

## Taxonomical studies of dragonfly nymphs (Odonata, Libellulidae) using their exuviae

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**ABSTRACT:** Taxonomic studies of dragonfly nymphs were done up to species level using their exuviae. Exuviae, being the last instar larval skin, possess all larval features. These features can be used for the identification of odonate nymphs up to species level. Seven species belonging to the family Libellulidae were identified using the features present on exuviae. This is a non-invasive method that can be used for the taxonomic studies of dragonfly nymphs without rearing them in the laboratory. The study describes the morphological features of seven species of dragonfly nymphs belonging to family Libellulidae using exuviae. A taxonomic key for the identified exuviae were also provided.

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**KEYWORDS:** Species identification, non-invasive method, taxonomic key

### INTRODUCTION

Odonata comprises dragonflies and damselflies, one of the most ancient insect groups. In Kerala, a total of 174 species of odonates have been reported of which 65 are endemic to the Western Ghats, and 10 to India (Gopalan *et al.*, 2022). The kole wetlands of central Kerala reported 44 species of odonates (Chandran *et al.*, 2021). Being amphibiotic and hemi-metabolous insects, adults are terrestrial, and nymphs have aquatic modes of life. Adult females, after mating, lay their eggs in different aquatic ecosystems. Egg hatch into nymphs. Dragonfly nymphs are strictly predacious, and by using their extensible labium, they capture aquatic insects, crustaceans, small molluscans, larvae of other insects, and oligochaetes. At the end of the aquatic life, the nymph finds a support, such as a rock or plant stem, where it can tear up its skin and

enter the aerial life as an adult. The skin that remains on the rock or plant stem is called exuviae, and they are nothing more than the dried skin of the last larval instar and are therefore vulnerable. Since the exuvia is the moulted skin of the penultimate instar, it possesses all the larval features (Adambukulam and Kakkassery, 2013a, b). The main features that can be used for the taxonomic identification larva includes shape of the labium, number and shape of antennal segments, number of premental and palpal setae, presence or absence of mid dorsal spines, and position of lateral abdominal spines, shape of caudal appendages etc. These features show variation within the family, genus and species level and hence can be used to study the larval taxonomy. Taxonomical studies of dragonfly nymphs were usually conducted either by collecting nymphs from the field or eggs from an ovipositing female in the field and rearing them

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in the laboratory. In Kerala, Nirmalakumari and Balakrishnan (1981, 1983) studied the life history of the nymphs of *Rhodothemis rufa* (Rambur, 1842) and *Urothemis signata* (Rambur, 1842) by rearing the larvae in the laboratory. No further studies on Odonate nymphs were reported from Kerala till 2013. Adambukulam and Kakkassery (2013a, b) conducted the larval description studies of dragonfly nymphs by using their exuviae. A typical libellulidae larvae can be identified by their spoon shaped labium and well-developed premental and palpal setae. Interspecific variations were seen in the number of setae. The presence or absence of mid dorsal abdominal and lateral abdominal spines can also be taken as a taxonomic feature. The present study describes the morphological features of seven species of dragonfly nymphs belonging to family Libellulidae using their exuviae.

## MATERIALS AND METHODS

During the exuvial survey in Thrissur district, exuvia were collected from a pond (10°27'50"N; 76°11'31"E) located in a paddy field and also from a man-made cement tank located in Ammadam, a small village, 8 km away from Thrissur. Collections were done during the early morning hours, because the process starts in late midnight to avoid predation pressure and hence the collector had the opportunity to see the adult just emerging from the exuviae, which helped to identify the species at the primary level in the field itself. The specimens (exuviae) were collected in plastic bottles of the photographic film and brought into the laboratory. Wet specimens were dried by placing them under an incandescent lamp and then they were pinned and preserved as dry specimens for analysis. The exuviae were dissected by observing through the Stereo Dissection Microscope (CZM 4, LABOMED) for analyzing the larval features present on exuviae. The description of the general morphology follows Tennessen (2019). The SEM images of the main features present on the exuviae (Figs. 1 A-F) were taken using JEOL 6390LA. Exuvia sample was smeared over the multisided adhesive carbon tape fixed on specimen stubs and over-coated with gold using JFC 1600. This ion-sputtering device performs rapid and efficient gold coating on the microscopic

specimen, allowing surface visualization. The SEM measurements were performed at 15 kV accelerating voltage. Different magnifications were used, as indicated on the images. The main taxonomic features present on exuviae includes shape of the labium, the number of premental and palpal setae, setaceous margin of palpus, crenations on palpus, presence or absence of mid dorsal protuberances, their shape (spine like or hook like), lateral spines, extension of wing pads, presence of hairs at base of the abdomen and length and size of caudal appendages. The features observed on exuviae were compared with original larval descriptions by Kumar (1984, 1988, 1990) and Hussain and Riaz (1999a, b). The final plates were prepared using Leica S80AP0 stereo microscope (Leica Microsystems, Germany) with an in-built camera (Leica MC 170 HD) and LAS auto-imaging software. The specimens were retained in the Entomology museum of Department of Zoology, St. Thomas College Thrissur.

## RESULTS AND DISCUSSION

A total of 105 exuviae were collected during the study. They include *Acisoma panorpoides* Rambur, 1842 (12 nos.), *Bradinopyga geminata* (Rambur, 1842) (22 nos.), *Neurothemis tulia* (Drury, 1773) (9 nos.), *Orthetrum sabina* (Drury, 1773) (26 nos.), *Rhyothemis variegata* (Linnaeus, 1763) (12 nos.), *Tholymis tillarga* (Fabricius, 1798) (11 nos.) and *Zyxomma petiolatum* Rambur, 1842 (13 nos.). All belong to the family Libellulidae.

