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Report of intersex individuals from a southeastern Chinese *Choroterpes facialis* (Gillies, 1951) population (Ephemeroptera: Leptophlebiidae)

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Abstract

Previously there were only three cases of mayfly gynandromorphism reported from Asia, occurring in the families Baetidae and Heptageniidae. Here, we report two intersex individuals of *Choroterpes facialis* (Gillies, 1951) (Ephemeroptera: Leptophlebiidae) from southeastern China. They have similar external morphologies (each having two different eyes, two shortened penes, and female sternum IX) but with one being predominately male and the other being predominately female (one with eggs in the abdomen, but the other apparently with sperm). We believe this to be the first report of a feminized male individual. This phenomenon implies their intersexuality is caused by some similar reasons, such as temperature or parasitism. Remarkably, two intersex specimens found among 1,736 normal individuals shows that gynandromorphism does occur rarely, and only six normal males in the sampling suggest the species *C. facialis* is partially parthenogenetic at least.

Key words: gynandromorph, intersex, mayfly, China, Oriental

Introduction

Although insect gynandromorphism is relatively rare, it has been found in 16 orders of Insecta, including the order Ephemeroptera (Santos *et al.*, 2019). Since Lestage (1922) reported the first case of this from mayflies, around 70 wild specimens with both male and female characters have been documented (Soldán and Landa, 1981; McCafferty and Bloodgood, 1986; Grant and Masteller, 1987; Wu *et al.*, 1993; Kluge, 2015; Pohe, 2019; Santos *et al.*, 2019; Nascimento *et al.*, 2021). However, most of them were found in Europe, or North and South America; only three cases were reported from Asia: one from Mongolia, one from Tajikistan, and one from eastern China (Soldán and Landa, 1981; Wu *et al.*, 1993; Kluge, 2015). More cases from this region will enlarge our understanding about this condition, in terms of its part of biodiversity and its biogeographic prevalence.

Soldán and Landa (1981) recognized three categories of mayfly gynandromorphism known at that time: true gynandromorphism (male and female parts of body normally developed, only exceptionally reduced in size, evenly or unevenly distributed), intersexuality (male and/or female parts of body intermediately developed, always unevenly distributed) and external genitalia teratology (deformations or unusual location of forceps and penis lobes). Among intersexes, however, only masculinized females were found. The subsequent related reports did not show other conditions (McCafferty and Bloodgood, 1986; Grant and Masteller, 1987; Wu *et al.*, 1993; Kluge, 2015; Santos *et al.*, 2019; Nascimento *et al.*, 2021). More reports on this subject will provide more types of gynandromorphism, if they exist.

The three most species-diverse mayfly families—Baetidae, Heptageniidae and Leptophlebiidae—contain the most numerous reports of gynandromorphism in the order Ephemeroptera. Three Asian cases were reported from the two former families: *Baetopus* Keffermüller, 1960 (Soldán and Landa, 1981) (Baetidae), *Cingmina* Kimmins, 1937 (now *Afronurus* Lestage, 1924) (Wu, 1993, Zhang *et al.*, 2021) and *Rhithrogena* Eaton, 1881 (Kluge, 2015) (Heptageniidae), but no case has been found in the third family so far.

Previously reported mayfly gynandromorphic specimens were usually found in mass collections, but no exact level of incidence has been calculated. Soldán and Landa (1981) reported this kind of abnormal individual was

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usually caught with ten to one thousand others. McCafferty and Bloodgood (1986) collected an *Ephoron leukon* Williamson, 1802 gynander (Polymitarcyidae) together with thousands of normal individuals. Grant and Masteller (1987) identified four cases among 18,731 mayfly specimens. Wu *et al.* (1993) detected one case in a "massive mayfly collection". Pohe (2019) noticed three gynandromorph imagos among almost 14,000 imaginal individuals (0.0002%). Funk *et al.* (2010) reported high autogenic gynandromorphs (8%–10%) in three cultivated mayfly populations. So, accurate data of gynandromorphism in one particular wild population will help to provide insight into its frequency in nature.

