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## Research article

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# A contribution to the genus *Afronurus* Lestage, 1924 in China (Ephemeroptera: Heptageniidae, Ecdyonurinae)

Wei ZHANG <sup>1</sup>, Zhi-Ming LEI <sup>2</sup>, Wen-Juan LI <sup>3</sup> & Chang-Fa ZHOU <sup>4,\*</sup>

<sup>1,2,3,4</sup>The Key Laboratory of Jiangsu Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing 210023, China.

\*Corresponding author: zhouchangfa@njnu.edu.cn

<sup>1</sup>Email: zyhnjnu@163.com

<sup>2</sup>Email: zrqz.lzm@qq.com

<sup>3</sup>Email: 1824201416@qq.com

<sup>1</sup> urn:lsid:zoobank.org:author:B7919DEB-15AA-4915-B155-9866D4ECFA06 <sup>2</sup> urn:lsid:zoobank.org:author:720E4C28-2AF3-4E95-B0A5-DA16506B0A24 <sup>3</sup> urn:lsid:zoobank.org:author:77E8C53E-8710-4C92-9283-7D99D2095765 <sup>4</sup> urn:lsid:zoobank.org:author:73E0090E-6A83-4238-893C-EFD79A2590DA

**Abstract.** The genus *Afronurus* has several very common mayfly species in China and they are widely distributed in this country. Some of them are quite similar to each other in both imaginal and nymphal stages. However, these species have not been systematically compared and reviewed so far. In this study, six species are recognized. All nymphs of them share the following characters: gills V–VI with additional arrow-like accessory lobes, branched dentisetae, two rows of bristles and setae on hindtibiae and spotted abdominal terga. The males have divergent penes and clearly expressed titillators. The nymphs of the new species *A. drepanophyllus* sp. nov. have sickle-like gills I, spotted and striped body color, and males have unique genitalia. The nymphal stages of *A. furcatus* and *A. hunanensis*, which are associated and described for the first time, have similar body color to *A. drepanophyllus* sp. nov., but their pale dots on the head capsules and the shape of the hypopharynx are different. Keys to males and nymphs of the six species are provided.

Keywords. Heptageniid mayfly, Afronurus drepanophyllus sp. nov., classification, morphology, insect.

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# Introduction

The mayfly genus *Afronurus* Lestage, 1924 was first established for the species *Ecdyonurus peringueyi* Esben-Petersen, 1913 based on male and female imagoes from South Africa. From then on, more species from the Afrotropical and Oriental regions were added or transferred to this genus by researchers, such as Flowers & Pescador (1984), Kang & Yang (1994), Wang & McCafferty (2004), Braasch (2005,

2011), Braasch & Boonsoong (2010), Braasch & Jacobus (2011) and Sartori (2014). As a result, it has become the third largest heptageniid genus with over 65 species currently known (Sartori & Brittain 2015; Yanai *et al.* 2017).

Kimmins (1937) erected an Asian genus, *Cinygmina*, to include the species *Cinygmina assamensis* Kimmins, 1937 based on adults from India. The generic characters were detailed by Zhou & Zheng (2003): nymphal gills V–VI with additional projections, penial lobes of male imago expanded laterally and presence of titillators (spine- or plate-like). However, Wang & McCafferty (2004) and Kluge (2004) synonymized *Cinygmina* with *Afronurus*. Webb & McCafferty (2008) agreed with them and provided the diagnostic features of *Afronurus* (including the Asian genus *Cinygmina*). Subsequently, the genus *Cinygmina*, which contains 24 Asian species, was recognized as the *Afronurus assamensis* group based on morphology, egg structures and zoogeography by Braasch & Boonsoong (2010), Braasch (2011) and Braasch & Jacobus (2011).

In China, 18 species under the name of *Afronurus* or *Cinygmina* have been reported (You *et al.* 1981; Wu & You 1986; Wu *et al.* 1986; You 1987; Zhang & Cai 1991; Kang & Yang 1994; Zhou & Zheng 2003; Braasch & Jacobus 2011; Sartori 2014). However, only 4 species were described at both nymphal and imaginal stages, and all original descriptions are very brief and simple. For instance, You (1987) described six *Afronurus* species from Tibet in brief: *A. kouandengensis* You, 1987, *A. linzhiensis* You, 1987, *A. yadongensis* You, 1987, *A. xiasimaensis* You, 1987, *A. yadongxiasimaensis* You, 1987 and *A. xizangensis* You, 1987. However, most of the types are subimagoes, and their exact status demands more research and material. Kang & Yang (1994) described four species from Taiwan based on nymphs. In contrast, Braasch & Jacobus (2011) reported two new species from Hong Kong based on adults. Sartori (2014) transferred another Hong Kong species, *Compsoneuria taipokauensis* Tong & Dudgeon, 2003, into *Afronurus*. All these species need to be clarified using more associated specimens.

Zhou & Zheng (2003) compared some species of *Cinygmina* from this country, but most species were known from imaginal stages at that time. In order to know the exact condition of this genus in China, we checked all related materials in our collection. As the first report of a serial study, here we compare and clarify the most common species on the Chinese mainland, leaving those species from the Chinese Hong Kong, Taiwan and Tibet areas for later reports.

As a result, six species are presented in this study, including a new one. They are: *A. furcatus* (Zhou & Zheng, 2003), *A. hunanensis* (Zhang & Cai, 1991), *A. obliquistriatus* (You *et al.*, 1981), *A. rubromaculatus* (You *et al.*, 1981), *A. yixingensis* (Wu & You, 1986) and *A. drepanophyllus* sp. nov. The eggs, nymphs and males of them are described and photographed. The nymphs of six described *Afronurus* species have the typical characters within *Afronurus* specified by Kluge (2004) and Webb & McCafferty (2008): anterior margin of head slightly thickened, mid- and hindtibiae with two rows of setae, maxillae with scattered setae on ventral surface and branched dentisetae. The male imagoes have titillators, which has been inferred by Braasch & Jacobus (2011) and considered as the main character of the *Afronurus assamensis* group (= *Cinygmina*) in Asia. In addition, the egg structures have small KCTs at the poles and large KCTs located equatorially, which is consistent with the theories of Belfiore *et al.* (2003), Kluge (2004) and Webb & McCafferty (2008).

## Material and methods

The nymphs were collected by hand net while adults were attracted by lights. Some adults were reared indoors from mature nymphs. All materials were stored in ethanol (more than 80%).

All specimens were photographed with a digital camera (Single Lens Reflex) and examined under a stereo microscope. Some small structures, like mouthparts, claws and gills, were observed and photographed with a microscope camera.

Eggs were dissected from female imagoes. All SEM samples were prepared with a standard protocol: fixed in 4% glutaraldehyde for 5–8 hours, rinsed with PBS 2–3 times (10–15 minutes each), dehydrated in a concentration gradient of acetone (30%, 50%, 70%, 80%, 90%, 100%, 10 to 15 minutes each), and coated with gold film in a vacuum.

All specimens used in this study are deposited in the Mayfly collection, College of Life Sciences, Nanjing Normal University (NNU).

