

On the natural history of the willow sawfly *Nematus oligospilus* (Hymenoptera, Tenthredinidae) inhabiting *Salix humboldtiana*, in Colombia

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Abstract

The willow sawfly, *Nematus oligospilus* Förster, has recently been recorded from Colombia. Natural populations were searched for in Bogotá City, where one of the host plants, *Salix humboldtiana*, grows as an ornamental tree. Larvae and cocoons were found, which thrive on the leaves of this plant. Parthenogenetic reproduction of this sawfly was confirmed. Fecundity of every female was estimated through counting the number of eggs laid on the leaves of the host. In addition, some variations in the color pattern of the adult are described and several of its morphological traits measured. For the larval stage, the body length of each instar was measured and their stadium (intermoult period or instar duration) calculated. Six larval instars were observed regularly; only one case of seven instars occurred.

Keywords

Symphyla, development, introduced species, Neotropics, Bogotá

Introduction

The presence of the willow sawfly, *Nematus oligospilus* Förster, was recently recorded for Colombia (Malagón-Aldana et al., in press); two female specimens were found deposited at different entomological collections (Entomological Museum UNAB and the biological collection from Alexander Von Humboldt Institute IAvH), both distributed in central region of Colombia, departments of Cundinamarca and Boyacá. In early 1980s, this species was recorded for the first time for southern South America, in Argentina and Chile (Smith 1983, González et al. 1986, Dapoto and Giganti 1994, Koch and Smith 2000), where this introduced species is a pest occurring at very high larval densities, causing severe defoliation, tree mortality, and economic losses on plantations of different species of *Salix* (Dapoto and Giganti 1994). Populations identified as *N. oligospilus* in different regions of the world could involve different members of a species group (Koch and Smith 2000, Smith 2003, Caron et al. 2013a).

The natural history of *N. oligospilus* has been addressed in several studies from different biogeographical regions (De Santis and De Sureda 1984, Dapoto and Giganti 1994, Urban and Eardley 1995, Ede et al. 2007, Alderete et al. 2010). Some common features regarding these studies include absence of males and reproduction by parthenogenesis (apomictic thelytoky) in the invasive populations (De Santis and De Sureda 1984, González et al. 1986, Ede et al. 2007, Caron et al. 2013b). Regarding immature forms, larvae hibernate as prepupae when their deciduous hosts lose their leaves; the larvae move from the host into the soil, where they build cocoons and dwell until spring when they pupate and adults eventually emerge (Ede et al. 2007). A small proportion of the population may pupate directly either on leaves or trunks within the summer and winter season (Dapoto and Giganti 1994, Alderete et al. 2010). Concerning their development, stadia (timing) of various stages have been estimated several times (Ede et al. 2007), the number of larval instars varies between four to seven (González et al. 1986, Ovruski 1991, Charles and Allan 2000). However, little information of the intermoult growth and stadia of larval instars has been reported. As to their natural enemies, several parasitoids of the families Ichneumonidae, Eurytomidae, Pteromalidae, Eulophidae, Eupelmidae, and Chalcididae, as well as predators of the families Pentatomidae and Vespidae have been recorded (De Santis and De Sureda 1984, González et al. 1986, Dapoto and Giganti 1994).

In Colombia, *Salix* is represented primarily by the species *S. humboldtiana* (Alford 2015), which is naturally distributed from Mexico to Argentina (STRI 2015). Likewise, though much less common, some introduced species, including *S. babylonica*, *S. viminalis*, and *S. purpurea* have also been recorded. Contrary to other countries in the region, in Colombia there are no extensive plantations of *Salix* spp.; the main purpose of urban plantings of the introduced species are ornamental, landscaping, soil conservation, and protection of water bodies. In cities such as Bogotá, the planted trees of *S. humboldtiana* are easily found, albeit scattered.

Based on a previous record of some specimens of *N. oligospilus* from localities in central Colombia, and the abundance of *Salix humboldtiana* thriving in Bogotá City,

the main goals of this study were to discover populations of *N. oligospilus* in the city, corroborate parthenogenetic reproducing populations, the presence/absence of males, and highlight some traits of the populations, including phenotypic variation of adults, larval development, oviposition behavior, and occurrence of parasitoids.

