

Life history and description of the larva of *Acrotaeniostola spiralis* (Diptera: Tephritidae: Dacinae: Gastrozonini), an Oriental fruit fly inhabiting bamboo twigs

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Abstract. The life history of the bamboo-infesting fruit fly *Acrotaeniostola spiralis* was studied in northern Thailand. *Acrotaeniostola spiralis* larvae fed on the apical meristem and surrounding soft tissue of bamboo twigs of *Pseudoxynthera albociliata*. Every infested twig was occupied by one larva. The larva created a feeding tunnel, 32 to 67 mm long and between 0.9 and 3.8 mm wide. Feeding activity of the larva caused the apical leaf of the twig to turn yellow and die. The females deposited their eggs under the margins of rolled apical leaves. The freshly emerged larvae squeezed their way through the folds of these leaves towards the growing point of the bamboo twig. Mature larvae abandoned their feeding sites for pupariation and flies emerged 10–11 days after pupariation. Flies were recorded in the field between the end of April and beginning of December and their larvae between mid-June and beginning of December, indicating that *A. spiralis* is probably multivoltine. A larva maturing at the end of December remained dormant during the hot season and the fly emerged at the end of April. The external morphology and cephalopharyngeal skeleton of the third instar larva of the genus *Acrotaeniostola* is described for the first time. The main distinguishing characteristics found for *A. spiralis* larvae are their very long and slim body shape and their reduced facial mask lacking oral ridges. These are probably adaptations to life in the very narrow bamboo twigs. Bamboo twigs displaying withered apical leaves were sometimes occupied by other flies (for example, Chloropidae), as well as beetle larvae or caterpillars.

Key words. apical meristem miner, natural history, *Pseudoxynthera albociliata*, Thailand

INTRODUCTION

Species of the Dacinae tribes Ceratitidini (*Ceratitis*) and Dacini (*Bactrocera*, *Dacus*) infest a broad host range of fruits and comprise some of the major agricultural pests, while larvae of the lesser known members of the dacine tribe Gastrozonini are specialised pests of grasses (Poaceae). Gastrozonini comprise more than 140 described species found in Asia and Africa. The majority of species are distributed in the Oriental region. All Oriental species with known host records breed in bamboo, while the Afrotropical species develop in other Poaceae (e.g., *Panicum*, *Sorghum*, and *Zea*, see Hancock, 1999; Hancock & Drew, 1999; Copeland, 2007).

Gastrozonini larvae usually feed on the meristem (undifferentiated tissue from which new cells are formed)

and neighbouring soft tissue of bamboo shoots, in which they occupy different niches. For example, some species breed in the bamboo wall, others develop in the hollow internode cavity of a bamboo shoot stems and still others on the bamboo surface below the protective internode sheath. The thickness of the internodes also plays a role, leading to a vertical zonation in bamboo shoot utilisation by different Gastrozonini species (Dohm et al., 2014).

In the present article, we report on larvae of *A. spiralis* Munro breeding in a new type of Gastrozonini microhabitat: namely twigs of the bamboo *Pseudoxynthera albociliata* (Munro) Nguyen. *Acrotaeniostola spiralis* is widely distributed in Bangladesh, India (Meghalaya), China (Yunnan), Laos, Malaysia (Sabah) and Indonesia (Sumatra) (Kovac et al., 2006) and is recorded here from Thailand for the first time. We characterise the habitat of *A. spiralis*, outline its biology and phenology, describe its third-instar larva, puparium and egg and compare its larvae with the five Gastrozonini larvae described to date.

MATERIAL & METHODS

Study site. Fieldwork was conducted in northwest Thailand (Province Mae Hong Son) in the mountainous Pangmapha district. Pangmapha has three distinct seasons: the rainy season (end of April to mid-October), the cool season (mid-October to mid-February) and the hot season (mid-February

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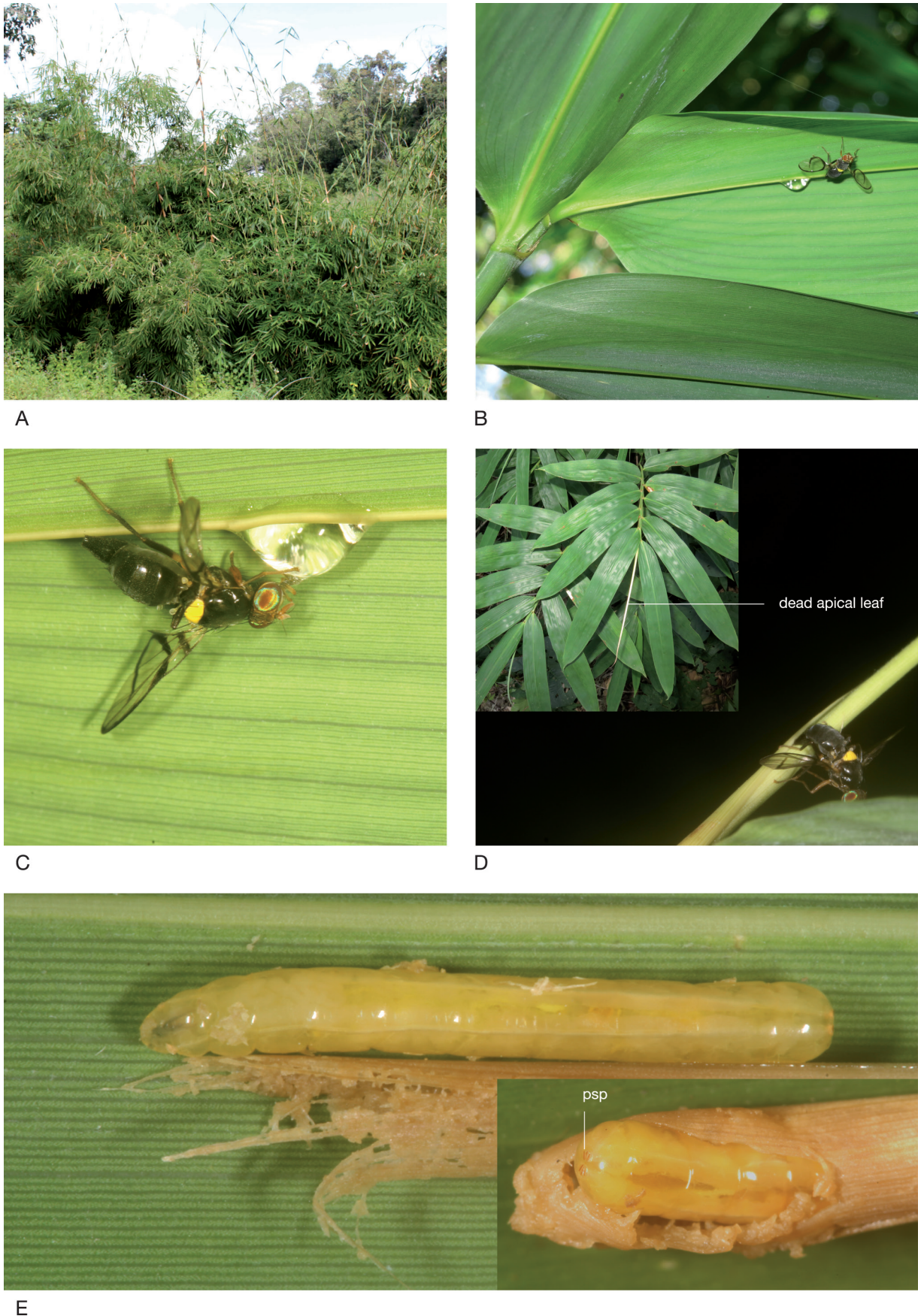


Fig. 1. *Acrotaeniostola spiralis*, habitat and biological traits. A, Bamboo stand of the host plant of *A. spiralis*, *Pseudoxystenantha albociliata*, in northern Thailand in November. Note the emerging bamboo shoots; B, Female on the underside of a bamboo leaf near the apex of a twig of *P. albociliata*; C, Female imbibing water; D, Female ovipositing under the margin of a rolled up apical leaf located at the apex of a bamboo twig. Inset: A twig infested by *A. spiralis* can be recognised by the yellow colour of the rolled up apical leaf (arrow); E, The oblong, yellow larva of *A. spiralis*. Inset: Larva sticking out the rolled up apical leaf, after being pulled out of the apical sheath. Note the feeding marks and the posterior spiracles (= psp) at the caudal end of the abdomen.

to end of April). The host plant of *A. spiralis*, the bamboo *Pseudoxytenanthera albociliata*, was common in areas devoted to farming. *Acrotaeniostola spiralis* was studied sporadically between 2010 and 2016 throughout all months of the year except January/February and August.

