BioControl (2014) 59:547–556
DOI 10.1007/s10526-014-9595-6

# Parasitism of the "Fuller's rose weevil" *Naupactus cervinus* by *Microctonus* sp. in Argentina

**Marcela S. Rodriguero · Daniel A. Aquino · Marta S. Loiácono · Agustín J. Elías Costa · Viviana A. Confalonieri · Analía A. Lanteri**

Received: 20 January 2014 / Accepted: 16 June 2014 / Published online: 1 July 2014


**Abstract** We report the occurrence of an unidentified species of the wasp *Microctonus* Wesmael (Hymenoptera: Braconidae) parasitizing adults of the Fuller's rose weevil *Naupactus cervinus* (Boheman) (Coleoptera: Curculionidae), a widespread pest of economically important crops included in the South American tribe Naupactini. Cytochrome *c* oxidase subunit I-based phylogenetic analysis indicates that the parasitoid is closely related to *Microctonus hyperodae* Loan. Their first instar larvae show slight morphological differences with this species. Superparasitism by first instar larvae occurred at low frequency. Some teratocytes were observed. *Microctonus* sp. and its host were infected with different strains of the reproductive parasite *Wolbachia pipientis* Hertig (Rickettsiales: Rickettsiaceae), although the bacterial lineage harbored by the wasp coincides with that infecting most parthenogenetic Naupactini. This multipartite association (weevil bearing both *Wolbachia* and *Microctonus*, and *Microctonus* bearing *Wolbachia*) opens challenging perspectives for future research on biological interactions and biological control.

**Keywords** Naupactini · Braconidae · Parasitoid · *Microctonus* · *Wolbachia* · Thelytoky

Handling Editor: Dirk Babedreier

**Electronic supplementary material** The online version of this article (doi:10.1007/s10526-014-9595-6) contains supplementary material, which is available to authorized users.

M. S. Rodriguero (✉) · A. J. Elías Costa · V. A. Confalonieri
Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires,- IEGEBA (CONICET-UBA), Av. Intendente Güiraldes y Av. Costanera Norte s/n, Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina
e-mail: rodriguero@ege.fcen.uba.ar

D. A. Aquino · M. S. Loiácono · A. A. Lanteri
División Entomología, Museo de La Plata, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n, 1900 La Plata, Argentina

## Introduction

The knowledge of natural enemies of weevils is crucial to develop biological control strategies (Lanteri et al. 1998). The available information is limited to pathogens and parasites of some introduced species that cause important damage to different crops and plantations, e.g. the "cotton boll weevil" *Anthonomus grandis* Boheman in the USA, the "*Eucalyptus* weevil" of the genus *Gonipterus* in Europe and the Americas, and the "Argentine stem weevil" *Listronotus bonariensis* (Kuschel) in New Zealand (Lanteri et al. 2002). Potential changes in geographic ranges and relative abundances of insect pests like *Naupactus cervinus*, wrought by modern production practices, crop expansion and climate change emphasize the

need to design sustainable pest management programs. Biological control of weevils harmful for alfalfa and other cultivated Fabaceae (e.g. *Hypera postica* and *Sitona discoideus*) have been applied with success in the USA, Europe and New Zealand using braconid species of the genus *Microctonus* (Goldson et al. 1993; Barratt 2004). The finding of a *Microctonus* species parasitizing the worldwide distributed "Fuller's rose weevil" opens a new perspective for its control in the countries where it has been introduced.

Natural enemies of weevils include bacteria, protozoans, fungi, mites and insects (Lanteri et al. 1998). Insect parasitoids are of major importance due to their diversity and potential as biological control agents. They exploit all developmental stages of the weevil, which is unable to complete its life-cycle and/or dies before reproduction. Most weevil parasitoids belong to the hymenopteran superfamilies Chalcidoidea and Ichneumonoidea (especially Braconidae), and to the dipteran family Tachinidae (Lanteri et al. 1998).

In Argentina, larvae and adults of weevil pests have been found parasitized by over 15 braconid species (Lanteri et al. 1998). Among these, the South American species *Microctonus hyperodae* Loan (Braconidae: Euphorinae) was imported into New Zealand in 1991 as a biocontrol agent of adult *L. bonariensis* (Goldson et al. 1993). Another species of this genus, the Palaearctic *Microctonus aethiopoides* Loan, was also released in New Zealand in 1982 for the biocontrol of the Mediterranean weevil *S. discoideus* Gyllenhall (Barratt 2004). Wasps of the latter species from Ireland were used for the control of the weevil *Sitona lepidus* Gyllenhall in New Zealand (McNeill et al. 2006). These *Microctonus* species are solitary endoparasitoids with similar morphology and life cycle (Loan and Holdaway 1961). Occasional superparasitism has been noted, with supernumeraries being eliminated in the first larval stage. Adult weevils usually die soon after the mature parasitoid larva emerges. Then, the larva spins a cocoon close to the soil surface and roots where the pupa develops, and the adult emerges between ten and 19 days (average 15.6 days). *Microctonus hyperodae* reproduces by thelytoky (males absent or extremely rare) and apomixis (parthenogenesis without meiosis) (Iline and Phillips 2004), *M. aethiopoides* is arrhenotokous in most European countries (unfertilized eggs produce males and fertilized eggs produce females) (McNeill et al. 1993), and the biotype of *M. aethiopoides* in Ireland is thelytokous and gregarious (McNeill et al. 2006). Moreover, *M. hyperodae* is monophagous to oligophagous (Goldson et al. 1992) whereas *M. aethiopoides* is polyphagous (Goldson et al. 2005). There is also an endemic *Microctonus* species in New Zealand, *Microctonus zealandicus* Shaw, which is gregarious (2–6 larvae per host capable of reaching the pupal stage), arrehenotokous, and specific to the native broad-nosed weevil *Irenimus aequalis* (Broun) (McNeill et al. 1993; Shaw and Huddleston 1991).

