher specimens of both the flies and fire ants are deposited in the collection of the South American Biological Control Laboratory.

Biochemical Analysis. Workers were placed in a glass vial containing enough hexane to cover the ants. After ≈24 h, the hexane was transferred to another glass vial via a pipette and allowed to passively evaporate. The vial was capped (cap liner was either metal foil or Teflon) and sent to Gainesville, FL (R.K.V.M.) for chemical analysis. A similar procedure was used to extract alkaloids and hydrocarbons from *S. geminata*, *S. invicta*, and *S. richteri*. Workers for these samples were collected from colonies found in the United States. The samples were reconstituted in hexane, and venom alkaloids and cuticular hydrocarbons were separated by gas chromatography (GC). GC analyses were carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector and a 30-m by 0.25-mm i.d. DB-1 fused silica capillary column (Agilent J&W Scientific, Folsom, CA). Hydrogen was used as the carrier gas, and detector sensitivity was increased by using nitrogen as the makeup gas. Venom alkaloids and cuticular hydrocarbons were analyzed in the same GC injection. The oven temperature started at 150°C, and then it was programmed to 285°C at 4°C per min. The temperature was held at 285°C until the components of interest eluted from the column. The injector and detector temperatures were 300°C. Venom alkaloid peak assignments were based on GC, GC-mass spectrometry (MS), and comparison with standard alkaloids from *S. invicta.* Mass spectral analyses were carried out using an Agilent mass selective detector. Compound separation was carried out on the same model GC and the same oven temperature program, but with helium as the carrier gas and no makeup gas. The GC-MS transfer line was set at 285°C. Hydrocarbons were separated from the extract using a Pasteur pipette, loaded onto a silica gel column, and then eluted with hexane to isolate the hydrocarbons. Hydrocarbon component identification was made based on GC retention time, mass spectral fragmentation patterns, and previously published data. All quantitation was carried out using the flame ionization detector and the Agilent data analysis software.

Molecular Analysis. Genomic DNA was extracted from individual phorid flies and their fire ant hosts by using the salting-out protocol of Sunnucks and Hales (1996). The mitochondrial cytochrome oxidase I (COI) gene from phorid flies was polymerase chain reaction (PCR) amplified using the primers C1-J-2183 (Jerry) and TL2-N-3014 (Pat), and from *Solenopsis* ants by using the primers C1-J-2183 and TL2-N-3014 (Pat, honey bee version; Simon et al. 1994). A portion of the mitochondrial cytochrome B (CytB) gene from *Pseudacteon obtusus* Borgmeier also was amplified using the either CB-J-10612 (CB1L) or CB-J-10933 (CB1) as the forward primers and CB-N-11367 (CB2) as the reverse primer (Simon et al. 1994). The thermal profile for all PCR reactions was one cycle at 94°C for 2 min; 35 cycles of 92°C for 30 s, 56°C or 58°C for 1 min, 72°C for 45 s; and a single final cycle at 72°C for 3 min. The total volume of each PCR reaction was 10 μl, and 1 μl of genomic DNA was used as the template in each reaction. The presence of a PCR product in each reaction was verified by running 5 μl on a 1.5% agarose gel and staining with ethidium bromide. PCR reactions were purified by digestion with exonuclease I and shrimp alkaline phosphatase. Sequencing reactions were then set up for both the forward and reverse directions by using the same primers used for PCR and adding either 1 or 2 μl of the PCR reaction as the template, depending on the apparent concentration of the PCR product. DNA sequencing was performed using the Applied BioSystems Big Dye Terminator 3.1 cycle sequencing kit, and the sequencing products ran on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) equipped with a 56-cm capillary array and POP4 polymer.

Fig. 1. Main sample locations in central Chile and central western Argentina (the numbers refer to localities in Table 1). Argentinean provinces: SJ, San Juan; MZ, Mendoza; SL, San Luis; LP, La Pampa; NQ, Neuquén; RN, Río Negro; and CH, Chubut.

