



Larval morphology, life cycle and nutritional values of *Lepidostoma abruptum* Banks 1931 (Trichoptera: Lepidostomatidae) from Lower-Hill Evergreen Forests of Southern Thailand

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Abstract

The larval morphology, life cycle, and nutritional values of *Lepidostoma abruptum* Banks 1931 are described and discussed. The larval case is rectangular in cross section, made of rectangular pieces of leaf. The larva of *L. abruptum* shares the morphological characteristics of other *Lepidostoma* spp. which have been described from different countries and continents. The head capsule widths of 74 specimens were measured and plotted to determine the instars of the larvae. This species is non-seasonal. In addition, the moisture (68.87%) and dry-weight nutritional values of the specimens were determined: protein (49.08%), total fat (27.13%), total dietary fiber (5.9%), omega-3 (0.15%), omega-6 (6.04%), and omega-9 (11.7%). Larvae of this species live in small debris pools dominated with leaf litter and small branches.

Key words: caddisflies, streams, shredder, Southeast Asia

Introduction

The Lepidostomatidae constitute a common family that occurs worldwide in all zoogeographic regions, including the Nearctic, Palearctic, Oriental, and Afrotropical Regions, and the northern areas of the Neotropical and Australasian Regions (Holzenthal *et al.* 2017; Weaver *et al.* 2010). Three genera, *Paraphlegopteryx*, *Zephyropsyche*, and *Lepidostoma*, have been recorded from Thailand, where 43 species are found (Malicky 2010). *Lepidostoma* includes more species than any other genus of the family in Thailand and is distributed throughout the country (Bunlue *et al.* 2012; Laudee & Malicky 2014; Laudee & Prommi 2011; Malicky 2010; Thapanya *et al.* 2004). Three species of *Lepidostoma*, namely *L. abruptum* Banks 1931, *L. brevipenne* Olh 1993, and *L. moulmina* Mosely 1949, have been recorded from the Nakhon Si Thammarat ranges. *Lepidostoma abruptum* is common and dominant in Tai Rom Yen National Park, and many samples of this species have been recorded from there (Laudee & Malicky 2014).

The larvae of several *Lepidostoma* species in Asia have been studied in recent years. Ito (2001) described the larva of *L. cornigerum* (Ulmer 1907). The larva of *L. flavum* (Ulmer 1926) was described by Yang & Yang (2002). The larvae of *L. fui* (Hwang 1957) and *L. arcuatum* (Hwang 1957) were described by Shan & Yang (2005) and that of *L. nuburagangai* Dinakaran & Anbalagan 2013 (in Dinakaran *et al.* 2013) was described by Dinakaran *et al.* (2013).

Genus *Lepidostoma* is considered to be a shredder and it has a univoltine life cycle (Grafius & Anderson 1980; Malicky 2021). Dinakaran *et al.* (2013) reported that the larvae live in slow-moving streams where leaf litter and woody debris are deposited. Ito (2011) reported that *Lepidostoma* larvae live in small streams with hygropetric habitats and semi-aquatic plants in mountainous areas. Karaouzas & Waringer (2016) and Terefe *et al.* (2018) reported that European *Lepidostoma* spp. inhabit springs and cool, slow streams in mountainous areas.

The life cycle of *L. nuburagangai* was reported from India by Dinakaran *et al.* (2013). Five instars of the larvae were observed, with the first instars building their cases of sand, the second and third instars making their cases of sand and pieces of leaves, and the fourth and fifth instars making cases of leaves only (Dinakaran *et al.* 2013).

More than 1,700 species of insects are reported to be edible for humans and animals and have been used for this purpose for at least 10,000 years (Riggi *et al.* 2016). An analysis of aquatic insect nutritional values reported that they may have high protein content as a percentage of dry weight, as in Ephemeroptera (66.26%), Odonata (40–65%), Hemiptera (42–73%) and Coleoptera (23–66%) (Xiaoming *et al.* 2010). Trichoptera are important to aquatic ecosystems because they are a food source for fish and other aquatic life. Nutrition values of ten species of Trichoptera were reported as healthy food with low fat, but high calcium, iron, zinc, and protein (Anankware *et al.* 2015). Reinecke & Owen (1980) reported that Trichoptera also have high nutritional values: protein 45.7%, fiber 8.8%.

The current study investigated the life cycle, some aspects of biology, larval morphology, and nutritional values of *Lepidostoma abruptum*. The nutritional values reported in this study estimate the potential importance of culturing the insect for aquatic animal food.

Materials and methods

Larval morphology study

Final instar larvae and mature pupae of *L. abruptum* were collected from Lumphum stream, Tai Rom Yen National Park, southern Thailand (8°50'35"N 99°28'38"E) during March 2019 and February 2020 (Fig. 1). The final instar larvae and mature male pupae of *L. abruptum* were associated with larval sclerites in the pupal case (metamorphotype method, Wiggins 1996). Features of the identified larvae were photographed by stereomicroscopy (Leica Stereo S series). An ocular micrometer was used to measure larval dimensions.



