ECOLOGY, BEHAVIOR AND BIONOMICS

Life History of *Aneugmenus merida* Smith (Hymenoptera: Tenthredinidae) in the Venezuelan Andes

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História Natural de *Aneugmenus merida* Smith (Hymenoptera: Tenthredinidae) nos Andes Venezuelanos

RESUMO - Detalhes do ciclo de vida de *Aneugmenus merida* Smith criados em laboratório são relatados. As fêmeas produzem ovos partenogenéticos e não partenogenéticos. Após a eclosão, as lagartas dos machos de *A. merida* passam cinco ínstares, a pré-pupa, pupa e imago. O ciclo de vida dura cerca de 44 dias. As larvas alimentam-se de samambaia [*Pteridium caudatum* (L.) e *Pteridium arachnoideum* (Kaulf)] (Dennstaedtiaceae). Neste estudo também são relatadas observações sobre a morfologia do *sinus sexualis*, mediante imagens obtidas em microscópio eletrônico de varredura, e detalhes dos comportamentos exibidos durante a cópula.

PALAVRAS-CHAVE: Samambaia, Symphyta neotropical, *Pteridium*, *sinus sexualis*, comportamento de cópula

ABSTRACT - Details of the life cycle of *Aneugmenus merida* Smith (Hymenoptera: Tenthredinidae) reared under laboratory conditions are reported. Females may produce parthenogenetic or non-parthenogenetic eggs. After eclosion, males of *A. merida* go through five larval instars, pre-pupa, pupa and imago. The complete life cycle lasts approximately 44 days. Larvae feed on bracken [*Pteridium caudatum* (L.) and *Pteridium arachnoideum* (Kaulf)] (Dennstaedtiaceae). We also report on morphological observations of the *sinus sexualis* in males by scanning electron micrographs and give details on mating behavior.

KEY WORDS: Bracken, Neotropical sawfly, *Pteridium*, *sinus sexualis*, mating behavior

Aneugmenus merida Smith is a neotropical sawfly species recently described by Smith (2005) from specimens caught in the Venezuelan Andes in Northern South America. *A. merida* adds to three other *Aneugmenus* species so far known for the new world, and even though Tenthredinid diversity has been estimated to reach as many as in 60 species in Venezuela (García 2003), *A. merida* is the second published report for the family and the first for the Selandrinae (see Smith 2003 and 2005).

Knowledge on the biology of Neotropical Symphyta is scarce. Contributions to the knowledge of three Brazilian species of Argidae are worth mentioning (Dias 1976, Penteado-Dias 1991, Boraschi & Del Lama 2004, Boraschi *et al*. 2005). Dutra (2003) studied cases of bovine intoxication caused by ingestion of sawfly larvae, and reported on the biology of *Perreyia flavipes* Konow (Hymenoptera: Pergidae). Smith & Janzen (2003) have contributed with important information to the life histories and hostplants of species of Argidae, Tenthredinidae and Pergidae of Costa Rica.

Knowledge on the Neotropical species of *Aneugmenus* Hartig is still scantier. The use of bracken fern *Pteridium aquilinum* (L.) (Dennstaedtiaceae) as the larval host plant of several paleartic and nearctic species has been reported by Smith (1969) on a global scale. Similar host associations have been reported for the South American species *A. merida* and *A. colombia* Smith (Hymenoptera: Tenthredinidae) (Smith 2005).

This paper is the first contribution to the knowledge of the natural history of *A. merida*, and reports on characteristics of its life cycle and on the morphological description of male abdominal structures which are involved in mating behavior.

Materials and Methods

Life cycle. Females of *A. merida* were collected in Cerro La Bandera, a mountain savannah located at La Hechicera, Mérida, Venezuela (8º31'N-71º13´W, 2100 m). Individuals were netted on the wing while flying near the abundant bracken fern which grows in association with grasses and woody species.

