THE DISAPPEARANCE OF MALE SECONDARY SEXUAL CHARACTERISTICS IN THE TYPICAL CAVE SPECIES IN *TRIPLOPHYSA*

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ABSTRACT

It was suggested that the darkness environment would influence the fishes' morphology, not only the barbells, snout, but also the male secondary sexual features. This paper reported that the three typical cave dwelling fish species of *Triplophysa* did not show these sexual morphological traits, such as *Triplophysa lewangensis*, *Triplophysa nasobartula* and *Triplophysa zhenfengensis* collected in Guizhou Province, meanwhile, there were not the description on the traits about the 11 typical cave dwelling species of this genus in Guangxi Province.

RESUMÉ: La disparition des caractères sexuels secondaires masculins dans l'espèce typique des cavernes du genre *Triplophysa*.

Il a été suggéré que l'environnement de l'obscurité influencerait la morphologie des poissons, non seulement les barbillons, le museau, mais aussi les caractéristiques sexuelles secondaires masculines. Cet article rapporte que les trois espèces de poissons troglodytes typiques de *Triplophysa* tels que *Triplophysa lewangensis*, *Triplophysa nasobartula* et *Triplophysa zhenfengensis* récoltés dans la province de Guizhou, n'ont pas présenté ces caractères morphologiques sexuels, qu même temps n'ayant pas de description sur les traits caractéristiques des 11 espèces vraies troglodytes de ce genre de la province de Guangxi.

REZUMAT: Dispariția caracterelor sexuale secundare masculine la speciile troglobionte tipice din genul *Triplophysa*.

S-a sugerat că mediul lipsit de lumină ar influența morfologia peștilor, nu numai la nivelul mustăților, rostrului, dar și caracteristicile sexuale secundare masculine. Articolul a concluzionat că cele trei specii de pești din genul *Triplophysa*, tipice pentru mediul cavernicol, nu au prezentat aceste trăsături morfologice sexuale, precum *Triplophysa lewangensis*, *Triplophysa nasobartula* și *Triplophysa zhenfengensis* colectate în provincia Guizhou, în timp ce nu există o descriere a trăsăturilor celor 11 specii troglobionte tipice din acest gen care sunt întâlnite în Provincia Guangxi.

INTRODUCTION

Rendahl (1933) established *Triplophysa hutjertjuensis* as a model species, and Bănărescu and Nalbant (1975) classified *Triplophysa* into two genera: *Hedinichthy* and *Triplophysa*. Zhu Songquan (1989) combined them in one genus, *Triplophysa*, based on the structural characteristics of the position of the male secondary sexual features of *Triplophysa* and the shape of the small spines. *Triplophysa* is the larger genus in *Nemacheilidae*. Till now 197 species have been described (Eschmeyer et al., 2017), of which more than 100 species have been distributed in China (Zhang Chunguang et al., 2015). In addition, there are 29 species of typical caves species in *Triplophysa* in China *Triplophysa* (Tab. 1), eight species in Yunnan, six species in Guizhou, 13 species in Guangxi, and one species in Chongqing and one species in Hunan.

There are not only surface species, but also 29 species that live in caves. However, the characteristics of male secondary sexual characteristics have not been found in the description of these cave species. Therefore, the species of Guizhou Province's caves (Fig. 1), such as *Triplophysa lewangensis*, *Triplophysa zhenfengensis*, and *Triplophysa nasobartula*, were inspected to determine if the male secondary sexual characteristics were present.

The surface species' adult males have the secondary sexual characteristics mainly on the sides of the head and the pectoral fins (Zhu Songquan, 1989; Hou Feixia, 2010). The phenotype could be divided into: 1. The upper and lower parts of the infraorbital line are thickened with spines (e.g. *T. minxianensis*). 2. No spines in the upper and lower parts of the infraorbital line, only thickened (e.g. *T. daqiaoensis* (Fig. 2-P1). 3. Only the upper part thickened, and no spike (e.g. *T. stenura* (Fig. 3-P2). 4. Only the upper part thickened and has spines (e.g. *T. stoliczkae*) (Fig. 4-P3) have changes in pectoral fins (Hou Feixia et al., 2010). Sexual characteristics are divided into: 1. The pectoral fin masks are thickened with pads with spiny spines (e.g. *T. daqiaoensis*. (Fig. 5-P11) 2. Thick padded pectoral fins on back, no spikes (e.g. *T. markehenensis*) (Fig. 5-P12). 3. The pad on the back of the pectoral fin is not obvious, and there are no spines (e.g. *T. markehenensis* (Fig. 5-P13). (Hou F. X. et al., 2010)

Figure 1: Studied areas.

Figure 2: *Triplophysa daqiaoensis*; Lateral view of head of *Triplophysa* males, showing the secondary sexual characters; T P = Thickened pad; S = Spines; T I L = The infraorbital line; Scalebars = five mm (photo Hou F. X. et al., 2010).

