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Notes on the retractabilty of gill tufts in *Pseudolestes mirabilis* (Zygoptera: Pseudolestidae)

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A detailed observation of living larva of *Pseudolestes mirabilis* was conducted, with a focus on the gill tufts, which were confirmed to be retractable. Photographs of the larva in life and video records are provided to demonstrate this finding.

Keywords: Odonata; Zygoptera; Pseudolestidae; *Pseudolestes mirabilis*; larva; gill tufts; retractable

Introduction

Pseudolestes mirabilis Kirby (1900), the only member of its genus, endemic to Hainan, continues to provoke speculation on its phylogenetic position. Based on its unique adult characters the species was originally placed in its own subfamily by Kirby (1900), and then in subfamily Lestinae (now Lestidae) by Calvert (1902) and Tillyard and Fraser (1938–1940). Fraser (1957) established the family Pseudolestidae to accommodate *Pseudolestes* and several other genera, an arrangement followed by Davies and Tobin (1984) and van Tol (2006). According to recent molecular studies it either nested in "Megapodagrionidae" (Bybee, Ogden, Branham, & Whiting, 2008) or deserves its own family Pseudolestidae (Dijkstra et al., 2014). Nevertheless some researchers have placed emphasis on larva characters. Kalkman, Choong, Orr, and Schütte (2010) divided megapodagrionid larvae into four groups and supposed that the larva of *P. mirabilis* would fall into group 1, i.e. having sack-like gills. The first detailed description of the larva of *P. mirabilis* was provide by Yu and Bu (2011) in which the sack-like caudal gills were confirmed. However this study also revealed the presence of a remarkable character, namely the gill tufts, which might implies that it may be close to Amphipterygidae, in the restricted sense defined by Novelo-Gutiérrez (1995) and Rehn (2003). It is in this traditional sense we will use the term amphipterigid henceforth. Unfortunately, Yu and Bu (2011) did not check if the gill tufts of *P. mirabilis* larva are retractable. Larvae of amphipterygids possess paired gill tufts (Corbet 1999; Watson 1966) which are retractable in life (Orr, 2008). Larvae of *P. mirabilis* also possess paired gill tufts which strongly resemble those of amphipterygids. If closer examination should prove them to be essentially the same as the structures known from the amphipterygids group (retractable), it would be strong evidence for a

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shared ancestry (Yu & Bu, 2011). The present study gives a detailed observation on gill tufts of living larvae of *P. mirabilis* and provides both photos and video files as evidence.

Methods

Larvae were found in both shady brooklets and open montane streams with shallow, slow flowing water and stony substrates in Wuzhishan, Hainan, China. Larvae were collected using a dip net with the removal of large stones by hand. Larvae were photographed and videoed soon after they were found in the field using a digital camera Canon Power Shot G12 (Japan) in case they should die or be inactive later. More photographs and videos were later taken when specimens were brought back to the campsite. In some cases artificial stimulation was conducted using small sticks or forceps to induce the larva to retract their gill tufts. Attempts were made to rear collected larvae in the laboratory in plastic containers for almost one month but these failed just before emergence.

Observations

The first observed larva (final stadium male, collected Wuzhishan, Hainan, China, 18°54′18″ N, 109°40′49″ E, 772 m alt., 8 January 2014, Xin Yu col.) was found in a very small brooklet, just under a dark coloured stone (Figure 1). We caught it with a dip net and turned it over to see the abdomen and gill tufts. First the gill tufts were extended but they were withdrawn immediately when disturbed by a stick, or even by the flashlight of the camera. When the larva relaxed in a plastic container the gill tufts gradually extended fully. However, it never pulsed tufts in and out rhythmically in our observation, like *Devadatta* larvae do. The second larva (final stadium male, collected Wuzhishan, Hainan, China, 18°45′27″ N, 109°38′25″ E, 471 m alt., 9 January 2014, Xin Yu col.) was caught at an open, steadily flowing stream. It hid under a stone the size of a volleyball where the water was almost still owing to being protected from the current. This larva clung tightly to the bottom surface of the stone even when removed from the water. It was taken back to the campsite and put into a plastic box in order to get better quality photographs and videos. It showed similar responses to the first larva when stimulated by forceps (Figures 2, 3).

Figure 1. Larva of *Pseudolestes mirabilis* in field, final stadium male, with algae on the body surface.

Figure 2. Larva of *Pseudolestes mirabilis* in a plastic box, ventral view showing gill tufts extended.

Figure 3. Same larva as Figure 2, ventral view showing gill tufts withdrawn when disturbed.

Discussion

Adult characters of *P. mirabilis*, which may simply be the result of rapid evolution and adaptation, are so aberrant that it is very difficult to place this monotypic genus by analysis of adult characters (Yu & Bu, 2011). Even comprehensive molecular studies (Bybee et al., 2008; Dijkstra et al., 2014) have not securely established its phylogenetic position. Many researchers believe that in odonates larval characters may often be less affected by adaptation and radiation than those of the adults, and may therefore yield insights into family level relationships. Thus more and more attention has recently been paid to larvae (Kalkman et al., 2010; Orr, 2008). On the basis of overall larval characters Yu and Bu (2011) suggested it best to place *P. mirabilis* in its own family, Pseudolestidae, the same result also suggested by Rehn (2003) and Dijkstra et al. (2014). However, the authors also mentioned that if the gill tufts of *P. mirabilis* larva are retractable it

will imply that it is closely related to or even belongs to Amphipterygidae (Yu & Bu, 2011). The present study has confirmed retractable gill tufts as well as some other behaviours of *P. mirabilis*, which have made the question even more complicated. The fact that the tufts in *P. mirabilis* do not show the obvious separation found in other amphipterigids suggests that they may have arisen independently in this species, but it is also possible that two paired structures have partially coalesced, and the condition observed in *P. mirabilis* is homologous with one or several or all of the other amphipterigids exhibiting paired gill tufts. Based on an extensive DNA analysis of zygopteran taxa, Dijkstra et al. (2014) recognise no close relationship between any of these genera, but while this study is currently the basis of a consensus classification provisionally accepted by most odonatologists (Dijkstra et al., 2013), most would also agree the position of all amphipterigid genera and *P. mirabilis* remains unclear at present. Firstly, detailed comparative morphological studies of the gill tufts of *P. mirabilis* and those of the various amphipterigid genera are needed to establish what their evolutionary relationship may be. At present we are being asked to accept that the very similar and highly specialised gill tufts have arisen independently five times. Future, additional molecular study is needed to better reveal the true phylogenetic position of *P. mirabilis* and its enigmatic sisters.

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