Abbreviations used in this study are as follows:

dd = distal dentisetadp = proximal dentiseta

KCT = knob-terminated coiled threads

MA = Medius anterior MP = Medius posterior PBS = physiological saline

Rs = Radial sector

 $R_1 = Radius$ 

SEM = scanning electronic microscope

## Results

# **Taxonomy**

Class Insecta Linnaeus, 1758
Order Ephemeroptera Hyatt & Arms, 1890
Family Heptageniidae Lestage, 1917
Subfamily Ecdyonurinae Jacobson & Bianchi, 1905
Genus *Afronurus* Lestage, 1924

Afronurus drepanophyllus sp. nov. urn:lsid:zoobank.org:act:8B78F047-E8D6-4149-9A2E-10D631959037 Figs 1–3, 4A, D, 5, 6A

## **Diagnosis**

Compared to the other five Chinese *Afronurus* species, this new species somewhat resembles *A. furcatus*, *A. hunanensis* and *A. yixingensis* in imaginal stages because of their similar abdominal color patterns (with longitudinal stripes and pale dots submedially). However, these species can easily be differentiated from each other by the shape of the penes and titillators: penial lobes of *A. drepanophyllus* sp. nov. are obviously divergent and form a U-shaped cleft; apex of each penis diverges into three lobes; titillators are thicker and stronger than in other congeners (Fig. 5D–E). Comparatively, *A. yixingensis* has a spine-like projection between the two penial lobes and divergent spine-like titillators (Fig. 13I–J), while *A. furcatus* and *A. hunanensis* have plate-like titillators. Furthermore, the penial lobes of *A. furcatus* are slightly divergent and connected by a foliated structure (Fig. 13A–B). In contrast, *A. hunanensis* has penial lobes obviously spread out and U-shaped deeply cleft (Fig. 13C–D).

Generally, the nymphs of the six Chinese *Afronurus* species are alike because of their yellowish-brown body with various pale dots or stripes. For the convenience of identification, we separate them into two groups here: anterior margin of head with pale dots (*A. drepanophyllus* sp. nov., *A. furcatus*, *A. obliquistriatus* and *A. rubromaculatus*) (Figs 1A, 7A, G, J) and without any dot (*A. hunanensis* and *A. yixingensis*) (Fig. 7D, M) (for detailed comparisons see remarks of each species below). Among the

first group, *A. drepanophyllus* sp. nov. and *A. furcatus* can be identified by the following characters: 1) glossae of the new species are slightly oblong (Fig. 2F), by contrast, glossae of *A. furcatus* are lobe-like (Fig. 8E); 2) gill I is sickle-like in the new species (Fig. 3F) but banana-shaped in *A. furcatus* (Fig. 9F).

Compared to other Asian congeners in the genus, this new species appears to be closely related to the Thailand species *A. namnaoensis* Braasch & Boonsoong, 2010 in both nymphal and imaginal stages, e.g., the structures of mouthparts, legs and the shape of genitalia. However, the nymph of *A. drepanophyllus* sp. nov. has larger body size (6.0–8.0 mm); head capsule has 2–5 pale dots on anterior margin and 4–5 pale dots on posterior margin, respectively; abdominal terga yellowish brown and with marked pale dots on terga I–IX; gill I sickle-like; caudal filaments slightly longer than body. In contrast, the nymph of *A. namnaoensis* has smaller body size (4.5–6.1 mm); head capsule without distinct dot; terga yellowish and with light markings on terga VII–IX; gill I leaf-like; caudal filaments 2× length of body. In male imago, *A. drepanophyllus* sp. nov. has larger body size (8.0–10.0 mm); abdominal terga I–IX with brown longitudinal stripe and a pair of small pale dots medially; styliger plate with projections laterally; forceps with segment III subequal in length to segment IV and combined segments III and IV about half of segment II. Comparatively, *A. namnaoensis* has smaller body size (6.2–6.9 mm); terga I–IX with median stripe only; lateral margins of styliger plate slightly expanded; forceps with segment IV half of segment III in length and combined segments III and IV ½× length of segment II.

#### **Etymology**

The Latin specific name 'drepanophyllus' means 'sickle'. It indicates the nymphs having sickle-like gills I.



**Fig. 1.** Nymph of *Afronurus drepanophyllus* sp. nov. (NNU). **A**. Nymphal habitus. **B**. Selected segments, enlarged, of terminal filament and right cercus (dorsal view). Scale bars: A = 2.0 mm; B = 0.1 mm.

## Material examined

## Holotype

CHINA •  $\hat{O}$ ; Yunnan Province, Meng-La County, Meng-Lun town; 21°56′7.12″ N, 101°14′55.72″ E; alt. 683 m; 22 Jan. 2019; X.H.Y. Zheng leg.; NNU.

# **Paratypes**

CHINA • 1  $\circlearrowleft$ , 1  $\circlearrowleft$  subimago, 1  $\circlearrowleft$ , 4 nymphs; same collection data as for holotype; NNU.

#### Additional material

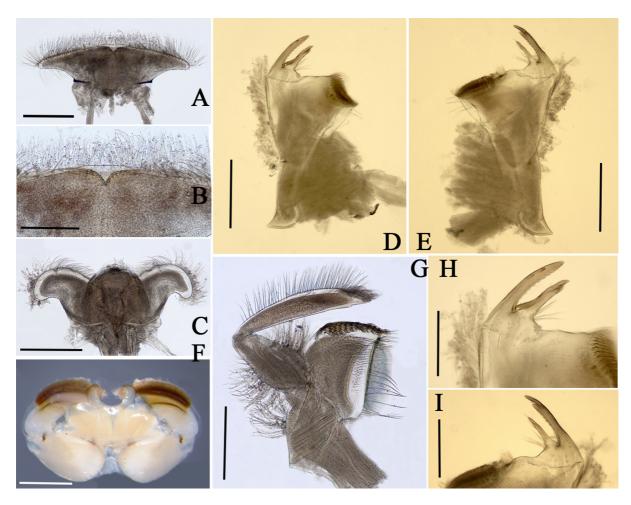
CHINA • 2 & 6, 50 nymphs; Yunnan Province, Liang-He County, Fen-Shui-Ling town; 24°46′44.11″ N, 98°16′5.92″ E; alt. 951 m; 26 Apr. 2018; W. Zhang and Z.X. Ma leg.; NNU.

# **Description**

# Nymph

Measurements. Body length 6.0–8.0 mm, caudal filaments 8.0–10.0 mm long.

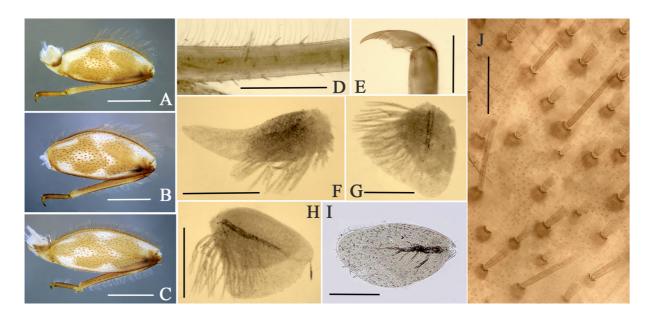
COLORATION. Body yellowish-brown to brown.



**Fig. 2.** Mouthparts of *Afronurus drepanophyllus* sp. nov. (NNU). **A.** Labrum (ventral view). **B.** Median bristles of ventral labrum. **C.** Hypopharynx (ventral view). **D.** Left mandible (ventral view). **E.** Right mandible (ventral view). **F.** Labium. **G.** Maxillae. **H.** Incisors of left mandible. **I.** Incisors of right mandible. Scale bars: A, C–G = 0.5 mm; B = 0.3 mm; H–I = 0.2 mm.