FIGURE 1. Characteristic pool zone for *Lepidostoma abruptum* Banks 1931 at the study site in Tai Rom Yen National Park, Thailand.

Life cycle studies

Seventy-four larval specimens of *L. abruptum* were handpicked bimonthly in one year from the study site. The head capsule widths of the larvae were measured with an ocular micrometer. The distribution of head capsule widths was plotted to determine the larval instars of the insect.

To determine the development time of *L. abruptum*, live larvae at different stages were collected and cultured in an aquarium (40x60x20cm) covered with a net. The illumination in the aquarium was managed by using a timed light, with 12 hours daylight and 12 hours darkness. The temperature was controlled within the range 22–25°C.

The rearing water was replaced every week with natural stream water. The head capsule widths of the larvae were observed and measured every week until the larvae developed to adult stage.

Nutritional values analysis

To analyze nutritional values, larvae were collected from January to June 2020. The specimens were dried in a vacuum freeze-dryer at -40°C for 24 hours and were thoroughly ground with mortar and pestle in liquid nitrogen. The ground samples were analyzed at the Laboratory of Food and Beverage Testing, Institute of Food Research and Product Development, Kasetsart University. The methods applied were as follows. Protein: In-house method based on AOAC (Latimer 2016) 991.20. Fat: In-house method based on AOAC (Latimer 2016) 2003.05. Fiber: In-house method based on AOAC (Latimer 2016) 985.29. Moisture: In-house method based on AOAC (Latimer 2016) 925.45. Omega-3, Omega-6, and Omega-9: In-house method based on The Compendium of Methods for Food Analysis (Department of Medical Sciences 2003).

Results

Description of the final instar larva: *Lepidostoma abruptum* Banks 1931

General aspects. Total length 8.0–12.0 mm ($n = 12$). Head and other sclerotized parts dark brown to yellow brown. Soft parts of thorax and abdominal segments white. Abdominal segments II–VII with single gills dorsally and ventrally, bifurcate.

Larval case. Case length of final-instar larva 9.5–12.5 mm ($n = 12$). Case quadrangular in cross-section, made of rectangular pieces of leaf (Figs 2A, 2B).



FIGURE 2. *Lepidostoma abruptum* Banks 1931. **2A**, habitus of final instar larva in its case, right lateral; **2B**, habitus of final instar case (left) and larva (right), right lateral.

Final instar larva. Head capsule nearly circular in dorsal view, with granulate surface; head capsule length 1.25–1.30 mm (n = 12); head capsule width 1.50–1.58 mm (n = 12). Dorsal view of head dark brown to yellow brown with numerous transversely elliptical yellow muscle scars on posterior half of head. Eyes black, round, enclosed in transparent halo. Frontoclypeus with anterior margin concave, anterior 1/6 somewhat trapezoidal and dark brown, anterior half with convex sides, 1.5 times as wide as posterior half, subposterior end with yellow marks. Labrum light brown, elliptical with numerous setae anteriorly (Fig. 3A). Venter of head light brown. Submentum small, short, rectangular. Ventral apotome yellowish brown, shaped as isosceles triangle, broad anteriorly and acute posteriorly. Medial ecdysial line shorter than ventral apotome (Fig. 3B).

