## Original Article

# A New Species with Two New Subspecies of *Rhinogobius* (Teleostei: Gobiidae) from Yaeyama Group, the Ryukyu Islands, Japan

Toshiyuki Suzuki<sup>1)</sup>, Naoharu Oseko<sup>2)</sup>, Yo Y. Yamasaki<sup>3)</sup>, Seishi Kimura<sup>4)</sup> & Koichi Shibukawa<sup>5)</sup>

**Abstract.** A new freshwater species with two new subspecies of the gobiid fish genus *Rhinogobius* is described from the Yaeyama Group of the Ryukyu Islands, Japan. One of the subspecies, Rhinogobius aonumai aonumai (29 specimens, 35.9-70.5 mm SL) known only from Iriomote-jima Island, is distinguished from all congeneric species-group taxa (species and subspecies) by having the following combination of features: 9-15 predorsal scales; 32-37 longitudinal scales; 11+15-17=26-28 vertebrae (mode 27); anteriormost two pterygiophores (proximal radials) of the second dorsal fin mounted over the neural spine of 10th vertebra; fifth segmented pelvic-fin ray divided into 3-4 (usually four) branches at the position where proximal-most segment of each branch alignes transversely; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with vertical rows of dark spots or forming dark zigzag bands. The other subspecies, Rhinogobius aonumai ishigakiensis (12 specimens, 33.3-56.5 mm SL) known only from Ishigaki-jima Island, is distinguished from all congeneric species-group taxa by having the following combination of features: 10–14 predorsal scales; 33–38 longitudinal scales; 10+16-18=26-28 vertebrae (mode 27); anteriormost two pterygiophores (proximal radials) of the second dorsal fin mounted over the neural spine of 9th vertebra; fifth segmented pelvicfin ray divided into 2-3 (usually two) branches at the position where the proximal-most segment of each branch alignes transversely; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with dark zigzag bands on the caudal fin.

**Key words:** description, fish taxonomy, freshwater resident, *Rhinogobius* sp. YB

#### Introduction

Rhinogobius Gill, 1859 comprises medium-sized freshwater gobies (reaching up to 30–100 mm in standard length) and is known from the East and Southeast Asian regions, including the Russia Far East, Japan, Korea, China, Taiwan, the Philippines, Vietnam, Laos, Cambodia, and Thailand (Chen & Miller, 2014). At least in the

insular habitats, a majority of the species of the genus are amphidromous; namely, adults spawn in the freshwater habitats, larvae just after hatching immediately go to the coastal marine waters, and after that, the juveniles enter the inland waters (Mizuno, 1960a). On the other hand, in the continental areas, many species of the genus are non-diadromous, freshwater inhabitants (e.g., lakes, ponds, and rivers) throughout their life cycle (Huang & Chen, 2007).

- 1) Osaka Museum of Natural History 1-23 Nagai Park, Higashi-Sumiyoshi-ku, Osaka 546-0034, Japan 大阪市立自然史博物館 〒546-0034 大阪府大阪市東住吉区長居公園1-23 Toshiyuki Suzuki: eviotatoshiyuki@gmail.com
- <sup>2)</sup> Ginowan, Okinawa 901-2212, Japan 〒901-2212 沖縄県宜野湾市
- <sup>3)</sup> National Institute of Genetics 1111 Yata, Mishima, Shizuoka 411-8540, Japan 国立遺伝学研究所 〒 411-8540 静岡県三島市谷田 1111

- 4) Graduate School of Bioresources, Mie University 1577 Kurimamachiya-cho Tsu, Mie 514-8507, Japan 三重大学大学院生物資源学研究科 〒 514-8507 三重県津市栗真町屋町 1577
- 5) Museum of Natural and Environmental History, Shizuoka 5762 Oya, Suruga-ku, Shizuoka, Shizuoka 422-8017, Japan ふじのくに地球環境史ミュージアム 〒 422-8017 静岡県静岡市駿河区大谷 5762

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Rhinogobius, originally described as a monotypic genus based on Rhinogobius similis Gill, 1859, is currently known as the most species-rich freshwater gobiid genus, comprising 86 described, valid species worldwide (Suzuki et al., 2020; Wanghe et al., 2020), although several additional species remain unnamed. In Japanese waters, 13 valid species hitherto have been confirmed: Rhinogobius biwaensis Takahashi & Okazaki, 2017, R. brunneus (Temminck & Schlegel, 1845), R. flumineus (Mizuno, 1960b), R. fluviatilis Tanaka, 1925, R. kurodai (Tanaka, 1908), R. mizunoi Suzuki, Shibukawa & Aizawa, 2017, R. nagoyae Jordan & Seale, 1906, R. ogasawaraensis Suzuki, Chen & Senou, 2012, R. simillis, R. telma Suzuki, Kimura & Shibukawa, 2019, R. tyoni Suzuki, Kimura & Shibukawa, 2019, R. yaima Suzuki, Oseko, Kimura & Shibukawa, 2020 and R. yonezawai Suzuki, Oseko, Kimura & Shibukawa, 2020. In addition, Akihito et al. (2013) reported five undescribed species from Japanese waters, all of which are distinguished by specific abbreviations as follows (each vernacular name in Japan is in parenthesis): Rhinogobius sp. BB (Aobarayoshinobori), Rhinogobius sp. KZ (Kazusa-yoshinobori), Rhinogobius sp. MO (Aya-yoshinobori), Rhinogobius sp. OM (Oumi-yoshinobori) and Rhinogobius sp. YB (Kibara-yoshinobori).

Yamasaki *et al.* (2020) reported that the speciation of *Rhinogobius* sp. YB from its amphidromous ancestor, *R. brunneus*, occurred in parallel across five islands groups (Amami-oshima Island, Tokuno-shima + Okinoerabu-jima islands, Okinawa-jima Island, Kume-jima Island, and Ishigaki-jima + Iriomote-jima islands in the Ryukyu Islands, Japan) using population genetic analysis.

We describe one of these populations from Ishigakijima and Iriomote-jima islands as a new species with two new subspecies of *Rhinogobius*. Namely, these new species/subspecies were known and confused under the name "*Rhinogobius* sp. YB" or "Kibara-yoshinobori" in the previous studies (e.g., Iwata, 1989; Akihito *et al.*, 2013; see "Discussion", below). The taxonomic status of the other populations of "*Rhinogobius* sp. YB" will be resolved elsewhere.

#### **Materials and Methods**

Yamasaki *et al.* (2020) reported that the populations of "*Rhinogobius* sp. YB" in the upper streams above the dam lakes in Ishigaki-jima Island contained individuals that have hybridized with the other congener *Rhinogobius* brunneus. For selecting type specimens in Ishigaki-jima Island, we thus used non-hybridized specimens with *R*.

brunneus in the upper streams of the dam lakes, judged from microsatellite markers and STRUCTURE analysis (Pritchard *et al.*, 2000) followed by Yamasaki *et al.* (2020). We also used the ones from the upper reaches above large waterfall(s), where *R. brunneus* is not or rarely found sympatrically. Note that no artificial dam lake is present in Iriomote-jima Island.

The specimens examined in this study are deposited in the following institutions: Kanagawa Prefectural Museum of Natural History, Odawara (KPM); National Museum of Natura and Science (NSMT); Osaka Museum of Natural History, Osaka (OMNH); Museum of Natural and Environmental History, Shizuoka (SPMN). The type series and comparative materials were collected at a time when no permission for the collection was required, or with permission from Okinawa or Kagoshima Prefectures.

All specimen lengths given are standard lengths (SL). Measurements were made point-to-point with calipers (in the case of the lengths more than 10 mm), or micrometer (10 mm or less) attached to the microscope to the nearest 0.1 mm. The methods for measurements followed those of Suzuki et al. (2020). The methods for counting followed Prince Akihito et al. (1984), except for scales between the origin of a dorsal fin and dorsal insertion of a pectoral fin (counting scales in an oblique row from the dorsalmost point of pectoral-fin base to the origin of the first dorsal fin). The information about squamation is based on specimens stained with Alizarin Red S. The count of scales was examined based on specimens temporary stained with cyanine blue or stained with Alizarin Red S. The observation of cephalic sensory system was examined based on specimens temporary stained with cyanine blue. The number of the first branches of the fifth segmented pelvic-fin ray was counted based on specimens stained with Alizarin Red S at the position where the proximal most segment of each branch alignes transversely (e.g., Fig. 3). Osteological features were observed from radiographs. The method of Akihito et al. (2013) is used in describing the pattern of the interdigitation of the dorsal-fin pterygiophores and neural spines ("P-V") with exceptions given below: "9&10" behind the second slash shows that anteriormost two pterygiophores (proximal radials) of the second dorsal fin mounted over the neural spine of the 9th and 10th vertebrae. The counts of vertebrae follow Akihito et al. (2013). Notations of cephalic sensory-canal pores and sensory-papillae rows followed Prince Akihito et al. (1984) and Suzuki et al. (2017), respectively. In the description, if there are differences between the holotype and paratypes data, data from the holotype are indicated

Table 1. Measurements for Rhingobius aonumai aonumai

| Cat. No.                       | OMNH-P     | OMNH-P     | OMNH-P     | KPM-NI     | SPMN-PI            | OMNH-P     | OMNH-P     | OMNH-P     |
|--------------------------------|------------|------------|------------|------------|--------------------|------------|------------|------------|
| Type status                    | Holotype   | 40254      | 40257      | 59988      | 46248<br>Paratypes | 40040      | 40043      | 43796      |
| Locality                       | Поготуре   | Hinai-gav  | wa River   |            | 1 aratypes         | Hranchi-o  | gawa River |            |
| Sex                            | Male       | Male       | Female     | Female     | Male               | Male       | Female     | Female     |
| SL (mm)                        | 65.9       | 66.0       | 61.5       | 56.5       | 48.0               | 46.3       | 46.0       | 47.5       |
| As % in SL                     | 05.9       | 00.0       | 01.5       | 30.3       | 46.0               | 40.3       | 40.0       | 47.3       |
| Head length                    | 34.9       | 34.1       | 30.1       | 31.2       | 33.3               | 33.5       | 32.6       | 33.7       |
| Predorsal length               | 41.0       | 41.7       | 38.4       | 38.4       | 40.2               | 40.4       | 39.1       | 40.6       |
| Length of snout to D2 origin   | 60.7       | 62.1       | 61.3       | 61.2       | 60.4               | 60.5       | 60.9       | 61.1       |
| Length of snout to anus        | 59.2       | 59.5       | 57.7       | 58.4       | 59.8               | 57.2       | 60.0       | 58.9       |
| Length of snout to A origin    | 63.1       | 63.6       | 62.8       | 62.1       | 66.5               | 63.9       | 65.2       | 63.2       |
| Prepelvic length               | 29.6       | 28.8       | 25.4       | 26.2       | 31.3               | 30.2       | 30.2       | 29.5       |
| Caudal peduncle length         | 25.2       | 23.5       | 24.4       | 26.5       | 24.0               | 23.8       | 23.9       | 22.9       |
| Caudal peduncle depth          | 12.3       | 12.1       | 12.2       | 12.6       | 13.1               | 13.4       | 13.0       | 13.5       |
| Length of D1 base              | 15.9       | 14.4       | 16.1       | 14.5       | 17.1               | 15.3       | 17.2       | 16.8       |
| Length of longest D1 spine*    | 15.0 (3rd) | broken     | 13.0 (2nd) | 12.7 (2nd) | 21.7 (2nd)         | 19.2 (2nd) | 14.8 (2nd) | 15.6 (3rd) |
| Length of D2 base              | 17.0       | 16.7       | 17.1       | 16.5       | 17.1               | 17.5       | 17.8       | 17.7       |
| Length of longest D2 ray*      | 12.9 (7th) | 14.2 (7th) | 13.7 (6th) | 14.0 (4th) | 17.7 (7th)         | 17.5 (8th) | 14.1 (4th) | 14.3 (2nd) |
| Length of last D2 ray          | 12.9       | 12.3       | 11.2       | 10.3       | 16.3               | 15.8       | 10.7       | 12.6       |
| Length of A base               | 13.5       | 14.8       | 13.5       | 13.3       | 14.6               | 15.1       | 13.5       | 15.4       |
| Length of longest A ray*       | 13.7 (7th) | 12.7 (5th) | 12.5 (6th) | 13.3 (6th) | 15.2 (5th)         | 14.9 (6th) | 13.7 (4th) | 13.1 (6th) |
| Caudal-fin length              | 22.8       | 21.2       | 22.0       | 20.4       | 22.9               | 23.3       | 23.5       | 21.7       |
| Pectoral-fin length            | 23.8       | 24.8       | 21.6       | 23.9       | 26.0               | 25.5       | 23.9       | 22.1       |
| Pelvic-fin length              | 15.9       | 16.4       | 16.3       | 15.9       | 17.9               | 17.3       | 18.9       | 16.4       |
| Body depth of A origin         | 15.3       | 14.7       | 15.9       | 15.6       | 15.8               | 16.6       | 16.5       | 17.5       |
| Body width of A origin         | 12.3       | 11.4       | 12.8       | 12.4       | 13.5               | 11.4       | 12.4       | 12.0       |
| Length of P2 origin to anus    | 30.3       | 32.7       | 32.8       | 35.2       | 29.2               | 31.5       | 32.2       | 31.8       |
| As % in HL                     |            |            |            |            |                    |            |            |            |
| Snout length                   | 35.7       | 35.6       | 32.4       | 31.3       | 37.5               | 34.8       | 32.7       | 31.3       |
| Eye diameter                   | 19.1       | 20.0       | 21.1       | 19.3       | 20.0               | 20.6       | 22.0       | 19.4       |
| Postorbital length             | 49.1       | 48.9       | 48.1       | 46.0       | 45.6               | 45.8       | 49.3       | 50.0       |
| Cheek depth                    | 28.3       | 28.4       | 27.6       | 28.4       | 31.3               | 29.7       | 26.7       | 26.9       |
| Head width in maximum          | 65.2       | 62.2       | 70.8       | 68.2       | 61.3               | 58.1       | 65.3       | 51.9       |
| Head depth in maximum          | 51.7       | 48.9       | 56.8       | 51.1       | 54.4               | 52.3       | 54.0       | 59.4       |
| Bony interorbital width        | 4.3        | 3.6        | 4.9        | 4.5        | 5.0                | 5.2        | 4.7        | 5.0        |
| Upper jaw length               | 40.9       | 44.0       | 37.8       | 35.8       | 45.6               | 41.9       | 38.0       | 38.8       |
| As % in Caudal peduncle length |            |            |            |            |                    |            |            |            |
| Caudal peduncle depth          | 48.8       | 51.6       | 50.0       | 47.3       | 54.8               | 56.4       | 54.5       | 58.7       |

