



Description of the larva of *Vestalis gracilis* (Rambur, 1842) (Zygoptera: Calopterygidae) from Thailand

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Abstract. *Vestalis gracilis* (Rambur, 1842) is a forest stream damselfly belonging to the family Calopterygidae. Its last-stadium larvae and exuviae are described and illustrated based on laboratory-raised specimens from Thailand, and observations of agonistic behavior are provided. The taxonomical characters of *V. gracilis* larvae are similar to those of *V. amoena*. They exhibit synapomorphic characters such as posterolaterally directed protuberances on the postocular lobes, posterior margin of median lamella obliquely truncate, and two setae on labial palps. *Vestalis gracilis* and *V. luctuosa* bear eight antennal segments whereas seven antennal segments are found in *V. amoena*. The most significant difference between *V. gracilis* and *V. luctuosa* is that *V. luctuosa* has posterolaterally directed protuberances on the postocular lobes and an obliquely truncate posterior margin of the median lamella.

Keywords. Odonata, damselfly, exuviae, larvae, raised specimen, Thailand

Research Article

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Data Availability Statement:

All relevant data are within the paper.

Introduction

The genus *Vestalis* Selys, 1853, comprises green metallic forest stream damselflies belonging to the family Calopterygidae (Dijkstra et al., 2014). Its distribution ranges from South to Southeast Asia and includes sixteen known species (Paulson & Schorr, 2021). Four species have so far been recorded from Thailand (i.e., *V. amethystina* Lieftinck, 1965, *V. amoena* Hagen in Selys, 1853, *V. anne* Hämäläinen, 1985, and *V. gracilis* (Rambur, 1842)) (Hämäläinen, 2017).

To date, two species have had their larvae described. *Vestalis amoena* was described from Malaysia by Lieftinck (1965), and *V. luctuosa* (Burmeister, 1839) was described from Indonesia by Ris (1912). Here, we provide a description of the last-stadium larvae of *V. gracilis* based on laboratory-raised specimens and their larvae are compared to those described of other congeneric species.

Methods

The larvae studied here were found in headwater streams in Thailand (sample sites see below) and collected by means of a D-frame net. Last-stadium larvae were transported to the laboratory for further raising in earthenware pots furnished with an oxygenator until the emergence of adults. Wooden chopsticks were provided as substrate and support during emergence. The specimens were then preserved in absolute alcohol. Adult identification was performed based on caudal ap-

pendages and genital ligula, following Asahina (1993). Measurements (mm) and photographs were taken with an Olympus CX41 compound microscope and an Olympus SZX16 stereo microscope with an Olympus DP25 Digital Camera (Olympus Corporation, Shinjuku-ku, [Tokyo], Japan) attached. Drawings based on a representative digital photograph were made on an iPad using the Procreate application (Savage Interactive Pty. Ltd., North Hobart, [Tasmania], Australia). Final plates were prepared using Adobe Photoshop CC 2017 (Adobe Inc., San Jose, California, USA). The descriptions of the caudal lamellae and the setae on the labium follow Kumar (1973) and the larval mandibular formula follows Watson (1956). Segments 1–10 and antennomeres 1–7 are indicated as S1–10 and A1–7, respectively. Preserved specimens were deposited in the Entomology Collection of the Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation, Bangkok, Thailand (ECNP).

Specimens examined. Thailand: 2 exuviae (collected last-stadium larvae raised in the laboratory); (1 ♂, emerged) 28 January 2020, Pha Kluai Mai Waterfall (14.43333° N, 101.41541° E, altitude 659 m), Nakhon Ratchasima Province, Keetapithchayakul leg. (1 ♀, emerged) 9 August 2020, Nang Rong Waterfall (14.31388° N, 101.30666° E, altitude 37 m), Nakhon Nayok Province, Keetapithchayakul leg. 4 final-stadium larvae; 1 ♀, 4/I/2019, Wang Jum Pee (14.44661° N, 101.12275° E, altitude 746 m) Nakhon Ratchasima Province, Rattanachan leg., 1 ♂ 17/XII/2019, Huay Mae Kae (18.74144° N, 99.817761° E, altitude 360 m) Lumpang Province, Rattanachan leg., 1 ♀, 8/III/2019, Erawon waterfall (14.50526° N, 99.14408° E, altitude 73 m), Kanchanaburi Province, Rattanachan leg. 1 ♂, 2/XI/2020, Tan ngam waterfall, (17.15534° N, 102.73714° E, altitude 207 m), Udonthani Province, Keetapithchayakul leg.