Collecting and rearing. Flies were collected using a sweep net. Larvae were obtained by selectively cutting off bamboo twigs of *Pseudoxytenanthera albociliata* (Munro) displaying dead, rolled yellow apical leaves. The leaf blades and the respective leaf sheaths from the base towards the apex of the twig were then torn off until only the apical leaf sheath, including the rolled yellow apical leaf, was left. Subsequently, the apical leaf was pulled out and the apical leaf sheath was carefully torn open using fingernails, starting at the apical (wider) end. The collected *A. spiralis* larvae were boiled in water for 2–3 minutes and finally preserved in 70% ethanol. Larvae selected for rearing were left in the apical leaf sheath, which was partly wrapped in moist tissue and placed in a plastic container (17 × 17 × 11 cm). Mature larvae leaving the substrate for pupariation were transferred to smaller plastic containers and allowed to pupariate between moistened paper tissues. The emerging adults were pinned or transferred to 70% ethanol three days post-hatching.

Slide mounting of the cephalopharyngeal skeleton and posterior spiracles. For the study of the cephalopharyngeal skeleton and the posterior spiracles larvae were transversely cut into three sections. The body was cut between the thorax and abdomen and between the 7th and 8th abdominal segments. Next, the larval sections were treated overnight in 10% KOH at room temperature, following Steck & Wharton (1986) and Steck et al. (1990). Subsequently, the cephalopharyngeal skeleton was further cleared in 10% KOH at 60°C for about one hour. The larval sections were then washed in distilled water for one hour and dehydrated in a graded series of ethanol solutions and terpeneol. Finally, the cephalopharyngeal skeleton and anterior and posterior spiracles were slide-mounted in Canada balsam together with the enclosing larval cuticle.

Scanning Electron Microscopy (SEM). Larvae were cut into three parts as described above, dehydrated in 96% ethanol and then transferred to hexamethyldisilazane (= HMDS; Nation, 1983) for one hour. Subsequently, the larval parts were transferred to fresh HMDS and then HMDS was allowed to evaporate under a fume hood. Dried specimens were mounted on stubs, coated with palladium gold and observed under a Hitachi scanning electron microscope (CamScan CS24).

Terminology. Entomological terminology follows Courtney et al. (2000), Frías et al. (2006), Teskey (1981), and White et al. (2000). Sensilla were classified according to Frías et al. (2006), Frías et al. (2009), and Kovac et al. (2013). Botanical terminology follows Recht & Wetterwald (1992) and Wong (1995).

Acrotaeniostola spiralis larvae used in the present study are deposited at the Senckenberg Natural History Museum,

Frankfurt am Main, Germany (SMF) and at the Steinhardt Museum of Natural History, Tel Aviv University, Tel Aviv, Israel (SMNHTAU).

RESULTS

Biology

Adult habitat and biology. *Acrotaeniostola spiralis* flies were commonly found on bamboo twigs of their host plant *P. albociliata*. *Pseudoxytenanthera albociliata* grew in open clearings, at the edges of fields (Fig. 1A) or in secondary forest at altitudes between 600–1200 m. Culms of mature bamboo stands reached up to 3 cm width at base and averaged about 5 m in height.

Adults were collected or observed in the field at the end of April (first record on 30 April), in May, June, July, September, October, November, and at the beginning of December (last record on 2 December). At the end of April and beginning of May, i.e., at the end of the dry season when the temperatures were still high, the flies remained in humid areas along streams. During the rainy season (around mid-May to mid-October) and the first half of the cool season (November, December) they occupied bamboo twigs.

Acrotaeniostola spiralis flies usually remained in the apical area of bamboo twigs of *P. albociliata*. The adults were not easy to detect, being usually hidden on the underside of the uppermost 1–2 apical leaves of a bamboo twig (Fig. 1B). Sometimes, the flies were also found walking on the leaf sheaths, between the leaves or on the rolled apical leaf. Occasionally, the adults were observed to dab at objects coated with sweat (backpack, walking stick, knife handle), on the sweep net lying on the ground or on leaf surfaces covered with urine; and once in a while they imbibed liquid from water drops adhering to leaves (Fig. 1C).

Females laid their eggs below the margins of the rolled apical leaves (Fig. 1D). The eggs were deposited in the central third of the visible part of the apical leaf, about 4–10 cm below the apex (median=4.5 cm, n=9). At the oviposition site the apical leaves did not always fit tightly onto the underlying leaf surface, perhaps due to tension forces during leaf growth. The egg was located 1.8–9.3 mm from the leaf margin (median=4.6 mm, n=6). There was always only one egg per leaf (n=7), except in one case in which three eggs were detected (two eggs 4.2 cm and one egg 10.4 cm below the apex of the rolled apical leaf).

Larval microhabitat and development. Larvae of *A. spiralis* developed in twigs of the bamboo *P. albociliata*. Each infested bamboo twig contained only one larva (n=40). The elongated body of younger larvae was pale yellow, while mature larvae were intense yellow (Fig. 1E). The larvae were collected in June (first record on 16 June), July, October, November, and December (last record on 5 December). Field data are not available for August, September, and the period between mid-December until mid-March.

The larvae fed in stems of the foliated apical parts of bamboo twigs. The apex of a bamboo twig possessed 5–11 alternate leaves, including the tightly rolled apical leaf (Figs. 1D, 2D). The leaves (proper term “leaf blade”) were 15–25 cm long, 2–3 cm wide and attached to the hard leaf sheaths by a stalk-like structure (“leaf stalk” in Figs. 2A, 2B). The leaf sheaths encircled the stem and were inserted at each node of the segmented axis of the twig (Fig. 2B). The leaf sheaths were much longer than the respective internodes and overlapped. The tightly rolled apical leaf protruded from the apical leaf sheath. The visible part of the leaf was 7–25.5 cm long (Median=15; n=37); and its lower part, hidden by the apical leaf sheath was 4–9 cm long (length measured from the apical edge of the apical sheath to the leaf stalk; Median=6.5 cm; n=9; Fig. 2B). The leaf stalk of the rolled apical leaf was connected to a leaf sheath, which was hidden below the apical leaf sheath (=“hidden leaf sheath”).

The feeding area of *A. spiralis* was located approximately between the 3rd and 8th leaf (Fig. 2D). It occupied roughly the lower two thirds enveloped by the apical sheath and did not quite reach the basal node where the apical leaf sheath was inserted (Fig. 2B; feeding area highlighted in grey). The apical sheath was slightly curved, 7.5–14 cm long (Median=13, n=11), with the upper end wider and flat, and the lower end narrower and circular (Fig. 2B). The feeding area was 3.2–6.7 cm long (Median=5.3 cm; n=13). At the upper end of the feeding area the leaf sheath measured $1.27\text{--}1.82 \times 2.2\text{--}3.8$ mm (n=10) and at the lower end from 0.9 mm in diameter (if the feeding channel reached the lower circular area) up to 1.42×2.8 mm (n=10). Thus, the width of the feeding tunnel of *A. spiralis* lay within the range of 0.9–3.8 mm.

Acrotaeniostola spiralis larvae fed on the apical meristem and surrounding soft tissues as well as on the soft basal 1–2 cm of the rolled apical leaf. Due to this feeding activity, the basal part of the apical leaf became almost detached from its base and could be easily pulled out of the apical sheath. Although under natural conditions the yellow apical leaves bent down after several weeks, they remained on top of the twig, being held in place by the enclosing leaf sheaths. The larvae abandoned their twigs for pupariation. In the lab they crawled on the ground using their mouth hooks and occasionally tried to skip: the larva raised the anterior part of its body off the substrate and tried to form an upright body loop as is known from other Gastrozonini (see Discussion). However, the larvae usually fell on their sides before being able to adopt the skipping posture. When lying on the ground the larvae continued to bend their bodies in order to form a loop, but they did not succeed and a proper jumping movement was not observed.