In the present contribution we study a parasitoid of adult females of the "Fuller's rose weevil" *N. cervinus* (Boheman) (Curculionidae: Entiminae: Naupactini), a parthenogenetic species native to South America and distributed worldwide due to its association with crops of economic importance, such as alfalfa, citrus and ornamental plants (Rodriguero et al. 2010a, b). Since *N. cervinus* is infected with the reproductive parasite *Wolbachia pipientis* Hertig (hereafter *Wolbachia*) (Rodriguero et al. 2010a), it is interesting to check if the parasitoid could be a potential vehicle for the horizontal transfer of this bacterium (Werren et al. 1995), thus contributing to its pandemic distribution.

## Materials and methods

### Sample collection

Adult females of *N. cervinus* were collected from 18 locations in Argentina and Brazil (N = 92) using sweeping nets during summer 2004–2013 (Table S1). They were dissected under a stereo microscope and examined for the presence of parasitoids. All larvae were preserved in 100 % ethanol and stored at 4 °C.

### Molecular identification

#### *PCR and sequencing*

The parasitoid was identified by sequencing a fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI), the standard DNA barcode for animal species (Hebert et al. 2003a, b). Total genomic DNA was extracted from two bulks of four larvae each using the REDExtract-N-Amp Tissue kit (Sigma-Aldrich). A segment of ca. 700 bp of the COI was amplified using the specific primers S1718 and A2442 (Rodriguero et al. 2010b). Amplification was carried out in a

50 µl-volume reaction with 50–100 ng of DNA used as template, 0.5 µM of each primer, 0.1 mM of each dNTP (Promega), 3.0 mM $MgCl_2$, 1.0 unit of Taq polymerase and 1× reaction buffer (Invitrogen). The reactions were performed in a Verity thermal cycler (Applied Biosystems), under the conditions described by Rodriguero et al. (2010b). PCR products were purified with a QIAquick Gel Extraction Kit (Qiagen Inc.). DNA was sequenced using a 3130-XL Automatic Sequencer (Applied Biosystems). Standard chromatographic curves of forward and reverse sequences were edited with the program BIOEDIT v. 7.0.5.3 (Hall 1999).

*Phylogenetic analysis*

For parasitoid identification we followed the procedure of Sasakawa et al. (2011). First, we identified the family to which this parasitoid belongs through comparison of the sequence obtained with previously published sequence data using BLASTn sequence analysis (Altschul et al. 1990, 1997). Second, we accomplished a genus-level analysis including COI sequences of our samples and other members of Braconidae. Third, we compared genetic distances of COI sequences between our samples and putative congeneric species and tested whether our genus-level identification was compatible in terms of genetic variability.

Cytochrome *c* oxidase subunit I sequences of Hymenoptera were retrieved from GenBank (NCBI) (see accession numbers in Fig. 2). The Clustal W software v. 2.0S was used for sequence alignments (Thompson et al. 1994), with default parameter settings. The best molecular evolution model was chosen on the basis of the Akaike information criterion (Posada and Buckley 2004) using MRMODELTEST software v. 2.2 (Nylander 2004).

Phylogenetic analysis included 50 sequences of five genera and one representative of Ichneumonidae was used as outgroup. Our complete dataset included 676 aligned nucleotide positions. The GTR + I + G model was selected as the best fit model of nucleotide substitution.

We applied Bayesian inference based on Metropolis coupled Markov chain Monte Carlo algorithm implemented in MRBAYES v. 3.2.2 (Ronquist et al. 2012). Two independent analyses were run with a random starting tree over 2,000,000 generations with a sample frequency of 500. The tree space was explored using one cold and three incrementally heated chains, with temperature set to 0.20. We assessed stationarity of the cold Markov chain for all MRBAYES analyses through the standard deviation of the split frequencies. All posterior samples of a run prior to the burn-in point (at 25 % of sampled topologies) were discarded. Remaining trees were used to obtain a 50 % majority-rule consensus tree with mean branch length estimates. Node support was assessed by posterior probability (Huelsenbeck and Ronquist 2001).

Genetic distances between genera and between congeneric species were estimated by pairwise comparisons using MEGA v. 6 (Tamura et al. 2013). Several sequences were used for each species, depending on data availability.

Morphological identification

First instar larvae were cleared in 10 % KOH for 24 h, slide-mounted in Canada Balsam and examined under a light microscope LeitzWetzlar SM/LUX. Shape and size of the head, capsule and mandibles and the pattern of hypopharyngeal sclerotization are the most reliable characters for specific identification of first instar larvae of *Microctonus* (Fuester 1970). However, parasitoids usually need to be reared to the adult stage for species identification (Phillips and Emberson 1999). Mature hymenopteran larvae were not described because they do not allow taxonomic identification beyond the order level (Sasakawa et al. 2011). Both first instar and mature larvae were photographed using a Leica DFC295 digital camera attached to a stereo microscope Leica S8 APO. Digital images were mounted using free software Combine ZM (Hadley 2011) and enhanced using Photoshop.

