Breeding Behaviour of *Paedocypris* sp. (Teleostei: Cyprinidae) from Kalimantan Tengah, comprising world’s smallest vertebrate

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ABSTRACT

*Paedocypris* is a genus of miniature cyprinid fish found in acidic waters of peat swamp forests, comprising one of the world’s smallest vertebrate animal. It possesses many unique characteristics especially the sexually dimorphic specialisation of the pelvic girdle of the male. This unique feature was hypothesised by Kottelat *et al.*, 2006 to be involved in reproduction. The aim of this study is to investigate the breeding behaviour of this unique genus. It is shown here that males perform a unique breeding behaviour by positioning themselves onto the underside of leaves to attract females. It is likely that the unique pelvic fins and hypertrophied muscles of the pelvic girdle aid the male fish in this unique behaviour. The males also possess an iridescent spot on dorsum of the head which is shown to be involved in fish recognition and signalling. Similarity of egg laying behaviour is seen with the genera *Boraras* and *Trigonostigma* but this is likely due to convergence of behaviour living in similar habitats. It is likely that the evolution of sexually dimorphic pelvic girdle is related to egg laying behaviour but whether this trait is novel or ancestral is yet to be discovered.

INTRODUCTION

*Paedocypris*, is a genus of miniature cyprinid described recently by Kottelat *et al.* (2006) from the blackwater peat swamps of Southeast Asia. Currently, there are two known species in this genus, *Paedocypris progenetica* and *P. micromegethes*, of which the former is the smallest fish and vertebrate species currently known at 7.9 mm SL (Kottelat *et al.*, 2006). Several possibly undescribed populations are known from various localities in Southeast Asia, namely Pulau Singkep, Pontianak, Pulau Banka and Kalimantan Tengah (Ruber *et al.*, 2007).

In the genus *Paedocypris*, individuals are sexually dimorphic and males possess a unique pelvic girdle consisting of highly modified pelvic fins and abductor muscles believed to play an important function in the breeding behaviour (Kottelat *et al.*, 2006). Here, the breeding behaviour of a population of *Paedocypris* from Kalimantan Tengah was studied and described. The possible role of the unique pelvic girdle of males in breeding behaviour was also investigated. Comparisons between the egg laying behaviour and sexually dimorphic characters of *Paedocypris* and its closely related congeners were also made.

MATERIALS AND METHODS

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Individuals of *Paedocypris* sp. “Kalimantan Tengah” were obtained from a local aquarium distributor and placed into separate tanks. A tank consisting of 50 healthy individuals was used for filming. A single leaf of an Amazon Sword plant was used as the spawning substrate. Sony Digital HD video camera recorder (HDR-SR8E) was used for filming. Filming was done for 1hr every morning just before the lights came on.

Eggs were removed from the leaf and placed in fixative solution (2.5% paraformaldehyde, 2% glutaraldehyde). They were further dehydrated with ethanol and critical point dried using BALZERS CPD030 critical point dryer. After gold coating using JEOL Fine ion sputter JFC-1100, the eggs were viewed under scanning electron microscope JEOL JSM T220A at 200X magnification.

**RESULTS**

**Spawning Behaviour**

Males started to show increased swimming activity in the tank a few minutes after the lights came on. They usually swim to the underside of the leaf to establish spawning territories. Fights between males were seen. The winner of the fights would position itself belly facing up in a quick motion, onto the underneath of the leaf (Figure 1). In this inverted position, the male either remained in the same position or moved slightly up or down. It was observed while in this position, that the abdomen was in contact with the underside of the leaf while the caudal and pectoral fins were not. After about 1-2s in this inverted position, the male fish detached itself from the leaf and returned to the normal swimming position. Males were seen performing the behaviour of positioning themselves on the underside of the leaves in the presence of females, presenting the iridescent spot on the heads to them. It was also observed that some male fish would perform this behaviour many times even in the absence of any female. Eventually, the interested female would join the male fish together at the same spot, both positioned belly side up, on the underside of the leaf and mating takes place. Mating was extremely rapid but due to the small size and often very transparent egg together with the lack of specialised equipment for taking of high speed photographs, the actual mating process could not be clearly captured on video.

