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Larval morphology of a Palearctic Rutelini, *Parastasia ferrieri* (Coleoptera, Scarabaeidae), with discussions on their feeding habits

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Abstract

Rutelini is one of the largest tribes of Rutelinae, widely distributed but primarily in the New World. Recently, both larvae and adults of *Parastasia ferrieri* had been discovered in Liaoning Province of northeastern China from the Palearctic realm. The third-instar larvae of *P. ferrieri* were described using light and scanning electron microscopy in order to discover more morphological characters for larval taxonomy. The larvae of *P. ferrieri* exhibit remarkable features, including four protuberances on labrum, no helus on epipharynx, two scissorial teeth on each mandible, five stridulatory teeth plus a blunt protuberance on each maxilla, and the obtuse claws on the thoracic legs. The correlation between morphological features and feeding habits is briefly discussed.

Key Words

saproxylic, shinning leaf chafer, SEM, ultramorphology, white grub

Introduction

Rutelinae, commonly known as shining leaf chafers, are so named due to the bright colors of most of their species and their adults feeding on plant leaves (Jameson and Ratcliffe 2002). Rutelinae encompass more than 4,000 species distributed among 235 genera (Krajčík 2007; Dietz et al. 2023). These species are further categorized into seven tribes: Adoretini, Alvarengiini, Anatistini, Anomalini, Anoplognathini, Geniatini, and Rutelini (Bouchard et al. 2011). On tribal level, in fact, Rutelinae exhibit great diversity on adult morphology, geographical distribution, diurnal/nocturnal rhythms, phototaxis, or even larval feeding habits (Frew et al. 2016; Ślipiński and Lawrence 2019).

Larvae of Rutelinae are commonly referred to as white grubs, exhibiting diverse feeding and living habits at the tribal level (Ritcher 1966; Johnson and Rasmann 2015; Frew et al. 2016). The larvae are described as root-feeding in some Anoplognathini, Geniatini, Adoretini and most of the Anomalini species (Habeck 1963; Ritcher 1966; McQuillan 1985; Fuhrmann 2013; Fang et al. 2018). However, the larval feeding habits remain generally unknown in Anatistini and Alvarengiini (Pardo-Locarno et al. 2006; Fuhrmann 2013; Rodrigues et al. 2017). Rutelini larvae predominantly exhibit saproxylic habits, characterized by their consumption of decaying wood, vegetation, roots, or other organic matter (Ritcher 1948), distinguishing them from the majority of other root-attacking larvae in the Anomalini (Ritcher 1966; Zhang 1984). It is unwise to generalize the ecological role of a specific Rutelinae species until their larvae have been accurately identified (Ślipiński and Lawrence 2019). Unfortunately, larval identification has been proven to be extremely challenging, with fewer than 2% of all known species having been described (Newton 1990; Śipek 2010; Lawrence et al. 2011).

The Rutelini encompass approximately a thousand species assigned into 93 genera, with a primary presence in the New World (Jameson 1997; Ślipiński and Lawrence 2019). They exhibit an array of distinctive morphological features, including enlarged mandibles, prominent thoracic horns, expanded hindlegs, and striking metallic coloration (Jameson and Ratcliffe 2002). Notably, Old World Rutelini typically display significant sexual dimorphism, with male adults possessing well-developed thoracic horns or mandibles (Jameson 1997; Ślipiński and Lawrence 2019). Regarding their immature stages, the larval morphology of Rutelini has been described for a limited subset, totaling 39 species in 24 genera (Ritcher 1948, 1966; Jameson and Morón 2001; Albertoni et al. 2014; Carvalho et al. 2019; Barria et al. 2020; Barria et al. 2021; Lugo-García et al. 2023).

