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Tephritidae flies associated with *Chuquiraga avellanedae* (Asteraceae) in Patagonia, Argentina

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Abstract: In Patagonia, knowledge about the interaction among tephritids and the native flora is very scarce. In this study we identified for the first time two tephritid species (*Cecidochares* sp. and *Neosphaeniscus m-nigrum*) associated with the capitula of *Chuquiraga avellanedae*. This is the first record of a host plant for the genus *Neosphaeniscus*. *Cecidochares* sp. was more abundant and had a shorter development time than *N. m-nigrum*. Also, two families of parasitoid wasps (Pteromalidae and Eurytomidae) were registered. Further studies are needed to understand the impact of these tephritids on *C. avellanedae* fitness and their potential to control its populations.

Key words: Biological control, encroachment, host plant, insect-plant interaction, non-frugivorous Tephritids.

INTRODUCTION

With 4968 recognized species, the Tephritidae are among the larger families of Diptera (Norrbom et al. 1999, Norrbom 2010, Savaris et al. 2016, Borkent et al. 2018, Brown et al. 2018). Larvae of most species of the subfamily Tephritinae feed almost exclusively on plants of the family Asteraceae (Prado et al. 2002, Norrbom 2010, Savaris et al. 2015). In Argentina, 171 species of Tephritidae belonging to 34 genera have been registered (A.L. Norrbom, unpublished data), but knowledge of the fauna is based primarily on old, incomplete surveys, and there are large gaps in knowledge of the host plant relationships, especially in the native flora (Bartolucci 2008).

Chuquiraga avellanedae Lorentz (Asteraceae) is an evergreen perennial shrub of Patagonian steppes (Bisigato et al. 2016). Some studies have indicated that *C. avellanedae* density has increased mainly as a consequence of overgrazing by sheep (Beeskow et al. 1995, Campanella et al. 2016). Understanding the potential impact of tephritid flies on *C. avellanedae* fitness could be a useful tool for the control and management of the populations of this shrub. The objective of this work was to identify for the first time tephritid fly species associated with capitula of *C. avellanedae*.

Sampling was made at Playa Paraná (42°47’43”S; 64°57’12”W; Fig. 1), a site belonging to the Patagonia Phytogeographical Province (Bisigato et al. 2016). Field work was carried out during one *C. avellanedae* blooming season (Dec 2016-Feb 2017). At the beginning of January, we randomly harvested branches with inflorescences. They were placed in plastic bags and transferred to the laboratory. Then, we searched for larvae and/or pupae in the capitula using a stereomicroscope. After corroborating the presence of immature stages of insects, we returned to the field at the end of January and beginning of February to collect inflorescences and transferred them to the

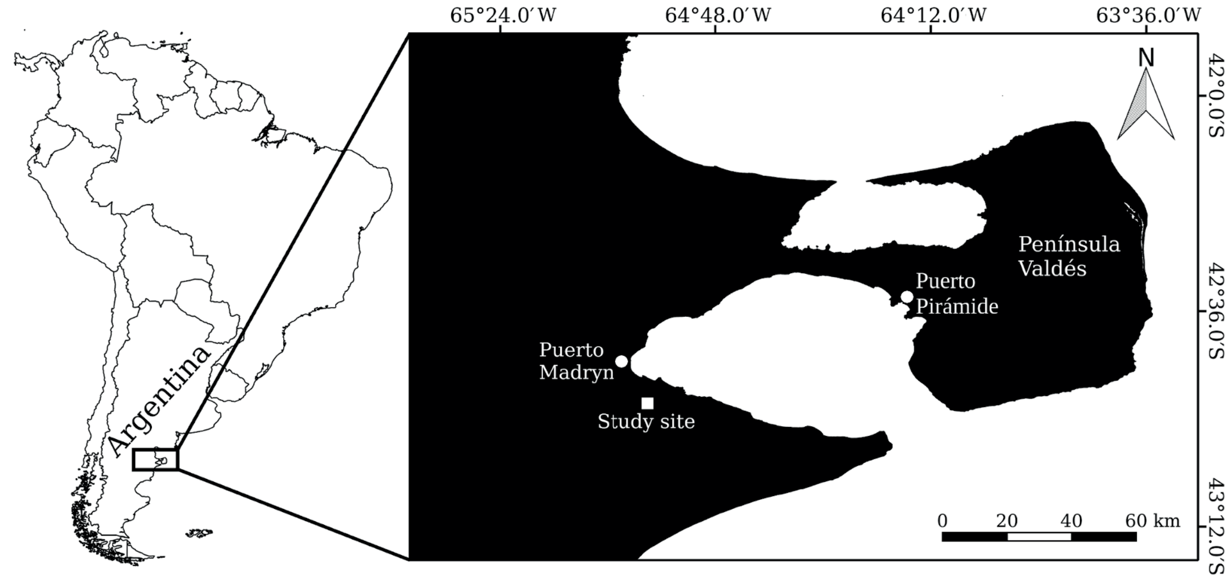

Figure 1. Location of the study site in the northeast of Patagonia, Argentina.

