



Abstract—Post-settlement juveniles of 3 greeneye species in the family Chlorophthalmidae, the blackedge greeneye (*Chlorophthalmus nigromarginatus*), humpback greeneye (*C. acutifrons*), and bigeyed greeneye (*C. albatrossis*), are described for the first time on the basis of 5, 2, and 9 specimens, respectively, collected from northern Japan. These identifications were well supported by an analysis of mitochondrial cytochrome *c* oxidase subunit I gene sequences of juveniles and adults of the 3 species and congeners. Among the 3 species, post-settlement juveniles of *C. albatrossis* are readily distinguishable by the following characters: inner pelvic-fin rays heavily pigmented (versus lightly pigmented or unpigmented), grayish blotches on body variegated and form a transverse band from the posterior end of the dorsal-fin base to the pelvic-fin origin (versus round or elliptical, not forming transverse bands), and 13–14 upper and 12–13 lower procurrent caudal-fin rays (versus 10–12 and 9–10). Although post-settlement juveniles of *C. nigromarginatus* and *C. acutifrons* are superficially very similar to each other, they are separable by the shape of grayish blotches and several proportional and meristic characters. On the basis of examination of post-settlement specimens, juveniles of the genus *Chlorophthalmus* appear to undergo a very rapid metamorphosis after settlement on the seafloor when they change from pelagic to benthic lifestyles.

Manuscript submitted 6 March 2024.
Manuscript accepted 21 August 2024.
Fish. Bull. 122:186–202 (2024).
Online publication date: 13 September 2024.
doi: [10.7755/FB.122.4.4](https://doi.org/10.7755/FB.122.4.4)

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Descriptions of post-settlement juveniles of 3 greeneye species, *Chlorophthalmus nigromarginatus*, *C. acutifrons*, and *C. albatrossis* (Teleostei: Chlorophthalmidae), from Japan, with notes on their rapid metamorphosis after settlement

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Greeneye species of the genus *Chlorophthalmus* Bonaparte, 1840, are demersal fish species distributed on the continental shelf and upper slope in tropical to temperate waters of all major oceans (Fricke and Durville, 2020). This group currently comprises 20 valid species (Fricke and Durville, 2020; Prokofiev, 2020) and several others that remain undescribed (Gomon et al., 2014). Although some species of this genus are rare and known from only a few type specimens (e.g., Fujiwara et al., 2019; Fricke and Durville, 2020), substantial amounts of other species are caught by bottom trawling and are important fishery resources (e.g., Honda et al., 2001; Yoshida, 2003; D’Onghia et al., 2006; Bineesh et al., 2014). Especially in Japan, the bigeyed greeneye (*Chlorophthalmus albatrossis* Jordan and Starks, 1904) (Teleostei: Chlorophthalmidae) is important as

a food fish caught in offshore bottom-trawl fisheries, and it recently has been designated as a target species for stock assessment in Japan (Hirakawa et al., 2007; Inoue and Horie, 2024). Chlorophthalmids, as well as other members of the suborder Alepisauroidae, are known to be simultaneous hermaphrodites (Davis and Fielitz, 2010), and the intricate reproductive biology of the shortnose greeneye (*Chlorophthalmus agassizi* Bonaparte, 1840) has been well investigated (e.g., D’Onghia et al., 2006; Parenti et al., 2015).

In contrast to the rich diversity of species and their large biomass, knowledge of the morphology of larvae and juveniles of members of *Chlorophthalmus* is scarce (Okiyama, 1984). To date, a complete developmental series from larvae to juveniles has been well documented only for *C. agassizi*, with descriptions based on specimens from

the Mediterranean Sea and Atlantic Ocean (Sanzo, 1915; Tåning, 1918; Ditty, 2006). Only brief morphological descriptions of a species common in the western Pacific Ocean, *C. albatrossis*, from larvae to juveniles, were reported by Saruwatari et al. (2005) and Saruwatari (2008). Other fragmentary accounts of the morphology of early life stages of this genus have been limited to larvae of the Brazilian greeneye (*Chlorophthalmus brasiliensis* Mead, 1958), robust greeneye (*Chlorophthalmus mento* Garman, 1899), blackedge greeneye (*Chlorophthalmus nigromarginatus* Kamohara, 1953), and Hawaiian greeneye (*Chlorophthalmus proridens* Gilbert and Cramer, 1897) and unidentified species (Ahlstrom, 1971; Pertseva-Ostroumova and Rass, 1973; Miller et al., 1979; Okiyama, 1984, 1988; Chiu, 1999; Ditty, 2006; Okiyama, 2014).

