

# 琉球大学学術リポジトリ

形態および遺伝的証拠から同定したルリメイシガキ  
スズメダイ (硬骨魚綱スズキ目スズメダイ科)  
の浮遊期稚魚

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## Pelagic juvenile of *Plectroglyphidodon johnstonianus* Flower & Ball, 1924 (Perciformes, Pomacentridae, Stegastinae) identified from morphometric and genetic evidence

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**Abstract.** The morphology of pelagic juveniles of *Plectroglyphidodon johnstonianus* (Perciformes, Pomacentridae) is reported on the basis of two specimens collected from the offshore region of Aguni Island, Okinawa, Japan. The juveniles were identified using morphometric and genetic evidence. The juveniles were characterized by their round body, elongated pectoral fin, and melanophore pigmentation pattern. In addition, fresh specimens had a large red spot on the lateral side of the body.

### Introduction

Family Pomacentridae is a relatively large group, including 28 genera and about 348 species (Nelson 2006). The great diversity and abundance of pomacentrids, especially around coral reefs, are indicative of their great ecological importance (Kavanagh et al. 2000). However, morphological knowledge on the early stages of the life history of pomacentrids is limited because of the lack of reliable diagnostic features. Genus *Plectroglyphidodon* contains 10 species from tropical to subtropical Indo-Pacific waters. Six species of the genus *Plectroglyphidodon* are widely distributed in Indo-Pacific waters, and other four species are endemic to Tuamotu, Hawaii, Marquesas and Mauritius, respectively (Allen 1991; Randall & Earle 1999; Aonuma et al. 2013). The early stage morphology of *Plectroglyphidodon* is known for *P. leucozonus* (Bleeker, 1859) and *P. johnstonianus* Fowler & Ball, 1924, only by Kinoshita (2014). Larvae and juveniles of those *Plectroglyphidodon* species can be distinguished from other pomacentrids by a combination of the characters of the fin counts, high body depth, and elongated pectoral fin (Kinoshita 2014). In his description, Kinoshita (2014) mentioned that the pigmentation of the examined specimen of *P. johnstonianus* was faded and not complete. Recently, we collected two fresh juveniles of *P. johnstonianus* from the offshore waters of Aguni Island. These specimens still retained their distinct color pattern when collected. The present study describes the morphological features of the

juveniles of *P. johnstonianus* on the basis of the fresh specimens.

### Material and methods

**Specimens.** Two pomacentrid juveniles were obtained with a scooping net and aquatic lamp from 34 km offshore of Aguni Island (26°53'54"N, 127°15'49"E) on the night of May 1, 2014 (Fig. 1). The specimens were fixed in 10% formalin for 15 hours and preserved in 70% ethanol thereafter. After taking photographs and removing tissue for genetic analysis, the specimens were again put into 10% formalin for 5 days to bleach the nonmelanistic chromatophores and preserved again in 70% ethanol thereafter. All specimens were deposited in the collection of the Okinawa Churashima Foundation (OCF).

**Observation, measurements and counts.** The specimens were stained with Cyanine 5-R (see Kinoshita 1987). Stained samples were examined under a microscope (Keyence VHX-1000). The following characters were measured: standard length (SL), body depth (BD), predorsal fin length (PDL), preanal length (PAL), head length (HL), eye diameter (ED), and snout length (SNL). The number of dorsal, anal, pectoral, and pelvic fin spines and soft rays, pored scales on the lateral line (LLp), and spines on the outer preopercular were

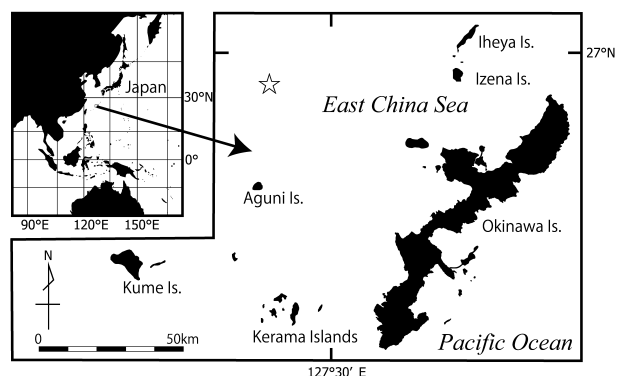


Fig. 1 Collection site of pelagic juveniles of *Plectroglyphidodon johnstonianus* (open star).