Figure 3: *Triplophysa stenura*; Lateral view of head of *Triplophysa* males, showing the secondary sexual characters; T P = Thickened pad; T I L = The infraorbital line; Scalebars = five mm (photo Hou F. X. et al., 2010).

Figure 4: *Triplophysa stoliczkae*; Lateral view of head of *Triplophysa* males, showing the secondary sexual characters; T P = Thickened pad; S = Spines; T I L = The infraorbital line; Scalebars = five mm (photo Hou F. X. et al., 2010).

Figure 5: Dorsal view of pectoral fin of *Triplophysa* males, showing secondary sexual characters; S = Spines; T P = Thickened pad; U = Unobvious; P 11 *Triplophysa daqiaoensis*; P 12-P 13 *Triplophysa markehenensis*; Scalebars = five mm (photo Feixia et al., 2010).

Table 1: The species distribution of the typical genus *Triplophysa* in China.

Species	Geographical distribution
<i>Triplophysa aluensis</i> (Li W. X. and Zhu Z. G., 2000)	Alu ancient cave, Luxi County, Yunnan Province
<i>Triplophysa gejiuensis</i> (Chu X. L., et al., 1979)	Gejiu City, Yunnan Province, China
<i>Triplophysa qiubeiensis</i> (Li W. X., et al., 2008)	Nijiao Village, Qiubei County, Yunnan Province
<i>Triplophysa shilinensis</i> (Chen Y. R., et al., 1992)	Boyi Village near Shilin in Lunan County, Yunnan Province
<i>Triplophysa xiangshuingensis</i> (Li W. X., 2004)	Shilin County, Yunnan Province
<i>Triplophysa yunnanensis</i> (Yang, 1990)	Yiliang County, Yunnan Province
<i>Triplophysa tianxingensis</i> (Yang H. F., et al., 2016)	Qiubei County, Yunnan Province
<i>Triplophysa xichouensis</i> (Liu S. W., et al., 2017)	Xichou County, Yunnan Province
<i>Triplophysa nasobartula</i> (Wang D., and Li D., 2001)	Wengang Township and Dongtang Township, Libo County, Guizhou Province
<i>Triplophysa zhenfengensis</i> (Wang D., and Li D., 2001)	Longchang Town, Zhenfeng County, Guizhou Province
<i>Triplophysa longliensis</i> (Ren Q., et al., 2012)	Longli County, Guizhou Province
<i>Triplophysa jiarongensis</i> (Lin Y., et al., 2012)	Libo County, Guizhou Province
<i>Triplophysa longibarbatula</i> (Chen Y. R., et al., 1998)	Libo County, Guizhou Province
<i>Triplophysa lewangensis</i> (unpublished)	Lewang Town, Wangmo County, Guizhou Province
<i>Triplophysa nandanensis</i> (Lan J. H., et al., 1995, 2013)	Mayang Village, Six Village Town, Nandan County, Guangxi Province
<i>Triplophysa tianeensis</i> (Chen X. Y., et al., 2004)	Number eight hole, Bala Township, Tiane County, Guangxi Province
<i>Triplophysa lingyunensis</i> (Liao J. W., et al., 1997)	Sha hole, Mawang Village, Sicheng Town, Lingyun County, Guangxi Province
<i>Triplophysa huapingensis</i> (Zheng L. P., 2012)	Huaping Village, Leye County, Guangxi Province
<i>Triplophysa longipectoralis</i> (Zheng L. P., 2009)	Xunle Township, Huanjiang County, Guangxi Province
<i>Triplophysa macrocephala</i> (Yang J., et al., 2012)	Bawei Township, Nandan County, Guangxi Province

Table 1 (continued): The species distribution of the typical genus *Triplophysa* in China.

<i>Triplophysa lihuensis</i> (Wu T. J., et al., 2012)	Lihu Town, Nandan County, Guangxi Province
<i>Triplophysa huanjiangensis</i> (Yang J., et al., 2011)	Chuanshan Town, Huanjiang County, Guangxi Province
<i>Triplophysa langpingensis</i> (unpublished)	Langping Township, Tianlin County, Guangxi Province
<i>Triplophysa fengshanensis</i> (unpublished)	Lintong Township, Fengshan County, Guangxi Province
<i>Triplophysa dongganensis</i> (unpublished)	Tonggan Village, Chuanshan Town, Huanjiang County, Guangxi Province
<i>Triplophysa tianlinensis</i> (Li J., et al., 2017a)	Tianlin County, Guangxi Province
<i>Triplophysa luochengensis</i> (Li J., et al., 2017b)	Luocheng County, Guangxi Province
<i>Triplophysa rosa</i> (Huang J., et al., 2013)	Stove Town, Wulong County, Chongqing City
<i>Triplophysa xiangxiensis</i> (He L., et al., 2006)	Huoyan Township, Longshan County, Xiangxi Autonomous Prefecture, Hunan Province

MATERIAL AND METHODS

Triplophysa lewangensis, collecting place Lewang Town, Wangmo County, Guizhou Province, specimen number 28.