Four captured females were taken to the laboratory and transferred into individual cages (24 cm diameter and 23.5 cm height) provided with a segment of a tender frond of *Pteridium caudatum* (L.) (Dennstaedtiaceae) and a piece of cotton soaked in an aqueous solution of honey (15% v/v). Those eggs oviposited by the four females which hatched synchronously in a period of less than 24h were used to establish the cohort of 15 first instar larvae used for our observations. The larvae were transferred individually to small rearing cages provided with tender fronds of *P. caudatum*, one of the local hostplants of the sawfly. The frond segments were changed for fresh material every 2-3 days. Growth was monitored daily, keeping records of dates of molting and mortality at each instar. The rearing cages were kept in a growth chamber with a mean temperature of $22^{\circ} C \pm 1^{\circ}C$ and a photoperiod of 12h. All adults reared were sexed and kept in cages provided with a diluted honey solution for food. These cages were kept under the same conditions of temperature and photoperiod of the rearing cages.

External morphology of the abdomen of adult *A. merida.*
Field collected specimens of both sexes were frozen at -18ºC, lyophilized, mounted on carbon conductive adhesive tape and examined under a Hitachi S-2500 scanning electron microscope.

Mating behavior of *A. merida.* Virgin couples of less than three days of age were confined into a round experimental arena (7 cm diameter) made with perforated aluminum plates (1.5 cm thickness) enclosed within two glass plates. The bottom of the arena was covered with a sheet of Whatman filter paper. Observations were conducted in diurnal hours under both, artificial fluorescent laboratory light and natural light. Different phases in mating behavior were recognized and photographed with a digital camera.

Results

Field behavior of *A. merida.* Adults of both sexes are usually found in the wild flying near the tender fronds of the host species and have occasionally been seen feeding from exudates on the fern's extrafloral nectaries. Field observations suggest that most daily activity occurs between 10AM and 4PM, especially on sunny days with little or no wind. Males always dominated over females in our captures in about 75%. Population abundance of *A. merida* follows a seasonal pattern: the first adult sawflies are found from April-May, when the local rainy season begins. A short dry period occurring between July and August is characterized by a severe drop in the number of larvae and adults in the field, until September-November when they reappear.

Life cycle. A total of 140 eggs were obtained from the four females (35 ± 7.3 eggs/female; $\bar{X} \pm SD$), 95% of the eggs hatched (33.3 ± 6.2 eggs). Table 1 shows the duration of the different developmental stages of *A. merida* measured for a cohort of 15 larvae. After egg eclosion, the species goes through five larval instars, pre-pupa, pupa and imago. In the laboratory, the isolated eggs are oviposited abaxially on the pinnae segments of *P. caudatum* and *P. arachnoideum* Kaulf.

Table 1. Time of development (days) of different stages of *A. merida* ($\bar{X} \pm SD$).

Stages	Time of development ($\bar{X} \pm SD$)[1]
Eggs	5.4 ± 0.55
L I	5.3 ± 0.45
L II	3.0 ± 0.51
L III	3.3 ± 0.49
L IV	3.2 ± 0.58
L V	7.3 ± 1.67
Pre-pupa	9.5 ± 0.71
Pupa	7.3 ± 0.50

[1]Eggs, n = 133; larvae, n = 15; prepupa and pupa, n = 4, other pupae could not be recorded as they remained underground until emergence.

(Dennstaedtiaceae) (Fig. 1), a behavior also observed in the field. Eggs are ovoid in shape and translucid at oviposition. The eruciform larvae lack ornamentation and are yellowish at eclosion, but turn bright green after ingestion of their first few meals, becoming quite criptic due to the matching of its color to the fronds of the host plant (Fig. 2).

The larvae locate underneath the pinnae where they feed. Fifth instar larvae abandon the frond before molting, burrowing into a substratum of organic soil provided with the rearing cage. In this emulated underground condition, the larva constructs a cocoon with soil particles and turns into a pre-pupa (Figs. 3-6). After emergence from the exarate pupa, the imago exits the cocoon by making a hole in the conglomerate of soil particles of the wall. A total of 12 adults were obtained, all male. The average development time from egg to adult for nine of the individuals was 44.3 ± 0.4 days, but three of the individuals in the cohort remained as pupae for several more days (20, 26 and 32 days respectively). We did not keep rigorous track of the longevity of adults in laboratory conditions, but our observations on reared adults and field collected adults fed with the honey solution, suggest a short life span of about 3-4 days. We also noted that captive females died shortly after oviposition. Females (6.5 ± 0.6 mm; n = 9) are slightly larger than males (5.6 ± 0.4 mm; n = 14).