For each template, Clustal X (Thompson et al. 1997) was used to align the contigs and to generate a consensus sequence. For the *Solenopsis* data set, the total sequence length (COI only) was 758 bases. The *P. obtusus* data set was made up of 1,400 bases of DNA sequence from nine individuals. The relationships among taxa were examined by constructing a maximum parsimony tree by using the program PAUP (Swofford 1993), and the support for the tree was estimated by performing 1,000 bootstrap replicates. For the analysis of *Solenopsis,* all *Solenopsis* COI sequences from GenBank were downloaded as well as sequences from *Myrmica rubra* (L.) (Hymenoptera: Formicidae) and *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae) as outgroups. The large number of sequences from the *Solenopsis saevissima*-group proved unwieldy, so we deleted sequences from each of the major *saevissima*-group clades, as defined in Shoemaker et al. (2006). The phylogenetic relationships among the *Solenopsis* species were reconstructed using maximum parsimony (and bootstrapping), as described above. The most likely substitution rate parameters were identified using the program ModelTest (Posada and Crandall 1998), and this model was used to reconstruct a maximum likelihood tree in PAUP (Swofford 1993). Support was estimated by using the program MrBayes (Huelsenbeck and Ronquist 2001) to calculate posterior probabilities. This analysis was conducted by running one cold and three hot Markov chains, sampling every 100 generations, and discarding the first 10,000 generations, after the likelihood values had stabilized.

Here, we confine out analyses to consideration of species-group membership. Because the *saevissima-*

Table 1. Fire ant decapitating flies in central Chile and central western Argentina

Country, region or province, locality[a]	No. collecting sites (w/flies)	No. mounds examined (w/flies)	No. *Pseudacteon* females[b] (males)				
			obtl	*noc*	*cur*	*tri*	*lit*
Chile							
Bio Bio							
Bulnes (1, LC28)	1	2 (2)	5 (4)				
Valparaiso							
Los Molles (2)	1	1					
Coquimbo							
Quilimari River (3)	1	2					
Rivadavia (4)	1	1					
Argentina							
San Juan							
El Encón	1	3					
Mendoza							
San Rafael (5)	2	5 (3)				5 (2)	
Uspallata (6)	1	6 (6)	3 (2)				
Others	3	7					
San Luis							
Villa Mercedes	1	1					
La Pampa							
La Reforma (7)	2	4 (1)		1			
Puerta Grande (8)	1	2 (1)		2			
Santa Isabel (9, LC18)	1	3 (1)	1				
Others	7	25					
Rio Negro							
Rio Colorado (10)	2	4 (2)	4	10			2
Choele-Choel (11)	1	5 (1)				(2)	
Chichinales (12)	1	3 (2)	1 (6)	1	2	1	
Maique (13, LC26)	1	3 (3)	1 (6)	2	8		
Allen (14)	1	3 (2)				1	
Corralito (15)	1	3 (3)	11 (3)				
Others	5	8					
Neuquén							
Neuquén (16)	1	2 (1)		1			
Picún Leufú (17)	1	3 (3)	3 (4)	3		(3)	
Piedra del Aguila (18)	1	2 (1)	(11)				
Alicura (19)	1	1 (1)	2				
Others	4	7					
Total	39 (16)	106 (33)	31 (36)	20	10	7 (7)	2

[a] Number in parentheses refer to localities shown in Fig. 1, whereas codes refer to labeled *Solenopsis* and *P. obtusus* individuals in Figs. 2 and 3, respectively.

[b] *Pseudacteon* species: *obtl*, large form of *P. obtusus; noc, P. nocens; cur, P. curvatus; tri, P. tricuspis;* and *lit, P. litoralis.*

group clades are typically not monophyletic, likely due to interspecific hybridization (Shoemaker et al. 2006), these data cannot be used to accurately identify species. Therefore, we only attempt to determine the relationship of the *Solenopsis* species of interest to the *saevissima*- and *geminata*-groups.

Results and Discussion

Survey in Chile. Six colonies of *Solenopsis gayi* were found at 4.7% (4/85) of the sites surveyed in January 2005. All colonies lacked an observable aboveground portion of the nest (cryptobiotic) as also occurs in other non-*Solenopsis* ant species in Chile (Snelling and Hunt 1975).