In a 2022 mayfly sampling from Fujian Province, southeastern China, nearly 1,740 individuals of *Choroterpes facialis* (Gillies, 1951) were identified. Among them, two specimens with a pair of different compound eyes drew our attention. After careful examination, they are regarded as two gynanders. They represent not only the first record of this phenomenon from the genus *Choroterpes* Eaton (1881), but also the first real Oriental case. In addition, they are the second report of mayfly gynandromorphism from China and the first report in Asian Leptophlebiidae. So, they are described and illustrated herein to show their uniqueness and expand our understanding of the diversity of these phenomena in mayflies.

Materials and methods

All specimens were attracted by a light trap (small road light set nearby water), collected, and stored in ethanol (more than 80%).

Identifications were made based on morphology, following Zhou (2006).

Digital photos were taken with a digital camera (Mshot MSX 11, single lens Reflex, Mingmei photoelectric, Guangzhou, China) coupled to a stereomicroscope (Mshot MZ81, Mingmei photoelectric, Guangzhou, China). Photos were edited and improved by Adobe Photoshop CC.

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

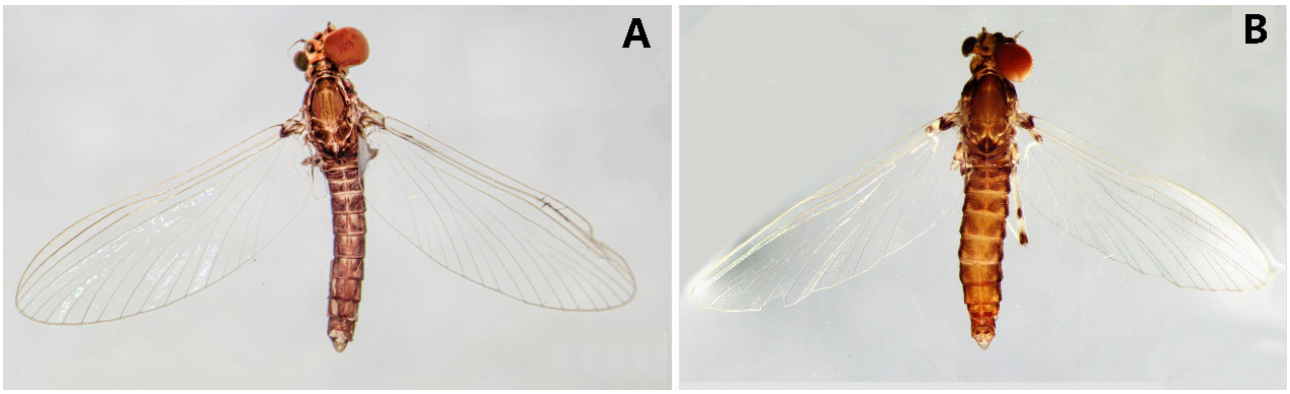

FIGURE 1 Two intersexes of *Choroterpes facialis* from China (dorsal view)
A: Predominate male (specimen 1); B: Predominate female (specimen 2)

Results

Description of specimen 1.

Predominately male intersex imago: body symmetrical except right compound eye with reddish brown turbinate upper portion and dark spherical lower portion, but left eye just dark, much smaller than right one (Figs. 1A, 2B), same as female eyes (Fig. 2D). Legs and caudal filaments broken and lost; color similar to normal male imago (Fig. 2A). Two pairs of symmetrical wings (Fig. 1A). Abdomen same as normal male in color and shape (Fig. 1A), mostly empty inside but with segments VIII–IX apparently filled with white (Fig. 3B). Genitalia: without forceps, subgenital plate similar to female sternum IX (Figs. 3B, 3D); two penis lobes present, slightly shorter than normal case (Figs. 3A, 3B).