### 1. *Acisoma panorpoides* (Figs. 2 A-F)

A small exuvia having dark brown colour; body length ranges between 16.2–18mm; head small, triangular with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of mid leg at rest; distal margin of prementum is triangular, and projecting; premental setae 11+11; palpal setae 8+8; distal margin of palpus formed into crenations with claviform setae; thorax bears long legs; hind leg extends beyond the body; wing pads diverging; hind wing pad extends up to the middle of the 7<sup>th</sup> segment; abdomen is more or less oval shaped; mid dorsal protuberances absent;

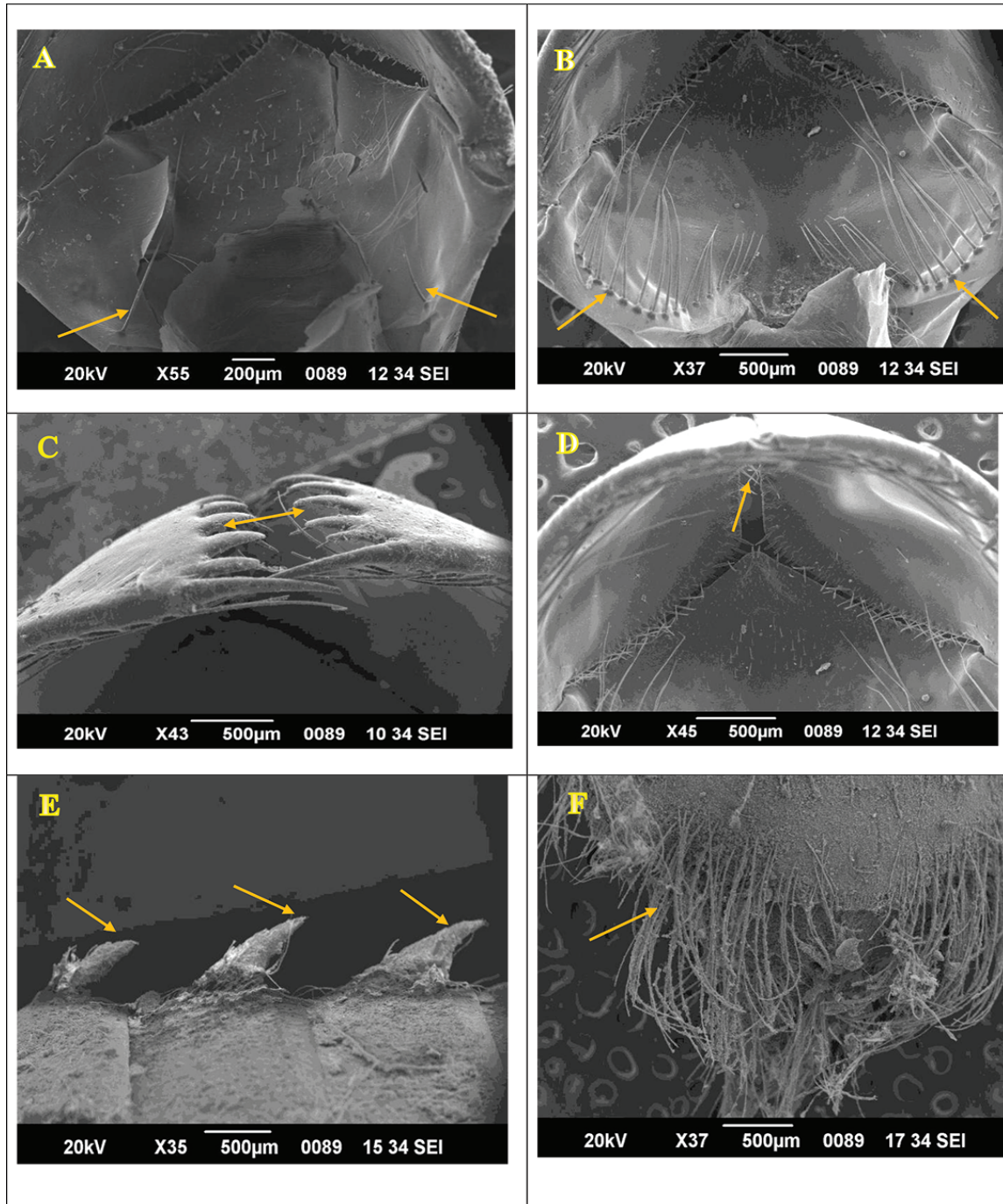


Fig. 1 Taxonomic features on Exuviae. A-Premental setae in *Rhyothemis variegata*; B-Premental setae in *Bradinopyga geminata*; C- Palpal crenations in *Pantala flavescens*; D- Palpal spines in *B. geminata* E- Mid dorsal protuberances in *Orthetrum sabina*; F- Abdominal hairs in *O. sabina*