Five females of the large form of *P. obtusus* were found attacking two colonies of *S. gayi* in the proximity of Bulnes (36° 52.389′ S, 72° 19.659′ W), Concepción department, Bio Bio region (Fig. 1; Table 1). Four male *P. obtusus* were among the five females at this site. This is the westernmost record for fire ant decapitating flies at present, and, to our knowledge, the first report of *Pseudacteon* flies occurring in Chile. This fly has previously been recorded as far west as 65° 46′ longitude in Argentina (Calcaterra et al. 2005). This is also the first report of a fire ant decapitating fly attacking *S. gayi.* All female flies collected in Chile were smaller (0.35 ± 0.03 mm) than the average size of large *P. obtusus* flies previously collected in northeastern Argentina and Paraguay (0.52 ± 0.04 mm) or northwestern Argentina and Bolivia (0.49 ± 0.05 mm) (Calcaterra et al. 2005). Thus, they matched with the size of the minor workers of *S. gayi.* The lateral lobes of the ovipositor were less rounded in the Chilean fly females than in the Argentine flies.

The flies were captured while hovering over or attacking workers. The fire ant colonies were found on the side of route 5, km 440. The flies were collected at 1830 hours, when the temperature was 21.5°C and relative humidity was 50%. Flies were attracted to the nests within 10 min of nest disturbance. The presence of flies did not seem to affect ant activity because the ants remained on the surface for >30 min. This behavior may indicate a recent *P. obtusus*–*S. gayi* relationship rather than a coevolutionary relationship.

Table 2. Identified alkaloid components and their percentage of composition from *S. gayi* and *S. geminata* (*S. geminata* species-group) and *S. invicta* and *S. richteri* (*S. saevissima* species-group)

Peak	Alkaloid	*S. gayi*	*S. geminata*	*S. invicta*	*S. richteri*
A	*Cis;* $C_{11:0}$	**33.3**	**29.5**	Trace	Trace
B	*Trans;* $C_{11:0}$	**54.0**	**69.7**	5.0	**15.4**
C	*Cis;* $C_{13:1}$	Trace			2.6
D	*Trans;* $C_{13:1}$	2.1	Trace	**25.0**	**70.0**
E	*Cis;* $C_{13:0}$	5.6	Trace		
F	*Trans;* $C_{13:0}$	3.3	Trace	**13.4**	8.3
G	*Cis;* $C_{15:1}$				Trace
H	*Trans;* $C_{15:1}$	Trace		**39.3**	2.1
I	*Trans;* $C_{15:0}$			**14.5**	Trace?
J	*Trans;* $C_{17:1}$			1.9	

Compounds are listed in order of gas chromatographic elution. Those components present in >10.0% of the total alkaloid components for a given species are in bold to aid in visualizing differences. The *cis* and *trans* refers to the configuration of the two substituents on the piperidine ring (see Fig. 2), and the C and subscript refer to the chain length of substituent R and whether the substituent contains a double bond, e.g. $C_{13:0}$ means R is a 13 carbon chain with no double bonds; C13:1 means R is a 13 carbon chain containing a single double bond. Identifiable alkaloids <1% of the total alkaloid composition are listed as a trace.

Chemical analysis of worker extracts from these six colonies showed that they had similar alkaloid and hydrocarbon compositions. The identified venom alkaloids and cuticular hydrocarbons are listed in Tables 2 and 3, respectively. The venom alkaloids are dominated by *cis*- and *trans*-2-undecyl-6-methylpiperidine. These compounds are also typically the dominant alkaloids found in the *geminata* species complex (see *S. geminata;* Table 2). The cuticular hydrocarbons were composed of a series of normal, methyl- and dimethyl-branched hydrocarbons that are characteristic of members of the *saevissama* group (see *S. invicta,* Table 3; Nelson et al. 1980, Thompson et al. 1981). In addition, the *S. gayi* hydrocarbon fraction contained pairs of monounsaturated and saturated straight chain hydrocarbons (Table 3) that dominate the *S. geminata* complex species (Cabrera et al. 2004; Attygalle et al. 2006; R.K.V.M., unpublished data), but it also may be found in *S. saevissama* complex species (Table 3).