Except for in the work by Saruwatari et al. (2005) and Saruwatari (2008), who identified larvae and juveniles using molecular techniques, the basis for species identification has been ambiguous because morphological characters adequate for distinguishing the species of *Chlorophthalmus* from congeners have not been provided from previous studies. Furthermore, because most previously reported larvae and juveniles were collected from pelagic waters with mid-water trawl nets or plankton nets, little is known about post-settlement juveniles. Settlement is an important event in the early life stages of demersal fishes. Fish of these species change lifestyles from pelagic to demersal during settlement, and metamorphosis occurs at or around the time of this shift, bringing changes to their morphology and pigmentation (McCormick et al., 2002). It is crucial, therefore, for exploration of the early life history and recruitment of greeneye species to accumulate fundamental information about post-settlement juvenile morphology and to enable accurate species identification.

Recently, 16 post-settlement juvenile specimens of *Chlorophthalmus* were collected during bottom-trawl surveys conducted off the Pacific coast of Tohoku District in northern Japan. Morphological examinations and an analysis of mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences revealed that these juveniles represent 3 species, *C. nigromarginatus*, the humpback greeneye (*Chlorophthalmus acutifrons* Hiyama, 1940), and *C. albatrossis*. Herein, we provide the first detailed morphological descriptions of post-settlement juveniles of these 3 species. In addition, we discuss the rapid metamorphosis after settlement of these species inferred from the specimens.

Materials and methods

All post-settlement juveniles described in this study were collected during bottom-trawl surveys conducted off the Pacific coast of Tohoku District, in northern Japan, from late September through late November of 2022 and 2023 (for details on sampling stations, see Misawa et al., 2020). Bottom otter trawl nets were deployed from the R/V *Wakataka-maru* belonging to the Japan Fisheries

Research and Education Agency. Developmental stages of the specimens were defined as follows: early metamorphosing, individuals with grayish blotches on body weakly developed but indistinct; late metamorphosing, individuals with grayish blotches moderately developed; and metamorphosed, individuals with grayish blotches well developed. Methods for counts and measurements followed Fujiwara et al. (2019). Pectoral-fin rays were counted on both sides. Gill rakers were counted on the right side of the first arch. A full morphological description is provided only for *C. nigromarginatus*, for which specimens of various developmental stages were obtained and little information about its early life stages exists; characters shared by the 3 species are omitted from descriptions of *C. albatrossis* and *C. acutifrons*. Standard length is abbreviated as SL. Measurements were made to the nearest 0.1 mm with a digital caliper. Stereomicroscopes with a camera lucida were used for making line drawings of the specimens. Institutional codes follow Sabaj (2020).

The following adult specimens (maturation not investigated) were used as comparative material for morphological examinations: *Chlorophthalmus acutifrons*: HUMZ 39774, 140 mm SL, Mimase fish market, Kochi, Japan, 29 February 1974; HUMZ 48685, 170 mm SL, Mimase fish market, Kochi, Japan, 6 October 1972; HUMZ 146581, 146597, 146609, 129–138 mm SL, Okinawa Trough, 2 August 1994; HUMZ 177868, 176 mm SL, Mimase fish market, Kochi, Japan, 15 January 2001; HUMZ 191229, 148 mm SL, off Java, Indonesia, 6 September 2004; HUMZ 193614–193616, 108–127 mm SL, off Java, Indonesia, 4 May 2005; HUMZ 193999, 139 mm SL, off Java, Indonesia, 11 May 2005; HUMZ 201365, 117 mm SL, Mimase fish market, Kochi, Japan, 11 February 2008; HUMZ 208026, 178 mm SL, off Katsurahama, Kochi, Japan, 1 March 2010. *Chlorophthalmus albatrossis*: HUMZ 207252, 207255, 101–142 mm SL, Numazu fish market, Shizuoka, Japan, 12 February 2010. *Chlorophthalmus nigromarginatus*: FAKU 148097, 114 mm SL, off Omaezaki, Shizuoka, Japan, 27 November 2020; HUMZ 39044, 39056, 39060, 39076, 39759, 119–184 mm SL, Mimase fish market, Kochi, Japan, 2 March 1974; HUMZ 48298, 48489, 48497, 128–180 mm SL, Mimase fish market, Kochi, Japan, 29 March 1974; HUMZ 48517, 127 mm SL, Mimase fish market, Kochi, Japan, 26 March 1974; HUMZ 148174, 100 mm SL, Okinawa Trough, 2 August 1994; HUMZ 177760, 124 mm SL, Saga fishing port, Kochi, Japan, 22 January 2001; HUMZ 199981, 213 mm SL, East China Sea, 4 June 2007. *Chlorophthalmus vityazi* Kobylansky, 2013: HUMZ 190747, 195 mm SL, off Sumatra, Indonesia, 3 October 2004. *Chlorophthalmus* sp.: HUMZ 190745, 67 mm SL, off Sumatra, Indonesia, 3 October 2004.