Parastasia is one of the largest genera within Rutelini, comprising approximately 105 species worldwide (Wada 2015; Zhao 2019; Hongsuwong et al. 2022). However, unlike many other Rutelini beetles, the adults of *Parastasia* do not display enlarged structures or vibrant metallic coloration (Zhao 2019). Recently, both larvae and adults of *Parastasia ferrieri* were observed in Liaoning Province of northeastern China, belonging to the Palearctic realm, similar to the previous records in Korea (Kim 2014). Larvae of *P. brevipes* have been morphologically described and reported to feed on dead wood (Ritcher 1966). However, most of the other larvae of *Parastasia* are not adequately described hitherto.

In this study, third instar larvae of *P. ferrieri* were obtained through rearing. Their morphology is described using light and scanning electron microscopy in order to better understand the morphological diversity within the group and help with the identification of larvae in this genus.

Materials and methods

Insect collection and rearing

Larvae of *P. ferrieri* were collected from Qipanshan Forest Park in Shenyang City, Liaoning Province of northeastern China, in late October 2019. A total of 13 adult *P. ferrieri* beetles (Fig. 1A, B) were obtained through rearing in the following May. Paired adults were kept in plastic boxes filled with moist, fermented sawdust (Beetle-Password Company, Shenyang, China), and a decayed wood log off-cut was provided to facilitate potential boring and egg-laying. Third instar larvae were collected from the sawdust in the following September.

Light and scanning electron microscopy

To conduct morphological observations, a total number of ten larvae were fixed in Dietrich's solution (formalin: 95% ethanol: glacial acetic acid: distilled water = 6: 15: 1: 80, v/v), which was heated up to 70 °C and then left to cool naturally for 12 h under a fume hood before being preserved in 75% ethanol (Jiang and Hua 2015).

Photographs were captured using a SONY ILCE–7RM4 digital camera. Scanning electron microscopy (SEM) was employed to examine third instar larvae. These larvae were dissected and examined in 75% ethanol using a Leica EZ4HD Stereoscopic Zoom Microscope. After a two-minute ultrasonic cleaning and two rinses in 75% ethanol, they were prepared for SEM. Dissected organs underwent serial dehydration using graded ethanol, followed by replace-

ment with tertiary butanol. They were then subjected to freeze-drying for 3 hours, sputter-coated with gold, and examined under a Hitachi S–3400N scanning electron microscope (Hitachi, Tokyo, Japan) at 5 kV. Nomenclature for larval morphology follows (Ritcher 1966).

Voucher specimens of both adults (Fig. 1A–E) and larvae (Fig. 1F) were deposited at the Entomological Museum of Shenyang Agricultural University (SYAU).

Results

General larval morphology

Larvae are of typically scarabaeiform shape, bearing three pairs of thoracic legs on the C-shaped body (Fig. 1F). The larval trunk is generally white in addition to the yellowish head capsule, thoracic legs and spiracles. The prothoracic spiracles are C-shaped, $0.41\pm0.05 \text{ mm} (N=20)$ in length. Spiracles on the anterior six abdominal segments are similar in sizes, approximate $0.22\pm0.05 \text{ mm} (N=20)$ in length. Whereas, the spiracles on the seventh and eighth abdominal segments are comparative larger, $0.27\pm0.05 \text{ mm} (N=20)$ and $0.28\pm0.05 \text{ mm} (N=20)$ respectively.

Head

The head capsules are 3.1 ± 0.15 mm (N = 10) in width. The larval head displays symmetrical adornment, boasting a total of 14 pairs of setae. These include two pairs aligned vertically on the clypeus, five pairs in the frontal region (comprising two pairs of posterior frontal setae and three pairs of anterior frontal setae), three pairs on the vertex, and four pairs in the genal area (Fig. 2A).

The antenna is elongated and slender, comprising four segments, with the second segment being the longest (Fig. 2C). The basal two segments of the antenna are adorned with one and four setae, respectively. The third segment is smooth, featuring two to four oval dorsal sensory spots. The distal segment of the antenna of conical shape with seven sensilla basiconica at its apex (Fig. 2D).

Mouthparts

The mouthparts are of a biting-chewing type, consisting of a labrum, a pair of mandibles, and a maxilla-labia complex.