Surveys in Argentina. In total, 100 fire ant colonies were found at 63.6% (42/66) of the sites surveyed in October 2004, January 2005, and January 2006; all in the phytogeographical province of the Monte, one of the most arid areas of Argentina with scarce vegetation (open woodland scattered over a matrix with cover of perennial shrubs and grasses, Cabrera and Willink 1980). The climate of the Monte is distinctive: dry and seasonal with warm (>20°C) and rainy (146 mm) summers and cold (<10°C) and dry (54 mm) winters (Cabrera and Willink 1980, De Fina 1992). However, the amount of precipitation can vary widely from year to year. Most of the colonies found in desert areas (mostly in La Pampa and Mendoza) lacked the aboveground portion of the nest. This matches the assessment of Hays (1958), who noted that fire ants are more widely dispersed and difficult to locate in the extreme south of their distributional range along the Río Negro. All the colonies found in wet areas, generally irrigated, presented visible mounds as in other regions of Argentina. It is important to note that no fire ant colonies were found in Chubut, or in areas of

Table 3. Identified hydrocarbon components and their percentage of composition from *S. gayi* and *S. geminata* (*S. geminata* species-group) and *S. invicta* and *S. richteri* (*S. saevissima* species-group)

Peak	Hydrocarbon	*S. gayi*	*S. geminata*	*S. invicta*	*S. richteri*
1	Heniecosane	Trace	**11.7**		Trace
2	Docosene	Trace	1.5		Trace
3	Docosane	Trace	1.6		Trace
4	Tricosene	Trace	**56.7**		3.0
5	n-Tricosane	1.2	**19.6**		**7.7**
6	11-Methyltricosane		Trace		**6.7**
7	3-Methyltricosane	3.6			**17.9**
8	n-Tetracosene	Trace	1.0		
9	n-Tetracosane	1.0	Trace		1.9
10	3,9- and 3,11-Dimethyltricosanes	1.2			4.7
	11- and 12-Methyltetracosane				2.0
11	n-Pentacosene	**13.0**	1.1		**12.8**
12	n-Pentacosane	**12.4**	1.9		**6.5**
13	11- and 13-Methylpentacosanes	3.25			**16.8**
14	3-Methylpentacosane	**18.1**			4.3
15	n-Hexacosane	1.0			
16	3,11- and 3,13-Dimethylpentacosanes	**13.9**			3.5
17	12- and 13-Methylhexacosane	1.0			
18	3- and 4-Methylhexacosane	**8.1**			
19	n-Heptacosane	**5.1**	Trace	**8.92**	1
20	11- and/or 13-Methylheptacosane	**6.4 (11 = minor)**		**26.5**	3.0 (13-methyl)
21	13,15-Dimethylheptacosane	2.1		**15.6**	4.8
22	3-Methylheptacosane	3.6		**15.8**	
23	3,9- and 3,11-Dimethylheptacosane	2.7 (no 3,9-)		**20.2**	
25	11- and 12- and 13-Methyloctacosane	1.6			
26	10,12- and 11,13-Dimethyloctacosane	1.0			

Compounds are listed in order of gas chromatographic elution. Those components present in >5.0% of the total hydrocarbon components for a given species are in bold to aid in visualizing differences. Identifiable hydrocarbons <1% of the total hydrocarbon composition are listed as a trace.

Neuquén and Río Negro belonging to the Patagonica phytogeographical province (Cabrera and Willink 1980). The climate in this province is dry and cold with snowfalls in winter, frosts during almost all the year, a mean temperature of 5–13.4°C, and an annual rainfall of 100–270 mm. These conditions seem to be unsuitable for most fire ants and could be an effective dispersal barrier for them.