The larvae pupariated shortly after being collected, i.e., they were already close to maturation. The period of time between collection of the larvae and emergence of the adults was 9–24 days (Median=14; n=11). The interval between pupariation and emergence of the adult was 11 and 12 days (n=2). In one case a dead mature larva was found in the area

between the outer surface of the rolled apical leaf and the inner wall of the apical leaf sheath.

Larvae collected between June and December pupariated immediately after leaving their bamboo twigs. The first larva collected on 16 June was reared to adulthood on 27 June and the last larva collected on 5 December was reared to adulthood on 23 December. However, one larva collected at the beginning of December remained in the larval stage for several months and the fly emerged on 30 April, i.e., at the end of the dry season.

Despite being protected from the outside world by the hard overlapping leaf sheaths, the larvae were sometimes infested by parasitic wasps that had emerged from *A. spiralis* puparia. Furthermore, some *A. spiralis* larvae were apparently preyed upon, because leaf sheaths of several twigs were seen to have been pecked open at the level of the apical meristem, probably by birds.

Causes of apical leaf damage and abundance of withered apical leaves. The damage of the apical meristem and withering of apical leaves was caused not only by *A. spiralis*, but also by other insects. Thus, larvae of three species of Chloropidae (Diptera) fed on the apical meristem of bamboo twigs and produced similar feeding marks to those of *A. spiralis*. In contrast with *A. spiralis* they pupariated inside the twigs. Other insects found feeding on the meristem of bamboo twigs were two species of unidentified caterpillars and at least two species of beetles (beetle larvae belonging to Chrysomelidae and Curculionidae). The caterpillars and beetle larvae were able to puncture the leaf sheaths in order to reach the twig meristem or create an exit hole. Twigs infested by caterpillars initially possessed a single yellow apical leaf, but later on up to four more leaves withered. In many cases feeding marks were lacking and the reason for apical leaf damage was not apparent. The basal parts of dead apical leaves were sometimes folded together inside the apical leaf sheaths.

Intact rolled apical leaves were always abundant during the periods covered in the present study; whereas twigs displaying withered apical leaves were rare and usually only 0–3 such twigs per bamboo bush or culm were found. An approximate four hour search in a *P. albociliata* bamboo area at an accessible height yielded 18–73 twigs displaying withered apical leaves. Occasionally, twigs possessing withered apical leaves were abundant at certain bamboo bushes, but the damage was not caused by insect infestation, since there were no feeding marks. During the present study, larvae of *A. spiralis* were initially found in October and November (end of the rainy and cool season). Attempts to detect larvae between May and July (beginning of the rainy season) failed for two years, but following an intensive search during the third year larvae were finally detected in June and July, although in low numbers.

The number of twigs containing *A. spiralis* or other insects during the rainy season was as follows: on 7 days between

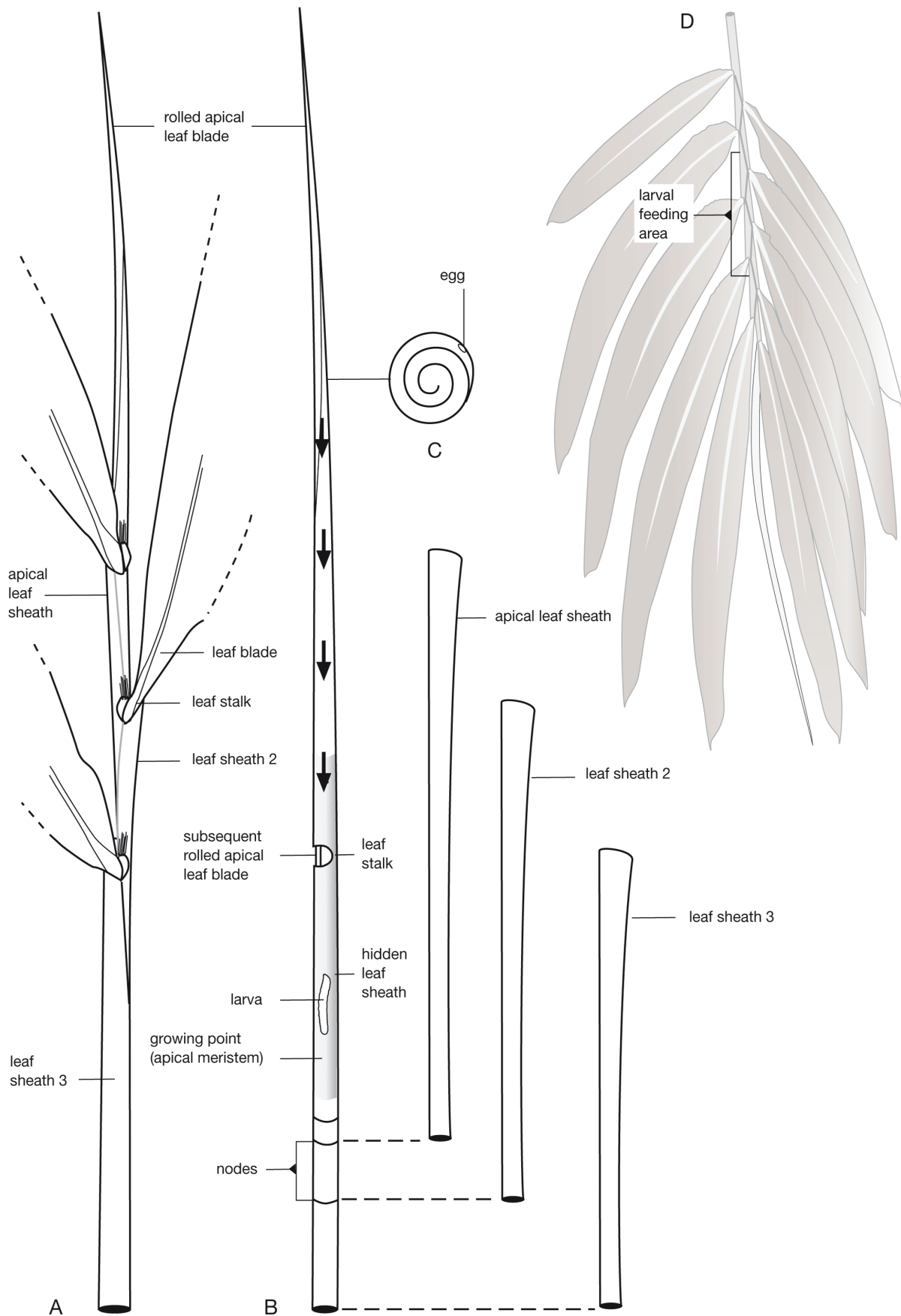


Fig. 2. Schematic illustration of a bamboo twig of *Pseudoxystenantha albociliata* showing the egg-laying and larval feeding site of *A. spiralis*. A, Intact apex of a bamboo twig; B, Visible sheaths of a bamboo twig removed and placed beside the stem in order to show their insertion locations and their degree of overlapping (leaf blades omitted). Bold arrows symbolise the penetration of the larva through the folds of the leaf towards the growing point of the bamboo branch. The larval feeding area is highlighted in grey; C, Cross-section of a rolled up apical leaf showing the location of an *A. spiralis* egg; D, Apical part of a bamboo twig hanging down from a bamboo stem showing the location of the feeding area of *A. spiralis*.

