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DNA barcoding and morphological identification of Argentine species of *Apanteles* (Hymenoptera : Braconidae), parasitoids of cactus-feeding moths (Lepidoptera : Pyralidae : Phycitinae), with description of a new species

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Abstract. The gregarious species of *Apanteles* that attack cactus-feeding phycitine moths (Lepidoptera : Pyralidae) from Argentina are identified using DNA barcodes and morphological data. Sequences of specimens from 10 different populations were generated. Corrected genetic divergences showed two clusters of specimens separated by COI divergences higher than 6%. Our morphological examinations were congruent with the molecular evidence and therefore two species were confidently identified: *Apanteles alexanderi* Brèthes and a new species, *Apanteles opuntiarum* Martínez & Berta, sp. nov., which we describe and illustrate. Despite these two taxa being considered to represent a single polyphagous species for at least 50 years, they can be easily distinguished by the length of their ovipositor sheaths. A key to the species of microgastrine parasitoids of cactus-feeding Lepidoptera in South America is provided. The recognition of the new species reveals a narrower host range for the species involved in this system, making it necessary to re-evaluate their potential as biocontrol agents of the prickly pear moth.

Additional keywords: *Apanteles alexanderi*, *Apanteles opuntiarum*, biological control, *Cactoblastis cactorum*, COI, *Opuntia*, prickly pear.

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Introduction

The parasitoid wasp genus *Apanteles* is one of the largest genera of Microgastrinae (Hymenoptera : Braconidae), currently containing ~1000 described species (Yu *et al*. 2005). Accurate identification of *Apanteles* species is very difficult even for specialists, and currently there are no keys for the Neotropical Region (Whitfield 1997). *Apanteles* species are endoparasitoids, mostly of microlepidoptera (Whitfield 1997), with one specialised species group containing gregarious endoparasitoids of cactus-feeding moths (Lepidoptera : Pyralidae : Phycitinae) in the New World. Only one species of this group has been described for the Neotropical Region, *Apanteles alexanderi* Brèthes. This species has been associated with many cactus-feeding moth species, including the prickly pear moth, *Cactoblastis cactorum* (Berg) (Lepidoptera : Pyralidae) (Pemberton and Cordo 2001).

The introduction of *Cactoblastis cactorum* into Australia in the early 20th century to control prickly pear (*Opuntia* spp.) represents one of the most successful and remarkable cases in classical biological control of weeds (Dodd 1940; Moran and Zimmermann 1984; Zimmermann *et al*. 2000). The successful control of *Opuntia* encouraged the introduction of *Cactoblastis* into other countries, including some Caribbean islands (Simmonds and Bennett 1966). In 1989, the Caribbean populations of *Cactoblastis* reached the peninsula of Florida and since then the species has become a major concern for the conservation of native species of *Opuntia* in North America:

Cactoblastis cactorum is spreading westwards at ~160 km per year along the Gulf of Mexico coast (Hight *et al*. 2002; Solis *et al*. 2004). More recently, this species has been recorded from Mexico on Isla Mujeres, a small island just 5 km off the coast of the Yucatan Peninsula, in the state of Quintana Roo (Zimmermann *et al*. 2007). This moth not only threatens to decimate the centre of *Opuntia* biodiversity in North America if it continues spreading westwards from its current range in eastern Louisiana, but might also become a serious pest affecting the *Opuntia* cultures that are widely consumed by Mexican people since prehispanic times.

During recent collecting trips conducted in Argentina to survey the parasitoid species associated with *Cactoblastis* in its native range, several gregarious specimens of *Apanteles* were obtained. All microgastrine species reared from *Cactoblastis* and other phycitine species on *Opuntia* during the 20th century were almost axiomatically assumed to be *A. alexanderi* without a thorough examination of the specimens. Zimmermann *et al*. (1979) reported that this species was responsible for 30% of larval mortality of *C. cactorum*, but because of its presumed polyphagy it has not been considered in biological control programs of the prickly pear moth. Nevertheless, we found that our recently collected specimens exhibited morphological variation, suggesting that more than one species is associated with phycitine moths in South America. The aim of this work was therefore to investigate the species boundaries among these specimens using DNA barcode sequences (Hebert *et al*. 2003), complemented by morphological comparison, and to describe any newly recognised species. The use of the COI marker to characterise economically important species has already shown its utility for identifing invasive insect species (e.g. Scheffer *et al*. 2006), host–parasitoid associations (e.g. Hrcek *et al*. 2011), as well as parasitoid wasp species employed in biological control of aphids and scale insects (e.g. Zhang *et al*. 2011; Derocles *et al*. 2012).

Material and methods

Extensive samplings were carried out from 2002 to 2012 in several localities along central and northern Argentina in order to survey the cactus-feeding phycitine moths and their parasitoids. Phycitine larvae were transported to the laboratory and fed with their original hosts. Once pupation occurred, parasitised larvae were placed in 5-mL plastic cups for emergence of adult parasitoids and were subsequently kept in 96% ethanol until they were processed for DNA sequencing. Specimens from more than 50 localities across 15 Argentine provinces were examined morphologically, including specimens obtained from various entomological collections. The examined material is listed alphabetically according to Argentine provinces.

Specimens from 10 different populations were selected to obtain barcode sequences to assess DNA sequence variation among populations (DNA voucher numbers CNIN 1106–25, GenBank accession numbers JX566772–90). DNA Barcode sequences (~650 bp of the cytochrome oxidase I (COI) mitochondrial DNA gene: Hebert *et al*. 2003) were generated using the same DNA extraction, amplification and sequencing protocols employed by Ceccarelli *et al*. (2012). Sequences from seven Costa Rican species belonging to the *A. leuscostigmus* complex (GenBank accession numbers DQ492267, DQ492269, DQ492272, DQ492274, DQ492275, DQ492283, DQ492286: Janzen *et al.* 2009) were included in the analysis for comparison. These species were chosen because our Argentine specimens are morphologically similar to *A. lecuostigmus* according to the available key to species (Muesebeck 1921). Corrected genetic divergences among the included sequences were calculated using the K2P distance model (Kimura 1980) and a Neighbour-Joining (NJ) tree was reconstructed with these distances using PAUP* ver. 4 (Swofford 2002).

