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Source: Folia Zoologica, 60(4) : 285-289

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/fozo.v60.i4.a3.2011>

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# White linear markings on the abdomen of river loaches (Nemacheilidae) – potential usage for the identification of individuals

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Received 16 October 2010; Accepted 25 March 2011

**Abstract.** Former studies demonstrated that the river loach species *Lefua* sp. and *Lefua echigonia* (Nemacheilidae) have thin white linear markings from the pectoral fin base to the ventral fin base on both right and left sides of the abdomen that can be used to identify individuals in laboratory and in the field. In the present study we demonstrate the existence of such markings and the uniqueness of their shape in reared individuals of a further six species of Nemacheilidae, but found them absent in three species of Balitoridae. Furthermore, the long-term stability of the shape of the markings was examined in reared individuals of the nemacheilid *Barbatula toni* over two years. The white line markings offer a non-invasive tool for the individual recognition of nemacheilid species, some of which are threatened with extinction.

**Key words:** individual identification, Balitoridae

## Introduction

The family Nemacheilidae has recently been demonstrated to be separate from the family Balitoridae (Šlechtová et al. 2007) and is known to science with at least 30 genera and 420 species (Nelson 2006). In individuals of the nemacheilid species *Lefua* sp. (sensu Hosoya 2002) and *Lefua echigonia*, dual white linear markings were externally observed from the pectoral fin base to the ventral fin base on the right and left sides of the abdomen (Aoyama 2000). Aoyama (2000) stated that the linear markings of *Lefua* sp. were formed of dense connective tissue. The markings are useful for the individual identification of specimens, since they meet the following criteria: 1) marks exist on all specimens collected, 2) each specimen has a unique marking pattern, 3) the shape of the marks on each specimen did not change with time (Aoyama 2000, Akada et al. 2005). It has been demonstrated, that this method is useful to obtain ecological information without killing captured individuals of both endangered species (Aoyama 2007, Moriyama et al. 2007). However, it is not known if other species of Nemacheilidae have such linear markings.

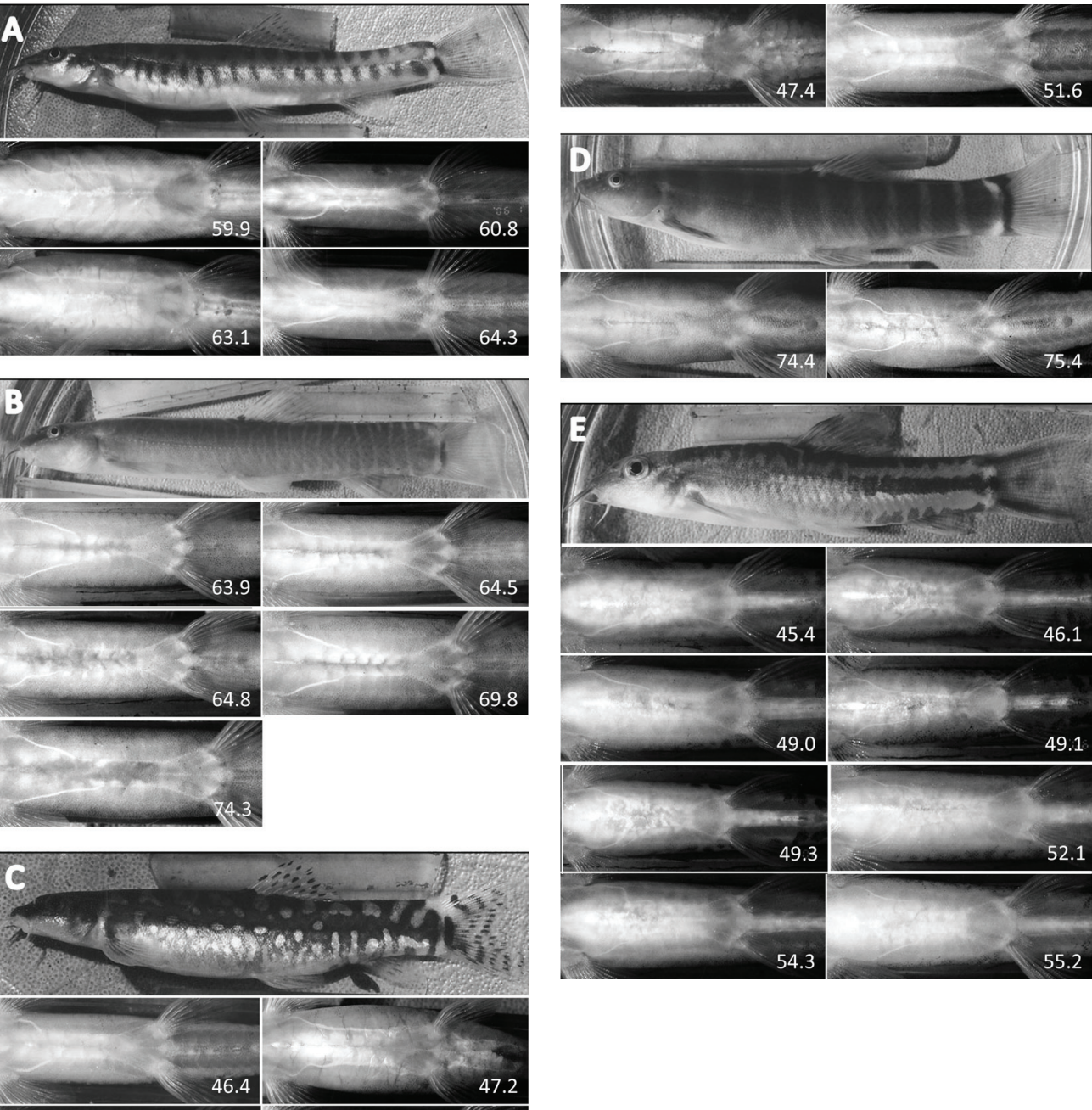
The aim of the present study was to examine more nemacheilid species for the presence of white line markings and to test their potential for identification of individuals in a similar way as formerly done with *Lefua*.

