Larval study revealed diversity and life-history traits of crypto-benthic eel gobies

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Abstract
Because adult and juvenile eel gobies usually hide within the burrows of muddy substrates, their diversity and life history have not yet been fully elucidated. We investigated larval specimens of the eel gobies collected on Okinawa Island in southern Japan. The genus *Trypauchenopsis* was previously thought to consist of only one species, but our larval collection identified two species, *Trypauchenopsis limicola* and *Trypauchenopsis intermedia*, distinguished by their species-specific melanophore arrangements and differences in their fin-ray counts. *Taenioides kentalleni* were previously known from only two specimens worldwide. A third specimen of this species has now been added from the larval collection. In addition to the three species above, *Taenioides gracilis* and *Caragobius urolepis* were identified and larval morphologies of the five species were described for the first time. All the larvae collected in the present study were at late postflexion stage. *Trypauchenopis limicola*, *T. intermedia*, and *T. gracilis* were presumably collected in the estuaries and beaches when approaching their adult habitats at the end of pelagic life. They were 8.5–10.3 mm in standard length, and otolith analysis suggests that their pelagic

larval durations are a little longer than 1 month (average 34–37 days). The larval occurrence suggested that the spawning season of *T. limicola* is May–December, when the water temperature is warmer than approximately 20°C. Our work reveals that studying the larval stage can provide new information on the taxonomy and life history of the elusive cryptobenthic fish.

KEYWORDS

larva, goby, morphology, pelagic larval duration, age

1 INTRODUCTION

Crypto-benthic fishes are difficult to study because they cannot be located without extensively excavating the substrate where they live. However, such fish, together with many other fish taxa, usually grow in pelagic habitats during the larval stage, and pelagic larvae can easily be collected (for example, Maeda and Tachihara, 2014; Maeda *et al.*, 2008). Here we provide typical examples from a larval study of eel gobies performed in marine habitats on Okinawa Island in the Ryukyu Archipelago, Japan.

Eel gobies used to be assigned to subfamily Amblyopinae (Nelson, 2006). However, recent molecular studies have revealed that eel gobies are paraphyletic and form a monophyletic lineage together with a polyphyletic group of mudskippers (subfamily Oxudercinae) (Agorreta *et al.*, 2013; Steppan *et al.*, 2022; Thacker, 2003). Adult eel gobies typically live in the burrows of tidal mudflats and the muddy bottoms of estuaries, but they are also found in trawls of muddy substrates from the sea at up to approximately 100 m in depth (Dôtu, 1957; Gonzales *et al.*, 2008; Itani and Uchino, 2003; Koreeda and Motomura, 2021; Murdy, 2003; Nayar, 1951; Prokofiev, 2015; Rao, 1939). A specimen of *Karsten totoyeinsis* (Garman 1903) was even collected from a depth of 1,122 m off Sulawesi, Indonesia (Murdy, 2002). Eel gobies have elongated, pink, purple, or red bodies, and very small to vestigial eyes (Murdy, 2011). As eel goby eggs have yet to be reported in either natural or aquarium conditions, their spawning habit is unknown, although the induced spawning and embryonic development of *Odontamblyopus lacepedii* (Temminck and Schlegel 1845) have been described and is suspected to lay eggs in the walls of burrows (Dotsu and Takita, 1967). Larvae have been collected by set nets, midwater trawling, small seine nets, and light traps and are therefore predicted to swim freely in water columns (Dôtu, 1957, 1958; Hanahara *et al.*, 2021; Leis and Carson-Ewart, 2000; Maeda and Tachihara, 2014). The larval morphologies, described in Dôtu (1957, 1958), Harada and Suharti (2000), Leis and Carson-Ewart (2000), Leis and Trnski (1989), Okiyama (2014), and Ruple (1984), show moderate-sized eyes at the mid-lateral position of the head, like other gobies, which reduce in size after settlement.

Three species of *Taenioides* Lacepède 1800, one of *Trypauchenopsis* Volz 1903, and one of *Caragobius* Smith and Seale 1906 are known to inhabit Okinawa Island (Kurita and Yoshino, 2012;

Nakabo, 2013), but the diversity and distribution of eel gobies on the island are not well understood. A record of *Taenioides kentalleni* Murdy and Randall 2002 from Okinawa was based on a single specimen (Kurita and Yoshino, 2012), and with no additional specimens reported in the region; the only other known specimen of this species is the holotype that was collected in Saudi Arabia. In Okinawa, *Taenioides anguillaris* (Linnaeus 1758) was previously only identified in Ohura Bay, although another habitat was recently found (Miyahira and Tachihara, 2022). Maeda and Tachihara (2006, 2014) reported occurrences of an additional species of *Trypauchenopsis* on Okinawa Island (as *Taenioides* in Maeda and Tachihara, 2006), but its morphology has not been described in detail, and its taxonomic status has not been determined. In the present study, we describe the morphologies of eel goby larvae collected on Okinawa Island to improve our knowledge of this fish and to better understand larval morphologies of eel gobies. We also describe their life-history traits based on the larval occurrences, sizes, and ages.

2 MATERIALS AND METHODS

2.1 Sampling

2.1.1 Ohura Bay

Collections were made every month in 1999, at three sites along Ohura Bay on the east coast of Okinawa Island in southern Japan (Figure 1). The sites were a sandy beach in Sedake (26°32'59"N 128°03'16"E), the mouth of the Teima Stream (26°33'10"N 128°03'54"E), and the middle reaches of the estuary of the Teima Stream (26°33'25"N 128°04'12"E). The larvae were collected using a small seine net (0.8 mm mesh; 0.8 m in height by 3.5 m in width; with a bag 1 m in length by 0.7 m in diameter at the centre; without a sinker). The net was hauled by two people using poles set at either side of the net along the shoreline or stream bank at a depth of 0.6–1.0 m. The samplings were conducted at night because nocturnal sampling usually yields more goby larvae in this method (Maeda and Tachihara, 2014). Details of the environments of the sampling sites and sampling methods are described in Maeda and Tachihara (2005, 2014). The net is illustrated in Maeda and Tachihara (2014: figure 2). Fish specimens were fixed in 10% formalin and preserved in 70% ethanol. The specimens collected in Ohura Bay in 1999 were used for morphological description but not for otolith analysis.

One specimen collected in Sedake in October 2003 was also used for morphological description as well as the otolith analysis in this study. The method of collection was the same as described above, although a different net was used; the net was the same as the one used at Aritsu (see below).

2.1.2 Aritsu

Collections were made at night from 2003 to 2007 (October and November in 2003; every month in 2004; August 2005; January, February, April, June, and August–December in 2006; and February, March, April, June, and November in 2007) on a stony beach in Aritsu, Arume Bay on the east coast of Okinawa Island (26°35'27"N 128°07'50"E; Figure 1). The collection site was adjacent to the mouth of the Aritsu Stream. The larvae were collected using another small seine net (1.0 mm mesh; 0.8 m in height by 3.5 m in width; with a bag 1 m in length by 0.7 m in diameter at the centre; sinkers attached along the bottom edge). The hauling method was the same as that used in Ohura Bay, but the site was often shallower (0.2–1.0 m in depth). Details of the environment of the sampling site and sampling methods are described in Maeda and Tachihara (2008) and Maeda *et al.* (2007). Larvae of the eel gobies were sorted soon after collection and were euthanized in ice water. Their standard lengths (SL) were measured using a Vernier calliper under a stereomicroscope. Many of these specimens were used for otolith analysis and morphological description, but a few selected specimens were used only for the morphological description after fixation in 5% buffered formalin without removal of the otoliths and preserved in 70% ethanol.

2.1.3 Nakagusuku Bay

One *T. kentalleni* larva was collected by a shirasu trawl off Nishihara, Nakagusuku Bay on the east coast of Okinawa Island (26°12'30.5"N 127°47'16.9"E; Figure 1) in the daytime on June 20, 2006. The site was 13.7 m in depth and the surface-water temperature was 28°C. The shirasu trawl is usually operated in commercial fishing to collect clupeoid larvae and juveniles, but it was used in Nakagusuku Bay for the purpose of a research. The net comprised 68 m-wide wing nets (21 m maximum height, 0.6 m mesh) on both sides of a 25.4-m long bag (mouth dimensions: 3.8 m in width and height; made by various meshes of 22, 4, 3, 2, and 0.33 mm, from the mouth to the end) and was horizontally towed below the water surface using 100 m ropes by a boat at approximately 3.7 km h^{-1} (2 knots) for 2 minutes. The specimen was fixed in 5% seawater formalin, preserved in 70% ethanol, and used for morphological description of *T. kentalleni*. Details of the sampling methods are described in Uehara and Tachihara (2020).

2.2 Otolith analysis for age estimation

Sagittal otoliths were extracted from fresh larval specimens under a stereomicroscope and were fixed on a glass slide with clear nail varnish. After extracting the otoliths, most of the fish specimens were fixed in 5% buffered formalin and preserved in 70% ethanol. The number of otolith increments was counted from the core to the margin using a light microscope. Because daily deposition of otolith increments has been observed for many goby taxa (Hernaman *et al.*, 2000; Hoareau *et al.*, 2007; Iglesias *et al.*, 1997; Maeda *et al.*, 2007; Radtke *et al.*, 1988; Shafer 2000; Taillebois *et al.*, 2012; Yamasaki *et al.*, 2007), the number of increments from the core to the margin of the otolith was considered to represent the daily age of the larva (number of days between hatching and sampling dates).