Species descriptions are organised following the basic format of recent taxonomic descriptions of *Apanteles* species (Whitfield *et al*. 2001) in order to facilitate comparisons. Absolute measurements of body and fore wing length are given for primary types, followed by observed paratype variation in parentheses. Photographs were taken and edited using a Leica® Z16 APO-A stereoscopic microscope, a Leica® DFC295/DFC290 HD camera, and the Leica Application Suite® program. Scanning electronic microscope images were obtained using a PHILIPS xl 30 instrument. Specimens for SEM images were coated with gold–palladium with a Thermo VG Scientific SC7620 coater. Morphological terminology follows Sharkey and Wharton (1997), surface sculpture terminology follows Harris (1979), and illustrations and descriptions of wing veins follow Mason (1986). Material examined is deposited at Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia', Buenos Aires, Argentina (MACN), Instituto y Fundación Miguel Lillo, San Miguel de Tucumán, Argentina (IFML) and Instituto de Biología de la Altura, Universidad Nacional de Jujuy, San Salvador de Jujuy, Argentina (INBIAL).

Results

Species boundaries using DNA barcodes

In total, 19 barcode sequences were generated for the specimens of *Apanteles* included in this work. A NJ tree based on corrected genetic distances among the included specimens and previously published sequences of the *A. leucostigmus* species complex is shown in Fig. 1. The sequenced specimens were collected from populations from most of the known geographic range of the *Apanteles* species group that attacks pyralid moths in Argentina (Fig. 2) and represent specimens covering all the observed morphological variation.

The NJ tree (Fig. 1) recovered two separate sequence clusters of *Apanteles* specimens originally assigned to *A. alexanderi*. This separation is congruent with the morphological variation observed among the two groups, demonstrating that two separate species were present, *A. alexanderi* and a new species that is described below, *A. opuntiarum*, sp. nov. Intraspecific genetic distances within *A. alexanderi* ranged from 0 to 0.98% (DNA voucher numbers CNIN 1114–1115, 1120–1122) whereas within *A. opuntiarum*, sp. nov, they varied from 0 to 0.16% (DNA voucher numbers CNIN 1106–1113, 1116–1119, 1123–1125). However, the interspecific variation between the above two species ranged from 6.8 to 8.1%, whereas variation among these two species and species of the *A. leucostigmus* complex ranged from 9.6 to 12.6%.

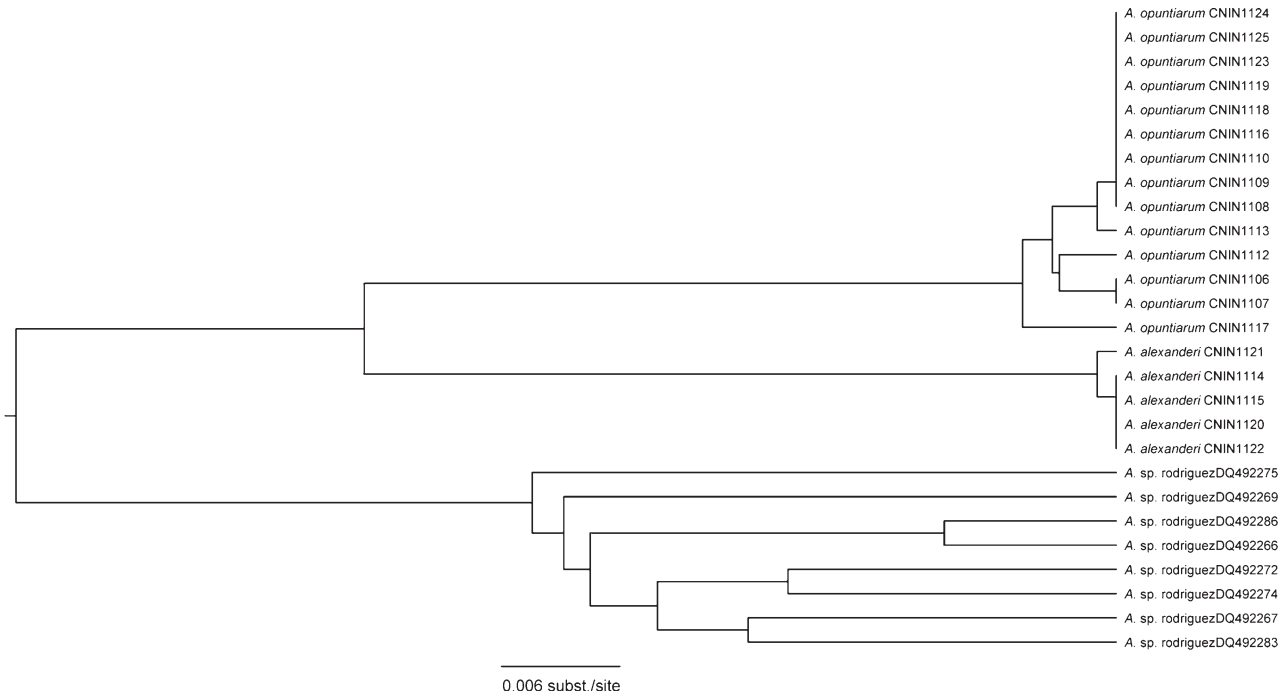


Fig. 1. NJ tree showing corrected genetic distances among *Apanteles alexanderi*, *Apanteles opuntiarum*, sp. nov. and species belonging to the *Apanteles leucostigmus* species complex.

Taxonomy

Apanteles alexanderi Brèthes

(Figs 3–10)

Apanteles (*Pseudapanteles*) *alexanderi* Brèthes, 1922: 19. Lectotype female (here designated), MACN, examined.