## Material and Methods

The individuals examined belonged to seven species of Nemacheilidae, *Nemacheilus* cf. *pallidus* (n = 4), *Nemacheilus* sp. (n = 5), *Mesonoemacheilus guentheri* (n = 4), *Schistura* cf. *similis* (n = 2), *Traccatichthys pulcher* (n = 8) and *Barbatula toni* (n = 20), and three species of Balitoridae, *Beaufortia kweichowensis* (n = 5), *Sewellia lineolata* (n = 7) and *Sewellia* sp. (n = 3) (Table 1). Specimens except *B. toni* were bought from ornamental fish shops and reared in a stock tank or an exhibition tank with a water temperature of 25–28 °C. To check and document linear markings, specimens were anesthetized in 0.0075 % 4-allyl-2-methoxyphenol, placed on their back in a vinyl tube or in a hose cut lengthwise and the abdominal region was photographed in November 2006. In the case of balitorid species, the abdominal region of the material individuals was also

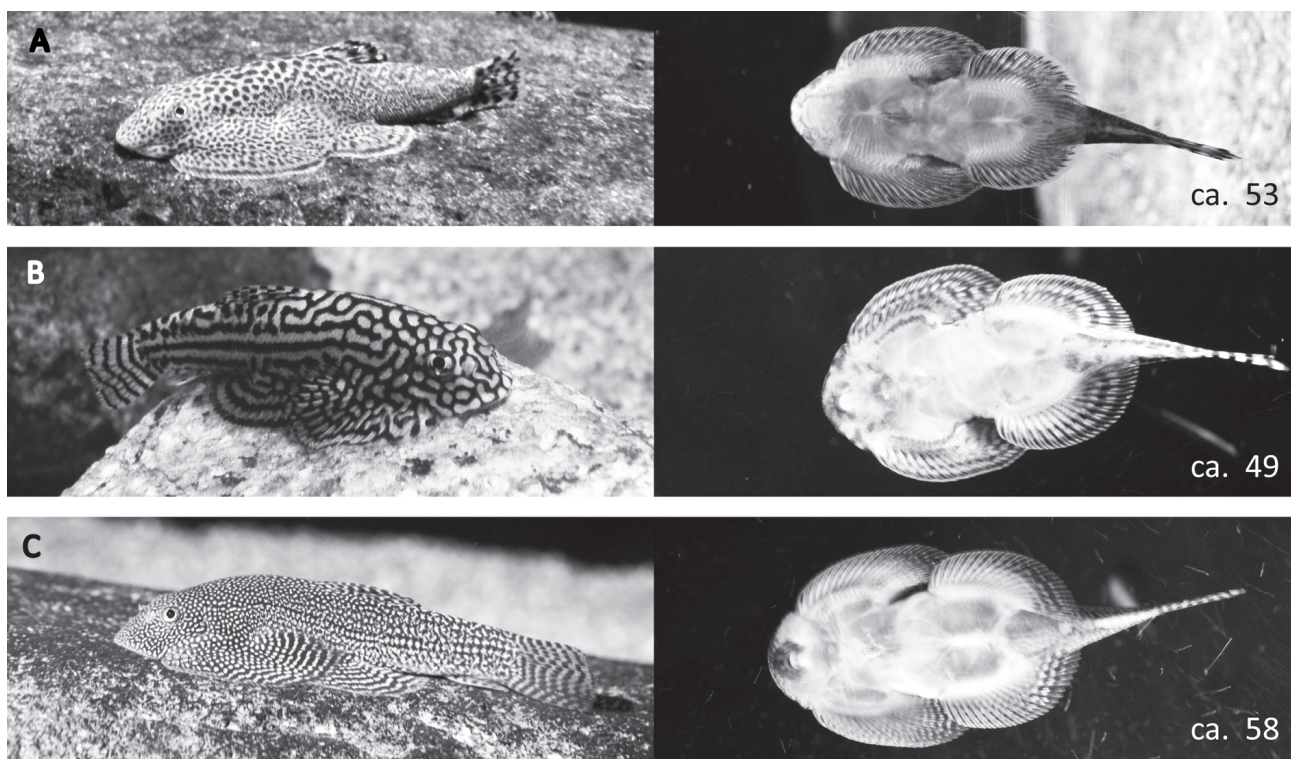
**Table 1.** Summary of observations of the white linear markings.