*Wolbachia* detection

*Wolbachia* infection was surveyed through amplification of genes 16S rDNA and gatB, using the primers designed by O'Neill et al. (1992) and Baldo et al. (2006) for the host and parasitoid, respectively. We randomly chose six individuals of *N. cervinus* from the locations where the parasitoid had been found to detect the occurrence of infection, as previously reported for many populations of this weevil (Rodriguero et al. 2010a, b). Thermal profiles and negative controls were

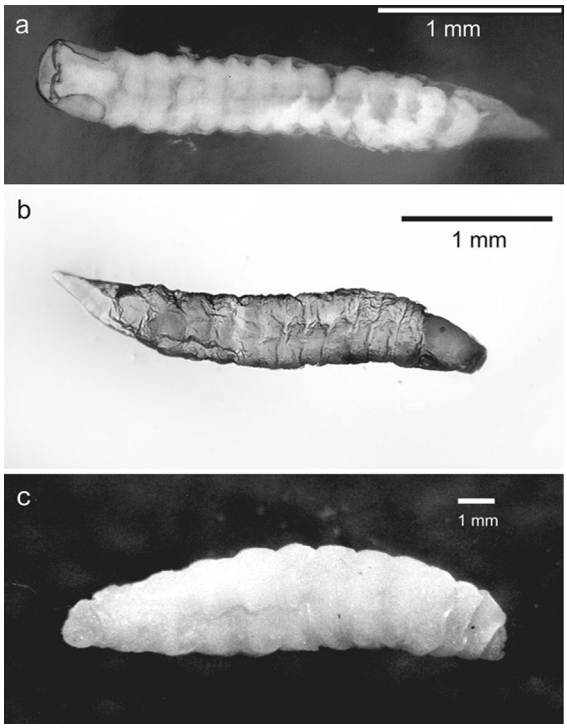


**Fig. 1** Larva of *Microctonus* sp.: **a** first instar, ventral view, scale 1 mm; **b** first instar, lateral view, scale 1 mm; **c** mature larva, lateral view, scale 1 mm

performed as previously described (Rodriguero et al. 2010a).

*Wolbachia* strain in the parasitoid was characterized by full MLST. Amplification and sequencing of coxA, fbpA, ftsZ, gatB and hcpA fragments followed those of Baldo et al. (2006). Allele number per locus was assigned after comparison with the *Wolbachia* MLST database (http://pubmlst.org/wolbachia). This strain was characterized by the combination of the MLST numbers (i.e. allelic profile or ST).

## Results

We dissected 92 *N. cervinus* adults collected from 18 localities of Argentina and Brazil, and found six first instar larvae parasitizing one weevil in La Paz (northwest of Entre Rios province, north-eastern Argentina) (Table S1; Fig. 1a, b). The percentage of parasitism in this population was 16.7 % (one of six weevils). first instar larvae had a mean length of 1.65 mm. In addition, 46 mature larvae emerged from 70 weevils collected in Tandil (southeast of Buenos Aires province, central-eastern Argentina) while being transported to the laboratory, and 22 % of them pupated, although no emergence of adults was seen under lab conditions (Fig. 1c). The percentage of parasitism in this population was 65 %. Mature larvae had a mean length of 11.4 mm. Neither first instar nor mature larvae showed signs of host immune response, such as melanization. Some teratocytes were observed in the body cavity of *N. cervinus*.

### Molecular identification of parasitoids

The larvae from *N. cervinus* share almost identical mitochondrial sequences, except for two ambiguous bases: A/G and A/G at positions 452 and 473, respectively. Because of the low number of unambiguous positions, we chose a consensus sequence to define their phylogenetic position within Hymenoptera (G at positions 452 and 473 based on the electropherogram peak heights, GenBank Accession Number KJ158158).

BLASTn homology search indicated that the COI sequence of the *N. cervinus* parasitoid shared 82–85 % nucleotide identity with the COI sequences of several Braconidae, including genera such as *Microctonus* (e.g. GenBank accession no. KJ158158.1, E-value = 0.0), *Opius* (e.g. GenBank accession no. KF474863.1 E-value = 1e-111), *Adialytus* (e.g. GenBank accession no. HQ724539.1 E-value = 3e-118), *Binodoxis* (e.g. GenBank accession no. FJ798201 E-value = 3e-118) and *Chelonus* (GenBank accession no. HQ106889.1 E-value = 3e-118). Thus, this analysis reveals that the parasitoid of *N. cervinus* belongs to Braconidae. Consequently, the subsequent genus-level analysis includes five genera of Braconidae.

The studied parasitoid falls within the genus *Microctonus*, with high nodal support (Fig. 2). The tree shows that it is more closely related to *M. hyperodae* than any other *Microctonus* species. Genetic distances between the parasitoid and other *Microctonus* spp. range between 13 and 17 % (Table 1), which is similar to those among remaining *Microctonus* species (e.g. 13–16 % between *M. zealandicus* and other *Microctonus* spp.). However, the parasitoid and other braconid genera are separated by a wider range of distance (20–26 %).

### Morphological identification of parasitoids

Based on the molecular identification of the first instar larvae parasitizing *N. cervinus*, we described the head