Materials and methods

Field work

To locate the larvae, cocoons, or any other indication of the occurrence of *N. oligospilus*, we inspected approximately 25 trees of *S. humboldtiana* scattered around Bogotá, including the campus of the Universidad Nacional de Colombia. Following recommendations from the literature (Charles and Allan 2000, Loetti et al. 2012), we collected branches with numerous leaves, on heights between 0 and 3 m on each tree, for 10 to 15 minutes per tree. Sampling was carried out during July and August, 2015, from 10 am to 4 pm. The foliage accumulated was placed into several plastic boxes and transported to the laboratory.

Laboratory procedures and data analysis

Laboratory work was conducted at the Entomological Museum UNAB (Universidad Nacional Agronomía Bogotá). Several specimens were mounted, curated, and housed in the Central Taxonomic Collection (CTC) of UNAB, following the curatorial standards of Martínez-Alava and Serna (2015). To confirm the identity of the collected specimens, we needed adults, because this is the main stage of development for which taxonomic keys, diagnosis, and descriptions are available (Benson 1958, Förster 1854, Koch and Smith 2000, Smith 2003). To obtain adults, the larvae and in-cocoon pupae were put individually in Petri dishes. Larvae were maintained with leaves of *S. humboldtiana*, while cocoons were placed on a filter paper that was the same size as the Petri dish, and into which 10 drops of water was daily applied to guarantee moisture. The breeding was kept at room temperature (with oscillations between 5–18 °C, between night and day).

All stages of development were observed under a Nikon SMZ-1 stereo microscope. Measurements were taken with a micrometric scale, and photographs were taken with a Canon EOS RebelXS camera, adapted to the stereo microscope. Adult specimens were preserved in 96%-ethanol. The ovipositors were cleared in 10% KOH for 12 hours, then cleaned in diluted acetic acid (3 drops into 30 ml of distilled water) for one minute, and subsequently rinsed in 75% and 96% ethanol for 5 minutes each; finally, they were slide-mounted, using Canada balsam partially diluted with Xylene. To observe details of the ovipositor, an Olympus CX31 microscope was employed. Morphological terminology was based on Snodgrass (1935), Gibson (1985, 1993) and Viitasaari (2002).

Results

Field collections

Larvae, pupae, and cocoons of *Nematus oligospilus* were found on the collected foliage of *S. humboldtiana*. Populations of *N. oligospilus* had low densities, 1-10 individuals per tree, including the larvae and pupae. We did not observe any adults in the field. The green color of eggs, larvae and cocoons of the sawfly made them cryptic on the willow leaves. Neither the individuals nor their damage were immediately noticeable in the trees examined. No symptoms from moderate to severe defoliation occurred.

