First pyraloid (Insecta, Lepidoptera) caterpillar from Dominican amber

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Abstract. Only three fossils in the Pyraloidea (Insecta, Lepidoptera) have been confirmed to date, two adults and one larva. The first confirmed larva, in the subfamily Pyraustinae (Crambidae), was described from Baltic amber. Recently, another pyraloid larva from Dominican amber has come to our attention. We describe this second confirmed larval fossil as *Penestola wichardi* Solis, Léger & Neumann, **sp. nov.**, based on larval morphological characters, such as setal patterns and the shape of their sclerotized bases or pinacula, and place it in the subfamily Spilomelinae (Crambidae).

Introduction

Lepidoptera are often said to be extremely rare in the fossil record, including their preservation in amber (Grimaldi and Engel 2005; Sohn et al. 2012). This general observation also applies to Dominican amber, where, among the Amphiesmenoptera, the Lepidoptera have thus far been recorded only sporadically (Penney 2010); in contrast, the Trichoptera are much more common, with 34 species (Wichard 2007; Wichard and Neumann 2021). Dominican amber is a fossil resin that was produced by an extinct *Hymenaea* L. (Fabaceae) tree species and is late Early Miocene to early Middle Miocene in age (20–15 Ma) (Iturralde-Vinent and MacPhee 1996, 2019). This amber is derived mainly from outcrops in the mountains north and northeast of Santiago, Dominican Republic. Weitschat and Wichard (2002) observed that in Baltic amber many lepidopteran inclusions involve caterpillars that most likely fed on the resin produced by the host plant and were embedded in resin flowing or dropping down the tree. The same process can be observed today in extant caterpillars (Fig. 1).

Heikkilä et al. (2018) reassessed the eleven known fossil specimens listed in Sohn et al. (2012) purported to belong to the superfamily Pyraloidea and concluded that only three could be confidently placed in this superfamily based on morphological characters (the fourth fossil from Mizunami amber was not located). Of the three, two were adults, *Eopyralis morsae* Simonsen, 2018, a compression fossil, and *Glendotricha olgae* Kusnezov, 1941. The latter was an inclusion in copal, not amber as had been reported. The third fossil, *Baltianania yantarnia* Solis, 2018, was the only known pyraloid larva in amber (Baltic). Recently, another pyraloid larval fossil in amber

Figure 1. A caterpillar in the act of getting trapped in the resin of a spruce tree near Berlin.

(Dominican) was brought to our attention. We describe this second known fossil crambid larva based on morphological characters such as setal patterns and the shape of the pinacula.

Material and methods

The holotype is a single finding and consists of an excellently preserved larva in Dominican amber. The specimen was provided by Prof. Wilfried Wichard (Bonn), who purchased it in 1974 from a commercial amber trading company (Ámbar del Caribe). It is now located in the amber collection of the Museum für Naturkunde Berlin (MfN), with the inventory number MB.I 11433 (former Wichard amber collection). Digital photographs of the holotype were taken at the MfN using Canon EOS 80D digital camera (Canon, Tokyo, Japan) mounted on a Carl Zeiss AxioScope A5 compound microscope (Carl Zeiss, Oberkochen, Germany), with incident and transmitted light simultaneously. The image of the *Penestola bufalis* (Guenée, 1854) specimen used for comparison was taken with the Visionary Digital imaging system at the National Museum of Natural History, Washington, D.C. (NMNH) (Fig. 2). Drawings of morphological details (such as setae) were produced using Adobe Illustrator CS6. The amber piece was cut and polished to enhance views of the lateral side.