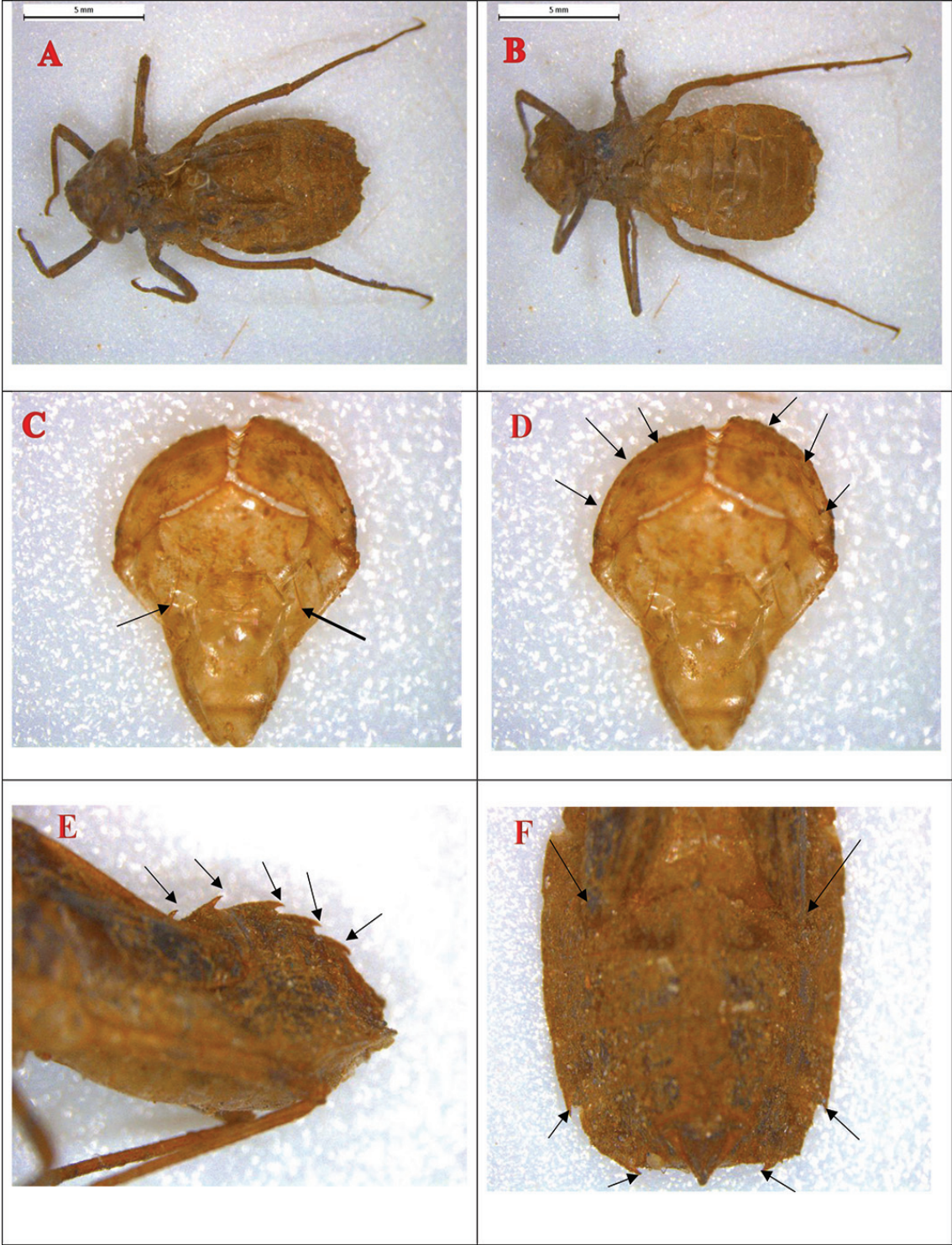


Fig. 2 *Rhyothemis variegata*. A-Dorsal view; B-Ventral view ; C-Premental setae D-Palpal setae; E- Mid dorsal spines F- Lateral spines and Wing pads

lateral spines on S8 and S9; S10 smaller than anal appendages; epiproct triangular, broad at the base; cerci shorter than epiproct and are pointed; paraproct equal length of epiproct. These features were compared with the larval features given (Kumar, 1984) by rearing the larvae in the laboratory.

Remarks: *A. panorpoides* is widely distributed in India, Nepal, China, Sri Lanka, Burma, Thailand, Malaysia, Indonesia, Japan, Taiwan, Philippines, Africa and Madagascar. In Kerala they usually associated with ponds and canals in paddy fields and also near to other stagnant water bodies and their oviposition occurs just after first monsoon rain. The emergence occurs at the end of monsoon season. The exuviae found to be attached to lower side of aquatic plants at a height of 10-20cm above water level.

### 2. *Bradinopyga geminata* (Figs. 3A-F)

A medium sized transparent exuvia; body length ranges between 19–22mm; head small, triangular with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 19+19; palpal setae 16+16; distal margin of palpus crenated with claviform setae; long slender legs with dark bands on femur and tibia; wing pads diverging; hind wing pad extends up to the middle of the 6<sup>th</sup> segment; abdomen is broader than head; dorsally convex; ventrally flattened; irregular dark spots present; abdominal segments S2–S8 almost equal sized; S9–S10 tapering; mid dorsal protuberances absent; well-developed lateral spines on S8 and S9; S10 smaller than anal appendages; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct equal length of epiproct. The exuvial features were compared with the larval features Sangal and Kumar (1970).

Remarks: This species is widely distributed throughout the oriental region. They usually breed in overhead tanks and garden ponds. The exuviae were usually found attached to manmade garden ponds. But in garden ponds in which ornamental

fishes are growing their emergence is found to be less. Sometimes they emerge in large numbers. It is a multivoltine species and emerges throughout the year.

### 3. *Neurothemis tulia* (Figs. 4 A-F)

A medium sized exuvia with brown colour; body length ranges between 11–14mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 13+13; palpal setae 8+8; distal margin of palpus crenated; long slender legs with dark bands on femur and tibia; wing pads diverging; hind wing pad extends up to the middle of the 5<sup>th</sup> segment; abdomen is broader than head; dorsally convex; ventrally flattened; more or less oval shaped; mid dorsal protuberances absent; lateral spines on S8 and S9; S10 smaller than anal appendages; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct equal length of epiproct. The features were compared with studies of Kumar (1988) and Begum *et al.* (1990).

Remarks: *N. tulia* is an extremely widespread common species, found throughout mainland tropical and subtropical Asia. In Kerala the species is distributed everywhere. They are also multivoltine and emergence occurs all the year around. They breed in small ponds, streams and rivers etc. A variation in the number of premental and palpal setae is observed in Indian species where it is 13 and 8 respectively (Kumar, 1988), for the Bangladesh specimen it is 10 and 9 from Bangladesh (Begum *et al.*, 1990) The difference in the number may be due to the geographical intra specific variations. The collected exuviae show features of Indian representative.