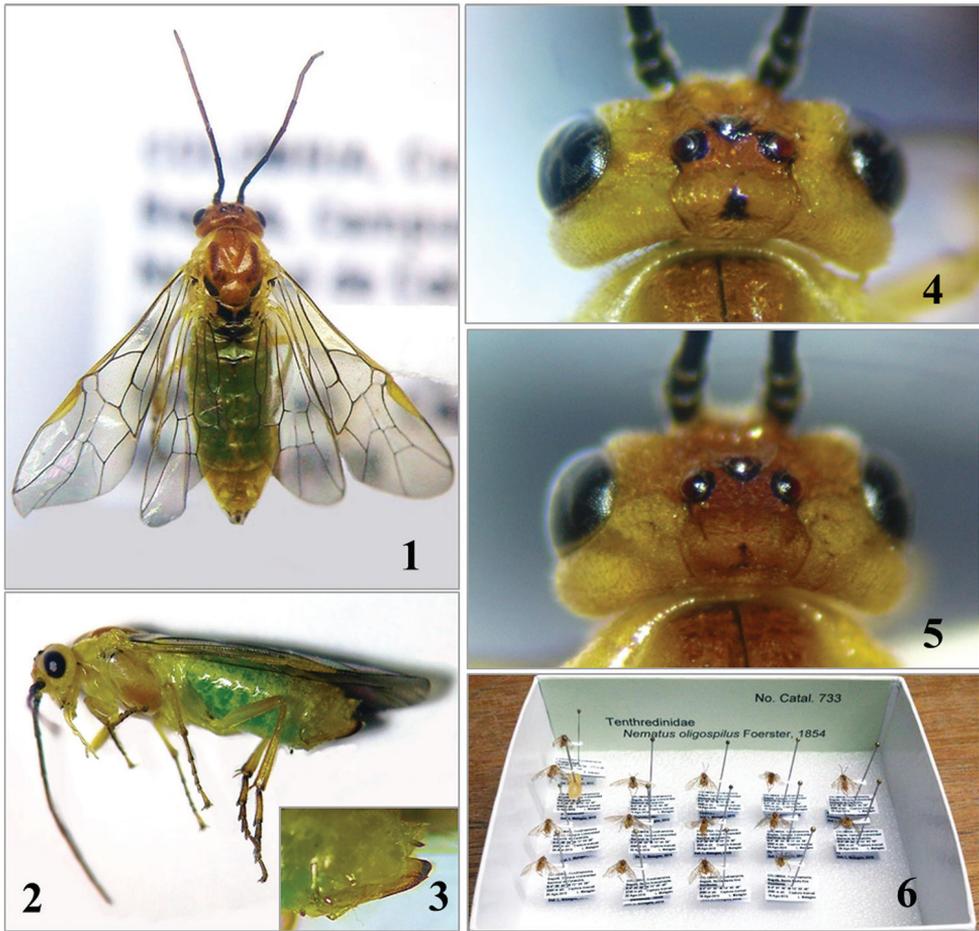
Fifteen adult females were obtained from rearing. These specimens are housed in the CTC, UNAB (Figs 1–6), with the following data: COLOMBIA, Cundinamarca, Bogotá, Campus Universidad Nacional de Colombia, N 4°38'34", W 74°04'58", 2600 m, Ago.-2015, L. Malagón (11 ♀); COLOMBIA, Cundinamarca, Bogotá, Barrio Guacamayas, N 4°33'09", W74°05'49", 2730 m, 22-Ago-2015, L. Malagón (2 ♀); COLOMBIA, Cundinamarca, Bogotá, Barrio Santa Ana Occidental, N4°41'36", W74°02'23", 2580 m, 19-Ago-2015, L. Malagón (2 ♀). All under catalogue number 733.

Phenotypic variation of females

Supplemental to the descriptions by Koch and Smith (2000) and Smith (2003), information about some variation may be useful in recognizing females of *N. oligospilus*.

Measurements (n = 15): Total length 5.3–7.8mm; ranges of metric ratios include antenna length / head width 3–3.2; lower interocular distance/ eye length 1.8–1.9; upper interocular distance/ eye length 1.7–1.8; width / length of postocellar area 2–2.5; malar space length / median ocellus diameter 1.4–1.6; cercus length / valvulae 3 length, in dorsal view, 0.7–0.8.

Color. Head (Figs 4, 5): vertex, genae, and frons above antennae dark yellow; facial area below antennae, including clypeus, labrum, and malar areas pale yellow; limit between vertex and occiput either with or without a mesal triangular black spot; areas surrounding ocelli containing linear black spots; frons lacking a black spot; in living individuals, all areas of head lack green color. Antenna black, being clearer distally to the third flagellomere. Mandible yellow, with a brown apical tooth. Thorax (Figs 1, 2): pronotum light yellow; propleuron yellow, with its anterodorsal margin from black to entirely yellow; basalare, tegula, and postspiracular sclerite yellow; mesoscutum reddish yellow, with lateral lobes either having or lacking black spots; antecostal suture and medial sulcus of mesoscutum black; axilla from entirely black to white, or with a black spot on the disc of its posterior margin; mesoscutellum yellow, with its posterior margin black to yellow entirely; mesopostnotum white with a black discal spot; middle of upper half of mesepisternum pale yellow, and middle of lateroventral lower area reddish yellow; epicnemium yellow; metascutum white, with cenchri yellow; metascutellum dark