Abbreviations: SL: standard length; D1: first dorsal-fin; D2: second dorsal-fin, A: anal fin, P2: pelvic fin. \*Longest ray is indicated in parentheses.

by asterisks. In the description of the counts, the frequency of each count is given in parentheses following the relevant count. Description of the coloration was based on digital images photographed on a white background. The names of colors follow those of the Japan Color Research Institute (1995). In "Variations between rivers", proportional characters were measured horizontally on the holotype and 21 paratypes images including all river populations in Iriomote-jima Island.

In the description of *R. aonuma aonumai*, proportional measurements on the holotype and seven paratypes are given in Table 1; The following observations of characteristics are based on the material in parentheses:

proportion and fins (holotype and seven paratypes), the first dorsal fin and length of pelvic fin (holotype and 23 paratypes), branching of the pectoral-fin rays, first branches of fifth segmented pelvic-fin ray, and sensory canal pores (holotype and 27 paratypes), squamation (20 paratypes), sensory-papillae rows and coloration when preserved in alcohol (holotype and a paratype), coloration when freshly collected (holotype and 19 paratypes), and coloration when alive (four underwater photographs). In the description of *R. aonumai ishigakiensis*, proportional measurements on the holotype and six paratypes are given in Table 2; the following observations of characteristics are based on the material in parentheses:

Table 2. Measurements for Rhingobius aonumai ishigakiensis

| Cat. No.                       | SPMN-PI<br>49269 | KPM-NI<br>65588 | SPMN-PI<br>49270 | OMNH-P<br>40912 | OMNH-P<br>40914 | OMNH-P<br>40911 | OMNH-P<br>40913 |
|--------------------------------|------------------|-----------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Type status                    | Holotype         |                 |                  | Para            | types           |                 |                 |
| Locality                       | Sal              | kuta-gawa Ri    | ver              | Miyara-ga       | awa River       | Sokobaru-       | gawa River      |
| Sex                            | Male             | Male            | Female           | Male            | Female          | Male            | Female          |
| SL (mm)                        | 51.5             | 55.9            | 45.0             | 38.0            | 38.4            | 39.9            | 33.3            |
| As % in SL                     |                  |                 |                  |                 |                 |                 |                 |
| Head length                    | 32.4             | 34.0            | 31.8             | 31.8            | 33.9            | 33.6            | 31.5            |
| Predorsal length               | 41.7             | 42.9            | 40.0             | 41.3            | 40.4            | 41.4            | 42.0            |
| Length of snout to D2 origin   | 60.4             | 61.7            | 61.1             | 62.1            | 59.9            | 60.7            | 60.1            |
| Length of snout to anus        | 57.1             | 60.6            | 59.8             | 61.1            | 59.9            | 59.1            | 58.6            |
| Length of snout to A origin    | 61.7             | 64.4            | 65.6             | 64.2            | 63.5            | 62.9            | 61.9            |
| Prepelvic length               | 30.3             | 32.2            | 29.1             | 29.2            | 26.0            | 28.8            | 24.9            |
| Caudal peduncle length         | 26.2             | 25.4            | 26.2             | 25.0            | 23.4            | 24.8            | 26.4            |
| Caudal peduncle depth          | 12.6             | 12.9            | 13.3             | 13.9            | 14.3            | 14.3            | 13.5            |
| Length of D1 base              | 15.9             | 14.0            | 15.8             | 15.5            | 14.8            | 15.0            | 15.0            |
| Length of longest D1 spine*    | 25.6 (3rd)       | 20.8 (2nd)      | 17.8 (2nd)       | 31.1 (2nd)      | 15.6 (2nd)      | 24.3 (2nd)      | 14.1 (3rd)      |
| Length of D2 base              | 16.5             | 15.4            | 16.4             | 17.4            | 17.2            | 16.8            | 15.0            |
| Length of longest D2 ray*      | 16.1 (8th)       | 19.7 (7th)      | 17.1 (2th)       | 21.1 (7th)      | 18.0 (3rd)      | 20.6 (7th)      | broken          |
| Length of last D2 ray          | 14.0             | 18.2            | 10.2             | 19.2            | 12.2            | 19.3            | 8.7             |
| Length of A base               | 14.2             | 12.0            | 12.0             | 16.3            | 16.4            | 15.8            | 13.5            |
| Length of longest A ray*       | 15.0 (7th)       | 17.2 (6th)      | 16.0 (6th)       | 17.6 (7th)      | 16.7 (6th)      | 17.5 (7th)      | 14.7 (4th)      |
| Caudal-fin length              | 24.3             | 23.3            | 23.3             | 27.4            | 26.0            | 26.3            | 24.0            |
| Pectoral-fin length            | 25.0             | 23.3            | 24.4             | 27.4            | 24.2            | 23.8            | 23.1            |
| Pelvic-fin length              | 15.5             | 15.7            | 18.0             | 18.2            | 16.9            | 16.8            | 16.2            |
| Body depth of A origin         | 15.9             | 16.8            | 17.3             | 16.6            | 17.4            | 16.8            | 18.0            |
| Body width of A origin         | 11.7             | 10.7            | 12.4             | 11.6            | 10.7            | 12.0            | 11.4            |
| Length of P2 origin to anus    | 29.1             | 30.1            | 30.7             | 31.8            | 33.9            | 30.1            | 34.5            |
| As % in HL                     |                  |                 |                  |                 |                 |                 |                 |
| Snout length                   | 34.1             | 36.8            | 35.0             | 37.2            | 30.0            | 35.8            | 28.6            |
| Eye diameter                   | 18.0             | 18.9            | 21.0             | 22.3            | 21.5            | 23.9            | 24.8            |
| Postorbital length             | 50.3             | 46.8            | 48.3             | 54.5            | 46.2            | 47.8            | 48.6            |
| Cheek depth                    | 26.3             | 28.9            | 25.9             | 29.8            | 23.1            | 28.4            | 22.9            |
| Head width in maximum          | 62.3             | 58.4            | 66.4             | 59.5            | 54.6            | 63.4            | 52.4            |
| Head depth in maximum          | 51.5             | 52.1            | 55.9             | 60.3            | 53.8            | 53.0            | 58.1            |
| Bony interorbital width        | 4.8              | 3.2             | 5.6              | 4.1             | 3.8             | 4.5             | 4.8             |
| Upper jaw length               | 41.3             | 44.7            | 37.1             | 46.3            | 33.1            | 43.3            | 37.1            |
| As % in Caudal peduncle length |                  |                 |                  |                 |                 |                 |                 |
| Caudal peduncle depth          | 48.1             | 50.7            | 50.8             | 55.8            | 61.1            | 57.6            | 51.1            |

Abbreviations: SL: standard length; D1: first dorsal-fin; D2: second dorsal-fin, A: anal fin, P2: pelvic fin. \*Longest ray is indicated in parentheses.

proportion and fins (holotype and six paratypes), the first dorsal fin, branching of the pectoral-fin rays, length of the pelvic fin, first branches of fifth segmented pelvic-fin ray and sensory canal pores (holotype and 11 paratypes), squamation (five paratypes), sensory-papillae rows and coloration when preserved in alcohol (holotype and a paratype), coloration when freshly collected (holotype and six paratypes), and coloration when alive (two underwater photographs).

The heights of waterfalls in Iriomote-jima Island refer to Kano *et al.* (2012), except for the Kanpire Fall of Urauchi-gawa River. The heights of Kanpire Fall

and waterfalls of Sakuta-gawa River in Ishigaki-jima Island was calculated using the GSI Maps (Geospatial Information Authority of Japan, 2021).

Comparative materials were treated as supplementary materials.

# Results

## Rhinogobius aonumai sp. nov.

(New Standard Japanese name: Painu-kibarayoshinobori) **Holotype.** OMNH-P 40256, male, 65.9 mm SL, Hinaigawa River, Iriomote-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan, upper reaches above Pinaisara Fall (total vertical height 58.7 m), 24°22′ 54.99″N 123°49′ 13.29″E, 104 m above sea level, 19 Aug. 2012, Fig. 1.

Paratypes. Total 28 specimens (15 males and 13 females, 35.9-70.5 mm SL), collected from Iriomotejima Island. Aira-gawa River: OMNH-P 40849 (Fig. 6B) and 40852 (Fig. 6A), female and male, 42.3 and 47.5 mm SL, stained with Alizarin Red S., 24°20'54"N 123°54'1"E, 54 m above sea level, 7 May 2014; Geta-gawa River: OMNH-P 40832 (Fig. 6C) and 40836 (Fig. 6D), male and female, 58.0 and 40.5 mm SL, stained with Alizarin Red S., upper reaches above Geta Fall (total vertical height 32.7 m), 24°23'18.59"N 123°51' 36.09"E, 100 m above sea level, 6 May 2014; Hinai-gawa River: KPM-NI 59987 (formerly OMNH-P 40254), and 59988 (formerly OMNH-P 40255) (Fig. 2), male and female, 66.0 and 56.5 mm SL, collected with the holotype; OMNH-P 40257, female, 61.5 mm SL, collected with the holotype; OMNH-P 43694, male, 70.5 mm SL, stained with Alizarin Red S., same locality with the holotype, 13 Aug. 1993; NSMT-P 138485 (Fig. 6E) and 138486 (Fig. 6F), male and female, 56.0 and 55.5 mm SL, stained with Alizarin Red S., collected with the holotype; Kuira-gawa River: KPM-NI 59984 (Fig. 6H), 59985 (Fig. 6G) and 59986, female, male and female, 38.5, 35.9 and 39.0 mm SL, two females are stained with Alizarin Red S., 24°17'23.18"N 123°45'4.25"E, 30 m above sea level, 23 July 2003; Kura-gawa River: OMNH-P 43162 (Fig. 6I) and 43165 (Fig. 6J), male and female, 42.0 and 36.0 mm SL, stained with Alizarin Red S., upper reaches above Kura Fall (total vertical height 7.6 m), 24°23'53.07"N 123°50' 47.73"E, 15 m above sea level, 28 July 2015; Nakara-gawa River: OMNH-P 43153 (Fig. 7A) and 43156 (Fig. 7B), male and female, 47.3 and 37.0 mm SL, stained with Alizarin Red S., upper reaches above Nakara Fall (total vertical height 27.9 m), 24°19'30.16"N 123°47' 9.93"E, 52 m above sea level, 31 July 2015; Nishida-gawa River: OMNH-P 40533 (Fig. 7C) and 40537 (Fig. 7D), male and female, 58.3 and 50.3 mm SL, stained with Alizarin Red S., upper reaches above Sangara Fall (total vertical height 7.2 m), 24°23'1.37"N 123°49' 58.81"E, 30 m above sea level, 1 Aug 2013; Urauchi-gawa River: OMNH-P 40040 (Fig. 7E), male, 46.3 mm SL, upper reaches above Kampire Fall (total vertical height 13.0 m), 24°21'17.18"N 123°48' 27.00"E, 65 m above sea level, 28 March 2012; SPMN-PI 46248 (formerly OMNH-P 40042) and SPMN-PI 46249 (formerly OMNH-P40043) (Fig. 7F), male and female, 48.0 and 46.0 mm SL, collected with OMNH-P 40040; OMNH-P 43689, 43690, 43691 and 43796, three males and female, 51.3, 47.5, 51.1 and 47.5 mm SL, three males are stained with Alizarin Red S., upper reaches above Mariudo Fall (total vertical height 19.4 m), 24°21'25.49"N 123°48' 12.27"E, 34 m above sea level, 10 Aug 1993; Yuchin-gawa River: OMNH-P 40323 (Fig. 7G) and 40327 (Fig. 7H), male and female, 63.0 and 49.2 mm SL, stained with Alizarin Red S., upper reaches above Yuchin Right Falls (total vertical height 44.2 m), 24°21'58.82"N 123°53' 2.39"E, 253 m above sea level, 27 March 2013.