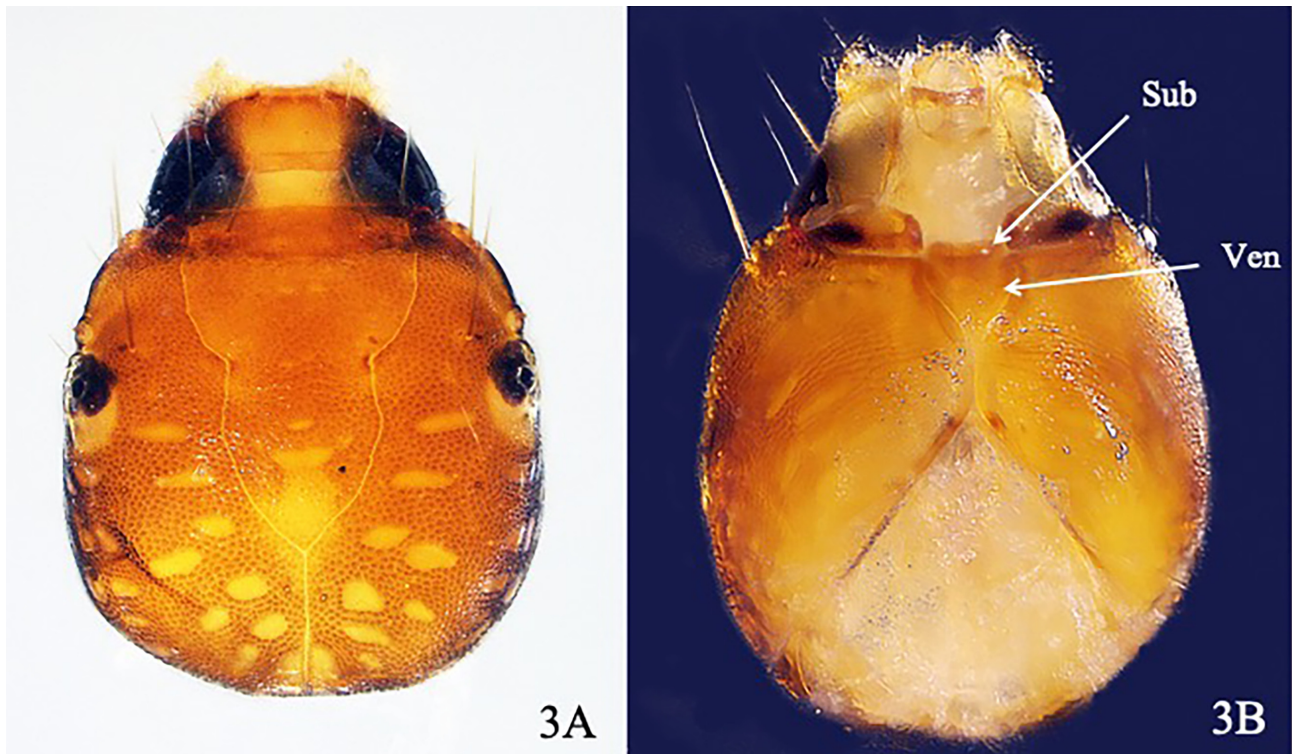


FIGURE 3. *Lepidostoma abruptum* Banks 1931, final instar larva. **3A**, head, dorsal; **3B**, head, ventral. Sub = submentum, Ven = ventral apotome.

Thoracic tergites yellowish brown, with moderately numerous long black setae. Pronotal sclerites dark brown, each with eleven black setae anteriorly, four setae arranged transversely near midlength, curved rows of muscle marks subposteriorly (Fig. 4A). Mesonotal sclerites yellowish brown, each with one anteromedial seta (*sa1*), three posteromedial setae (*sa2*), two lateral setae (*sa3*). Metanotum membranous, light yellow, without anteromedial setae (*sa1*), two pairs of posteromedial setae (*sa2*), four pairs of lateral setae (*sa3*) (Fig. 4A).

Forelegs stout and short, each with coxa trapezoidal, trochanter somewhat rectangular, femur stout, tibia short, and tarsal claw with basal seta. Midlegs and hind legs of somewhat similar shape, each with coxa, trochanter, femur, and tibia slender and long, and tarsal claw with basal seta (Figs 4B).

Abdominal segments cylindrical, membranous, cream-colored, with dorsal and ventral single gills. Abdominal segment I dorsal hump absent, with lateral hump on each side (Fig. 5A, arrow and dashed circle). Abdominal segments II–VIII each with single posterodorsal and posteroventral gills, with lateral line, number and positioning of gills and the extent of lateral line as in Fig 8. Abdominal tergite IX dark brown, semicircular, and with four long setae on its posterior edge (Fig. 6A). Anal prolegs short with long setae (Figs 6B, 7A, 7B). Anal claws (Fig. 7A, An) dark brown, each with dorsal accessory hook (Fig. 7A, Ac).