Figures 1–6. *Nematus oligospilus*, ♀, reared from immatures collected on *Salix humboldtiana* (Salicaceae) in Bogotá, Colombia. **1** Dorsal view **2** Lateral view **3** Ovipositor sheath, lateral view **4** Vertex with a conspicuous black spot **5** Vertex with a reduced black spot **6** Housed specimens at the Entomological Museum UNAB (Catal. Number 733).

yellow with posterior margin black to entirely black; metapleuron and metasternum yellow; metapostnotum black mesally and white laterally. Abdomen: Green in living individuals, segments VII–X yellow (green color sometimes preserved (Figs 1, 2), but the entire abdomen usually turns yellow in mounted specimens); urotergites (segmental abdominal terga) I, II and III with or without black wide medial spot, which when present may be very narrow and anteroposteriorly extended on dorsum, as a longitudinal medial line. Legs (Fig. 2): yellow, darker on each segment apically. Genitalia: valvulae 1 (= lancet) and 2 (= lance) hyaline yellow, with annuli dark yellow; valvula 3 (= sheath) (Fig. 3) dark yellow, with posteroventral margin black; cerci dark brown. Wings hyaline (Fig. 1); Costal vein and pterostigma yellow; remaining veins dark brown.

Table 1. Fecundity and longevity of *N. oligospilus* in laboratory. Females, n= 15; pupae, n = 15; larvae, n= 11 (from several instars); eggs, n= 490.

	Fecundity			longevity (days)							
	number of eggs/female	number of eggs/leaf		Larval instar					Pupae (in cocoon)	Adult	
				1	2	3	4	5			6
Maximum	53	17	Maximum	8	2	3	3	4	4	11	9
Average	30	3.7	Average	6.5	2	3	3	4	3	9.78	7.1
Minimum	9	1	Minimum	5	2	3	3	4	2	9	4

Notes on the development of *N. oligospilus* on *S. humboldtiana* in Bogotá

From the immature forms reared in the lab, we obtained only females. Immediately after emergence, a female lays an average of 31 eggs (n = 15) on the leaves of the host plant (Table 1). Postembryonic development lasts from 32 to 44 days (Table 1). The larval stage has the longest time span, between 19 and 24 days. Most eggs obtained in the lab did not hatch, a few viable eggs lasted between six and eight days before hatching.

Six larval instars (n = 10) were recognized; each instar varied in size; most instars did not overlap in body length, except 5th and 6th where overlap was close to 0.15mm. Regarding intermoult size increment (Gullan and Cranston 2014), 3rd instar had the lowest (0.1 mm) and the 5th instar the highest (0.55mm). Concerning stadia (Gullan and Cranston 2014), intermoult duration was highest in the 1st instar, varying between five and eight days, while 2nd instar had a two-day shortest stadium (Table 1). One larva went through seven instars.

Darkening of the head capsule of the larvae in early instars marks the initiation of the moulting process (Fig. 8). Right after completing each ecdysis, the new larvae are light green (except for eyes and mandibles) (Fig. 9). Prepupae build the cocoon and pupate directly on foliage of *Salix humboldtiana* (Figs 12, 13). In the lab, larval ecdysis tends to occur during night hours, whereas adults emerge around noon. No parasitoids were obtained from the reared larvae or pupae.

Oviposition behavior

For oviposition, the first and second valvulae work together as a sharp structure tapering distally. With the teeth of ventral margin of the 1st valvula of the ovipositor, the sawfly produces an incision in the cuticle of the leaf, generating a small swelling (Fig. 14) in the epidermis or between the epidermis and the chlorenchyma (Braccini et al. 2013). During oviposition, the valvulae are inserted in the leaf initially in a position perpendicular to the body axis; as the valvulae penetrate deeper, they rotate anteriorly, being parallel to the body axis at the end of oviposition. This forms a kidney-shaped pocket, which is immediately filled with an egg, turning back the ovipositor in the opposite direction.