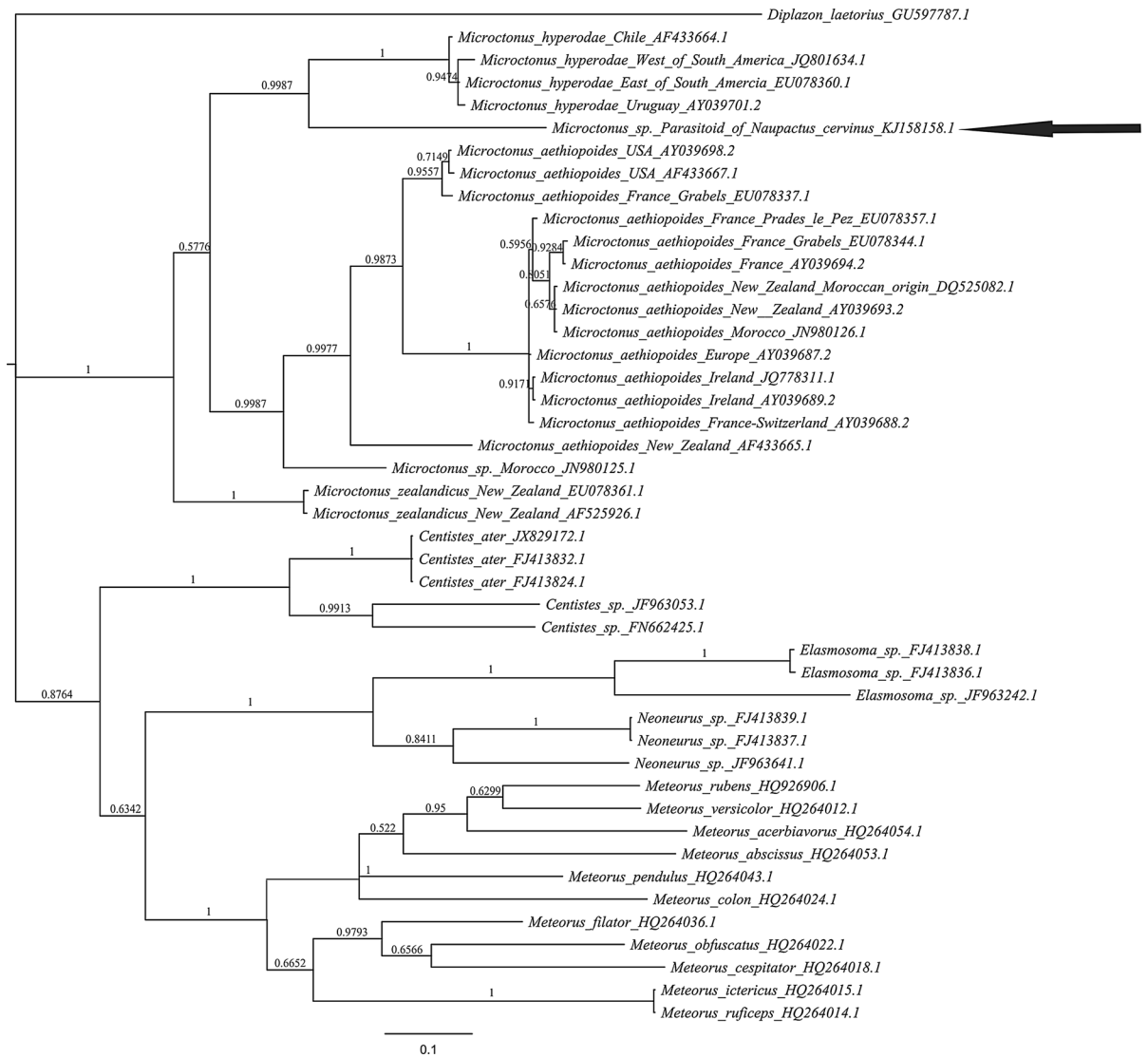


**Fig. 2** Phylogenetic position within Braconidae of parasitoid larvae from *Naupactus cervinus* adults on the basis of COI sequences indicated with an arrow. Numbers above the branches indicate node support (posterior probability). The scale bar represents the number of substitutions per site

capsule, mandibles and hypopharynx of this species of the genus *Microctonus*.

Diagnosis of *Microctonus* sp.

First instar larva 1.0–2.1 mm long, of caudate-mandibulate type (Fig. 1a). Head capsule pale-yellow, about 0.9 × as long as wide, with sides nearly straight (Fig. 1b). Mandibles large, with lateral walls basally thickened. Length/width ratio of mandibles greater than 2.5 (Fig. 3). Hypopharynx parallel-sided, not tapering from front to rear. Hypopharyngeal sclerites poorly developed (Fig. 3). Measurements taken from slide-mounted larva (μm): total body length 1,645; mandible length 100–106; mandible width 40–42; head capsule length 232; head capsule width 264.

Comparison with other *Microctonus* larvae

The first instar larva described herein is similar to that of *M. hyperodae*, except for the size of the head capsule (shorter in *M. hyperodae*: 0.82× as long as wide) and the

**Table 1** Pairwise genetic distances between species of the genus *Microctonus* and other genera of Braconidae

| Taxa | Genetic distances | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Larva from *N. cervinus* | | | | | | | | | |
| *M. hyperodae*[a] East | 0.139 | | | | | | | | |
| *M. hyperodae*[a] West | 0.145 | 0.006 | | | | | | | |
| *M. zealandicus* | 0.160 | 0.136 | 0.140 | | | | | | |
| *M. aethiopoides* from *Hypera* sp.[b] | 0.160 | 0.147 | 0.153 | 0.146 | | | | | |
| *M. aethiopoides* from *Sitona* sp.[b] | 0.171 | 0.159 | 0.163 | 0.165 | 0.075 | | | | |
| *Centistes* | 0.224 | 0.223 | 0.225 | 0.211 | 0.221 | 0.227 | | | |
| *Elasmosoma* | 0.258 | 0.249 | 0.251 | 0.243 | 0.256 | 0.257 | 0.242 | | |
| *Meteorus* | 0.206 | 0.195 | 0.199 | 0.198 | 0.186 | 0.201 | 0.225 | 0.243 | |
| *Neoneurus* | 0.243 | 0.219 | 0.221 | 0.225 | 0.227 | 0.236 | 0.241 | 0.215 | 0.206 |