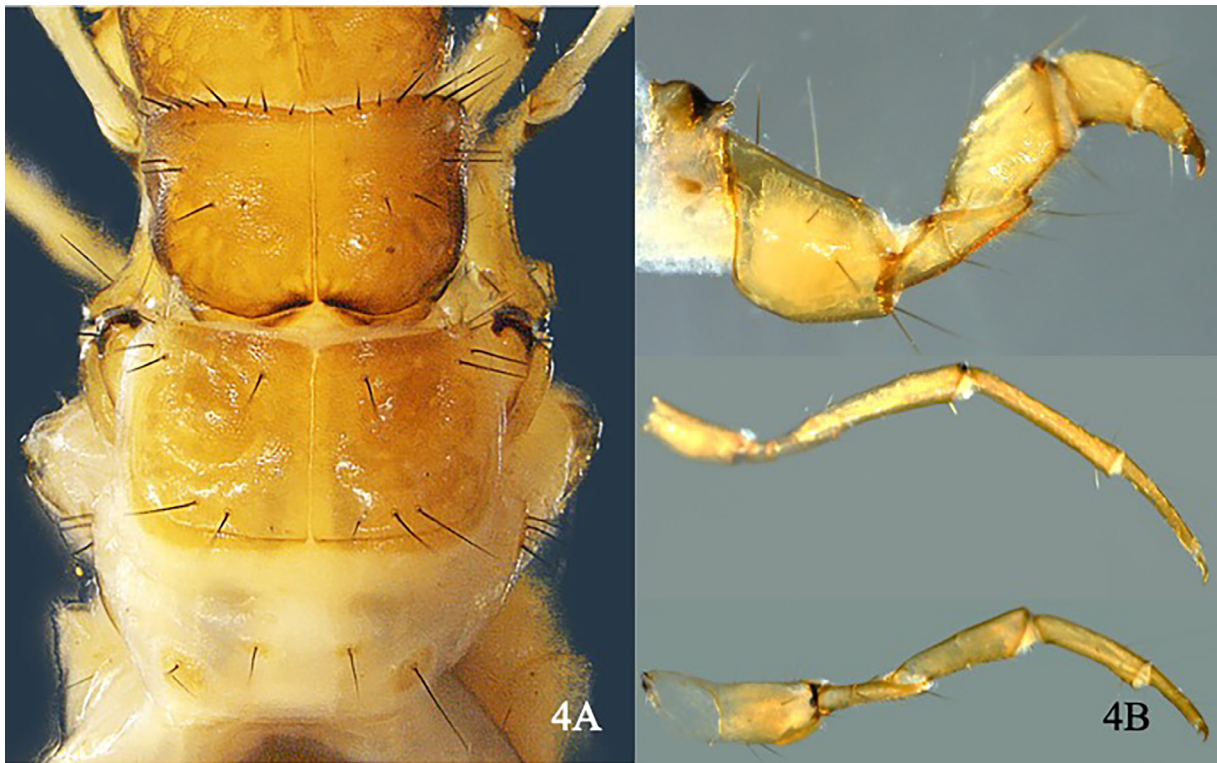


FIGURE 4. *Lepidostoma abruptum* Banks 1931, final instar larva. **4A**, thorax (pronotum, mesonotum, and metanotum), dorsal; **4B**, left front leg, anterior; left middle leg, anterior; left hind leg, anterior.

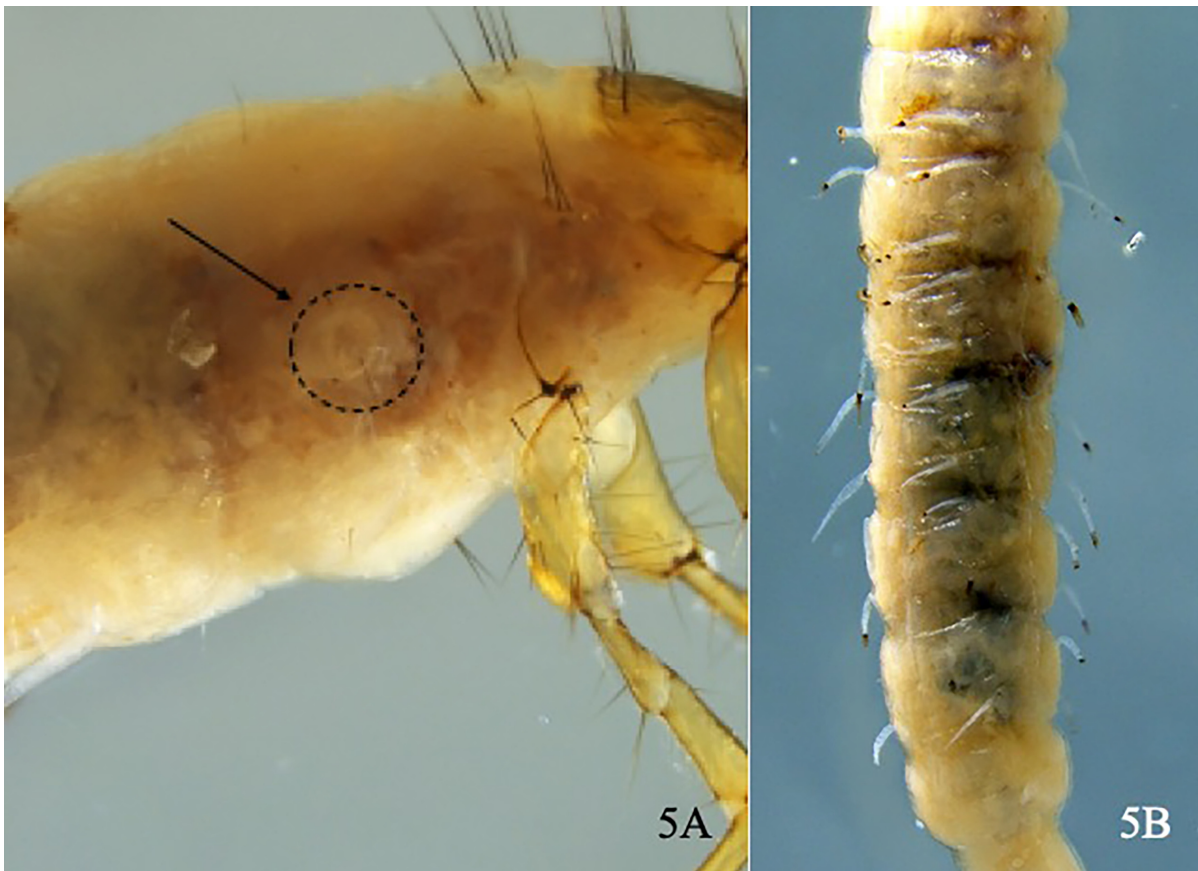


FIGURE 5. *Lepidostoma abruptum* Banks 1931, final instar larva. **5A**, mesothorax, metathorax, abdominal segments I and II, right lateral (arrow and dashed circle indicating abdominal segment I lateral hump); **5B**, gills of abdomen segments I–VII, right lateral.