Family	Species	No. of individuals observed	Existence of the white linear markings	Uniqueness of the shape of the linear markings
Nemacheilidae	<i>Nemacheilus</i> cf. <i>pallidus</i>	4	Yes	Yes
	<i>Nemacheilus</i> sp.	5	Yes	Yes
	<i>Mesonoemacheilus guentheri</i>	4	Yes	Yes
	<i>Schistura</i> cf. <i>similis</i>	2	Yes	Yes
	<i>Traccatichthys pulcher</i>	8	Yes	Yes
	<i>Barbatula toni</i>	20	Yes	Yes
Balitoridae	<i>Beaufortia kweichowensis</i>	5	No	-
	<i>Sewellia lineolata</i>	7	No	-
	<i>Sewellia</i> sp.	3	No	-



**Fig. 1.** Photos of the abdominal region of each individual of nemacheilid loaches, showing existence of unique linear markings. A: *Nemacheilus* cf. *pallidus*, B: *Nemacheilus* sp., C: *Mesonoemacheilus guentheri*, D: *Schistura* cf. *similis*, E: *Traccatichthys pulcher*. The numerical number in each photo shows the individual body length (mm).





**Fig. 2.** Photos of the abdominal region of individuals of balitorid loaches, all individuals were observed not to show the linear markings. A: *Beaufortia kweichowensis*, B: *Sewellia lineolata*, C: *Sewellia* sp. The numerical number in each photo shows the individual body length (mm).

photographed through the glass of the tank onto which they adhered on in October 2009.

Specimens of *B. toni* were reared from eggs spawned on 15 May 2007 by parental fish caught from the River Abira, Astuma, Hokkaido, Japan. Seven individuals were introduced into a glass tank (90 cm × 45 cm × 40 cm) at the beginning of the observation in November 2007 and a further 13 individuals were added in April 2008. The existence and shape of linear white markings were documented photographically as described above at intermittent intervals until May 2010. Water temperature during the rearing period changed between ca. 10 °C in winter and ca. 25 °C in summer.

All individuals were measured for their body size at photographing of the abdominal region and were subsequently returned to their rearing tank after they had completely recovered from the anesthetization.