**Diagnosis**. *Rhinogobius aonumai* is distinguished from all congeneric species by having the following combination of features: 9–15 predorsal scales; 32–38 longitudinal scales; 26–28 vertebrae, mode 27; sensory-papillae rows on cheek arranged longitudinally, with no transverse rows; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with 4–14 and 3–10 vertical rows of dark spots or forming dark zigzag bands in males and females, respectively; a pair of short, vertically aligned, rod-shaped dark mark on caudal-fin base in females.

## Rhinogobius aonumai aonumai subsp. nov.

(New Standard Japanese name: Iriomote-painukibara-yoshinobori) (Figs. 1–8 & 17A; Tables 1 & 3)

Rhinogobius brunneus (not of Temminck & Schlegel): Hayashi, 1984: 259 (in part: Medium Egg Type, Iriomote-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan).

Rhinogobius sp. YB: Iwata, 1989: 598 (in part: Iriomote-jima Island); Akihito et al., 1993: 1082 (in part: Iriomote-jima Island); Akihito et al., 2000: 1255 (in part: Iriomote-jima Island); Akihito et al., 2002: 1255 (in part: Iriomote-jima Island); Suzuki et al., 2004: 460 (in part: Iriomote-jima Island); Akihito et al., 2013: 1461 (in part: Iriomote-jima Island); Suzuki et al., 2021: 466 (in part: Iriomote-jima Island).

**Holotype**. As for *Rhinogobius aonumai* sp. nov. **Paratypes**. As for *Rhinogobius aonumai* sp. nov.

**Photograph Records from Image Database of Fishes.** Arabara-gawa River: KPM-NR 211716–211721, 3 males and 3 females, 28.0–43.0 mm SL, 24°22'14.43"N 123°45' 47.16"E, 118 m above sea level, 18 February 2013, Yo Y. Yamasaki; Hora-gawa River: KPM-NR 211722–211733, 4 males and 8 females, about 40–55 mm SL, upper reaches above Hora Fall (total vertical height 23.3 m), 24°22'10.71"N 123°54' 3.61"E, 174 m above sea

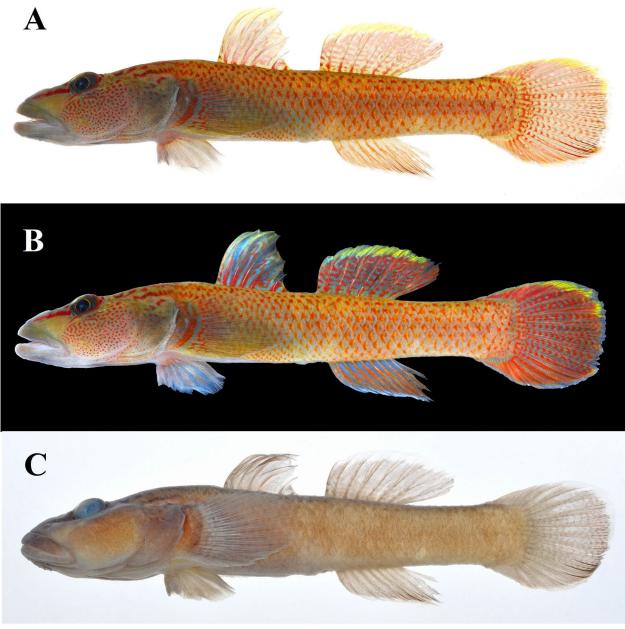


Fig. 1. Holotype of *Rhinogobius aonumai aonumai* (OMNH-P 40256, male, 65.9 mm SL) collected from Hinai-gawa River, Iriomote-jima Island, the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

level, 12 June 2011, Yuichi Kano; Namure-gawa River, a tributary of the Nakama-gawa River System: KPM-NR 211734–211738, 3 males and 2 females, about 40 mm SL, 24°17'50.7"N 123°49' 43.4"E, 15 m above sea level, 15 March 2020, Yoshiyuki Akatuka; Nishifunatsuki-gawa River, a tributary of the Nakama-gawa River System: KPM-NR 211739 and 211740, male and female, about 40 mm SL, 24°18'33.9"N 123°51' 31.0"E, 87 m above sea level, 27 June 2020, Yoshiyuki Akatuka.

**Diagnosis**. Rhinogobius aonumai aonumai is distinguished from all congeneric species-group taxa (species and subspecies) by having the following

combination of features: 9–15 predorsal scales; 32–37 longitudinal scales; 11+15–17=26–28 vertebrae, mode 27; anteriormost two pterygiophores (proximal radials) of second dorsal fin mounted over neural spine of tenth (sometimes nineth and tenth) vertebra; fifth segmented pelvic-fin ray divided into 3–4 (usually four) branches at the position where proximal most segment of each branch alignes transversely; sensory-papillae rows on cheek arranged longitudinally, with no transverse rows; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with 4–14 and 3–10 vertical rows of dark spots or forming dark zigzag bands in males and females, respectively; a pair of short, vertically aligned,

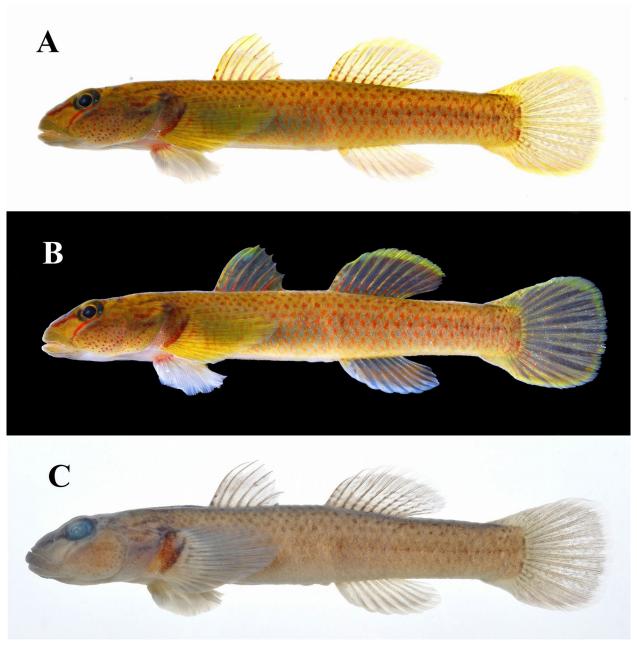


Fig. 2. Female paratype of *Rhinogobius aonumai aonumai* (KPM-NI 59988, 56.5 mm SL) collected from Hinai-gawa River, Iriomote-jima Island, the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

rod-shaped dark mark on caudal-fin base in females.

**Description**. First dorsal-fin rays V(2), VI\*(25) or VII (2); second dorsal-fin rays I, 7\* (1), I, 8 (21) or I, 9 (7); anal-fin rays I, 8\* (21) or I, 9 (8); pectoral-fin rays (left/right) 18/19 (2), 19/18 (1), 19/19 (16), 20/19 (2) or 20/20\* (7); pelvic-fin rays I, 5 (28); segmented caudal-fin rays (upper part + lower part) 8+8 (1) or 9+8\* (27); branched caudal-fin rays (upper part + lower part) 6+6 (1), 7+7\* (15), 7+8 (2), 8+7 (5), 8+8 (4) or 9+8 (1); longitudinal scales 32 (1), 33 (6), 34 (7), 35 (6), 36\* (7) or 37 (1); transverse scales 9 (4), 10 (12), 11 (8) or 12\* (3); scales between origin of dorsal fin and dorsal insertion of pectoral fin 7 (8), 8 (9), 9\* (9), or 10 (2); predorsal scales 9 (2), 10 (3), 11\* (9), 12 (5), 13(5), 14(3) or 15 (1); number of

the first branches of fifth segmented pelvic-fin ray (left/right) at the position where proximal most segment of each branch alignes transversely (Fig. 3A) 3/3 (7), 3/4 (1), 4/3 (2) or 4/4\* (18); P-V 3/122000/10 (1), 3/122100/10 (1), 3/122110/10 (1), 3/122111/10(1), 3/21210/9&10 (3), 3/212100/10 (1), 3/212101/10 (1), 3/212100/10 (1), 3/221010/10 (1), 3/221100/10 (8); vertebrae (abdominal + caudal = total vertebrae) 11+15=26 (1), 11+16=27\* (18) or 11+17=28 (2).

Head slightly large and slightly depressed; body slightly slender, slightly compressed anteriorly, and compressed posteriorly (see "Variations between rivers"). Snout nearly pointed, long and longer than eye diameter; snout length of males greater than that of females. Eye large, dorsolateral

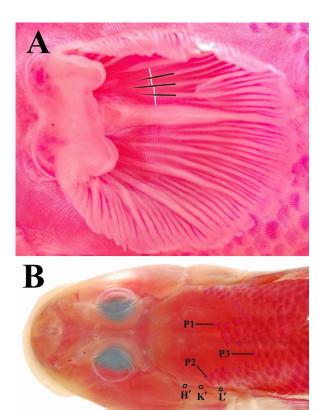


Fig. 3. Ventral view of pelvic fin (A) and dorsal view of head (B), in *Rhinogobius aonumai aonumai* stained with Alizarin Red S. OMNH-P 43694, paratype, male, 70.5 mm SL. White lines indicate position where proximal most segment of each branch alignes transversely with the fifth segmented ray. Black wedge indicates slits between branches. Black circles with black letters H', K', and L' indicate sensory-canal pores. P1, P2, and P3 indicate anteriormost point of anterior extension of scaly area along predorsal midline, anteriormost point of anterior extensions of scaly area on temporal region, and greatest concaved point of scaly area between P1 and P2, respectively. Photographed and annotated by T. Suzuki.

on head, and located slightly behind a vertical through midpoint between snout tip and posterior margin of preopercle. Cheek somewhat bulbous and fleshy. Lips thick and fleshy; anterior tips of both lips even or usually lower lip slightly protruding anteriorly\*; gape slightly oblique; posterior margin of lower jaw extending posteriorly to a vertical through anterior margin of eye in females and slightly beyond it\* in males. Anterior naris a short tube without skin flap at its tip, and its base located slightly behind the midpoint between snout tip and anterior margin of eye. Posterior naris a round pore with low rim and located at the midpoint between base of anterior naris and anterior margin of eye. Gill opening extending anteriorly to a vertical through posterior margin of preopercle. Gill membranes broadly attached to isthmus. No fleshy papillae or finger-like projections on lateral margin of shoulder

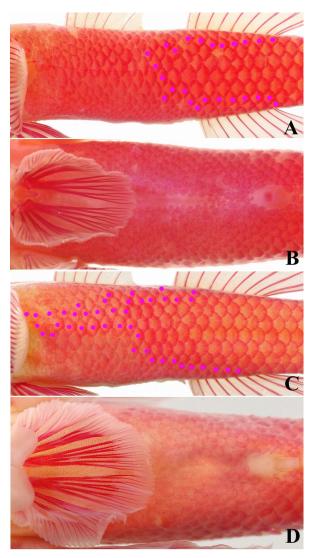


Fig. 4. Lateral view of body (A, C) and ventral view of belly (B, D) in *Rhinogobius aonumai aonumai* stained with Alizarin Red S. A and B: NSMT-P 138485, paratype, male, 56.0 mm SL, Hinai-gawa River; C and D: OMNH-P 40852, paratype, male, 47.5 mm SL, Aira-gawa River. Pink spots indicate the lateral anterior margin of ctenoid scale area. Photographed and annotated by T. Suzuki.

girdle. Tongue free from floor of mouth, with rounded anterior margin. Genital papillae cone-shaped\* in males and oval in females.

