# Notes on protandry in the creediid fishes Limnichthys fasciatus and L. nitidus (Teleostei: Creediidae)

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News and Comments

Six pages

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Fishes of the family Creediidae occur mainly on sandy bottom of tropical and temperate shallow waters in Indo–West Pacific Ocean. Eight genera and 18 valid species are known (Fricke and Golani 2012; Nelson et al. 2016). For ecological information, Leis (1982) and Reader et al. (2000) describe egg and larval development of *L. fasciatus* and *L. nitidus*, respectively. Cozzi and Clark (1995) reports darting behavior, exhibited by *L. nitidus* as quick movements out and back in the sand, suggesting that it was an escape behavior when they were disturbed.

Biological information on reproduction of Creediidae has been very limited. Langston (2004) histologically studies sexuality of 10 species of creediids and shows that *Crystallodytes cookei* and *Limnichthys nitidus*, identified as *L. donaldsoni* in the original paper, which is a junior synonym of *L. nitidus* (Yoshino et al. 1999; Shimada 2013), are regarded as protandry (sex change from male to female) by histological observation of gonads and size distributions of both sexes (female>male). In addition to these two species, *Chalixodytes tauensis*, *Crystallodytes pauciradiatus* and *L. fasciatus* have gonads comprised of ovarian and testicular parts divided by connective tissue in functional males, whereas those of females consist of only ovarian part.

Sadovy de Mitcheson and Liu (2008) indicates that functional hermaphroditism is confirmed in 27 families of teleost fishes in their review on hermaphrodite fishes. However, Creediidae is not included among these families. Namely, hermaphroditism of Creediidae has been overlooked for a long time. In order to provide evidential data of functional hermaphroditism, we made histological observations on gonads of the two creediid fishes *L. fasciatus* and *L. nitidus*.

Forty–two specimens of *L. fasciatus* were collected by hand net using SCUBA at Banda Beach, Tateyama, Chiba, Japan (34 58′ N, 139 46′ E) on 13 April (n = 1), 14–15 May (n = 7), 17 June (n = 9), 18–16 July (n = 13) and 18–21 August (n

= 12), 2013. The fish were brought to the laboratory, anesthetized in 100 ppm MS-222, measured for standard length (SL) to the nearest 0.1 mm with a digital caliper, fixed in Bouin’s solution for 24 hours, and then preserved in 70 % ethanol. The abdominal parts of the specimens were embedded in paraffin, sectioned to 5 μm, and stained with haematoxylin and eosin.

We also examined gonads of seven *L. nitidus* specimens deposited at the National Science Museum, Tokyo: NSMT–P 71438 (n = 4, 16.0–20.5 mm SL, collected at Ambon Isl., Indonesia on 5 December 1998) and NSMT–P 77532 (n = 3, 14.9–26.8 mm SL, Okinoshima Isl., Kochi, Japan on 24 July 2007). We dissected and extracted the abdominal organs containing the gonads and prepared the tissues following the methods outlined above.

The gonads of 21 specimens of *L. fasciatus* were comprised of both testis and ovary (Fig. 1a), which were apparently divided by connective tissue. The oocytes of all 21 hermaphroditic specimens were immature, while the testicular parts of seven specimens collected in July and August were developed and sperm and spermatids were detected. Therefore, those individuals were regarded as functional male. The gonads of other 21 specimens were comprised of only vitellogenic oocytes (Fig. 1b). These individuals were identified as functional female. The females (mean ± SD = 39.8 ± 7.0 mm SL, range = 25.4–47.5 mm SL) were significantly larger than the males (31.3 ± 5.1 mm SL, 24.3–39.1 mm SL) ($t$-test, $t = -4.4$, $df = 40$, $P < 0.01$) (Fig. 2). These results strongly suggest that *L. fasciatus* is protandrous.

The gonadal structure of *L. nitidus* also indicated bisexuality (Fig. 1c, d), as in *L. fasciatus*. The gonads of the three individuals (14.9, 16.4 and 17.8 mm SL) were comprised of both testicular ovarian parts. However, only ovarian tissue was detected in the other four specimens (16.0, 20.5, 23.5 and 26.8 mm SL). The former and latter samples were regarded as males and females, respectively. Although we did not have enough data on sizes for a statistical analysis, there was a tendency that the females were larger than the males. These results suggest that *L. nitidus* is also protandrous.