HEAD. Head capsule subquadrangular, anterior margin slightly thickened and with 2–5 small pale dots (some immature individuals less than 5 dots), posterior margin slightly concave; additional 4–5 pale dots located between compound eyes (Fig. 1A). Labrum expanded laterally, with slightly rounded apices; anterior margin with shallow median groove, long and dense setae on dorsal and ventral surfaces, those on dorsal surface longer and denser than others (Fig. 2A-B); an additional row of short bristles on ventral anterior margin (Fig. 2B). Both mandibles covered with numerous setae on outer margins; prostheca with 3–5 fimbriate bristles (Fig. 2D–E, H–I); outer incisor of left mandible with serrated margin and one larger terminal denticle; inner incisor shorter than outer incisor and with trifid apex (Fig. 2D, H); outer incisor of right mandible serrated and with 2 apical terminal denticles; inner one divided into 2 sharp denticles (Fig. 2E, I). Hypopharynx: apex of superlinguae strongly curved and extended into round lobelike structures, row of long hair-like setae on lateral margins from base to apex; lingua bell-like, subequal to superlinguae in length and with tuft of setae at apex (Fig. 2C). Maxillae with scattered fimbriate setae on ventral surface (Figs 2G, 4D), row of 13-15 comb-shaped setae on crown of galea-lacinia, middle combs with 9-13 teeth, distal dentisetae branched, proximal dentisetae bifid and fringed (Fig. 4A); maxillary palpi 3-segmented, first segment with setae on outer margin and basal half of inner margin; second segment obviously longer than basal one, outer margin with long setae, terminal segment with dense setaceous brush (Fig. 2G). Labium: slightly oblong, inner margins of glossae slightly expanded and with tuft of long setae; paraglossae expanded greatly into palpable lobes, with dense setae and bristles on dorsal and anterior margins; labial palpi broad, 2-segmented; ventral surface and free margin with setae brushes; dorsal surface with setae (Fig. 2F).

THORAX. Pronotum slightly extended laterally, subequal to head in width (Fig. 1A–B). Supracoxal spurs rounded. Femora of all legs with long setae on outer margins, dorsal surfaces and inner margins with two kinds of spatulate setae, the shorter setae more abundant than longer ones (Fig. 3J). Foretibia subequal in length to femur, base of outer margin with sparse hair-like setae (Fig. 3A), dorsal surface with rows of fine setae and bristles, inner margin with row of bristles only; foretarsi slightly shorter than ½ tibia, outer and inner margins with tiny setae. Midleg similar to foreleg, except tibia 0.7× length of femur, with



**Fig. 3.** Nymphal structures of *Afronurus drepanophyllus* sp. nov. (NNU). **A.** Foreleg. **B.** Midleg. **C.** Hindleg. **D.** Hindleg tibia. **E.** Foreleg claw. **F.** Gill I. **G.** Gill II. **H.** Gill VI. **I.** Gill VII. **J.** Foreleg femur, bristles on dorsal surface. Scale bars: A-C = 1.0 mm; D, F, H = 0.5 mm; E, G = 0.3 mm; I = 0.2 mm; I = 0.1 mm.

row of hair-like setae on outer margin (Fig. 3B). Hindleg similar to midleg except tibia  $0.65 \times$  length of femur, outer margin with rows of dense hair-like setae and bristles, dorsal surface with short and long bristles (Fig. 3C–D). Claws of all legs with 3–4 subapical denticles (Fig. 3E).

ABDOMEN. Abdominal terga I–II pale, terga III–VII brown with 2 median pale dots and 2 lateral dots; pale dots on terga IV–V fused together and forming pale stripes; terga VIII–IX with fused pale dots and tergum VIII always with longitudinal brown stripe medially; tergum X dark brown (Fig. 1A); posterolateral angles of terga III–VII extended into small acute projections. Gill I sickle-like, inner margin slightly curved (Fig. 3F); gills II–VI heart-like and lamellae expanded laterally (Fig. 3G), gills V–VI with apical arrow-like accessory lobes (Fig. 3H); gill VII round, with fine marginal setae, appreciably asymmetrical (Fig. 3I). Basal of caudal filaments pale and other parts yellowish brown, with whorled spines on articulations (Fig. 1A–B).

## Male imago

MEASUREMENTS. Body length 8.0–10.0 mm, forewing 8.0–10.0 mm, hindwing 2.0–3.0 mm, cerci 19.0–22.0 mm long. Compound eyes contiguous, upper portion hoar and lower portion dark blue.

COLORATION. Body generally pale to yellowish (Fig. 5A, 5F).

Thorax. Mesonotum with apparent transverse suture, and medial depression of furcasternum parallel. Foreleg: length of femur 3.0 mm, length of tibia 3.5 mm, length of tarsi 4.5 mm, tarsal segments from basal to apical = 0.9:1.4:1.2:0.6:0.4. Midleg: length of femur 2.0 mm, length of tibia 1.3 mm, length of tarsi 1.0 mm, tarsal segments arranged in decreasing order as 1, 5, 2, 3, 4. Hindleg: length of femur 2.0 mm, length of tibia 1.5 mm, length of tarsi 0.8 mm, tarsal segments arranged in decreasing order as 5, 1, 2, 3, 4. All claws of legs similar, one blunt and one hooked. Forewing hyaline, costal, subcostal and  $R_1$  fields semitransparent; Rs and MP forked at same level at wing base, MA forked at over  $\frac{1}{2}$  of distance from base of wing to margin (Fig. 5B). Hindwing transparent, with costal projection at base, MA forked in middle, MP forked much more basally than MA (Fig. 5C).

ABDOMEN. Pale but with a longitudinal light brown stripe medially, terga I–VII with pair of pale dots, posterior margin of each tergum with dark brown stripe (Fig. 5F).

GENITALIA. Posterior margin of styliger plate convex, lateral margins with two distinct lobes (Fig. 5D–E). Forceps of segment III subequal to those of segment IV in length, combined segments III–IV approximately half length of segment II. Penial lobes divergent and with U-shaped cleft between them; apices of penial lobes divided into three parts or apices, the median one much broader than inner and outer lobes. Robust but blunt titillators at base of penes, covered by styliger plate and invisible in ventral view (Fig. 5D–E). Cerci pale with dense tiny setae on surface and brown dots on articulations.

# Egg

Oval, chorion decorated with small KCTs at poles, large KCTs located equatorially (Fig. 6A).

#### Distribution

China (Yunnan, Guizhou, Zhejiang, Guangxi).

*Afronurus furcatus* (Zhou & Zheng, 2003) Figs 4B, D, 6B, 7A–C, 8–9, 12A, 13A–B

*Cinygmina furcata* Zhou & Zheng, 2003: 755, figs 1–6. Types: male and female adults, from Tian-Mu Mountain, Zhejiang Province, China.

Afronurus furcatus - Braasch & Jacobus 2011: 64.

## Material examined

### Holotype

CHINA • &; Zhejiang Province, Lin-An city, Tian-Mu Mountain, San-Mu-Ping; 30°21′53.06″ N, 119°25′53.99″ E; alt. 780 m; 29 Jul. 1998; M.S. Zhao leg.; NNU.

### **Paratypes**

CHINA • 2  $\lozenge\lozenge\lozenge$ , 4  $\lozenge\lozenge\lozenge$ ; same collection data as for holotype; NNU.