Biotypes of *Microctonus* species are distinguished when sequences were available

[a] East and West biotypes of *M. hyperodae* after Iline and Phillips (2004)

[b] Biotypes of *M. aethiopoides* after their host weevil, Vink et al. (2003)

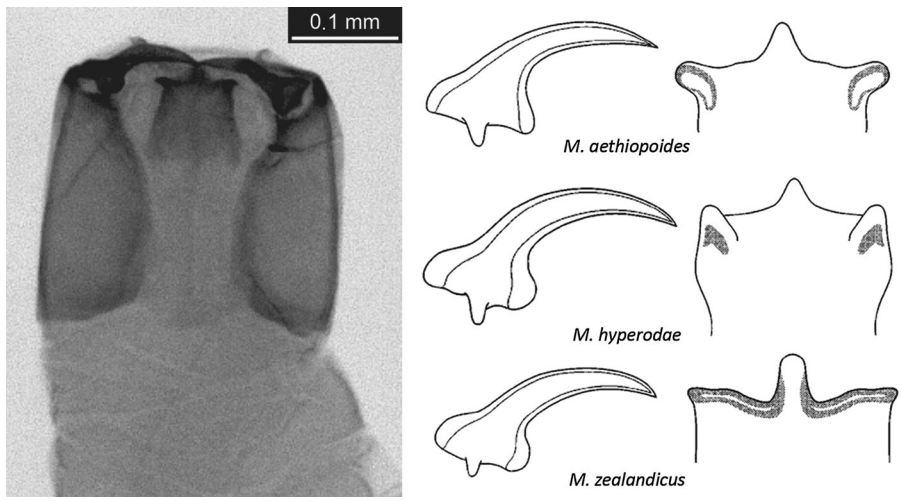


**Fig. 3** *Left* detail of head capsule (mandibles and hypopharinx) of first instar larva of *Microctonus* sp., ventral view. *Right* Drawings of mandibles and hypopharynx of other *Microctonus* species taken from Phillips and Emberson (1999), with permission

length/width ratio of mandibles (smaller in *M. hyperodae*: 2.1–2.5). When viewed ventrally, after being compressed by the microscope slide coverslip, the basal third of the mandible is similar to that of *M. hyperodae* and *M. zealandicus*, namely flared or vase-like in shape with a distinct sinuation on the outer margin (Fig. 3). However, it differs from the latter two species and resembles *M. aethiopoides* in having poorly developed hypopharyngeal sclerites, which protrude laterally from the hypopharynx (Fig. 3). The hypopharynx of the studied *Microctonus* sp. differs from that of *M. zealandicus* in the lack of a very distinctive band of sclerotization, which is effaced medially and extends across the anterior margin (Fig. 3).

## Molecular detection and identification of *Wolbachia*

We positively diagnosed *Wolbachia* infection for the parasitoids of *N. cervinus* and hosts from La Paz (N = 6) and Tandil (N = 6). The sequencing of MLST genes of the parasitoid strain yields alleles 14, 181 and 9 for loci coxA, fbpA and gatB, respectively. Unfortunately, the ftsZ and hcpA genes could not be amplified. This partial sequence type (ST) does not coincide with the one infecting *N. cervinus* (ST = 14-9-127, strain *w*Nau5), but closely resembles to the *Wolbachia* strain from most of the parthenogenetic Naupactini weevils (ST = 14-181-9, strain

*w*Nau1). It belongs to *Wolbachia* Supergroup B (Rodriguero et al. 2010a).

## Discussion

This is the first report of a parasitoid of the genus *Microctonus*, family Braconidae, infesting the weevil pest *N. cervinus*. At present, the only known parasitoids of this species are the braconid *Centistes* sp., a parasitoid of the larval stage (Artigas 1994), and the platygastrid *Fidiobia citri* (Nixon), an egg parasitoid (Morse et al. 1988). The presence of *Centistes* sp. has also been reported for another weevil pest native to South America, the "fruit weevil" *Naupactus xanthographus* (Germar) (Artigas 1994; Lanteri et al. 1998).

The genus-level analysis indicated that the parasitoid recovered from adult *N. cervinus* clusters with *M. hyperodae* with a high support. We have no hesitation to assign the species to the genus *Microctonus* since it is included within the *Microctonus* clade.

The following four species of *Microctonus* are native to South America: *M. hyperodae*; *Microctonus audax* Muesebeck from Argentina, parasitoid of *Listroderes* sp.; *Microctonus berryi* Muesebeck from Central Chile, parasitoid of Chrysomelidae; and *Microctonus brasiliensis* (Szepligeti) from Brazil, of unknown host (Loan and Lloyd 1974). *M. hyperodae* is the best known and most widespread species of the genus in South America (Loan and Lloyd 1974). It has two biotypes differentiated by genetic markers based on variation in malate dehydrogenase allozymes, originating from east (Argentina, Brazil and Uruguay) and west (Chile) of the Andes mountains (Iline and Phillips 2004; Winder et al. 2005).