Labrum exhibits symmetry and is slightly wider than it is long. The outer surface of the labrum displays symmetrical features, including four prominent protuberances and seven pairs of setae (Fig. 2B). The distal margin of the labrum is equipped with numerous sensory setae pointed distally (Fig. 3A).

Epipharynx, membranous, situated on inner surfaces of labrum and clypeus. Epipharynx is further divided into distinct functional areas (Fig. 3A), including a heptomeron at the apex, a pair of plegmatium on the lateral margin, nesium in the central portion, and glabrous gymnoparia on



Figure 1. Adults and a larva of *Parastasia ferrieri*. A. Male adult, dorsal view; B. Male adult, ventral view; C. Male genitalia, dorsal view; D. Male genitalia, lateral view; E. Male genitalia, ventral view; F. Third instar larva.



Figure 2. Larval head of *Parastasia ferrieri*. **A.** Head; **B.** Labrum; **C.** Antenna; **D.** Sensilla on the apex of antenna. AFS, anterior frontal seta; AT, antenna; CLP, clypeus; DES, dorsoepicranial setae; ES, epicranial stem; F, frons; FCS, frontoclypeal suture; LB, labrum; LP, labral protuberance; PFS, posterior frontal seta; SB, sensillum basiconicum.



Figure 3. Larval epipharynx of *Parastasia ferrieri*. A. Epipharynx; B. Magnification of haptomerum; C. Magnification of plegma; D. Basal part of the epipharynx. ACP, acanthoparia; GP, gymnoparia; PL, plegma; PE, pedium; SP, sclerotized plate; S, sensillum; Z, zygum.

both sides (Fig. 3A). The heptomeron exhibits a slight curvature and is composed of four minute sensilla basiconica, lacking helus (Fig. 3B). The plegmatium consists of eight acanthoparia, intricately intertwined with an equal number of plegma (Fig. 3C). The chaetoparia displays asymmetry and is composed of numerous sensilla chaetica, with a higher density on the right side than on the left. Adjacent to the right acanthoparia, the sensory nesium is situated, featuring four micro sensilla basiconica at its apex (Fig. 3D).

The paired mandibles are heavily sclerotized, bearing two anterodorsal setae and a row of 11 setae on the lateral surface (Fig. 4A, B). The paired mandibles each bear a shuttle-shaped stridulatory area on ventral surface (Fig. 4C, D). The mandibular incisor is equipped with two apical teeth curved inward. The molar region exhibits asymmetry, featuring a prominent molar tooth accompanied by an acia on the left and a group of four ridged molar teeth on the right (Fig. 4C, D). The ventral process is relatively narrow on the left mandible (Fig. 4D), while it is generally wider on the right mandible (Fig. 4C).

The maxillae, labia, and hypopharynx are fused together to form a structural complex (Fig. 4E). Each of the paired maxillae comprises a cardo, a stipes, a maxillary palp, and a lobe fused from the galea and lacinia (Fig. 4E). The maxillary stridulatory area comprises a row of five stridulatory teeth and an anterior truncate process (Fig. 4F). The maxillary palp consists of four segments, with the longest distal segment bearing a digitiform organ on its lateral surface and ten sensilla basiconica on its apex (Fig. 4E). The labia comprise a mentum, a prementum, a pair of two-segmented labial palps, and a sensory glossa (Fig. 4E). Dorsally, the glossa is adorned with numerous sensilla (Fig. 4E). The hypopharynx is specialized, forming a hypopharyngeal sclerome that bears a sclerotized truncate process, two tufts of microtrichia on the left, and a pair of membranous lobes on the lateral margin (Fig. 4F).

Thoracic legs

Each of the thoracic legs is composed of five segments: coxa, trochanter, femur, tibiotarsus, and a distal single claw (see Fig. 5A, B). The coxa exhibits a long and slender shape. The trochanter possesses a slight curvature. The femur is covered with numerous setae on its surface. The tibiotarsus is originally a fusion of the tibia and tarsus, and it bears medium-sized setae on its dorsal, ventral, and lateral sides. The distal claw is heavily sclerotized, featuring an obtuse distal end and bearing three short setae.