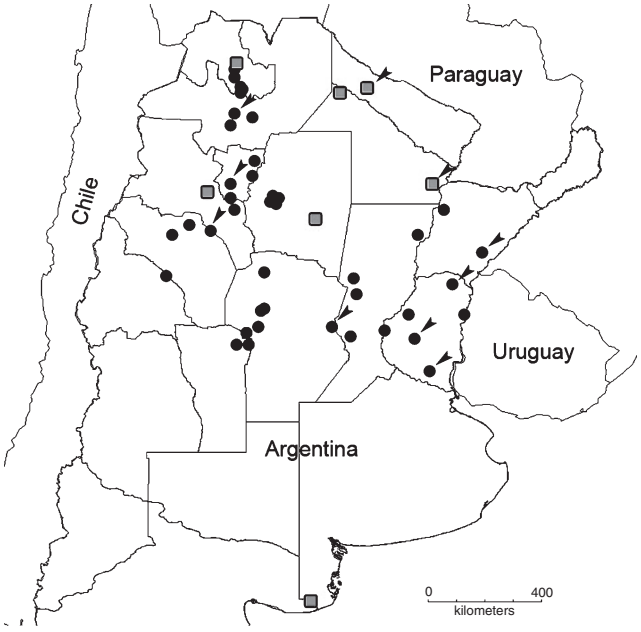


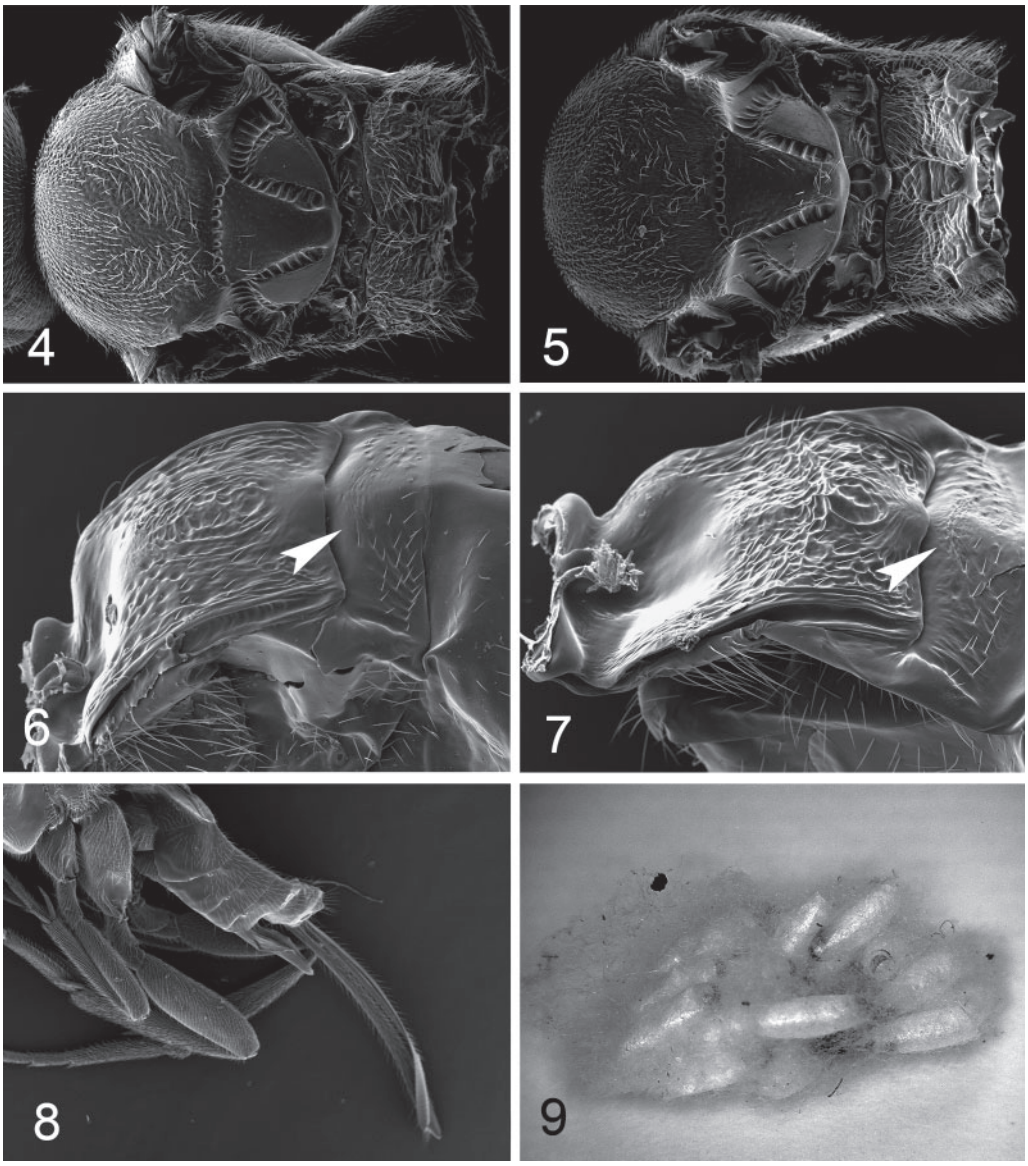
Fig. 2. Distributional records of populations of *Apanteles alexanderi* (squares) and *Apanteles opuntiarum* (circles). Arrows indicate populations from which DNA barcode sequences were obtained.

Material examined

Type series. Lectotype female (here designated): ARGENTINA, Buenos Aires, Carmen de Patagones, 23.i.1921, V. B. Alexander; *Apanteles* (*Pseudapanteles*) *alexanderi* Brèthes; Lectotype *Apanteles* (*P.*) *alexanderi* (Brethès) by P. Marsh '79 (MACN); Paralectotype, one female with the same data and on the same pin.

Other material examined. ARGENTINA. **Buenos Aires:** 4 females, Carmen de Patagones (one coated for SEM images), 17.i.1921, Blanchard col. (MACN). **Catamarca:** 2 females, Andalgalá, Mar. 1921 (MACN). **Chaco:** 8 females, 1 male, Puerto Tirol II, 27°21′49.7″S, 59°03′59.2″W, 12.xi.2010, Logarzo, Varone, from larvae of *Tucumania* sp. on *O. cardiosperma* (DNA voucher nos

Fig. 3. *Apanteles alexanderi*, habitus of female in lateral view.



Figs 4–9. *Apanteles alexanderi. 4*, *5*, mesosoma in dorsal view; *6*, *7*, first and second metasomal median tergites in dorsolateral view; *8*, hind leg and metasoma showing ovipositor sheaths in lateral view; *9*, cocoon mass. Figs *4*, *6*: specimen from Blanchard's collection collected in the type locality. Figs *5*, *7*: newly reared specimen. Arrows indicate rugosities on second metasomal tergite.