図 1. ルリメシガキスズメダイ浮遊期稚魚の採集場所 (星印).

counted. Each measurement and count followed the definition of Leis and Carson-Ewart (2000).

**Genetic analyses.** The right eyeballs of two juveniles in their first preservation in ethanol were used for genetic analyses. The tissue samples were dissolved in 18  $\mu$ l of 50 mM NaOH, incubated at 95 °C for 10 min, and neutralized by 1 M Tris-HCL (pH 8.0, 2  $\mu$ l). The templates for DNA amplification were used from the supernatant liquid from the centrifugal process (12000 rpm, 5 min) of the lysate. A partial sequence of the mitochondrial DNA 16S rRNA region (ca. 580 bp) was amplified by the polymerase chain reaction (PCR) using KOD FX Neo (Toyobo Inc.) and the following primers: L2510 (5'-GCCTGTTTAACAAAAACAT-3') and H3059 (5'-CGGTCTGAACTCAGATCACGT-3') (Miya & Nishida 1996). PCR amplifications were carried out with a Gene Atlas 322 (Astec Inc.) Thermal Cycler at 94 °C for 2 min, followed by 15 cycles of denaturation at 98 °C for 10 s, annealing at 56 °C for 15 s and 68 °C for 20 s, 20 cycles of denaturation at 98 °C for 10 s, annealing at 53 °C for 15 s and 68 °C for 20 s, and extension at 68 °C for 2 min. Sequence reactions were analyzed on an ABI 3730XL sequencer (Applied Biosystems) using the BigDye terminator v3.1 Cycle Sequencing Kits (Applied Biosystems). The two sequences obtained were submitted to the GenBank nucleotide sequence databases under the accession numbers AB979701 and AB979702. The partial sequences of 16S (547–564 bp) of all six widely distributed species of *Plectroglyphidodon* were downloaded from GenBank (accession numbers in brackets): *P. dickii* (n = 3: FJ616469, JF457555, JF457556), *P. imparipennis* (n = 4: JF457557–JF457560), *P. johnstonianus* (n = 5: JF457561–JF457565), *P. lacrymatus* (n = 7: AF285955, FJ616470, JF457566–JF457570), *P. leucozonus* (n = 1: FJ616471), and *P. phoenixensis* (n = 2: JF457571, JF457572). All sequences were aligned using the program ClustalW (Higgins et al. 1994). Neighbor-joining and distance matrices were generated using MEGA6 (Tamura et al. 2013) in an analysis of Kimura two-parameter distances (Kimura 1980) based on the common sequences.

## Results and discussion

**Species identification.** Kinoshita (2014) indicated that juveniles of *Plectroglyphidodon* could be distinguished from other pomacentrids by the combination of the dorsal, anal, and pectoral fin ray counts, relatively high body depth, and elongated pectoral fin. Since the collected juveniles agree