Description of the larva of *Vestalis gracilis*

(Figures 1–6, 8C, 9B, 10–11)

Larva yellowish brown to dark brown, long, slender, length of body 7.43× as long as maximum body width (Figure 1).

Head: subpentagonal, with marked pattern, 1.18× wider than head length, head wider than thorax and abdomen. Distal half of labrum covered with dense setae, anterior margin flattened ventrally with sparse setae. Clypeus with small, sparse setae. Frons and vertex glabrous. Occiput concave, mostly glabrous. Postocular lobes curvilinear in outline with several scattered spiniform setae, with posterolaterally directed protuberance at the middle of each side. Compound eyes narrow and rounded, protruding posterolaterally. Antennae (Figure 2A) filiform, 1.87× longer than head length, 8-segmented with A1 (scape) the longest; relative lengths of antennomeres 1.00 (0.5 mm) : 0.17 : 0.60 : 0.48 : 0.24

: 0.20 : 0.11 : 0.13; A1 concave laterally, triangular, its maximum width 2.20 and 2.75 × A2 and A3 width, respectively, with small setae on lateral side and distally on mesal side, and long setae on the proximal to middle inner side; A3 with small scattered setae; A3–A8 with small setae. Genae (Figure 2B) with scattered setae. Labium: prementum-postmentum articulation extended at level of anterior margin of mesocoxae; prementum (Figure 2C) with scattered setae, lobes of ligula separated by a deep, ovate, median cleft, maximum width between lobes 0.53× the cleft length, tips of lobes truncated and touching each other mesally (Figure 2D); two setae on mesal side of each lobe of ligula, and 4–5 minute scattered setae in basal area; lateral margin of ligula lobe finely serrate; sub-quadrangular postmentum with scattered setae on ventral side. Labial palp (Figure 3A) as long as 0.38× prementum length, with two short palpal setae, one seta near articulation of movable hook, the other one close to the palp's articulation, mesal margin serrulate, a row of delicate setae along lateral margin, distal end with three hooks (Figure 3C), outer and middle hook approximately of the same size and inner hook

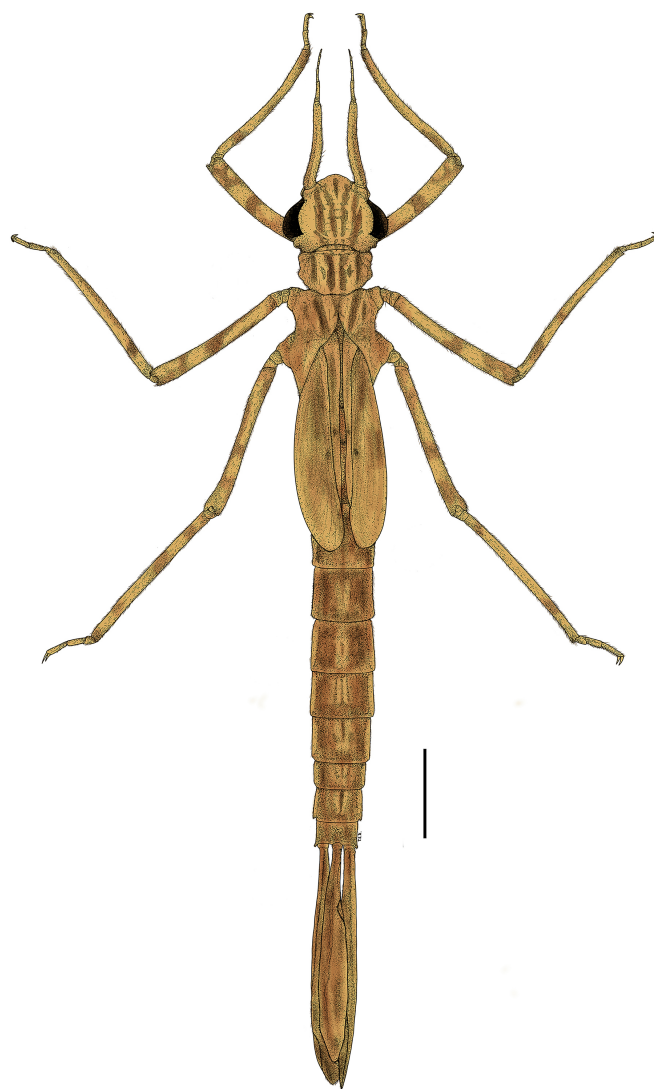


Figure 1. Larva of *Vestalis gracilis*, dorsal habitus. Scale bar = 2 mm.