CNIN1120, CNIN1121, CNIN1122; GenBank accession nos JX566787–88, JX566790) (MACN, IFML). **Formosa:** 4 females, Laguna Yerma, 24°14′47.5″S, 61°14′01.2″W, 28.ii.2009, Varone, Logarzo, from larvae of *Tucumania* sp. on *O. cardiosperma* (DNA voucher nos CNIN1114, CNIN1115; GenBank accession nos JX566786, JX566789) (MACN, IFML); 6 females, 8 km N Pozo del Mortero, 24°21′42.2″S, 62°05′32.8″W, 28.ii.2009, Logarzo, Varone, from larvae of *Tucumania* sp. on *O. anacantha* (MACN, IFML). **Jujuy:** 2 females, 2 males, Pucará-Tilcara, 4.x.1990, E. Neder, on 'oruga azul' ex/*Trichocereus pasacana* (INBIAL). **Santiago del Estero:** 31 females, 6 males; no locality, 08.ii.1935, from larvae of *Tucumania tapicola* (MACN); 14 females, 17 males, Añatuya, Nov. 1933, from larvae of *Tucumania tapicola* (MACN).

Diagnosis

Apanteles alexanderi can be easily distinguished from other *Apanteles* species associated with cactus-feeding moths by the weak but distinct rugosity on the second metasomal tergite (Figs 6, 7).

Female (Fig. 3)

Body length (excluding ovipositor and sheaths) 2.9 mm (2.9–3.7 mm). Fore wing length 2.9 mm (2.9–3.8 mm).

Colour. General body colour black, except palpi which are whitish; antenna, tibia and tarsus of hind leg and ovipositor

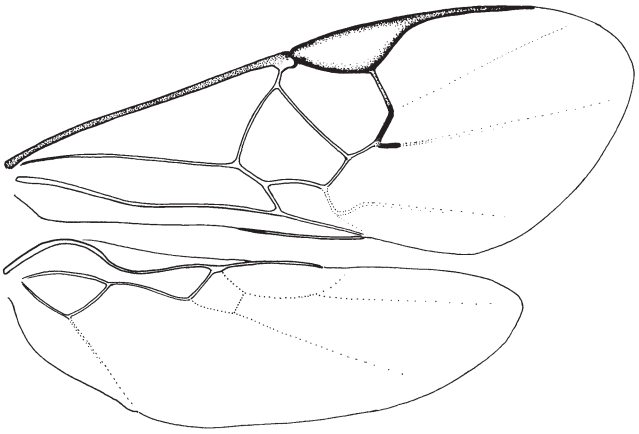


Fig. 10. *Apanteles alexanderi*, wings.

sheaths dark brown; fore and mid legs, trochanter, trochantellus and femur of hind leg and ovipositor yellowish brown. Laterotergites of anterior metasomal tergites dark brown. Tegula pale, light brown. Wings hyaline, most veins unpigmented except C-Sc-R, pterostigma, R1, r, 2RS, 2M, 1Cua-b, which are brownish. Pterostigma pigmented only marginally, pale translucent medially.

Head. Face shallowly punctate, wider than high. Malar space as short as, or shorter than, width of mandible base, with distinct malar suture. Vertex coarsely but indistinctly punctate, surface smoother between ocelli. Ocelli reddish, forming an obtuse angle, lateral ocelli more than 2 times as far from one another as either is from anterior ocellus. Antennae clearly shorter than body; first flagellomere 2.6 times longer than apically wide and about as long as second, distal flagellomeres 2.0 times as long as broad and with only a single rank of placodes.

Mesosoma (Figs 4, 5). Lateral areas of pronotum with two transverse slightly scrobiculate furrows, median area and dorsolateral and ventrolateral corners rugulose, otherwise smooth. Mesonotum densely and distinctly punctate, punctures becoming coarser in anterior and posterior courses of notauli. Scutoscutellar scrobe formed by 9–10 sometimes partially confluent pits. Scutellum triangular, largely smooth, except for hair punctures; lunules subsemicircular to subtriangular, entirely smooth and shining, delimited anteriorly by strongly scrobiculate curved furrows. Dorsolateral areas of propodeum weakly sculptured and densely setose, posterolateral areas rugulose and largely devoid of setae; areola well defined posteriorly, open anteriorly but indicated by convergent rugae; with transverse rugae or carinae on its inner surface. Hind tibiae on outer surface with scattered spines. Inner hind tibial spurs 1.4–1.5 times longer than outer and 0.4–0.5 times as long as hind basitarsus.

Wings. Fore wing (Fig. 10) with pterostigma 2.5–2.6 times longer than wide and mostly pale translucent, pigmented only on its margins. R1 ~0.8–0.9 times the length of pterostigma and 0.6 times the length between apex of pterostigma and end of 3RS fold. Vein r distinctly longer than 2Rs, 1.3–1.4 times its length and weakly curved, meeting 2RS at a distinct obtuse angle.

Metasoma. Tergite I approximately as long as broad, to only slightly longer, almost equally broad anteriorly as posteriorly, anteriorly smooth and rugose to rugulose posteromedially. Second tergite largely smooth, with a very weakly but distinctly sculptured curved area (Figs 6, 7), 3.1–3.2 times broader than medially long. Remaining tergites entirely smooth. Hypopygium about two-thirds as long as ovipositor sheaths, strongly acuminate apically, medially desclerotised over its entire length. Ovipositor sheaths (Fig. 8) about as long as metasoma and 1.4–1.5 times as long as hind tibia, weakly decurved and hairy over most of length.

Male

Similar to female but with slightly smaller body size, 2.3–3.2 mm long; with relatively longer antennae and first metasomal tergite slightly tapering apically.

Biology

Apanteles alexanderi has been recorded as parasitising several species of phycitine moths in South America, including *C. cactorum*. Almost all specimens assigned to this species during the current study were reared from larvae of the phycitine genus *Tucumania*, with a few exceptions for which there are no host associations. Larvae pupate in a whitish ovoid mass (Fig. 9). The previously reported associations of *A. alexanderi* with *Cactoblastis doddi* Heinrich, *Salambona analamprella* (Dyar), *Plutella xylostella* (Linné), *Argyrotaenia loxonephes* (Meyrick) and *Argyrotaenia sphaleropa* (Meyrick) (Pemberton and Cordo 2001) need to be revisited.

Distribution

Specimens of *A. alexanderi* studied here are distributed from central to northern Argentina (Fig. 2).