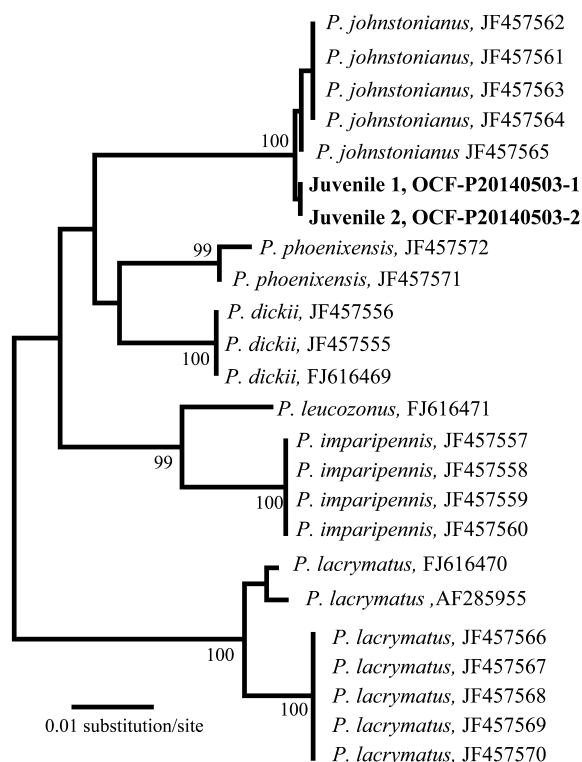


Fig. 2 Neighbor-joining tree based on sequences of 16S rRNA partial region of mitochondrial DNA of *Plectroglyphidodon* species with wide distribution in the Indo-Pacific waters (6 species); distances were calculated using Kimura-two parameter model of base substitution. The numbers beside internal branches indicate bootstrap probabilities (>90%) based on 1,000 pseudoreplicates.

図 2. インド-太平洋域の広範囲に生息するイシガキスズメダイ属6種のミトコンドリア DNA 16S 領域の塩基配列の類似性に基づいた近隣結合樹状図。遺伝的距離は木村の 2 変数法を用いて算出した。分枝の数値は 90%以上のブートストラップ値 (1000 回反復) を示す。

with *Plectroglyphidodon* in their fin counts, relatively high body depth (45.1–46.8% to BL, Table 1), and elongated pectoral fins, they could be identified to *Plectroglyphidodon*. From comparison with fin counts between two juveniles (Table 1) and all *Plectroglyphidodon* species (Table 2), juvenile 1, which has 16 anal fin soft rays, resembled *P. dickii*, *P. flaviventris* and *P. johnstonianus*, and juvenile 2, which has 17 anal fin soft rays, corresponded with the counts of *P. johnstonianus*.

A neighbor-joining tree based on the genetic sequences is shown in Fig. 2. The six species inhabiting Japanese waters clearly each formed a separate clade with very high bootstrap values, and the two juveniles were contained in the clade of *P. johnstonianus*. Accordingly, the two juveniles were identified as *P. johnstonianus* based on their

Table 1 Meristic and proportional measurements of two pelagic juveniles of *Plectroglyphidodon johnstonianus* collected from offshore of Aguni Island.

表 1. 粟国島沖で採集されたルリメイシガキスズメダイの浮遊期稚魚の計数形質および計測部位の相対比.

	Juvenile 1 OCF-P20140503-1	Juvenile 2 OCF-P20140503-2
Standard length (SL: mm)	12.6	14.2
標準体長 (mm)		
Proportional measurements (%)		
計測値相対比 (%)		
Body depth to SL	46.8	45.1
体高体長比		
Predorsal fin length to SL	34.6	32.5
背鰭前長体長比		
Preanal length to SL	59.5	58.4
肛門前長体長比		
Head length (HL) to SL	37.5	35.2
頭長体長比		
Eye diameter to HL	33.4	35.4
眼径頭長比		
Snout length to HL	25.4	27.4
吻長頭長比		
Meristic characters		
計数形質		
Dorsal fin rays	XII, 18	XII, 18
背鰭鰭条数		
Anal fin rays	II, 16	II, 17
臀鰭鰭条数		
Pectoral fin rays	19	19
胸鰭鰭条数		
Pelvic fin rays	I, 5	I, 5
腹鰭鰭条数		
Pored lateral line scales	19+	19+
側線有孔鱗数		
Spines on outer preopercular	6	5
前鰓蓋骨外縁棘数		

meristic counts and DNA sequences.

**Morphological description.** Proportions and meristic counts are listed in Table 1. Photograph and line drawing are indicated in Fig.3. Body laterally compressed. Gut coiled and compact, with virtually no gap between anus and anal fin. Head rounded, compressed laterally, large. Snout less than eye diameter. Mouth moderately oblique. Scales covering whole body, pore scale forming lateral line except for caudal peduncle. Head spination poorly developed; 5–6 small spines on outer preopercular edge. Counts of all fins completed. Pectoral fin elongate at level of middle

of dorsal, anal soft rays base. In fresh specimens (before breaching), guanophores spreading between eye and caudal peduncle of lateral body, remarkable large red spot (erythrophore) present on posterior portion of body. These colors faded after five days formalin preservation. Melanophores present on tip of upper and lower jaws, dorsal portion of head and anterior portion of trunk, dorsal side of abdominal cavity, posterior portion of lateral body, dorsal soft ray base, pectoral fin, anterior edge of dorsal fin.