# **Additional material**

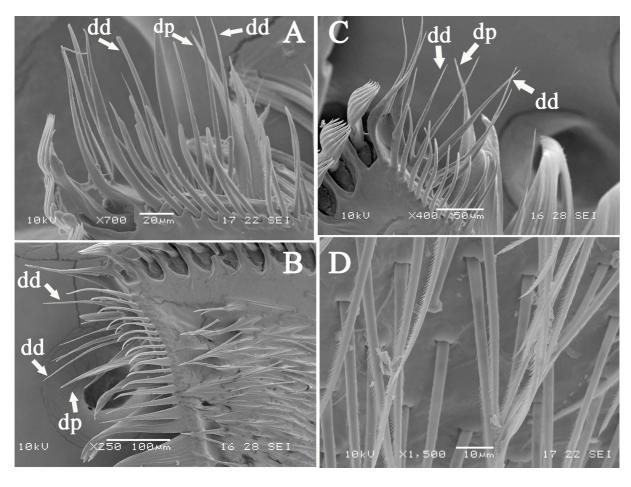
CHINA • 7  $\lozenge\lozenge\lozenge$ , 15  $\lozenge\lozenge\lozenge$ , 5 nymphs; Zhejiang Province, Long-Quan city, Shuang-Xi village; 27°53′16.34″ N, 119°12′55.17″ E; alt. 1391 m; 3 Aug. 2020; Z.X. Ma and X.H.Y. Zheng leg.; NNU.

## **Description**

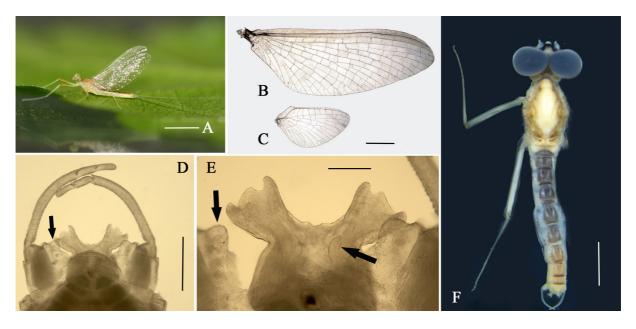
Nymph (first description, Figs 7A–C, 8–9)

Measurements. Body length 7.0–10.0 mm, caudal filaments 13.0–18.0 mm long.

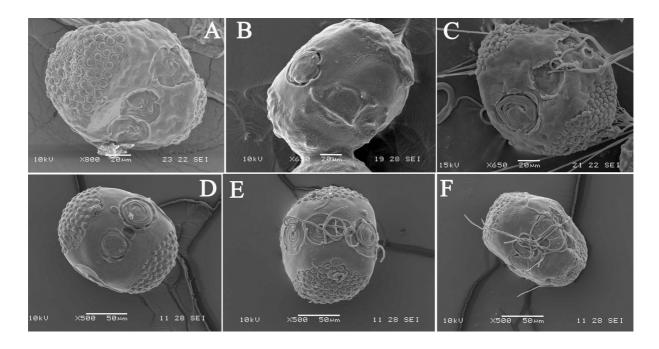
COLORATION. Generally yellowish to brown.



**Fig. 4.** SEM photos of nymphal maxillae. **A.** *A. drepanophyllus* sp. nov. (NNU). **B.** *A. furcatus* (Zhou & Zheng, 2003) (NNU). **C.** *A. hunanensis* (Zhang & Cai, 1991) (NNU). **A–C.** Dentisetae. **D.** Enlarged scattered setae on ventral surface; the three species have similar structures (labels show distal dentisetae and proximal dentisetae respectively). Abbreviations: see Material and methods.



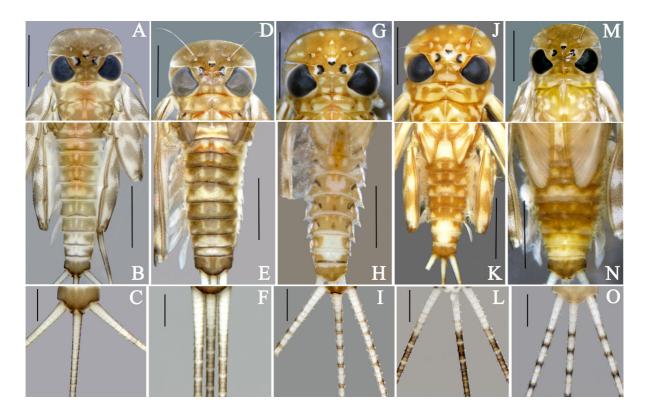
**Fig. 5.** Male structures of *Afronurus drepanophyllus* sp. nov. (NNU). **A.** Male imago habitus. **B.** Forewing. **C.** Hindwing. **D.** Genitalia. **E.** Penes enlarged (arrows show lobe on lateral margin and titillators, respectively). **F.** Male imago, showing abdominal terga. Scale bars: A = 5.0 mm; B-D = 1.0 mm; E = 0.2 mm; E = 0.2 mm.



**Fig. 6.** SEM photos of eggs. **A.** Afronurus drepanophyllus sp. nov. (NNU). **B.** A. furcatus (Zhou & Zheng, 2003) (NNU). **C.** A. hunanensis (Zhang & Cai, 1991) (NNU). **D.** A. obliquistriatus (You et al., 1981) (NNU). **E.** A. rubromaculatus (You et al., 1981) (NNU). **F.** A. yixingensis (Wu & You, 1986) (NNU).

HEAD. Head capsule subquadrate, anterior margin of head with 4 small pale dots and basal part of antennae with 2 pale dots; posterior margin of head slightly concave and with 4 pale dots between the compound eyes (Fig. 7A). Labrum 0.5× width of head, laterally tapering and slightly curved; anterior margin with shallow emargination; an additional row of short bristles on ventral anterior margin (Fig. 8A). Both mandibles covered with numerous setae on outer margins; prostheca with 6–8 fimbriate bristles (Fig. 8C– D, G-H); outer incisor of left mandible with serrated margin and one larger terminal denticle; inner incisor shorter than outer incisor and with 3 blunt denticles (Fig. 8C, G); outer incisor of right mandible serrated and with 2 apical terminal denticles; inner one divided into 2 sharp denticles (Fig. 8D, H). Hypopharynx: apex of superlinguae strongly curved and extended into round lobe-like structures, long hair-like setae up to the lower part of superlinguae; lingua bell-like, subequal to superlinguae in length and with tuft of setae at apex (Fig. 8B). Maxillae with scattered fimbriate setae on ventral surface (Figs 4D, 8F), row of 17-20 comb-shaped setae on crown of galea-lacinia, middle combs with 10-15 teeth, distal dentisetae branched, proximal dentisetae bifid (Fig. 4B); maxillary palpi with setae on outer margin and basal half of inner margin of first segment; segment II longer than the former one and outer margin with long setae, terminal segment with dense setal brush (Fig. 8F). Labium: glossae lobe rounded, inner margin slightly expanded, with tuft of long setae; paraglossae expanded greatly into palpable lobes, with dense setae and bristles on dorsal and free margins; labial palpi 2-segmented, ventral surface and free margin of second segment with setal brush, dorsal surface with scattered golden setae (Fig. 8E).