Comments

According to Brèthes (1922) the type series was collected in Carmen de Patagones, Argentina, in January 1921 and, based on his original labels, it was collected by V. B. Alexander. Later, Blanchard (1935) mentioned that he himself collected the type series sent to Brèthes for study. It is possible that both Blanchard and Alexander conducted the collecting trip together since both of them were involved in the survey of potential enemies of prickly pear in Argentina to be introduced into Australia. Fortunately, Blanchard kept some specimens collected in the same locality and probably during the same collecting trip in his personal collection. After Blanchard's death, his collection (including these non-type specimens of *A. alexanderi*) was donated to the entomological collection of the Museo Argentino de Ciencias Naturales. These specimens, collected one week before the type series, were used for comparison with the new specimens applying methodologies which are unsuitable for type specimens (i.e. gold-palladium coating for SEM images). The types examined by Brèthes were labelled as lectotype and paralectotype by P. Marsh; however, to our knowledge the designation was never published.

Apanteles opuntiarum Martínez & Berta, sp. nov.

(Figs 11–16)

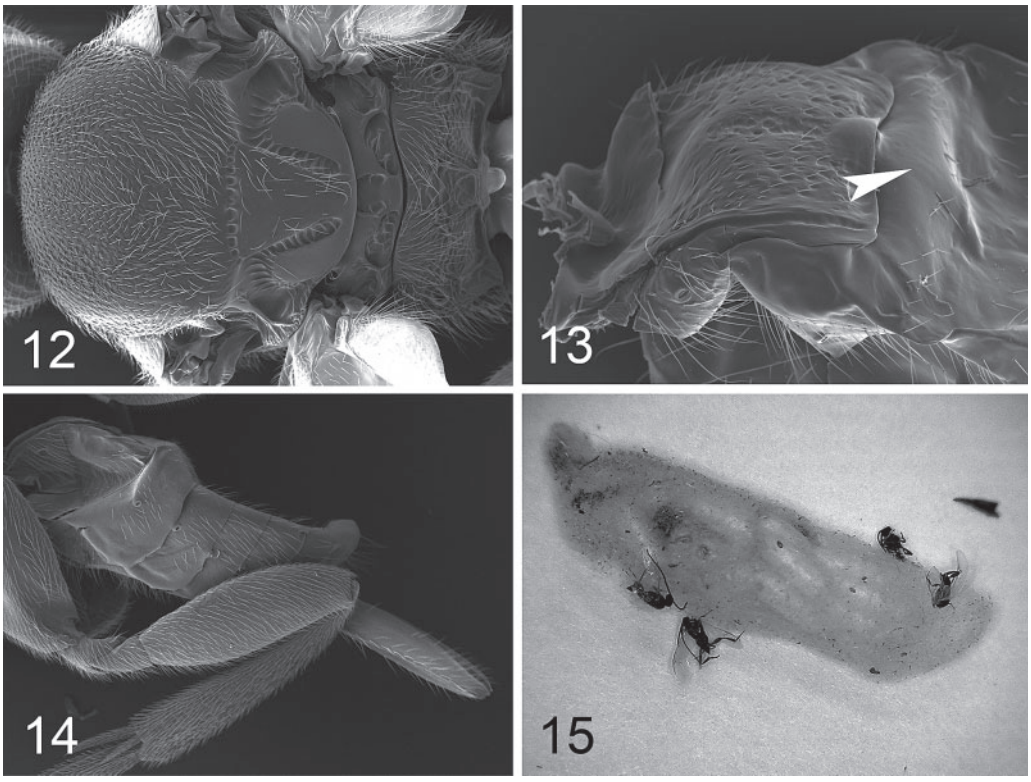
Material examined

Holotype female. ARGENTINA, Corrientes, Rt Nac. 123, 32 km S San Salvador, 29°33′29.8″S, 57°30′07.2″W; 26.ii.2009; Varone, Logarzo, from *C. cactorum* larvae on *O. elata* var. *elata* (DNA voucher no. CNIN1107; GenBank accession no. JX566773) (MACN).

Paratypes. ARGENTINA. **Catamarca:** 2 females, Chumbicha, 28°51′25.6″S, 66°13′50.2″W, 27.viii.2007, Logarzo, Palottini, from larvae of *C. cactorum* on *O. ficus-indica* (DNA voucher nos CNIN1124, CNIN1125; GenBank accession nos JX566780–81) (MACN, IFML). **Córdoba:** 1 female, El Fortín, 31°57′45.3″S, 62°20′15.1″W, 03.ii.2008, Logarzo, Palottini, from larvae of *C. cactorum* on *O. megapotamica* (DNA voucher no. CNIN1123; GenBank accession no. JX566779) (MACN). **Corrientes:** 1 female, 1 male, same data as holotype (DNA voucher nos CNIN1106, CNIN1108; GenBank accession nos JX566772, JX566774) (MACN, IFML). **Entre Ríos:** 3 females, 9.1 km W Médanos, 33°23′09.8″S, 59°10′18.4″W, 24.ii.2009, Logarzo, Varone, from *C. cactorum* larvae on *O. elata* var. *elata* (DNA voucher nos CNIN1109, CNIN1110, CNIN1111; GenBank accession nos JX566775–76) (MACN, IFML); 1 female, Conquistadores, Rt 127, 30°36′01.7″S, 58°28′00.3″W, 25.ii.2009, Logarzo, Varone, from larvae of *C. cactorum* on *O. elata* var. *elata* (DNA voucher no. CNIN1116; GenBank accession no. JX566782) (MACN); 2 females, Nogoyá, 32°22′14.3″S, 59°37′34.0″W, 27.x.2009, Logarzo, Moore, from unidentified lepidopteran larvae on *O. megapotamica* (DNA voucher nos CNIN1118, CNIN1119; GenBank accession nos JX566784–85) (MACN, IFML). **Salta:** 1 female, 5 km NO El Carril, Rt 68, 25°02′58.5″S, 65°29′47.4″W, 15.xii.2008, Varone, Logarzo; from larvae of *C. cactorum* on *O. ficus-indica amyclaea* (DNA voucher no. CNIN1117; GenBank accession no. JX566783) (MACN). **Tucumán:** 2 females, near Concepción, 27°23′19.7″S, 65°35′45.3″W, 06.iii.1009, Logarzo, Varone, from larvae of *C. cactorum* on *O. ficus-indica* (DNA voucher nos CNIN1112, CNIN1113; GenBank accession nos JX566777–78) (MACN, IFML).