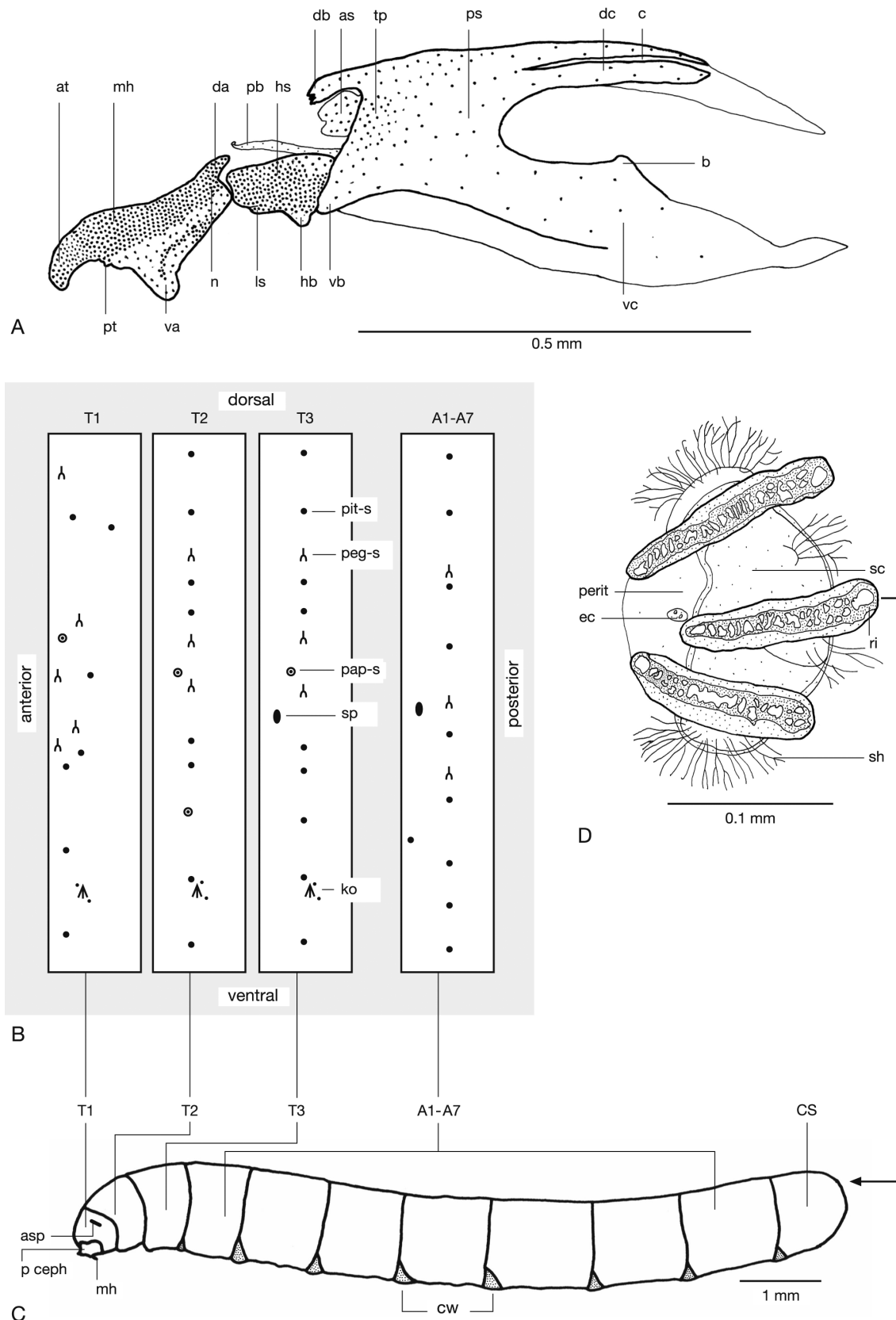


Fig. 3. Cephalopharyngeal skeleton, posterior spiracle, larval habitus (light microscopy) and sensillae on thoracic and first abdominal segments (SEM microscopy) of *A. spiralis*. A, Cephalopharyngeal skeleton in lateral view; B, Schematic illustration of the location of sensillae distributed on thoracic and abdominal segments; C, Larval habitus, length about 8–10 mm; D, Posterior spiracle. Abbreviations: A1–A7 = abdominal segments, asp = anterior spiracle, as = anterior sclerite, at = apical tooth, b = bulge, CS = caudal segment, c = cleft, cw = creeping welt, da = dorsal apodeme, db = dorsal bridge, dc = dorsal cornu, ec = ecdysial scar, hb = hypopharyngeal bridge, hs = hypopharyngeal sclerite, ko = keilin's organ, ls = labial sclerite, mh = mouth hook, n = neck, pb = parastomal bar, pap-s = papilla-sensillum, p ceph = pseudocephalon, peg-s = peg-sensillum, perit = peritreme, pit-s = pit-sensillum, ps = pharyngeal sclerite, pt = praecipal tooth, ri = rima, sc = spiracular chamber, sh = spiracular hairs, sp = (rudimentary) spiracle, tp = tentoreal phragma, T1–T3 = thoracic segments, va = ventral apodeme, vb = ventral bridge, vc = ventral cornu.

15 June and 13 July 2016, 238 twigs displaying withered apical leaves were collected, of which only 12 twigs were occupied by *A. spiralis* larvae (5%). The remaining twigs were inhabited by larvae or puparia of Chloropidae (25 twigs; 10.5%), caterpillars (18 twigs; 7.6%), larvae of Chrysomelidae (28 twigs; 11.8%) or larvae of Curculionidae (2 twigs; 0.8%). One hundred and twelve twigs (47.1%) possessing withered apical leaves showed no feeding marks, and in 26 of them the basal parts of apical leaves (enclosed by the apical sheaths) were folded together. Finally, in 41 twigs (17.2%) feeding marks probably caused by Tephritidae or Chloropidae were found, but the twigs were already deserted (9 twigs in the last two weeks of June and 32 twigs in the first two weeks of July).

Thirty eight twigs were collected on 31 October and 1 November 2015, i.e., during the cool season. They contained *A. spiralis* larvae (9 twigs; 23.7%) and Chloropidae larvae or puparia (5 twigs; 13.1%). Twelve twigs (= 31.6%) were already deserted and showed feeding marks of Tephritidae or Chloropidae. The deserted cavities were often occupied by secondary inhabitants, such as tiny fly larvae or ants. Finally, 12 twigs (31.6%) showed no feeding marks, i.e., the apical leaves had died for other reasons.

Larval Description

Acrotaeniostola spiralis (third instar larva)

Material examined. 1 larva, T55/13b, between Soppong and Ban Nam Rin, Mae Hong Son, Thailand, coll. D. Kovac, 6 November 2013; 2 larvae, Z75/1/2013b, between Soppong and Ban Nam Rin, coll. D. Kovac, 15 November 2013; 1 larva, T73/13b, between Soppong and Ban Nam Rin, coll. D. Kovac, 22 November 2013; 1 larva, NV130/14, between Soppong and Ban Nam Rin, coll. D. Kovac, 2 December 2014; 5 larvae, Z52/1/14, between Soppong and Ban Nam Rin, coll. D. Kovac, 6 December 2014. All larvae collected from twigs of the bamboo *Pseudoxytenanthera albociliata*. Seven larvae used for habitus measurements, 3 larvae for preparing slides of the cephalopharyngeal skeleton/posterior spiracle and 5 larvae for the SEM study.

Habitus. (Figs. 1E, 3C). Length: 8.0–10.8 mm (Median: 9.4 mm; n=7), width: 1.0–1.2 mm (Median: 1.1 mm; n=7). Mature larvae intense yellow, slender, length 7.2–9.6× the width of the body (Median: 8.5; n=7), broadest in the area of the first and second abdominal segments, slightly tapering towards the posterior end, conical anteriorly, truncate posteriorly, caudal end somewhat convex.

Pseudocephalon. (Fig. 4A–F). Facial mask covering a narrow area on both sides of the mouth opening (Fig. 4A) or sometimes extending dorsally with 1–3 rows of plates up to the level of the antenna (Fig. 4B, 4C). In one specimen extended facial mask present only on one side of the body (Fig. 4B). Shape of plates of facial mask variable, if facial mask extended then plates usually shorter and non-serrated (slightly serrated in one specimen), if only a narrow area

covered then plates neighbouring the edge of mouth opening somewhat larger and distinctly to slightly serrated.

Antenna and maxillary sense organs located on slightly protruding cephalic lobes (Fig. 4D). Antenna two-segmented. Basal segment approximately cylindrical, apical segment cylindrical with rounded tip. Maxillary sense organ consisting of maxillary palpus and dorsolateral group of sensilla. Maxillary palpus enclosed by a cuticular fold, bearing three papilla-sensilla and two knob-like sensilla. Dorsolateral group of sensilla containing two papilla-sensilla.