Virgin females in laboratory cultures oviposit non-fertilized eggs which only yield males, clear evidence that parthenogenesis, and haplodiploidy, are part of its reproductive characteristics as would be expected for most Hymenoptera (Lee *et al*. 1998).

External morphology of the abdomen of *A. merida*.
Scanning electron microphotographs show a dorsal view of male and female abdomen respectively (Figs. 7-8). The male abdomen shows a profound transversal cavity on tergum VII (Fig. 9) which is absent in the female abdomen. Tergum V and VI in the male are divided in two halves by a central suture, and the posterior margin of tergum VI projects backwards towards the cavity in two pointed projections. Tergum VIII is ovoid and somewhat concave at the center.
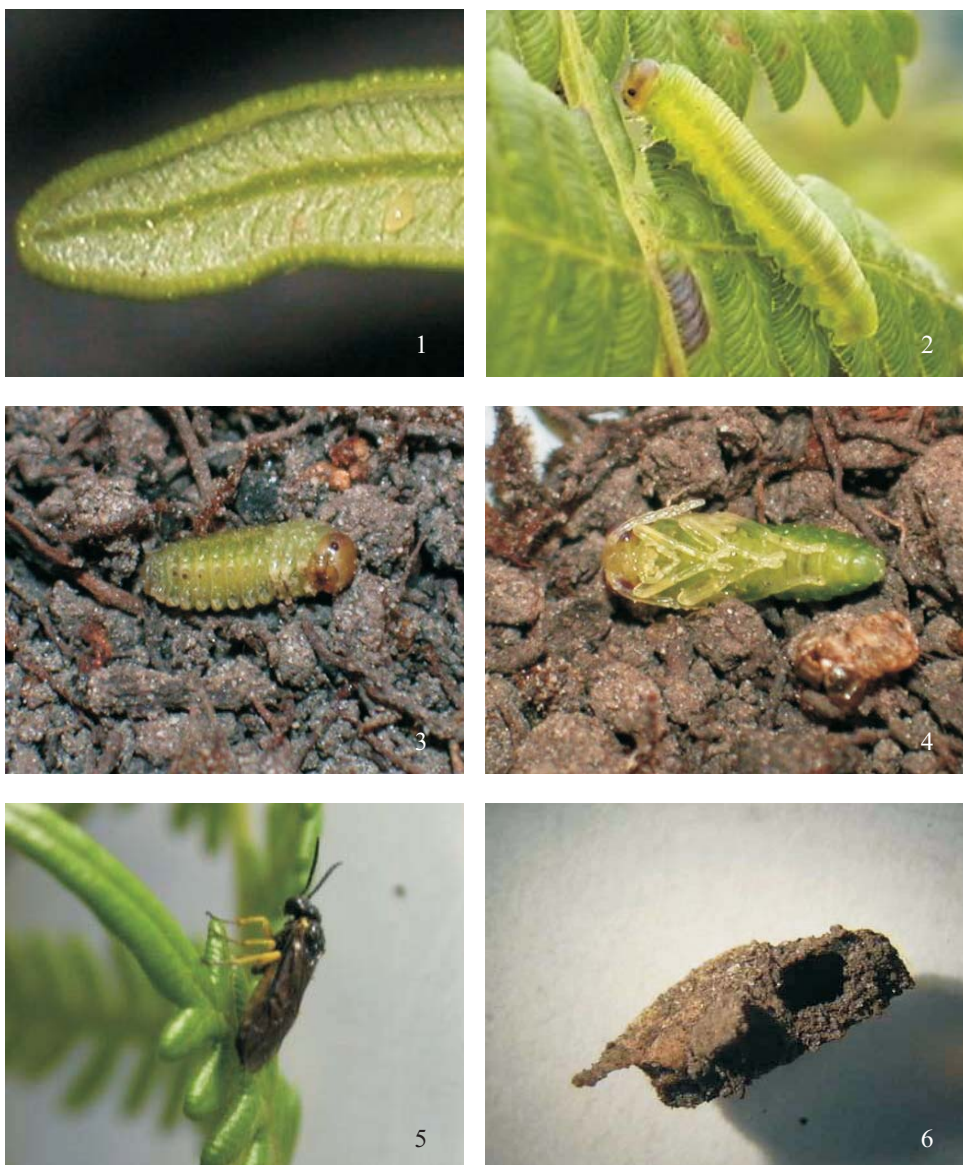
Figs. 1-6. *A. merida*. 1, Egg on *P. caudatum*. 2, Larva on *P. caudatum*. 3, Pre-pupa. 4, Pupa. 5, Male. 6, Cocoon.