Specimens from Indonesia were collected during 2004 and 2005 in the Japan–Indonesia Deep Sea Fisheries Resources Joint Exploration Project conducted by the Overseas Fishery Cooperation Foundation of Japan and the Agency for Marine and Fisheries Research, Indonesia Ministry of Marine Affairs and Fisheries.

In addition to the 16 post-settlement juvenile specimens described herein, 6 adult specimens (maturation

not investigated) from 5 species of *Chlorophthalmus* and 1 specimen of *Paraulopus filamentosus* Okamura, 1982, were used for DNA barcoding (Table 1). Total DNA was extracted from an eye or muscle tissue stored in 99% ethanol by using a Wizard Genomic DNA Purification Kit¹ (Promega Corp., Madison, WI). The partial COI gene sequence was amplified by using polymerase chain reaction (PCR) with the primers Fish F1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and Fish R1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA3') (Ward et al., 2005). Amplification through PCR was done with a single reaction volume of 10 µL containing 1.0 µL of a template DNA solution,

5.0 µL of KAPA2G Robust HotStart ReadyMix with dye (Roche CustomBiotech, Penzberg, Germany), 1.0 µL of forward and reverse primers (5 µM), and 2.0 µL of dissolved water. The following protocol was performed for PCR: pre-denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 15 s, annealing at 54°C for 15 s, extension at 72°C for 30 s, and final extension at 72°C for 7 min. The PCR products were purified by using Applied Biosystems ExoSAP-IT Express reagent (Thermo Fisher Scientific, Waltham, MA) and sequenced by the DNA sequencing service Eurofins Genomics (Tokyo, Japan) with primers used in PCR.

All sequences in this study were deposited in the DNA Data Bank of Japan, which is part of the International Nucleotide Sequence Database Collaboration, under accession numbers LC799382–LC799404. Sequences

¹ Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Details about the specimens of *Chlorophthalmus* and *Paraulopus* for which gene sequences were analyzed in this study. Juveniles were collected during bottom-trawl surveys from late September through late November in 2022 and 2023 in northern Japan. Adults used as comparative material were collected from Japan and Emperor Seamounts during 2016–2022 or from waters of Indonesia in 2004. Asterisks (*) indicate sequences previously published in databases of the International Nucleotide Sequence Database Collaboration (INSDC).

Species	INSDC accession no.	Voucher specimen	Stage	Locality
<i>Chlorophthalmus nigromarginatus</i>	LC799382	HUMZ 233382	Juvenile	Off Ibaraki Prefecture, Japan
	LC799383	HUMZ 234649	Juvenile	Off Miyagi Prefecture, Japan
	LC799384	HUMZ 234783	Juvenile	Off Ibaraki Prefecture, Japan
	LC799385	HUMZ 234792	Juvenile	Off Fukushima Prefecture, Japan
	LC799386	HUMZ 234796	Juvenile	Off Miyagi Prefecture, Japan
	LC799387	FAKU 148097	Adult	Off Omaezaki, Japan
	LC799388	FAKU 149265	Adult	Sea of Japan off Yamaguchi Prefecture, Japan
<i>Chlorophthalmus acutifrons</i>	KF714739*	NMV A 29713-007	Unknown	Western Australia
	KJ190053*	FMNH 120838	Unknown	Yilan County, Taiwan
	LC799389	HUMZ 234650	Juvenile	Off Miyagi Prefecture, Japan
<i>Chlorophthalmus albatrossis</i>	LC799390	HUMZ 234793	Juvenile	Off Fukushima Prefecture, Japan
	LC799391	FAKU 147423	Adult	Off Fukushima Prefecture, Japan
	KF714716*	NMV A 29672-020	Unknown	Western Australia
<i>Chlorophthalmus borealis</i>	LC799392	HUMZ 234534	Juvenile	Off Iwate Prefecture, Japan
	LC799393	HUMZ 234780	Juvenile	Off Fukushima Prefecture, Japan
	LC799394	HUMZ 234787	Juvenile	Off Fukushima Prefecture, Japan
	LC799395	HUMZ 234788	Juvenile	Off Fukushima Prefecture, Japan
	LC799396	HUMZ 234789	Juvenile	Off Fukushima Prefecture, Japan
	LC799397	HUMZ 234803	Juvenile	Off Fukushima Prefecture, Japan
	LC799398	HUMZ 234804	Juvenile	Off Fukushima Prefecture, Japan
	LC799399	HUMZ 234805	Juvenile	Off Fukushima Prefecture, Japan
	LC799400	HUMZ 234806	Juvenile	Off Fukushima Prefecture, Japan
	LC799401	FAKU 148899	Adult	Off Iwate Prefecture, Japan
	KF714717*	NMV A 26661-003	Unknown	Taiwan
	KF714735*	NSMT-P 66506	Adult	Off Ibaraki Prefecture, Japan
	KF714741*	AMS I.36455-016	Unknown	Philippines
	LC799402	HUMZ 190747	Adult	Off Sumatra, Indonesia
LC799403	FAKU 141991	Adult	Off Nii-jima Island, Japan	
<i>Paraulopus filamentosus</i>	LC799404	FAKU 146791	Adult	Emperor Seamounts