Origin of first dorsal fin about an eye diameter behind a vertical through dorsal insertion of pectoral-fin; first dorsal fin in males near falcate shape, pentagon (becoming slightly narrower distally) shape or semioval\*, and higher than second dorsal fin, whereas, in females, semicircular or semioval, and slightly higher than, or slightly lower than second dorsal fin in height; usually second, or third\* spine longest; all dorsal-fin spines slender and flexible; usually not filamentous\*, or distal tip of second or third spine slightly filamentous; posterior tip (= distal tip of second, usually third, or fourth\* spines) of first dorsal fin

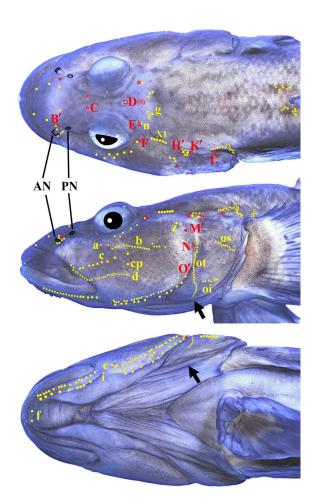


Fig. 5. Dorsal (top), lateral (middle), and ventral (bottom) views of head of *Rhinogobius aonumai aonumai* stained with cyanine blue showing cephalic sensory pores and papillae. OMNH-P 40256, holotype, male, 65.9 mm SL Red circles with red letters indicate sensory canal pores (letters with prime marks indicate terminal openings of sensory canals); rows of yellow spots indicated by yellow letters represent sensory papillae rows; black arrows show ventralmost positions of gill opening. Abbreviations: AN, anterior narial pore; PN, posterior narial pore. Photographed and annotated by T. Suzuki.

in males extending between front of origin\* and base of third segmented ray of second dorsal fin when adpressed, whereas, in females, posterior tip (= distal tip of third, fourth, fifth or usually sixth spines) not extending to origin of second dorsal fin when adpressed. First and second dorsal fins not connected by membrane (see "Variations between rivers"); all segmented rays of second dorsal fin branched; seventh\* or eighth, and second, fourth or sixth segmented rays longest in males and females, respectively; posterior tip (= distal tip of last\* or penultimate segmented ray) of second dorsal fin usually not extending\* to caudal fin, or to it when adpressed; posterior end of second dorsal-fin above posterior end of anal-fin base. Origin of anal fin below between bases of first and second

segmented rays of second dorsal fin; anal fin equal to\*, or usually slightly lower than second dorsal fin in height; all segmented anal-fin rays branched; fifth, sixth or seventh\* and fourth or sixth segmented rays longest in males and females, respectively; posterior tip (= distal tip of last or penultimate\* segmented ray) of anal fin not extending to caudal fin when adpressed. Pectoral fin oval, extending posteriorly to a vertical between base of sixth spine and posterior end of base\* of first dorsal fin; all pectoralfin rays branched, except for dorsalmost ray usually not branched (left side\*), or branched once (right side\*); ventralmost ray not branched (both side\*), or branched once. Pelvic fins fused medially by well-developed frenum (between spines) and connecting membrane (between innermost rays), forming a circular cup-like disc; pelvic fins extending posteriorly to a vertical between bases of second and fifth spines of first dorsal fin (base of third spine\*) (see "Variations between rivers"); pelvic fins not reaching to anus; pelvic-fin spine with a rounded membranous lobe at its tip; all segmented rays of pelvic fin branched. Caudal fin elliptical or fan-shaped\*.

Scales on body largely ctenoid, becoming smaller anteriorly; anterior edge of scaled areas with ctenoid scales on lateral, dorsal and ventral sides of body reaching respectively to a vertical between a little behind pectoralfin axilla and origin of second dorsal fin, to second dorsalfin base, and to anal-fin base (see "Variations between rivers"; Figs. 4A, 4C). Small cycloid scales on anterodorsal part of body before the area with ctenoid scales. Predorsal squamation with trifurcate anterior edge; its mid-anterior extension (Fig. 3B: P1) extending anteriorly to between transverse lines through sensory-canal pores H' and L'; anterior extensions of lateral sides (Fig. 3B: P2) extending anteriorly to posterior oculoscapular canal; greatest concaved point of anterior margin of scaled area of predorsal region between P1 and P2 (Fig. 3B: P3) extending posteriorly to above between sensory-canal pore H' and upper end of pectoral-fin axilla. Pectoral-fin base usually naked, or with one or two small cycloid scales. Prepelvic area usually naked, or with some small cycloid scales. Scaled area on belly with small cycloid scales usually extending anteriorly to, or not, side of prepelvic area; belly above pelvic fin naked, and its following anterior part of ventral midline of belly usually naked, or with some small cycloid scales (see "Variations between rivers"; Figs. 4B, 4D).

Cephalic sensory systems are illustrated in Fig. 5. Nasal extension of anterior oculoscapular canal with terminal pore B' located above both nares. Anterior interorbital sections of the anterior oculoscapular canal separated

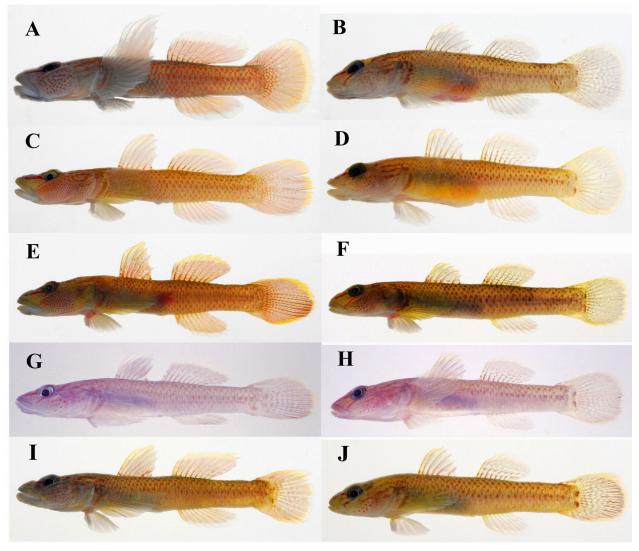


Fig. 6. Freshly-collected paratypes of *Rhinogobius aonumai aonumai* from Iriomote-jima Island, the Ryukyu Islands, Japan. Airagawa River: A (OMNH-P 40852, male, 47.5 mm SL) and B (OMNH-P 40849, female, 42.3 mm SL); Geta-gawa River: C (OMNH-P 40832, male, 58.0 mm SL) and D (OMNH-P 40836, female, 40.5 mm SL); Hinai-gawa River: E (NSMT-P 138485, male, 56.0 mm SL) and F (NSMT-P 138486, female, 55.5 mm SL); Kuira-gawa River G: (KPM-NI 59985, male, 35.9 mm SL) and H (KPM-NI 59984, female, 38.5 mm SL); Kura-gawa River: I (OMNH-P 43162, male, 42.0 mm SL) and J (OMNH-P 43165, female, 36.0 mm SL). Photographed by T. Suzuki.

bilaterally, with paired pore C and a single pore D. Pore E present just behind posterior edge of eye. Lateral section of anterior oculoscapular canal with anterior pore F and terminal pore H'. Posterior oculoscapular canal with two terminal pores K' and L'. A gap between anterior and posterior oculoscapular canals much narrower than the length of the posterior oculoscapular canal. Preopercular canal present, with three pores M', N, and O'. In the three paratypes, there is an accessory pore on the canal between pores C and D, pores D and E, and pores M' and N. Sensory-papillae row "a" oblique and uniserial, composed of loosely-arranged papillae, and extending anteriorly to a vertical through midpoint between anterior margin of eye and anterior margin of pupil. Row "b" longitudinal, composed of densely-arranged papillae, extending anteriorly to a vertical through the center of pupil, and

longer than eye diameter in length. Row "c" composed of loosely-arranged papillae, extending posteriorly to a vertical through posterior margin of the eye. Row "d" composed of densely-arranged papillae, extending posteriorly to a vertical through posterior margin of the pupil. Row "cp" comprising a single papilla. Row "f" comprising paired papillae. Anterior end of row "oi" a little separated from a vertical row "ot".

Coloration of male when freshly collected (Figs. 1A, 1B, 6A, 6C, 6E, 6G, 6I, 7A, 7C, 7E, 7G). Ground color of head and body usually reddish yellow. Belly pale yellow, and its side greenish. Head grayish; cheek, operculum and gill membrane usually with densely-arranged, many small reddish orange dots (see "Variations between rivers"). Snout with a broad oblique reddish orange stripe between anterior margin of eye and anteriormost part

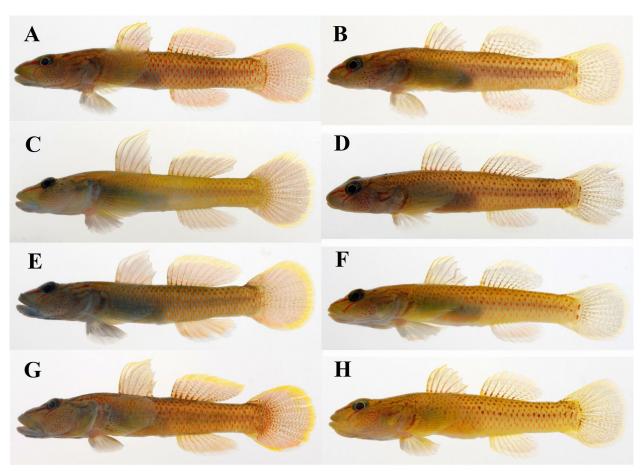


Fig. 7. Freshly-collected paratypes of *Rhinogobius aonumai aonumai* from Iriomote-jima Island, the Ryukyu Islands, Japan. Nakaragawa River: A (OMNH-P 43153, male, 47.3mm SL) and B (OMNH-P 43156, female, 37.0 mm SL); Nishida-gawa River: C (OMNH-P 40533, male, 58.3 mm SL) and D (OMNH-P 40537, female, 50.3 mm SL); Urauchi-gawa River: E (OMNH-P 40040, male, 43.6 mm SL) and F (SPMN-PI 46249, female, 46.0 mm SL); Yuchin-gawa River: G (OMNH-P 40323, male, 63.0 mm SL) and H (OMNH-P 40327, female, 49.2 mm SL). Photographed by T. Suzuki.

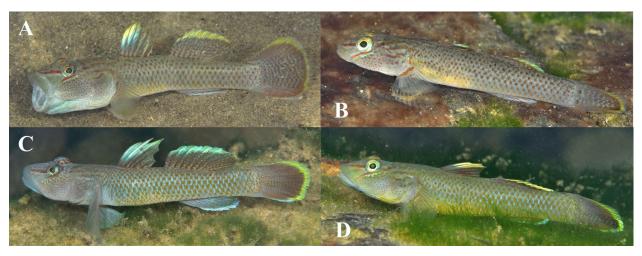


Fig. 8. Underwater photographs of *Rhinogobius aonumai aonumai* taken at Iriomote-jima Island, the Ryukyu Islands, Japan. Hinaigawa River: A (male, about 60 mm SL) and B (female, about 45 mm SL); Urauchi-gawa River: C (male, about 50 mm SL) and D (female, about 40 mm SL). Photographed by M. Suzuki.

of snout. Several irregular-shaped, short reddish orange stripes and minute spots on dorsal surface of snout and interorbital space, occipital region and nape; anterodorsal margin of cheek with a narrow reddish orange line edged ventrally by a bluish green line; temporal region above operculum with usually a short reddish orange stripe\*, or dashed line; upper part of operculum with usually an oblique reddish orange stripe, or dashed line\*. Almost all scale pockets on body with reddish orange spots. Ground color of fin membranes pale yellow, partially with bluish

sheen. Spines and segmented rays gray. Anterodorsal part of first dorsal fin and distal margin of second dorsal bright yellow; distal margin of anal fin white; distal margin of caudal fin usually bright yellow, or ventral margin white\*; membranes around spines and segmented rays of first dorsal and anal fins, and upper and lower parts of caudal fin reddish orange. Second dorsal fin with usually 1–6 (5\*) longitudinal rows of reddish orange spots\* or forming zigzag stripes; indistinct pale orange mottles above these spots or stripes. Central part of caudal fin with usually 4–14 (13\*) vertical rows of reddish orange\* or pale orange spots, or forming zigzag bands. Pectoral-fin base with a distinct reddish orange bow-shaped mark, and one or two\* vertical rows of reddish orange spots behind it.

Coloration of female when freshly collected (Figs. 2A, 2B, 6B, 6D, 6F, 6H, 6J, 7B, 7D, 7F, 7H). Resembles that of male, except as follows. Orange marks of head and body darker. Orange dots on cheek fewer in number; on operculum fewer in number or absent; gill membranes with no orange dots. Ventral side of body usually with no orange dots. Spines and segmented rays of fins except for those of first dorsal and pelvic fins yellowish. Second dorsal fin with 3-5 longitudinal rows of reddish orange spots or forming zigzag stripes; membrane above these spots or stripes pale pink. Caudal-fin base with a pair of short, vertically aligned, rod-shaped deep reddish orange mark; central part of caudal fin with 3-10 vertical rows of pale pink spots or forming zigzag bands, membrane behind these spots or bands usually pale pink, or forming indistinct mottles. Pectoral-fin base with a deep reddish orange band or a vertical row of or reddish orange spots behind a bowshaped mark.

Coloration when alive based on underwater photographs (Fig. 8). Coloration when alive in the populations of Hinai-gawa and Urauchi-gawa rivers in Iriomote-jima Island resembles that of freshly-collected coloration, except as follows. In the population of Hinai-gawa River, ground color of head and body light grayish green; belly bright yellow. In the population of Urauchi-gawa River, head and body greenish; belly bright yellow; orange and pink color darker; anterodorsal part of first dorsal fin and distal margin of second dorsal fin bright greenish in males.