Primary preoral lobe rounded, bearing preoral organ. Primary preoral lobe in some specimens surrounded by plates, while in other specimens plates anterior to preoral organ lacking, or in one specimen only flat elevations visible (Fig. 4A–C, 4E). Preoral organ bearing one large peg-sensillum, one very small peg-sensillum, one papilla-sensillum, and two pit-sensilla. Labial lobe elongate, widened at apex; basal surface covered with tubercles. Labial organ consisting of two small openings (Fig. 4F). Five pairs of pseudocephalic isolated pit-sensilla present. Pseudocephalic pit-sensilla distributed as follows: On each side of the pseudocephalon one sensillum located medial to the antenna, one medial to the maxillary sense organ, one posteroventral to the antenna, and two lateral to the facial mask.

Cephalopharyngeal skeleton (Fig. 3A). Total length of cephalopharyngeal skeleton 0.7–0.8 mm (n=3). Mouth hooks dark brown, indentation between tips of apical tooth and ventral apodeme 0.16–0.19 times as deep as mouth-hook long; apical tooth dark brown, curved, ventral surface slightly toothed (visible under high magnification); neck well developed; one small preapical tooth present; ventral apodeme large and stout, oriented posteroventrally; dorsal apodeme prominent, oriented posterodorsally. No dental sclerites visible. Hypopharyngeal sclerite about 2.5 times as long as high, anterior part dark brown, small posterodorsal part lighter brown. Hypopharyngeal bridge large, brown, located between central and posterior third of the length of the hypopharyngeal sclerite. Labial sclerite dark brown, flat. Pharyngeal sclerite light brown, darker brown in the area of the tentorial phragma and the dorsal bridge; dorsal and ventral cornua brown, hyaline tissues adjoin the posterior tips of both cornua and ventral margin of ventral cornu; brown areas of both cornua equally long. Dorsal cornu cleft; ventral cornu with a bulge at the dorsal margin. Dorsal bridge distinctly protruding; ventral bridge only slightly bulging. Parastomal bars light brown, approximately as long as hypopharyngeal sclerite, tips hooked in one specimen. Anterior sclerite small, light brown.

Thoracic segments (Figs. 3B, 4A, 4C, 5A). Anterior spiracles on thoracic segment I with 13–20 approximately cylindrical tubules arranged in a single row (Fig. 5A). Each tubule with a slit-like opening at the apex. A rudimentary spiracular opening located ventrolaterally near the anterior margin of third thoracic segment. Anterior margin of first thoracic segment covered with numerous rows of multidentate scales

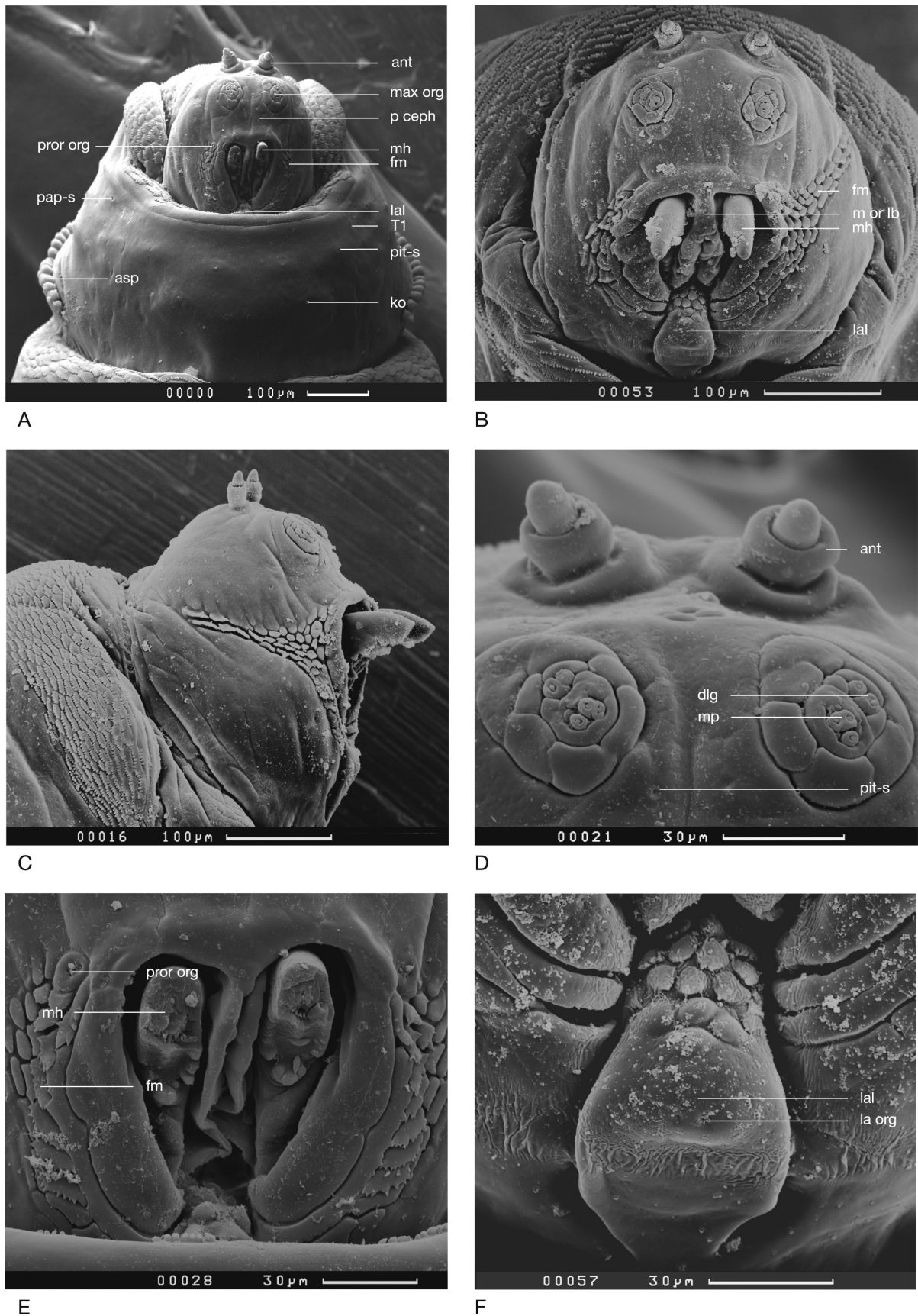


Fig. 4. Pseudocephalon and first thoracic segment of the larva of *A. spiralis* (SEM photographs). A, Pseudocephalon and first thoracic segment, ventral view; B, Pseudocephalon, frontoventral view, showing two types of facial masks on the same individual; C, Pseudocephalon and part of the first thoracic segment, lateral view; D, Antennae and maxillary palpus; E, Area surrounding the mouth opening showing the preoral organ, facial mask and the mouth hook (tips broken off); F, Labial lobe. Abbreviations: ant = antenna, asp = anterior spiracle, dlgs = dorsolateral group of sensilla, fm = facial mask, ko = keilin's organ, lal = labial lobe, la org = labial organ (arrow pointing to left opening), max org = maxillary organ, mh = mouth hook, m or lb = median oral lobe, mp = maxillary palp, p ceph = pseudocephalon, pap-s = papilla-sensilla, pit-s = pit-sensilla, pror org = preoral organ, T1 = first thoracic segment.