2.3 Morphological observations

Terminology and measurements principally followed that of Leis and Carson-Ewart (2000). Standard length, pre-dorsal-fin length (PDL), preanal length (PAL), head length (HL), snout length (SnL), eye diameter (ED), and body depth at the pectoral-fin base (BD) were measured using a micrometer or a calliper under a stereomicroscope and were expressed as proportions of SL. Fin-ray counts of dorsal, anal, pectoral, and pelvic fins were made under a stereomicroscope.

Larval morphologies of *Trypauchenopsis limicola* (Smith 1964), *Trypauchenopsis intermedia* Volz 1903, *Taenioides gracilis* (Valenciennes 1837), and *Caragobius urolepis* (Bleeker 1852) were described mainly based on the preserved specimens collected from Ohura Bay in 1999 and *T. kentalleni* was described based on a specimen from Nakagusuku Bay. Many specimens collected in Sedake and Aritsu from 2003 to 2007 had been damaged during otolith extraction and were therefore only used for the fin-ray counts. Two *T. gracilis* specimens from Aritsu (otolith was extracted from only one of the specimens) were used for proportional measurements in addition to the fin-ray counts.

The following adult specimens of *Trypauchenopsis*, from Ishigaki Island, Japan, borrowed from Yokosuka City Museum (YCM), Japan were observed to determine the Japanese names of the species of *Trypauchenopsis*: YCM-P 2639, two specimens, 68.9–74.3 mm SL, Shiiugawa River, May 1, 1976; YCM-P 3945, five specimens (there were originally eight specimens in this lot but only five were available; Hayashi and Ito 1978; Kiyoshi Hagiwara at YCM, personal communication), 56.3–85.9 mm SL, Shiiugawa River, March 19, 1977; YCM-P 4029, two specimens, 50.9–77.6 mm SL, Tsuurogawa River, May 2, 1977.

2.4 Ethical Statement

This study was conducted using old preserved larval specimens collected in 1999–2007 when all coauthors belonged to the University of the Ryukyus, Japan. The authors confirm that all specimens were obtained in accordance with the relevant laws and regulations of Japan. Samplings were conducted in accordance with the university's current regulations on animal experimentation, although no approval number was issued as there was no committee at the time of the study. The authors did not handle living animals in later examinations. This study also followed the "Guidelines for the use of fishes in research" of the Ichthyological Society of Japan.

3 RESULTS

3.1 *Trypauchenopsis limicola* (Smith 1964)

3.1.1 Material examined

Sixty-one postflexion larvae (8.3–10.3 mm SL) collected by small seine nets on Okinawa Island, Japan. URM-P 48889 (sketched, Figure 2a), 9.0 mm SL, mouth of the Teima Stream, September 18, 1999; URM-P 48892, 8.8 mm SL, Sedake, August 7, 1999; URM-P 48893, 8.5 mm SL, mouth of the Teima Stream, August 7, 1999; URM-P 48894, 8.8 mm SL, middle reaches of the estuary of the Teima Stream, August 10, 1999; URM-P 48895, three specimens, 9.0–9.4 mm SL, mouth of the Teima Stream, August 10, 1999; URM-P 48896–48897, seven specimens, 8.4–8.8 mm SL, Sedake, August 10, 1999; URM-P 48898, 9.2 mm SL, middle reaches of the estuary of the Teima Stream, August 17, 1999; URM-P 48899–48900, two specimens, 8.3–9.0 mm SL, Sedake, September 12, 1999; URM-P 48901, 9.2 mm SL, mouth of the Teima Stream, September 18, 1999; URM-P 48902, two specimens, 8.7–8.7 mm SL, Sedake, September 19, 1999; URM-P 48903, 8.7 mm SL, Sedake, October 19, 1999; URM-P 48904, 9.7 mm SL, Sedake, December 15, 1999. The following specimens were only used for the fin-ray counts: URM-P 49675, 10.3 mm SL, Aritsu, January 11, 2004; URM-P 49676–49680, five specimens, 8.8–9.3 mm SL, Aritsu, June 22, 2004; URM-P 49682, two specimens, 9.4–9.7 mm SL, Aritsu, July 10, 2004; URM-P 49683–49687, five specimens, 9.6–10.1 mm SL, Aritsu, July 22, 2004; URM-P 49688–49689, two specimens, 9.0–9.1 mm SL, Aritsu, August 14, 2004; URM-P 49690–49691, two specimens, 9.4–9.5 mm SL, Aritsu, September 11, 2004; URM-P 49692–49698, seven specimens, 9.8–10.2 mm SL, Aritsu, October 31, 2004; URM-P 49701–49705, five specimens, 9.5–9.8 mm SL, Aritsu, August 9, 2005; URM-P 49707, 9.2 mm SL, Aritsu, June 22, 2006; URM-P 49708–49711, four specimens, 8.7–9.3 mm SL, Aritsu, August 29, 2006; URM-P 49718–49721, four specimens, 8.7–9.1 mm SL, Aritsu, September 20, 2006; URM-P 49722, 9.1 mm SL, Aritsu, October 29, 2006.

3.1.2 Morphology

The body is elongated (BD = 12.1–15.4% of SL, Table 1) and compressed. The head is round (HL = 21.6–26.3% of SL) with a slightly convex snout. The mouth is oblique and exceeds the anterior margin of the eye. The lower jaw protrudes slightly beyond the upper jaw. The eye is situated at the mid-lateral part of the head and is small (ED = 2.5–3.9% of SL), elliptical, and down-slanting toward the posterior end. The anterior nostril is near the tip of the snout, and the posterior nostril is anterior to the eye. No head spination is present. A large gas bladder is situated at the posterior part of the abdominal cavity. The anus is located slightly posterior to the middle of the body (PAL = 51.9–56.5% of SL). The dorsal fin originates posterior to the pectoral fin and anterior to the gas bladder (PDL = 31.6–35.8% of SL), with the first and second dorsal fins connected by a membrane. The first dorsal fin has six spines; the first to fifth spines are regularly spaced while the sixth spine has a wider interval from both the fifth spine and the first soft ray of the second dorsal fin. The second dorsal and anal fins are higher than the first dorsal fin and comprise 28–31 and 26–30 soft rays, respectively (Tables 2 and 3). The anal fin begins just posterior to the anus. The second dorsal and anal fins are confluent with the caudal fin, which has 9 + 8 (dorsal + ventral) thick rays and a few thin procurrent rays. The posterior margin of the caudal fin is round. The pectoral fin is fan-shaped with 17–20 rays (Table 4). The pelvic fin has one spine and five soft rays; the left and right fins join together to form a cup-like disc with a frenum, and is small with its tip slightly exceeding the position vertically below the origin of the first dorsal fin.

3.1.3 Pigment

The left and right sides of the base of the second-dorsal-fin both display 4–20 melanophores, although their positions on the left and right sides are not identical. Along the anal-fin base, melanophores occurred on the left and right sides of the soft rays from the second or third ray to the penultimate or last ray, although a few of the soft rays lack the left or right melanophore (the number of melanophores on one side is 22–28). One larva had fewer melanophores (11 on the left and nine on the right). All melanophores along the bases of the dorsal and anal fins are the same size and not enlarged. One to three melanophore(s) are aligned along the lateral midline of the posterior-most part of the body. Melanophores form a vertical line along the caudal-fin base, but this vertical line breaks in the middle. On the caudal fin, melanophores also occur along the proximal part of the middle ray. Melanophores are seen at the ithmus and pre-pelvic region along the ventral midline of body, but they do not always occur; of the 22 specimens, nine had melanophores at both the ithmus and pre-pelvic region, seven had only the former, two had only the latter (Figure 2a), and four lacked both. Melanophores pigment the anterodorsal surface of the gas bladder.

3.2 *Trypauchenopsis intermedia* Volz 1903

3.2.1 Material examined

Ten postflexion larvae (8.5–9.7 mm SL) collected by small seine nets on Okinawa Island, Japan. URM-P 42755 (sketched, Figure 2b), 9.7 mm SL, mouth of the Teima Stream, September 18, 1999; URM-P 48891, 9.3 mm SL, Sedake, September 19, 1999. The following specimens were only used for the fin-ray counts: URM-P 49673, 9.0 mm SL, Aritsu, October 4, 2003; URM-P 49674, 9.0 mm SL, Sedake, October 25, 2003; URM-P 49700, 9.7 mm SL, Aritsu, August 9, 2005; URM-P 49706, 9.5 mm SL, Aritsu, June 22, 2006; URM-P 49712–49715, four specimens, 8.5–9.5 mm SL, Aritsu, August 29, 2006.

3.2.2 Morphology

While the general shape is the same as that of *T. limicola* (Figure 2b; Table 1), the second dorsal and anal fins have more rays (31–35 and 29–33 rays, respectively; Tables 2 and 3), and the pectoral fin has fewer rays (16 or 17; Table 4).

3.2.3 Pigment

The left and right sides of the base of the second-dorsal-fin both display 3–9 melanophores, although their positions on the left and right sides are not identical. There are enlarged melanophores at the two or three successive rays of the posterior part of the anal fin (sixth to seventh or sixth to eighth rays from the last ray) that combine to form a single blotch. One or two additional melanophores occur at the base of the anterior and/or middle anal-fin rays. The positions of the melanophores on the left and right sides are usually different. Melanophores form a vertical line along the caudal-fin base, which does not break in the middle. One specimen had a melanophore at base of the fifth soft ray of the left pelvic fin but no melanophore on the ventral midline of the pre-pelvic region. Melanophores pigment the anterodorsal surface of the gas bladder.