### 4. *Orthetrum sabina* (Figs. 5 A-F)

A medium sized exuvia with muddy brown colour; body length ranges between 19–22mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven

segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 7+7; palpal setae 7+7; distal margin of palpus crenated; legs robust and covered with hairs; wing pads diverging; hind wing pad extends up to the middle of the 6<sup>th</sup> segment; abdomen is broader than head; dorso-ventrally flattened; more or less oval shaped; spine like mid dorsal protuberances present from S4–S9; setae along posterior margin of all abdominal terga; lateral spines absent; sternum of S9 bears a bunch of hairs; S10 smaller than anal appendages; anal appendages are hairy; epiproct triangular, acuminate broad at the base shorter than paraprocts; cerci less than half length of epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: *O. sabina* is widely distributed in Ethiopian, Oriental and Australian region. Kumar (1973) studied the life history of *O. sabina*, and in his studies that the final instar has 11+11 premental setae and 7+7 palpal setae respectively. But the exuvia collected by the authors show variation in the number of premental and palpal setae 7+7 premental and 7+7 palpal setae and this may be due to geographical variation. In Kerala it is multivoltine species and emerges in large number during pre and post monsoon seasons.

#### 4. *Rhythemis variegata* (Figs. 6 A-F)

A small sized exuvia with grayish black colour; body length ranges between 11–13mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 1+1; palpal setae 5+5; distal margin of palpus crenated; legs long and slender; hind leg extending beyond the abdomen; wing pads diverging; hind wing pad extends up to the middle of the 5<sup>th</sup> segment; abdomen is broader than head; dorso-ventrally flattened; oval shaped; spine like mid dorsal protuberances present from S3–S9; lateral spines present on S8–9; S10 smaller than anal appendages; anal appendages are hairy;

epiproct triangular, acuminate broad at the base shorter than paraprocts; cerci less than half length of epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: This species is distributed throughout the Oriental region. The larvae have been described by Chowdhury and Akhteruzzaman (1981). In their description the larvae possess 2+2 premental setae but in the collected exuviae it is 1+1. In Kerala it usually emerge during post monsoon season in large numbers.

#### 6. *Tholymis tillarga* (Figs. 7A-F)

A small sized exuvia with grayish black colour; body length ranges between 11–13mm; head small, pentagonal in outline; with compound eyes projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 7+7; palpal setae 5+5; distal margin of palpus crenated; long, slender legs with dark bands; hind leg extending beyond the abdomen; wing pads diverging; hind wing pad extends up to the middle of the 5<sup>th</sup> segment; abdomen is broader than head; dorso-ventrally biconvex; tapering towards the distal end; hook like mid dorsal protuberances present from S3–S9; lateral spines present on S8–9; S10 smaller than anal appendages; anal appendages are hairy; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: This species has a wide distribution in all parts of the world except Europe and the Americas. The larvae are sluggish and bottom dwellers and were described (Kumar, 1973; Chowdhury and Akhteruzzaman, 1981). In Kerala the emergence occurs during post monsoon season in large numbers.

#### 7. *Zyxomma petiolatum* (Figs. 8 A-F)

A medium sized exuvia with blackish grey colour; body length ranges between 17–20mm; head small, pentagonal in outline; with compound eyes

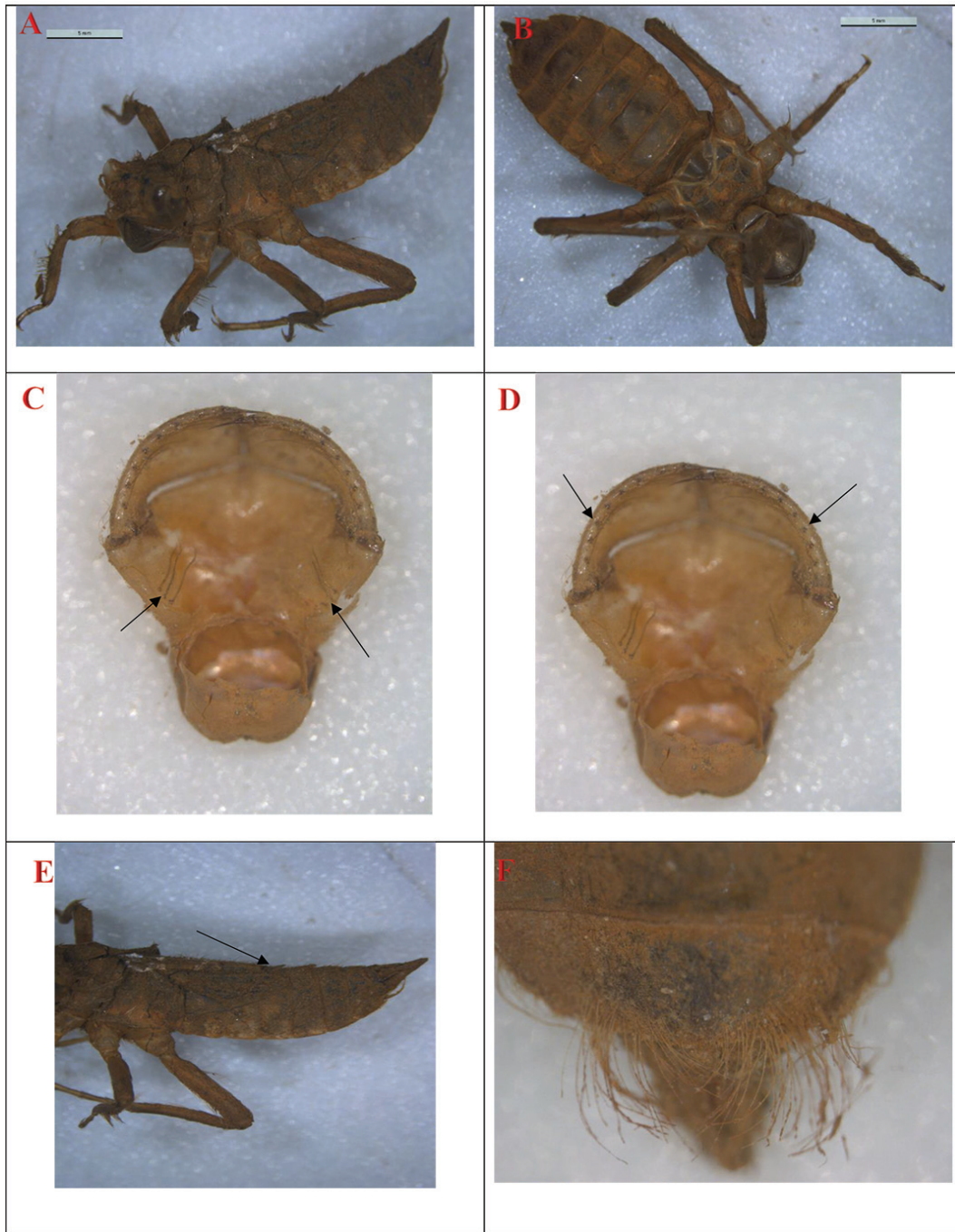


Fig.3 *Orthetrum sabina*. A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal spines F- Hairs on the abdomen