THORAX. Pronotum slightly expanded laterally, wider than head (Fig. 7A). Supracoxal spurs rounded. Femora of all legs with row of long setae on outer margins, dorsal surfaces with spatulate setae and inner



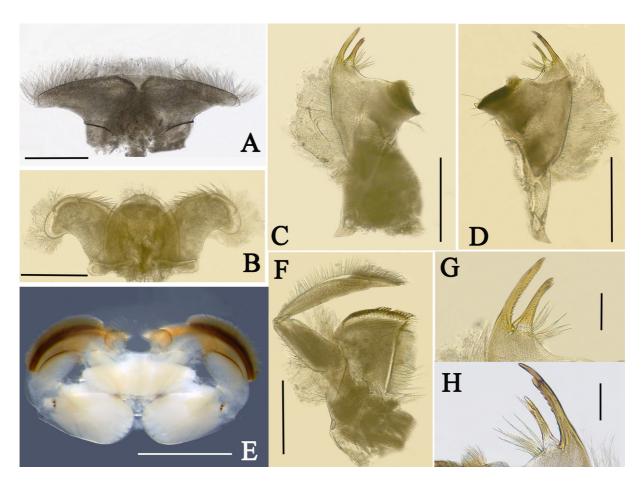
**Fig. 7.** Nymphal habitus of five species of *Afronurus* Lestage, 1924. **A–C**. *A. furcatus* (Zhou & Zheng, 2003) (NNU). **D–F**. *A. hunanensis* (Zhang & Cai, 1991) (NNU). **G–I**. *A. obliquistriatus* (You *et al.*, 1981) (NNU). **J–L**. *A. rubromaculatus* (You *et al.*, 1981) (NNU). **M–O**. *A. yixingensis* (Wu & You, 1986) (NNU). **A, D, G, J, M**. Head capsule. **B, E, H, K, N**. Abdominal terga. **C, F, I, L, O**. Caudal filaments. Scale bars: A–B, D–E, G–H, J–K, M–N = 2.0 mm; C, F, I, L, O = 1.0 mm.

margins with short bristles. Foretibia subequal in length to femur, outer margin with sparse hair-like setae near base, inner margin with row of bristles; foretars  $0.4 \times 10.4 \times 10.4$ 

ABDOMEN. Abdominal terga I–VII yellowish to brown, with 3 pale dots medially and 2 pale dots laterally; terga VIII–IX with fused pale dot medially; tergum X dark brown, anterior margin with 3 light pale dots (Fig. 7B–C). Gill I banana-shaped, inner margin slightly curved (Fig. 9F); gills II–IV heart-like and with lamellae expanded laterally (Fig. 9G); gills V–VI oval, with additionally arrow-like accessory lobes (Fig. 9H); gill VII oval, with fine marginal setae, trachea clearly visible (Fig. 9I). Caudal filaments pale, with whorled spines on articulations (Fig. 7C, as in Fig. 1B).

## Male imago

See Zhou & Zheng (2003: 755, figs 1–6, original description).



**Fig. 8.** Mouthparts of *Afronurus furcatus* (Zhou & Zheng, 2003) (NNU). **A.** Labrum (ventral view). **B.** Hypopharynx (ventral view). **C.** Left mandible (ventral view). **D.** Right mandible (ventral view). **E.** Labium. **F.** Maxillae. **G.** Incisors of left mandible. **H.** Incisors of right mandible. Scale bars: A, C–D, F = 0.5 mm; B = 0.3 mm; E = 1.0 mm; G–H = 0.1 mm.

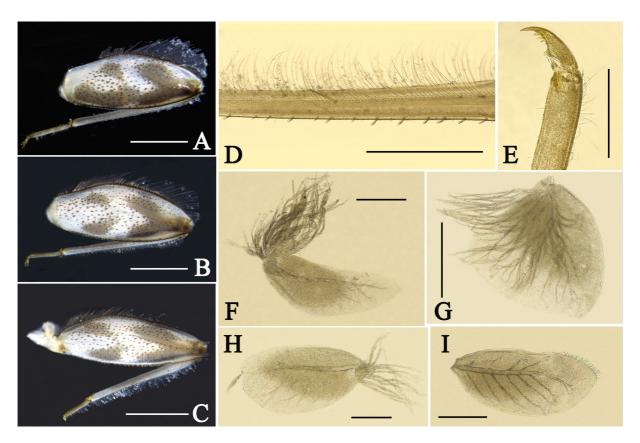
#### Egg

Oval, chorion mostly smooth and decorated with small KCTs at each pole, larger KCTs located equatorially (Fig. 6B).

#### Remarks

The male imago of *A. furcatus* is similar to the one of *A. hunanensis* due to color pattern of abdominal terga, plate-like titillators and bifurcated apices of penial lobes. However, they can be separated by the following characters: 1) the body color of *A. furcatus* is bright yellow when it is alive (Fig. 12A) while reddish brown in *A. hunanensis* (Fig. 12B); 2) *A. furcatus* has slightly divergent penial lobes, which are connected by a foliated structure (Fig. 13A–B), while penes of *A. hunanensis* are obviously divergent and with deeply cleft (Fig. 13C–D); 3) the plate-like titillators of *A. hunanensis* are smaller than in *A. furcatus* (Fig. 13A–D).

In the nymph, *A. furcatus* resembles *A. drepanophyllus* sp. nov., *A. obliquistriatus* and *A. rubromaculatus* because of marking patterns on head capsules. However, the following combination of characters can help to separate them: 1) head capsule of *A. furcatus* has one row (4 dots) of pale dots on anterior margin (Fig. 7A), while *A. drepanophyllus* sp. nov. has two rows (generally 2–5 dots) of pale dots on anterior margin (Fig. 1A), *A. obliquistriatus* and *A. rubromaculatus* have two rows (generally 8–9 dots) on anterior margins (Fig. 7G, J); 2) linguae and superlinguae are approximately the same length in *A. furcatus*, *A. drepanophyllus* sp. nov. and *A. rubromaculatus* (Fig. 2C, 8B); however, the lingua of *A. obliquistriatus* is much shorter than superlinguae (as in Fig. 10B); 3) number of comb-shaped setae



**Fig. 9.** Nymphal structures of *Afronurus furcatus* (Zhou & Zheng, 2003) (NNU). **A.** Foreleg. **B.** Midleg. **C.** Hindleg. **D.** Hindleg tibia. **E.** Foreleg claw. **F.** Gill I. **G.** Gill II. **H.** Gill VI. **I.** Gill VII. Scale bars: A–D = 1.0 mm; E–I = 0.3 mm.

on maxillae: 13–15 in *A. drepanophyllus* sp. nov. (middle combs with 9–13 teeth), 17–20 in *A. furcatus* (middle combs with 10–15 teeth), 11–13 in *A. obliquistriatus* (middle combs with 8–10 teeth) and 14–16 in *A. rubromaculatus* (middle combs with 10–12 teeth); 4) caudal filaments of *A. furcatus* are pale (Fig. 7C) whereas in *A. drepanophyllus* sp. nov. they are pale near the base and yellowish-brown in other parts (Fig. 1A); *A. obliquistriatus* and *A. rubromaculatus* have dark brown dots on articulation (Fig. 7I, L).

#### **Distribution**

China (Jiangxi, Zhejiang).

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Afronurus hunanensis (Zhang & Cai, 1991)
Figs 4C–D, 6C, 7D–F, 10–11, 12B, 13C–D
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*Cinygmina hunanensis* Zhang & Cai, 1991: 237, figs 1–9 (male, male subimago). Types: male, male subimago, from Hunan, China.

Cinygmina hunanensis – You & Gui 1995: 54, fig. 53 (male). — Zhou & Zheng 2003: 756, figs 11, 15 (male).

Afronurus hunanensis - Braasch & Jacobus 2011: 64.

#### Material examined

## Holotype

CHINA • ♂; Hunan Province, Zhang-Jia-Jie city, Jin-Bian creek; 29°20′45.14″ N, 110°32′46.36″ E; alt. 253 m; 18 Jun. 1986; J. Zhang and S.S. She leg.; NNU.

## **Paratypes**

CHINA • 9  $\lozenge\lozenge$ , 3  $\lozenge\lozenge$ ; same collection data as for holotype; NNU.

#### **Additional material**

CHINA • 25 ♂♂, 22 ♀♀, 16 nymphs; Hunan Province, Chen-Zhou city, Mang-Shan National Forest Park; 24°58′55.92″ N, 112°51′22.32″ E; alt. 511 m; 7 Aug. 2020; Z.X. Ma leg.; NNU.