Branch lengths suggest high divergence between the studied *Microctonus* sp. and *M. hyperodae* biotypes. In fact, genetic divergence between these entities are similar to values for interspecific comparisons between *Microctonus* species (Sasakawa et al. 2011). In addition, genetic distances between the parasitoid of *N. cervinus* and other *Microctonus* species are smaller than those between the former and species of other genera. The species studied here is distinguished from *M. hyperodae* by the following criteria: (1) Morphological characters at larval level are compatible with species differentiation, although the species status should be confirmed with the taxonomic study of adult specimens. On the opposite, the two highly divergent biotypes of *M. hyperodae* are morphologically identical (Phillips and Emberson 1999). (2) *M. hyperodae* seems to be a species-specific parasitoid of *L. bonariensis* since it is its only weevil host so far reported for South America. Host-preference studies carried out in New Zealand revealed that *M. hyperodae* wasps preferred to lay eggs in *L. bonariensis*, only 3 % of the parasitoids emerged from other weevil species, and the proportion of melanized larvae (signs of a host immune response) was over 40 % in the alternative hosts and about 8 % in *L. bonariensis* (Barratt 2004). (3) *M. hyperodae* and *N. cervinus* are partially sympatric in South America. However, the latter is more widespread in subtropical areas of Brazil and *L. bonariensis* in arid environments of central and southern Argentina, including Patagonia. (4) Genetic divergences between the studied parasitoid and both *M. hyperodae* biotypes exceed the limits of intraspecific variation (<4.0 % for COI sequences, Danforth et al. 1998). Moreover, this divergence is higher than that observed between the two biotypes of *M. aethiopoides* (9 %), which is a consequence of the specialization of the wasps to different host weevils, although they are still considered a single species (Vink et al. 2003, 2012; Phillips et al. 2008).

Based on the current evidence and until more detailed studies are made (e.g. lab rearing of parasitoids to the adult stage for consistent identification of species), we assume that the studied *Microctonus* sp. is a new or an already known South American species that can only be identified on the basis of adult characters. The possible species could be *M. audax* or *M. brasiliensis*, whose geographic distributions are within the natural geographic range of *N. cervinus*. Phylogeny estimates from morphological and molecular data are congruent in the sense that both suggest a close relationship between the putative new species of *Microctonus* and *M. hyperodae*.

The finding of several first instar larvae of *Microctonus* sp. in a single female of *N. cervinus* may suggest superparasitisism, but it is necessary to determine if only one larva outcompetes siblings within the host. Although *M. hyperodae* and *M. aethiopoides* are solitary endoparasitoids, they may initially be supernumerary, what has been proposed as a strategy to suppress host defences (Barrat and Johnstone 2001). Moreover, the number of supernumeraries is likely to

depend on host size. The available information precludes us from drawing firm conclusions about whether the studied *Microctonus* sp. is solitary as *M. hyperodae* and *M. aethiopoides*, or gregarious as *M. zealandicus*.

Some teratocytes were observed in the body cavity of *N. cervinus*. These cells, which are derived from the serosal membrane of parasitoid eggs, may be nutritive, immunosuppressive, or secretory and can be involved in regulating host development (Dahlman and Vinson 1993). In Euphorinae they are thought to have a clear trophic role, and are extensively fed upon by middle-instar larvae, whose mandibles are reduced or absent (Shaw and Huddleston 1991). Further studies are needed to elucidate the function of teratocytes for the parasitoid of *N. cervinus*.

The probability of success as a biological control agent in exotic locations is greater when parasitism rates are high in the native range (McNeill et al. 2006). On this account, the population of Tandil has good potential for controlling *N. cervinus* in countries where it has been accidentally introduced, such as Australia, New Zealand or the USA. Further research is needed on this issue.

Parasitoids have been suggested as one of the potential vehicles of *Wolbachia* between species. This possible route of inter-taxon transfer has received both supporting (e.g. Werren et al. 1995, i.e. highly similar *Wolbachia* strains in both host and parasitoid) or refuting (e.g. West et al. 1998, i.e. highly dissimilar *Wolbachia* strains in both host and parasitoid) evidence. In the case under study, the host and parasitoid were found to have different strains, but the strain isolated from the wasp, *w*Nau1, infects most of the parthenogenetic Naupactini species studied so far (Rodriguero et al. 2010a). Although this result makes horizontal transfer of *Wolbachia* between *Microctonus* sp. *and N. cervinus* unlikely, such transmission between the studied *Microctonus* sp. and other Naupactini hosts cannot be ruled out. It may parasitize more than one Naupactini species (same as *Centistes* sp.), thus spreading the strain *w*Nau1 among species such as *Aramigus conirostris* (Hustache) or *Atrichonotus taeniatulus* Berg. The infection of *N. cervinus* by the strain *w*Nau5 could have been acquired from another parasitoid or through other means used by *Wolbachia* to overcome interspecific barriers (e.g. Kittayapong et al. 2003).

There is a noteworthy relationship among hosts, parasitoids, environment and *Wolbachia* infection. Broad-nosed weevils of the subfamilies Entiminae and Cyclominae are among the main hosts for euphorine braconids, such as *M. hyperodae*, *M. aethiopoides* and *M. zealandicus* (Shaw 1988). Some of them are flightless species associated with native or exotic grasslands, pastures and alfalfa, and reproduce by parthenogenesis (Lanteri and Normark 1995; Marvaldi et al. 2012), same as their parasitoids. This kind of reproduction facilitates the dispersal and colonization of new areas outside the species' natural range (Guzmán et al. 2012; Lanteri et al. 2013). In the context of the relationship among weevil pests, braconid parasitoids and *Wolbachia* infection, our finding of a *Microctonus* species parasitizing the parthenogenetic species *N. cervinus* opens challenging perspectives for future research on biological interactions. Their better understanding is crucial for a more effective biological control of the "Fuller's rose weevil" and other weevil pests.