The respiratory plate is composed of numerous minute openings that encircle the oval bulla, along with a slightly curved spiracular slit (refer to Fig. 5B). The prothoracic spiracles are slightly larger than the abdominal ones. The abdominal spiracles are similar in size (see Fig. 1).



Figure 4. Mandibles and maxillae of *Parastasia ferrieri*. A. Left mandible, dorsal surface; b Right mandible, dorsal surface; C. Right mandible, ventral surface; D. Left mandible, ventral surface; E. Maxillae and labia, ventral surface, insert showing the magnification of maxillary palpus; F. Maxilla, labia, and hypopharynx, dorsal surface. AC, acia; CAR, cardo; DC, dorsal carina; DO, digitiform organ; GL, glossa; HS, hypopharyngeal sclerome; IC, incisor; LL, lateral lobe; LP, labial palpus; M, mola; MP, maxillary palpus; PC, penicillus; PM, postmentum; PR, precoila; PRM, prementum; SC, scobis; ST, stridulatory teeth; STA, stridulatory area; SP, stipes; TP, truncate process; VP, ventral process.

Raster

The raster is furnished with paralleled palidia, each composed of a longitudinal patch of mesal directed pali, surrounding a prominent septula. The palidia are submerged in a large number of setae, which are slightly longer and distributed at the lateral region.

Discussion

In this study, larvae of *P. ferrieri* were described using scanning electron microscopy for the first time. The larvae of *P. ferrieri* are remarkable for the following structures: bearing four protuberances on labrum; no helus on epipharynx; two scissorial teeth on each mandible; five stridulatory teeth and a blunt protuberance on each maxilla; obtuse claws on thoracic legs, and 12 pairs of pali on raster. By the combination of these characters, larvae of *P. ferrieri* can be readily distinguished from most of the other larvae in Rutelini (Ritcher 1948, 1966; Jameson and Morón 2001; Albertoni et al. 2014; Carvalho et al. 2019; Barria et al. 2020; Barria et al. 2021; Lugo-García et al. 2023).

The labrum exhibits a wide range of morphological features within families or subfamilies of Scarabaeidae

(Ritcher 1966; Grebennikov and Scholtz 2004). The labrum is typically fan-shaped in Passalidae and Lucanidae (Hayes 1929; Qu et al. 2019), trilobed in Aphodiinae, Scarabaeinae, some Cetoniinae, and Pleocomidae (Grebennikov and Scholtz 2004; Li et al. 2019; Dong et al. 2020), or bearing an apical protuberance in some Sericinae (Sipek and Ahrens 2011). In the genus Apogonia (Melolonthinae), the labrum forms a dorsal ridge (Jia et al. 2023), while in some Hybosoridae, the labrum possesses serrations (Grebennikov and Scholtz 2004; Grebennikov et al. 2004). The labrum has varying numbers of setae or display glabrous, wrinkled, or humped dorsal surfaces in different lineages (Qu et al. 2019; Jia et al. 2020; Jia et al. 2021; Jia et al. 2023). In this study, the larval labrum of P. *ferrieri* is atypical for the presence of four protuberances on its dorsal surface, which is not mentioned in the congeneric larvae of P. brevipes (Ritcher 1966).