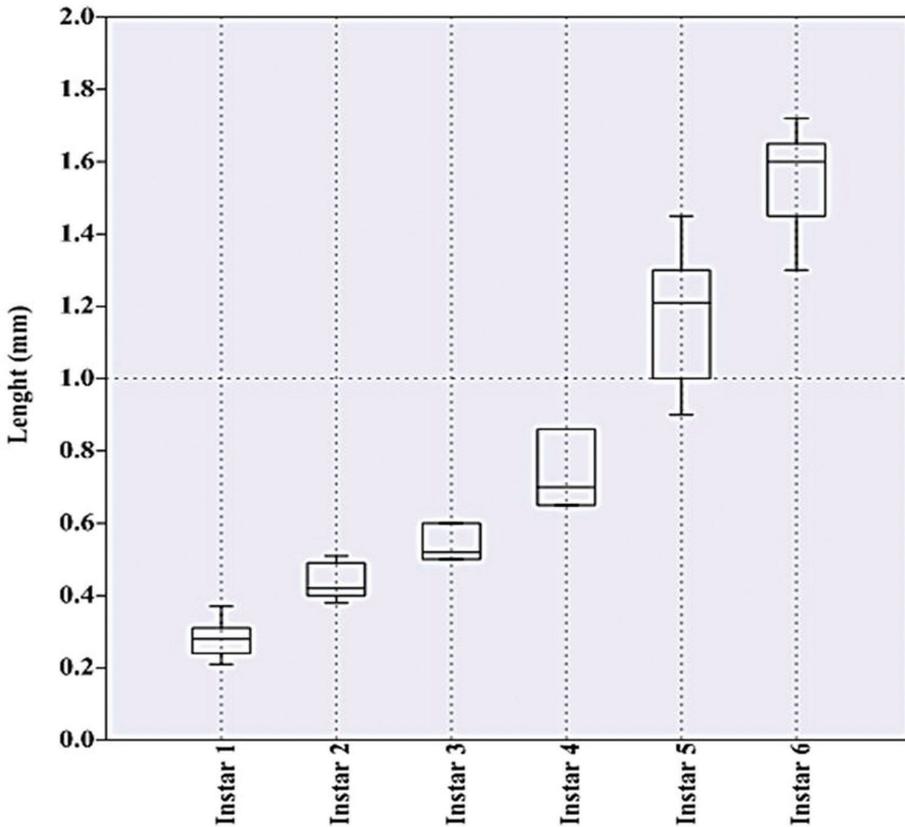
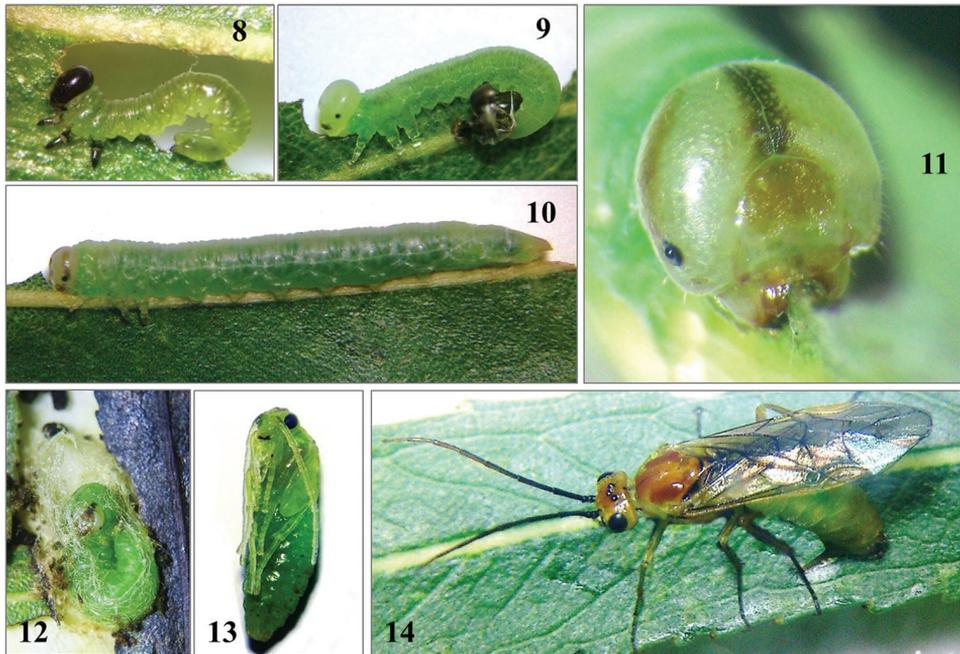