3.2.4 Remarks

Shibukawa and Murdy (2012) redescribed the genus *Trypauchenopsis* and synonymized *Taenioides jacksoni* Smith 1943 and *Taenioides limicola* Smith 1964 with *Trypauchenopsis intermedia* Voltz 1903. They suggested the possibility that *Brachyamblyopus burmanicus* Hora 1926 is another synonym of *T. intermedia*, but they could not confirm this as they did not examine syntypes of *B. burmanicus*. Thus, *Trypauchenopsis* is currently considered a monotypic genus, with *T. intermedia* the only known member. The status of *B. burmanicus* remains unclear.

This study identified two species of *Trypauchenopsis* clearly distinguished by morphology, indicating that *Trypauchenopsis* is composed of at least two species and that these species coexist on Okinawa Island. These two species are principally distinguished by differences in their melanophore arrangements, with one displaying small melanophores at the bases of most of the anal-fin rays (sp. 1; Figure 3a) while the other has a larger blotch formed by enlarged melanophores at the two or three successive rays of the posterior part of the anal fin and a lack of melanophores at the bases of most of the other anal-fin rays (sp. 2; Figure 3b). There are a few additional differences in their pigment patterns; for example, sp. 1 has a vertical line along the caudal-fin base that breaks at the centre and a short horizontal line on the proximal part of the middle of the caudal fin (Figure 3a), whereas sp. 2 has a complete vertical line and lacks the horizontal line (Figure 3b). Furthermore, sp. 1 often has melanophores at the ithmus and/or pre-pelvic region which are not present on sp. 2.

We compared the second dorsal-, anal-, and pectoral-fin-ray counts of the two species after identification based on the melanophore patterns along the anal-fin base. Sp. 1 has fewer second-dorsal- and anal-fin rays than sp. 2 (28–31 and 26–30 vs. 31–35 and 29–33, respectively; Tables 2 and 3) and more pectoral-fin rays (17–20 vs. 16–17; Table 4). The ranges of counts slightly overlapped between the two species; specimens having 31 second-dorsal-fin rays, 29–30 anal-fin rays, or 17 pectoral-fin rays were found in both species. However, no specimen had a combination of the counts composed only of the overlapped values. This indicates that the two species can be distinguished by a combination of the counts of these three fins. While the pigment patterns are only applicable to the larvae, adults and juveniles can be identified by their fin-ray counts.

The holotype of *T. limicola* has 29 rays in the second dorsal fin, 27 rays in the anal fin, and 18 rays in the pectoral-fins (Smith, 1964). Because these correspond to the most or second most frequent counts of sp. 1 (Tables 2–4), sp. 1 can be identified as *T. limicola*. The type locality is Guam.

Although Volz (1903) described *T. intermedia* as having 28 rays in the second dorsal fin and 27 rays in the anal fin (the pectoral-fin-ray count was not provided in the original description), Shibukawa and Murdy (2012), who examined the holotype of *T. intermedia*, stated that it actually has 32 rays in the second dorsal fin, 30 rays in the anal fin, and 16 and 17 rays in the left and right pectoral fins, respectively. These counts correspond to the mode of each count of sp. 2, except for the left pectoral-fin-ray count (but is within the range; Tables 2–4). Therefore, sp. 2 can be identified as *T. intermedia*. The type locality is Sumatra, Indonesia. Our data supports the hypothesis of Shibukawa and Murdy (2012) that *T. jacksoni* is a synonym of *T. intermedia* as the fin-ray counts of the holotypes of these species are almost same (Tables 2–4).

The second-dorsal-, anal-, and pectoral-fin-ray counts of the *B. burmanicus* syntypes described in Hora (1926) are almost out of the ranges of the specimens of *Trypauchenopsis* examined in this study and Shibukawa and Murdy (2012) (Tables 2–4). However, the counting of fin rays of *Trypauchenopsis* is not easy and counts reported in historical literature often have errors, as suggested by Shibukawa and Murdy (2012). Therefore, further examination of the *B. burmanicus* syntypes will be necessary to confirm its status.

Incidentally, we were able to count the fin rays of the undamaged larvae directly under the microscope, because the larval fins had not yet become too thick and the surfaces of the bodies and fins were yet to be covered by pigment, although Shibukawa and Murdy (2012) mentioned the necessity of radiography for accurate counting.

Shibukawa and Murdy (2012) examined 485 specimens of *Trypauchenopsis* (18.3–102.8 mm SL) and determined the ranges of the second-dorsal-, anal-, and pectoral-fin-ray counts. The ranges they determined are almost the same as the ranges of the two species presented here (Tables 2–4). Therefore, it is plausible that the material examined in Shibukawa and Murdy (2012) also contained at least two species.

In Japan, Hayashi and Ito (1978) first reported eel gobies of the genus *Trypauchenopsis*. They listed 15 specimens collected at two estuaries on Ishigaki Island (western part of the Ryukyu Archipelago) in 1976 and 1977 and identified them as "*Taenioides* cf. *jacksoni* Smith". Hayashi *et al.* (1981) later re-identified them as "*Taenioides limicola* C. L. Smith 1964" and provided a new Japanese name, "Hige-warasubo". Japanese *Trypauchenopsis* gobies were subsequently referred to as "Hige-warasubo, *Taenioides limicola*" (e.g., Masuda *et al.*, 1984; Nakabo, 1993; Suzuki and Senou, 1982) until Shibukawa and Murdy (2012) identified them as *Trypauchenopsis intermedia*. Hige-warasubo is currently used as the standard Japanese name for *T. intermedia* (Nakabo, 2013).

We examined nine of the 15 adult and juvenile specimens of *Trypauchenopsis* listed in Hayashi and Ito (1978). At the time of writing, the remaining six specimens are missing (Kiyoshi Hagiwara at YCM, personal communication). Based on their fin-ray counts, two of them were identified as *T. limicola* and seven as *T. intermedia* (Table 5); thus, the name-bearing specimens of "Hige-warasubo" are in fact composed of two species. In order to avoid future confusion, we propose that the name "Hige-warasubo" should be replaced with the new standard Japanese names "Hoshidome-hige-warasubo" for *T. limicola*, based on URM-P 48889 (Figure 2a), and "Mabara-hige-warasubo" for *T. intermedia*, based on URM-P 42755 (Figure 2b). Both names reflect the characteristic melanophore patterns along the anal-fin base. The Japanese generic name should remain as "Hige-warasubo-zoku", as proposed by Shibukawa and Murdy (2012).

Since many of the distribution records reported to date do not distinguish between the two species, little is known about their distribution. Investigations based on reliable identification are necessary to understand distribution of each species.

The following records are reidentified here; *Trypauchenopsis* sp. 1 and sp. 2 in Maeda and Tachihara (2014) are *T. limicola* and *T. intermedia*, respectively. *Taenioides limicola* and *Taenioides* sp. in Maeda and Tachihara (2006) are *T. limicola* and *T. intermedia*, respectively. *Trypauchenopsis* sp. in Hanahara *et al.* (2021; figure 1b) is identified as *T. limicola.* Miyake *et al.* (2019) used a single adult specimen of *Trypauchenopsis* (URM-P 48087) and referred to it as *T. intermedia* in the main text. However, in table S1 of the paper, the specimen is referred to as *T. limicola*, and its mitochondrial genome sequence was registered in the DNA Data Bank of Japan as "Trypauchenopsis sp. G341" (accession number AP019362). Based on the fin-ray counts, the specimen can be identified as *T. limicola*.

3.3 *Taenioides gracilis* (Valenciennes 1837)

3.3.1 Material examined

Three postflexion larvae (9.5–9.9 mm SL) collected by small seine nets on Okinawa Island, Japan. URM-P 48888 (sketched, Figure 4a), 9.9 mm SL, Sedake, May 15, 1999; URM-P 48906 and 48907, two specimens, 9.5–9.5 mm SL, Aritsu, June 22, 2004. Most pigments of URM-P 48906 and 48907 were faded and were not used for pigment assessment.

3.3.2 Morphology

The body is elongated (BD = 12.6–13.9% of SL) and compressed. The head is moderate in size (HL = 23.7–25.5% of SL). The dorsal profile of the snout is straight or slightly concave. The mouth is oblique and barely reaches to the anterior margin of the eye. The lower jaw protrudes beyond the upper jaw. The eye is situated at the mid-lateral part of the head and is small (ED = 3.3–3.9% of SL), elliptical, and down-slanting toward the posterior end. The anterior nostril is near the tip of the snout, and the posterior nostril is anterior to the eye. No head spination is present. A large gas bladder is situated at the middle of the trunk. The anus is located slightly anterior to the middle of the body (PAL = 46.5–48.4% of SL). The dorsal fin originates posterior to the pectoral-fin (PDL = 33.3–34.2% of SL), with the first and second dorsal fins connected by a membrane. The first dorsal fin has six spines; the first to fifth spines are regularly spaced while the sixth spine has a wider interval from both the fifth spine and the first soft ray of the second dorsal fin. The second dorsal and anal fins are higher than the first dorsal fin and comprise 47 or 48 soft rays (Table 6) and 45–48 soft rays (Table 7), respectively. The anal fin originates just posterior to the anus. The second dorsal and anal fins are confluent with the caudal fin, which has 9 + 8 (dorsal + ventral) thick rays and four or five thin procurrent rays each on the dorsal and ventral sides. The posterior margin of the caudal fin is round. The pectoral fin is fan-shaped with 16–18 rays (Table 8). The pelvic fin has one spine and five soft rays; the left and right fins join together to form a cup-like disc with a frenum, and is large with its tip reaching slightly anterior to the anus. The total number of vertebrae is 29 (counted directly from a translucent specimen or photographs taken of fresh specimens; Table 9). The number of myomeres is 29 or 30, although the myomeres are difficult to define with confidence at the anterior-most and posterior-most regions.