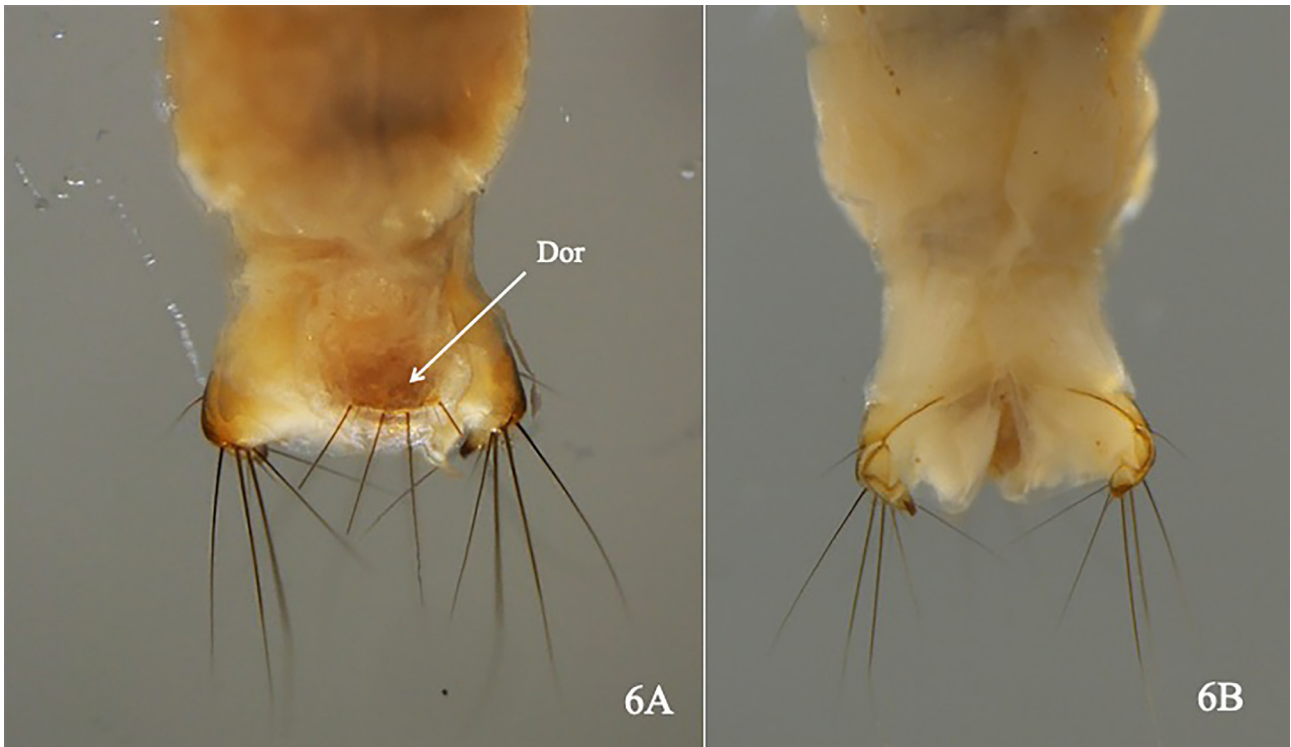


FIGURE 6. *Lepidostoma abruptum* Banks 1931, final instar larva. **6A**, abdominal segments VIII, IX, and anal prolegs, dorsal; **6B**, abdominal segments VIII, IX, and anal prolegs, ventral. Dor = dorsal tergite.