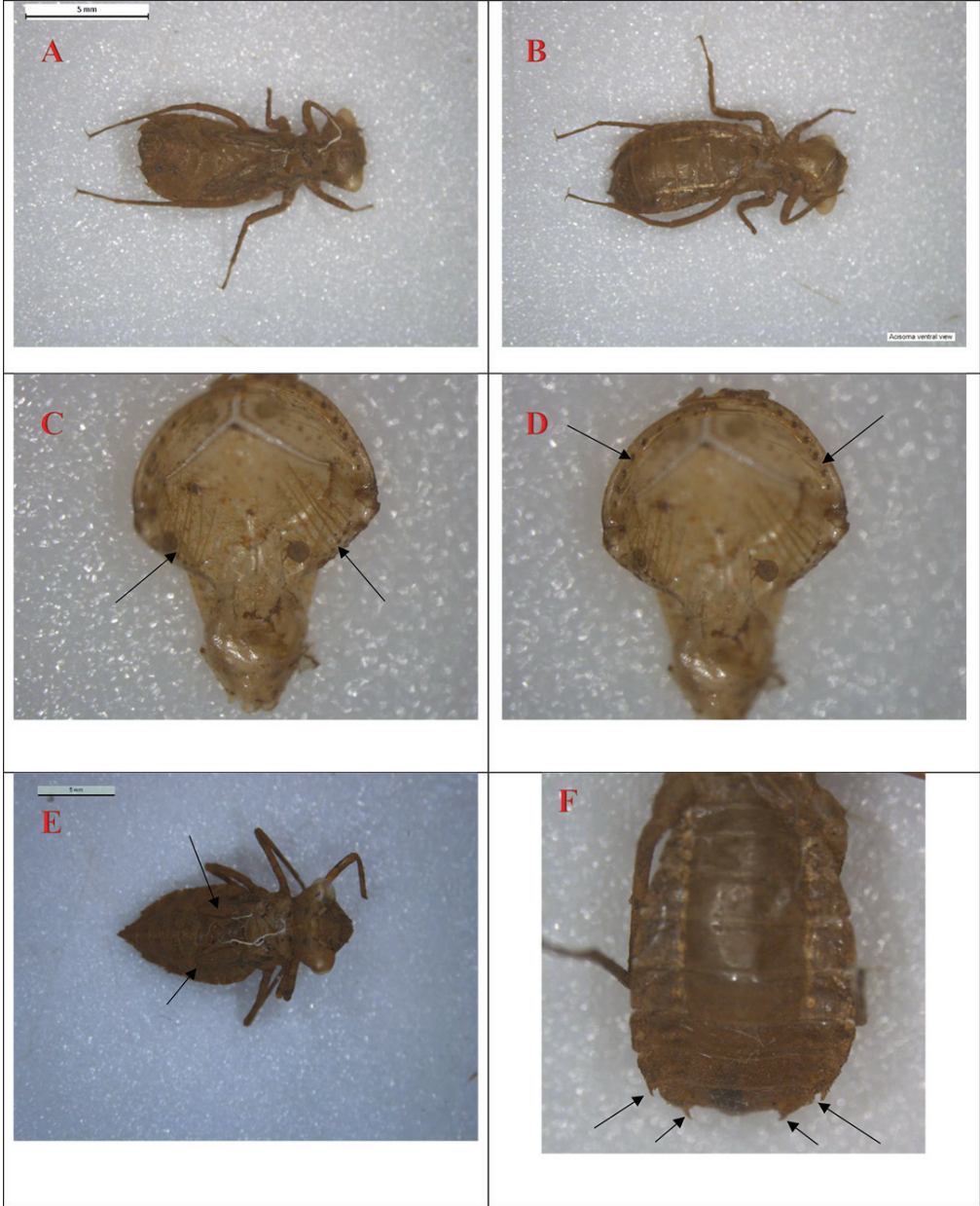


Fig. 4 *Acisoma panorpoides*. A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Wing pads; F- Lateral spines on the abdomen



