

Dorsal spinule patch variations in the puffer Lagocephalus spadiceus from Japan; revisited evidence for the existence of "spadiceus"- and "wheeleri"-forms.

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1	Dorsal spinule patch variations in the puffer Lagocephalus spadiceus from Japan; revisited evidence for
2	the existence of "spadiceus"- and "wheeleri"-forms
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20	Running head: Two forms of Lagocephalus spadiceus
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22	Short report, 6 text pages including title page, 1 page of figure captions, 3 figures, 3 tables.
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27 Abstract

28	Examination of the dorsal spinule patch in Lagocephalus spadiceus specimens revealed the existence of two
29	intraspecific forms, the "wheeleri"-form, characterized by an elliptical patch, and the "spadiceus"-form, with a
30	rhomboidal patch with a continuous or intermittent posterior extension. The forms were not indicative of sexual
31	dimorphism, although the appearance of a posterior extension of the dorsal patch was apparently growth-related.
32	Ontogenetic studies of both forms, including genetic analyses and cross breeding experiments, should be future
33	considerations.
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37	Keywords Tetraodontidae · Lagocephalus · Dorsal spinule patch · Morphology
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38 Introduction

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40	Due to the generally toxic nature of most Lagocephalus species, only three of the nine species currently
41	recognized are permitted for consumption in Japan (MHLW 1983; Harada and Abe 1994), viz. kuro-sabafugu [L.
42	cheesemanii (Clarke)], kanafugu [L. inermis (Temminck and Schlegel)], and shiro-sabafugu [L. spadiceus
43	(Richardson)]. The last mentioned (Fig. 1) was for a time recognized in Japanese waters as L. wheeleri Abe,
44	Tabeta and Kitahama [characterized by an elliptical dorsal spinule patch and referred to here as "wheeleri-form",
45	Figs. 1b, 2a], separate from L. spadiceus [characterized by a rhomboidal patch with a posterior extension, and
46	referred to here as "spadiceus-form", Figs. 1a, 2c] which was accorded the Japanese name moto-sabafugu (not
47	permitted for culinary use). However, the culinary status of moto-sabafugu changed with the synonymization of
48	L. wheeleri under L. spadiceus by Matsuura (2010), due to variations in the dorsal patch making them
49	specifically inseparable (Fig. 2).
50	In fact, both forms, including variations, can be caught during the same fishing operation, their appearance
51	being almost inseparable (Fig. 1), according to local fishermen. However, the degree and significance, including
52	any sex specific relationship, of the spinule patch variations have remained unclear, due to the small number of
53	specimens (43 individuals) examined by Matsuura (2010). During the present study, variously sized specimens
54	of L. spadiceus were examined in order to determine any significant variation patterns in the dorsal spinule
55	patch.
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57	Materials and methods
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59	A total of 423 individuals unloaded at ports in Yamaguchi and Fukuoka Prefectures in 2010, 2011 and 2017,
60	including 109 individuals used for sex determination and 314 individuals for morphological observations were
61	examined (Table 1). Originally, Abe et al. (1984) described Lagocephalus wheeleri as "the dorsal spinulose area
62	narrows posteriorly and its rearward extension varies from individual to individual, sometimes being interrupted,

63 but never reaching to the dorsal-fin origin", based on only three specimens, and it was synonymized with L.

spadiceus by Matsuura (2010) because of their inseparable dorsal spinule patch extension variation. Therefore,
variations in the dorsal spinule patch were grouped expediently here as follows: typical *wheeleri*-form (Fig. 2a),
with an elliptical dorsal spinule patch without posterior patch extension, *spadiceus*-form (Fig. 2b), with a wide
variation of intermittent posterior patch extension, and typical *spadiceus*-form (Fig. 2c), with continuous
posterior patch extension.