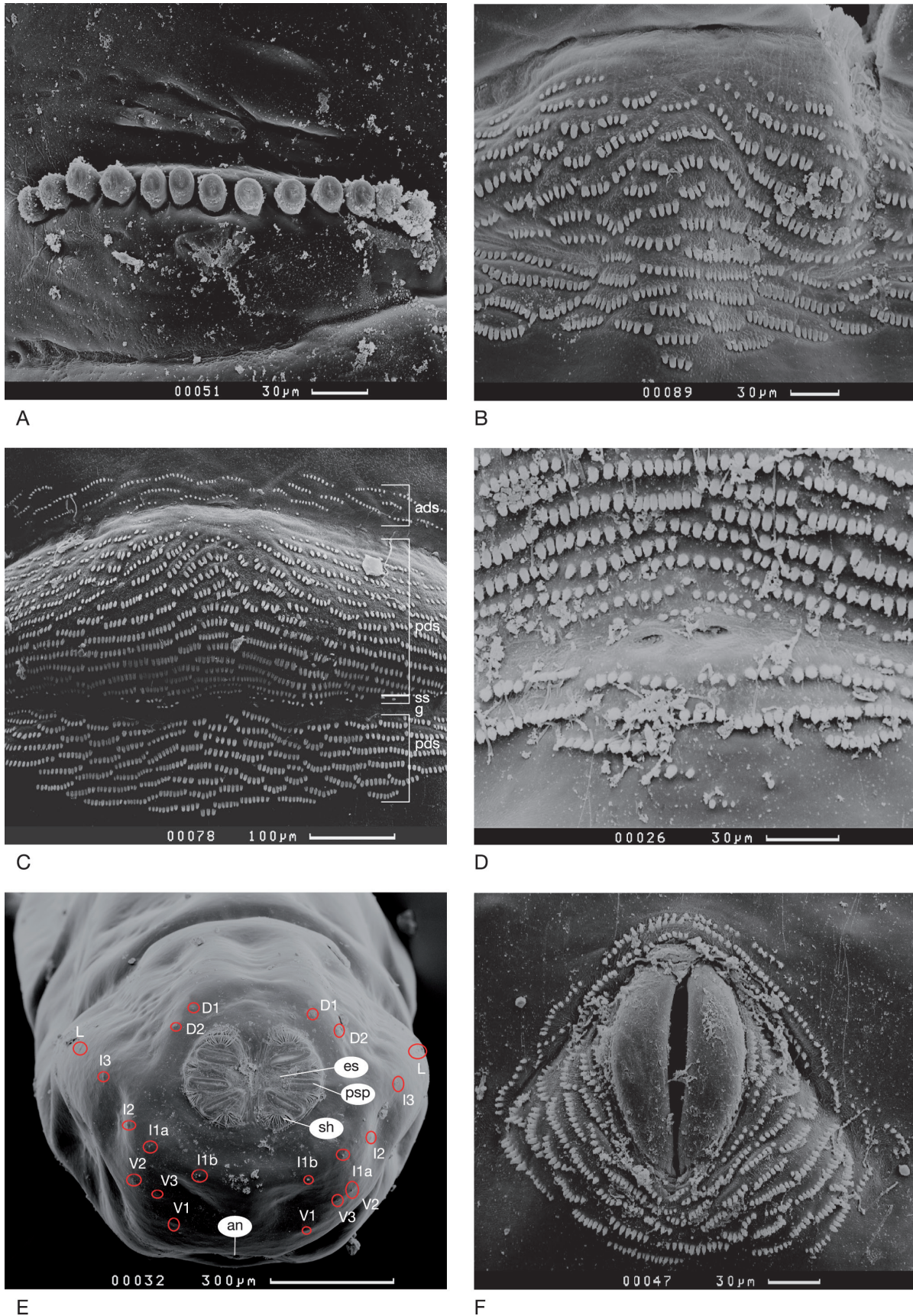


Fig. 5. Anterior spiracle and caudal segment showing the posterior spiracle, anus and the locations of the sensilla of *A. spiralis* (SEM photographs). A, Anterior spiracle; B, Creeping welt of first abdominal segment; C, Creeping welt of the fifth abdominal segment; D, Part of the creeping welt of the caudal segment showing two openings; E, Caudal segment; F, Anal lobe. Abbreviations: ads = anteriorly directed spinules, an = anal lobe, D1 = dorsal sensillum 1, D2 = dorsal sensillum 2, es = ecdysial scar, g = gap, I1a = intermediate sensillum 1a, I1b = intermediate sensillum 1b, I2 = intermediate sensillum 2, I3 = intermediate sensillum 3, L = lateral sensillum, pds = posteriorly directed spinules, psp = posterior spiracle, sh = spiracular hairs, ss = small spinules, V1 = ventral sensillum 1, V2 = ventral sensillum 2, V3 = ventral sensillum 3.

(Fig. 4A, 4C). Second and third thoracic segments bearing 5–6 rows of stout spinules at anteroventral margin; lateral and dorsal segment areas lacking spinules. Each thoracic segment bearing 15 sensilla on each side of the body, location of sensilla depicted in Fig. 3B. Paired Keilin's organs shaped as trifid sensilla located ventrally on each thoracic segment (Figs. 3B, 4A).

Abdominal segments I–VII (Figs. 3B, 5B–C). Abdominal segments I–VII each bearing 13 pairs of sensilla, one on each side of the body (Fig. 3B). Rudimentary spiracles present near the anterior margins of abdominal segments I–VII. Single opening present medioventrally on the sixth abdominal segment, in two specimens single opening also present on seventh abdominal segment.

Creeping welts present ventrally on all abdominal segments (Fig. 5B, 5C); first abdominal creeping welt consisting of about 12 rows of large, blunt, posteriorly directed spinules (Fig. 5B); creeping welts of abdominal segments II–VII consisting of rows of spinules arranged as follows (from anterior to posterior): 1–3 irregular rows of small anteriorly directed spinules (in some cases strongly reduced); 11–14 rows of large, blunt, posteriorly directed spinules; 1 irregular row of upwards to anteriorly directed spinules followed by a gap; 3–8 rows of large, blunt, posteriorly directed spinules (Fig. 5C).

Caudal segment (Figs. 3D, 5D–F). Posterior spiracles located slightly above midline, closest distance between the two spiracles about 0.4 times as long as the longest spiracle opening (Figs. 3D, 5E). Peritreme hyaline; spiracular chamber yellow; ecdysial scar visible. Central opening located more lateral than dorsal or ventral openings; angle between dorsal and central openings 18° – 27° , about one third the length of the longest opening apart; angle between central and ventral openings 23° – 30° , at the medial tip of the central opening one fifth to one sixth the length of the longest opening apart. Rima visible; spiracular openings 7.3–9.2 times as long as wide. Spiracular hairs arranged in four groups, located on small protuberances; hairs up to 0.4 times the length of the spiracular openings, spiracular hairs branched up to 2 times; dorsal bundle containing 12–20 hairs, ventral bundles containing 10–18 hairs, dorsolateral and ventrolateral bundles containing 4–9 hairs each.

Caudal ridges and dark transverse line on caudal segment absent. Anal lobes slightly protruding, of slender oval shape, surrounded anteriorly by 2–3 rows of stout spinules and posteriorly by about 6 rows of similar spinules (Fig. 5F). Spinules lateral to anal lobes lacking in one specimen. Constant dorsal, intermediate, lateral and ventral sensilla present, sensilla I1a and I1b located unusually far apart from each other and sensillum I3 unusually far dorsally. A row of three pit-sensilla present on each body side between anterior margin of anal lobes and lateral sensillum of caudal area. Creeping welt present, rows of spinules arranged as in abdominal segments I–VII, in the middle of the creeping welt two openings visible (Fig. 5D).

Eggs (empty shells). Length: approximately 2–2.5 mm (Median: 2.1 mm; $n=5$); width: approximately 0.4–0.6 mm (Median: 0.5 mm; $n=5$) wide. Eggs elongate and approximately parallel-sided over most of their length; posterior end rounded; anterior end with distinct pedicel (visible in only one examined shell). Pedicel slightly wider (0.16 mm) than long (0.14 mm), somewhat pointed at anterior end; no aerophyles visible. Surface of egg shell entirely covered by net-like sculpture. Intact eggs white in color.

Puparia. Length: 5.3–6.8 mm ($n=2$), width 1.3–1.8 mm ($n=2$). Coloration brown, thoracic segments strongly tapering towards anterior end, abdominal segments more parallel sided, broadest at the fourth abdominal segment.

DISCUSSION

Biology. In the present study we report on *A. spiralis* larvae inhabiting bamboo twigs, a newly recorded habitat and niche for the Gastrozonini. The observed *A. spiralis* larvae possessed a remarkably elongate body shape adjusted to the dimensions of their feeding tunnel, which was approximately 50 mm long and only about 1–3 mm wide. They fed on the apical meristem and neighbouring soft bamboo tissue, which provided just enough food for a single larva, while in most other Gastrozonini many larvae are found in a bamboo shoot or a single internode (Dohm et al., 2014).

