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New records and new host-parasitoid interactions of phorids (Diptera: Phoridae) parasitizing leafcutter ants (Hymenoptera: Formicidae) in Argentina

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Nuevos registros y nuevas interacciones hospedador-parasitoide de fóridos (Diptera: Phoridae) que parasitan hormigas cortadoras de hojas (Hymenoptera: Formicidae) en Argentina

RESUMEN. Las hormigas cortadoras de hojas son una de las plagas más importantes de la región Neotropical. En el presente estudio, fueron recolectados representantes de cuatro provincias de Argentina, con el objetivo de identificar las especies de fóridos que las parasitan. Como resultado, se registraron siete especies de fóridos, *Apocephalus neivai* Borgmeier, *Ap. noetingerorum* Disney, *Neodohrniphora unichaeta* Disney, *Myrmosicarius catharinensis* Borgmeier, *M. cristobalensis* Disney, *M. crudelis* Borgmeier y *M. gracilipes* Borgmeier, obtenidos en hormigas cortadoras de hojas de las especies *Acromyrmex ambiguus* Emery, *Ac. crassispinus* Forel, *Ac. heyeri* Forel, *Ac. lobicornis* Emery y *Ac. lundii* Guérin. Asimismo, son reportados todos los registros previos de hospedadores y su distribución geográfica en Argentina para las siete especies de fóridos. Se citan nuevos registros en todas las especies estudiadas, ampliando así sus rangos geográficos y estableciendo once nuevas interacciones hospedador-parasitoide. Nuestros datos amplían el conocimiento de este poco estudiado sistema y proveen importantes consideraciones para el uso potencial de estos parasitoides como controladores biológicos de hormigas cortadoras de hojas.

PALABRAS CLAVE. Enemigos naturales. Rango geográfico. Sistema hormiga-parasitoide.

ABSTRACT. Leafcutter ants are one of the most important crop pests in the Neotropics. In the present study, several specimens were collected from four provinces in Argentina in order to determine the species of phorid flies parasitizing them. We report our findings on seven phorid species, *Apocephalus neivai* Borgmeier, *Ap. noetingerorum* Disney, *Neodohrniphora unichaeta* Disney, *Myrmosicarius catharinensis* Borgmeier, *M. cristobalensis* Disney, *M. crudelis* Borgmeier and *M. gracilipes* Borgmeier, over the leafcutter ants *Acromyrmex ambiguus* Emery, *Ac. crassispinus* Forel, *Ac. heyeri* Forel, *Ac. lobicornis* Emery and *Ac. lundii* Guérin. All the known hosts and geographic ranges for these seven phorids in Argentina are provided. The seven phorid species represent new records for all the provinces studied, expanding their geographical ranges; furthermore, we establish a total of eleven new host-parasitoid interactions. Our data expand the knowledge of this little studied system and provide important considerations for the potential use of these parasitoids as biological control candidates of the leafcutter ants.

KEYWORDS. Ant-parasitoid system. Geographic ranges. Natural enemies.

Leafcutter ants in the genera *Atta* Fabricius and *Acromyrmex* Mayr are considered the most important agricultural and forestry pests in the Neotropics (Wirth et al., 2003), consuming more plant biomass than any other herbivore (Cherret, 1986). Their complex social organization and their ability to circumvent most plant's defense mechanisms make controlling them a complex task (Montoya Lerma et al., 2012; Della Lucia et al., 2014). Chemical control, although effective in the short term, poses serious threats to human health and the environment, and more chemicals are added to the lists of banned or restricted pesticides every year, so that producers intending to sell their crops to the European and North American markets have limited options when controlling these pests (Della Lucia et al., 2014). Therefore, alternative methods of pest control have received increased attention, with researchers focusing mainly on biological control either by microorganisms or by other insects (Montoya Lerma et al., 2012; Della Lucia et al., 2014). Amongst the latter, the flies in the family Phoridae have been proposed as promising candidates for biological control of leafcutter ants (Folgarait, 2013) because of two main reasons: their high specificity (Elizalde & Folgarait, 2011) and the indirect effects they have on their hosts, which widely exceed the mortality they inflict (Elizalde & Folgarait, 2012; Guillade & Folgarait, 2014). Research on leafcutter ant phorids in Argentina is relatively new, and therefore the information on their distribution and the ant species they attack is still somewhat limited (Folgarait, 2013; Elizalde et al., 2017). In this paper, we present an updated distribution of seven species in three genera of phorids attacking *Acromyrmex* leafcutter ants in Argentina: *Apocephalus neivai* Borgmeier, *Ap. noetingerorum* Disney, *Neodohrniphora unichaeta* Disney, *Myrmosicarius catharinensis* Borgmeier, *M. cristobalensis* Disney, *M. crudelis* Borgmeier and *M. gracilipes* Borgmeier. We also expand the range of phorid species attacking several ant hosts. Because we reared all the parasitoids obtained, the phorid-host relationships found are not dubious data as sometimes happens from observations of ovipositions only.