Chemical analysis of the fire ants collected revealed the presence of eight chromatogram patterns (chromatotypes) (R. K.V.M. and L.A.C., unpublished data). The first chromatotype from La Pampa and Río Negro corresponded morphologically to the biotype *Solenopsis quinquecuspis* Forel, which occurs mainly in Buenos Aires province, but also in Córdoba (Fig. 2, LC22). Another two chromatotypes from La Pampa, Mendoza, Río Negro, San Juan, and San Luis corresponded to a second lighter biotype of *S. quinquecuspis* (Fig. 2, LC26) and a biotype variant of *S. invicta.* This second chromatotype of *S. quinquecuspis* may be the result of gene flow between *S. quinquecuspis* and *S. invicta* or *S. richteri* as reported recently by Ross and Shoemaker (2005) for *S. richteri* and *S. quinquecuspis* in Buenos Aires. Alternatively, this form could be a new cryptic *Solenopsis* species with a cryptobiotic life form (which could explain its lighter color) or the cryptic species previously reported by Ross and Trager (1990) for central Argentina, because it is morphologically indistinguishable from *S. quinquecuspis.* Further genetic studies may be necessary to clarify the identity of this fire ant. The presence of a biotype variant of *S. invicta* at the latitude of San Luis is surprising, because it has previously been described only from regions of Argentina with >1,200 mm of annual rainfall, a mean temperature of >18°C, and an absolute minimum temperature of more than −8°C (Trager 1991, Pitts 2002). One possibility is that it is a separate cryptic species or another hybrid. If not, this would be the southernmost record for *S. invicta.* The fourth chromatotype from Neuquén and Mendoza is morphologically similar to *Solenopsis interrupta* Santschi, but the chemical pattern was similar to (introduced) *S. richteri* from the United States. This difference also may indicate some level of historical gene flow. The fifth pattern from Río Negro and Neuquén corresponded to a variant of *S. interrupta* profile. The sixth chromatotype only found in Neuquén corresponded to an undetermined fire ant species close to *S. interrupta.* Based on mtDNA sequences, the seventh pattern from Mendoza may corresponded to *Solenopsis electra* Forel (D. D. Shoemaker, personal communication), although this species was previously recorded only in Santiago del Estero (Ross and Trager 1990) and recently detected in Salta (L.A.C., J.P.P., and R.K.V.M., unpublished data). The eighth chromatotype from La Pampa corresponded to an *S. richteri* biotype from Buenos Aires province, but its morphology is more similar to the biotype of *S. quinquecuspis* found in Buenos Aires.

In total, 113 flies (70 females and 43 males) belonging to five *Pseudacteon* species were collected in La Pampa, Mendoza, Neuquén, and Río Negro (Table 1). No flies were collected in San Juan and San Luis. Phorid flies were much more common and abundant in central western Argentina than in central Chile (Table 1). They were found in 42.9% of the sites with fire ants and at 31% of the mounds. The large form of *P. obtusus* was the most abundant species with 44.3% of all the female flies found (70), followed by *Pseudacteon nocens* Borgmeier (28.6%), *Pseudacteon curvatus* Borgmeier (14.3%), and *Pseudacteon tricuspis* Borgmeier (10%). The shape of the ovipositor of the *P. tricuspis* females collected from *S. quinquecuspis* and the probable new fire ant species in Río Negro and Mendoza, respectively, was similar to those of the flies parasitizing *S. invicta* in northeastern Argentina and Brazil (Porter and Pesquero 2001). Males were not considered for estimating relative abundance because males of the large form of *P. obtusus* and *P. tricuspis* are the only males normally attracted to disturbed fire ant colonies (Porter and Pesquero 2001, Calcaterra et al. 2005). *P. obtusus, P. tricuspis,* and *P. curvatus* abundances would have been overestimated because most of the collections were conducted next to the midday, when they are more abundant because the humidity is lower and temperature higher (L.A.C., unpublished data). However, *P. nocens* and *P. litoralis* abundances may have been underestimated, because sampling was not conducted at sunrise when they are usually more active because the humidity is higher and temperature lower.

