Female mimicry in a freshwater goby Rhinogobius sp. OR

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Abstract We found female-mimic males in a freshwater goby, *Rhinogobius* sp. OR, from Kamo River, Ehime, Japan. Of 171 adult males collected, three had a female appearace. A discriminant analysis showed that they had a spectrum of morphology from a wholly female morph, through a neutral morph, to a somewhat malelike morph. These three males made an extremely higher gametic investment than did the normal males, suggesting that they have the potential for sneaking fertilizations. Among *Rhinogobius* species, female mimicry might be more common than previously known.

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A lternative reproductive tactics among males are common in fishes (Taborsky, 1994). In species in which male mating competition is severe, dominant males can monopolize a large proportion of females, while some subordinate males will gain little or no access to mates. In such a situation, the latter males may alternatively exploit the former's mating effort by sneaking fertilizations, thereby increasing their fitness. Female mimicry is one of such alternative tactics by which subordinate males assuming the appearance of females can have easy access to spawning by dominant males. Although female mimicry has been reported for many species, it was often judged from the researcher's subjective view, without taking morphological measurements (but see Gonçalves et al., 1996).

The study species, *Rhinogobius* sp. OR (sensu Akihito et al., 2002), is a freshwater goby in which males care for multiple clutches in a nest (Okuda et al., 2002). In the present study, we found female-mimic males from Kamo River, Ehime, Japan. Here we report the morphological features of these female-mimic males.

Materials and Methods

We captured 518 individuals of *Rhinogobius* sp. OR from Kamo River, Ehime, Japan, prior to and during the breeding season of 2001. In the laboratory, we sexed fish, based on traditional criteria of sexual dimorphism in this genus (male having longer first dorsal fin, longer head, and wider mouth; Suk and Choe, 2002), and assigned them separately to stock tanks. Of 347 individuals who were judged females by their appearances, three proved to be males on gonadal inspection under dissection. Although they assumed female body coloration in life (Fig. 1), such coloration disappeared after their death, becoming dark as males.

We took morphological measurements for these three female-mimic males, comparing them with 14 females and 14 normal males. We used 23 morphometric characteristics (Fig. 2). To remove the size effect, we selected the females and normal males from a 1-year-old cohort that matches the female mimics for body size. This attempt was, however, unsuccessful because there was a significant difference in SL among three groups (ANOVA, $F_{2, 28} = 4.91$, P < 0.02): normal males ($\bar{x} \pm$ SD = 38.52 \pm 2.46 mm, n = 14) were as large as female-mimic males $(39.71 \pm 2.06 \text{ mm}, n = 3;$ Scheffé's test, P = 0.66) but were significantly smaller than females $(40.92 \pm 1.46 \text{ mm}, n = 14, P < 0.02)$. This difference is considered an artifact resulting from a sampling bias because males are larger than females in Rhinogobius sp. OR (Suk and Choe, 2002). We performed a discriminant analysis to examine morphometric characteristics effective in discriminating between the sexes and to evaluate to what extent female-mimic males constitute a morphological feature of females on the basis of these discriminants. We adopted a stepwise method with a linear discriminant function, incorporating 22 log-transformed morphometric variables except the SL.

We also took anatomical measurements to compare gametic investment between female-mimic and normal males. We dissected male specimens used for morphological measurements, separating these into their liver, gonad, and eviscerated body. We dried these organs at 60°C for 24 h and weighed them to the nearest 0.1 mg. For each specimen, we calculated gonadosomatic index (GSI = 100 × gonad weight/total body weight) as an indicator of gametic investment, and hepatosomatic index (HSI = 100 × liver weight/total body weight) and the condition factor (K = 10³ × total body weight/TL³) as indicators of somatic condition.