# **Description**

Nymph (first description, Figs 7D–F, 10–11)

Measurements. Body length 7.0–8.5 mm, caudal filaments 15.0–17.0 mm long.

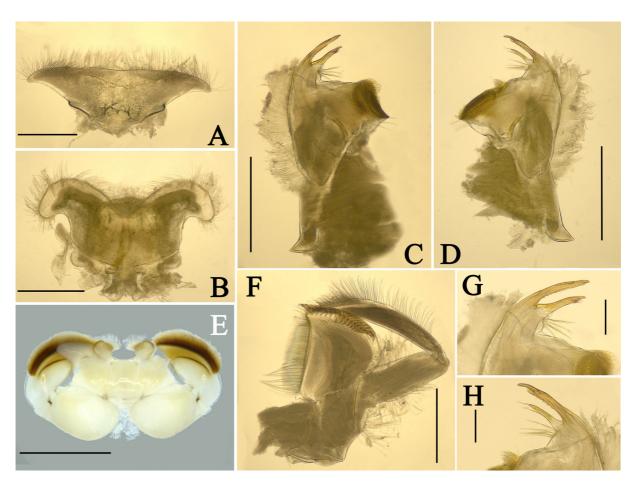
Coloration. Body brown to dark brown.

HEAD. Head capsule ellipsoid, anterior margin unicolor, posterior margin slightly concave and with 4 pale dots in the middle of the compound eyes (Fig. 7D). Labrum ca half width of head, lateral margins expanded slightly; free margin nearly straight, both surfaces with setae but those on dorsal surface relatively longer and denser; ventral surface with shallow median groove, an additional row of bristles on ventral anterior margin (Fig. 10A). Both mandibles covered with numerous long setae on outer margins; prostheca with 5–7 fimbriate bristles (Fig. 10C–D, G–H); outer incisor of left mandible with serrated margin and a large terminal denticle; inner incisor divided into 3 blunt denticles (Fig. 10C, G); outer incisor of right mandible serrated with 2 apical terminal denticles; inner one divided into 2 sharp denticles (Fig. 10D, H). Hypopharynx: apex of superlinguae extended into round lobe-like structures, row of long hair-like setae on lateral margins from base to apex; lingua bell-like, much shorter than superlinguae, apex with tuft of setae (Fig. 10B). Maxillae with fimbriate setae on ventral surface (Figs 4D, 10F), row of 16–18 comb-shaped setae on crown of galea-lacinia, middle combs with

10–13 teeth, distal dentisetae branched, proximal dentisetae bifid and fringed (Fig. 4C); maxillary palpi 3-segmented, basal segment with scattered setae on both outer and inner margins, second segment with long setae on outer margin and terminal segment with setal brush (Fig. 10F). Labium: glossae lobe rounded, inner margins slightly expanded, with tuft of long setae; paraglossae expanded into distinct lobes, with dense setae and bristles on dorsal surface and outer margin; labial palpi 2-segmented, ventral surface and free margin of second segment with setal brush (Fig. 10E).

Thorax. Pronotum slightly extended laterally, subequal in length to head (Fig. 7D). Supracoxal spurs rounded. Femora of all legs with acute and spatulate setae on dorsal surfaces, outer margins with long setae and inner margins with short bristles. Foretibia subequal in length to femur, outer margin with tiny setae at base; foretarsi approximately ½ length of tibiae (Fig. 11A). Midleg similar to foreleg, but tibia 0.89× of femur in length, outer margin and dorsal surface with rows of sparse setae (Fig. 11B). Hindleg similar to midleg except tibia 0.7× of femur in length and dorsal surface of tibia with long and short bristles (Fig. 11C–D). Claws of all legs with 4 subapical denticles (Fig. 11E).

ABDOMEN. Abdominal terga I pale or with transverse brown band; terga II–VII brown with pair of pale dots medially, and terga IV–V, VII with adlateral dots; terga VIII–IX pale but with pale thin stripe anteriorly; tergum X brown with 2 small pale longitudinal stripes (Fig. 7E); terga II–VIII with weakly



**Fig. 10.** Mouthparts of *Afronurus hunanensis* (Zhang & Cai, 1991) (NNU). **A.** Labrum (ventral view). **B.** Hypopharynx (ventral view). **C.** Left mandible (ventral view). **D.** Right mandible (ventral view). **E.** Labium. **F.** Maxillae. **G.** Incisors of left mandible. **H.** Incisors of right mandible. Scale bars: A–D, F = 0.5 mm; E = 1.0 mm; G–H = 0.1 mm.

developed posterolateral angles. Gill I banana-shaped, trachea visible (Fig. 11F); gills II–VI heart-like and lamellae expanded laterally (Fig. 11G), gills V–VI with arrowlike accessory lobes (Fig. 11H); gill VII slightly asymmetrical, with fine marginal setae (Fig. 11I). Caudal filaments with whorls of spines on articulations, proximal part pale and other parts yellowish brown (Fig. 7F, as in Fig. 1B).

## Male imago

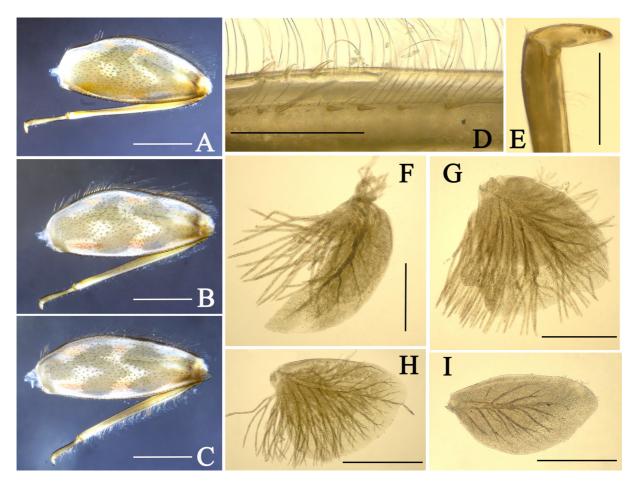
See Zhang & Cai (1991: 237, figs 1–9, original description) and Zhou & Zheng (2003: 756, figs 11, 15).

## Egg (Fig. 6C)

Ovoid, small KCTs concentrated at each pole, chorion smooth, large KCTs and oval micropyles located equatorially.

#### Remarks

The nymphs of *A. hunanensis* and *A. yixingensis* can be separated into one group due to the anterior margins of head capsules being without any dot. However, the following characters can help differentiate them: 1) lingua of *A. hunanensis* is much shorter than superlinguae (Fig. 10B) but in *A. yixingensis* it is subequal in length (as in Fig. 8B); 2) glossae of *A. hunanensis* are round (Fig. 10E) while slightly oblong in *A. yixingensis* (as in Fig. 2F); 3) number of comb-shaped setae on maxillae: 16–18 in *A. hunanensis* 



**Fig. 11.** Nymphal structures of *Afronurus hunanensis* (Zhang & Cai, 1991) (NNU). **A.** Foreleg. **B.** Midleg. **C.** Hindleg. **D.** Hindleg tibia. **E.** Foreleg claw. **F.** Gill I. **G.** Gill II. **H.** Gill VI. **I.** Gill VII. Scale bars: A–C = 1.0 mm; D–F = 0.5 mm; G–I = 0.3 mm.

(middle combs with 10–13 teeth) and 12–14 in *A. yixingensis* (middle combs with 7–10 teeth); 4) gill VII of *A. hunanensis* is broad (Fig. 11I) but narrow, leaf-like in *A. yixingensis* (as in Fig. 9F); 5) caudal filaments of *A. hunanensis* are pale at base and yellowish-brown in other parts (Fig. 7F), whereas caudal filaments of *A. yixingensis* have dark brown dots on every two or three segments (Fig. 7O).