These data are the first reports of these five fly species in this region of Argentina (Monte), and the first report of them using *S. quinquecuspis* as a host. Although *P. curvatus* and *P. tricuspis* have been reported previously to attack *S. quinquecuspis* in laboratory oviposition tests (Folgarait et al. 2002), they have never been reported attacking *S. quinquecuspis* in natural conditions. A fire ant species with a chemical pattern close to *S. interrupta* were found from the Río Negro Valley until 40° 43.786′ S latitude in Corralito, Río Negro. Conditions at this site are particularly harsh. Corralito area has a mean temperature for the warmest (January) and coldest (July) months of 16.8 and 3.1°C, respectively (with an absolute minimum temperature of around −15°C), and an annual rainfall of around 255 mm (De Fina 1992). This site is, to our knowledge, the southernmost locale ever reported for fire ant decapitating flies, in this case the large form of *P. obtusus. Pseudacteon* flies have been previously reported up to 38° south latitude (Folgarait et al. 2005). Fire ants have been previously reported up to 42° south latitude (Hays 1958); however, the identity of the *Solenopsis* species was not clear because, at the time, all the fire ant species were considered *S. saevissima.*

Together, the data from this and previous studies indicate that the large form of *P. obtusus* has the widest distribution (Calcaterra et al. 2005, Folgarait et al. 2005). Its latitudinal and altitudinal (up to 2,300 m) distribution suggests that it is the most cold tolerant species and could survive under low-moisture conditions. This agrees with the opinion of Folgarait et al. (2005), who mentioned that *Pseudacteon* species with