Tough internode sheaths are the main hindrance preventing Gastrozonini from reaching the nutritious bamboo shoot substrate, because the flat aculeus of the females is apparently unsuitable for piercing the sheaths. Instead, females usually deposit their eggs below the margins of the internode sheaths. Freshly emerged larvae thus have to squeeze between the sheath and the bamboo surface (or overlapping sheaths) in order to reach their food source. Bamboo shoots protected by many overlapping sheaths (for example, the apical area of thick bamboo shoots), form an impenetrable barrier for the larvae. Colonisation of such shoots is only possible if the protective sheaths are damaged, e.g., by falling trees, large animals or human activity. Species belonging to *Cyrtostola* and *Paraxarnuta* are even entirely dependent on the egg-laying holes created by the weevil *Cyrtotrachelus* sp. in order to lay their own eggs (Kovac & Azarae, 1994; Dohm et al., 2014). A similar behaviour is also known from some Tephritidae breeding in wood (see Kovac et al., 2010).

The apical area of the bamboo twigs was also protected by tough overlapping sheaths (Fig. 2B). In order to bypass these sheaths females of *A. spiralis* laid their egg under the edge of the tightly rolled apical leaf (Fig. 1D). The freshly hatched larvae squeezed through the folds of the leaf towards the soft tissue of the growing point of the twig (Fig. 2B). Mature larvae appeared to leave the twigs by squeezing towards the apex of the apical sheath between the outer surface of the rolled up apical leaf and the inner wall of its sheath, as supported by two observations: (1) a dead mature larva was found in the area between the apical leaf and the apical sheath, suggesting that the larva had not managed to squeeze out of the feeding area, as was observed in some

larvae of *Anoplomus rufipes* Hardy (see Kovac 2015); and (2) sheaths of abandoned *A. spiralis* twigs never displayed any holes, in contrast with twigs inhabited by caterpillars and beetle larvae.

Gastrozonini larvae of most species abandon their bamboo feeding sites and pupariate in the soil, probably because their habitat is short-lived and decomposes fast (for example, 2–3 weeks for dead bamboo shoots lying on the ground; Dohm et al., 2014). Conversely, *Ichneumonopsis burmensis* Hardy larvae, inhabiting long-lived thin bamboo shoot stems, pupariated in their feeding tunnels and remained there during the hot season (Kovac et al., 2013). The habitat of *A. spiralis* was also relatively long-lived (several months) and pupariation in bamboo twigs was possible in principle, as reflected in Chloropidae dwelling in the same habitat always pupariating in their feeding tunnels. Furthermore, the puparial stage of *A. spiralis* lasted only 10–11 days. Nevertheless, *A. spiralis* larvae left the twigs for pupariation, perhaps avoiding parasitoids, predators, extreme temperatures or for other reasons.

Mature Gastrozonini larvae are known to skip after leaving their feeding sites, especially if disturbed. The larvae can leap more than 10 cm making it difficult for predators or parasitoids such as ants or parasitic wasps to catch or relocate them. Typically, the Gastrozonini larva raises the anterior part of its body off the substrate and then bends it downwards and brings the head in contact with the caudal segment, thus forming an upright loop. The mouth hooks are firmly anchored to the caudal segment and the muscles develop tension in the body wall to power the jump (Maitland, 1992). Larvae of *A. spiralis* also tried to form a jumping loop when disturbed, but they always fell over. Perhaps, the larvae were not able to keep balance or build up tension in this unstable position due to their elongated body shape.

Acrotaeniostola spiralis adults were present in the field between the end of April and mid-December. There are no collection data for August, but we presume that *A. spiralis* flies were present during this month as well, because reared flies emerged in July. Larvae were found from June to December (no collecting in August and September) and adults reared from larvae emerged during all of these months. Since the period from egg-laying to adult emergence lasts about 3–5 weeks in Gastrozonini (Dohm et al., 2014) it is likely that *A. spiralis* is normally multivoltine.

Mature *A. spiralis* larvae collected between June and beginning of December pupariated immediately after they had abandoned the twig. However, one larva collected in mid-December remained in the larval stage for several months after leaving the twig, and the respective fly emerged only on 30 April, i.e., at the beginning of the rainy season. This suggests that at least some larvae reaching maturity after the beginning of December do not pupariate but, rather, remain dormant during the dry season. The advantage of remaining in the larval stage is probably that larvae can relocate if the environmental conditions in the soil become unfavorable.

The same phenomenon was also observed in the gastrozonine *Anoplomus rufipes* (Kovac, 2015).

In June and July larvae of *A. spiralis* were hard to find and relatively rare (only 5% of collected twigs colonised by *A. spiralis*), while in November and December they were more abundant (25% of collected twigs colonised by *A. spiralis*). Thus, it appears that the population density of *A. spiralis* increases over the course of most of the year and then drops during the dry season due to a possible increased mortality. Bamboo twigs inhabited by *A. spiralis* were recognisable through the presence of withered rolled apical leaves. However, apical leaves could also have died due to other reasons. Thus, some apical leaves were destroyed by the feeding activities of various insects (Diptera, Lepidoptera, Coleoptera), while others have died for unknown reasons since feeding marks were lacking. Possible reasons for the latter deaths were diseases, lack of minerals or disturbed growth, since the basal parts of some apical leaves were occasionally folded together inside the apical leaf sheaths. Despite being protected by the tough bamboo sheaths *A. spiralis* larvae were, like other Gastrozonini, parasitised by parasitic wasps and, judging from injuries to the leaf sheaths possibly also preyed upon by birds. Feeding tunnels abandoned by *A. spiralis* served as a habitat for other insects, such as small fly larvae and ants.

In summary, the following overall picture of the life cycle of *A. spiralis* emerges: *A. spiralis* adults emerge from puparia at the beginning of the rainy season (end of April/May) and gather in moist places along streams as long as it remains hot. When rainfall increases the flies spread to the surrounding areas and occupy apical parts of bamboo twigs. Eggs are deposited below the margin of rolled apical leaves of twigs and the larvae feed on the apical twig meristem. Mature larvae leave the twigs for pupariation. The flies probably mate and lay their eggs in May/June and the first generation of flies emerges at the end of June and in July. *Acrotaeniostola spiralis* is probably multivoltine and the population density seems to increase throughout the year but probably decreases during the hot season. The greater number of larvae developing in December probably remains dormant until the beginning of the next rainy season in April.

Larval morphology. To date, larvae of five Gastrozonini species belonging to four genera are known. Elson-Harris (1992) described *Chaetellipsis atrata* Hardy (= *Chaetellipsis paradoxa* Bezzi), *Chaetellipsis* n. sp. (= *Chaetellipsis maculosa* Hancock & Drew), *Gastrozona fasciventris* (Marquart) and *Taeniostola limbata* Hendel (= *Cyrtostola limbata* (Hendel), see Hancock & Drew, 1999) and Kovac et al. (2013) described the atypical Gastrozonini *Ichneumonopsis burmensis* Hardy.

The most obvious morphological difference between the larva of *A. spiralis* and larvae of other Gastrozonini lies in its elongate body shape and the reduced facial mask lacking oral ridges. The facial mask of *A. spiralis* consists of plates, which are sometimes confined to a narrow area

flanking the preoral cavity. The plates vary considerably in shape among different specimens. In most other Gastrozonini (*Chaetellipsis*, *Gastrozona*, *Cyrtostola*) the facial mask consists of long parallel rows of dentated oral ridges flanking the preoral cavity, followed by a few plates (= "accessory plates") situated at the edges of the facial mask. *Ichneumonopsis burmensis* is similar to *A. spiralis*, with the extensive facial mask of the former consisting mainly of plates, while the oral ridges are non-dentate, short and indistinct (Kovac et al., 2013).

Dentate oral ridges are common in saprophagous frugivorous tephritids (White & Elson-Harris, 1992). The grooves between the oral ridges are thought to direct food-bearing liquids to the mouth. In truly phytophagous, predatory or parasitoid larvae, the facial mask features plates rather than oral ridges or the ridges may be strongly reduced (see Courtney et al., 2000). Thus, the reduction of the oral ridges in *A. spiralis* and *I. burmensis* is probably due to feeding in living, relatively dry plant tissue, in contrast with Gastrozonini species living in soft, decaying bamboo shoots. The high variability in size of the facial mask and shape of accessory plates among *A. spiralis* (in one specimen even differing between the two sides, see Fig. 4B) may indicate that the evolutionary process of reduction of the facial mask is still in progress.