In male imago, *A. hunanensis* has plate-like titillators and bifurcated apices of penial lobes (Fig. 13C–D, I–J). In contrast, *A. yixingensis* has spine-like titillators and a remarkable projection between divergent penial lobes.

## **Distribution**

China (Hunan, Jiangxi, Guizhou).

# *Afronurus obliquistriatus* (You, Wu, Gui & Hsu, 1981) Figs 6D, 7G–I, 12C, 13E–F

*Cinygmina obliquistriata* You, Wu, Gui & Hsu, 1981: 26, pl. 1 figs 1–13 (male, female). Types: male, female, from Jiangsu, China.

*Cinygmina obliquistriata* – Gui 1985: 86. — Wu *et al.* 1986: 65, figs 12–22 (nymph). — Zhang & Cai 1991: 237. — You & Gui 1995: 51, fig. 50 (male). — Zhou & Zheng 2003: 756, figs 9, 12, 16 (male and nymph).

Afronurus obliquistriatus – Braasch & Jacobus 2011: 65.

### **Material examined**

## Holotype

CHINA • &; Jiangsu Province, Yi-Xing city, Ming-Ling village; 31°10′5.02″ N, 119°40′17.52″ E; alt. 67 m; 11 Aug. 1980; J. Zhang and S.S. She leg.; NNU.

#### **Paratypes**

CHINA • 13 &; same collection data as for holotype; NNU.

### **Additional material**

CHINA • 15  $\circlearrowleft \circlearrowleft$ , 18  $\circlearrowleft \circlearrowleft$  (some reared from mature nymphs), 20 nymphs; Zhejiang Province, Lin-An city, unnamed creek in front of Tian-Mu Hotel; 30°19′16.7″ N, 119°26′55.7″ E; alt. 479 m; 3–6 Apr. 2019; W. Zhang and Z.X. Ma leg.; NNU.

## **Description**

#### Nymph

See Zhou & Zheng (2003: 757, fig. 9, first description).

## Male imago

See You et al. (1981: 26, figs 1–13, original description).

## Egg (Fig. 6D)

Ovoid, small KCTs concentrated at each pole, chorion decorated with small KCTs, large KCTs and oval micropyle located equatorially.

#### Remarks

In China, the nymphs of A. obliquistriatus and A. rubromaculatus are the most difficult to distinguish, especially when they are young or typical stripes are faded. Herein, the following combination of

characters are differentiable: 1) lingua is much shorter than superlinguae in *A. obliquistriatus* (as in Fig. 10B), while that in *A. rubromaculatus* is subequal in length (as in Fig. 2C); 2) number of comb-shaped setae on maxillae: 11–13 in *A. obliquistriatus* and 14–16 in *A. rubromaculatus*; 3) *A. obliquistriatus* has lateral oblique stripes on terga I–X (Fig. 7H) and *A. rubromaculatus* has pale dots on terga I–X only (Fig. 7K); 4) posterolateral projections of *A. obliquistriatus* are moderately developed on segments III–VIII (Fig. 7H) but weakly developed in *A. rubromaculatus* (as in Fig. 7E); 5) caudal filaments of *A. obliquistriatus* have brown stripes on every two or three articulations (Fig. 7I), but *A. rubromaculatus* has dark brown stripes on some articulations (Fig. 7L).

In male imago, the color pattern of abdominal terga and shape of genitalia can be used as valid characters to distinguish them: 1) *A. obliquistriatus* has unique oblique stripes on abdomen (Fig. 12C), while *A. rubromaculatus* has reddish longitudinal stripes laterally (Fig. 12D); 2) penial lobes of *A. obliquistriatus* are slightly divergent (Fig. 13E–F), whereas *A. rubromaculatus* has a distinct conelike projection between obviously divergent penial lobes (Fig. 13G–H).

#### **Distribution**

China (Shaanxi, Henan, Hubei, Hunan, Anhui, Guizhou, Jiangsu, Zhejiang, Guangxi, Fujian, Guangdong, Hainan).

*Afronurus rubromaculatus* (You, Wu, Gui & Hsu, 1981) Figs 6E, 7J–L, 12D, 13G–H

*Cinygmina rubromaculata* You, Wu, Gui & Hsu, 1981: 28, pl. 2 figs 14–24 (male, female). Types: male, female, from Jiangsu, China.

*Cinygmina hainanensis* She, Gui & You, 1995: 72, fig. 1 (male, female, male subimago). Types: male, female subimago from Hainan (synonymized by Zhou & Zheng 2003: 758).

*Cinygmina rubromaculata* – Gui 1985: 86. — Wu *et al.* 1986: 65, figs 1–11 (nymph). — Zhang & Cai 1991: 237. — You & Gui 1995: 52, fig. 51 (male).

Ecdyonurus rubromaculatus - Tshernova et al. 1986: 114.

Afronurus rubromaculatus - Braasch & Jacobus 2011: 65.



**Fig. 12.** Male abdominal terga of five species of *Afronurus* Lestage, 1924. **A.** *A. furcatus* (Zhou & Zheng, 2003) (NNU). **B.** *A. hunanensis* (Zhang & Cai, 1991) (NNU). **C.** *A. obliquistriatus* (You *et al.*, 1981) (NNU). **D.** *A. rubromaculatus* (You *et al.*, 1981) (NNU). **E.** *A. yixingensis* (Wu & You, 1986) (NNU). Scale bars = 1.0 mm.

#### Material examined

## Holotype

CHINA • &; Jiangsu Province, Yi-Xing city, Ming-Ling village; 31°10′5.02″ N, 119°40′17.52″ E; alt. 67 m; 15 Jul. 1980; T. Wu leg.; NNU.

### **Paratypes**

CHINA • 48  $\lozenge\lozenge$ , 27  $\lozenge\lozenge$ ; same collection data as for holotype; NNU.

## Additional material

CHINA • 6  $\circlearrowleft$  (reared from mature nymphs), 7  $\circlearrowleft$   $\circlearrowleft$  subimagoes, 10  $\circlearrowleft$   $\circlearrowleft$ , 8  $\circlearrowleft$  subimagoes, 40 nymphs; Hunan Province, Zhang-Jia-Jie city, Jin-Bian creek; 29°20′45.14″ N, 110°32′46.36″ E; alt. 253 m; 12 Aug. 2019; R. Lo and W. Zhang leg.; NNU.

## **Description**

## Nymph

See Zhou & Zheng (2003: 758, fig. 7, first description).

## Male imago

See You et al. (1981: 28, figs 14–24, original description).

**Egg** (Fig. 6E)

Ovoid, small KCTs concentrated at each pole, large KCTs and oval micropyle located equatorially.

#### Remarks

Compared to other Chinese congeners, *A. rubromaculatus* resembles *A. obliquistriatus* in the nymph because of the similar color patterns of the head and abdomen. However, the nymphal and imaginal stages of these two species can be identified by other characters, as mentioned in remarks for *A. obliquistriatus*.

Additionally, the genitalia of *A. rubromaculatus* have an obvious projection between divergent penial lobes (Fig. 13G–H), which makes it seems closer to the Vietnamese species *A. cervina* Braasch & Soldán, 1984. Nevertheless, the particular color pattern (terga pale medially and reddish laterally) of *A. rubromaculatus* is unique and distinguishable in the genus (Fig. 12D). The nymphal characters of *A. rubromaculatus*, such as the pale dots on the head capsule and larger body size (8.0–12.0 mm), can help differentiate them effectively.