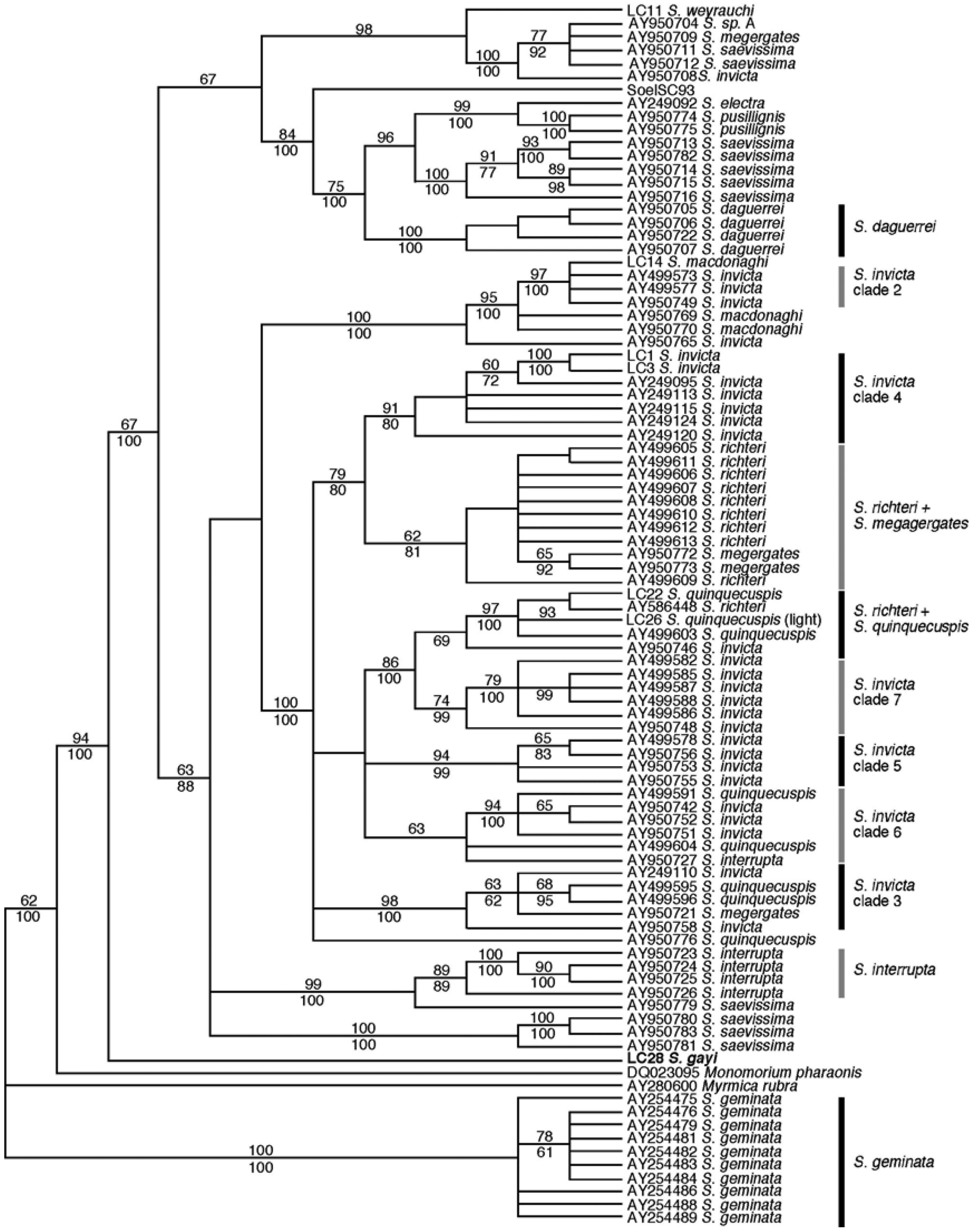

Fig. 2. Relationships among *Solenopsis* fire ants. Each sequence from GenBank is labeled with the GenBank accession number and the species name. Numbers above the branches indicate the level of bootstrap support for the maximum parsimony tree (1,000 replicates); numbers below each branch indicate the Bayesian posterior probabilities. The labeling of individual ants corresponds to the labels of their phorid fly parasitoids in Fig. 3, and the labeling of *Solenopsis* clades (far right) follows that of Shoemaker et al. (2006).

broader geographical distribution (as *P. obtusus*) would have greater climatic tolerance and thus could survive in a continental climate, very dry and with extreme temperatures. The high tolerance of the large form of *P. obtusus* to severe climatic conditions makes this candidate very promising for introduction into the

United States with an increased probability for good establishment and dispersal.

The new host record for the large form of *P. obtusus* reported in Chile would indicate that it is not limited to the *S. saevissima* species-group, because *S. gayi* was included by Trager (1991) and later confirmed by Pitts (2002) within *S. geminata* species-group. However, an alternative explanation could be that *S. gayi* is indeed closer to the *S. saevissima* species-group than to the *S. geminata* group. Some cuticular hydrocarbon components found in *S. gayi,* which are characteristic of the *S. saevissima* species-group, suggests that this scenario might be possible.

Phylogenetic Analyses of *Pseudacteon* Flies and *Solenopsis* Fire Ants. Our phylogenetic analyses, using mitochondrial COI sequence data, indicate that *S. gayi,* the Chilean fire ant host for *P. obtusus,* falls within the *S. saevissima* species-group rather than the *S. geminata*-group (Fig. 2; 94% maximum parsimony [MP] bootstrap support, 100% posterior probability). However, within the *S. saevissima*-group, *S. gayi* seems to be distantly related to the other species, and it is basal relative to the other species within this group.

Intraspecific phylogenetic analyses of the *P. obtusus* showed that the seven populations sampled formed a well supported monophyletic clade (Fig. 3, bootstrap support = 80% for the MP tree, 77% for the maximum likelihood [ML] tree). Within *P. obtusus,* the Chilean form seemed to be more similar to the clade including flies from Formosa, Santa Fe and La Pampa, Argentina (bootstrap support = 60% for the MP, 72% for ML). The small morph of *P. obtusus,* from Formosa, formed a well supported monophyletic clade with the large form *P. obtusus* from Santa Fe and La Pampa (100% bootstrap support for both trees). That the Chilean *S. gayi* cuticular hydrocarbon pattern seems to be more similar to that of *S. richteri* (eastern Argentina) supports the idea that the Chilean *P. obtusus* flies seem to be more similar to the *P. obtusus* occurring in eastern Argentina than *P. obtusus* in western Argentina.