determined in this study and those previously published in databases of the International Nucleotide Sequence Database Collaboration (Table 1) were aligned by using the program Clustal W, vers. 2.1 (Thompson et al., 1994). *Paraulopus filamentosus* was used as an outgroup of the phylogenetic analysis because the family Paraulopidae has been placed more basally in Aulopiformes than Chlorophthalmidae (e.g., Davis, 2010). The phylogenetic tree was constructed by using the neighbor-joining method as implemented in MEGA11 (vers. 11.0.13; Tamura et al., 2021), with the Kimura 2-parameter model (Kimura, 1980) being selected to calculate genetic distances. The robustness of each node was tested by using the bootstrap method with 1000 replications.

Results

DNA barcoding

On the basis of the phylogenetic analysis of COI gene sequences (Fig. 1), we placed the juvenile specimens in 3 clades, in which sequences of *C. nigromarginatus*, *C. acutifrons*, or *C. albatrossis* and *C. borealis* Kuronuma and Yamaguchi, 1941, are included. Within the 3 clades, distances calculated with the Kimura 2-parameter model were 0.00–1.02%, 0.00–0.51%, and 0.00–0.67%, values that are lower than or within intraspecific genetic distances of the genus (0.10–1.56%) reported by Gomon et al. (2014). Although *C. borealis* was recently treated as a