3.3.3 Pigment

No melanophores are seen along the dorsal-fin base. Three melanophores occur each side of the anal-fin base; the positions of the first melanophores on the left and right sides are same, while the positions differ for the posterior two. There are melanophores around the bases of the middle rays of the caudal fin that somewhat expand posteriorly. Melanophores are seen at the ithmus, pre-pelvic region, pelvic-fin base along the ventral midline of body, and angle of the lower jaw. Melanophores pigment the anterodorsal surface of the gas bladder.

3.3.4 Remarks

Kurita and Yoshino (2012) revealed that four species of *Taenioides* are distributed in Japan and that they are distinguished from each other by their morphological differences, such as the number of fin rays, vertebrae, and barbels, and the degree of development of dermal folds on the head. They tentatively identified the four species as *T. anguillaris* (Linnaeus 1758), *T. snyderi* Jordan and Hubbs 1925, *T. gracilis* (Valenciennes 1837), and *T.* cf. *kentalleni* Murdy and Randall 2002, but they recognized that their identifications required verification with examinations of the type specimens. Murdy (2018) agreed with Kurita and Yoshino (2012) after examination of the type specimens of the four species. Three of the four species, *T. anguillaris*, *T. gracilis*, and *T. kentalleni*, are distributed on Okinawa Island (Kurita and Yoshino, 2012). Our larval collection contained two species of *Taenioides*. One of them was identified as

T. kentalleni (see remarks on *T. kentalleni* for details), and the other was identified as *T. gracilis*, as explained below.

We examined three larval specimens and found that their dorsal-, anal-, and pectoral-fin-ray counts are within the ranges of those established for *T. snyderi* and *T. gracilis* in Koreeda and Motomura (2021), Kurita and Yoshino (2012), and Miyahira and Tachihara (2022) (Tables 6–8). The dorsal- and anal-fin-ray counts are almost the same as those established for the paralectotype of *T. snyderi* and holotype of *T. gracilis* (total dorsal-fin elements, 53–54 in larvae vs. 54 in *T. snyderi* and *T. gracilis*; anal-fin rays, 45–48 vs. 47 in *T. snyderi* and 48 in *T. gracilis*; Tables 6 and 7). Our larvae had 16–18 pectoral-fin rays, which overlaps with 15 and 16 rays on the left and right sides, respectively, of the holotype of *T. gracilis* (Table 8). We were not able to compare our larvae with the type specimen of *T. snyderi* as its pectoral-fin-ray count has yet to be examined (Murdy, 2018). On the other hand, the counts for our larval specimens were almost out of the ranges of the dorsal-, anal-, and pectoral-fin-ray counts established for *T. anguillaris* and *T. kentalleni*, except for the pectoral-fin-ray count of *T. anguillaris* (Tables 6–8). Thus, the fin-ray counts of our larvae correspond to those of both *T. snyderi* and *T. gracilis*. These two species can be distinguished by the total number of vertebrae, with 31or 32 in *T. snyderi* (32 in the paralectotype) vs. 28–30 in *T. gracilis* (29 in the holotype) (Table 9; Koreeda and Motomura, 2021; Kurita and Yoshino, 2012; Murdy, 2018). Because our larvae had 29 vertebrae, they were identified as *T. gracilis*. While many adult specimens of *T. gracilis* have been collected on Okinawa Island, there is no record of *T. snyderi* from the Ryukyu Archipelago (Koreeda and Motomura, 2021; Kurita and Yoshino, 2012). This supports the identification of the larvae collected on Okinawa Island to be *T. gracilis*.

The larvae of *T. gracilis* and *T. kentalleni* differ from other eel goby larvae collected in the present study (*T. limicola*, *T. intermedia*, and *C. urolepis*) in that they have more second-dorsal- and anal-fin rays (>40 in both fins of *T. gracilis* and *T. kentalleni* vs. <36 in *Trypauchenopsis* and *Caragobius*), pointed snouts (vs. more round snouts), preanal lengths of 39–49% of SL (vs. >51% in *Trypauchenopsis* and 32% in *Caragobius*), larger pelvic fins (tips reach slightly anterior to or exceed the anus vs. not extending to middle of the trunk), having a melanophore at the angle of the lower jaw (vs. no melanophore), and no dorsal melanophores (*Trypauchenopsis* has melanophores along the dorsal-fin base; *Caragobius* also lacks them).

Dôtu (1958) and Dotsu in Okiyama (2014:1236–1237) described larvae and juveniles of *Taenioides* (9.3–15.5 mm in total length) collected from the Ariake Sound and the Seto Inland Sea, western Japan. They were identified as *T. snyderi* based on their localities and the higher number of myomeres. The specimens described in these previous studies are very similar to the *T. gracilis* larvae we observed but lack melanophores on the majority of the body surface except for the caudal-fin base. Care should be taken when observing this difference because melanophores on the body surfaces of eel gobies are small and can disappear after preservation. Indeed, it is for this reason that we were not able to use the two specimens collected at Aritsu in 2004 for description of melanophore arrangements. Conversely, the larvae of *Odontamblyopus lacepedii* showed similar melanophore patterns along the anal-fin base and a general body shape similar to that of the *T. gracilis* larvae presented here (Dôtu 1957; Dotsu in Okiyama 2014:1235–1236). The larvae of *T. gracilis* are also similar to larva of "*Taenioides anguillaris*" (10.7 mm SL) collected in Lombok Island, Indonesia, described in Harada and Suharti (2000), but the latter has three melanophores along each side of the second dorsal fin that are absent in *T. gracilis*.

3.4 *Taenioides kentalleni* Murdy and Randall 2002

3.4.1 Material examined

One postflexion larva collected by a shirasu trawl off Nishihara, Nakagusuku Bay on Okinawa Island, Japan on June 20, 2006. URM-P 48905 (sketched, Figure 4b), 12.9 mm SL.

3.4.2 Morphology

The body is elongated (BD = 14.3% of SL) and compressed. The head is of a moderate size (HL = 22.9% of SL). The snout is slightly concave. The mouth is oblique and barely reaches to the anterior margin of the eye. The lower jaw protrudes beyond the upper jaw. The eye is situated at the mid-lateral part of the head and is small (ED = 4.1% of SL), elliptical, and down-slanting toward the posterior end. The anterior nostril is near the tip of the snout, and the posterior nostril is anterior to the eye. No head spination is present. A large gas bladder is situated in the middle to posterior part of the trunk. The anus is located at the anterior part of the body, and the tail is long (PAL = 39.6% of SL). The dorsal fin originates posterior to the pectoral-fin (PDL = 29.8% of SL) with the first and second dorsal fins connected by a membrane. The first dorsal fin has six spines; the first to fifth spines are regularly spaced while the sixth spine has a wider interval from both the fifth spine and the first soft ray of the second dorsal fin. The second dorsal and anal fins are higher than the first dorsal fin and comprise 67 and 65 soft rays, respectively (Tables 6 and 7). The anal fin originates just posterior to the anus. The second dorsal and anal fins are confluent with the caudal fin, which has 9 + 8 (dorsal + ventral) thick rays and four and three thin procurrent rays on the dorsal and ventral sides, respectively. The middle of the caudal fin is pointed. The pectoral fin is fan-shaped with 20 rays (Table 8). The pelvic fin has one spine and five soft rays; the left and right fins join together to form a cup-like disc with a frenum, and is large with its tip exceeding beyond the anus to the origin of the anal fin. The number of myomeres is 44 (anterior-most and posterior-most parts are not shown in Figure 4b). The number of vertebrae is uncountable but estimated to be approximately 44 based on the myomere count.

3.4.3 Pigment

No melanophores are seen on the dorsal-fin base. There are seven and 13 melanophores at the left and right sides of the anal-fin base, respectively, which are distributed between the twelfth and sixty-first soft rays. Melanophores at the caudal-fin base form a vertical line. Melanophore also occur at the ithmus, pre-pelvic region along the ventral midline of the body, and the angle of the lower jaw. Melanophores pigment the anterior surface of the gas bladder.