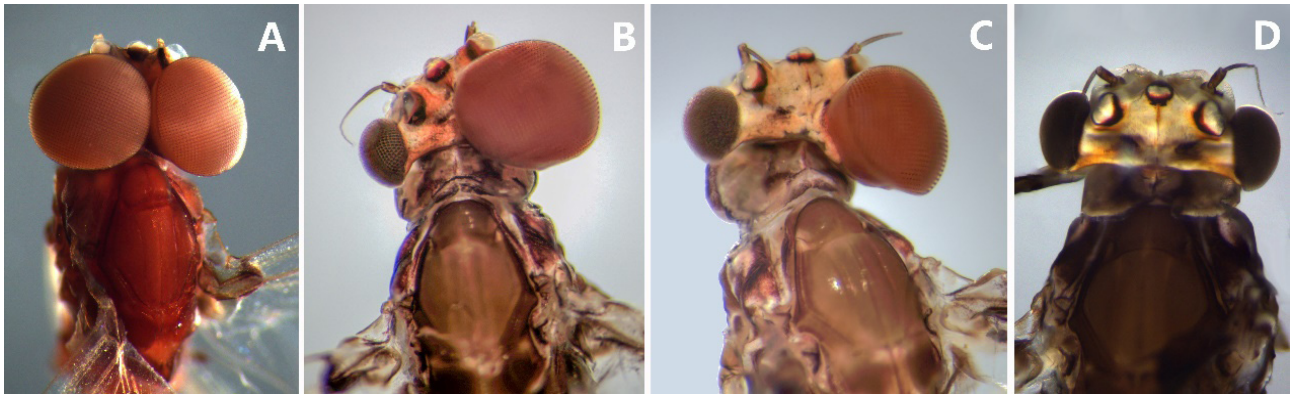

FIGURE 2 Head of two intersexes of *Choroterpes facialis* and two normal imagos (dorsal view)
A: Normal male; B: Predominately male intersex; C: Predominately female intersex; D: Normal female

Description of specimen 2.

Predominately female intersex imago: body, wing and eye patterns similar to specimen 1 except body color slightly duller (same as normal female, Figs. 2C, 2D), remaining two midlegs same as normal female (Fig. 1B); abdomen filled with eggs inside (Fig. 3C); sternum IX same as normal female (Figs. 3C, 3D); two relic penes present (Fig. 3C), more vestigial than those of specimen 1 (Figs. 3B, 3C).

FIGURE 3 Abdominal terminals of two intersexes of *Choroterpes facialis* and two normal imagos
A: Normal male (showing normal penes and forceps, dorsal view, dorsal portion of abdominal segment IX removed); B: Predominately male intersex (showing penes, sternum IX and white interior, dorsal view); C: Predominately female intersex (showing penes, sternum IX and eggs inside, dorsal view); D: Normal female (showing sternum IX, ventral view). Arrows in A–C indicating the penis.

Materials examined:

Choroterpes facialis (Gillies, 1951): 1 male imago, 5 male subimagos, 1,727 female imagos, 3 female subimagos, 2 intersex imagos, Fujian Province, Nanping municipality, 201 county road, 27.837329°N, 117.969880°E, alt. 283.4 m,VII-20-2022, Xin-He Qiang.

Discussion

In the Leptophlebiidae, gynandromorphs have been reported from the genera *Habrophlebiodes* Ulmer, 1920, *Habrophlebia* Eaton, 1881, *Leptophlebia* Westwood, 1840, *Paraleptophlebia* Lestage, 1917 (Soldán and Landa, 1981; Grant and Masteller, 1987), *Farrodes* Peters, 1971 (Santos *et al.*, 2019) and *Thraulodes* Ulmer, 1920 (Nascimento *et al.*, 2021). The present report is the first one of the *Choroterpes*, a very common taxon in China and Asia.