Other material examined. ARGENTINA. **Catamarca:** 6 females, 3 males, Chumbicha, 28°51′25.6″S, 66°13′50.2″W, 27.viii.2007, Logarzo, Palottini, from larvae of *C. cactorum* on *O. ficus-indica* (MACN). **Córdoba:** 4 females, Trompa de Elefante, 31°24′14″S, 64°35′26.9″W, 07.v.2009, Logarzo, Guala, from larvae of *C. cactorum* on *O. megapotamica* (MACN); 11 females, 2 males, Villa Quilino, 30°12′15″S, 64°28′32″W, 15–17.xii.2008, Varone, Logarzo, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 7 females, El Fortín, 31°57′45.3″S, 62°20′15.1″W, 03.ii.2008, Logarzo, Palottini, from larvae of *C. cactorum* on *O. megapotamica* (MACN); 16 females, 2 males, Santa Coloma, Tanti, 31°20′54″S, 64°31′56″W, 10.i.2008, Logarzo, from larvae of *C. cactorum* on *O. ficus-indica amyclaea* (MACN); 2 females, 2 males, between Athos Pampa and La Cumbrecita, 31°59′04″S, 64°40′15.3″W, 1062 m, 26.x.2008, Zamudio, from *Cactoblastis* sp. larvae on *O. ficus-indica* (IFML); 2 females, 2 males, Quilino, 30°12′54.8″S, 64°29′25.4″W, 416 m, 27.x.2008, Zamudio, from *Cactoblastis* sp. larvae from *O. ficus-indica* (IFML); 2 females, 2 males, Luyaba, 32°09.193′S, 65°03.761′W, 687 m, 15.iii.2011, Zamudio, from *Cactoblastis* sp. larvae, on *O. ficus-indica* (IFML). **Corrientes:** 20 females, 5 males, 32 km S San Salvador, Rt 123, 29°33′29.8″S, 57°30′07.2″W, 26.ii.2009, Varone, Logarzo, from larvae of *C. cactorum* on *O. elata* var. *elata* (MACN); 3 males, Rt Nac. 12 near Empedrado, 28°13′11″S, 58°43′37″W, 62 m, 3.v.2008, Zamudio, from *Cactoblastis* sp. larvae on *O. discolor* (IFML) **Entre Ríos:** 3 females, 2 males, Nogoyá, 32°22′14.3″S, 59°37′34″W, 27.x.2009, Logarzo, Moore, from unidentified lepidopteran larvae on *O. megapotamica* (MACN); 6 females, 17 km S Concordia, 31°33′10″S, 59°04′44.6″W, 10.xii.2008, Logarzo, Varone, from larvae of *C. cactorum* on *O. cardiosperma* (MACN); 27 females, 9 males, 9.1 km W Médanos, 33°23′09.8″S, 59°10′18.4″W, 24.ii.2009, Logarzo, Varone, from larvae of *C. cactorum* on *O. elata* var. *elata* (MACN); 10 females, 7 males, 2.2 km E El Pingo, 31°34′46.5″S, 59°52′16.7″W, 25.ii.2009, Logarzo, Varone, from larvae of *C. cactorum* on *O. elata* var. *elata*? (MACN); 10 females, 3 males, Conquistadores, Rt 127, 30°36′01.7″S, 58°28′00.3″W, 25.ii.2009, Logarzo, Varone, from larvae of *C. cactorum* on *O. elata* var. *elata*? (MACN); 18 females, 2 males, Diamante-Strobel, 19.vi.2003, Zamudio, Briz, from *Cactoblastis* sp. larvae on *O. elata* (IFML); 16 females, 1 male, Diamante, 32°3′43.7″S, 60°38′39.61″W, 27.iii–3.vi.2006, Campos, on *C. cactorum* (IFML). **Jujuy:** 6 females, 4 males, Rt9, 1 km S El Carmen, 24°23′37.7″S, 65°16′16.6″W, 15.xii.2008, Logarzo, Varone, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 7 females, 1 male, Purmamarca, 23°44′46.7″S, 65°28′10.1″W, 2225 m, 18.i.2012, Varone, Cuadra, from larvae of *C. doddi* on *O. sulfurea* (MACN); 2 females, 2 males, Los Alisos, Jan. 1991, Neder, Arce, from *C. cactorum* larvae (INBIAL-UNJu); 1 female, Hornillos, 19.vi.2010, Neder, from *Cactoblastis* sp. larvae on *O. sulfurea* (INBIAL-UNJu); 2 females, 2 males, near Tumbaya, 23°52′34.3″S, 65°27′12.3″W, 2031 m (INBIAL-UNJu); 2 females, 2 males, near Maimará, 23°39′40.6″S, 65°26′12.1″W, 2420 m (INBIAL-UNJu); 2 females, 2 males, near Purmamarca, 23°40′30″S, 65°26′40″W, 2369 m, 20–23.iii.2010, Zamudio, from *Cactoblastis* sp. larvae on *O. sulfurea* (IFML); 2 females, 2 males, El Carmen, A° Severino, 24°19′45.6″S, 65°14′58.7″W, 1196 m, 21.iii.2010, Zamudio, from *Cactoblasitis* sp. larvae on *O. sulfurea* (IFML). **La Rioja:** 7 females, 2 males, San Pedro, 28°39′49.6″S, 66°55′14″W, Varone, Logarzo, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 4 females, 5 males, Rt Nac 40 between Famatina and Chilecito, 29°00′05″S, 67°28′37″W, 1226 m, 10.iv.2008, Zamudio, from *Cactoblastis* sp. larvae on *O. sulfurea* (IFML). **Salta**: 1 female, Rt 9, 10 km SE Juramento, 25°12′27.6″S, 64°56′45.5″W, 13.xii.2008, Varone, Logarzo, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 6 females, 5 km NO El Caril Rt 68, 25°02′58.5″S, 65°29′47.4″W, 15.xii.2008, Varone, Logarzo, from larvae of *C. cactorum* on *O. ficus-indica amyclaea* (MACN); 5 females, 9 males, between Salta and Cafayate, Arroyo Ayuza, 25.viii.2002,

Fig. 11. *Apanteles opuntiarum*, habitus of female in lateral view.



Figs 12–15. *Apanteles opuntiarum. 12*, mesosoma in dorsal view; *13*, first and second metasomal median tergites in dorsolateral view; *14*, hind leg and metasoma showing ovipositor sheaths in lateral view; *15*, cocoon mass. Arrow indicates the entirely smooth second metasomal tergite.