3.4.4 Remarks

This larva had 67 and 65 rays in the second dorsal and anal fins, respectively. The only nominal species of *Taenioides* with more than 60 rays in these fins is *T. kentalleni* (see Kurita and Yoshino, 2012; Murdy and Randall, 2002). Only two specimens had previously been identified for this species; Murdy and Randall (2002) described this species based on an adult holotype collected in the Persian Gulf of Saudi Arabia and Kurita and Yoshino (2012) reported the identification of an adult specimen on Okinawa Island, although their identification was uncertain as they did not examine the holotype. Murdy (2018), who did examine the holotype, mentioned differences in the fin-ray counts and arrangements of the barbels on the ventral surfaces of the heads between these two specimens. The numbers of second-dorsal- and anal-fin rays of the larval specimen examined in this study are closer to those of the holotype from Saudi Arabia than those of the Okinawan adult specimen (Tables 6 and 7). There was a large difference in pectoral-fin-ray counts between the holotype (20–21) and Okinawan adult specimen (16), whereas the count for the larva identified here (20) corresponds to that of the holotype (Table 8). Thus, our specimen bridges the gap in fin-ray counts between the populations in Okinawa and Saudi Arabia. We are not able to comment on the barbels as they are not developed in larva. We agree with Kurita and Yoshino (2012) and Murdy (2018) in provisionally identifying the Okinawan specimen as *T. kentalleni*, and we believe our larva to be this species.

3.5 *Caragobius urolepis* (Bleeker 1852)

3.5.1 Material examined

Two postflexion larvae (6.9–7.9 mm SL) collected by small seine nets on Okinawa Island, Japan. URM-P 48890 (sketched, Figure 4c), 6.9 mm SL, Sedake, August 10, 1999; URM-P 49699, 7.9 mm SL, Aritsu, August 9, 2005. URM-P 49699 was only used for the fin-ray counts.

3.5.2 Description

The body is elongated (BD = 15.8% of SL) and compressed. The head is round and of a moderate size (HL = 22.1% of SL) and has a slightly concave snout. The mouth is oblique and reaches to the anterior margin of the eye. The lower jaw protrudes beyond the upper jaw. The eye is situated at the mid-lateral part of the head and is small (ED = 4.0% of SL), with a dent at the ventral side. The anterior nostril is located at the tip of the snout, and the posterior nostril is anterior to the eye. No head spination is present. A large gas bladder fills most of the small abdominal cavity. The trunk is shorter than half of the head

(PAL = 32.4% of SL). The dorsal fin originates posterior to the pectoral fin (PDL = 28.7% of SL), and the first and second dorsal fins are connected by a membrane. The first dorsal fin has six spines in both specimens; the first to fifth spines are regularly spaced, while the sixth spine has a wider interval from both the fifth spine and the first soft ray of the second dorsal fin. The second dorsal and anal fins are higher than the first dorsal fin. The second dorsal fin has 33 soft rays in both specimens, while the anal fin has 32 and 34 soft rays in the respective specimens. The anal fin originates just posterior to the anus and slightly anterior to the base of the first soft ray of the dorsal fin (i.e., the origin of the second dorsal fin). The second dorsal and anal fins are confluent with the caudal fin, which has 7 + 6 (dorsal + ventral) thick rays and one and two thin procurrent rays on the dorsal and ventral sides, respectively. The pectoral fin is small and fan-shaped, with 18 rays in both left and right fins of one of the specimens (uncountable in the other specimen). The pelvic fin is very small, comprising one spine and five soft rays, and lacks frenum. The left and right fins are barely connected by a low membrane between the fifth soft rays. The third soft rays are the longest, and the posterior margin of the pelvic fin is remarkably concave between the left and right third soft rays.

3.5.3 Pigment

No melanophores are seen along the dorsal- and anal-fin bases. Melanophores form a vertical line along the caudal-fin base, but this line breaks in the middle. There are two melanophores behind the pelvic-fin base. Melanophores pigment the anterodorsal surface of the gas bladder.

3.5.4 Remarks

The second-dorsal-, anal-, and pectoral-fin-ray counts of the larvae correspond to the mode of these counts of *C. urolepis* described in Murdy and Shibukawa (2003). Because no other Japanese eel goby species have the same combination of these counts, the larvae were identified as *C. urolepis*.

The anus and gas bladder of the larvae of this species are located more anteriorly than those of the other species examined in this study. Furthermore, the pelvic fin is smaller than those of other species. Larvae of *Paratrypauchen microcephalus* (Bleeker 1860) (8.5–9.9 mm in total length) from western Japan (figure 2A, B of Dôtu, 1958) and *Trypauchen* sp. (8.8 mm SL) from the Great Barrier Reef Lagoon (figure 66C of Leis and Trnski, 1989; figure 172C of Leis and Carson-Ewart, 2000) are similar to the larvae of *C. urolepis*, but these larvae have more second-dorsal- and anal-fin rays than *C. urolepis*, have melanophore on the hindgut above the anus (not present in *C. urolepis*), and lack melanophores behind the pectoral-fin base (present in *C. urolepis*).

3.6 Occurrence

Larvae of *T. limicola* were collected from June to January, *T. intermedia* larvae were collected in June and August to October, *T. gracilis* larvae were collected in May and June, *T. kentalleni* larva was collected in June, and *C. urolepis* larvae were collected in August.

3.7 Age estimation

The age estimations based on the number of otolith increments were 27–52 (mean ± standard deviation = 34.3 ± 4.6, n = 62) days for *T. limicola*, 29–40 (34.0 ± 5.4, n = 4) days for *T. intermedia*, 37 days (n = 1; 9.5 mm SL) for *T. gracilis*, and 28 days (n = 1; 7.9 mm SL) for *C. urolepis*. The SLs of *T. limicola* and *T. intermedia* were 8.7–10.3 mm (n = 80) and 8.5–9.7 mm (n = 7), respectively, including specimens whose otoliths were uncountable.

The hatching dates, estimated from the collection dates and the ages, were from May 14 to September 30 for the *T. limicola* larvae (n = 62) and from May 16 to July 31 for the *T. intermedia* larvae (n = 4). The *T. gracilis* larva was estimated to hatch on May 16, and the *C. urolepis* larva was estimated to hatch on August 12.

Semi-monthly changes of SL, daily age, and growth rate of *T. limicola* are shown in Figure 5. The approximate curve of the growth rate ($Y = -0.0057X^2 + 0.01002X - 0.1392$, $R^2 = 0.0848$) followed the changes in seawater temperature with a half to one month delay. The daily age (i.e., number of increments) ($Y = 0.8303X^2 – 14.03X + 90.962$, $R^2 = 0.1501$) showed an opposite trend from the growth rate, and the SL ($Y = 0.0355X^2 - 0.5069X + 11.246$, $R^2 = 0.253$) also increased from August and September to November with decreasing seawater temperature.

4 DISCUSSION

All the larvae collected in the present study were at the late postflexion stage. They had complete sets of fin rays, but their bodies were translucent and pigment had not yet developed over their body surfaces. Maeda and Tachihara (2014) demonstrated that estuarine mouths and beaches adjacent to the mouths are temporary habitats along the migration routes of many amphidromous and estuarine goby species from their marine larval habitats to the freshwater or estuarine adult habitats. The larvae occur there at the late postflexion stage, just before metamorphosis and settlement. Out data also suggests such migration patterns for *T. limicola*, *T. intermedia*, and *T. gracilis*. For *C. urolepis*, the larvae were slightly undeveloped as shown by the fact that the pelvic fins had not formed a cup-like disc. However, it is difficult to comment on the larval habitat and migration of *C. urolepis* because only two specimens were collected.

The larva of *T. kentalleni* is unique among specimens in that it was not collected by the coastline. The only Japanese adult specimen of this species was obtained at the bottom of Ohura Bay at a depth of 15 m and had already died when found by the diver (Kurita and Yoshino, personal communication). We made considerable larval collection efforts at a beach and estuaries along the coast of Ohura Bay (Maeda and Tachihara, 2005, 2006, 2014, this study) but were unsuccessful in finding *T. kentalleni*. The larva was collected in Nakagusuku Bay in the present study. Although several researchers have made larval collections efforts at beaches along the coast of Nakagusuku Bay (Kanou and Uehara, 2015; Shimose and Tachihara, 2005; Uehara and Tachihara, 2020; Uehara *et al.*, 2016; Tachihara *et al.*, unpublished data), no *T. kentalleni* larva has been found there. Maeda and Tachihara (2014) reported that larvae of coral reef gobies are rarely found on beaches, which is in contrast to the abundance of amphidromous and estuarine gobies. This suggests that the migration routes of marine gobies are different from those of amphidromous and estuarine gobies. The absence of *T. kentalleni* larva from beaches and estuaries may indicate that its larvae do not approach the shore when they move to the marine adult habitat.

As the larvae used to estimate ages in this study were assumed to have been collected when they were approaching their juvenile and adult habitats, their ages represent the pelagic larval duration and their body sizes represent the size at recruitment. The pelagic larval durations of *T. limicola*, *T. intermedia*, and *T. gracilis* were estimated to be a little longer than 1 month (average 34–37 days). The age of *C. urolepis* was younger (28 days), but this specimen may have been at a slightly earlier stage, as mentioned above.

Analysis on *T. limicola*, the only abundant species in this study, suggests that the larvae grow faster in warmer water (July and August) and that they metamorphose and settle at a younger age and smaller body size (Figure 5). With decreasing water temperature (September and October), their growth rates decrease, and the larvae became larger and older. No data was available after the water temperature dropped further (no sample was available in late November and December, and the otolith of the only larva collected in January was uncountable), but the same trend is expected to continue.

The same fluctuation patterns in SL, age at recruitment, and larval growth rate accompanied with temperature shift was reported for *Eleotris acanthopoma* Bleeker 1853 on Okinawa Island (Maeda *et al.*, 2007), as well as for *Lentipes concolor* (Gill 1860) in Hawaii (Radtke *et al.*, 2001) and *Galaxias maculatus* (Jenyns 1842) in New Zealand (McDowall *et al.*, 1994). This pattern is thought to be common among amphidromous and estuarine gobies on Okinawa, which grow in seawater that varies in temperature seasonally.