Other morphological structures found in the pseudocephalon of *A. spiralis* such as the two-segmented antenna, the maxillary sense organs and the preoral organ, are similar to other Gastrozonini (Elson-Harris, 1992; Kovac et al., 2013). The rounded primary preoral lobe of *A. spiralis* (bearing the preoral organ) is similarly shaped to that in the four Gastrozonini species described by Elson-Harris (1992). Conversely, the primary preoral lobe of *I. burmensis* is distinctly oval (Kovac et al., 2013). While the preoral organs of the previously described Gastrozonini larvae are surrounded by well-developed preoral lobes (Elson-Harris, 1992), or in the case of *I. burmensis*, by ordinary accessory plates (Kovac et al., 2013), the preoral lobes of *A. spiralis* are, as the remaining facial mask, fairly reduced and were hardly developed at all in one examined specimen. The labial lobe is similarly shaped in most known Gastrozonini larvae. In *A. spiralis* the basal surface of the labial lobe is covered by rounded protuberances. In contrast, the labial lobe of *I. burmensis* is covered by elongate spines (Kovac et al., 2013), whereas Elson-Harris (1992) did not mention any protuberances on the surface of the labial lobes of the Gastrozonini species described by her. The median oral lobe of *A. spiralis* is slightly protruding, while considerably protruding in other Gastrozonini (Elson-Harris, 1992; Kovac et al., 2013).

The cephalopharyngeal skeleton of *A. spiralis* is similar to those of other Gastrozonini. Thus, the mouth hooks exhibit well-developed necks and a slender appearance (exception: *I. burmensis*), the preapical teeth are present (exception: *C. limbata*, but according to our observations they are present in specimens from North Thailand) and the dental sclerites are lacking. The hypopharyngeal sclerite of *A. spiralis* (and also *I.*

burmensis (Kovac et al., 2013)) is short (about 2.5× as long as high), while in other Gastrozonini the hypopharyngeal sclerite is longer (3–4× as long as high; measurements taken from drawings by Elson-Harris (1992)). The pharyngeal sclerite of *A. spiralis* is similarly shaped as in other Gastrozonini species (Elson-Harris, 1992); however, *I. burmensis* differs in having a window rather than a cleft in the dorsal cornu (Kovac et al., 2013). *Ichneumonopsis burmensis* is also atypical among the Gastrozonini in possessing ventral sclerites (Kovac et al., 2013).

The anterior spiracles of *A. spiralis* consist of 13–20 tubules arranged in one row. This is very similar to the arrangement of the anterior spiracles of *Chaetellipsis* species described by Elson-Harris (1992) and most dacine species examined to date (for example, Phillips, 1946; Exley, 1955; Malan & Giliomee, 1969; Khan, 1980; Carroll, 1992; Elson-Harris, 1992; White & Elson-Harris, 1992; Carroll, 1998). In some other Gastrozonini the anterior spiracles are arranged in two rows (*C. limbata* and *G. fasciventris*; Elson-Harris, 1992) or in radiating branches (*I. burmensis*; Kovac et al., 2013). The third thoracic segment and the first seven abdominal segments of *A. spiralis* bear a pair of rudimentary spiracular openings (Fig. 3B). Such rudimentary spiracular openings have not been recorded in other Gastrozonini, but may have been overlooked, since rudimentary spiracles are common in Brachycera (Courtney et al., 2000) and are present in larvae of many tephritid species (see, for example, Goeden & Teerink, 1999a, 1999b; Goeden, 2000a, 2000b, 2000c, 2001a, 2001b, 2002a, 2002b, 2002c, 2002d).

The openings of the posterior spiracles of *A. spiralis* are slender in shape as in other Gastrozonini. According to Elson-Harris (1992), the slender openings of the posterior spiracles may be a common character of the bamboo-infesting Gastrozonini. Another morphological structure sometimes found on the caudal segment is the caudal ridges (White et al., 1999). In *A. spiralis* and *I. burmensis* (Kovac, 2013) caudal ridges are lacking, while in *Chaetellipsis*, *Gastrozona* and *Cyrtostola* they are present (unpublished observations). In addition to the larger morphological structures mentioned above, we also examined the tiny sensilla distributed on the pseudocephalon, thorax and abdomen. On the pseudocephalon of *A. spiralis* we have detected ten isolated pit-sensilla (see description and Fig. D). In contrast, *I. burmensis* larvae possess only two pairs of sensilla, located on flat, raised areas of the integument so-called "pad organs" (Kovac et al., 2013). Elson-Harris (1992) did not investigate sensilla on the larval surface of her specimens, but her SEM pictures reveal the presence of isolated sensilla as well as pad organs. In *Chaetellipsis maculosa* (in Elson-Harris text referred to as *C. n. sp.*) one pit-sensillum is visible posteroventral to the antenna. Furthermore, there is one pad organ located anterodorsally and one pad organ posterodorsally to the oral ridges (Elson-Harris, 1992: plates 312, 313). *Gastrozona fasciventris* bears at least one pit-sensillum anterodorsal to the oral ridges (Elson-Harris, 1992: plates 341, 343).

On the thoracic and abdominal segments of *A. spiralis* isolated sensilla are distributed in encircling rows (Fig. 3B).

The arrangement of sensilla on the first thoracic segment somewhat differ from the two following segments, while on abdominal segments 1–7 the arrangement is identical. The sensilla of *A. spiralis* are more or less isolated, while in *I. burmensis* they are often arranged in pairs of one pit- and one papilla- or peg-sensilla. Elson-Harris (1992) does not mention isolated sensilla on the larval body surface, but some sensilla are visible on her SEM-pictures. On the last abdominal (caudal) segment of *A. spiralis* several sensilla are present on the larval surface surrounding the posterior spiracles (Fig. 5E).

Comparative data on sensilla located on the larval body surface are fragmentary, but it seems that the sensilla are distributed in a similar manner in different species of Gastrozonini or even in other Dacinae or Tephritidae. Thus, the sensilla and pad-organs of Gastrozonini seen on pictures provided by Elson-Harris (1992: plates 312–314+341+343) are located in the same areas as in *A. spiralis* or *I. burmensis*. The arrangement of the sensilla found on the caudal segment of *A. spiralis* (Fig. 5E) is the same as in *Bactrocera* (White et al., 2000) and the arrangement of pit-sensilla found on the pseudocephalon of *A. spiralis* is similar to their arrangement in *Ceratitis rosa* Karsch and the Trypetinae, *Anastrepha ludens* Loew (Carroll & Wharton, 1989; Carroll, 1998). These fixed patterns of pit-, papilla- and peg-sensilla arranged in approximately one surrounding row at each segment occur in other Diptera groups as well, such as in Calliphoridae, Oestridae and Sciaridae (Green & Hartenstein, 1997).

The arrangement of spinules on the creeping welts in *A. spiralis* differs from that in other described Gastrozonini larvae. In the Gastrozonini described by Elson-Harris (1992), the arrangement of spinules is similar (7–13 rows of spinules; posterior rows composed of distinctly larger spinules). *Acrotaeniostola spiralis* differs from those species by a higher number of rows of spinules (13–25), the lack of distinctly larger posterior spinules, and the presence of a central gap between rows. While *A. spiralis* bears creeping welts with more or less pointed spinules on all eight abdominal segments, *I. burmensis* lacks creeping welts on the first and last abdominal segment. Additionally, the creeping welts of *A. spiralis* bear spinules with rounded tips, while those of *I. burmensis* are truncate. *Acrotaeniostola spiralis* is also the only member of the Gastrozonini known to bear orifices of unknown function on the ventral surface of the last three abdominal segments.

Morphological characteristics of *A. spiralis* larvae such as the elongated body shape, the reduced facial mask, the shape of the creeping welts or other structures such as the presence of orifices on abdominal segments are possibly an adaption to the very special habitat of this species.

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