Laboratory of Terrestrial Fauna of the Instituto Patagónico para el Estudio de los Ecosistemas Continentales (IPEEC-CONICET). Inflorescences were carefully dissected and pupae were put in plastic containers with a fine mesh in the top. The containers were kept at room temperature, under ambient conditions for nine months. Fly or parasitoid emergence was periodically checked, and after emergence adults were killed in 70% ethanol. Voucher specimens were deposited in the Entomological Collection of the IPEEC-CONICET (voucher numbers: CNP-CE 1476/1485). Tephritid species were identified by Allen L. Norrbom. The map was made with QGIS 2.14.18 (QGIS Development Team 2016). All figures were edited with GIMP 2.8.20 (GIMP Development Team 2017).

Among 42 inspected inflorescences, 54.76% were found to have immature tephritid larvae, 9.53% had lepidopteran larvae, and 35.71% had no insects. The number of tephritid specimens per capitula were 1 larva (43.48%), 2 larvae (34.78%) or 3 larvae (21.23%).

The capitula were infested by two tephritid species. From the total of 110 incubated tephritid pupae, adults of an undescribed species of *Cecidochares* (Fig. 2a) emerged from 24.5%, and adults of *Neosphaeniscus m-nigrum* (Hendel) (Fig. 2b) emerged from 2.7%. The others died or were parasitized. The average time of emergence for *Cecidochares* sp. was 209 days (7 ± SE; n=27) and for *N. m-nigrum* was 269 days (13 ± SE; n=3).

About seven percent of the tephritid pupae were parasitized by solitary parasitoid wasps belonging to the families Pteromalidae and Eurytomidae. The Pteromalidae species emerged in two periods, during the end of summer (18 days ± 8) and the next spring (295 days ± 18) after the emergence of tephritid species (Fig. 2c). Eurytomidae was represented by a single individual of the genus *Eurytoma,* which emerged the next spring (230 days) (Fig. 2d).

The results of this study report for the first time two tephritid flies associated with *C. avellanedae*, a representative shrub of the Patagonian Phytogeographical region. These tephritid species were also reared from *C. avellanedae* near the study area (Chubut: Puerto Pirámide, Península Valdés; Fig. 1) during

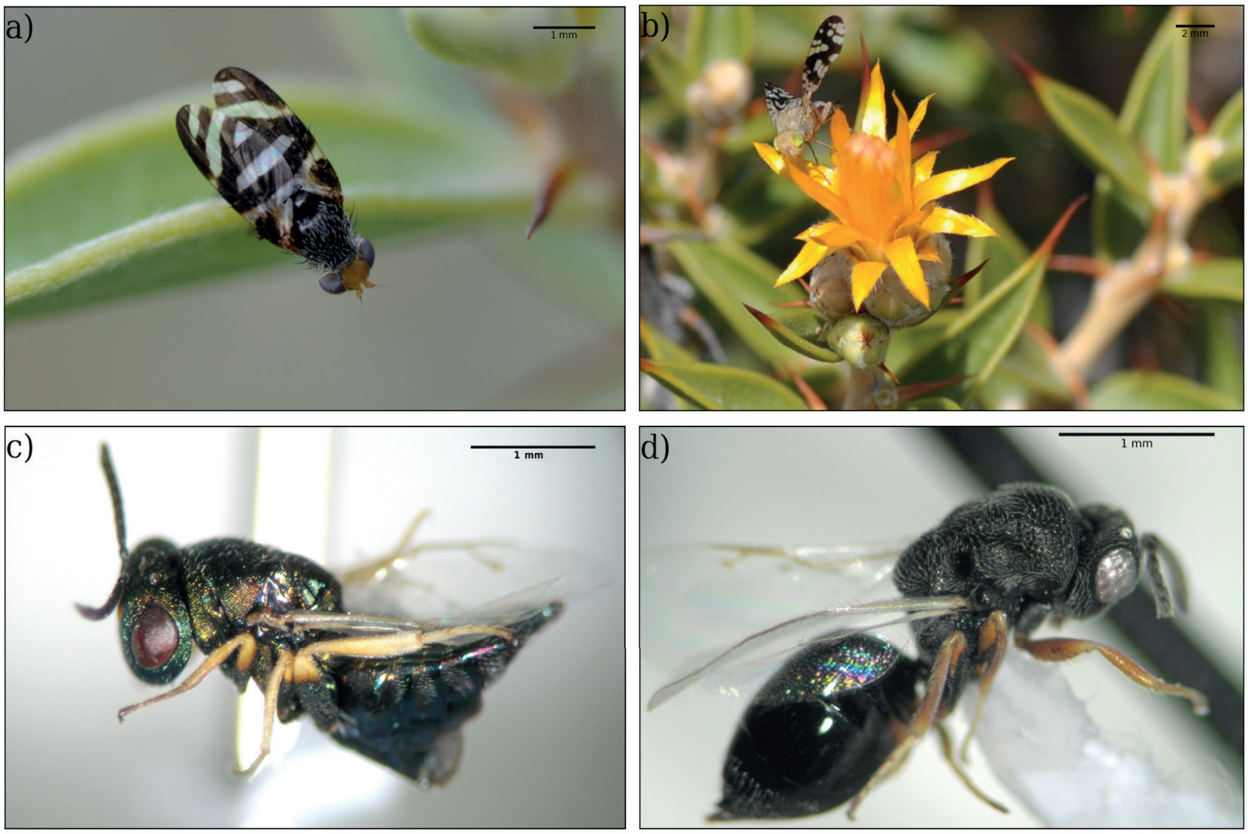

Figure 2. ***Cecidochares*** **sp. on foliage of** ***Chuquiraga avellanedae*** **(a),** ***Neosphaeniscus m-nigrum*** **on capitula of** ***C. avellanedae*** **(b), adult of Pteromalidae (c), and** ***Eurytoma*** **sp. (d).**