shortest, outer hook with a row of 5–6 small protuberances (Figure 3B); movable hook slender and sharply pointed, 0.71× as long as labial palp length. Maxilla (Figures 3D, E): galeolacinia covered with dense long setae, with seven teeth, three small ventral teeth of different size and robustness, apical tooth largest, three incurved dorsal teeth of similar size. Mandibles (Figures 4A–D) asymmetrical, brawny, with well-developed large teeth, with molar crest; mandibular formula:

L 1+1'234 0 a(m¹⁻⁶)b / R 1+1'234 y a(m⁰)b,
a > b in left mandible, a = b in right mandible.

Thorax: Pronotum subtrapezoidal with scattered setae, lateral margins almost concave, pronotal disc smooth with a small shallow groove. Pterothorax with scattered setae. Wing sheaths parallel; anterior and posterior wing sheaths reaching posterior margin of abdominal S4. Legs almost flat and very long: femora thin with a dark band on the posterior side and small setae, the hind femora 1.62× and 1.24 × longer than the fore and mid femora; tibial comb (Figure 4E) with dense spiniform setae on its distal ends; tarsi with two rows of scattered setae, tarsal formula 3–3–3, with 2 simple claws and pulvilliform empodium.

Abdomen: slender and cylindrical, narrowed caudally, abdominal terga S1–3 smooth, abdominal terga S4–10 scattered setae; abdominal sterna S1–10 with a pale

network of tracheoles, with scattered setae; postero-lateral ends of S9–10 with blunted spines. Male gonapophyses (Figure 5A, C) triangular, roundly pointed, widely divergent in ventral view, extending to anterior margin of S10; gonopore O-shaped, embossed, and with a fissure from its middle to its posterior end. Male cerci digitiform, concave on its inner surface and roundly tipped. Female gonapophyses (Figure 5B, D) almost reaching posterior margin of S10; lateral valves covered with small setae, parallel in lateral view (Figure 5D), di-

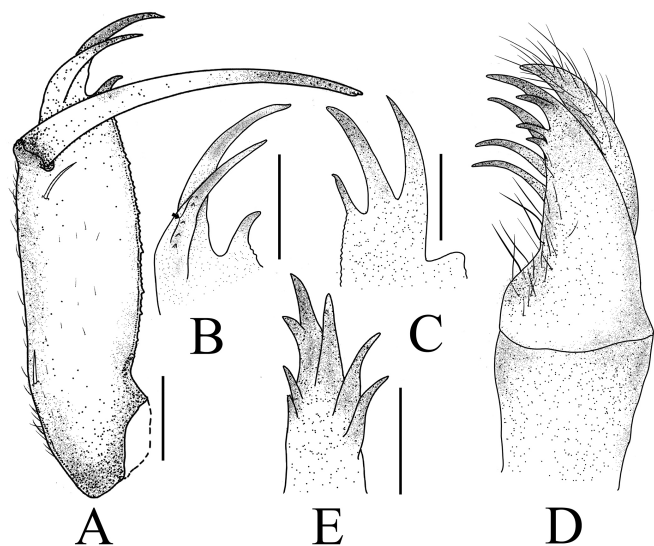


Figure 3. Mouthparts of *Vestalis gracilis*: (A) labial palp, dorsal view; (B) distal end of labial palp, dorsal view (arrow = row of protuberance); (C) distal end of labial palp, lateroventral view; (D) left galeolacinia, ventral view; (E) teeth on maxilla, ventral view. Scale bars = (E) 0.1 mm, (B, C, D) 0.25 mm, (A) 0.5 mm.