Images of the fossil larval were compared with the following extant larval taxa at the NMNH: *Pyrausta* Schrank, 1802, and *Achyra* Guenée, 1849 in the closely related Pyraustinae, and genera in several tribes of the Spilomelinae (Mally et al. 2019), including *Diaphania* Hübner, 1818, *Terastia* Guenée, 1854 (Margaroniini), *Herpetogramma* Lederer, 1863 (Herpetogrammatini), *Spoladea* Guenée, 1854 (Hymeniini), *Desmia* Westwood, 1832, *Nomophila* Hübner, 1825 (Nomophilini), *Udea* Guenée, 1845 (Udeini), *Penestola* Möschler, 1890, and *Duponchelia* Zeller, 1867 (Steniini). The following larval specimens of *Penestola bufalis* were examined: U.S.A., Florida, Key Largo, 17-I-1974, J.B. Heppner (1 larva), identified by D. M. Weisman 1985 [USNMENT01363016]; U.S.A., Florida, Alachua Co., Gainesville, ex. colony reared on soy-wheat germ diet, 2-VII-2022, J. E. Hayden (7 larvae), identified by J. E. Hayden 2023 [USNMENT01895254].

Morphological terminology for larval characters follows Hasenfuss (1960), Hinton (1946), and Neunzig (1987).

Acronyms and abbreviation of depositories

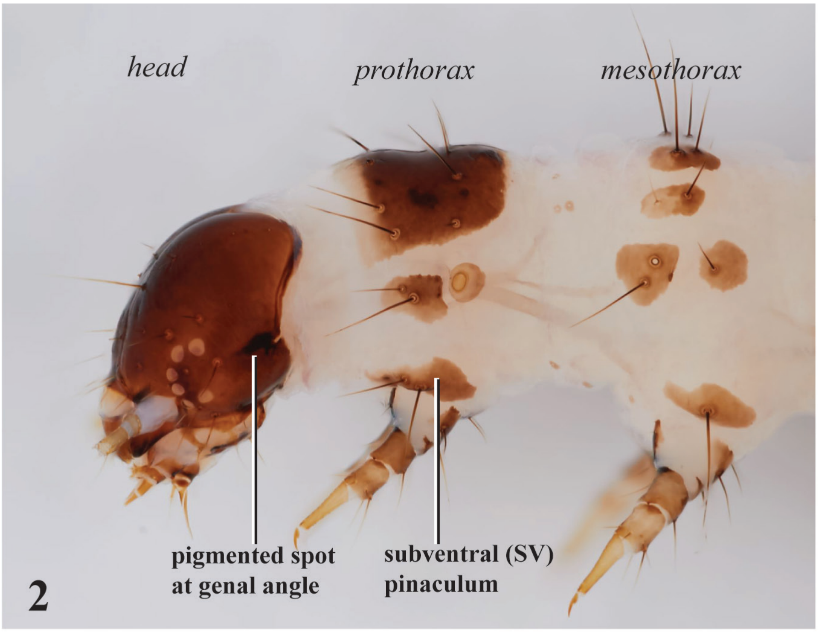
A	Abdominal segment;
D	Dorsal seta;
L	Lateral seta;
MfN	Museum für Naturkunde, Berlin, Germany;
NMNH	National Museum of Natural History, Washington, D.C., United States;
SD	Subdorsal seta;
SV	Subventral seta;
T	Thoracic segment;
V	Ventral seta;
XD	Prothoracic seta.