Coloration when preserved in alcohol (Figs. 1C, 2C). Ground color of head and body turned to yellowish gray; blue, green, pink, yellow and white color faded; orange and red color turns to brown or gray. Upper half of body with 1–2, and 3–4 longitudinal row(s) of brown spots in males and females, respectively.

Variations between rivers. In females of the population

of Hinai-gawa River, head length is shorter [28.1–28.9 % in SL (average 28.6 %) vs 29.0–31.6 % (average 30.8 %) than those of the other populations]; head, body, and caudal peduncle are lower in depth [head depth 14.2–14.8 % (average 14.5 %) vs 16.4–18.7 % (average 17.5 %), body depth at origin of pelvic-fin 14.2–14.8% (average 14.5 %) vs 16.4–18.7 % (average 17.6 %), caudal peduncle depth 11.0–11.4 % (average 11.2 %) vs 10.7–12.8 % (average 12.2 %)]; interspace between dorsal fins longer [8.2–8.6 % (average 8.5 %) vs 4.9–8.1 % (average 6.5 %)] than females of the other populations.

In the population of Hinai-gawa River, pelvic fins are shorter, extending usually posteriorly to a vertical between bases of the second and third spines of the first dorsal fin, whereas the fins extend posteriorly to a vertical between bases of the third and fifth spines of the first dorsal fin in the other populations. In the populations of Kuira-gawa and Aira-gawa rivers, anterior margin of the ctenoid scale area extends to a little behind pectoral-fin axilla (Fig 4A), whereas the area extends to a vertical between origins of first and second dorsal fins in the other population (Fig. 4C); ventral midline of belly largely covered with small cycloid scales (Fig. 4D), although naked except posterior part in other populations (Fig. 4B).

Furthermore, in the population of Kuira-gawa River, some small reddish orange dots on the cheek, the lower half of operculum, and gill membrane in males (Fig. 6G), but many densely-arranged dots are present in those of the other populations; females lack orange spots on the lower half of body, whereas many spots are seen in those of the other populations (Fig. 6H).

**Distribution and habitat.** Rhinogobius aonumai aonumai is known only from Iriomote-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. In this island, it is seen in the upper reaches of freshwater streams above waterfalls in montane areas of Geta-gawa, Hinai-gawa, Kura-gawa, Nakara-gawa, Nishida-gawa, Urauchi-gawa and Yuchin-gawa rivers, and the upper reaches of swift freshwater streams without waterfalls of Aira-gawa and Kuira-gawa rivers. In addition, we identified the gobies in the photographs taken from Arabara-gawa, Hora-gawa, and Nakama-gawa rivers registered in the Image Database of Fishes, as R. a. aonumai. The subspecies is usually found in small pools of streams, called "Fuchi" in Japanese.

**Etymology.** The specific/subspecific name, *aonumai*, refers to Dr. Yoshimasa Aonuma, who was the pioneer in the taxonomic study of the genus *Rhinogobius* in the Ryukyu Islands. In his master's thesis, he was the first to suggest that the number of vertebrae in *Rhinogobius* sp.

Table 3. Comparisons of 55 species-group taxa (species and subuspecies) of *Rhinogobius* with high or unknown vertebral counts

| Species            | Vertebrae | Longitudinal scales | Dark spot of<br>D1 | Bands or vertical rows of dark spots on C. | Reference  |  |  |
|--------------------|-----------|---------------------|--------------------|--|--|--|--|
| R. albimaculatus   | 28        | 29–31               | present            | present                                    | Chen et al., 1999a   |  |  |
| . aonumai aonumai  | 26–28     | 32–37               | absent             | present                                    | This study   |  |  |
| . a. ishigakiensis | 26–28     | 33–38               | absent             | present                                    | This study   |  |  |
| . boa              | 27        | 30–33               | present            | present                                    | Chen & Kottelat, 2005                                      |  |  |
| bucculentus        | unknown   | 40-44               | absent             | absent                                     | Herre, 1927  |  |  |
| carpenteri         | 28-29     | 34-40               | absent             | absent                                     | Endruweit, 2017; Seale, 1910                               |  |  |
| changtinensis      | 27        | 28-30               | present            | present                                    | Huang & Chen, 2007   |  |  |
| cheni              | 27        | 34–36               | present            | present                                    | Chen et al., 2008; Koumans, 1940; Nichols, 1931            |  |  |
| chiengmaiensis     | 28        | 26-30               | present            | present                                    | Chen et al., 2008; Fowler, 1934; Koumans, 1940             |  |  |
| cliffordpopei      | unknown   | 28–29               | present            | present                                    | Nichols, 1925; Wu & Chen, 2008                             |  |  |
| coccinella         | 28        | 27–30               | present            | present                                    | Endruweit, 2018  |  |  |
| davidi             | 28        | 30–31               | present            | absent in male;<br>present in female       | Chen & Miller, 1998;<br>Sauvage & Dabry de Thiersant, 1874 |  |  |
| duospilus          | 27        | 29–31               | present            | present                                    | Herre, 1935; Huang & Chen, 2007                            |  |  |
| filamentosus       | 27        | 30–33               | present            | present                                    | Chen et al., 2008; Wu, 1939                                |  |  |
| flumineus          | 27–28     | 30–36               | absent             | absent or present                          | Akihito et al., 2013 & Errata; Mizuno, 1960b; Th study     |  |  |
| fukushimai         | unknown   | 30-31               | absent             | present                                    | Mori, 1934; Wu & Chen, 2008                                |  |  |
| genanematus        | 27        | 27–29               | absent             | present                                    | Chen & Fang, 2006; Zhong & Tzeng, 1998                     |  |  |
| henryi             | 28        | 30–32               | present            | present                                    | Chen et al., 2008; Herre, 1938                             |  |  |
| honghensis         | 28        | 32–34               | present            | present                                    | Chen et al., 1999c   |  |  |
| houheensis         | 30        | 37–40               | present            | absent                                     | Wanghe <i>et al.</i> , 2020                                |  |  |
| imfasciocaudatus   | 27–28     | 30–31               | present            | absent                                     | Endruweit, 2018; Nguyen & Vo, 2005                         |  |  |
| immaculatus        | 27–28     | 29–31               | absent             | present                                    | Li et al., 2018  |  |  |
| lentiginis         | 27        | 30–32               | present            | absent in male;                            | Chen & Miller, 1998; Wu & Zheng, 1985;                     |  |  |
| 1: 11              | 27.20     | 20. 22              |                    | present in female                          | Wu & Chen, 2008  |  |  |
| lindbergi          | 27–28     | 30–32               | present            | present                                    | Berg,1933; Sakai <i>et al</i> ., 2000                      |  |  |
| lineatus           | 28        | 29–32               | present            | present                                    | Chen et al., 1999a   |  |  |
| linshuiensis       | 27–28     | 27–31               | present            | present                                    | Chen & Miller, 2014; Chen et al., 2002                     |  |  |
| liui               | 29        | 35–39               | present            | present                                    | Chen & Wu, 2008  |  |  |
| longyanensis       | 27        | 30–32               | present            | absent                                     | Chen et al., 2008  |  |  |
| lungwoensis        | 28        | 31–34               | present            | present                                    | Huang & Chen, 2007   |  |  |
| maculagenys        | 27        | 32–34               | present            | present                                    | Wu et al., 2018  |  |  |
| maculicervix       | 28        | 29–32               | present            | present                                    | Chen & Kottelat, 2000                                      |  |  |
| maxillivirgatus    | 27        | 28-30               | present            | present                                    | Xia et al., 2018   |  |  |
| mekongianus        | 28        | 29-31               | present            | present                                    | Chen et al., 1999a; Pellegrin & Fang 1940                  |  |  |
| milleri            | 27        | 28-31               | present            | present                                    | Chen & Kottelat, 2003                                      |  |  |
| multimaculatus     | 29        | 33–37               | present            | present                                    | Chen et al., 1999b; Wu & Zheng, 1985                       |  |  |
| nammaensis         | 28        | 30-32               | present            | present                                    | Chen & Kottelat, 2003                                      |  |  |
| nanophyllum        | 28        | 29–32               | present            | present                                    | Endruweit, 2018  |  |  |
| ngutinhoceps       | 29        | 30–32               | present            | present                                    | Endruweit, 2018  |  |  |
| niger              | 27–28     | 35–37               | present            | absent in male;<br>present in female       | Huang et al., 2016   |  |  |
| parvus             | 27        | 28–30               | absent             | absent in male;<br>present in female       | Huang et al., 2016; Luo, 1989                              |  |  |
| philippinus        | unknown   | 36–40               | absent             | absent absent in male;                     | Herre, 1927  |  |  |
| phuongae           | 28–29     | 28–32               | present            | present in female                          | Endruweit, 2018  |  |  |
| ponkouensis        | 28        | 32–34               | present            | present                                    | Huang & Chen, 2007   |  |  |
| rubromaculatus     | 27–28     | 29–32               | present            | present                                    | Lee & Chang, 1996; Wu & Chen, 2008                         |  |  |
| shennongensis      | unknown   | 31–33               | absent             | present                                    | Wu & Chen, 2008; Yang & Xie, 1983                          |  |  |
| sowerbyi           | unknown   | 35–36               | absent             | absent                                     | Ginsburg, 1917   |  |  |
| sulcatus           | 27–28     | 26–29               | present            | present                                    | Chen & Kottelat, 2005                                      |  |  |
| szechuanensis      | 27        | 30–34               | absent             | present                                    | Chen et al., 2008; Tchang, 1939; Wu & Chen, 20             |  |  |
| taenigena          | 27        | 29                  | present            | present                                    | Chen et al., 1999a   |  |  |
| vermiculatus       | 28        | 32–34               | present            | present                                    | Chen & Kottelat, 2003                                      |  |  |
| wangchuangensis    | 27        | 28–30               | present            | absent in male;<br>present in female       | Chen & Miller, 2014; Chen et al., 2002                     |  |  |
| wangi              | 27        | 26–27               | present            | present                                    | Chen & Fang, 2006  |  |  |
| wuyanlingensis     | 27        | 30–32               | present            | present                                    | Yang et al., 2008  |  |  |
| xianshuiensis      | 27        | 29–32               | present            | present                                    | Chen et al., 1999b; Wu & Chen, 2008                        |  |  |
| yaoshanensis       | 28        | 29-31               | present            | present                                    | Chen et al., 2008; Luo 1989                                |  |  |

Abbreviations: D1, first dorsal fin; C, caudal fin

YB from the Yaeyama Group of the Ryukyu Islands was higher than those from other islands of the Ryukyu Islands (Aonuma, 1992).

## Rhinogobius aonumai ishigakiensis subsp. nov.

(New Standard Japanese name: Ishigaki-painukibara-yoshinobori) (Figs 9–15 & 17B; Tables 2 & 3)

Rhinogobius brunneus (not of Temminck & Schlegel): Hayashi, 1984: 259 (in part: Medium Egg Type, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan).

Rhinogobius sp. YB: Iwata, 1989: 598 (in part: Ishigaki-jima Island); Akihito et al., 1993: 1082 (in part: Ishigaki-jima Island); Akihito et al., 2000: 1255 (in part: Ishigaki-jima Island); Akihito et al., 2002: 1255 (in part: Ishigaki-jima Island); Suzuki et al., 2004: 460 (in part: Ishigaki-jima Island); Akihito et al., 2013: 1461 (in part: Ishigaki-jima Island); Suzuki et al., 2021: 466 (in part: Ishigaki-jima Island).

Holotype. SPMN-PI 49269, male, 51.5 mm SL, Sakuta-

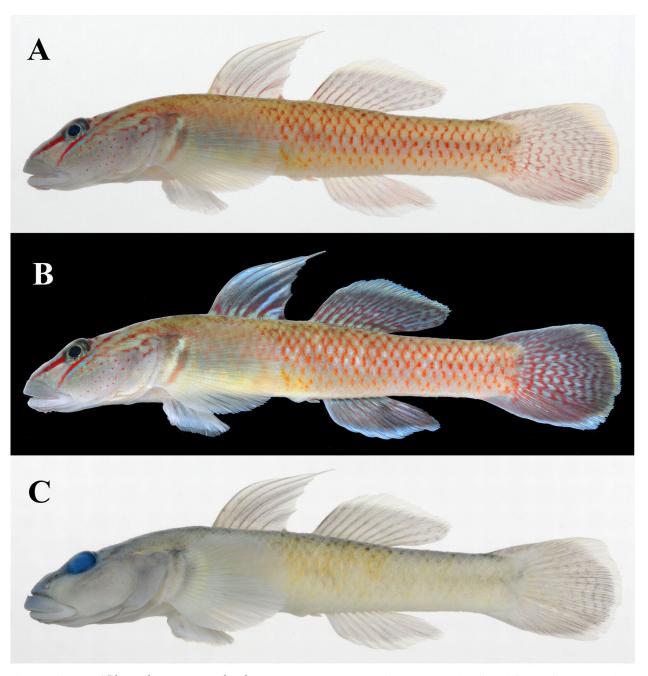


Fig. 9. Holotype of *Rhinogobius aonumai ishigakiensis* (SPMN-PI 49269, male, 51.5 mm SL) collected from Sakuta-gawa River, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

gawa River, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan, upper reaches above some falls (total vertical height about 100 m), 165 m above sea level, 26 Oct. 2020, Fig. 9.