69 Sex was determined by direct gonad observation. It was difficult to say from where the posterior extension 70 of the rhomboidal dorsal spinule patch starts especially in the typical spadiceus-form. Differences in dorsal 71spinule patch shape, therefore, were determined by comparing the distance from the patch posterior end to the 72 origin of the dorsal fin base (D in Fig. 2) as % of standard length (SL) (d-value hereafter), following Matsuura 73 (2010). Head length, pre-dorsal fin length, pre-anal fin length, and pectoral fin length as measurements, and 74numbers of vertebrae, dorsal fin rays, and fin rays, and pectoral fin rays were also compared among three forms. The Chi-square (X^2) test was used to consider form frequencies, and differences in actual measurements and 75 76 d-values (%) were tested by analysis of co-variance (ANCOVA), and counts by analysis of variance (ANOVA).

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78 **Results and Discussion**

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80 The three groups (typical wheeleri-, spadiceus- with intermittent extension, and typical spadiceus- with 81 continuous extension) occurred in all the samples (Table 1), although their frequencies differed significantly among samples ($X^2 = 67.874$, p < 0.0001). Both sexes were represented in all three groups [Table 2, also see 82 83 Electronic supplementary material (ESM) Table S1], males slightly outnumbering females, although statistically insignificant ($X^2 = 1.550$, p = 0.213), indicating that spinule patch shape was unrelated to gender. 84 85 All measurements and counts examined (ESM Table S2) were significantly different among the three groups other than the distance from the patch posterior end to the origin of the dorsal fin base (F = 421.103, p < 10086 87 0.0005) (Table 3). D-values of all individuals were plotted on a scatter diagram (Fig. 3), values also being significantly different among the three groups (F = 768.962, p < 0.0005), although declining gradually with 88 89 growth in each. Some smaller, intermittent patch extension *spadiceus*-form overlapped typical *wheeleri*-form,

but most of the former overlapped typical *spadiceus*-form. Consequently, two overall groups were apparent, the *wheeleri*-form and *spadiceus*-form, the intermittent-extension individuals conforming with typical continuous extension *spadiceus*-form. The distance from the patch posterior end to the origin of the dorsal fin base also differed significantly between the two overall groups (actual values, F = 326.773, p < 0.0005; *d*-values, F =631.229, p < 0.0005), which were clearly independent of gender, despite the comment by Matsuura (2010) that "the two forms cannot be separated by the shape of the spinule patch distribution on the back".

It is possible that the posterior extension of the dorsal patch appears gradually with growth, the smallest
 individuals examined (< 50 mm SL, five individuals) all being *wheeleri*-form, with no extension. In addition,

98 typical *spadiceus*-form individuals comprised proportionately fewer of the smaller specimens, whereas

99 intermittent-extension *spadiceus*-form individuals were fewer of the larger specimens, with the distance from the

100 patch to the dorsal fin gradually decreasing with growth (Fig. 3). Clearly, an ontogenetic study of the dorsal

spinule patch of *L. spadiceus* is necessary, in view of the single brief comment by Fujita (1962) that dorsal

spinules had already appeared in a 28 mm total length juvenile.

103 The genetic characteristics of the dorsal spinule patch of *L. spadiceus* are unknown to date. A future 104 genetic study, possibly involving a cross breeding experiment between the two forms, should provide some 105 clarification. If an inherited characteristic, the dorsal spinule patch shape may in fact be controlled by a small 106 number of alleles, since only two forms of *L. spadiceus* exist (with or without a posterior extension of the 107 spinule patch).

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142	Figure captions
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144	Fig. 1 Photos of <i>Lagocephalus spadiceus</i> , both ca. 250 mm SL; a, <i>wheeleri-</i> form; b, <i>spadiceus-</i> form
145	
146	Fig. 2 Dorsal spinule patch forms of <i>Lagocephalus spadiceus</i> as schematic drawings; a , typical <i>wheeleri</i> -form;
147	b , <i>spadiceus</i> -form with intermittently extended spinule patch, some representatives of a wide variation; c ,
148	typical spadiceus-form with continuously extended patch. D, distance from dorsal spinule patch tail to
149	origin of dorsal fin; SL, standard length
150	
151	Fig. 3 Scatter diagram of relative distance from dorsal spinule patch tail to dorsal fin (as % SL); closed circles,
152	typical wheeleri-form; cross symbols, spadiceus-form with intermittently extended spinule patch, open