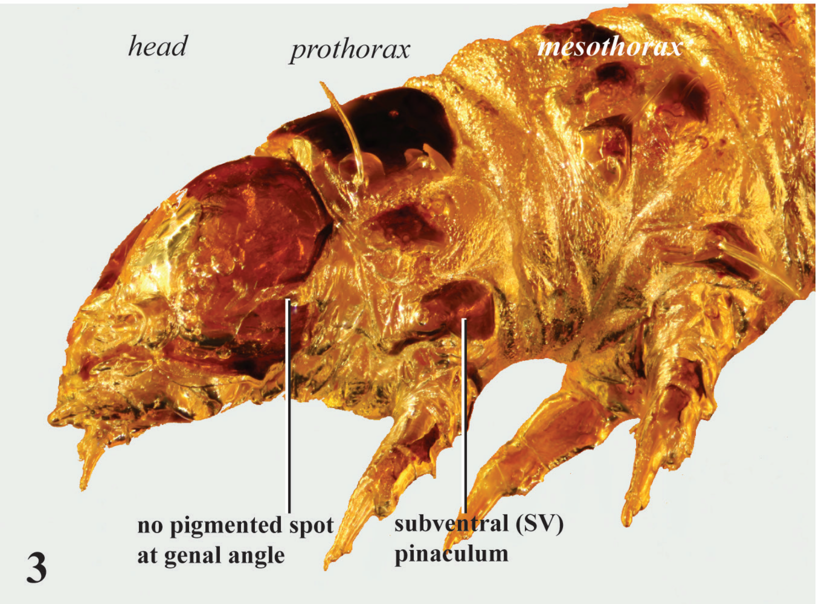
Taxonomic account

Class: Insecta Linnaeus, 1758
Order: Lepidoptera Linnaeus, 1758
Superfamily: Pyraloidea Latreille, 1809
Family Crambidae Latreille, 1810
Subfamily Spilomelinae, Guenée, 1854
Genus: *Penestola* Möschler, 1890

***Penestola wichardi* Solis, Léger & Neumann, sp. nov.**
https://zoobank.org/102BCE60-30D2-49AE-8D80-DBEFBAC51ED2
Figs 3–5

Type material. *Holotype*. The holotype is a larva preserved in Dominican amber of mid Miocene age. It is located in the amber collection of the MfN, inventory number: MB.I 11433 (former Wichard amber collection) (Figs 3, 4, 6).





Figures 2, 3. Lateral view of larval head and thoracic segments. **2.** *Penestola bufalis* (Guenée, 1854) (NMNH); **3.** *Penestola wichardi* Solis, Léger, and Neumann, sp. nov., holotype (MfN).

Condition. Excellently (completely) preserved caterpillar (length 9.7 mm) exposing lateral, ventral, and dorsal views. Size of amber piece after preparation: 3.7 cm.

Diagnosis. The holotype of *Penestola wichardi* differs by the absence of a pigmented spot at the genal angle of the head that occurs in *Penestola bufalis* (Figs 2, 3).

Generic placement. *Penestola wichardi* shares with the larva of the extant *Penestola bufalis* a setal number and a placement on pinacula for setae that can be observed. Most obviously, the holotype shares with *P. bufalis*, on the lateral view of the thoracic segments, a distinctive shape of the SV pinacula, a line thin anteriorly and broader posteriorly after the SV seta (Figs 2, 3). On the abdominal segments, the D1 pinacula is round, the D2 pinacula is elongate, and the D2 seta is situated near the lateral edge of pinacula. On the abdominal segments on A1 they share 3 SV setae. Most especially distinctive is the shape of the SV pinacula, a line thin anteriorly and broader posteriorly after the SV seta (this character does not occur in this shape in other spilomeline genera examined). This character also occurs in another taxon in the tribe Steniini, *Duponchelia fovealis* Zeller, 1847, but this species was originally distributed in Europe, and is an exotic species introduced this century into the Western Hemisphere. We should note that the morphological description is not complete due to inability to observe some features in the fossil, for example, the dorsal pinacula on abdominal segments A7–A9, the exact crochet pattern on the proleg, and ventral setal patterns (see Hayden and Burnette 2022 for comparison to *D. fovealis*).