**Paratypes**. Total 11 specimens (6 males and 5 females, 33.3–56.5 mm SL), collected from Ishigaki-jima Island. Miyara-gawa River: OMNH-P 40912 (Fig. 14A) and 40914 (Fig. 14B), male and female, 38.0 and 38.4 mm SL, upper stream above Maezato Dam, 93 m above sea level, 19 June 2014; Sakuta-gawa River: OMNH-P 48920, 48922, 48923 and 48924, three males and female, 38.0, 37.2, 35.5 and 35.5 mm SL, stained with Alizarin Red S., the same locality as holotype, 18 Sep 2020, KPM-NI

65589 and 65588 (Fig. 14C), female and male, 56.5 and 55.9 mm SL, female is stained with Alizarin Red S., the same locality as holotype, 19 Oct. 2020, SPMN-PI 49270 (Figs. 10, 14D), female, 45.0 mm SL, collected with the holotype; Sokobaru-gawa River, a tributary of the Miyaragawa River System: OMNH-P 40911 (Fig. 14E) and 40913 (Fig. 14F), male and female, 39.9 and 33.3 mm SL, upper stream above Sokobaru Dam, 159.6 m above sea level, 22 June 2014.

**Photograph Records from Image Database of Fishes.** Ayamashi-gawa River, a tributary of the Miyaragawa River System: KPM-NR 217906, male, about 40 mm SL, 24°23′47.59″N 124°11′07.28″E, 34 m above sea

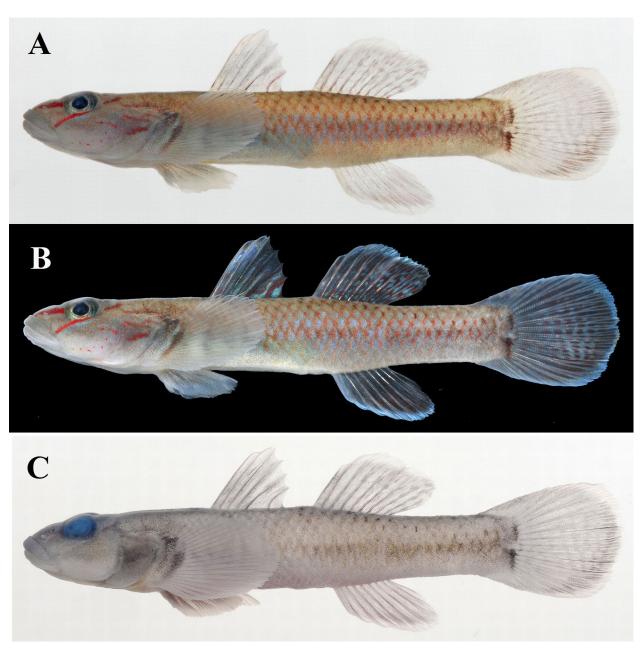


Fig. 10. Female paratype of *Rhinogobius aonumai ishigakiensis* (SPMN-PI 49270, female, 45.0 mm SL) collected from Sakutagawa River, Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. A and B: freshly-collected; C: alcohol-preserved. Photographed by T. Suzuki.

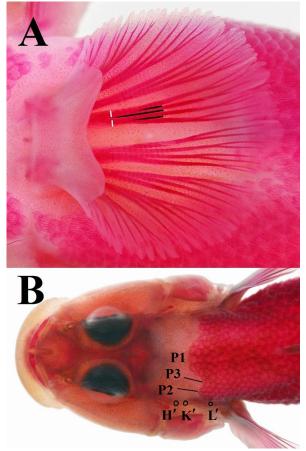


Fig. 11. Ventral view of pelvic fin (A) and dorsal view of head (B), in *Rhinogobius aonumai ishigakiensis* stained with Alizarin Red S. OMNH-P 48923, paratype, male, 35.5 mm SL, Sakuta-gawa River. White lines indicate position where proximal most segment of each branch alignes transversely with the fifth segmented ray. Black wedge indicates slits between branches. Black circles with black letters H', K', and L' indicate sensory-canal pores. P1, P2, and P3 indicate anteriormost point of anterior extension of scaly area along predorsal midline, anteriormost point of anterior extensions of scaly area on temporal region, and greatest concaved point of scaly area between P1 and P2, respectively. Photographed and annotated by T. Suzuki.

level, 17 September 2021, Naoharu Oseko; Isobe-gawa River: KPM-NR 217904 and 217905, male and female, about 35 mm SL, upper stream above Ishigaki Dam, 24°22′28.39″N 124°09′59.25″E, 113 m above sea level, 17 September 2021, Naoharu Oseko; Pensann-kara River, a tributary of the Uratabaru-gawa River System: KPM-NR 217910 and 217911, male and female, about 40 and 35 mm SL, 24°22′37.00″N 124°09′39.45″E, 90 m above sea level, 7 March 2018, Naoharu Oseko; Todoroki-gawa River: KPM-NR 217907 and 217908, male and female, about 35 mm SL, 24°23′52.60″N 124°13′36.59″E, 25 m above sea level, 10 March 2021, Naoharu Oseko; A tributary of the Todoroki-gawa River: KPM-NR 217909, male, about 35 mm SL, 24°23′37.23″N 124°13′04.84″E,

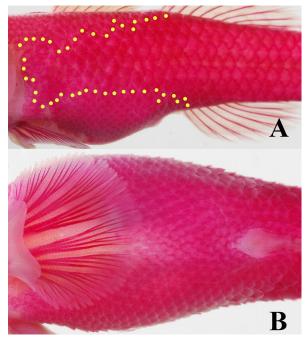


Fig. 12. Lateral view of body (A) and ventral view of belly (B) in *Rhinogobius aonumai ishigakiensis* stained with Alizarin Red S. OMNH-P 48923, paratype, male, 35.5 mm SL, Sakuta-gawa River. Yellow spots indicate the lateral anterior margin of ctenoid scale area. Photographed and annotated by T. Suzuki.

25 m above sea level, 10 March 2021, Naoharu Oseko.

**Diagnosis**. *Rhinogobius aonumai ishigakiensis* is distinguished from all congeneric species-group taxa by having the following combination of features: 10–14 predorsal scales; 33–38 longitudinal scales; 10+16–18=26–28 vertebrae (mode 27); anteriormost two pterygiophores (proximal radials) of second dorsal fin mounted over neural spine of nineth vertebra; fifth segmented pelvic-fin ray divided into 2–3 (usually two) branches at the position where proximal most segment of each branch alignes transversely; sensory-papillae rows on cheek arranged longitudinally, with no transverse rows; yellow-colored body in freshly-collected; no dark spot on first dorsal fin; caudal fin with 7–9 and 3–6 dark zigzag bands in males and females, respectively; a pair of short, vertically aligned, rod-shaped dark mark on caudal-fin base in females.

**Description**. First dorsal-fin rays V (1) or VI\* (11); second dorsal-fin rays I, 8\* (9) or I, 9 (3); anal-fin rays I, 7 (2), I, 8\* (2) or I, 9 (8); pectoral-fin rays (left/right) 18/18 (1), 18/19 (1), 19/18 (1), 19/19 (4), 19/20 (3), 20/19\* (1) or 20/20 (1); pelvic-fin rays I, 5 (12); segmented caudal-fin rays (upper part + lower part) 9+8\* (10) or 9+9 (2); branched caudal-fin rays (upper part + lower part) 7+7 (6), 8+7\* (5) or 9+7 (1); longitudinal scales 33 (2), 34 (1), 35 (6), 36 (1), 37\* (1) or 38 (1); transverse scales 9 (7) or 10\*(5); scales between origin of dorsal fin and dorsal

insertion of pectoral fin 8 (5) or 9\* (7); predorsal scales 10 (1), 11 (4), 12 (2) or 14\* (5); number of the first branches of fifth segmented pelvic-fin ray (left/right) at the position where proximal most segment of each branch alignes transversely (Fig. 11A) 2/2\* (10), 2/3 (1) or 3/2 (1); P-V 3/21210/9 (1), 3/22100/9 (1), 3/22110/9 (9) or 3/22200/9\* (1); vertebrae (abdominal + caudal = total vertebrae) 10+16=26 (3), 10+17=27 (7) or 10+18=28\* (2).

Head slightly large and slightly depressed; body slightly slender, slightly compressed anteriorly, and compressed posteriorly. Snout nearly pointed, long, and longer than eye diameter; snout length of males greater than that of females. Eye large, dorsolateral on head, and located slightly behind a vertical through midpoint between snout tip and posterior margin of preopercle. Cheek somewhat

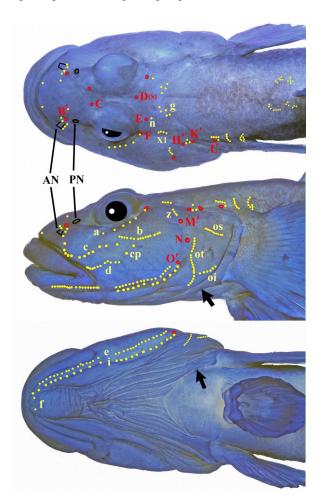


Fig. 13. Dorsal (top), lateral (middle), and ventral (bottom) views of head of *Rhinogobius aonumai ishigakiensis* stained with cyanine blue showing cephalic sensory pores and papillae. KPM-NI 65588, paratype, male, 55.9 mm SL. Red circles with red letters indicate sensory canal pores (letters with prime marks indicate terminal openings of sensory canals); rows of yellow spots indicated by yellow letters represent sensory papillae rows; black arrows show ventralmost positions of gill opening. Abbreviations: AN, anterior narial pore; PN, posterior narial pore. Photographed and annotated by T. Suzuki.

bulbous and fleshy. Lips thick and fleshy; anterior tips of both lips even\* or lower lip slightly protruding anteriorly; gape slightly oblique; posterior margin of lower jaw extending posteriorly to a vertical through anterior margin of eye in females and slightly beyond it in males. Anterior naris a short tube without skin flap at its tip, and its base located slightly behind the midpoint between snout tip and anterior margin of eye. Posterior naris a round pore with low rim and located the midpoint between base of anterior naris and anterior margin of eye. Gill opening usually extending anteriorly to center of operculum\*, or to a vertical through posterior margin of preopercle. Gill membranes broadly attached to isthmus. No fleshy papillae or finger-like projections on lateral margin of shoulder girdle. Tongue free from floor of mouth, with rounded anterior margin. Genital papillae cone-shaped in males and oval in females.

Origin of first dorsal fin about an eye diameter behind a vertical through dorsal insertion of pectoral-fin; first dorsal fin in males usually near falcate shape, and higher than second dorsal fin in height, whereas, in females, semicircular or semioval, and usually slightly higher than, or slightly lower than second dorsal fin in height; usually second, or third\* spine longest; all dorsal-fin spines slender and flexible; distal tip of first dorsal fin in males usually second or third\* spine filamentous, or with non-filamentous spines, whereas, in females with nonfilamentous spines; posterior tip (= distal tip of second or third\* spines) of first dorsal fin in males extending between bases of first and fifth segmented ray of second dorsal fin (forth ray\*) when adpressed, whereas, in females, posterior tip (= distal tip of third, fourth or sixth spine) usually not, or extending to origin of second dorsal fin, when adpressed. First and second dorsal fins not connected by membrane; all segmented rays of second dorsal fin branched; seventh or eighth\* and second or third segmented rays longest in males and females, respectively; posterior tip (= distal tip of last or penultimate\* segmented ray) of second dorsal fin not extending to caudal fin when adpressed; posterior end of second dorsal-fin base above posterior end of anal-fin base. Origin of anal fin below between origin and base of second segmented ray of second dorsal fin (between bases of first and second segmented rays\*); anal fin slightly lower than second dorsal fin in height; all segmented analfin rays branched; sixth or seventh\*, and fourth or sixth segmented rays longest in males and females, respectively; posterior tip (= distal tip of last or penultimate\* segmented ray) of anal fin not extending to caudal fin when adpressed. Pectoral fin oval, extending posteriorly to a vertical between base of sixth spine of first dorsal fin and origin of

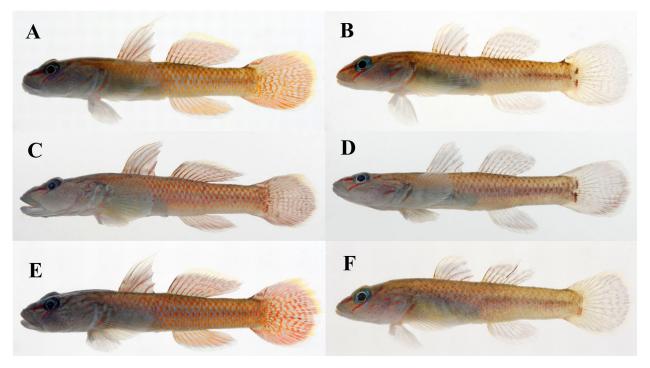


Fig. 14. Freshly-collected paratypes of *Rhinogobius aonumai ishigakiensis* from Ishigaki-jima Island, the Ryukyu Islands, Japan. Miyaragawa River: A (OMNH-P 40912, male, 38.0 mm SL) and B (OMNH-P 40914, female, 38.4 mm SL); Sakuta-gawa River: C (KPM-NI 65588, male, 55.9 mm SL) and D (SPMN-PI 49270, female, 40.5 mm SL); Sokobaru-gawa River: E (OMNH-P 40911, male, 39.9 mm SL) and F (OMNH-P 40913, female, 33.3 mm SL). Photographed by T. Suzuki.