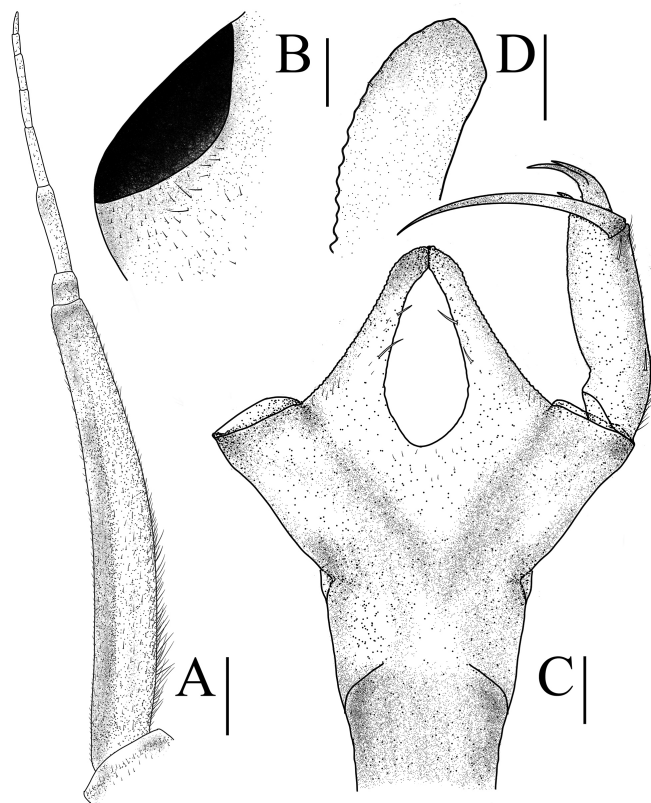


Figure 2. Details of morphology of *Vestalis gracilis*: (A) left antenna, dorsal view; (B) right gena, ventral view; (C) premen-tum, dorsal view; (D) left distal margin end of ligula. Scale bars = (D) 0.2 mm, (A, B, C) 0.5 mm.

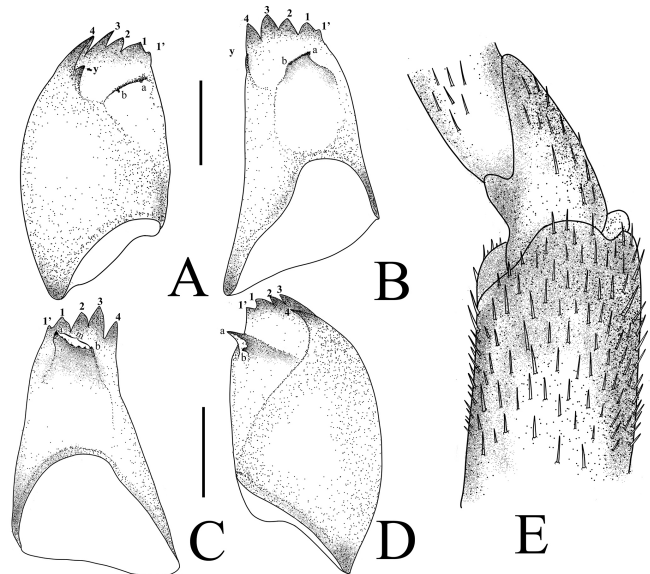


Figure 4. Mandible and tibial comb of *Vestalis gracilis*: (A) right mandible, ventral view; (B) right mandible, ventrointernal view; (C) left mandible, ventrointernal view; (D) left mandible, ventral view; (E) tibial comb. Scale bars = (E) 0.25 mm, (A, B, C, D) 0.5 mm.

vided into two lobes, the dorsal lobe digitiform, bluntly tipped, largest, the ventral lobe short, spine-like, sharply pointed, central valves slender, apically rounded, and slightly shorter than lateral valves; female cerci small, cone-shaped and bluntly tipped. Caudal lamellae (Figure 6) long triquetral, covered with small setae along their margins, lateral lamellae longer than median lamellae, median tracheae largest, distinct, reaching 80% of caudal lamellae, indistinct secondary branches, irregularly branched, a little undulate and extending to the distal margin, tertiary branches arising from margin; median lamellae obliquely truncated, subtrapezoid, with a row of 17–21 spiniform setae in the center on both sides; lateral lamellae oblong, apex with a small pointed tip, with a row of 14–16 spiniform setae on the lateral side of the median trachea.