A magnified view of the bottom of the cavity in tergum VII shows an area with small rod like structures arranged in lines which lay nearly perpendicular to the longitudinal axis of the abdomen (Fig 10).

Mating behavior of *A. merida*. The behavior of isolated couples of *A. merida* (n = 7) inside the experimental arena allows us to discriminate behavioral items which seem characteristic of *A. merida* and perhaps other species in the genus. The male, of smaller size (see Fig. 11), exposes the abdominal terga arching its abdomen upward and rapidly vibrating its wings. In response, the female approaches the male approximating its head and gnathal appendages to the exposed area in the male abdominal terga (n = 7). The male then grabs the female using the hind pair of legs. Male and female turn back to back and join in copula (n = 3) (Fig. 12). While in copula, the male abdominal apex lays below the female, his wings keep vibrating and his hind legs lay on the female's wings. It is worth noticing that copulae were always preceded by a nuptial gift, but not all nuptial gifts always led to a copula. This characteristic behavior was observed under both natural and artificial light conditions.

Discussion

All life stages of *A. merida* have a close association with its *Pteridium* hosts, as also occurs with other species of the subfamily Selandrinae, and more specifically with species of the genus *Aneugmenus* (Smith 1969). The association begins with the selection of the plant as a substratum for oviposition by females. It provides the food for the development of the larvae, and is required by adults for mate finding. It is also a likely food source for adults which feed from the extrafloral nectaries.
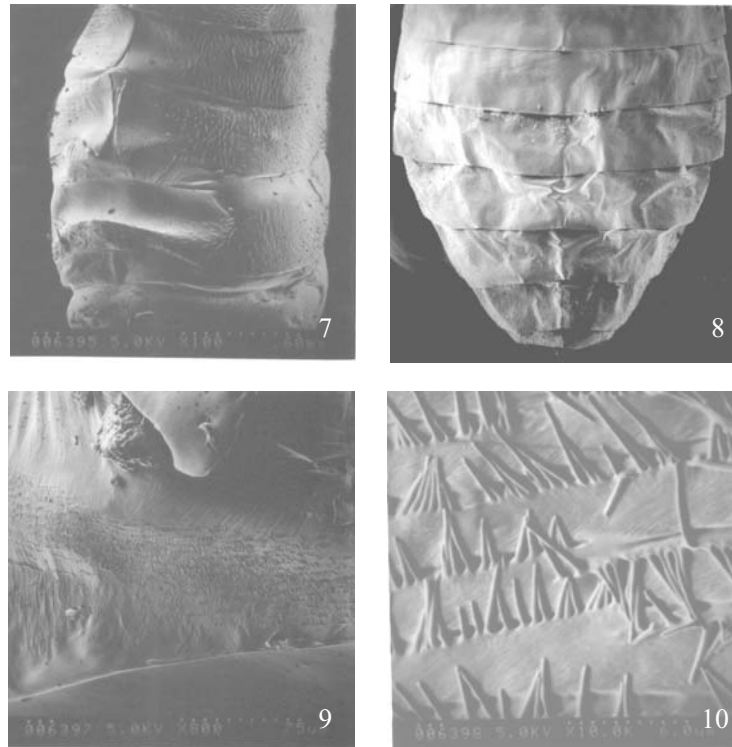
Figs. 7-10. *A. merida*. 7, Dorsal view of male abdomen showing the *sinus sexualis* in tergum *VII*. 8, Dorsal view of female abdomen. 9, Enlarged view of *sinus sexualis*. 10. Detail of area shown in 9.