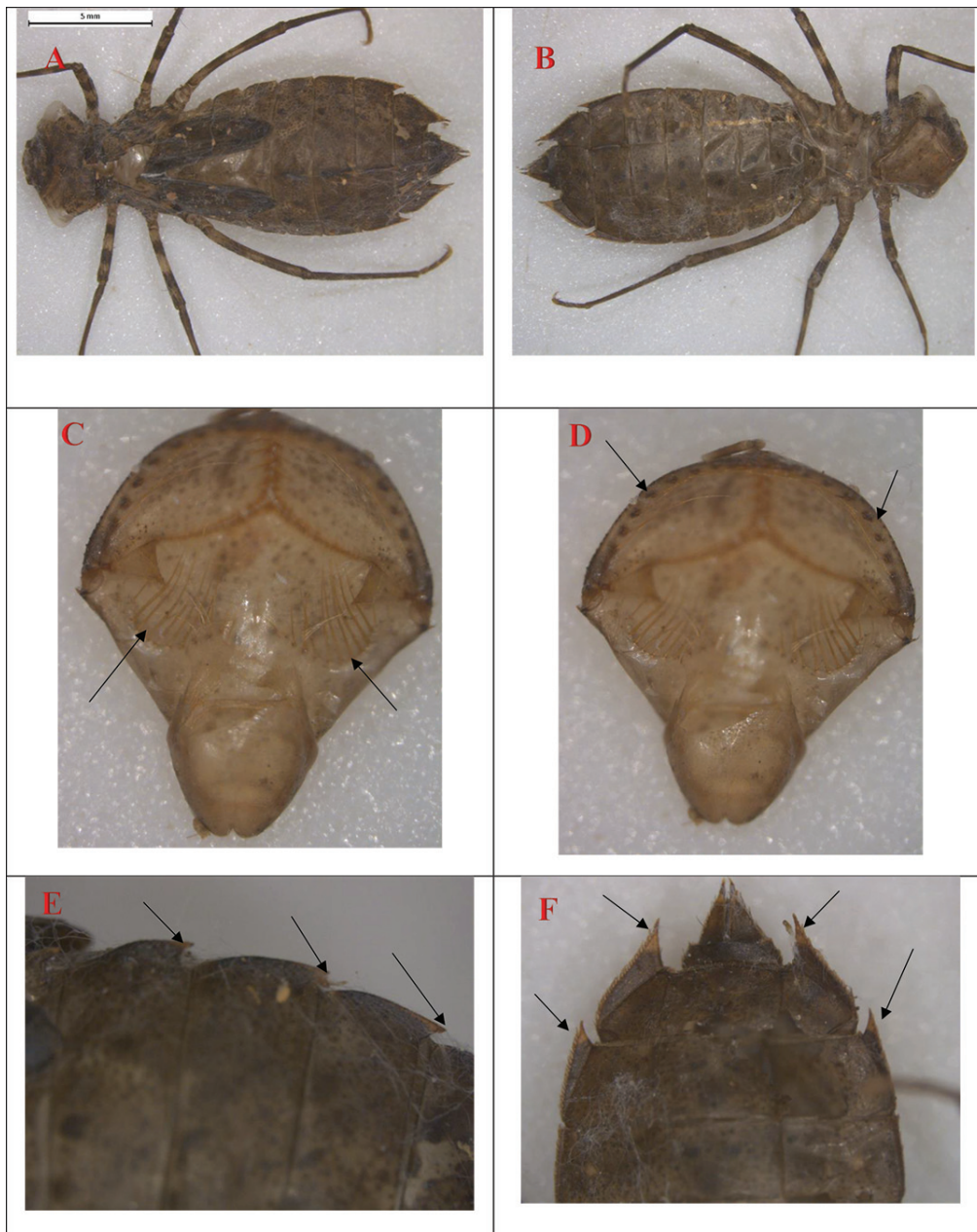


Fig 5: *Zyxomma petiolatum*. A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal protuberances; F- Lateral spines on the abdomen

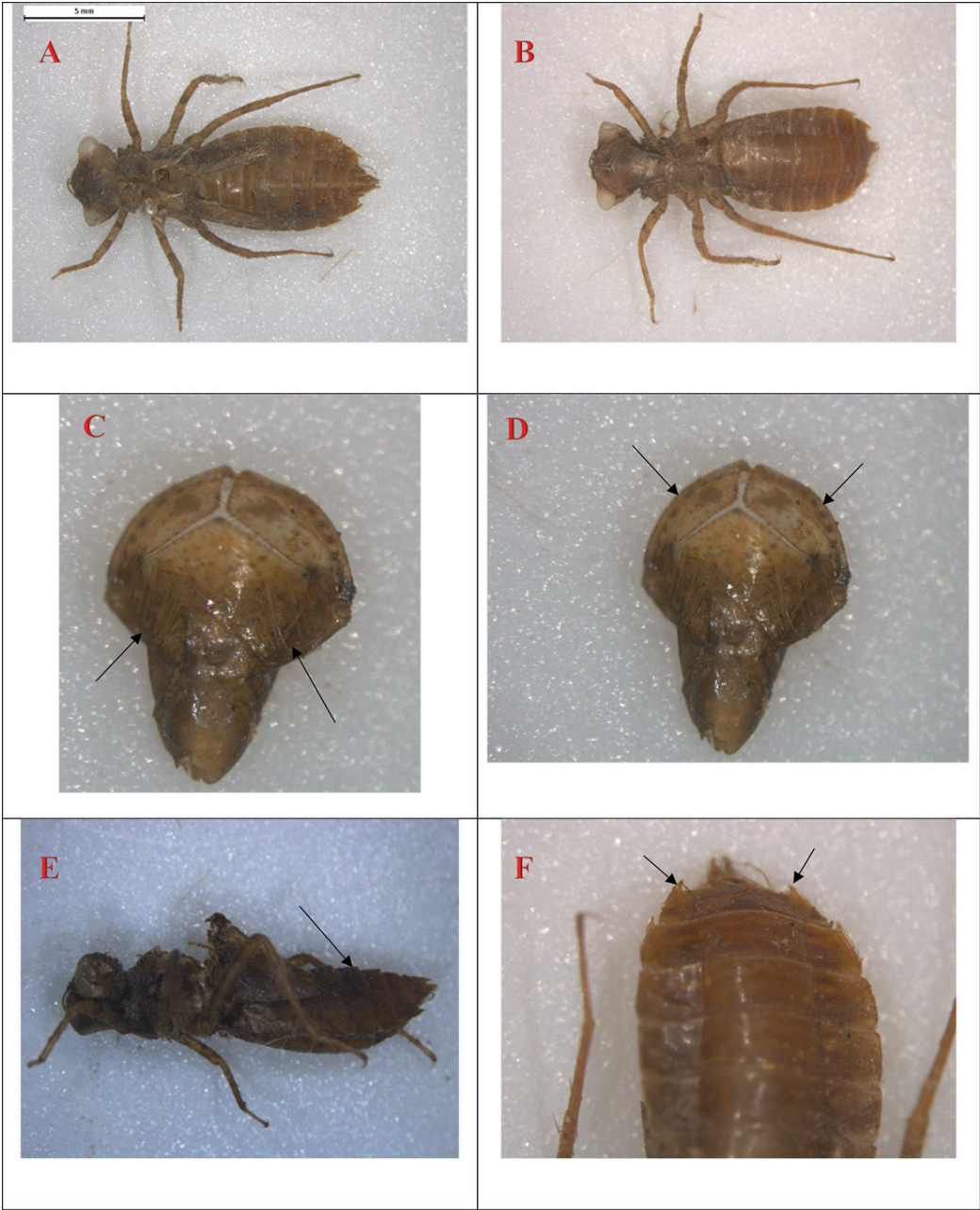


Fig. 6 *Neurothemis tulia*. A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal protuberances; F- Lateral spines on the abdomen