Our samplings took place monthly between August 2013 and August 2015 in a commercial pine plantation in Concordia, Entre Ríos province (31°38′56″ S, 58°00′54″ W), as well as at several urban localities in Buenos Aires province: Bernal (34°42′00″ S, 58°17′00″ W), Hudson (34°47′25″ S, 58°08′55″ W), surroundings of La Plata [Parque Pereyra (34°50′00″ S, 58°06′00″ W), Parque Ecológico Villa Elisa (34°51′12″ S, 58°04′45″ W) and Manuel B. Gonnet (34°51′00″ S, 58°01′00″ W)], and La Plata (4°56′00″ S, 57°57′00″ W). We also collected samples from Dina Huapi (41°04′12″ S, 71°09′54″ W), Río Negro province, in March 2015. From November 2015 to December 2016 we carried out monthly samplings in a commercial pine plantation in Esquina, Corrientes province (30°01′01″ S, 59°32′02″ W). Every nest sampled was geo-referenced and the ants identified to the species level following the keys by Gonçalves (1961) and Kusnezov (1978). Samplings were carried out through the Larval Parasitoid Collection method (Elizalde & Folgarait, 2011), which consists in collecting every ant that passes a fixed point in the foraging trail returning to the nest, until between 300 and 500 ants have been caught. Ants were then transported to the laboratory and kept in climatically controlled chambers at 24 °C and 70% RH, which according to Guillade & Folgarait (2015) are the optimal rearing conditions for leafcutter ant parasitoids. Ants were fed with sugar-water and water *ad libitum*, and dead ants were periodically removed, placed in smaller containers and inspected under a Nikon SMZ800 stereoscopic microscope. Parasitized ants were transferred to individual containers and observed daily until the emergence of adult parasitoids, at which time we identified them to the species level following the keys by Brown (1997), Brown et al. (2010) and Disney et al. (2006, 2008, 2009).

Leafcutter ants in the sites we sampled in Buenos Aires province were *Ac. heyeri* Forel and *Ac. lundii* Guérin. In Concordia, Entre Ríos province, we found four leafcutter species: *Ac. ambiguus* Emery, *Ac. crassispinus* Forel, *Ac*. *heyeri* and *Ac. lundii*. The only leafcutter species we recorded in Dina Huapi, Río Negro province, was *Ac. lobicornis* Emery. In Esquina, Corrientes province, we collected mostly *Ac. ambiguus* and *Ac. heyeri*, with *Ac. crassispinus* and *Ac. lundii* appearing only very rarely.

Table I summarizes all the current knowledge on geographic distribution and host parasitoid associations of the seven parasitoid species discussed here (Brown, 1997; Brown et al., 2010; Disney et al., 2006, 2008, 2009; Elizalde & Folgarait, 2010, 2011, 2012; Elizalde et al., 2017), as well as the new geographic records and host-parasitoid interactions registered in this work. We reared *Ap. neivai* from workers of *Ac. lundii*, a known host of this parasitoid, over a new range of localities in Buenos Aires province. We also recorded this parasitoid species for the first time in Entre Ríos province over three different hosts, one of which, *Ac. ambiguu*s, is a novel host. *Apocephalus noetingerorum* was collected for the first time in surroundings of La Plata, as well as in Entre Ríos province, where we also found two novel hosts, *Ac. ambiguus* and *Ac. lundii*, for this parasitoid.