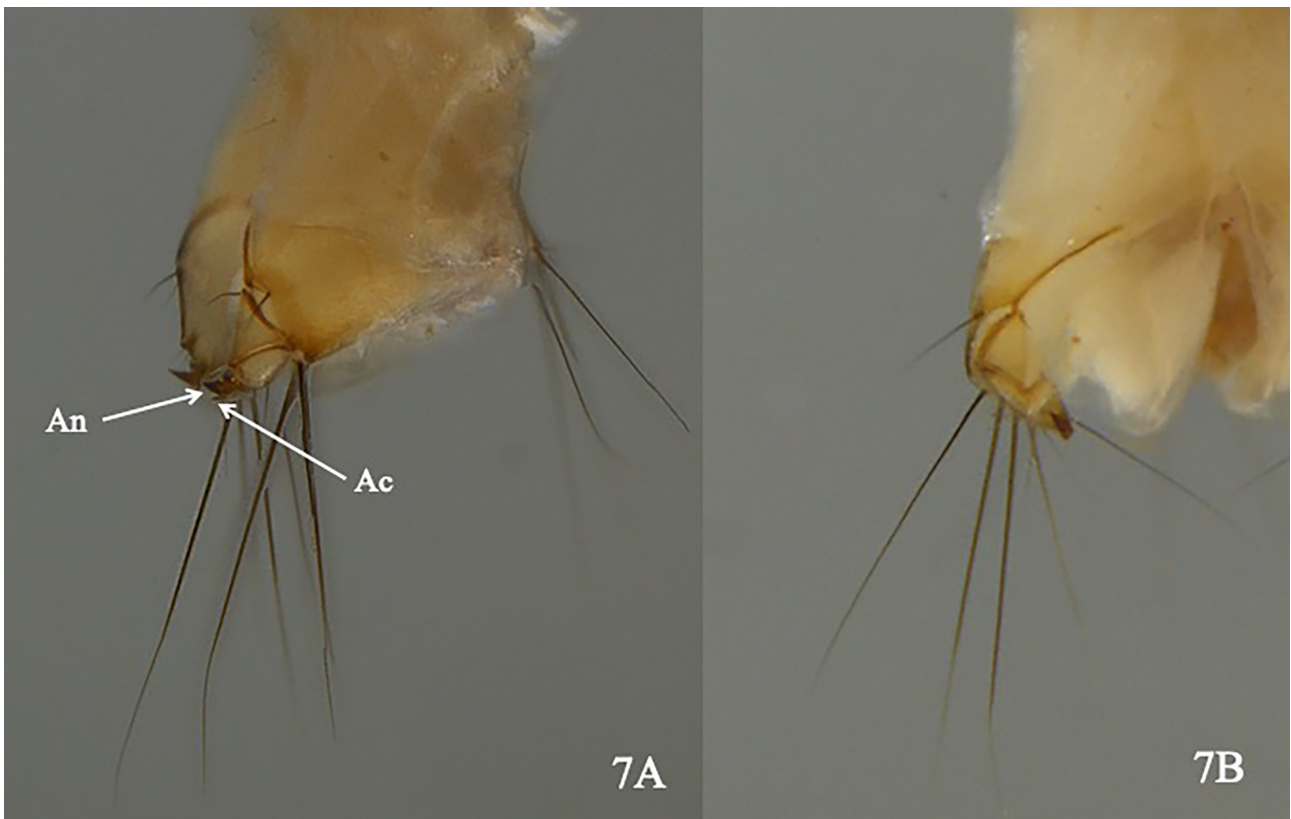


FIGURE 7. *Lepidostoma abruptum* Banks 1931, final instar larva. **7A**, left anal claw and accessory hook, left lateral; **7B**, right anal claw, ventral. An = anal claw, Ac = accessory hooks.

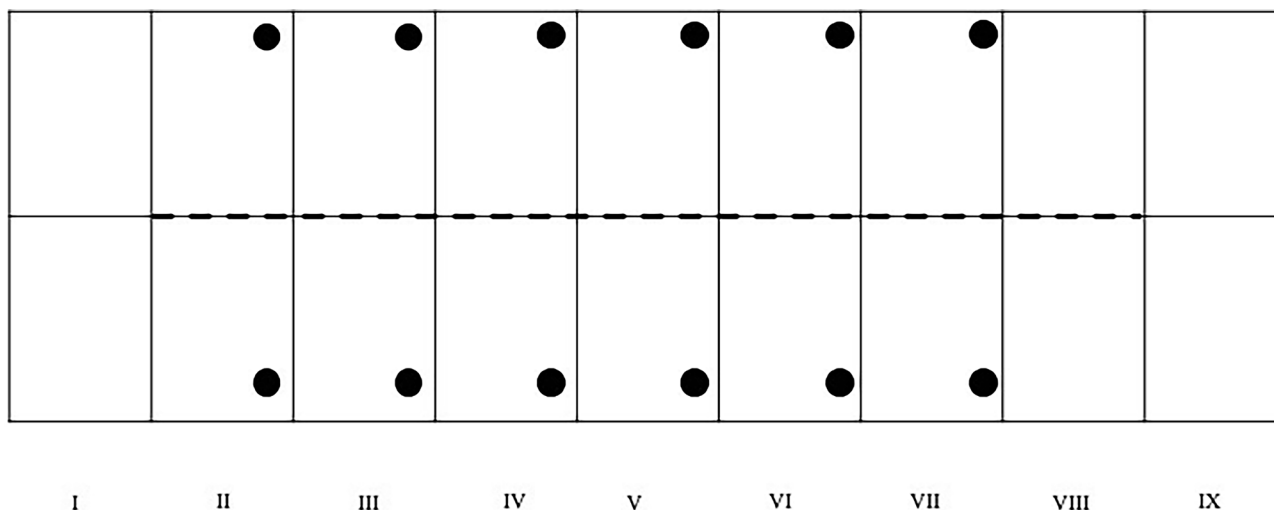


FIGURE 8. *Lepidostoma abruptum* Banks 1931, final instar larva, diagram of posterodorsal and posteroventral gills and extent of lateral line (dashed bold line along the middle), left lateral.