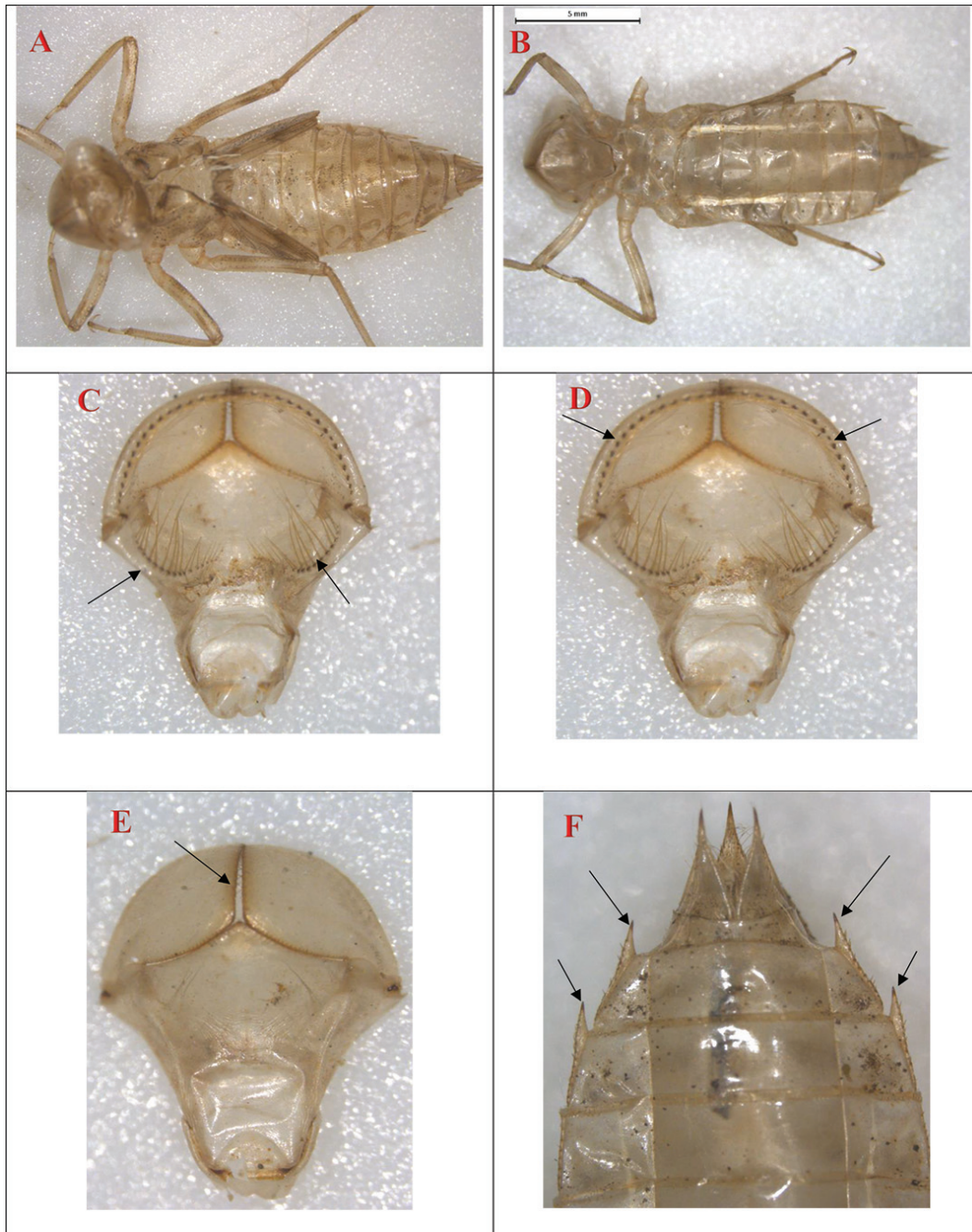


Fig. 7 *Bradinopyga geminata*. A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Palpus with crenations; F- Lateral spines on the abdomen