circles, typical *spadiceus*-form with continuously extended patch







		Number of individuals				
		wheeleri-form	spadiceus-form			
		elliptical dorsal patch	dorsal patch with posterior extension		_	Standard length (mm)
Landing localities	Collection date		intermittent	continuous	Total	mean (min, max)
For gender observations						
Shimonoseki, Yamaguchi Prefecture	10th Oct. 2017	48	44	17	109	191.0 (157.0, 262.0)
For morphplogical observations						
Murotsu, Yamaguchi Pref.	22nd Jul. to 20th Sep. 2011	90	48	12	150	89.7 (31.0, 132.8)
Hagi and Tokuyama, Yamaguchi Pref. Kanesaki, Fukuoka Pref.	9th Nov. 2010	22	41	51	114	199.1 (150.2, 241.5)
Kawatana, Yamaguchi Pref.	15th Nov. 2010	30	9	11	50	272.4 (227.4, 314.0)

Table 1. Landing locarlities, collection dates, number of individuals and standard length data of Lagocephalus spadiceus examined

	Number of individuals		
Form	Female	Male	
wheeleri-form			
eliptical dorsal patch	23	25	
spadiceus-form			
intermittent extension	19	25	
continuous extension	6	11	
Total	48	61	

Table 2. Sex ratios of two forms of Lagocephalus spadiceus

Table 3. Some relative lengths and counts for *wheeleri*-form, *spadiceus*-form with intermittent and continuous dorsal spinule patch posterior extension of *Lagocephalus spadiceus*, and their difference among forms based on actual values, ANCOVA (analyses of co-variance) for measurements and ANOVA (analyses of variance) for counts

	mean \pm standard deviation (range)				
	wheeleri-form	spadiceus-form			
	elliptical dorsal patch	dorsal patch with posterior extension			
		intermittent	continuous	<i>F</i> -value	р
In % of standard length					
Head length	31.2 ± 1.4 (28.0, 38.2)	30.5 ± 0.9 (28.8, 33.8)	30.5 ± 1.0 (28.5, 34.0)	0.096	0.192
Predorsal length	64.0 ± 1.6 (60.7, 70.0)	63.6 ± 1.2 (61.0, 66.8)	63.4 ± 1.2 (60.4, 66.2)	1.390	0.251
Preanal length	66.0 ± 1.2 (63.6, 70.0)	66.0 ± 1.4 (62.7, 70.2)	65.7 ± 1.3 (63.1, 69.5)	0.126	0.881
Pectoral fin length	15.5 ± 1.4 (13.1, 19.6)	15.7 ± 1.4 (13.1, 18.9)	16.4 ± 1.1 (12.8, 18.6)	1.365	0.257
Spinule patch end to dorsal fin base (<i>d</i> -value)	23.1 ± 2.8 (15.4, 29.9)	11.8 ± 4.2 (5.4, 24.5)	7.7 ± 2.1 (4.2, 16.7)	421.103	< 0.0005
Vertebrae	19.1 ± 0.4 (18, 20)	19.0 ± 0.4 (18, 20)	19.1 ± 0.4 (18, 20)	1.083	0.340
Pectoral rays	15.0 ± 0.5 (14, 16)	15.0 ± 0.5 (14, 16)	15.1 ± 0.4 (14, 16)	0.031	0.970
Dorsal rays	$12.3 \pm 0.5 (11, 13)$	12.4 ± 0.5 (11, 13)	12.4 ± 0.5 (12, 13)	0.934	0.394
Anal rays	11.0 ± 0.4 (9, 12)	11.0 ± 0.3 (10, 12)	11.0 ± 0.3 (10, 12)	0.987	0.374