Life cycle study

The larvae were taken from a pool (Fig. 1). The substrate was dominated by fine sand, fine gravel, leaves, and other dead plant material. Habitat and water physico-chemical parameters were measured during day time, bimonthly during February–December 2018 as follows: air temperature $19\pm 0.84^{\circ}\text{C}$, water temperature $23\pm 1.41^{\circ}\text{C}$, pH 7.85 ± 0.36 , current velocity 0.18 ± 0.25 m/s, stream width 4 ± 0.63 m, stream depth 0.5 ± 0.8 m, alkalinity 55.78 ± 2.24 mg/l, dissolved oxygen 6.03 ± 0.20 mg/l, electrical conductivity $506.84\pm 18.35\mu\text{S/cm.}$, total dissolved solids 211.56 ± 19.53 mg/l. The current velocity and stream depth were high during rainy season.

The seasonal light trapping for the hot season (March–May) and the rainy season (September–October) for the adults of *L. abruptum* showed that the *L. abruptum* has a non-seasonal life cycle. A total of 74 *L. abruptum* larvae were measured for head capsule width, then analyzed for frequency distribution. The results indicate the presence of 5 instars of larvae. The ranges and means \pm SD of head capsule widths (HCW) of each instar were: Instar I, HCW = 0.50–0.63 mm, 0.56 ± 0.05 mm (n=11); Instar II, HCW = 0.75–0.90 mm, 0.83 ± 0.05 mm (n= 17); Instar III, HCW = 1.00–1.06 mm, 1.06 ± 0.05 mm (n=27); Instar IV, HCW = 1.25–1.38 mm, 1.31 ± 0.05 mm (n=7); and Instar V, HCW= 1.5–1.63 mm, 1.58 ± 0.05 mm (n=12)(see Fig. 9).

Larval rearing of instars I–V in the laboratory showed that development time of Instar I was 2 weeks, Instar II 5 weeks, Instar III 8 weeks. Development times for instars IV and V were not recorded. Pupae lived for about 3 or 4 weeks. The postemergent adults lived for 1 or 2 weeks (Table 1).

TABLE 1. Mean and range of head capsule widths (mm) for larval instars along with times spent at each stage

Larval instar	Range of head capsule width (mm)	Development times (week)
Instar I	0.50–0.63	2 weeks
Instar II	0.75–0.90	5 weeks
Instar III	1.00–1.06	8 weeks
Instar IV	1.25–1.38	-
Instar V	1.50–1.63	-
Pupae	-	3–4 weeks (n = 5)
Adult	-	1–2 weeks (n = 5)