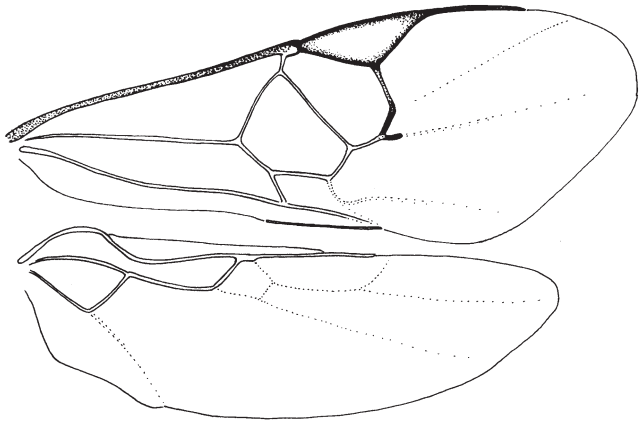


Fig. 16. *Apanteles opuntiarum*, wings.

Zamudio, from *Cactoblastis* sp. larvae on *O. sulfurea* (IFML). **San Juan:** 36 females, 10 males, Balde del Rosario, Rt Prov. 510, 30°18′58″S, 67°41′56″W, 1174 m, 11.iv.2008, Zamudio, from *Cactoblastis* sp. larvae on *O. sulfurea* (IFML). **San Luis:** 11 females, 25 km S Merlo, 24.xii.2008, Logarzo, from larvae of *C. cactorum* on *O. ficus-indica* (MACN). **Santa Fe:** 6 females, 6 males, 14 km NW El Trebol, 32°12′56.0″S, 61°41′19.2″W, 03.ii.2008, Logarzo, Palottini, from larvae of *C. cactorum* on *O. ficus-indica amyclaea* (MACN); 11 females, 2 males, Rt Nac. 11 between Las Garzas and Reconquista, 28°58′51″S, 59°34′52″W, 59 m, 4.v.2008, Zamudio, from *Cactoblastis* sp. larvae on *O. elata* (IFML); 5 females, 5 males, Sunchales, 30°54′49.1″S, 61°33′14.2″W, 61 m, 25.iii.2009, Zamudio, from *Cactoblastis* sp. larvae on *O. elata* (IFML); 5 females, 5 males, Curupaity, 30°23′38.7″S, 61°39′02.7″W, 93 m, 25.iii.2009, Zamudio, from *Cactoblastis* sp. larvae on *O. ficus-indica* (IFML). **Santiago del Estero:** 5 females, 1 male, El Virqui, 28°11′55.6″S, 65°28′06.7″W, 23.viii.2007, Logarzo, Palottini, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 4 females, 1 male, El Zanjón, 23.viii.2007, Logarzo, Palottini, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 8 females, 7 males, Rt Nac. 34, 2 Km east Vilmer, 27°47′25″S, 64°07′05″W, 175 m, 6.v.2008, Zamudio, from *Cactoblastis* sp. larvae on *O. discolor* (IFML); 8 females, 6 males, Robles, 27°46′30″S, 64°08′44″W, 178 m, 17.vii.2008, Zamudio, from *Cactoblastis* sp. larvae on *O. ficus-indica* (IFML); 2 females, 2 males, Vilmer, 27°45.967′S, 64°09.566′W, 190 m, 3.iv.2011, Zamudio, from *C. cactorum* larvae on *O. ficus-indica* (IFML). **Tucumán:** 6 females, near Concepción, 27°23′19.7″S, 65°35′45.3″W, 06.iii.2009, Logarzo, Varone, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 13 females, near La Cocha, 27°47′53.9″S, 65°35′17.3″W, 28.ii.2009, Logarzo, Varone, from larvae of *C. cactorum* on *O. ficus-indica* (MACN); 43 females, 46 males, Burruyacu, Gdor. Piedrabuena, 26°44′18.48″S, 64°38′26.57″W, 6.ii.2005, Zamudio, from *C. cactorum* larvae (IFML); 15 females, 15 males, Burruyacu, La Virginia, 17.xii.2002, Zamudio, from *C. cactorum* larvae on *O. ficus-indica* (IFML); 4 females, 3 males, Leales, Las Tusquitas, 27°7′0.46″S, 64°55′37.53″W, 7.vii.2007 from *Cactoblastis* sp. on *O. ficus-indica* (IFML); 29 females, 10 males, 11.i.–12.ii.2007, Zamudio (IFML); 3 females, Leales, Tres Pozos, 18.x.2006, Zamudio, from *C. cactorum* larvae (IFML).

Diagnosis

Apanteles opuntiarum can be easily distinguished from *A. alexanderi* and all other species of *Apanteles* that attack phycitine moths on *Opuntia* by the length of the ovipositor sheaths, which are distinctly shorter than the metasoma (Fig. 14). Among other South American species of *Apanteles*, it is morphologically close to *A. haywardi* Blanchard, from which it can be distinguished by the propodeum sculpture, with a well defined areola with transverse rugae in *A. haywardi*. Additionally, specimens of *A. haywardi* behave as solitary endoparasitoids of microlepidoptera attacking *Ceroplastes* (Hemiptera : Coccidae) (Blanchard 1947). Following the only available key to North American species of *Apanteles* (Muesebeck, 1921), *A. opuntiarum* runs to *A. leucostigmus*, currently known to be a complex of cryptic species parasitic on hesperiid butterflies (Janzen *et al*. 2009). Although we were not able to study specimens of the *A. leucostigmus* complex, *A. opuntiarum* differs by its distinct biology and at least 9.6% divergence in COI sequences (Fig. 1).

Female (*Fig.* 11)

Body length (excluding ovipositor and sheaths) 3.0 mm (2.4–3.7 mm). Fore wing length 2.9 mm (2.3–3.7 mm).

Colour. General body colour black, except for lighter brown palps, fore leg beyond coxa, mid leg beyond proximal 0.8 of femur, proximal 0.7 of hind tibia, and hind tarsus. Laterotergites of first and second metasomal segments brown. Tegulae pale yellow. Wings hyaline, most veins almost unpigmented except C-Sc-R, stigma, R1, r, 2RS, 2M, 1Cua-b, which are distinctly darker, brown. Pterostigma pigmented only marginally, pale translucent centrally.