![Figure 1. Male on Underside of leaf in the presence of female.](image1.png)

![Figure 2. SEM of a fertilised egg; Projection is marked by Red circle.](image2.png)
Description of eggs and larvae

Single spherical and adhesive eggs were always found and were normally attached to the underside of leaves. SEM images of the fertilised egg showed an apparent projection from the egg surface but this is not seen in the unfertilised egg (See Figure2). No attachment organs were seen. Hatching of the eggs took roughly 30 hrs at ~27 °C. Newly hatched larvae measured about 1.3mm and lie motionless, attached to sides of the tanks or underside of leaves.

DISCUSSION

Breeding Behaviour

The unique breeding behaviour of this species of *Paedocypris* could be similar for all members of the genus as most features including the sexually dimorphic pelvic girdle reported by Kottelat *et al.* (2006) is also seen in this particular species. It was observed that the males displayed the inverted position repeatedly on the underside of the leaves to attract receptive females as females were seen approaching male territory after these displays. This behaviour was also noted by Perrin and Beyer (2008) on a possibly different species of *Paedocypris* indicating the possibility that this behaviour is exhibited throughout this genus. The repetitive behaviour of positioning itself onto the underside of leaf requires a unique physical feature in the male fish. According to Kottelat *et al.* (2006), the pelvic girdle of the male fishes in this genus possessed highly modified pelvic fins with hypertrophied muscles. It was likely that the pelvic fins together with hypertrophied muscles allowed males to clasp the leaf surface and remain upside down.

The suggestion that male *Paedocypris* might use its pelvic girdle and fin to manipulate spawned eggs (Kottelat *et al.*, 2006) could not be observed due to the lack of specialised equipment. However, the suggestion that males use their pelvic girdle to clean the surface or coat it with sperms (Perrin and Beyer, 2008) could be plausible as males were seen to stay in the inverted position for prolonged periods of time. In some cases, the males were seen to move for short distances, while remaining in contact with the leaf in the inverted position.

Kottelat *et al.* (2006) also speculated the role of the iridescent spot on head of males as a means of male/ female recognition or male display. Observations suggested that this might be possible as when the male was in the inverted position, the iridescence spot on the top of the head could be clearly seen by the female.

Single eggs were always found after spawning and the absence of large egg clusters probably suggests that eggs are laid individually which was hypothesized by Kottelat *et al.* (2006). However, Perrin and Beyer (2008) reported that more than one egg is laid in each spawning seems to be not the case in this population. The SEM of a fertilised egg showed an apparent projection from the egg surface (Figure2). This projection was also seen in eggs obtained from the ovaries of pregnant females (Tan Heok Hui, personal communication). This projection could likely be used to attach eggs to undersides of leaves and possibly the reason for the adhesiveness of eggs.

Comparisons of egg laying behaviour and sexual dimorphic characters between closely related genera
Table 1 shows that only *T. heteromorpha* shares similar egg laying behaviour but lacks the sexually dimorphic pelvic girdle that *Paedocypris* has. This similarity could be due to convergence of egg laying behaviour in similar habitats. In addition, the behaviour of repeated positioning underside of leaves by males is not seen which could be the reason for the lack of the sexually dimorphic pelvic girdle. It would be interesting to look at the reproductive behaviour of the closest relative of *Paedocypris*, *Sundadanio*, which also has sexually dimorphic pelvic girdle.

<table>
<thead>
<tr>
<th>Species</th>
<th>Egg laying Behaviour</th>
<th>Sexual dimorphism of pelvic girdle</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Paedocypris</em></td>
<td>Underside of leaves⁴</td>
<td>Present¹</td>
</tr>
<tr>
<td><em>Sundadanio axelrodi</em></td>
<td>Not known</td>
<td>Present⁴</td>
</tr>
<tr>
<td><em>Danio rerio</em></td>
<td>Scatters on leaves and substratum⁵</td>
<td>Absent³</td>
</tr>
<tr>
<td><em>Danionella mirifica</em></td>
<td>Not known</td>
<td>Present⁴</td>
</tr>
<tr>
<td><em>Boraras maculatus</em></td>
<td>Top of leaves⁵</td>
<td>Absent⁶</td>
</tr>
<tr>
<td><em>Trigonostigma</em></td>
<td>Underside of leaves⁷</td>
<td>Absent⁷</td>
</tr>
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REFERENCES