## Results

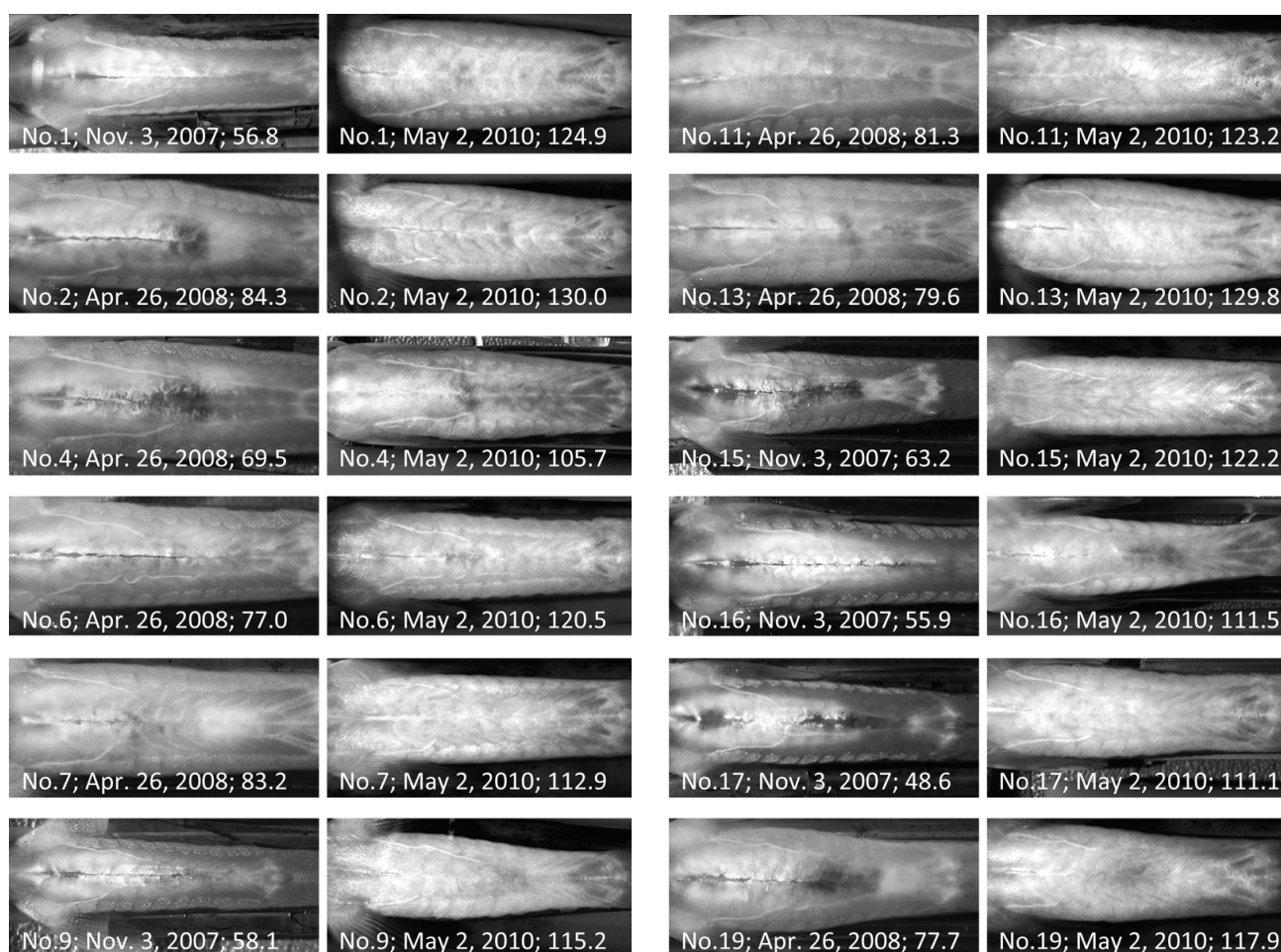
Unique linear markings were observed in all six species of Nemacheilidae, but no linear markings could be observed in any of the three species of Balitoridae (Fig. 1, 2, 3; Table 1). The shapes of the linear markings, including curving, bending, splittings

and confluence again, varied from fish to fish within each species of Nemacheilidae (Fig. 1, 3).

In the first observation of each individual of *B. toni* on November 2007 or April 2008, based on the shape of the unique linear markings, each individual was identified and they were named from no. 1 to no. 20. During a period of more than two years until May 2010, all individuals of *B. toni* could be identified by the shapes of their linear markings (Fig. 3). During the period, mean body size (FL: fork length) of the reared and surviving individuals at each observation was 56.1 mm (n = 7) in November 2007, 73.6 mm (n = 20) in April 2008, 116.8 mm (n = 19) in October 2009 and 118.4 mm (n = 17) in May 2010, respectively.

## Discussion

In the present study, the unique linear markings within each species were observed in all six examined nemacheilid species, although the number of observed individuals was small in each species. This suggests that the existence of unique linear markings is expected to be a common characteristic to nemacheilid loaches. Furthermore, the reared individuals of *B. toni* had grown larger over the two-year observation period and the individuals could be continuously identified



**Fig. 3.** Photos of the abdominal region of both the first and last observations in each individual of *B. toni*, showing long-term stability of linear markings. The label in each photo shows the individual number, observation date and fork length (mm).

by the shapes of the linear markings during the period. Therefore, shapes of the linear markings did not change with the long-term growth in this species as in the cases in both *Lefua* sp. (Aoyama 2000) and *L. echigonia* (Akada et al. 2005). These observations indicate that shapes of the linear markings are also expected to be stable. The linear markings could be observed only in live specimens, but not in specimens fixed in ethanol or formaldehyde, because the skin of

them turns white or opaque. Therefore, the method for individual identification of live specimens using the linear markings is a potentially useful non-intrusive method for nemacheilid species, some of which are threatened with extinction. More observations on other species of nemacheilids are expected to allow successive individual identification-recapture investigation in their natural environment.

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