Description. ***Body*:** (Figs 3–6) Elongate, cylindrical with a distinct head, thorax, and abdomen. ***Head*:** Hypognathous, height and width subequal, sclerotized light brown; posterior margin of gena dark brown, pigmented spot at genal angle absent; epicranial suture, frontoclypeus, labrum, and mandible not visible (specifically, the frontoclypeal area of the head capsule is missing, probably damaged, and appears white in color rather than light brown); stemmata 1–5 visible, 1–2 dorsal, 3–5 posterior to antennal base, stemma 6 only slightly visible. Hypopharyngeal complex with aciculate spinneret, prementum only slightly visible. Maxillary palpus and antenna visible. Chaetotaxy difficult to see with the exception of the presence of P1 and the socket of P2 on the frontal area of the head. ***Thorax*:** T1 dorsally with black pinacula; SD1, XD1 and D1 present; XD2, D2 and SD2 sockets present (seta missing); sockets of L1, L2 present on a sclerotized pinaculum, extending slightly below spiracle; sockets SV1, SV2 present on sclerotized pinaculum, less wide anteriorly. Ventrally T1 with sockets of V1 visible on a single triangular pinaculum. T2 with D1, D2 present on the same pinaculum, circular to subrectangular; SD1 and SD2 sockets present; L1 and L3 present; L2 present on a separate pinaculum; SV1 present, less wide anteriorly. T3 with D1, D2 present on separate pinacula; SD1 present; SD2 socket present; L1 present, L2 socket visible; SV1 present, shorter than in T1 and T2 and less-wide anteriorly. Ventrally T2 and T3 with V1 setae on separate pinacula (shape variable due to preservation distortion). ***Abdomen*:** A1 with D1 and D2 present on separate pinacula; D1 at same level as D2; SD1 present; L1 present, L2 pore present, L3 not visible; SV setae present, one long, conspicuous seta and one thin, short seta; one long V seta. A2 with D1, D2 present; SD1 socket visible; L1 present, L2 socket visible; 3 SV setae present, one V seta. A3–A6 with D1, D2; SD1 present, clearly visible; L1, L2 present, L3 present on A5, A6; SV1, SV2, SV3 setae visible at base of proleg. Prolegs present. Crochets in a biordinal mesal penellipse. A7 with D1, D2 (setae on separate pinacula), SD1 present; L1, L2 pores present; SV1, SV2, SV3 setae visible. A8 with D1 and D2 on separate pinacula, although D1 does not appear to be directly on the pinacula which could be an artefact; SD1 present; sockets L1, L2 present; 1 SV seta. A9 with D1 present, possibly D2 also present, but difficult to see; SD1 pore visible; L1, L2 setae present; 1 SV seta. A10 with D1, D2 setae present; SD1 present; L1, L2 setae present; SV1 present.

Remarks. The putative placement of this fossil in the Crambidae is based on a unisetose (sometimes bisetose) L group on A9, crochets in an incomplete circle (penellipse), and, most specifically, the lack of a pinaculum ring at the base of SD1 on A8 or any other segments (Hasenfuss 1960;

Figures 4–6. *Penestola wichardi* Solis, Léger, and Neumann, sp. nov. holotype, preserved in Dominican amber. **4.** Ventral view; **5.** Setal chart based on lateral view; **6.** Lateral view.

Neunzig 1987) which is present in the Pyralidae. Although Allyson (1984) was unable to find specific larval characters to define the subfamilies Pyraustinae and Spilomelinae, she noted that many Spilomelinae have the pinacula with setae D and SD fused (this larva does not have D and SD fused as in, for example, *Nomophila*, *Udea*, *Desmia*), and the pinaculum bearing SD1 reduced in abdominal segments 2 and 7, a character that is exhibited by this fossil larva.

The extant *Penestola bufalis* species (Fig. 2), which the fossil caterpillar resembles, is distributed throughout the Western Hemisphere, from Florida and Mexico south to northern South America and islands in the Caribbean, including the Dominican Republic, where its habitat consists of coastal mangrove swamps and shorelines. The Miocene forest biome producing Dominican amber has also been interpreted as a coastal (periodically flooded) swamp forest, as indicated by the occurrence of marine biota such as boring bivalves of the family Pholadidae (Mayoral et al. 2020).

Etymology. It is named in honor of the palaeoentomologist Wilfried Wichard (Bonn) who donated the specimen.

Discussion

Setae and pinacula, or small flat, usually sclerotized areas bearing setae that vary in size, shape, sclerotization, and placement on the larval body, have proven to be taxonomically stable and are used in larval descriptions and classification (Hinton 1946; Stehr 1987). Additionally, significant characters of the head also include setae, tonofibrillary platelets (place where muscles attach to the head), and/or simple sclerotization of various structures such as mandibles or the clypeus (Allyson 1984). In this paper, we define the fossil species and separate it from the extant *P. bufalis* based on the lack of a pigmented or sclerotized spot at the genal angle of the head (Fig. 2). Spots of various sizes and degree of sclerotization can occur in the genal angle of the head, or more commonly, a spot does not occur in the genal angle. In the Spilomelinae, there is an excellent example of the use of the presence and absence of the genal spot to separate larvae of two economically important species that feed on the potato or nightshade family. The Pickleworm *Diaphania nitidalis* (Stoll) has a dark spot at the genal angle, and the Melonworm *Diaphania hyalinata* (L.), does not exhibit the spot (see description and figs 26.218 a–e and 25.219 a–c in Neunzig 1987, and fig. 104 in Solis 1999). The lack of this spot is not an artifact of preservation in the fossil described here, although it appears to be slightly damaged in this area in the image (Fig. 3). Only the caudal edge of the head is sclerotized dark brown, and the spot is lacking on the genal angle on both sides of the caterpillar head.