**Remarks.** The morphological features of the juveniles of *Plectroglyphidodon johnstonianus* examined in the present study closely correspond to

Table 2. Dorsal, anal, and pectoral fin ray counts of all *Plectroglyphidodon* species. Data sources as follows. Al: Allen (1991); R: Randall & Earle (1999); Ao: Aonuma et al. (2013).

表 2. イシガキスズメダイ属全種の背・臀・胸鰭条数と分布域. 出典は以下の略語で示した. Al: Allen (1991); R: Randall & Earle (1999); Ao: Aonuma et al. (2013).

Species name 種名	Dorsal 背鰭	Anal 臀鰭	Pectoral 胸鰭	Distribution 分布	Source 出典
<i>P. dickii</i>	XII, 16–18	II, 14–16	17–19	Widespread Indo-Pacific インド-太平洋の広範囲	Al, Ao
<i>P. flaviventris</i>	XII, 18–19	II, 15–16	18–19	Tuamotu (endemic) トゥアモトゥ (固有種)	Al
<i>P. imparipennis</i>	XII, 14–16	II, 11–12	19–21	Widespread Indo-Pacific インド-太平洋の広範囲	Al, R, Ao
<i>P. johnstonianus</i>	XII, 18–19	II, 16–18	19	Widespread Indo-Pacific インド-太平洋の広範囲	Al, Ao
<i>P. lacrymatus</i>	XII, 15–18	II, 13–15	18–20	Widespread Indo-Pacific インド-太平洋の広範囲	Al, Ao
<i>P. leucozonus</i>	XII, 14–16	II, 11–13	19–21	Widespread Indo-Pacific インド-太平洋の広範囲	Al, Ao
<i>P. phoenixensis</i>	XII, 16–17	II, 13–14	20–21	Widespread Indo-Pacific インド-太平洋の広範囲	Al, Ao
<i>P. randalli</i>	XIII, 15–16	II, 12	19–20	Mauritius (endemic) モーリシャス (固有種)	Al
<i>P. sagmarius</i>	XII, 14–15	II, 11–12	19–21	Marquesas (endemic) マルキーズ (固有種)	R
<i>P. sindonis</i>	XII, 19–20	II, 15	21	Hawaii (endemic) ハワイ (固有種)	Al

those of Kinoshita's (2014) description of a similar-sized specimen (14.3 mm SL) of *P. johnstonianus*. The previous description differs from our observations in the head pigmentation. The melanophores in the present study were densely present all over the body, and head pigmentation was also observed. Kinoshita (2014) already mentioned the possibility that the pigmentation of his specimen may have faded. Although the number of outer preopercular small spines in the present study (5–6) was smaller than that of Kinoshita (2014) (10), this difference is an allowable variation among individuals. The distinctive red spot on the lateral body of pelagic juvenile had been unknown in the settlement stage (ca. 2.5–3.0 cm in total length) of *Plectroglyphidodon*, including *P. johnstonianus* (Masuda & Kobayashi 1994; Kato 2011). Thus, this spot appears to be a temporal feature during the pelagic stage.