Measurements. [in mm; n = 8; 2 exuviae and 6 larval specimens in alcohol] total length of body without caudal lamellae = 22.99–23.88; length of lateral lamellae = 8.26–8.50; length of median lamellae = 7.4–7.78; width of head = 2.85–3.00; length of antenna = 4.76–5.10;

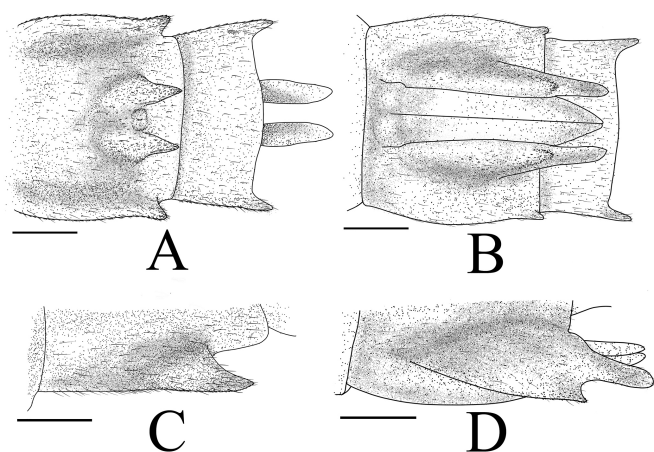


Figure 5. Detail of S8–10 (caudal lamella detached) of *Vestalis gracilis*: (A) male gonapophyses, ventral view; (B) female gonapophyses, ventral view; (C) male gonapophyses, lateral view; (D) female gonapophyses, lateral view; Scale bars = 0.5 mm.

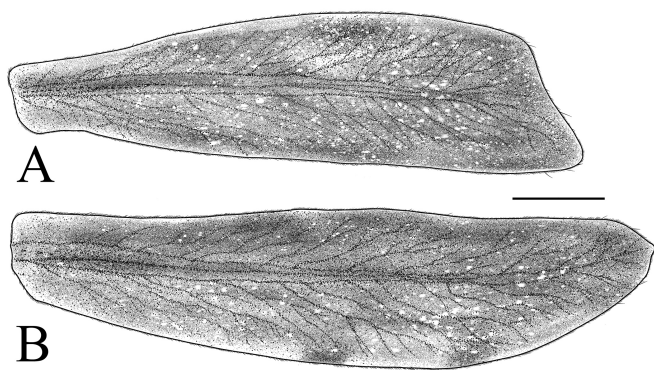


Figure 6. Caudal lamellae of *Vestalis gracilis*: (A) median lamella; (B) lateral lamella. Scale bars = 1 mm.

width and length of prementum = 2.90–3.00 and 3.90–4.15; length of labial palp = 1.50–1.55; length of movable hook = 1.60–1.70; length of inner and outer wing sheaths = 5.82–7.31 and 5.97–7.01; length of femora (fore: mid: hind) = 3.73–3.75: 4.63–5.08: 5.82–6.27; length of tibiae (fore: mid: hind) = 4.78–4.93: 5.81–5.84: 6.27–6.72; length of tarsi (fore: mid: hind) 1.05–1.19: 1.34–1.49: 1.34–1.64.

Diagnosis. The larva of *V. gracilis* is similar to that of *V. amoena* (Table 1). They share posterolaterally directed protuberance on their postocular lobes, obliquely truncate on the posterior margin of median lamella, and two setae on the labial palp, but *V. gracilis* bears eight antennal segments whereas seven antennal segments are found in *V. amoena*. *Vestalis luctuosa* shares eight antennal segments with *V. gracilis*. Moreover, *V. luctuosa* differs from the other two *Vestalis* spp. by having an upward-directed protuberance on its postocular lobe and a pointed posterior margin of its median lamella. Lieftinck (1965) mentioned a possibly diagnostic character of *V. amoena* on A2, which has an extra joint between the pedicel and the second segment of the antenna (Figure 10), but did not provide sufficient characteristics for comparisons with other species. This character on A2 might have been overlooked in other calopterygid species. The larva of *V. amoena* should be