Maeda and Tachihara (2014) reported that the late postflexion larvae of *Trypauchenopsis* sp. 1 were collected from a beach in Sedake and estuary of the Teima Stream in August to October and December. These specimens were reexamined in this study and were identified as *T. limicola* as noted above. We also collected the larvae of this species in June, July, November, and January, indicating that the 1-month-old larvae of *T. limicola* are found from June to January and that the spawning season is from May to

December. The spawning seasons of several other goby species, such as *Eleotris acanthopoma*, *Redigobius bikolanus* (Herre 1927), and *Stiphodon percnopterygionus* Watson and Chen 1998 (Maeda and Tachihara, 2010; Maeda *et al.*, 2007; Yamasaki and Tachihara, 2006), are also known to be from May to December. This corresponds to the season in which stream water is warmer than approximately 20°C (Maeda and Tachihara, 2010), and it can be assumed that *T. limicola* stops reproductive activity during the colder seasons.

The larvae of *T. intermedia* were found in June and August to October. Although we could not determine the entire spawning season, it is predicted to be similar to that of *T. limicola* because all of the larvae were collected together with *T. limicola*. The larvae of *T. gracilis* were found in May and June. This sample size is insufficient to show the spawning season, but it seems to start earlier than *Trypauchenopsis* as we did not find *Trypauchenopsis* larvae in May. This study also indicates that *C. urolepis* spawn in July at the latest.

Adult and juvenile eel goby live within muddy substrates, making it difficult to determine their biomasses; however, estimates can be made from their larval abundances. In the present study, *T. limicola* was remarkably more abundant than the other species; we collected 22 *T. limicola* in Ohura Bay in 1999, while only two *T. intermedia*, one *T. gracilis*, and one *C. urolepis* larvae were collected. Among the collection in Aritsu from 2003 to 2007, including the specimens not used for the otolith and morphological studies, 141 *T. limicola*, eight *T. intermedia*, two *T. gracilis*, and one *C. urolepis* were found, although the samplings were not conducted regularly each month. If we postulate that these species have common larval habitats and behaviours, this suggests that *T. limicola* is more than 10 times more abundant than *T. intermedia*, with *T. gracilis* and *C. urolepis* being less abundant than *T. intermedia*, at least around the study sites.

Although eel goby larvae were collected from a beach adjacent to the mouth of the Aritsu Stream, the estuary and coastline around the mouth of the stream are covered with stones and/or gravel. As a result, there are no muddy environments to provide habitats for the eel goby adults. The nearest adult habitat is the estuary of the Gesashi Stream; this was where Tachihara *et al.* (2003) identified "*Taenioides limicola*" (i.e., *Trypauchenopsis limicola* or *T. intermedia* in the present study). It is located approximately 2 km (in a straight line) from Aritsu across Arume Bay. Since there is no adult habitat around Aritsu, the larvae recruited there are probably unable to reach a suitable habitat to settle. It is likely that the larvae born in the Gesashi Stream estuary grow in Arume Bay, and a proportion of them approach Aritsu Beach at the end of the pelagic larval life after being attracted by the low salinity water. It is also possible that some larvae are transported from farther away. It would seem that the eel goby larvae do not typically stay within estuaries and disperse to some extent.

Taenioides kentalleni has only been found in the Persian Gulf of Saudi Arabia and Okinawa Island in Japan. Because the two known locations are very far apart, their populations may be genetically different or may even be separate species. However, the current gap of the distribution could be due to the difficulty in finding adult fish of this species. We hope that future larval studies will fill the gaps in our knowledge.

The present study revealed that in Okinawa, *T. kentalleni* is distributed in Nakagusuku Bay in addition to Ohura Bay. These two bays on the east side of the island are two of the only three known habitats for this species of the world. Both Nakagusuku Bay and Ohura Bay have undergone environmental changes because of large-scale reclamation projects in the past and present, which may have had a significant impact on the habitat of this species. In order to conserve and protect this species, a better understanding of their larval and adult habitats is required.

Because eel gobies have elongated bodies with species-specific meristic characters that present in the late postflexion stage, the larvae are not difficult to identify. Melanophore patterns are also useful to identify the species. Adults and juveniles are difficult to locate because of their hidden life styles, while the larvae are pelagic and are more likely to be collected. In this study, we have provided examples of eel gobies, but we believe similar examples will be found for other taxa.

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CONTRIBUTIONS

All authors contributed to the study conception and design. Small-seine-net samplings were conducted by K. M. and N. H., Shirasu-trawl sampling was directed by M. U. and K. T.. Morphological observation was made by K. M.. Otolith analysis was performed by N. H. and K. M.. The first draft of the manuscript was written by K. M., and all authors commented on previous versions of the manuscript. All authors have read and approve the final manuscript.

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TABLE 1 Morphometrics of the eel goby larvae expressed as a percentage of the standard length (SL)

	SL (mm)	PDL	PAL	HL	SnL	ED	BD
T. limicola (n=22)	8.3–9.7	31.6–35.8	51.9–56.5	21.6–26.3	3.9–6.7	2.5–3.9	12.1–15.4
T. intermedia (n=2)	9.3–9.7	32.0–33.3	51.5–55.9	21.6–22.6	4.4–5.0	2.8–3.6	13.4–15.5
T. gracilis (n=3)	9.5–9.9	33.3–34.2	46.5–48.4	23.7–25.5	5.3–6.6	3.3–3.9	12.6–13.9
T. kentalleni (n=1)	12.9	29.8	39.6	22.9	6.3	4.1	14.3
C. urolepis (n=1)	6.9	28.7	32.4	22.1	5.9	4.0	15.8

Note: PDL: pre-dorsal-fin length; PAL: preanal length; HL: head length; SnL: snout length; ED: eye diameter; BD: body depth at the pectoral-fin base.

TABLE 2 The number of soft rays in the dorsal fin of the *Trypauchenopsis limicola* and *Trypauchenopsis intermedia* examined in the present study in comparison with those of the type and non-type specimens of related species published in literature

	28	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**Broken**	**Total**
T. limicola (present study)	9	27	15	3	-	-	-	-	-	-	-	-	7	61
T. intermedia (present study)	-	-	-	3	3	3	-	1	-	-	-	-	-	10
T. limicola (holotype)[†]	-	1	-	-	-	-	-	-	-	-	-	-	-	1
T. intermedia (holotype)[‡]	-	-	-	-	1	-	-	-	-	-	-	-	-	1
T. jacksoni (holotype)[‡]	-	-	-	-	1	-	-	-	-	-	-	-	-	1
B. burmanicus (syntypes)[§]	-	-	-	-	-	-	-	-	1	-	-	1	-	2
Trypauchenopsis spp.[¶]	4	16	9	10	25	20	13	1	-	-	-	-	-	98

Note: [†]Smith (1964); [‡]Shibukawa and Murdy (2012); [§]Hora (1926); [¶]all material examined by Shibukawa and Murdy (2012).

TABLE 3 Anal-fin-ray counts for the *Trypauchenopsis limicola* and *Trypauchenopsis intermedia* examined in the present study in comparison with those of the type and non-type specimens of related species published in literature

	26	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**Broken**	**Total**
T. limicola (present study)	3	17	27	7	1	-	-	-	-	-	6	61
T. intermedia (present study)	-	-	-	1	5	2	1	1	-	-	-	10
T. limicola (holotype)[†]	-	1	-	-	-	-	-	-	-	-	-	1
T. intermedia (holotype)[‡]	-	-	-	-	1	-	-	-	-	-	-	1
T. jacksoni (holotype)[§]	-	-	-	-	1	-	-	-	-	-	-	1
B. burmanicus (syntypes)[¶]	-	-	-	-	-	-	-	1	-	1	-	2
Trypauchenopsis spp.[#]	-	8	17	13	24	25	6	3	-	-	-	96

Note: [†]Smith (1964); [‡]Shibukawa and Murdy (2012); [§]Smith (1943); [¶]Hora (1926); [#]all material examined by Shibukawa and Murdy (2012).

TABLE 4 Pectoral-fin-ray counts for the *Trypauchenopsis limicola* and *Trypauchenopsis intermedia* examined in the present study in comparison with those of the type and non-type specimens of related species published in literature

	14	**15**	**16**	**17**	**18**	**19**	**20**	**Broken**	**Total**
T. limicola left (present study)	-	-	-	2	21	24	1	13	61
T. limicola right (present study)	-	-	-	2	20	26	1	12	61
T. intermedia left (present study)	-	-	1	3	-	-	-	6	10
T. intermedia right (present study)	-	-	2	5	-	-	-	3	10
T. limicola left (holotype)[†]	-	-	-	-	1	-	-	-	1
T. limicola right (holotype)[†]	-	-	-	-	1	-	-	-	1
T. intermedia left (holotype)[‡]	-	-	1	-	-	-	-	-	1
T. intermedia right (holotype)[‡]	-	-	-	1	-	-	-	-	1
T. jacksoni (holotype)[§]	-	-	-	1	-	-	-	-	1
B. burmanicus (syntypes)[¶]	2	-	-	-	-	-	-	-	2
Trypauchenopsis spp. left[#]	-	-	6	23	7	2	-	-	38
Trypauchenopsis spp. right[#]	-	-	6	22	7	3	-	-	38

Note: [†]Smith (1964); [‡]Shibukawa and Murdy (2012); [§]Smith (1943); [¶]Hora (1926): [#]all material examined by Shibukawa and Murdy (2012).