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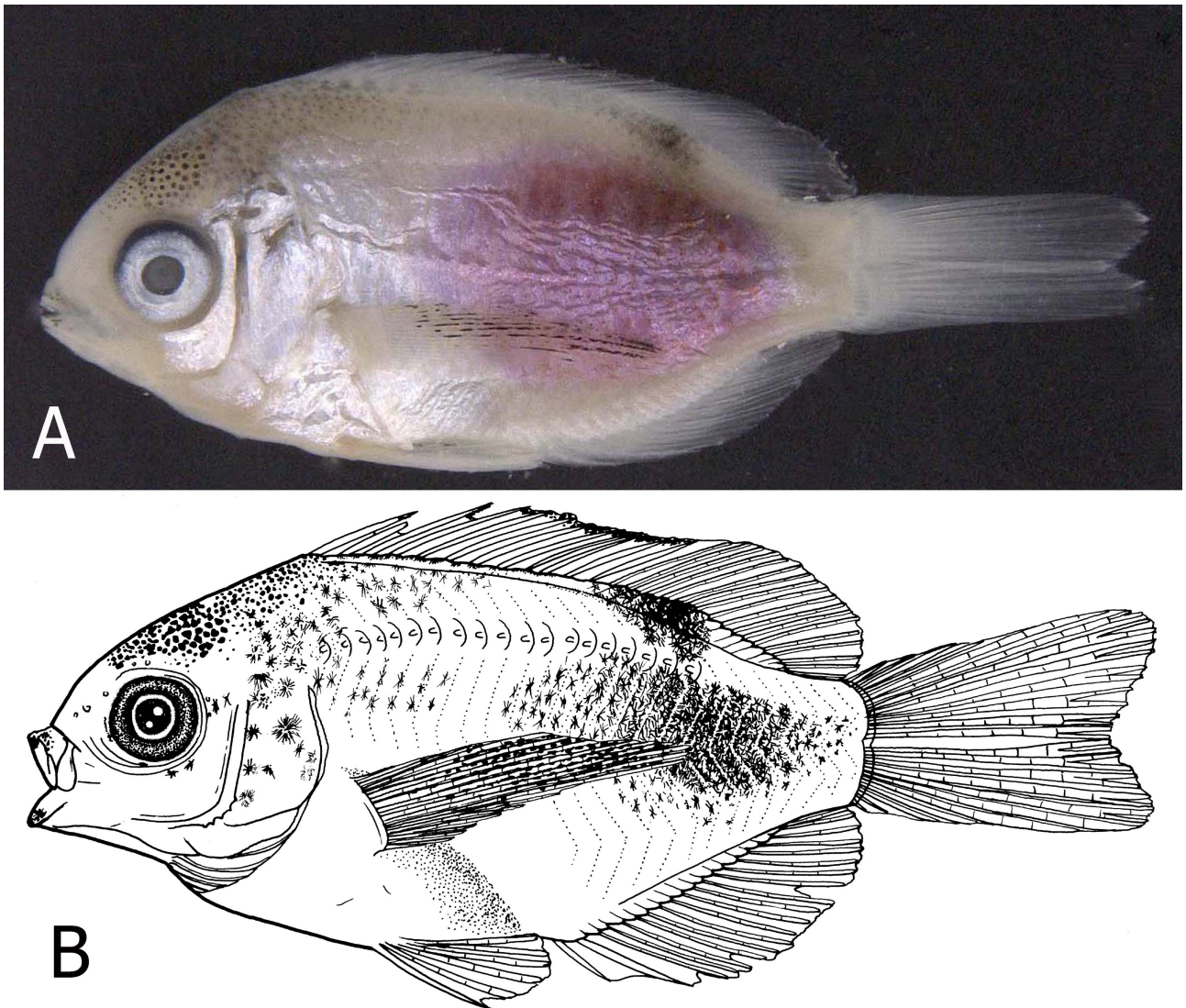


Fig. 3 Lateral view of a pelagic juvenile of *Plectroglyphidodon johnstonianus*. A: specimen before bleaching nonmelanistic chromatophores (Juvenile 1: OCF-P20140503-1, 12.6 mm in standard length); B: specimen after bleaching (Juvenile 2: OCF-P20140503-2, 14.2 mm in standard length). Specimen is fully scaled, but scales are shown only along lateral line.

図 3. ルリメシガキスズメダイ浮遊期稚魚の側面図。A: 黒色色素以外の色素脱色前 (標準体長 14.2 mm, OCF-P20140503-1); B: 色素脱色後 (標準体長 14.2 mm, OCF-P20140503-2) . 当標本は全身が鱗で覆われていたが、ここでは側線上のもののみを示した。

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## 形態および遺伝的証拠から同定したルリ メイシガキスズメダイ（硬骨魚綱スズキ 目スズメダイ科）の浮遊期稚魚

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**要旨.** 沖縄県粟国島沖で採集されたルリイシ  
ガキスズメダイの浮遊期稚魚（2 個体）を形態  
的および遺伝的証拠から同定し、その形態を記  
載した。当稚魚の形態は丸く寸詰まりの体形、  
伸長する胸鰭条および黒色色素の分布パター  
ンに特徴付けられる。加えて、色素（黒色色素  
を除く）脱色前の標本の体側には特徴的な赤色  
大斑が認められた。

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