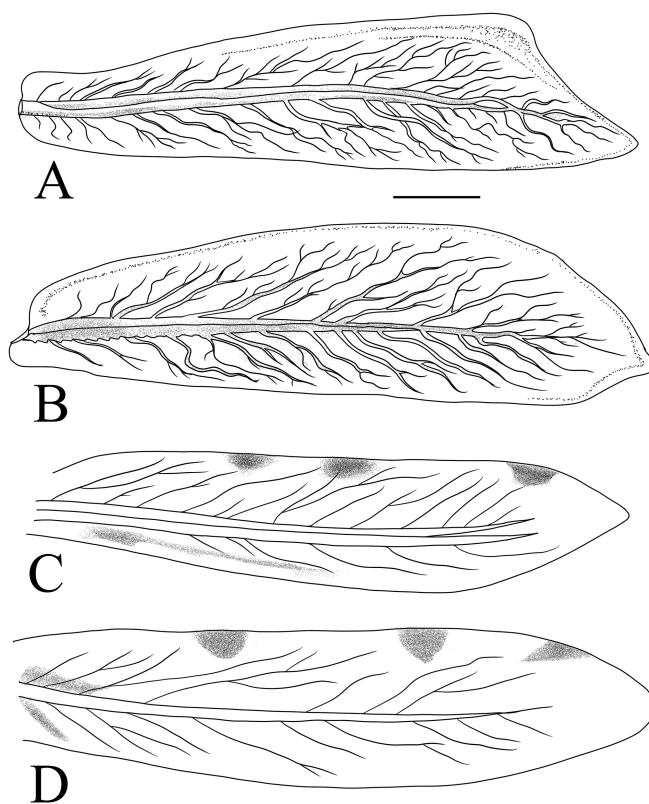


Figure 7. Caudal lamellae of *Vestalis* spp.: (A, B) *V. amoena*, (A) median lamella, (B) lateral lamella, redrawn from Lieftinck (1965); (C, D). *V. luctuosa* (median or lateral lamella are not mentioned in the original description), redrawn from Ris (1912), Scale bars = (A, B) 1 mm, (C, D) without scale.

Table 1. Comparison of characters of known *Vestalis* larvae (? = not mentioned in original description).

Characters	<i>V. luctuosa</i>	<i>V. amoena</i>	<i>V. gracilis</i>
Number of antennal segments (Figure 8)	8	7	8
Direction of protuberances on postocular lobe (Figure 9)	upward	posterolateral	posterolateral
Setae on labial palp	absent	2	2
Setae on basal region of ligula	?	?	present
Outer hook of labial palps with row of small protuberances	?	?	present
Blunt posterolateral spine on S9	absent	?	present
Posterior margin of median lamella (Figures 6–7)	point	oblique, truncated	oblique, truncated
References	Ris (1912)	Lieftinck (1965)	this study

reanalyzed from the original specimens to confirm the character described above and add more diagnostic characters.

Biological notes. The larvae of *V. gracilis* inhabit forest streams. They are usually concealed among vegetation in riffle zones (Figure 10) and are generally found to-

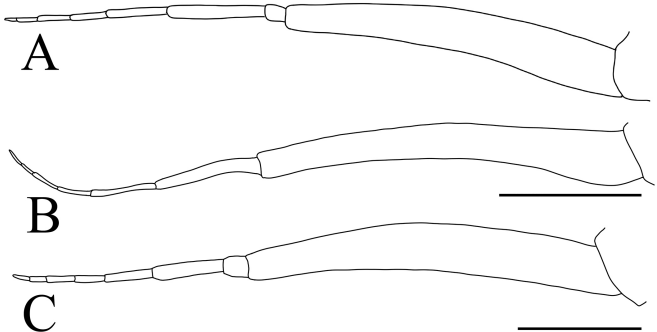


Figure 8. Antennae of *Vestalis* spp.: (A) *V. luctuosa*, redrawn from Ris (1912); (B) *V. amoena*, redrawn from Lieftinck (1965), (C) *V. gracilis*. Scale bars = (B, C) 1 mm, (A) without scale.

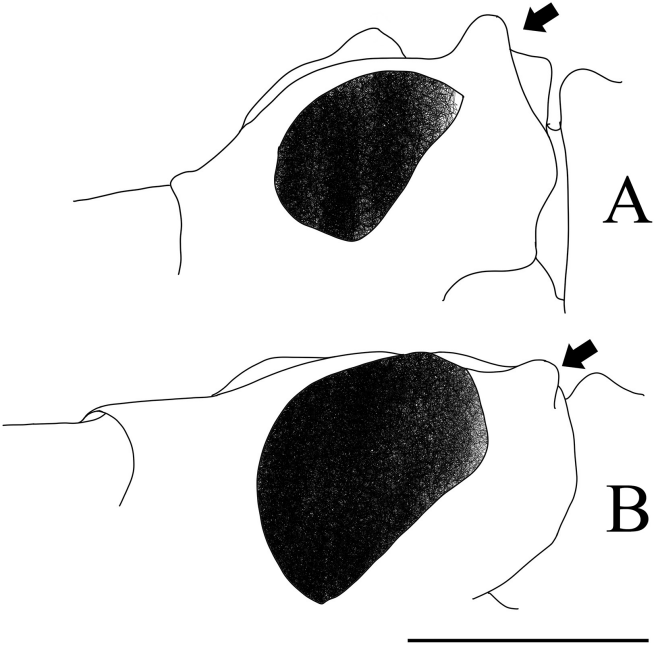


Figure 9. Lateral view of head of *Vestalis* spp. (arrow = protuberance): (A) *V. luctuosa*, redrawn from Ris (1912); (B) *V. gracilis*. Scale bar = (B) 1 mm, (A) without scale.