Neodohrniphora unichaeta was recorded in Entre Ríos province over three novel host ants: *Ac. ambiguus*, *Ac. heyeri* and *Ac. lundii.* It is interesting that, although its previously known host, *Ac. crassispinus*, was present in our sampling site in Entre Ríos, we never reared *N. unichaeta* from ants of this species.

The records for *M. catharinensis* were expanded to several new localities in Buenos Aires province. Besides, we broadened its host range in Entre Ríos province, where it was known to parasitize both *Ac. ambiguus* and *Ac*. *crassispinus*, given that we reared it from those two species, as well as from *Ac. heyeri* and *Ac. lundii*.

Province	Locality	Phorid species						
		Ap. neivai	***Ap. noetingerorum***	***N. unichaeta***	***M. catharinensis***	***M. cristobalensis***	***M. crudelis***	***M. gracilipes***
Buenos Aires	Otamendi				*A. ambiguus, A. heyeri, A. lundii*			
	Azul				*A. ambiguus, A. lundii*			
	Carmen de Areco				*A. lundii*			
	Magdalena	*A. lundii*			*A. ambiguus, A. heyeri, A. lundii*			
	Hudson	***A. lundii***			*A. lundii*			
	La Plata	***A. lundii***			*A. heyeri,* ***A. lundii***			
	surroundings of La Plata	***A. lundii***	***A. lundii***		***A. heyeri, A. lundii***	***A. lundii***		***A. lundii***
	Bernal	***A. lundii***			***A. lundii***	***A. lundii***		
	Castelar		*A. lundii*					
	Moreno		*A. lundii*					
	Isla Martín García						*A. lundii*	
Entre Ríos	El Palmar			*A. crassispinus*	*A. ambiguus, A. crassispinus*			
	Concordia	***A. ambiguus, A. crassispinus, A. lundii***	***A. ambiguus, A. crassispinus, A. lundii***	***A. ambiguus, A. heyeri, A. lundii***	***A. ambiguus, A. crassispinus, A. heyeri, A. lundii***	***A. crassispinus, A. heyeri, A. lundii***	***A. ambiguus, A. heyeri, A. lundii***	***A. ambiguus, A. heyeri, A. lundii***
Corrientes	Mercedes					*A. heyeri*		
	Esquina				***A. ambiguus, A. heyeri***	***A. ambiguus, A. heyeri***	***A. ambiguus, A. heyeri***	***A. ambiguus, A. heyeri***
Santa Fe	San Cristóbal	*A. crassispinus, A. heyeri, A. hispidus, A. lobicornis, A. lundii*	*A. crassispinus, A. lundii*	*A. heyeri, A. lundii*	*A. heyeri, A. lobicornis, A. lundii, A. ambiguus*	*A. crassispinus, A. fracticornis, A. lobicornis. A. lundii*	*A. crassispinus, A. lundii*	

Table I. Geographic ranges and host-parasitoid associations between phorids (*Apocephalus neivai*, *Ap. noetingerorum*, *Neodohrniphora unichaeta*, *Myrmosicarius catharinensis*, *M. cristobalensis*, *M. crudelis*, *M. gracilipes*) and leafcutter ants of the genus *Acromyrmex* from Argentina. Ant species given in bold indicate a new geographic record, whereas bold and underlined indicate a novel host for that ant species.