TABLE 5 Second-dorsal- (D_2), anal- (A), and left and right pectoral-fin-ray counts (P_1-l and P_1-r, respectively) of adult and/or juvenile *Trypauchenopsis* collected on Ishigaki Island

	SL	**D_2**	**A**	**P_1-l**	**P_1-r**	**Species**
YCM-P 2639	74.3	33	31	17	17	*T. intermedia*
YCM-P 2639	68.9	32	30	18	18	*T. intermedia*
YCM-P 3945	85.9	32	30	17	17	*T. intermedia*
YCM-P 3945	74.4	33	31	17	17	*T. intermedia*
YCM-P 3945	69.5	31	30	17	17	*T. intermedia*
YCM-P 3945	56.3	32	31	17	16	*T. intermedia*
YCM-P 3945	79.4	34	32	17	17	*T. intermedia*
YCM-P 4029	77.6	31	28	19	19	*T. limicola*
YCM-P 4029	50.9	29	26	18	19	*T. limicola*

Note: SL: standard length (mm).

TABLE 6 The number of dorsal-fin elements (spines and soft rays) for the larvae of *Taenioides* examined in the present study in comparison with those of Japanese adult and juvenile specimens of the four Japanese species and those of the type specimens published in literature

	47	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**Total**
T. gracilis (present study)	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
T. kentalleni (present study)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
T. anguillaris (Japan)†‡§	1	2	4	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
T. anguillaris (holotype)¶	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. snyderi (Japan)†‡	-	-	1	1	5	6	14	18	13	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	62
T. snyderi (paralectotype)¶	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. gracilis (Japan)†	-	-	-	7	19	36	38	31	15	9	3	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	166
T. gracilis (holotype)¶	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. kentalleni (Japan)†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
T. kentalleni (holotype)¶	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1

Note: †Kurita and Yoshino (2012); ‡Koreeda and Motomura (2021); §Miyahira and Tachihara (2022); ¶Murdy (2018).

TABLE 7 The number of anal-fin elements for the larvae of *Taenioides* examined in the present study in comparison with those of Japanese adult and juvenile specimens of the four Japanese species and those of the type specimens published in literature

	40	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**Total**
T. gracilis (present study)	-	-	-	-	-	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
T. kentalleni (present study)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
T. anguillaris (Japan)[†‡§]	1	2	3	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
T. anguillaris (holotype)[¶]	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. snyderi (Japan)[†‡]	-	-	-	1	6	13	16	18	5	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	62
T. snyderi (paralectotype)[¶]	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. gracilis (Japan)[†]	-	1	-	5	11	32	44	27	20	13	3	4-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	160
T. gracilis (holotype)[¶]	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. kentalleni (Japan)[†]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
T. kentalleni (holotype)[¶]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1

Note: [†]Kurita and Yoshino (2012); [‡]Koreeda and Motomura (2021); [§]Miyahira and Tachihara (2022); [¶]Murdy (2018).

TABLE 8 Pectoral-fin-ray counts for the larvae of *Taenioides* examined in the present study in comparison with those of Japanese adult and juvenile specimens of the four Japanese species and those of the type specimens published in literature

	15	**16**	**17**	**18**	**19**	**20**	**21**	**Broken**	**Total**
T. gracilis left (present study)	-	-	1	1	-	-	-	1	3
T. gracilis right (present study)	-	1	-	1	-	-	-	1	3
T. kentalleni left (present study)	-	-	-	-	-	1	-	-	1
T. kentalleni right (present study)	-	-	-	-	-	1	-	-	1
T. anguillaris (Japan)[†‡§]	-	2	7	2	-	-	-	-	11
T. anguillaris left (holotype)[¶]	-	-	1	-	-	-	-	-	1
T. anguillaris right (holotype)[¶]	-	-	1	-	-	-	-	-	1
T. snyderi (Japan)[†‡]	-	-	11	29	24	2	-	-	66
T. gracilis (Japan)[†‡]	18	64	71	10	2	1	-	-	166
T. gracilis left (holotype)[¶]	1	-	-	-	-	-	-	-	1
T. gracilis right (holotype)[¶]	-	1	-	-	-	-	-	-	1
T. kentalleni (Japan)[†]	-	1	-	-	-	-	-	-	1
T. kentalleni left (holotype)[¶]	-	-	-	-	-	1	-	-	1
T. kentalleni right (holotype)[¶]	-	-	-	-	-	-	1	-	1

Note: [†]Kurita and Yoshino (2012); [‡]Koreeda and Motomura (2021); [§]Miyahira and Tachihara (2022); [¶]Murdy (2018).

TABLE 9 The number of vertebrae in the larvae of *Taenioides* examined in the present study in comparison with those of Japanese adult and juvenile specimens of the four Japanese species and those of the type specimens published in literature

	28	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**Unknown**	**Total**
T. gracilis (present study)	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
T. kentalleni (present study)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
T. anguillaris (Japan)[†‡§]	-	10	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
T. anguillaris (holotype)[¶]	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. snyderi (Japan)[†‡]	-	-	-	7	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	57
T. snyderi (paralectotype)[¶]	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. gracilis (Japan)[†‡]	10	126[#]	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	148
T. gracilis (holotype)[¶]	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
T. kentalleni (Japan)[†]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1
T. kentalleni (holotype)[¶]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1

Note: [†]Kurita and Yoshino (2012); [‡]Koreeda and Motomura (2021); [§]Miyahira and Tachihara (2022); [¶]Murdy (2018), [#]Koreeda and Motomura (2021) did not indicate the number of individuals for the vertebral counts of the Okinawan specimens in their table 2, but it is based on one individual (Koreeda, personal communication).

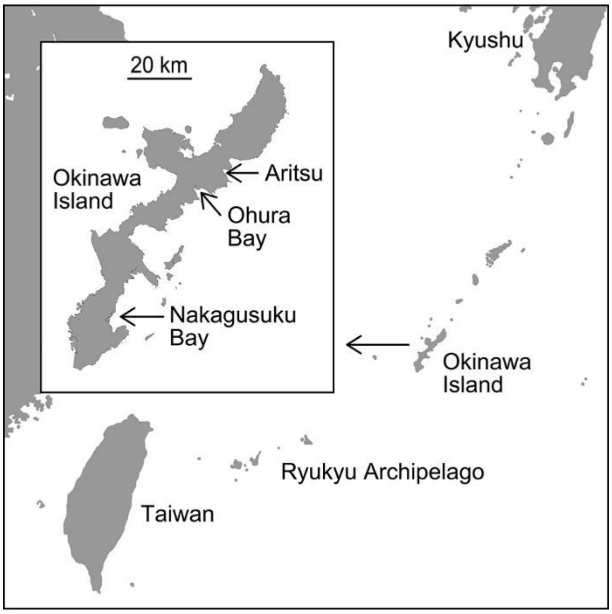

FIGURE 1 Map showing location of Okinawa Island and the sampling sites

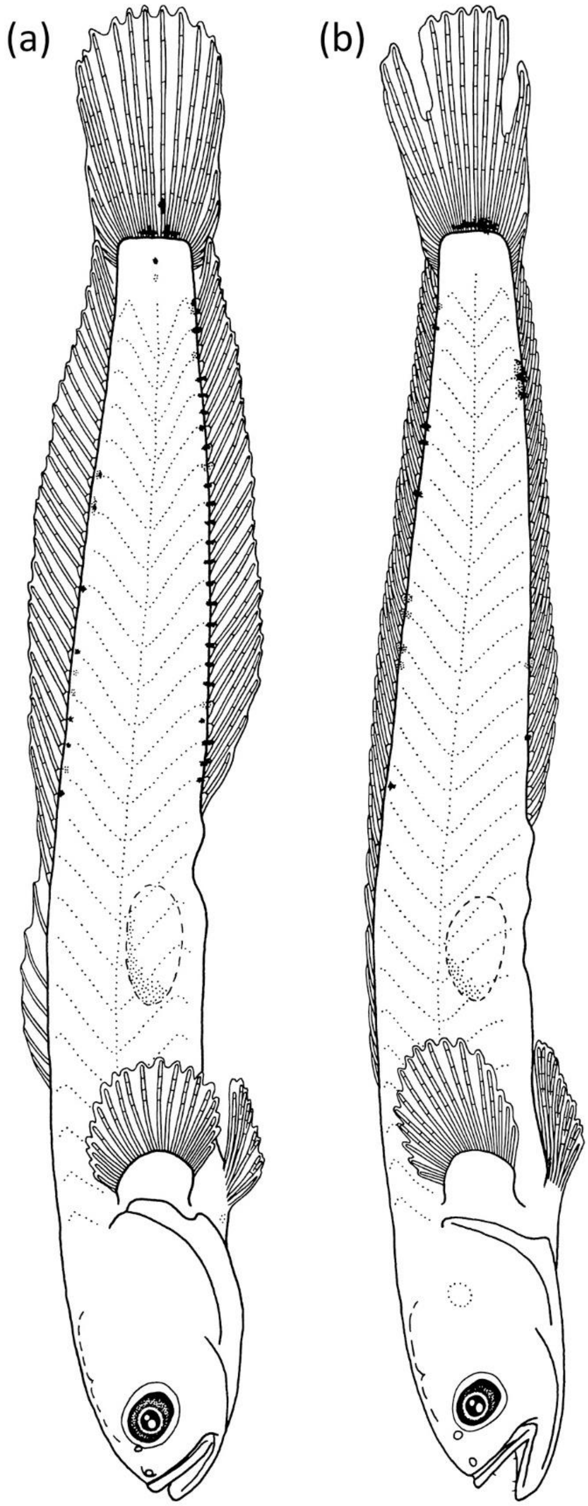

FIGURE 2 Larvae of *Trypauchenopsis* species collected at mouth of the Teima Stream in Okinawa Island on September 18, 1999. (a) *Trypauchenopsis limicola* (URM-P 48889, 9.0 mm SL); (b) *Trypauchenopsis intermedia* (URM-P 42755, 9.7 mm SL)