#### **Distribution**

China (Jilin, Liaoning, Hebei, Henan, Hubei, Hunan, Shaanxi, Sichuan, Guizhou, Jiangsu, Zhejiang, Guangdong, Hainan).

Cinygmina yixingensis Wu & You, 1986: 280, figs 1–13 (male, female). Types: male, female, from Jiangsu, China.

*Cinygmina yixingensis* – Gui 1985: 86. — Wu *et al.* 1986: 66, figs 23–33 (nymph). — Zhang & Cai 1991: 237. — She *et al.* 1995: 79. — You & Gui 1995: 53, fig. 52 (male). — Zhou & Zheng 2003: 758, figs 10, 14, 18 (adults and nymph).

Afronurus yixingensis – Braasch & Jacobus 2011: 65.

## Material examined

## Holotype

CHINA • &; Jiangsu Province, Yi-Xing city, Ming-Ling village; 31°10′5.02″ N, 119°40′17.52″ E; alt. 67 m; 15 Jul. 1980; T. Wu leg.; NNU.

## **Paratypes**

CHINA • 10  $\circlearrowleft$  2  $\circlearrowleft$  3 subimagoes, 6  $\circlearrowleft$  ; same collection data as for holotype; NNU.

## **Additional material**

CHINA • 12  $\circlearrowleft \circlearrowleft$  (some reared from mature nymphs), 15  $\circlearrowleft \circlearrowleft$  subimagoes, 40 nymphs; Hainan Province, Chang-Jiang County, Ba-Wang-Ling National Forest Park; 19°07′16.28″ N, 109°05′0.56″ E; alt. 304 m; 12–14 Nov. 2014; Q. Si, J.Z. Sun and J.Y. Luo leg.; NNU.

## **Description**

## Nymph

See Zhou & Zheng (2003: 758, fig. 10, first description).

## Male imago

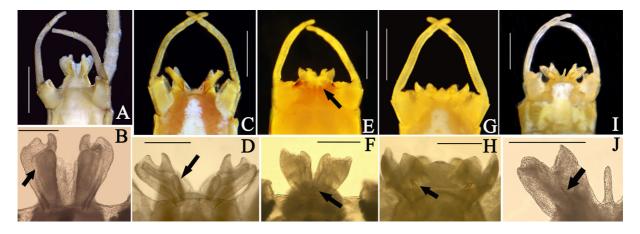
See Wu & You (1986: 280, figs 1–13, original description).

## Egg (Fig. 6F)

Ovoid, small KCTs concentrated at each pole, large KCTs and oval micropyles located equatorially.

#### Remarks

Among the six valid Chinese *Afronurus* species, *A. yixingensis* and *A. hunanensis* are separated into one group because of the similar color patterns of the head capsule in nymphs. However, as stated above for *A. hunanensis*, the nymphs can be separated by shape of hypopharynx, glossae, gills VII, number of comb-shaped setae on maxillae and color patterns of caudal filaments. In the male imago, they can easily be differentiated by shape of penes, titillators and projection between penial lobes.



**Fig. 13.** Genitalia of five species of *Afronurus* Lestage, 1924. **A–B**. *A. furcatus* (Zhou & Zheng, 2003) (NNU). **C–D**. *A. hunanensis* (Zhang & Cai, 1991) (NNU). **E–F**. *A. obliquistriatus* (You *et al.*, 1981) (NNU). **G–H**. *A. rubromaculatus* (You *et al.*, 1981) (NNU). **I–J**. *A. yixingensis* (Wu & You, 1986) (NNU). **A, C, E, G, I**. Genitalia. **B, D, F, H, J**. Penes enlarged; arrows show titillators. Scale bars: A, C, E, G, I = 0.5 mm; B, D, F, H, J = 0.2 mm.

## **Distribution**

China (Hebei, Hubei, Hunan, Anhui, Jiangxi, Guizhou, Jiangsu, Zhejiang, Fujian, Hainan).

## **Ecology**

Among the six Chinese *Afronurus* species studied in this paper, *A. obliquistriatus*, *A. rubromaculatus* and *A. yixingensis* are more common than the others and they are frequently found at the collecting sites. The last instar nymphs of *A. obliquistriatus* are recorded molting at about 10:00–12:30 AM or 17:00–19:30 PM while the last instar nymphs of *A. rubromaculatus* and *A. yixingensis* molted at about 16:00–19:00 PM local time, and these three species are observed molting underwater. The subimagoes persisted for 1–2 days and molted at 12:00–15:00 PM, the whole process lasting about 2–4 minutes. Moreover, in another observed species, *A. drepanophyllus* sp. nov., the last instar nymph molted at about 17:00–18:00 PM and the male subimago molted at 15:00–16:00 PM, the process lasting about 90 seconds.

# Key to six common Chinese Afronurus species (nymph)

1. -	Anterior margin of head capsule without pale dot
2.	Lingua and superlinguae subequal in length (as in Fig. 8B); crown of galea-lacinia with 12–14 comb-shaped setae; caudal filaments pale but with brown dots on articulations (Fig. 7O); glossae slightly oblong (as in Fig. 2F)
-	Lingua obvious shorter than superlinguae (Fig. 10B); crown of galea-lacinia with 16–18 comb-shaped setae; proximate part of caudal filaments pale but other parts yellowish brown (Fig. 7F); glossae lobe rounded (Fig. 10E)
3.	Head capsule with two rows of pale dots (8–9) on anterior margin (Fig. 7G, J); caudal filaments with brown stripes on articulations (Fig. 7I, L, O)
-	Head capsule with 2–5 pale dots on anterior margin (Figs 1A, 7A); caudal filaments pale to yellowish-brown, without any stripe (Figs 1A, 7C)
4.	Abdominal terga with oblique stripes laterally (Fig. 7H); crown of galea-lacinia with 11–13 comb-shaped setae; posterolateral projections of segments III–VIII moderately developed (Fig. 7H); lingua shorter than superlinguae (as in Fig. 10B)
5. -	Gill I sickle-like (Fig. 3F); glossae slightly oblong (Fig. 2F)
Key to six common Chinese Afronurus species (male imago)	
1. -	Penes with plate-like titillators 2 Penes with spine-like titillators 3
	Penes connected by foliated structure at ½ length from base; titillators about ½ of penes in length (Fig. 13A–B)

## **Discussion**

The nymphal gills V–VI with additional lobes, the widely separated penes and presence of titillators (spine- or plate-like) were considered as the autapomorphies of *Cinygmina* by Zhou & Zheng (2003). All six species in the present study have this kind of genitalia and gills. However, Braasch & Jacobus (2011) indicated that some *Afronurus* species in South Africa (e.g., *A. barnardi* Schoonbee, 1968 and *A. scotti* Schoonbee, 1968) have projections on the gills. Further, this character is also found in some *Notacanthurus* Tshernova, 1974 species, such as *N. baekdu* Bae, 1997, *N. baei* Braasch & Boonsoong, 2009 and *N. maculatus* Zhang *et al.*, 2020. Thus, this character is not considered autapomorphic.

The presence of titillators on the penes is another typical generic character, which is also found in the six Chinese species, in the type species *C. assamensis* and almost all *assamensis*-group species (Belfiore *et al.* 2003; Braasch & Jacobus 2011). By contrast, all African species lack titillators (Schoonbee 1968; Belfiore *et al.* 2003). Considering this imaginal difference between the previous *Cinygmina* and *Afronurus*, keeping the former as a subgenus or species-group in the latter genus is an acceptable arrangement, as already proposed by Kluge (2004), Wang & McCafferty (2004), Webb & McCafferty (2008) and Braasch & Jacobus (2011).

## **Acknowledgments**

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