1993-1995 (D.E. Gandolfo, unpublished data, specimens in National Museum of Natural History, Smithsonian Institution, Washington, DC). This is the first host plant record for the genus *Neosphaeniscus*. Females of *Cecidochares* sp. and *Neosphaeniscus m-nigrum* lay eggs in developing flowerheads of *C. avellanedae*. Generally, no achenes are produced in infested capitula.

There is evidence of Tephritinae species affecting plant fitness (Edwards et al. 2009), and some species are used in programs of biological control of weeds (Woodburn 1993, Turner et al. 1994, Van Driesche et al. 2008). Moreover, the majority of nonfrugivorous species used for biological control belong to the subfamily Tephritinae associated with Asteraceae (Karimpour 2011). For example, the stem gall fly *Cecidochares connexa* (Macquart) has been used as a successful biocontrol agent of the invasive weed *Chromolaena odorata* (L.) R. M. King & H. Rob. in Africa, Asia and the Pacific (McFadyen et al. 2003, Zachariades et al. 2009, Aigbedion-Atalor et al. 2018).

Other tephritid species also have been found associated with local species of Asteraceae; *Plaumannimyia valdesiana* (Gandolfo & Norrbom) was reared from capitula of *Gutierrezia solbrigii*, whereas *Rachiptera baccharidis* (Rondani) produced stem galls on *Gutierrezia* spp., *Baccharis* spp. and *Grindelia chiloensis* (Cornel.) Cabrera (Cordo & DeLoach 1992, Gandolfo & Norrbom 1997). *Trupanea patagonica* Brethes was also recorded in Chubut; larvae of this species were collected from capitula of *G. solbrigii* Cabrera and *Grindelia* spp. (Cordo & DeLoach 1992). In this study, a *Trupanea* sp. was observed in the field, foraging from capitula of *C. avellanedae,* but no adults of that fly emerged from the pupae collected.

The tephritid species reared from *C. avellanedae* are clearly univoltine. At the end of summer larvae pupated inside the capitula. Before spring the capitula detached from the shrubs and fell to the ground, and adults emerged the next spring. Generally, in temperate regions univoltine species predominate (Bartolucci 2008). Pupal diapause can be conceived as an overwintering strategy to cope with an extended period of adverse conditions (Kostal 2006).

Cecidochares sp. and *N. m-nigrum* differed in emergence time under laboratory conditions. In accordance with this, *Cecidochares* sp. was observed in the field earlier in the season F.J. Martinez & M.V. Campanella, personal observation. Thus, *Cecidochares* sp. could attack flowerheads earlier than *N. m-nigrum*. This pattern could be related to different strategies for the exploitation of resources (Dzul-Cauich et al. 2014, Straw 1989a). There is evidence that when two species share the same host, they usually differ in oviposition time and larval establishment using distinct phases of flowerheads development (Straw 1989b). Moreover, *Cecidochares* sp. was reared from two other species of *Chuquiraga* (*C. aurea* Skottsb. and *C. erinacea* D. Don; Gandolfo D.E., Velazquez and Calcaterra, unpublished data, specimens in National Museum of Natural History, Smithsonian Institution, Washington, DC) suggesting that *Cecidochares* sp. uses a broader range of host plant species than *N. m-nigrum*.

The two families, Pteromalidae and Eurytomidae, have been registered as parasitoids of tephritid species in other studies (Mena-Correa et al. 2008, Ovruski & Schliserman 2012). The Pteromalidae species was the most abundant and apparently has a bivoltine strategy, with two generations, one of rapid development or short cycle and another with a winter diapause. This strategy is frequently observed in parasitoids from temperate regions (He et al. 2010). The emergence of some parasitoids later than their adult tephritid hosts observed in this study has been observed in other studies (Ovruski et al. 2007).

Additional studies are needed to understand infestation patterns of *Cecidochares* sp. and *N. m-nigrum* and more exhaustive sampling of other host plants is also required for better knowledge of the biology of these species. A significant proportion (>54%) of *C. avellanedae* inflorescences were occupied by tephritid larvae. This appears to reduce seed production and subsequent dispersion, possibly acting as a constraint on the abundance of *C. avellanedae*. However, more studies are needed to provide a comprehensive view of the impact of these flies on the reproductive success of *C. avellanedae*.

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FJM and MVC conceived the idea, collected data and wrote the first draft of the manuscript. ALN identified specimens. FJM, ALN, PS and MVC contributed substantially to revisions.