Implications of the Findings in Chile. The previously confirmed natural host species of the large form of *P. obtusus* were the fire ants *S. invicta* and *S. richteri.* The geographic distribution of the new hosts found in this work (the lighter *S. quinquecuspis, S. interrupta,* and the form close *S. interrupta*) and those found by Calcaterra et al. (2005) is closer to Chile than that of *S. invicta* or *S. richteri.* Of these new hosts, the genetically closest to the Chilean fire ant was found by Calcaterra et al. (2005) in Tucumán province, up to 3,300-m altitude (LC 11, Fig. 2). This species may be *S. weyrauchi* Trager, which has only been reported for several widely spaced locations at elevations up to 3,900 m in the Peruvian and Bolivian Andes (Trager 1991; Pitts 2002; L.A.C.., unpublished data). According to Trager (1991), the range of *S. weyrauchi* could extend up to Argentina and Chile.

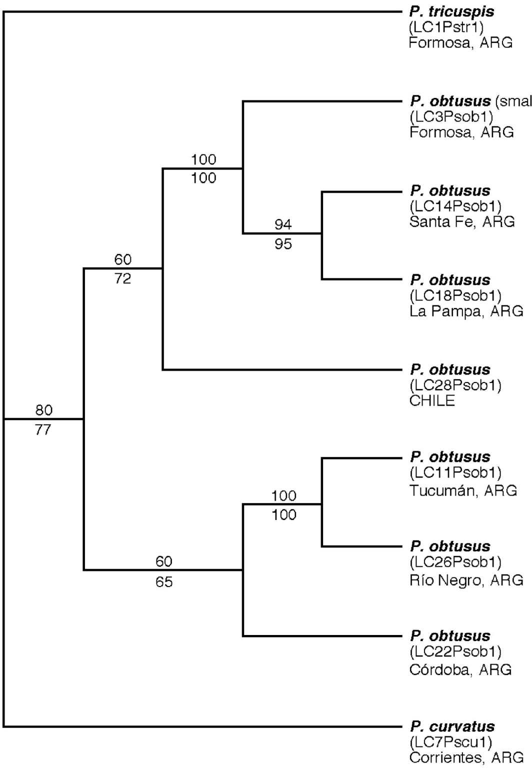

Fig. 3. Phylogenetic relationships among populations of *P. obtusus* from Argentina and Chile by using 1,400 bp of DNA sequence data from the mitochondrial COI (731 bp) and CytB (669) genes. Each sample is labeled with the general collection location; the small morph of *P. obtusus* is noted in parentheses, and the individual sample identification is provided on the second line, in parentheses. Bootstrap support for the maximum parsimony tree (1,000 replicates) is indicated shown above each branch, and bootstrap support for the maximum likelihood tree (1,000 replicates) is shown below each branch. *P. tricuspis* from Formosa, Argentina, and *P. curvatus* from Corrientes, Argentina, collected by Calcaterra et al. (2005) were used as outgroups.

Based on this phylogeographical evidence, it is possible that the discovery of the large form of *P. obtusus* in Chile is a consequence of the natural spread of parasitized fire ant species from Argentina. There are many potential routes of entry or corridors through various mountain passes in the southern portion of the Andes that connect Chile with Argentina, all of which are lower than 2,000-m altitude. The large form of *P. obtusus* was found previously in Argentina up to 2,300-m altitude (Calcaterra et al. 2005) and in this study up to the 70° 45′ longitude close to the Alicurá dam in Neuquén. It was also found in the Uspallata valley (69° 21′) in Mendoza, 90 km from Los Libertadores (the pass to Chile). Thus, it is very probable that some of these routes were invaded by fire ants and through them *P. obtusus* crossed the Andes into Chile. However, given that Chilean flies were genetically closer to flies from eastern Argentina, we feel that they also could have been subsequently introduced from eastern Argentina into Chile by human transport. We consider these two scenarios as the most likely explanations for the presence of *P. obtusus* in Chile, because *P. obtusus* has never been reported as a parasite of members of the *S. geminata* species-group.

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