

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

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

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Running title: Protandry in *Limnichthys*

News and Comments

Six pages

Two figures

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

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

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

54 Forty–two specimens of *L. fasciatus* were collected by hand net using SCUBA  
55 at Banda Beach, Tateyama, Chiba, Japan (34 58' N, 139 46' E) on 13 April (n =  
56 1), 14–15 May (n = 7), 17 June (n = 9), 18–16 July (n = 13) and 18–21 August (n

57 = 12), 2013. The fish were brought to the laboratory, anesthetized in 100 ppm  
58 MS-222, measured for standard length (SL) to the nearest 0.1 mm with a digital  
59 caliper, fixed in Bouin's solution for 24 hours, and then preserved in 70 %  
60 ethanol. The abdominal parts of the specimens were embedded in paraffin,  
61 sectioned to 5  $\mu$ m, and stained with haematoxylin and eosin.

62 We also examined gonads of seven *L. nitidus* specimens deposited at the  
63 National Science Museum, Tokyo: NSMT-P 71438 (n = 4, 16.0–20.5 mm SL,  
64 collected at Ambon Isl., Indonesia on 5 December 1998) and NSMT-P 77532 (n  
65 = 3, 14.9–26.8 mm SL, Okinoshima Isl., Kochi, Japan on 24 July 2007). We  
66 dissected and extracted the abdominal organs containing the gonads and  
67 prepared the tissues following the methods outlined above.

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

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

85 was a tendency that the females were larger than the males. These results  
86 suggest that *L. nitidus* is also protandrous.

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

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

100

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106

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142

Figure legends

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144

145 **Fig. 1** Gonad structure of *Limnichthys fasciatus* (male: **a**, female: **b**) and *L.*  
146 *nitidus* (**c**, **d**). *O*–ovarian tissue; *T*–testicular tissue. *Scale bars* 100  $\mu\text{m}$  (**a**) and  
147 300  $\mu\text{m}$  (**b**, **c**, **d**)

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



Fig. 1

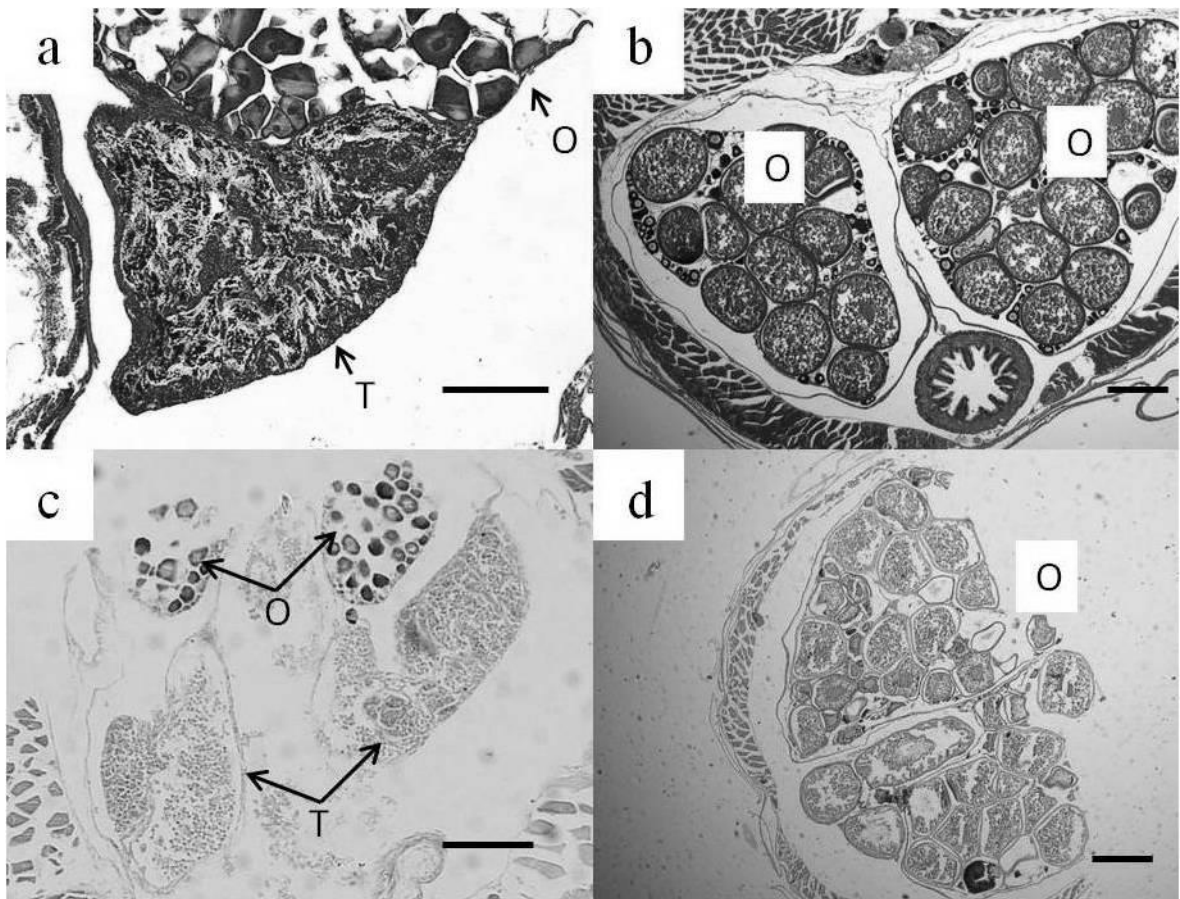


Fig. 2