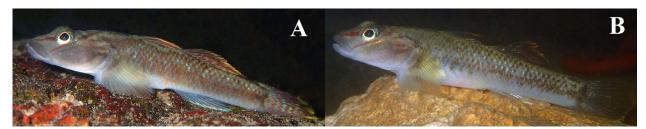


Fig. 15. Underwater photographs of *Rhinogobius aonumai ishigakiensis* taken at Ishigaki-jima Island, the Ryukyu Islands, Japan. Sakutagawa River: A (male, about 40 mm SL) and B (female, about 40 mm SL). Photographed by N. Oseko.

second dorsal fin (posterior end of first dorsal-fin base\*); pectoral-fin rays branched, except for dorsalmost and ventralmost rays unbranched. Pelvic fins fused medially by well-developed frenum (between spines) and connecting membrane (between innermost rays), forming a circular cup-like disc; pelvic fins extending posteriorly to a vertical between bases of second and fifth\* spines of first dorsal fin, not reaching to anus; pelvic-fin spine with a rounded membranous lobe at its tip; all segmented rays of pelvic fin branched. Caudal fin elliptical or fan-shaped\*.

Scales on body largely ctenoid, becoming smaller anteriorly; anterior edge of scaled areas with ctenoid scales on lateral, dorsal and ventral sides of body reaching respectively to a little behind pectoral-fin axilla, to between end of first dorsal fin and end of second dorsal fin, and to anal-fin base (Fig. 12A). Small cycloid scales on anterodorsal part of body before the area with ctenoid

scales. Predorsal squamation with trifurcate anterior edge; its mid-anterior extension (Fig. 11B: P1) extending anteriorly to between transverse lines through sensorycanal pore H' and K'; anterior extensions of lateral sides (Fig. 11B: P2) extending anteriorly to posterior oculoscapular canal; greatest concaved point of anterior margin of scaled area of predorsal region between P1 and P2 (Fig. 11B: P3) extending posteriorly to above between sensory-canal pore K' and upper end of pectoralfin axilla. Pectoral-fin base usually naked, or with one small cycloid scale. Prepelvic area usually naked, or with some small cycloid scales. Scaled area on belly with small cycloid scales usually extending anteriorly to, or not side of prepelvic area; belly above pelvic fin naked, and its following ventral midline of belly usually with small cycloid scale, or its anterior part naked (Fig. 12B).

Cephalic sensory systems are illustrated in Fig. 13. Nasal

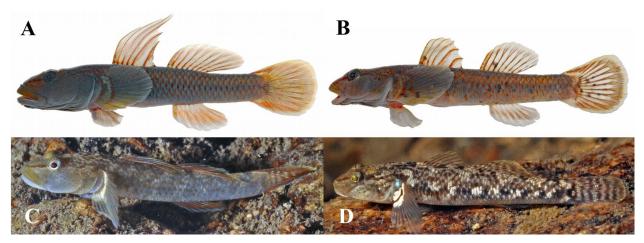


Fig. 16. *Rhinogobius flumineus* at Ina-gawa River, Ina-gawa, Hyogo Prefecture, Japan. Freshly-collected: A (OMNH-P 43228, 52.3 mm SL, male) and B (OMNH-P 43229, 50.6 mm SL, female), photographed by T. Suzuki.; underwater photographs: C (male, about 40 mm SL) and D (female, about 40 mm SL), photographed by M. Suzuki.

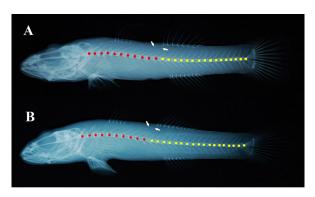


Fig. 17. Soft X-ray nagatives of holotypes of two subspecies of *Rhinogobius aonumai*. A: *Rhinogobius aonumai aonumai*, B: *Rhinogobius aonumai ishigakiensis*. Red and yellow spots indicate abdominal and caudal vertebrae, respectively; white arrows show anteriormost two pterygiophores (proximal radials) of second dorsal fin. Photographed and annotated by T. Suzuki.

extension of anterior oculoscapular canal with terminal pore B' located above between both nares. Anterior interorbital sections of anterior oculoscapular canal separated bilaterally, with paired pore C and a single pore D. Pore E present just behind posterior edge of eye. Lateral section of anterior oculoscapular canal with anterior pore F and terminal pore H'. Posterior oculoscapular canal with two terminal pores K' and L'. Gap between anterior and posterior oculoscapular canals much narrower than length of posterior oculoscapular canal. Preopercular canal present, with three pores M', N, and O'. As unusual variations, there is an accessory pore on the canal between pores F and H' in a paratype, and pore N is absent in two paratypes. Sensory-papillae row "a" oblique and uniserial, composed of loosely-arranged papillae, and extending anteriorly to a vertical through midpoint between anterior margin of eye and anterior margin of pupil. Row "b" longitudinal, composed of densely-arranged papillae, extending anteriorly to a vertical through center of pupil, and equal to eye diameter in length. Row "c" composed of loosely-arranged papillae, extending posteriorly to a vertical through posterior margin of eye. Row "d" composed of densely-arranged papillae, extending posteriorly to a vertical through midpoint of pupil. Row "cp" comprising a single papilla. Row "f" comprising paired papillae. Anterior end of row "oi" a little separated from a vertical row "ot".

Coloration of male when freshly collected (Figs. 9A, 9B, 14A, 14C, 14E, 14G). Ground color of head and body usually reddish yellow. Belly pale yellow and its side greenish. Head grayish; cheek, lower half of operculum and gill membrane with sparsely-arranged, some small purplish red dots. Snout with a broad oblique purplish red stripe between anterior margin of the eye and anteriormost part of snout. Several irregular-shaped, short purplish red stripes and minute spots on dorsal surface of snout and interorbital space, occipital region and nape; anterodorsal margin of cheek with a narrow purplish red line edged ventrally by a bright blue line; temporal region above operculum with a short purplish red stripe; upper part of operculum with two oblique purplish red stripes. Almost all scale pockets on body with reddish orange spots. Lateral side of body below dorsal-fin base with two longitudinal rows of blue spots. Ground color of fin membranes yellowish gray, partially with bluish sheen. Spines and segmented rays gray. Anterodorsal part of first dorsal fin and distal margin of second dorsal fin pale yellow; distal margin of anal fin white; dorsal and middle margins of caudal fin pale yellow and ventral margin white; membranes around spines and segmented rays of first dorsal and anal fins, and upper and lower parts of caudal

fin purplish red. Second dorsal fin with three longitudinal rows of reddish orange\* or purplish red spots, or usually forming zigzag stripes; a reddish orange or purplish red\* mottles above these spots or stripes. Central part of caudal fin with 7–9 (8\*) reddish orange or purplish red\* zigzag bands. Upper end of pectoral-fin base with a black triangle mark; pectoral-fin base with a reddish orange\* or purplish red bow-shaped mark, and in some specimens (except holotype), a vertical row of spots behind it.

Coloration of female when freshly collected (Figs. 10A, 10B, 14B, 14D, 14F). Resembles that of male, except as follows. Red or orange marks of head and body darker. Purplish red dots on cheek and operculum fewer in number, usually absent on operculum; gill membrane with no orange dots. Ventral side of body usually with no reddish orange spots. Second dorsal fin with 0–4 longitudinal rows of purplish orange spots or forming zigzag stripes. Caudalfin base with a pair of short, vertically aligned, rod-shaped grayish brown mark; central part of caudal fin with 3–6 indistinct purplish red zigzag bands. Pectoral-fin base with no vertical rows of spots behind a bow-shaped mark.

Coloration when alive based on underwater photographs (Fig. 15). Coloration when alive in the populations of Sakuta-gawa River, in Ishigaki-jima Island resembles that of freshly-collected coloration, except as follows. Ground color of head and body grayish; upper half of body with some pale blue spots; caudal-fin base with a Y-shaped, reddish gray mark in male.

**Coloration when preserved in alcohol** (Figs. 9C, 10C). Ground color of head and body turned to light gray; blue, green, yellow and white color faded; orange and red color turns to brown or gray. Dorsal side of body with 1–2 longitudinal row(s) of dark gray spots; mid lateral side of trunk with a brownish gray broad stripe in female.

**Distribution and habitat.** *Rhinogobius aonumai ishigakiensis* is known only from Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan. In this island, it is seen in the upper reaches of freshwater streams above waterfalls in montane areas of Sakuta-gawa River, and the upper streams above Maezato Dam of Miyara-gawa River and Sokobaru Dam of Sokobaru-gawa River of Miyara-gawa River System. In addition, we identified the gobies in the photographs taken from Isobe-gawa, Todoroki-gawa and Uratabaru-gawa rivers registered in the Image Database of Fishes, as *R. a. ishigakiensis*. The subspecies is usually found in small pools of streams, called "Fuchi" in Japanese.

**Etymology.** The subspecific name, *ishigakiensis*, refers to the type locality (Ishigaki-jima Island, the Yaeyama Group of the Ryukyu Islands, Japan).

#### Discussion

Subgroups of *Rhinogobius* sp. YB. *Rhinogobius* includes both amphidromous and freshwater resident species (Huang & Chen, 2007; Mizuno, 1960a). The latter is divided further into fluvial, lake-river migrating and lentic species (Takahashi & Okazaki, 2002).

Nishijima (1968) named a fluvial species of *Rhinogobius* collected from Okinawa-jima Island of Okinawa Group of the Ryukyu Islands as the Medium-egg type ("Chuurangata" in Japanese) with a note that it was characterized by its intermediate size and number of eggs between the small eggs of amphidromous species of Japanese Rhinogobius and the large eggs of Rhinogobius flumineus, and that it can spend its whole life in freshwater stream. Nakayama (1975) reported that there were two morphotypes of the Medium-egg type with yellow or blue bellies from Okinawa-jima Island. Hayashi (1984) reported that the Medium-egg type with a yellow belly was distributed from Amami-oshima Island (Amami Group of the Ryukyu Islands) to Taiwan. Iwata (1989) assumed this as a distinct unnamed species, tentatively named it as "Rhinogobius sp. YB" with a new Japanese vernacular name "Kibarayoshinobori"; he stated that it was distributed in the Ryukyu Islands, and that its distribution in the area outside of Japan was unknown. Akihito et al. (1993, 2000, 2002, 2013) reported Rhinogobius sp. YB occurred in the Ryukyu Islands. Suzuki et al. (2004) reported Rhinogobius sp. YB was distributed Amami-oshima, Kakeroma-jima, Tokuno-shima and Okinoerabu-jima islands of Amami Group, Okinawa-jima and Kume-jima islands of Okinawa Group, and Ishigaki-jima and Iriomote-jima islands, the Yaeyama Group of the Ryukyu Islands, Japan. Suzuki et al. (2021), an updated and revised version of Suzuki et al. (2004), added Tokashiki-jima Island of Okinawa Group to its distribution areas.

Nishida (1994) hypothesized that *Rhinogobius* sp. YB speciated multiple times from *Rhinogobius brunneus* as an ancestor species in the Ryukyu Islands, Japan. Kano *et al.* (2012) collected *Rhinogobius* sp. YB and *R. brunneus* from multiple rivers in Iriomote-jima Island. They showed that mtDNA divergence between *Rhinogobius* sp. YB populations in respective rivers was larger than between *Rhinogobius* sp. YB and *R. brunneus* populations. They estimated that *Rhinogobius* sp. YB populations in respective rivers independently speciated from a common migratory ancestor in the rivers where they inhabited. However, this hypothesis was rejected by a subsequent analysis using multiple nuclear gene markers (Yamasaki *et al.*, 2020). Yamasaki *et al.* (2020) revealed that the

speciation of *Rhinogobius* sp. YB from its migratory ancestor, *R. brunneus*, occurred in parallel across five islands or islands groups (Amami-oshima Island, Tokunoshima + Okinoerabu-jima islands, Okinawa-jima Island, Kume-jima Island, and Ishigaki-jima + Iriomote-jima islands) using population genetic analysis.