Biogeographically, this is the first report of mayfly gynandromorphism from the Oriental realm (Fig. 4). Two of the other three Asian cases were found in Mongolia and Tajikistan (Palearctic realm, Fig. 4), and the other from the transitional region of Palaearctic and Oriental realms (Anhui Province, China, Fig. 4). Our intersexes show that just like in the Americas, mayfly gynandromorphism can also happen from north to south in Asia (Fig. 4). Together with European, American and New Zealand findings (Soldán and Landa, 1981; Pohe, 2019; Santos *et al.*, 2019; Nascimento *et al.*, 2021), we can see that mayfly gynandromorphism is a cosmopolitan phenomenon.

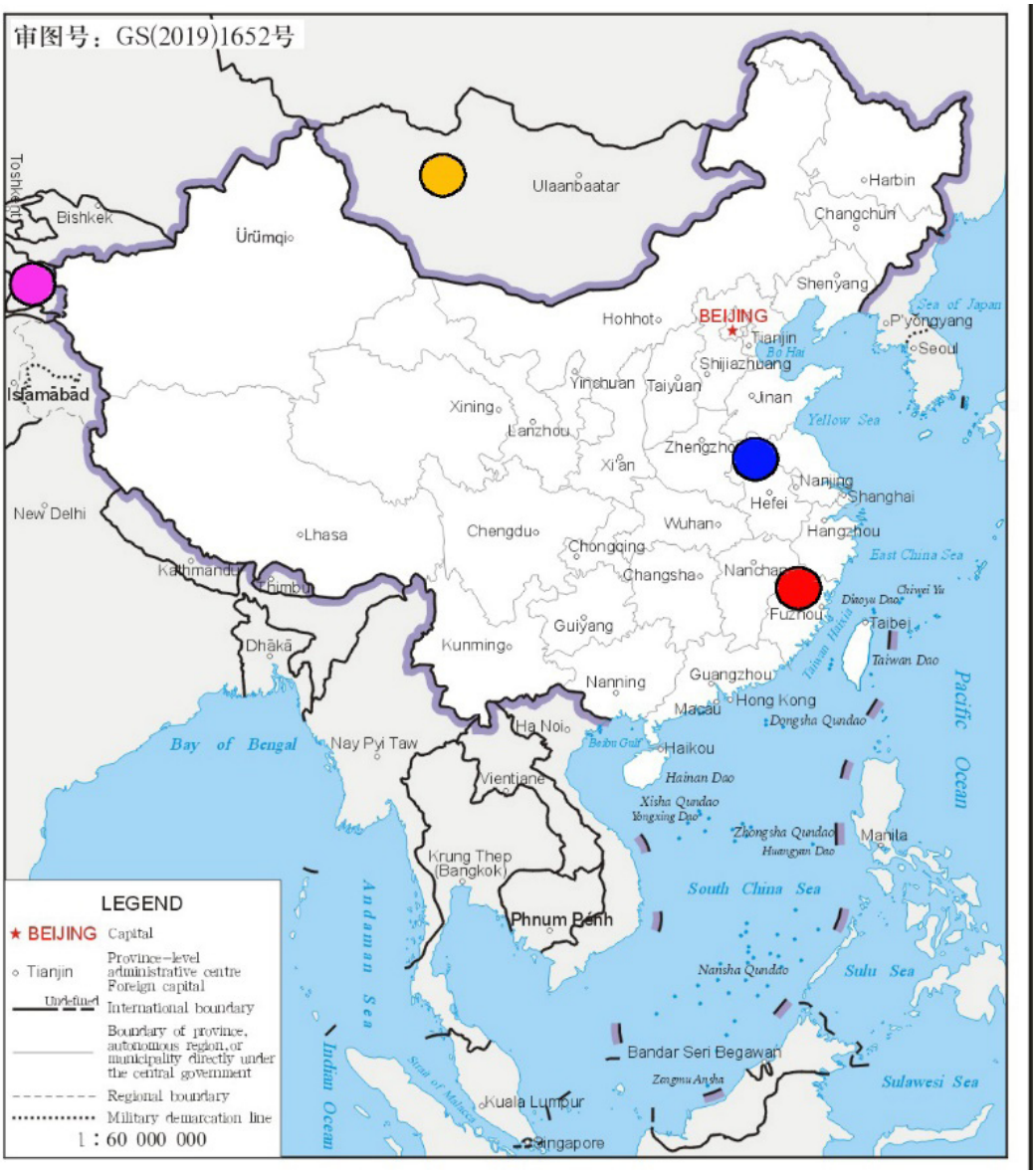

FIGURE 4 Locations of four mayfly gynandromorphisms found in Asia
Yellow dot: *Baetopus* sp., Chövsgöl, Mongolia (after Soldán and Landa, 1981); Pink dot: *Rhithrogena nepalensis* Braasch, 1984, Alay mountain range, Tajikistan (after Kluge, 2015); Blue dot: *Cingmina* sp., Anhui Province, China (after Wu *et al.*, 1993); Red dot: *Choroterpes facialis*, Fujian Province, China.

This is the first leptophlebiid gynandromorphism found in Asia. Along with one baetid and two heptageniids, this reinforces the notion that those three families host most gynandromorphism, or are at least the most likely candidates for finding this phenomenon (Soldán and Landa, 1981).

From our sampling, 1,736 individuals of *Choroterpes facialis* were collected, and only two intersexes were found. Although the data here is based on a random sampling, it shows clearly that gynandromorphism happens quite rarely in the wild population, about one in 860 individuals from the same population. This also indicates that massive sampling is necessary to increase the chances of finding possible gynandromorphism.

The two intersexes here have somewhat similar external morphologies but opposite predominate sexes (Fig. 1).

The predominate male (with left female compound eye and female sternum IX, Figs. 1A, 2B, 3B) is different from all previous cases. Soldán and Landa (1981) reported and compiled "masculinized females" only in their category intersex of gynandromorphism. The intersexes found by Grant and Masteller (1987), Kluge (2015) and Santos *et al.