Province	Locality	Phorid species						
		Ap. neivai	***Ap. noetingerorum***	***N. unichaeta***	***M. catharinensis***	***M. cristobalensis***	***M. crudelis***	***M. gracilipes***
Córdoba	Noetinger	*A. crassispinus, A. heyeri, A. lobicornis, A. lundii*	*A. crassispinus, A. lundii*		*A. ambiguus, A. heyeri, A. lundii*	*A. fracticornis, A. lobicornis*	*A. crassispinus, A. lundii*	*A. crassispinus*
Formosa	Formosa					*A. hispidus*		
	Parque Nacional Pilcomayo				*A. lundii*			
	Reserva Natural Formosa					*A. lobicornis, A. striatus*		
Chaco	Parque Nacional Chaco				*A. hispidus*	*A. lundii, A. subterraneus*		
Río Negro	Parque Nacional Nahuel Huapi				***A. lobicornis***			

Table I (cont.). Geographic ranges and host-parasitoid associations between phorids (*Apocephalus neivai*, *Ap. noetingerorum*, *Neodohrniphora unichaeta*, *Myrmosicarius catharinensis*, *M. cristobalensis*, *M. crudelis*, *M.gracilipes*) and leafcutter ants of the genus *Acromyrmex* from Argentina. Ant species given in bold indicate a new geographic record, whereas bold and underlined indicate a novel host for that ant species.

We also found this phorid for the first time in Corrientes province, parasitizing both *Ac. ambiguus* and *Ac. heyeri* as well as in Río Negro province, over *Ac. lobicornis*. *Myrmosicarius cristobalensis* was recorded for the first time in Buenos Aires province over a novel host, *Ac. lundii*. We reported it for the first time in Entre Ríos province over three different leafcutter species, one of which was the novel host *Ac. lundii*. *Myrmosicarius crudelis* was collected for the first time in Entre Ríos province over two novel hosts, *Ac. ambiguus* and *Ac. heyeri*. This new host-parasitoid interaction was also present in Corrientes province, which is a new geographic record. *Myrmosicarius gracilipes* was collected for the first time in Buenos Aires province at the localities around La Plata, over a new host, *Ac. lundii*. It was also recorded for the first time in Entre Ríos province, over the novel hosts *Ac. ambiguus*, *Ac. heyeri* and *Ac. lundii*, and in Corrientes province over the new hosts *Ac. ambiguus* and *Ac. heyeri*.

Geographic ranges of phorid species were expanded in all the provinces where we collected samples, with two new records for Buenos Aires province, five for Entre Ríos, three for Corrientes and one for Río Negro, which is also the first record of leafcutter phorids for this province. Furthermore, we found a total of eleven new host-parasitoid interactions. Elizalde & Folgarait (2011) have proposed that leafcutter ant phorids constitute a well-defined guild, given that they do not parasitize other ant genera. In addition, they observed that phorids parasitizing *Atta* ants do not parasitize *Acromyrmex*, and vice versa. Our results provide further evidence to that hypothesis, because none of the phorid species that we found on *Acromyrmex* ants have ever been reported attacking or being reared from *Atta* ants. Over the thousands of ants reared, we never collected any *Eibesfeldtphora* Disney (Diptera, Phoridae), providing further evidence that this genus specializes in attacking only *Atta* ants (Folgarait, 2013). On the other hand, all the phorid species we recorded have, at least, three confirmed *Acromyrmex* hosts, suggesting a low degree of host specificity for these parasitoids within host genus. Moreover, *M. catharinensis* reached up to nine hosts, which is likely the reason for its wide distribution, with records in all the provinces studied so far. In addition, our new records show that *M. crudelis* and *M. gracilipes*, two phorid species specialized to attack

Acromyrmex ants at refuse dumps (Elizalde & Folgarait, 2011), were here reared from foraging ants. It is rather difficult for a worker involved in disposing refuse to change tasks and become a forager after being exposed to all the hazards involved in refuse piles, when only old ants are supposed to be allocated to that microhabitat. Our records for these two phorids confirmed the pattern previously found of obtaining them from sites with relatively lower light intensities (Folgarait, 2013). In fact, we believe that further studies of these parasitoids will result in confirming the patterns already observed and even broadening geographic ranges, as well as new host-parasitoid interactions.

Taking into account that the hosts reported in this study correspond to ant species that have pest status in Argentina and Brazil (authors, pers. obs.) and they are attacked by four to seven parasitoid species including nocturnal ones, with several ovipositional strategies and sites on the worker body (Folgarait, 2013), our data further contributes for considering these natural enemies as promising candidates for biological control of leafcutter ants.

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