Figure 7. Box-whisker plot of total length variation with reference to each larval instar (n=10).

Discussion

The presence of *N. oligospilus* in Colombia was confirmed by finding specimens feeding on the foliage of *Salix humboldtiana*, in the City of Bogotá. According to records from other parts of South America, *N. oligospilus* is distributed between 0 and 1700m above sea level (Koch and Smith 2000); in Colombia it reaches higher elevations (2600m) in Bogotá.

Regarding phenotypic characters of this species, contrary to what was written by Koch and Smith (2000) for the lectotype, we did not find black spots on the frons or on the interocellar area; additionally, we observed intraspecific variation in the coloration of the individuals reared in the lab. Whereas the specimens housed in different collections have black color pattern characteristic of the species, the adults we reared lack or have the triangular spot on the vertex reduced, whereas the spots on the mesoscutum and abdominal terga are retained. Similar variations regarding the reduction of spots and black color patterns are found in specimens from several countries around the world (Koch and Smith 2000). Characteristics of the ovipositor agree with other descriptions (Malagón-Aldana et al., in press); similarly, the coloration of the larva coincides with what is described in the literature.



Figures 8–14. *Nematus oligospilus*. **8** Larval instar 1 immediately before ecdysis **9** Larval instar 3 immediately after ecdysis **10** Larval instar 6 feeding on a leaf of *Salix humboldtiana* **11** Frontal view of head of 6th larval instar **12** A sixth larval instar spinning its own cocoon **13** Pupa **14** A female ovipositing.

Parthenogenetic reproduction is confirmed in *N. oligospilus*. For the first time, we observed and compared the different intermolt larval instar stadia and growth; likewise, the number of larval instars coincides with the data in Ovruski (1991) and Charles and Allan (2000); other development stages agree with data recorded in different studies (Ede et al. 2007). Contrary to what was previously reported for other parts of the Southern Hemisphere (Ede et al. 2007), the larvae of *N. oligospilus* in Colombia make cocoons directly on leaves of *S. humboldtiana* and not in the soil. This difference is probably due to climate seasonality in other regions, usually subtropical (Argentina, South Africa, Australia), where the photoperiod and temperature change abruptly from one season to the next (especially winter and spring), and favoring strategies as winter diapause (Ede et al. 2007), and pupation underground.

Compared to other countries in the Southern Hemisphere, population densities of *N. oligospilus* are low in Bogotá. It is noteworthy that in different studies *S. humboldtiana* and its varieties exhibit lower levels of both oviposition and phytophagy by the willow sawfly (Cerillo et al. 2011, Braccini et al. 2013), compared to exotic species such as *S. nigra*, *S. babylonica*, *S. alba*, and *S. matsudana*. Moreover, in the other countries where the introduction of *N. oligospilus* has been recorded, there are extensive commercial plantations, or additional natural communities of *Salix* spp., which favor establishing sawfly populations.

In Colombia, the larvae seldom devour the leaves entirely. This behavior is similar to that described by Carr et al. (1998) in native populations of *N. oligospilus* in Arizona, on *Salix lasiolepis* (Arroyo Willow), the sawfly displays moderate movements between leaves, and populations remain low and stable.

Concerning the expansion of the distribution of this sawfly from Argentina, and the wide distribution of *S. humboldtiana* in South America, it is likely that *N. oligospilus* is present in neighboring countries. In Colombia, it is necessary to determine whether this sawfly is also present on other species of planted *Salix*.

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