Triplophysa nasobartula, collecting place Lijiangshui Cave, Jiarong Town, Libo County, Guizhou Province, specimen number 22.

Triplophysa zhenfengensis, collecting place Shuangrufeng Scenic, Zhenfeng County, Guizhou Province, specimen number 12.

Determination and selection of adult individuals, according to the report of Wang and Li (2001), the total length of 34 mm, males with atypical cave types over 28 mm have secondary sexual characteristics. Therefore, this study selected specimens with body length greater than 30 mm as comparatively mature individuals.

Three types of *Triplophysa* specimens with body length greater than 28 mm were examined.

No padding and spines were found on the lateral side of all *Triplophysa* crickets. Pectoral fins also found no thickened pads and spines. It was found that there are 17 males in *Triplophysa lewangensis*. Eleven *Triplophysa nasobartula* has nine males. 12 *Triplophysa zhenfengensis* has eight males, and four females. Three *Triplophysa* body length measurements were as is following: *Triplophysa lewangensis* body length 27.71-54.81 mm, full length 42.33-56.32 mm; *Triplophysa nasobartula* body length 50.70-91.31 mm, full length 62.46-109 mm; *Triplophysa zhenfengensis* body length 57.47-101.50 mm, full length 65.79-123.18 mm.

RESULTS AND DISCUSSION

Comparative results of morphological characteristics of the infraorbital line side of three species of genus *Triplophysa* in Guizhou Province.

Figure 6: Infraorbital line; 1. *Triplophysa lewangensis*; 2. *Triplophysa nasobartula*; 3. *Triplophysa zhenfengensis*; Scalebars = five mm; TIL = the infraorbital line.

Comparison of morphological characteristics of pectoral fins of three kinds of genus *caves* in Guizhou Province.

Figure 7: Pectoral fins; 4. *Triplophysa lewangensis*; 5. *Triplophysa nasobartula*; 6. *Triplophysa zhenfengensis*; Scalebars = five mm.

The secondary sexual characteristics of male individuals in three cave species of *Triplophysa* in Guizhou Province.

Among the three *Triplophysa* cave species with typical male individuals we observed: 1. *Triplophysa lewangensis* individual had smaller white transparent back with a little stain that does not recede, compared with the first two types of both sides of head lateral morphology. The subaxillary line tube is not obvious. There was no thickening of the upper and lower leaves of the male zygomatic line at the cheeks of the male, and no spines were distributed (Fig. 6). The thickened pectoral fins have disappeared. The spines have also disappeared (Fig. 7). 2. The upper and lower mesophyll pads of the male *Triplophysa nasobarbatula*, and the inferior lateral line of male cheeks have disappeared. No spines distribution (Fig. 6). The thickened pectoral fins have disappeared, and the spines have disappeared (Fig. 7). 3. *Triplophysa zhenfengensis* male genital cheeks under the lateral line of the upper and lower leaves of the pad have disappeared; also, no spines distribution was observed (Fig. 6). The pectoral fin cushion has disappeared along with the spines (Fig. 7).

The sex determination of fish is very complicated. The current study on the sex determination of fish is still at an exploratory stage. For those who have obvious secondary sexual characteristics, wait until sexual maturity. And in the breeding season, we can accurately distinguish their sex by morphology. The disappearance of male secondary sexual characteristics in cave species will undoubtedly bring great difficulties to the population ecology studies of cave fishes. Therefore, it is necessary to study a new technical means to identify the gender of cave fish. At present, SD genes in fish with XX/XY sex determination system, five other SD-related genes, such as *gsdf* in *Oryzias luzonensis*, *sox3* in *Oryzias dancena*, *amhr2* in *Takifugu rubripes*, *Amhy* in *Odontesthes hatchery* and *sdY* in *Oncorhynchus mykiss*, were respectively identified from several teleost fish with XX/XY sex determination system (Mei and Gui, 2015). It is gradually progressing during the study of fish sex determination. But until now, the discovery of such a gene, which is a very stable characteristic of the sex determination of male fish, has not yet been established. Therefore, this work is very worthwhile to carry out.

According to Zhu Songquan's research (1989), the surface species of the genus *Triplophysa* have the habit of chasing after breeding. It is presumed that the special structure of the male and pectoral fins contributes to the effective attachment of females in the flowing water environment and improves the fertilization rate. However, the disappearance of male secondary sexual characteristics in the typical cave species in *Triplophysa* is a reaction to the influence of the environment on the morphological characteristics of *Triplophysa*. Therefore, courtship behavior for typical cave fish is a very interesting issue.

CONCLUSIONS

Triplophysa cave fish species have lost the secondary sexual characteristics because of long-term living in the dark cave environment.

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