Fossils in the Pyraloidea are rare, and the fossil described here represents only the second confirmed pyraloid caterpillar. The Order Lepidoptera is one of the larger, successful orders of Insecta, yet the fossil record is comparatively scant in comparison to other insect orders (e.g., Labandeira and Sepkoski 1993; Grimaldi and Engel 2005; Kristensen et al. 2007; Sohn et al. 2015). A comprehensive survey found 4,593 lepidopteran fossil specimens in the literature, of which only eleven fossils were purported to belong to the superfamily Pyraloidea (Sohn et al. 2012, 2015). When Heikkilä et al. (2018) reassessed these fossils, only three could be confirmed to belong to the Pyraloidea, one of which was a caterpillar in Baltic amber (a new genus and species were described for this fossil). And although it is not common to describe extant species based on larvae, we describe this second fossil caterpillar as new to distinguish it from the larva of its extant congener, *Penestola bufalis*. Our current discovery of another caterpillar in amber provides optimism that there are other undiscovered lepidopteran larvae in collections worldwide that have yet to be recognized or critically examined by lepidopterists.

Sohn et al. (2015) also noted that over 78% of lepidopteran fossils remain unidentified. Identification of fossil larvae requires comparison to extant taxa based on the details of morphology. Most of the species of lepidopteran groups such as Noctuoidea or Pyraloidea with larger, free-living larvae have less distinctive overall morphologies, and are usually described as naked or worm-like by non-specialists, without reference to setal or pinacular morphologies in comparison to extant taxa, as was done in this paper and in Heikkilä et al. (2018). A recent paper on lepidopteran caterpillars in Dominican and Mexican amber (Haug et al. 2022) illustrates the obstacles in determining the identity of fossil larvae. They reported 19 caterpillar specimens in the literature, with some identified to the family level, but these were larvae whose general morphologies were obvious, e.g., Geometridae, or inchworm caterpillars.

Fischer (2021) stated that it is common practice not to compare fossils with extant taxa because it is often difficult to study internal morphological characters or to use molecular approaches. Additionally, in extant Pyraloidea, the larval stage has only been described for a small proportion of genera. But where pyraloid larval diagnostic morphologies have been examined and compared (e.g., Allyson 1984; Neunzig 1987; Hasenfuss 1960), morphological diagnostic characters are clearly expressed at the genus level. Although we place the caterpillar putatively in the genus *Penestola* and describe it as a new species, it could possibly belong to another genus within the subfamily Spilomelinae whose larval stage has yet to be discovered. There is insufficient knowledge about the morphologies of modern lepidopteran caterpillars, and we emphasize that more research in this field would undoubtedly improve identification of larval lepidopteran fossils.

The sister taxa Pyraustinae and Spilomelinae are the two largest subfamilies in the Crambidae (Mally et al. 2019). The Spilomelinae is the most speciose extant subfamily with over 4,126 valid species and the Pyraustinae less so with over 1,284 valid species (Nuss et al. 2003–2023). The superfamily Pyraloidea is estimated to have appeared in the Late Cretaceous, about 93 million years ago (Walhberg et al. 2013), and in a recent analysis, 91.6 to 77.6 million years ago (Kawahara et al. 2023). The two recently described caterpillars, the pyraustine from Baltic amber *Baltianania yantarnia* Solis, about 48–34 million years old and the spilomeline caterpillar described here from Dominican amber, *Penestola wichardi* Solis, Léger & Neumann, sp. nov., about 20–15 million years old, could be used to fine-tune diversification events and their genesis, as more accurately identified pyraloid fossils are discovered.

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