This insect-plant association is of special interest because *Pteridium* is an invasive cosmopolitan weed which shows little susceptibility to herbivory by arthropods (Lawton 1976, Hendrix 1977, Jones 1983) due to the enormous variety of bio-active compounds it produces (Alonso-Amelot *et al*. 2001). Other non -*Aneugmenus* old world Symphyta have also been quoted as bracken specialists: *Strongylogaster* spp., *Tenthredo* spp. and *Stromboceros delicatulus* (Fall.) (Hymenoptera: Tenthredinidae) (Lawton 1976, Balick *et al*. 1978).

It is not possible to compare the life history of *A. merida* with that of other species due to the lack of information available on other neotropical *Aneugmenus*, this in spite of the fact that Tenthredinidae is the family with the largest number of species in the new world (Smith 2003). Just like other neotropical tenthredinid species, the pre-pupa and the pupa occur under the ground inside a cocoon made with soil particles but no silk. In laboratory conditions, metamorphosis was completed in approximately 44 days, suggesting that there could be 3-4 generations in one year, this considering that the rainy season extends from April to November with some short intercalated dry periods. A diversity of patterns of population dynamics have been observed in other Neotropical sawfly species, e.g. *Digelasinus diversipes* (Kirby) (Hymenoptera: Argidae) is an univoltine species which can go into diapause for the whole 7-9 month dry season of Estaç{\~a}o Ecológica Jataí in São Paulo State, Brazil (Boraschi *et al*. 2005), while *Dielocerus diasii* Smith (Hymenoptera: Argidae) can go through four developmental cycles in one year (Días 1976).

Notwithstanding, soil humidity does not seem to be the only controlling factor of diapause. We had some individuals emerge after a longer period of time, an adaptation which allows the overlapping of several generations in one year.

Parthenogenetic reproduction is common in Hymenoptera and in Symphyta as well. Virgin females of *A. merida* were able to lay non-fertilized eggs which produced viable adults. It was not possible to confirm if this species has an arrhenotokous sex determination due to the short longevity of adults in captivity. Arrhenotoky has been confirmed in Tenthredinidae and in other Hymenoptera where females have the capacity of determining the sex of their progeny by means of controlling the fertilization of eggs at oviposition (Craig *et al*. 1992, Lee *et al*. 1998). We must point out that the overall length of development in *A. merida* could be longer than we report, had we obtained females from our rearings. The larvae of females in other Selandrinae go through six instars, while males will only go through five (Smith *et al*. 2002). In our case, all individuals reared were males.

The presence of a dorsal cavity in abdominal tergum VII has also been reported for other species of Selandrinae. Smith & Marshall (2003) describe a similar structure in *Aneugmenus flavipes* Norton (Hymenoptera: Tenthredinidae) and suggest that it may house glandular structures which produce a substance used during courtship. They also point out that it has been found by other authors in species of the genera *Aneugmenus* and *Neostromboceros*. It was in species of *Neostromboceros* where the structure was first described and named with the term *sinus sexualis,* which identifies it as a structure likely to be involved as an auxiliary organ in reproductive behavior (Smith & Marshall 2003).
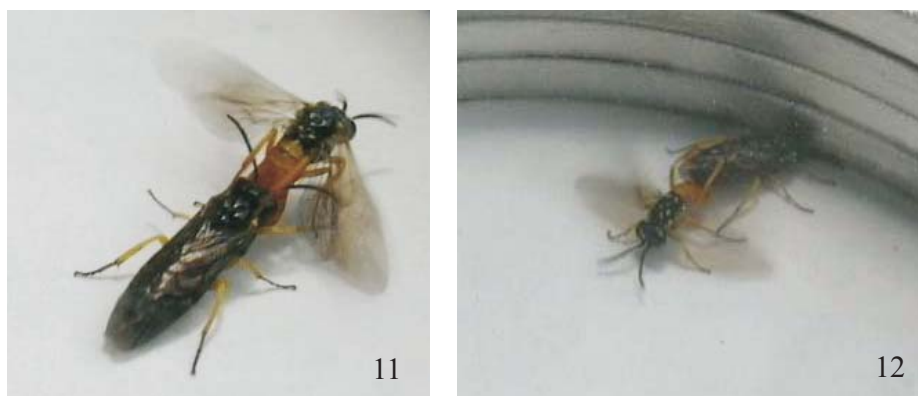
Figs. 11-12. *A. merida*. 11, Female approaching the exposed *sinus sexualis* in male. 12. Copula.