**Acknowledgments** We specially thank María Guadalupe del Río, Pablo Colombo, Jerson Guedes and Mauricio Lovadina for fieldwork collaboration. We also wish to thank two anonymous reviewers for comments that helped to improve this paper. This work was supported by Grants of Agencia Nacional de Promoción Científica y Tecnológica (PICT 0962 awarded to MSR and PICT 480 awarded to VAC), Universidad de Buenos Aires (20020100100186 awarded to VAC), Universidad Nacional de La Plata (N261 awarded to MSL and N595 awarded to AAL), and Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 1324 awarded to VAC, PIP 0458 awarded to MSL, and PIP 1272 awarded to AAL). MSR, MSL, VAC and AAL are members of Carrera del Investigador Científico of Consejo Nacional de Investigaciones Científicas y Técnicas.

## References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Artigas J (1994) Entomología económica, vol 2. Universidad de Concepción, Chile

Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MCJ, Tettelin H, Werren (2006) Multilocus sequence typing system for the

endosymbiont *Wolbachia pipientis*. Appl Environ Microbiol 72:7098–7110

Barrat BIP, Johnstone PD (2001) Factors affecting parasitism by *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) and parasitoid development in natural and novel host species. Bull Entomol Res 86:101–108

Barratt BIP (2004) *Microctonus* parasitoids and New Zealand weevils: comparing laboratory estimates of host ranges to realized host ranges. In: van Driesche RG, Reardon R (eds) Assesing host ranges of parasitoids and predators used for classical biological control: a guide to best practice, USDA Forest Health Technology Enterprise Team, Morgantown, West Virginia, USA, FHTET-2004-03, pp 103–120

Dahlman DL, Vinson SB (1993) Teratocytes: developmental and biochemical characteristics. In: Beckage N, Federici BA, Thompson SN (eds) Parasites and pathogens of insects, vol 1. Academic Press, New York, USA, pp 145–165

Danforth BN, Mitchell PL, Packer L (1998) Mitochondrial DNA differentiation between two cryptic *Halictus* (Hymenoptera: Halictidae) species. Ann Entomol Soc Am 91:387–391

Fuester RW (1970) Separation of first-stage larvae of two species of *Microctonus* (Hymenoptera: Braconidae) which attack the alfalfa weevil. Ann Entomol Soc Am 63(6):1777–1778

Goldson SL, McNeill MR, Proffitt JR, Barker GM, Addison PJ, Barratt BIP, Ferguson CM (1992) Host specificity testing and suitability of the parasitoid *Microctonus hyperodae* (Hym.: Braconidae, Euphorinae) as a biological control agent of *Listronotus bonariensis* (Col.: Curculionidae) in New Zealand. Entomophaga 37(3):483–498

Goldson SL, McNeill MR, Proffitt JR, Barker GM, Addison PJ, Barratt BIP, Ferguson CM (1993) Systematic mass rearing and release of *Microctonus hyperodae* (Hym.: Braconidae, Euphorinae), a parasitoid of the argentine stem weevil *Listronotus bonariensis* (Col. Curculionidae) and records of its establishment in New Zealand. Entomophaga 38(4):527–536

Goldson SL, McNeill MR, Proffitt JR, Barratt BIP (2005) Host specificity testing and suitability of a European biotype of the braconid parasitoid *Microctonus aethiopoides* sloan as a biological control agent against *Sitona lepidus* (Coleoptera: Curculionidae) in New Zealand. Biocontrol Sci Technol 15:791–813

Guzmán N, Lanteri AA, Confalonieri VA (2012) Colonization ability of two invasive weevils with different reproductive modes. Evol Ecol 26:1371–1390

Hadley A (2011) http://www.hadleyweb.pwp.blueyonder.co.uk/CZM/combinezm.htm. Cited 27 Dec 2013

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hebert PDN, Cywinska A, Ball SL, Waard JR (2003a) Biological identification through DNA bar codes. Proc R Soc Lond B Biol Sci 270:313–321

Hebert PDN, Ratnasingham S, de Waard JR (2003b) Barcoding animal life: cytochrome oxidase subunit 1 divergences among closely related species. Proc R Soc Lond B Biol Sci 270(Suppl. 1):S96–S99

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755

Iline II, Phillips CB (2004) Allozyme markers to help define the South American origins of *Microctonus hyperodae* (Hymenoptera: Braconidae) in Canterbury, New Zealand. Bull Entomol Res 94:229–234

Kittayapong P, Jamnongluk W, Thipaksorn A, Milne JR, Sindhusake C (2003) *Wolbachia* infection complexity among insects in the tropical rice-field community. Mol Ecol 12:1049–1060

Lanteri AA, Normark BB (1995) Parthenogenesis in the tribe Naupactini (Coleoptera: Curculionidae). Ann Entomol Soc Am 88(6):722–731

Lanteri AA, Loiácono MS, Díaz NB, Marvaldi AE (1998) Natural enemies of weevils (Coleoptera: Curculionidae) harmful to crops in Argentina. Rev Bras Entomol 41(2–4):517–522

Lanteri AA, Marvaldi AE, Suárez SM (2002) Gorgojos de la Argentina y sus plantas huéspedes, Tomo I: Apionidae y Curculionidae. Publicación Especial de la Sociedad Entomológica Argentina, La Plata, Argentina, p 98

Lanteri AA, Guzmán NV, del Río MG, Confalonieri VA (2013) Potential geographic distributions and successful invasions of parthenogenetic broad-nosed weevils (Coleoptera: Curculionidae) native to South America. Environ Entomol 42(4):677–687

Loan CC, Holdaway FG (1961) *Microctonus aethiops* (Nees) auctt and *Perilitus rutilus* (Nees) (Hymenoptera: Braconidae) European parasites of *Sitona* weevils (Coleoptera: Curculionidae). Can Entomol 93:1057–1079