* (2019) were all predominate females with some male structures. In contrast, our predominately male intersex has penes and the usual internal tissues in the abdomen but is without forceps and a left male eye. It is a clear male individual with select female characters. To show its opposite sex to the "masculinized females" of Soldán and Landa (1981), it can be defined as "feminized male". The present finding not only enriches our understanding of the diversity of the intersexual types of mayflies, but also demonstrates the first case of a feminized male.

Furthermore, the predominately female intersex in our present study is also somewhat unique. In the previous cases, the female intersexes usually have one male turbinate eye and one forceps (see Santos *et al.*, 2019). In our case, it has one male eye (Fig. 1B) and two vestigial penes, but it is without male forceps or subgenital plate (Fig. 3C).

Soldán and Landa (1981) and Santos *et al.* (2019) listed several reasons possibly regulating mayfly gynandromorphism, like genetic disorder, disrupted cleavage of fertilized cell, poly-spermic fertilization or mermithid parasitism. Kluge (2015) found a female gynandromorph caused by parasite. However, Funk *et al.* (2010) found that in three different cultivated baetid species, evidently male, female and gynandromorph individuals can be reproduced automatically via parthenogenesis. This means their eggs can develop into male or female individuals. This is a genetic potentiality of some mayflies, especially those parthenogenetic species, to keep their genetic diversity. Gynandromorphs may be just the imperfect or maldeveloped male or female caused by some unknown reasons. Our two individuals, different predominate sexes but with similar structures, especially the same type of eyes and penes (Figs. 1–3), seem to imply that there may be a particular factor causing this same phenotype. Considering the arguments mentioned above, we regard environmental factors, such as temperature or parasites, as the most possible reason.

In our 1,736 normal adults, there are only one male imago and five male subimagos. If this sample represents nature at a certain level, we can say that it is impossible for so few males to mate so many females. Just as with the parthenogenetic mayflies reported by Funk *et al.* (2010), this species is assumed as a parthenogenetic one, too, at least in part.

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