Our investigation further revealed that the population of *Rhinogobius* sp. YB of the Yaeyama Group can be morphologically differentiated from the congeners, including the population of *Rhinogobius* sp. YB from the other island groups of the Ryukyu Islands, and that the population of *Rhinogobius* sp. YB of Ishigakijima and Iriomote-jima islands can be morphologically distinguished. Considering together with the result of the molecular studies made by Yamasaki *et al.* (2020), therefore, we here recognize the population of *Rhinogobius* sp. YB of the Yaeyama Group as a distinct species (*R. aonumai*), comprising two subspecies (*R. a. aonumai* from Iriomote-jima Island and *R. a. ishigakiensis* from Ishigakijima Island).

Comparisons with the congeners. Rhinogobius is currently known as the most specious freshwater gobiid genus, comprising 88 valid species-group taxa (Suzuki et al., 2020; Wanghe et al., 2020; present study). As indicated by Chen & Shao (1996) and Suzuki et al. (2015), the genus is divided into two distinct groups; one comprises only a single species R. similis, whereas the other includes all the remaining species. Rhinogobius similis differs from the other congeners by having large ctenoid scales on the nape (vs. nape naked or with cycloid scales in the others) and several short transverse rows of sensory papillae on the cheek (vs. no distinct transverse rows of sensory papillae on the cheek). Suzuki et al. (2020) assigned all species of the genus but R. similis to the "Rhinogobius brunneus complex", following Chen & Shao (1996). Rhinogobius aonumai aonumai and R. a. ishigakiensis described here also belong to the *R. brunneus* complex.

Furthermore, Suzuki *et al.* (2020) attempted to divide the *R. brunneus* complex into two subgroups: one almost always has 27 or more vertebrae (named as "Group I"), whereas the others have lower counts of vertebrae (25–27, almost always 26) ("Group II"). The groups I and II, both of which appear to be phylogenetic grades merely assembled by the vertebral counts (Suzuki *et al.*, 2020), hitherto comprise at least 47 and 32 described species, respectively. Unfortunately, Suzuki *et al.* (2020) failed to assign the remaining six species to these subgroups due to the lack of information on their vertebral counts. *Rhinogobius aonumai aonumai* and *R. a. ishigakiensis*, having 26–28 vertebrae (usually 27), belong to Group I, making the total

number of species-group taxa in the group 49.

Species of Group I and the assemblage with no information about vertebral counts including 49+6=55 species-group taxa are compared in Table 3. Within 55 species-group taxa, R. a. aonumai and R. a. ishigakiensis are most similar to Rhinogobius flumineus (Mizuno, 1960) and R. szechuanensis (Tchang, 1939), by having the following combination of characters: the number of longitudinal scales (32-37 in R. a. aonumai; 33-38 in R. a. ishigakiensis; 30–36 in R. flumineus; 30–34 in R. szechuanensis); no dark spot on first dorsal fin; vertical rows of dark spots or dark bands on caudal fin (absent or vertical rows of dark spots in R. flumineus). Rhinogobius aonumai aonumai and R. a. ishigakiensis are, however, distinguished from R. flumineus and R. szechuanensis by having more than 9 predorsal scales (9–15 in R. a. aonumai; 10-14 in R. a. ishigakiensis vs. 2-9 in R. flumineus; 0 in R. szechuanensis). Furthermore, R. aonumai aonumai and R. a. ishigakiensis are distinguished from R. flumineus by having a yellow-colored body in freshly-collected (vs. gray in R. flumineus), and a pair of short, vertically aligned, rod-shaped mark on the caudalfin base in females (vs. single rod-shaped mark in both sexes) (Fig. 16). The previously-recognized "Rhinogobius sp. YB" (including R. a. aonumai and R. a. ishigakiensis) and Rhinogobius flumineus are distinguishable by genetic features, and the genetic distance between them is as large as that between the species of Rhinogobius (Yamasaki et al., 2015). Rhinogobius aonumai aonumai and R. a. ishigakiensis are distinguished from R. szechuanensis by having sensory canals on the head (vs. absent in R. szechuanensis). In the above comparisons, data on R. flumineus follow Akihito et al. (2013) and its errata (Nakabo & Tokai University Press, 2014), Mizuno (1960), as well as the comparative materials examined here (see below), and data on R. szechuanensis follow Chen et al. (2008), Tchang (1939) and Wu & Chen (2008).

Rhinogobius aonumai aonumai and R. a. ishigakiensis are distinguished from the populations of Rhinogobius sp. YB from the other islands by having usually 27 vertebrae (vs. 26 in Rhinogobius sp. YB from the other islands). Furthermore, Rhinogobius sp. YB populations showed a large divergence between the Middle Ryukyu and South Ryukyu (including the Yaeyama Group), and Rhinogobius sp. YB has speciated in five islands in the Ryukyu Islands (Yamasaki et al., 2020). The Yaeyama Group is one of them.

Rhinogobius aonumai aonumai is distinguished from R. a. ishigakiensis by having 11 abdominal vertebrae (vs. 10 in R. aonumai ishigakiensis), the anteriormost two pterygiophores

(proximal radials) of second dorsal fin mounted over the neural spine of the tenth vertebra (vs. ninth vertebra) (Fig. 17), fifth segmented pelvic-fin ray usually divided into four branches (vs. usually two branches) at the position where proximal most segment of each branch alignes transversely, no filamentous spine of first dorsal fin (vs. usually second spine filamentous in males), around the midline of belly usually naked except posterior part (vs. usually scaly), cheek, the lower half of operculum and gill membrane usually with many small orange spots densely (vs. some red spots sparsely), and the lower margin of caudal fin usually bright yellow (vs. white).

Current statuses of the populations. Rhinogobius aonumai aonumai and R. a. ishigakiensis are the endemic subspecies of Iriomote-jima Island and Ishigaki-jima Island, respectively, and are the second and third known species-group taxa of the genus Rhinogobius from Japan, with a high vertebral count. In the Red List 2020 of the Ministry of the Environment of Japan, Rhinogobius sp. YB (including R. a. aonumai and R. a. ishigakiensis) is ranked as EN "Endangered" (Ministry of the Environment Government of Japan, 2020). Both R. a. aonumai and R. a. ishigakiensis are the noteworthy species-group taxa (species and subspecies) with the smallest distribution area and population size among the congeners hitherto known from Japan. Gobies of the previously-recognized "Rhinogobius sp. YB" has diversified independently in five islands groups of the Ryukyu Islands (Yamasaki et al., 2020), but the taxonomic assessment of all but the populations from the Yaemyama Group (herein described as R. a. aonumai and R. a. ishigakiensis) has not yet made properly. Resolving the taxonomic statuses and proper naming of the populations of the "Rhinogobius sp. YB" are urgently needed for assessing respective population statuses and developing their conservation measure adequately, and this paper is the first step for the task.

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## Supplementary materials

Supplementary Material #1 is shown in the last page of the article.

## 摘要

鈴木寿之・大迫尚晴・山崎 曜・木村清志・渋川浩一, 2022. 琉球列島八重山諸島から得られたハゼ科 ヨシノボリ属魚類の2新亜種を含む1新種. 神奈川県立博物館研究報告(自然科学), (51): 9–34. [Suzuki, T., N. Oseko, Y. Y. Yamasaki, S. Kimura & K. Shibukawa, 2022. A New Species with Two New Subspecies of *Rhinogobius* (Teleostei: Gobiidae) from Yaeyama Group, the Ryukyu Islands, Japan. *Bull. Kanagawa Pref. Mus.* (*Nat. Sci.*), (51): 9–34.]

琉球列島八重山諸島の河川渓流域に生息するハゼ科ヨシノボリ属魚類の2新亜種(Rhinogobius aonumai aonumai と Rhinogobius aonumai ishigakiensis)をふくむ1新種 Rhinogobius aonumai(新標準和名パイヌキバラヨシノボリ)を記載した。Rhinogobius aonumai aonumai(新標準和名イリオモテパイヌキバラヨシノボリ)は西表島のみに分布し、背鰭前方鱗数9–15、縦列鱗数32–37、脊椎骨数11+15–17=26–28(モードは27)、第2背鰭前端の2個の坦鰭骨は第10脊椎骨の神経棘をまたぐ、腹鰭第5軟条は最初に3または4分岐(ふつう4分岐)する、頬の孔器列は縦列する、生鮮時の体の地色は黄色系である、第1背鰭に暗色斑はない、尾鰭に暗色の横点列かジグザグ横線が並ぶ、雌の尾鰭基底に垂直に並ぶ1対の暗色の短い棒状斑があるなどの特徴で同属の他種階級タクソン(種及び亜種)から区別できる。Rhinogobius aonumai ishigakiensis(新標準和名イシガキパイヌキバラヨシノボリ)は石垣島のみに分布し、背鰭前方鱗数10–14、縦列鱗数33–38、脊椎骨数10+16–18==26–28(モードは27)、第2背鰭前端の2個の坦鰭骨は第9脊椎骨の神経棘をまたぐ、腹鰭第5軟条は最初に2または3分岐(ふつう2分岐)する、頬の孔器列は縦列する、生鮮時の体の地色は黄色系である、第1背鰭に暗色斑はない、尾鰭に暗色のジグザグ横線が並ぶ、雌の尾鰭基底に垂直に並ぶ1対の暗色の短い棒状斑があるなどの特徴で同属の他種階級タクソン(種及び亜種)から区別できる。

#### Supplementary Material 1. Comparative materials

Rhinogobius flumineus: 9 specimens (41.0–53.5 mm SL): OMNH-P 43227–43230, 2 males and 2 females, 47.5–53.5 mm SL, Ina-gawa River, Inagawa, Hyogo Prefecture, Japan, 34°54'42.93"N 135°22'8.05"E, 10 October 2015; OMNH-P 42905, male, 48.1 mm SL, stained with Alizarin Red S., Ina-gawa River, 34°55'0.54"N 135°21'18.62"E, 29 August 2014; OMNH-P 48836 and 48838, male and female, 41.0 and 46.5 mm SL, stained with Alizarin Red S., Kumagawa River, Kumakogen, Ehime Prefecture, Japan, 33°36'55.65"N 132°57'16.91"E, 20 February 2020; OMNH-P 48840 and 48841, male and female, 42.0 and 44.5 mm SL, stained with Alizarin Red S., Shimantogawa River, Nakatosa, Kochi Prefecture, Japan, 33°21'52.10"N 133°8'4.99"E, 20 February 2020.

Rhinogobius sp. YB: 38 specimens: (27.0–61.0 mm SL). Amami-oshima Island (Amami Group of the Ryukyu Islands, Japan): OMNH-P 43030–43033, three males and a female, 38.3–42.3 mm SL, Kawauchi-gawa River, 28°20'0.88"N 129°25'13.9"E, 27 July 2014; OMNH-P 42870–42872, two males and a female, 37.0–42.8 mm SL, Akina-gawa River, 28°24'52.3"N 129°33'36.5"E, 28 July 2014. Tokunoshima Island (Amami Group): OMNH-P 43021 and 43023, male and female, 41.0–50.8 mm SL, Agon-gawa River, 27°42'34.93"N 128°55'6.28"E, 30 January 2015; OMNH-P 43024 and 43026, male and female, 41.5 and 46.5 mm SL, Uwanaru-gawa River, 27°44'12.25"N 128°55'37.15"E, 27 January

2015; OMNH-P 43016 and 43017, male and female, 48.0 and 53.6 mm SL, Akigiyan-gawa River, 27°46'43.33"N 128°57'28.61"E, 28 January 2015; OMNH-P 43027, male, 38.0 mm SL, Oose-gawa River, 27°43'53.78"N 128°59'38.25"E, 30 January 2015. Okinoerabu-jima Island (Amami Group): OMNH-P 43011-43014, two males and two females, 38.0-53.5 mm SL, Amata-gawa River, 27°21'46.1"N 128°36'40.9"E, 22 or 24 January 2014. Sampling in Kagoshima Prefecture was approved by Kagoshima Prefecture. Okinawa-jima Island (Okinawa Group of the Ryukyu Islands, Japan): OMNH-P 38191-39194, two males and two females, 44.5-61.0 mm SL, Sukuta-gawa River, 26°33'41.6"N 127°59'18"E, 11 December 2011; OMNH-P 43048 and 43050, male and female, 40.0 and 45.5 mm SL, Okukubigawa River, 26°28'59.24"N 127°54'20.25"E, 2 March 2015; OMNH-P 43051-43054, two males and two females, 39.3-49.1 mm SL, Yofuke-gawa River, 26°33'31.1"N 128°00'23.3"E, 2 March 2015. Tokashiki-jima Island (Okinawa Group): OMNH-P 48916-48919, three males and a female, 36.5-38.0 mm SL, a small stream with an unknown name, 26°10'07.8"N 127°21'18.1"E, 27 February 2018. Kume-jima Island (Okinawa Group): OMNH-P 42910-42912, two males and female, 27.0-32.0 mm SL, Suhara-gawa River, 26°18'57.87"N 126°47'27.41"E, 5 October 2014; OMNH-P 42913-42915, two males and female, 28.0-38.4 mm SL, Shirase-gawa River, 26°21'31.04"N 126°46'22.62"E, 6 or 8 October 2014.