Loan CC, Lloyd DC (1974) Description and field biology of *Microctonus hyperodae* loan n.sp. (Hymenoptera: Braconidae, Euphorinae) a parasite of *Hyperodes bonariensis* in South America (Coleoptera: Curculionidae). Entomophaga 19(1):7–12

Marvaldi AE, Lanteri AA, del Río MG, Oberprieler RG (2012) Entiminae Schoenherr 1823. Chapter 4.7.5. In: Beutel R, Leschen R (eds) Handbook of zoology, coleoptera II. The Gruyter Press, Berlin, Germany, pp 219–238

McNeill MR, Phillips CB, Goldson SL (1993) Diagnostic characteristics and biology of three *Microctonus* spp. (Hymenoptera: Braconidae: Euphorinae) parasitoids of weevils (Coleoptera: Curculionidae) in New Zealand pasture and Lucerne. N Z Entomol 16:39–44

McNeill MR, Proffitt JR, Gerard PJ, Goldson SL (2006) Collections of *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) from Ireland. N Z Plant Prot 59:290–296

Morse JG, DeMason DA, Arpaia MS, Phillips PA, Goodell PB, Haney PB, Smith DJ (1988) Options in controlling the Fuller rose beetle. Citrograph 73(7):135–140

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden

O'Neill SL, Giordano R, Colbert AME, Karr TL, Robertson HM (1992) 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc Natl Acad Sci USA 89:2699–2702

Phillips CB, Emberson RM (1999) Morphological discrimination between first instar larvae of four euphorinae parasitoids. BioControl 44:159–169

Phillips CB, Vink CJ, Blanchet A, Hoelmer KA (2008) Hosts are more important than destinations: what genetic variation in *Microctonus aethiopoides* (Hymenoptera: Braconidae)

means for foreign exploration for natural enemies. Mol Phylogenet Evol 49:467–476
Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst Biol 53(5):793–808
Rodriguero MS, Confalonieri VA, Guedes JVC, Lanteri AA (2010a) *Wolbachia* infection in the tribe Naupactini: association between thelytokous parthenogenesis and infection status. Insect Mol Biol 19(5):599–705
Rodriguero MS, Lanteri AA, Confalonieri V (2010b) Mitonuclear genetic comparison in the *Wolbachia* infected weevil *Naupactus cervinus*: insights on reproductive mode, infection age and evolutionary forces shaping genetic variation. BMC Evol Biol 10:340. doi:10.1186/1471-2148-10-340
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542
Sasakawa K, Ikeda H, Sutou M, Dobata S, Ito M (2011) Parasitism of adult *Poecilus versicolor* (Coleoptera: Carabidae) by hymenopteran larvae. Can Entomol 143:211–223
Shaw SR (1988) Euphorine phylogeny: the evolution of diversity in host-utilization by parasitoid wasps (Hymenoptera: Braconidae). Ecol Entomol 13:323–335
Shaw MR, Huddleston T (1991) Classification and biology of braconid wasps. Handbooks for the identification of British insects. Vol. 7, part 11. Royal Entomological Society of London, UK
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729
Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22(22):4673–4680
Vink CJ, Phillips CB, Mitchell AD, Winder LM, Cane RP (2003) Genetic variation in *Microctonus aethiopoides* (Hymenoptera: Braconidae). Biol Control 28:251–264
Vink CJ, Barratt BIP, Phillips CB, Barton DM (2012) Moroccan specimens of *Microctonus aethiopoides* spice our understanding of genetic variation in this internationally important braconid parasitoid of adult weevils. BioControl 57:751–758
Werren JH, Zhang W, Guo LR (1995) Evolution and phylogeny of *Wolbachia* reproductive parasites of arthropods. Proc Roy Soc B 261:55–63
West SA, Cook JM, Werren JH, Godfray HCJ (1998) *Wolbachia* in two insect host parasitoid communities. Mol Ecol 7:1457–1465
Winder LM, Phillips CB, Williams CL, Cane RC, Paterson K, Vink CJ, Goldson SL (2005) PCR-based DNA analysis corroborates phenotypic evidence of trans-Andean variation in the South American parasitoid *Microctonus hyperodae*. Bull Entomol Res 95:289–298

**Marcela S. Rodriguero** is a junior research scientist at Institute of Ecology, Genetics and Evolution-CONICET and University of Buenos Aires, Argentina. Her work focuses on evolution of asexuality in insects and their interaction with reproductive parasites.

**Daniel A. Aquino** is a postodoctoral fellow of CONICET at Entomology Division of "Museo de La Plata", National University of La Plata, Argentina. His work focuses on the systematics and biology of parasitic wasps, especially Chalcidoidea.

**Marta S. Loiácono** is a senior research scientist of CONICET at Entomology Division of the "Museo de La Plata", National University of La Plata, Argentina. Her work focuses on the systematics and biology of parasitic wasps, especially Proctotrupoidea.

**Agustin J. Elias Costa** is an undergraduate student of biology at University of Buenos Aires, Argentina. His work focuses on evolutionary transitions between sexuality and asexuality in weevils.

**Viviana A. Confalonieri** is a senior research scientist at Institute of Ecology, Genetics and Evolution-CONICET and University of Buenos Aires, Argentina. She is also a professor teaching courses in molecular ecology at the Department of Ecology, Genetics and Evolution, University of Buenos Aires. She is a specialist in phylogeography and molecular systematics interested in the study of invasive insect pests.

**Analia A. Lanteri** is a senior research scientist of CONICET at Entomology Division of "Museo de La Plata", University of La Plata, Argentina. She is also a professor teaching courses of taxonomy and systematics at the University of La Plata. She is a weevil specialist, working on systematics and evolution of the tribe Naupactini.