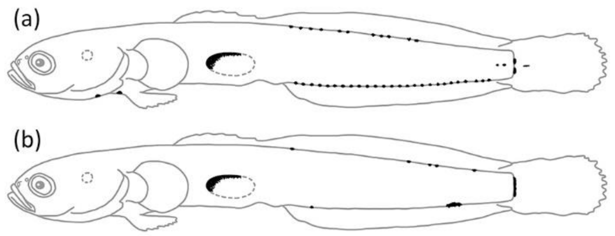

FIGURE 3 Schematic illustrations showing typical arrangement of melanophores of *Trypauchenopsis*. (a) sp. 1 (= *T. limicola*); (b) sp. 2 (= *T. intermedia*)

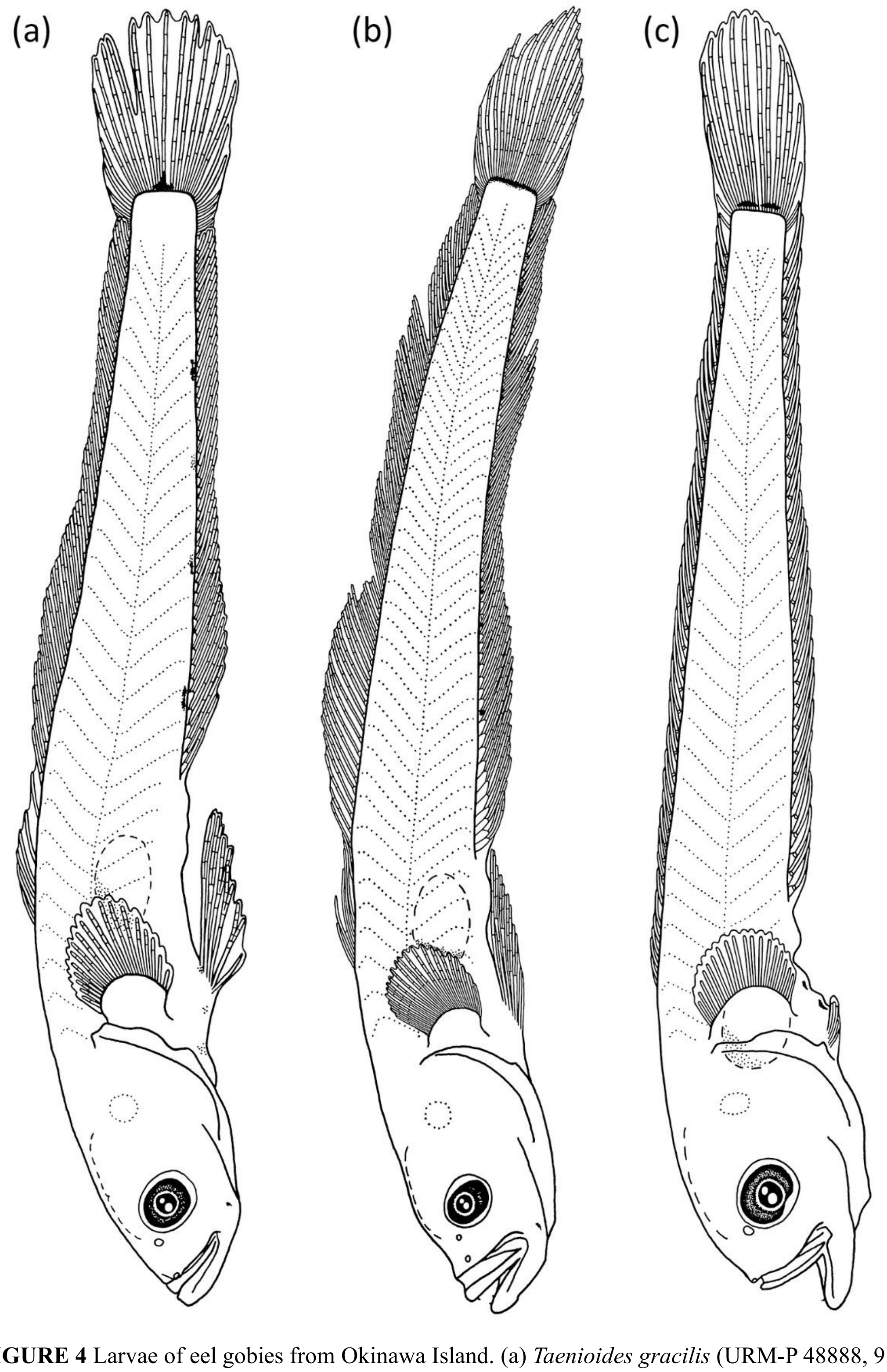

FIGURE 4 Larvae of eel gobies from Okinawa Island. (a) *Taenioides gracilis* (URM-P 48888, 9.9 mm SL) collected at Sedake Beach on May 15, 1999; (b) *Taenioides kentalleni* (URM-P 48905, 12.9 mm SL) collected at Nakagusuku Bay on June 20, 2006; (c) *Caragobius urolepis* (URM-P 48890, 6.9 mm SL) collected at Sedake Beach on August 10, 1999

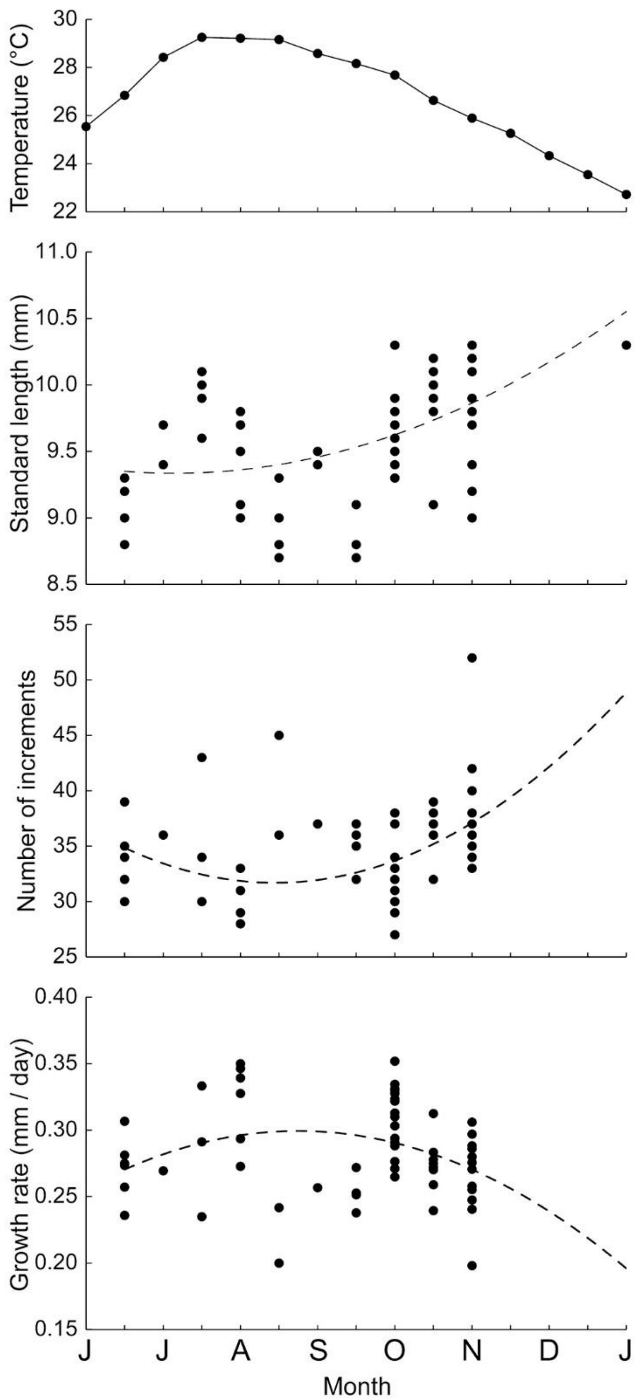

FIGURE 5 Semi-monthly changes of the standard length, daily age (number of otolith increments), and growth rate of *T. limicola* larvae collected at Aritsu Beach on the east coast of Okinawa Island, Japan in 2003–2006. Scales on the horizontal axis with initials of the months indicate values for the 1st to 15th of the month and scales between them indicate values for the 16th to end of the month. Water temperatures on the top are average surface temperatures on the east coast of Okinawa Island (around 26°23'N

128°06'E) from 2003 to 2006 (obtained from a database of the Japan Meteorological Agency; https://www.jma-net.go.jp/okinawa/know/kaiyo/engan.html)