Previous comprehensive studies (Ritcher 1966; Zhang 1984; Sawada 1991) have indicated that heli on the epipharynx often serve as valuable taxonomic characteristics for larval identifications in Scarabaeidae (Fang et al. 2018; Jia et al. 2021). In Rutelinae, the larval epipharynx is equipped with two to four heli in Anomalini (Micó et al. 2003), six to eight in Anoplognathini (Neita-Moreno and Morón 2017), six to nine in Adoretini (Fang et al. 2018),



Figure 5. Thoracic legs, spiracles and raster of *Parastasia ferrieri*. A. Prothoracic leg, lateral view; B. Prothoracic spiracle; C. Raster; D. Magnification of the anterior palidia; E. Magnification of the hamate seta. BU, bulla; CL, claw; CX, coxa; FM, femur; RSP, respiratory plate; SS, spiracular slit; TT, tibiotarsus; TR, trochanter.

or entirely absent in Geniatini and Rutelini (Jameson and Morón 2001; Fuhrmann 2013). In this study, the larval epipharynx of *P. ferrieri* is devoid of heli, consistent with previous descriptions in Rutelini (Ritcher 1966; Jameson and Morón 2001; Barria et al. 2020; Barria et al. 2021; Lugo-García et al. 2023).

Mandibles, being the most heavily sclerotized structures on mouthparts, are structurally correlated with feeding habits (Zhang 1984). The larval mandibular incisor typically varies: straight and apically sharp in some wood-consuming species of Lucanidae and Passalidae (Hayes 1929; Katovich and Kriska 2002; Richards and Spencer 2014; Qu et al. 2019), sharp but inwardly curved in dung decomposers within Aphodiinae and Scarabaeinae (Jerath 1960; Edmonds and Halffter 1978; Frolov 2009; Li et al. 2019), or blade-like and specialized to form a cutting edge in phytophagous Melolonthinae (Jia et al. 2020; Jia et al. 2021; Jia et al. 2023). Within Rutelinae, the mandibular incisors are typically blade-like in some phytophagous larvae of Anomalini and Adoretini (Micó et al. 2003; Fang et al. 2018), and are apically sharp and curved in some saproxylic larvae of Rutelini (Jameson and Morón 2001). Within Rutelini, larval mandibles are usually asymmetric, equipped with three teeth on the left and two teeth on the right (Jameson and Morón 2001; Albertoni et al. 2014; Carvalho et al. 2019). In this study,

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however, the larval mandibular incisor is symmetric, each bearing two scissorial teeth, similar to the previously described larvae of *P. brevipes* (Ritcher 1966).

The claws of thoracic legs show considerable morphological diversity across lineages (Zhang 1984). In most species of Melolonthinae and Cetoniinae, the claw is typically curved, sharp, and adorned with paired setae (Sousa et al. 2018; Jia et al. 2020; Jia et al. 2021; Jia et al. 2023). Conversely, it is usually blunt in some wood-decomposing species within Lucanidae (Katovich and Kriska 2002; Qu et al. 2019) and remnant in certain dung-feeding larvae of Geotrupidae (Grebennikov and Scholtz 2004). In Rutelinae, thoracic claws are typically sharp in Adoretini (Fang et al. 2018), Geniatini (Pardo-Locarno et al. 2006; Fuhrmann 2013), and Anomalini (Micó and Galante 2005). However, they display morphological heterogeneity among the pro-, meso-, or metathoracic legs in some species of Rutelini (Carvalho et al. 2019). In this study, the claws of P. ferrieri are generally blunt on all three pairs of thoracic legs, congruent with other saproxylic larvae in Lucanidae (Richards and Spencer 2014; Qu et al. 2019).

Rutelinae usually attract attention due to their exquisite adult appearances or the economic losses caused by their larval stages (Jameson and Ratcliffe 2002). In the Palearctic realm, particularly in northeastern China, larvae of Rutelinae are frequently recognized as agricultural pests, because they mostly belong to the phytophagous Anomalini or Adoretini (Zhang 1984; Sawada 1991). This scarcity record of *P. ferrieri* may be attributed to their small, nocturnal adults, or the fact that their larvae never attack living organs of crops or trees. Given their significance of biogeographical distribution, the larvae of *P. ferrieri* warrant increased attention for conservation purposes.

Author contributions

Conceived and designed the experiments: LJ, XYS. Performed the experiments: XYS, XMD. Analyzed the data: XYS and LJ. Wrote the paper: XYS, XMD and LJ.

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