Figure 10. Natural habitats of *Vestalis gracilis* larva.



Figure 11. Larva of *Vestalis gracilis* displaying agonistic behavior.

gether with other calopterygid larvae such as those of *Neurobasis chinensis* (Linnaeus, 1758). When the larvae felt threatened, they displayed agonistic behavior in that they lifted the abdomen and pointed the caudal lamellae forward in a scorpion-like posture (Figure 11). These agonistic displays are similar to those observed in other calopterygid species (Ryazanova & Mazokhin-Poshnyakov, 1992) and seem to be a characteristic behavior of damselfly larvae (Rowe, 1992, 2002, 2004; Saetung et al., 2020).

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References

- Asahina, S. (1993). *A list of the Odonata from Thailand (Parts I–XXI)*. Bangkok: Bosco Offset.
- Dijkstra, K-D. B., Kalkman, V. J., Dow, R. A., Stoks, F. R. & Van Tol, J. (2014). Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata). *Systematic Entomology*, 39(1), 68–96. doi:10.1111/syen.12035

- Hämäläinen, M. (2017). The Caloptera damselflies of Thailand—Distribution maps by provinces (Odonata: Calopterygoidea). *Faunistic studies in South-East Asian and Pacific Island Odonata*, 19, 1–28.
- Kumar, A. (1973). Descriptions of the last instar larvae of Odonata from the Dehra Dun Valley (India), with notes on Biology I. (sub-order Zygoptera). *Oriental Insects*, 7(1), 83–118. doi:10.1080/00305316.1973.10434207
- Lieftinck, M. A. (1965). The species-group of *Vestalis amoena* Selys, 1853, in Sundaland (Odonata, Calopterygidae). *Tijdschrift voor Entomologie*, 108(11), 325–364.
- Paulson, D. & Schorr, M. (2021, September 6). *World Odonata List*. [Online] Retrieved September 6, 2021, from <https://www2.pugetsound.edu/academics/academic-resources/slater-museum/biodiversity-resources/dragonflies/world-odonata-list/>.
- Ris, F. (1912). Über Odonaten von Java und Krakatau. *Tijdschrift voor Entomologie*, 55, 157–183.
- Rowe, R. J. (1992). Ontogeny of agonistic behavior in the territorial damselfly larvae, *Xanthocnemis zealandica* (Zygoptera: Coenagrionidae). *Journal of Zoology, London*, 226, 81–93. doi:10.1111/j.1469-7998.1992.tb06128.x
- Rowe, R. J. (2002). Agonistic behaviour in final-instar larvae of *Agriocnemis pygmaea* (Odonata: Coenagrionidae). *Australian Journal of Zoology*, 50(2), 215–224. doi:10.1071/ZO01024
- Rowe, R. J. (2004). Agonistic behaviour in final-instar larvae of *Epi-synlestes cristatus*, *Synlestes tropicus* and *Chorismagrion risi* (Odonata: Synlestidae), and relationships within the ‘Lestoidea’. *Australian Journal of Zoology*, 50(2), 215–224. doi:10.1071/ZO02023
- Ryazanova, G. I. & Mazokhin-Poshnyakov, G. A. (1992). Spatial interactions between conspecific and heterospecific *Calopteryx* larvae (Zygoptera: Calopterygidae). *Odonatologica*, 21(1), 105–110.
- Saetung, T., Makbun, N., Sartori, M. & Boonsoong, B. (2020). The subfamily Platycnemidinae (Zygoptera: Platycnemididae) in Thailand, with description of the final stadium larva of *Copera chantaburii* Asahina, 1984. *International Journal of Odonatology*, 23(3), 219–237. doi:10.1080/13887890.2020.1755377
- Watson, M. C. (1956). The utilization of mandibular armature in taxonomic studies of anisopterous nymphs. *Transactions of the American Entomological Society*, 81(3/4), 155–202.