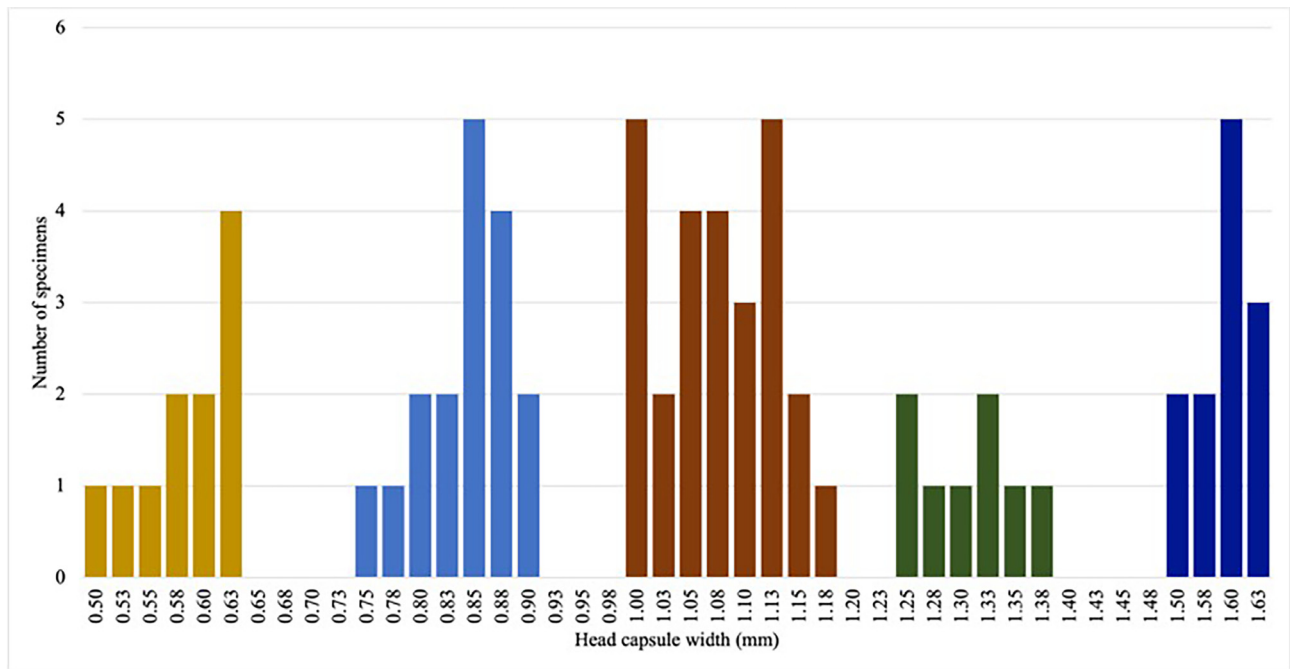


FIGURE 9. The frequency distributions of head capsule widths (mm) by larval instar stage of *Lepidostoma abruptum* Banks 1931.

Nutritional values

The nutrient composition determined from a total of 70 g of pooled *L. abruptum* larval stages, as dry matter (after removing 68.87% moisture), is presented in Table 2. The proximate analysis determined protein, fat, fiber, omega 3, omega 6, and omega 9 based on dry weight of the larval stage.

TABLE 2. Nutritional values of the *L. abruptum* larvae in the current study

Proximate analysis	Nutrients (% dry weight)
1) Protein	49.08
2) Total Fat	27.13
3) Total dietary fiber	5.90
4) Omega-3	0.15
5) Omega-6	6.04
6) Omega-9	11.70
	(% wet weight)
7) Moisture	68.87

Discussion and conclusions

Lepidostoma abruptum is a common species that has been recorded nationwide in Thailand; however, the larva has not been previously described (Thapanya *et al.* 2004; Malicky 2010; Bunlue *et al.* 2012; Laudee & Malicky 2014). The larva of *L. abruptum* shares similar characteristics with other of *Lepidostoma* species found in the Oriental Region. The characteristics that those species share are that the frontoclypeus is narrow and elongate with the anterior margin concave, the ventral apotome is longer than the median ecdysial line, the cases of final instar larvae are rectangular tubes made of leaves or/and sand, and a dorsal hump is absent from abdomen segment I (Dinakaran

et al. 2013; Ito 2011; Karaouzas & Waringer 2016; Morse *et al.* 2017; Terefe *et al.* 2018). The final-instar larva of *L. abruptum* can be distinguished from others known in the Oriental Region by the head capsule width. The head capsule width of a last instar larva of *L. abruptum* is 1.5–1.63 mm, but is 1.2–1.3 mm, 0.9 mm, and 0.7–0.9 mm in *L. arcuatum*, *L. fui*, and *L. fulvum* from China, respectively. Also, the number of setae on each side of the mesonotum can differentiate them: *L. abruptum* has one anteromedial seta (*sa1*), three posteromedial setae (*sa2*), and two lateral setae (*sa3*); but both *L. fui* and *L. arcuatum* have on each side one anteromedial seta (*sa1*), four posteromedial setae (*sa2*), and a tuft of lateral setae (*sa3*). Moreover, each anal proleg claw of *L. abruptum* and *L. arcuatum* has 2 accessory hooks, but each has only one accessory hook in *L. fui*.

Malicky (2021) reported that *Lepidostoma* species in northern Thailand, including *L. moulmina* and *L. doligung*, have non-seasonal life cycles with the adults observed year-round. In this study, the adults of *L. abruptum* were collected both in the hot season (March–May 2019) and in the rainy season (September–October 2019). We conclude that *L. abruptum* also has a non-seasonal life cycle. The life cycle of *L. abruptum* has 5 larval instars, which is similar to *Lepidostoma* species in other parts of the world (Dinakaran *et al.* 2013).

The *Lepidostoma* spp. in Thailand are inhabitants of first- and second-order streams, with substrate dominated by bedrock, boulders, cobbles, pebbles, and sand, but they have not yet been found in big rivers (Laudee & Prommi 2011; Laudee & Malicky 2014; Malicky 2021). Dinakaran *et al.* (2013) found that the larva of *L. nuburagangai* lives in slow-moving streams in pool and riffle areas, where the woody debris and leaf litter accumulate. Moreover, Terefe *et al.* (2018) reported that, in general, larvae of the genus *Lepidostoma* are shredders living in forested streams. We found the habitat of *L. abruptum* in a pool of a small stream where the substrate is dominated by fine sand, fine gravel, dead leaves and other plant material.

Trichoptera constitute one of the largest groups of primary consumers in streams and rivers (McCafferty 1981; Holzenthal *et al.* 2007). Their high concentration of nutrients could justify development of sustainable cultures of caddisflies for human and animal food (Williams & Williams 2017).

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