The gonadal structure of *L. fasciatus* and *L. nitidus* are well corresponded with the previous study by Langston (2004), being divided into testicular and ovarian parts by the connective tissue in functional male and comprised of only ovarian part in functional female. In some protandrous species, the structure of ovotestis is divided by connective tissue (Sadovy and Shapiro 1987). This type of gonad structure is similar to those of the other protandrous species like *Thysanophrys celebica* (Platycephalidae) (Sunobe et al. 2015), genus *Amphiprion* (Pomacentridae) (Moyer and Nakazono 1978) and *Acanthopagrus schlegelii* (Sparidae) (Chang and Yueh 1990).

Protandry has been known in Centropomidae, Gonostomidae, Latidae, Muraenidae, Platycephalidae, Pomacentridae and Sparidae (Sadovy de Mitcheson and Liu 2008). Creediidae is the eighth family, which protandry is confirmed.

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## Figure legends

**Fig. 1** Gonad structure of *Limnichthys fasciatus* (male: **a**, female: **b**) and *L. nitidus* (**c**, **d**). *O*–ovarian tissue; *T*–testicular tissue. *Scale bars* 100 μm (**a**) and 300 μm (**b, c, d**)

**Fig. 2** Size frequencies of male and female *Limnichthys fasciatus*

Fig. 1

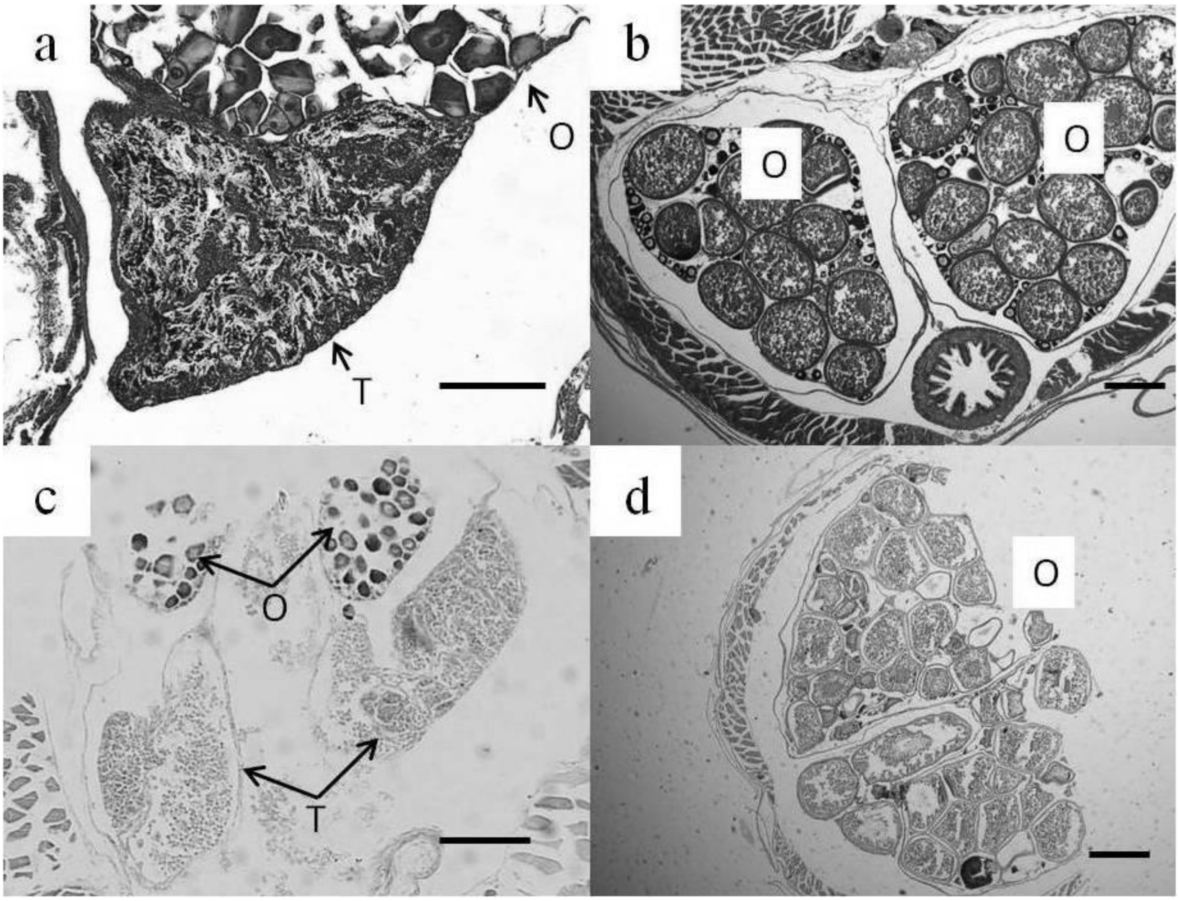
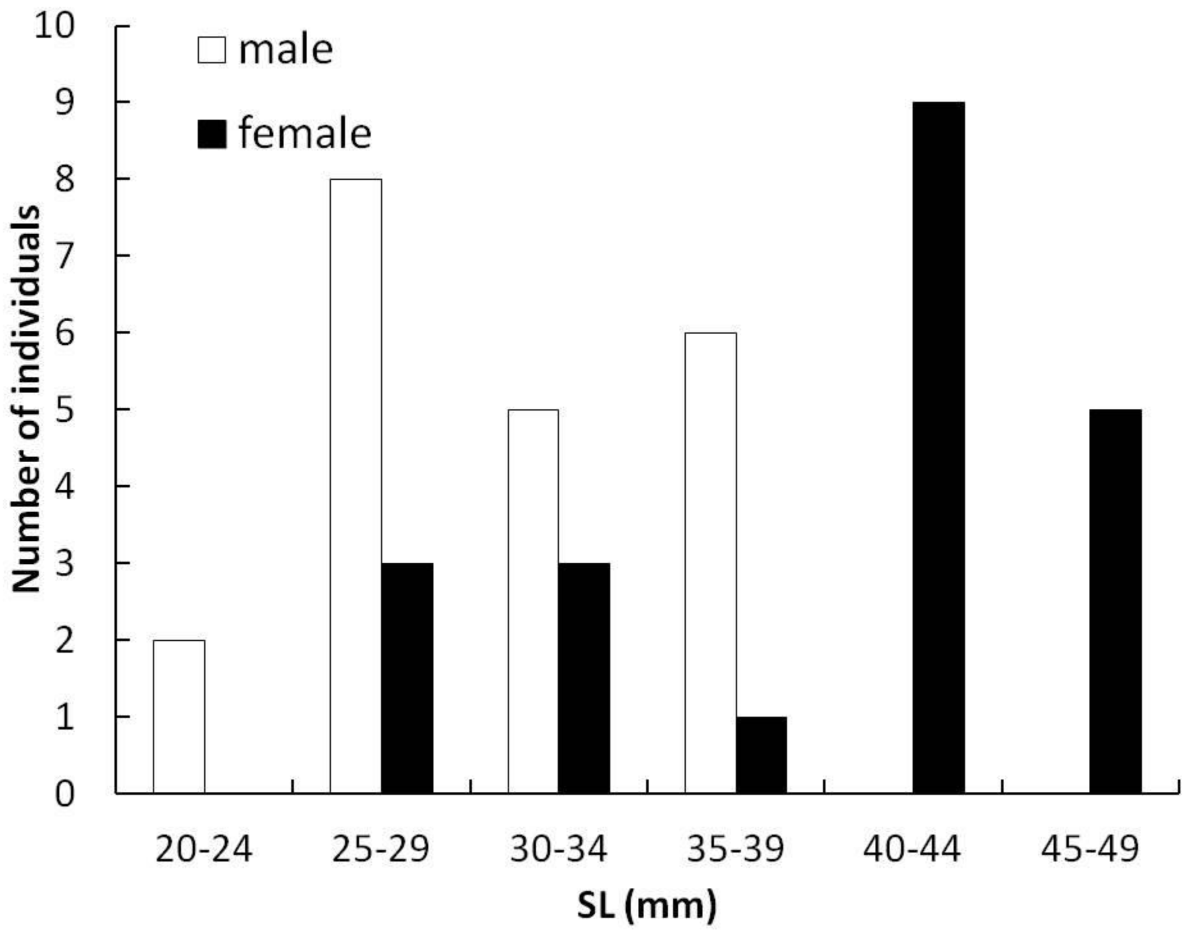


Fig. 2



10
9
8
7
6
5
4
3
2
1
0
male
female
Number of individuals
20-24
25-29
30-34
35-39
40-44
45-49
SL (mm)