Head. Face shallowly punctate, wider than high, separated from clypeus by a furrow, clypeus with a few poorly defined longitudinal rugae. Malar space as short as, or shorter than, width of base of mandible, with distinct malar suture. Vertex punctate-rugose, somewhat slightly transversely sculptured. Ocelli reddish brown, in a strongly obtuse angle, lateral ocelli more than 2 times as far from one another as either is from anterior ocellus. Antennae clearly shorter than body; first flagellomere 2.6 times longer than apically wide and 1.1–1.2 times longer than second, distal flagellomeres 2.0 times as long as broad and with only a single rank of placodes.

Mesosoma (Fig. 12). Lateral areas of pronotum with two transverse slightly scrobiculate furrows, median area and dorsolateral and ventrolateral corners rugulose, otherwise smooth. Mesonotum densely and distinctly punctate, punctures becoming coarser in anterior and posterior courses of notauli. Scutoscutellar scrobe formed by 9–10 sometimes partially confluent pits. Scutellum triangular, largely smooth, except for hair punctures; lunules subsemicircular to subtriangular, entirely smooth and shining, delimited anteriorly by strongly scrobiculate curved furrows. Dorsolateral areas of propodeum weakly sculptured and densely setose, posterolateral areas rugulose and largely devoid of setae; areola well defined posteriorly, open anteriorly but indicated by convergent rugae; with transverse rugae or carinae on its inner surface. Hind tibia with scattered spines on outer surface. Inner hind tibial spur ~1.5 times longer than outer spur and ~0.4 times as long as hind basitarsus.

Wings. Fore wing (Fig. 16). Pterostigma 2.5 times longer than wide and mostly pale translucent, pigmented only on its margins. R1 ~0.8 times the length of pterostigma and 0.6 times the length between apex of pterostigma and end of 3RS fold. Vein r distinctly longer than 2Rs, 1.7–1.8 times its length and weakly curved, meeting 2RS at a distinct obtuse angle.

Metasoma. Tergite I approximately as long as broad, to only slightly longer, almost equally broad anteriorly as posteriorly, punctate and sometimes rugulose medially, with two apicolateral transverse depressions. Second tergite entirely smooth and shining (Fig. 13), 3.2 times as broad as medially long and with weakly convex posterior margin. Remaining tergites also entirely smooth. Hypopygium about as long as ovipositor sheaths, medially desclerotised into a series of expandable folds over entire length. Ovipositor sheaths (Fig. 14) about as long as hind femur, hairy over most of length, somewhat thick and stout.

Male

Similar to female but slightly smaller body size, 2.3–3.2 mm long, with relatively longer antennae and first metasomal tergite slightly tapering apically.

Biology

Apanteles opuntiarum is a gregarious endoparasitoid of cactus-feeding moths, specially *C. cactorum*, with a single record from *Cactoblastis doddi*. Almost all specimens studied in this work were reared from larvae of *Cactoblastis*, except one sample which was reared from an unidentified lepidopteran larva. Larvae of *Cactoblastis* were collected from at least six species of *Opuntia.* Pupation occurs in ovoid yellowish cocoon masses that are slightly more compact than those of *A. alexanderi* (Fig. 15).

Distribution

Based on the specimens studied here, *A. opuntiarum* occurs in north and central Argentina (Fig. 2). However, this species is expected to occur more extensively within the distribution of *C. cactorum* in Argentina, Paraguay, Uruguay and southern Brazil.

Comments

The specimens chosen to be the type series of *A. opuntiarum* are those from which COI sequences were obtained.

Etymology

The specific epithet refers to the plant genus (*Opuntia*) where the host is commonly encountered, meaning ‘from the prickly pears’.

Key to *Apanteles* species parasitic on cactus-feeding moths in South America

Ovipositor sheaths shorter than metasoma and about as long as hind tibia (Figs 11, 14), second metasomal tergite smooth and polished (Fig. 13) ..... *Apanteles opuntiarum* Martínez & Berta, sp. nov.

Ovipositor sheaths about as long as metasoma and distinctly longer than hind tibia (Figs 3, 8), second metasomal tergite with weak but distinct rugosities (Figs 6, 7) ..... *A. alexanderi* Brèthes

Discussion

According to the excellent review provided by Pemberton and Cordo (2001), *A. alexanderi* has been recorded as parasitising at least eight microlepidopteran species on *Opuntia*. Almost all specimens that were confidently identified in this work as *A. alexanderi* were reared from specimens of the genus *Tucumania*. The first mention of *A. alexanderi* associated with *C. cactorum* is that of Parker *et al*. (1953), who reported this association from Uruguay. Subsequent records mentioning this association (De Santis 1967; Mann 1969; Zimmermann *et al*. 1979) are species lists with no original information and are probably based on the first report. All specimens reared from *Cactoblastis* were identified as *A. opuntiarum*. These results suggest a strong host preference for *Tucumania* and *Cactoblastis* by *A. alexanderi* and *A. opuntiarum*, respectively.

Our morphological observations were entirely congruent with the information provided by the barcoding data, with ~6% interspecific and less than 1% intraspecific divergence for both species. The arbitrary limit of 2% divergence traditionally employed to separate 'barcoding species' (Hebert *et al*. 2003) thus seems to easily apply for this species complex.

The host range of both *A. alexanderi* and *A. opuntiarum*, as they relate to other cactus-feeding moths in South America, remains uncertain. The association of *A. alexanderi* with other phycitine moths needs to be confirmed. A recent study of what was thought to be a polyphagous species, *Apanteles leucostigmus*, revealed that it actually constitutes a complex of cryptic and oligophagous species associated with several hesperiid butterflies in tropical America (Janzen *et al*. 2009). Similar results have recently been obtained for the genus *Anicetus* (Hymenoptera : Encyrtidae) associated with scale insects (Hemiptera : Coccidae) (Zhang *et al*. 2011) and for tachinid parasitoid flies (Smith *et al*. 2006). It is possible that the complex of cactus-feeding Lepidoptera supports a similar and richer community of microgastrine parasitoids than has been previously assumed.

The finding of a new species, *A opuntiarum*, attacking the prickly pear moth, *Cactoblastis cactorum*, in Argentina is significant for our understanding of the potential of microgastrine species as possible control agents of *C. cactorum* outside its native distribution. A re-evaluation of the potential of these species to control the prickly pear moth where it has become a serious concern for endemic populations or economically important species of *Opuntia* is therefore strongly suggested.

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