Modifications of abdominal terga are also found in aculeate hymenopterans. Social bees such as *Apis mellifera* L. and *Bombus terrestris* (L.) (Hymenoptera: Apidae) have pheromone producing glands distributed on the terga of abdominal segments, and include a special modification of tergum VII which is known as the "gland of Nasanov". The structure is known to emit warning signals in foraging bees (Dornhaus *et al*. 2003). Surprisingly similar structures are found in males of some cockroach species (Brossut & Roth 1977). It has been pointed out that *Blatella germanica* (L.) (Dictyoptera: Blatellidae) emits pheromonal compounds made of olygosaccharids and phospholipids which play the role of sexual attractors during courtship (Nojima *et al*. 1999, Kugimiya *et al*. 2002). Considering the phylogenetic distance between these groups, it is impossible not to draw attention towards the enormous similarity of the associated structures in the sawflies and the ones described and illustrated for Blattaria by Brossut & Roth (1977).

Female *A. merida* showed a behavioral item which occurs immediately previous to copula, in which she seems to use her gnathal appendages in the search of some sort of secretion in the *sinus sexualis* of the male. Such behavior was first described by Smith & Marshall (2003) for Symphyta and Hymenoptera in general. They proposed that during the interaction occurs a transference of glandular secretions which play the role of a nuptial gift for the attraction of the female. Nuptial gifts where the male provides food during courtship and copula have been recorded in many arthropod species (Vahed 1998). Surprisingly again, the description of courtship and copula for the male of the blaberid *Nauphoeta cinerea* Olivier (Dictyoptera: Blaberidae) match well with our observations on *A. merida*. The behavior of *N. cinerea* includes vibrating of the wings and exposure of abdominal terga by the male, an aproximation of the female's gnathal appendages towards a glandular area located on terga VII and VIII, and a subsequent copula.

At least two important chemical fractions have been identified in the interaction: a volatile fraction which stimulates the aproximation of the female, and an aphrodisiac fraction which promotes the receptiveness of the female toward copulation (Sreng 1990). We ignore if such kind of chemical signals occur in *A. merida*. Little knowledge is available on the mating behavior and the involved chemistry of Symphyta. The best information available comes from several genera of Diprionidae (Hallberg & Lofqvist 1981, Ayasse *et al*. 2001, Östrand & Anderbrant 2001) but in these cases, no mention is made of substances or associated structures which might play a role during courtship.

Gordh (1975) described the mating behavior of *Hemitaxonous dubitatus* (Norton) (Hymenoptera: Tenthredinidae) in captivity. The male *H. dubitatus* climbs on the dorsum of the female continually vibrating his wings and doing successive intents of engaging in copula. Once in copula, the two sexes were joined by their abdominal apices with their heads looking in opposite directions. This behavioral display shows two common items with *A. merida*, the vibrating wings of the male and the opposing position of the individuals. This position has been denominated "strophandrous" and is exhibited by males of species that have developed the ability of rotating their genitalia in 180º angle over its middle axis. Copula in other males that do not show this genital torsion is known as "orthandrous" (Gordh 1975). Schulmeister (2001) points that the "strophandrous" condition is characteristic of the Tenthredinidae, highlighting the fact that the male will frequently lay one or both hind legs over the wings of the female, apparently as a means of subjection during copula as observed in *A. merida* (Fig. 12). We hope this paper is a worthy contribution to the knowledge of Neotropical sawfly species.

Acknowledgments

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