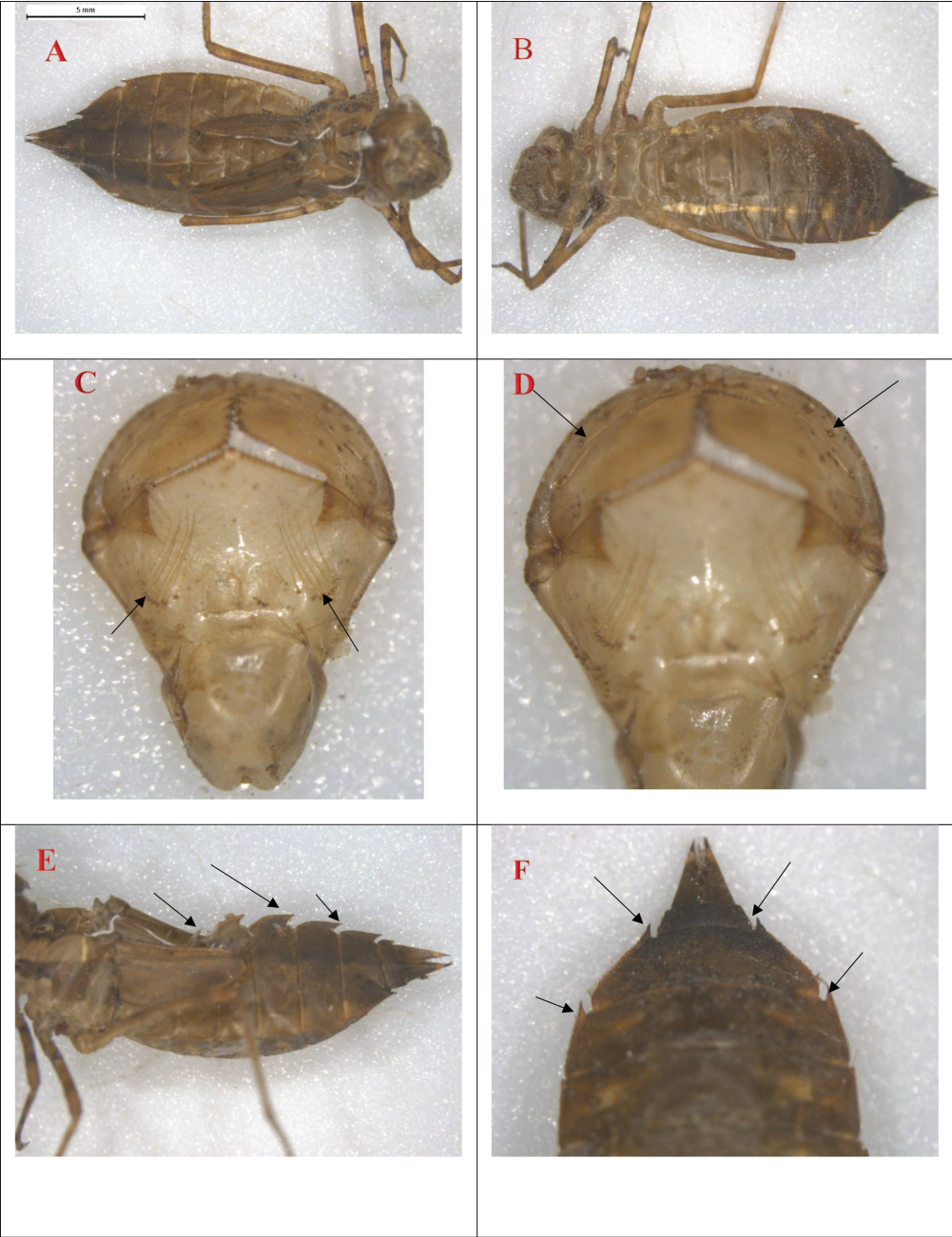


Fig. 8 *Tholymis tillarga*. A-Dorsal view; B-Ventral view; C-Premental setae; D-Palpal setae; E- Mid dorsal protuberances; F- Lateral spines on the abdomen

projecting antero-laterally; antennae seven segmented and filiform; labium is spoon shaped; extends up to the coxae of first pair of leg at rest; distal margin of prementum triangular, and projecting; premental setae 12+12; palpal setae 8+8; distal margin of palpus crenated; legs long and slender ; hind leg extending beyond the abdomen; wing pads diverging; hind wing pad extends up to the middle of the 5<sup>th</sup> segment; abdomen is broader than head; dorso-ventrally biconvex; tapering towards the distal end; hook likemid-dorsal spines present from S1–S10, spines on S1–S3 and S10 were very small, S4–S9 prominent, sharp and pointed; postero lateral spines on S8–9, spine on S9 is longest and reaching up to the length of epiproct; S10 smaller than anal appendages; anal appendages are hairy; epiproct triangular, acuminate broad at the base; cerci shorter than epiproct and acuminate; paraproct acuminate equal length of epiproct.

Remarks: It is a common species in India, Myanmar and Sri Lanka and is a crepuscular dragonfly appearing shortly before dusk and coming often to house lights. Breed in small stagnant pools and even in domestic wells. The larvae was studied by (Kumar, 1973: Chowdhury and Akhteruzzaman, 1981).The breed in natural water boides or man-made water bodies.They are multivoltine species and emerge in all seasons throughout the year.

**Key to the 7 species of Libellulidae**

- 1. Mid dorsal abdominal spines absent.....2
  - Mid dorsal abdominal spines present.....5
- 2. Body length <15mm; palpal setae<10.....3
  - Body length >15mm; palpal setae >10.....4
- 3. Wing pads extending up to middle of S5; premental setae 13+13; palpal setae 8+8; lateral spines on S8–9;.....*Neurothemis tulia*

Premental setae 11+11; palpal setae 7+7; lateral spines on S9–10; wing pads extending up to middle of S7.....*Acisoma panorpoides*
- 4. Wing pads extending to S6; premental setae

17+17; palpal setae 15+15; lateral spines on S8–9; .....*Bradinopyga geminata*

5. Abdominal lateral spines absent on S8–9.....6

Abdominal lateral spines present on S8–9.....7

6. Premental setae 7+7; palpal setae 7+7; long tapering abdomen with tuft of hairs at S9.....*Orthetrum sabina*

7. Abdomen with dark mid dorsal stripe from S3–9.....8

Abdomen without mid dorsal stripe form S3–9...9

8. Premental setae 7+7; palpal setae 5+5; mid dorsal protuberances from S4 9.....*Tholymis tillarga*

9. Mid dorsal protuberance from S3–9 are spine like; premental setae 1+1; palpal seate5+5; .....*Ryothemis variegata*

Mid dorsal protuberances from S1-10 are hook like; premental setae 12+12; palpal setae8+8; .....*Zyxomma petiolatum*

This present work was focused on morphological features of exuvia (final instar exoskeleton) for identifying dragonfly nymphs up to species level. Most of the nymphal studies of dragonflies were based on the rearing of larvae that directly collected from the field or by collecting the eggs from ovipositing females from the field. Both methods are time consuming Thus studies, using exuviae can be considered as a noninvasive method for nymphal diversity. This methodology of exuvia collection from the field is suggested for the those species which are difficult to observe as adult and also for identifying larval features endemic and endangered dragon fly species. The presence of exuviae also indicates the successful breeding sites of dragonflies and thus health of the aquatic ecosystem can be assessed. The counts of exuviae may give a clear picture of real dragonfly abundance and diversity than do male biased counts, and that numbers emerging from a small or medium sized water body can usually be monitored by a single researcher (Corbet and Hoess, 1998).

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