Discriminant score

Fig. 3. The frequency distribution of discriminant scores for females (*white bars*), female-mimic males (*slant bars*), and normal males (*black bars*)

Results and Discussion

Fig. 1. A female (**A**), a female-mimic male (**B**), and a male (**C**) of *Rhinogobius* sp. OR. Males assume a dark body color with a bright yellow caudal fin base, whereas females and female-mimic males assume a light body color with lateral dark bands and an opaque caudal fin with a spotted pattern



Fig. 2. Morphometric characteristics of *Rhinogobius* sp. OR. *AFB*, length of anal fin base; *AFL*, anal fin length; *AHD*, anterior head depth; *AHW*, anterior head width; *CFH*, caudal fin height; *CFL*, caudal fin length; *DL*, dentary length; *ED*, eye diameter; *FDFB*, length of first dorsal fin base; *FDFL*, first dorsal fin length; *HL*, head length; *IFW*, infraorbital width; *ML*, maxillary length; *MW*, mouth width; *PFB*, length of pectoral fin base; *PFL*, pectoral fin length; *PHD*, posterior head depth; *PHW*, posterior head width; *SDFB*, length of second dorsal fin base; *SDFL*, second dorsal fin length; *SL*, standard length; *SNL*, snout length; *VFL*, ventral fin length

A stepwise discriminant analysis showed that six morphometric characteristics [first dorsal fin length (FDFL), second dorsal fin length (SDFL), pectoral fin length (PFL), head length (HL), dentary length (DL), and eye diameter (ED)] were effective in discriminating between the sexes (z =43.25FDFL + 16.46SDFL - 4.36PFL - 34.76HL + 22.19DL - 43.49ED, trait values log-transformed; eigenvalue = 36.2, canonical correlation = 0.99, Wilks' λ = 0.03, $\chi^2 = 83.2$, P < 0.001; consider it male if z > 0 and female if z < 0). Using this discriminant equation, we classified fish into either sex, without incorrectness (Fig. 3). Judging from discriminant scores, two of three female-mimic males were classified as female and the remaining one as male (Fig. 3). One fish was wholly constituted of characteristic features of females, while the other two showed an intermediate score. Of these two, the somewhat malelike one was found receiving and brooding eggs from females in the stock tank 10 days before the morphological measurements.

It was also found that the female-mimic males all had a genital papilla characteristic of males, regardless of their appearances. This character is therefore effective in discriminating between females and female-mimic males. Genital inspection will enable us to detect female mimicry in the field without killing the fish.

Three female-mimic males also showed an extremely high GSI (6.31%, 6.53%, and 7.62%), compared to the normal males ($\bar{x} \pm SD = 0.66 \pm 0.29\%$, n = 14; $t_{15} = -26.12$, P < 0.001), but their somatic condition did not differ from that of the latter (HSI: $t_{15} = -1.54$, P = 0.14; K: $t_{15} = -1.02$, P = 0.33). This result suggests that these female-mimic males have the potential for sneaking fertilizations, as is often the case in fishes with male alternative reproductive tactics (Taborsky, 1994). In this goby, the female mimics had a spectrum of morphology from a wholly female morph through a neutral morph to a somewhat malelike morph; the latter was the one that was brooding eggs in the stock tank. In a peacock blenny (*Salaria pavo*) in which nesting males care for eggs, female-mimic males ready to become a nest holder develop their testicular gland, which is involved in androgen production to induce the expression of male secondary sexual characters (Gonçalves et al., 1996; Oliveira et al., 2001). In *Rhinogobius* sp. OR, female-mimic males may change themselves into a male morph as they have a chance to become nest brooders, implying that female mimicry shows phenotypic plasticity.

Female mimicry is ubiquitous among nest-brooding fishes (e.g., tessellated darter Etheostoma olmstedi: Constantz, 1979; bluegill sunfish Lepomis macrochirus: Dominey, 1980; European wrasse Symphodus ocellatus: Taborsky et al., 1987; peacock blenny: Gonçalves et al., 1996). In contrast, there have hitherto been no reports for a genus Rhinogobius except the subject species, in spite of intensive work on their reproductive biology. This lack may be because most species studied are fluvial. For fluvial species in whose habitat boulders are usually abundant, subordinate males can afford to have a nest and thus access to mates, resulting in a decreased benefit from becoming a female mimic. Nevertheless, some recent studies suggest the occurrence of female mimicry in Rhinogobius [e.g., Rhinogobius sp. MO (sensu Akihito et al., 2002): M. Kunisato, personal communication; Rhinogobius sp. OR: Suk and Choe, 2002; S. Ito, unpublished data]. In some of these study populations, fish inhabit a downstream niche (M. Kunisato, personal communication) or a small pond (S. Ito, unpublished data) where boulder nests are relatively scarce. Among Rhinogobius species, female mimicry may be more common than was previously known.

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