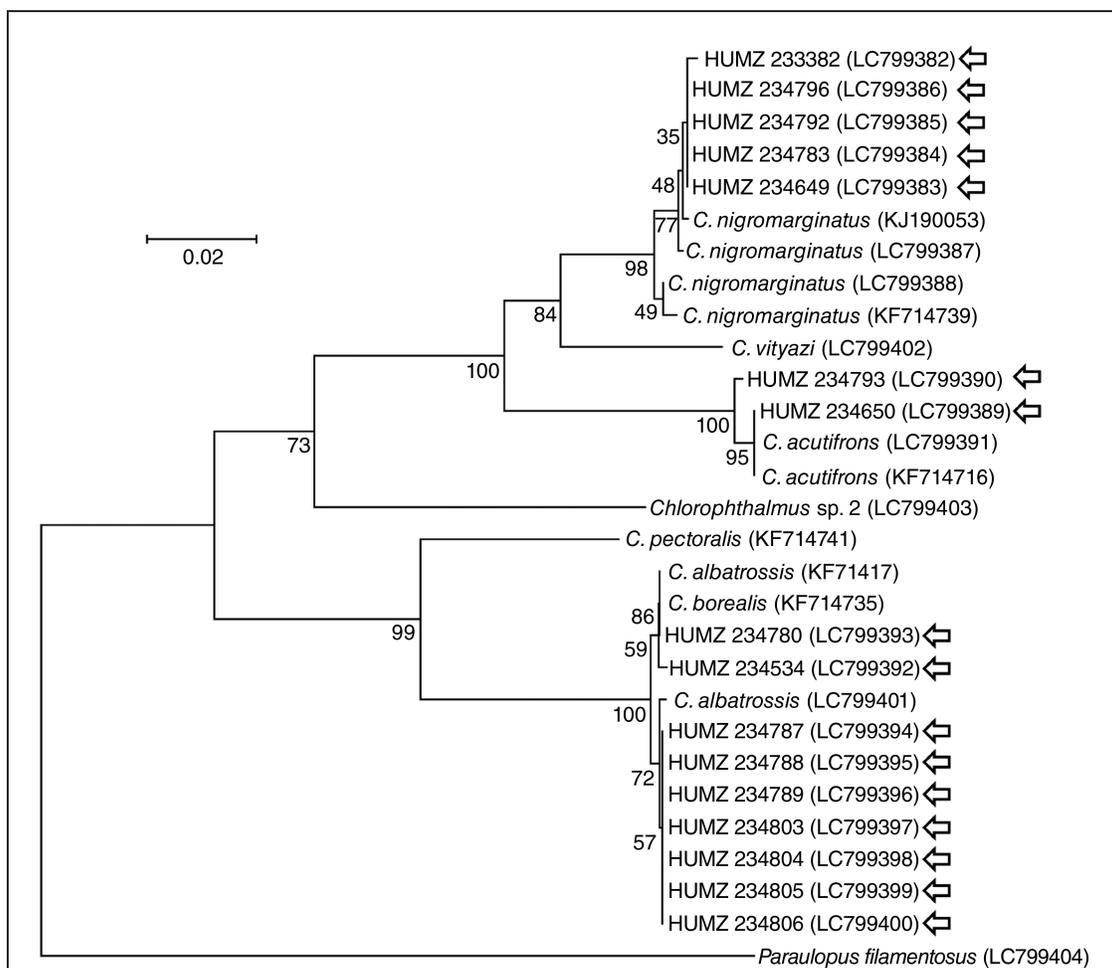


Figure 1

Neighbor-joining tree based on mitochondrial cytochrome *c* oxidase subunit I gene sequences of specimens of species of *Chlorophthalmus*, with *Paraulopus filamentosus* as an outgroup. Juvenile specimens were collected during bottom-trawl surveys from late September through late November in 2022 and 2023. Adults used as comparative material were collected from waters of Japan and Emperor Seamounts during 2016–2022 or from waters of Indonesia in 2004. International Nucleotide Sequence Database Collaboration accession numbers are provided in parentheses. Arrows indicate sequences of the post-settlement juveniles of *Chlorophthalmus* reported in this study. Numbers below branches indicate bootstrap values from testing with 1000 replications.

valid species in many studies (e.g., Nakabo and Kai, 2013; Gomon et al., 2014; Fricke and Durville, 2020), its possible synonymy under *C. albatrossis* has long been pointed out because of its morphological similarity with that species (e.g., Okamura, 1984a; Nakabo and Kai, 2013; Prokofiev, 2020). Results of molecular studies also indicate that these 2 species are not distinguishable on the basis of 16S rRNA (Saruwatari et al., 2005; Saruwatari, 2008) and COI gene sequences (Gomon et al., 2014; this study). Although the validity of *C. borealis* should be carefully assessed through a further taxonomic study with adequate materials, we identified all 9 juveniles included in the third clade, the one for *C. albatrossis* and *C. borealis*, as the former species because the juveniles were not separable morphologically. Consequently, in this study, 5 post-settlement juveniles were identified as *C. nigromarginatus*, 2 specimens were identified as *C. acutifrons*, and 9 specimens were identified as *C. albatrossis*.

The COI gene sequence of *C. vityazi* determined in this study (accession number: LC799402; voucher specimen: HUMZ 190747) differed from the previously registered sequence of the same specimen (accession number: KF714725) and completely matched the registered sequence of another specimen (accession number: KF714718; voucher specimen: HUMZ 190745); these specimens were identified by Gomon et al. (2014) as “*C. alb-like* IO” and *C. vityazi*, respectively. On the basis

of examinations of voucher specimens of these sequences and a personal communication with the author (Gomon²), it is likely that the catalog numbers of vouchers (HUMZ 190745 and HUMZ 190747) of the 2 previously published sequences were mistakenly swapped.

Descriptions of post-settlement juveniles

Chlorophthalmus nigromarginatus Kamohara, 1953

Blackedge greeneye

Standard Japanese name: Tsumaguro-aome-eso

Figures 2A and 3, Tables 2 and 3

Materials examined

Early metamorphosing: HUMZ 233382, 38.3 mm SL, off Hitachi, Ibaraki Prefecture, 36°31'19"N, 140°58'8"E–36°29'49"N, 140°56'58"E, 154 m depth, bottom temperature 13.2°C, station (stn.) H150, 28 October 2022; HUMZ 234796, 38.8 mm SL, off Minamisanriku, Miyagi Prefecture, 38°39'8"N, 142°0'53"E–38°39'24"N, 142°0'57"E, 344–346 m depth, bottom temperature 5.0°C, stn. DE350, 16 November 2023.

² Gomon, M. F. 2023. Personal commun. Museums Victoria, GPO Box 666, Melbourne, Victoria 3001, Australia.

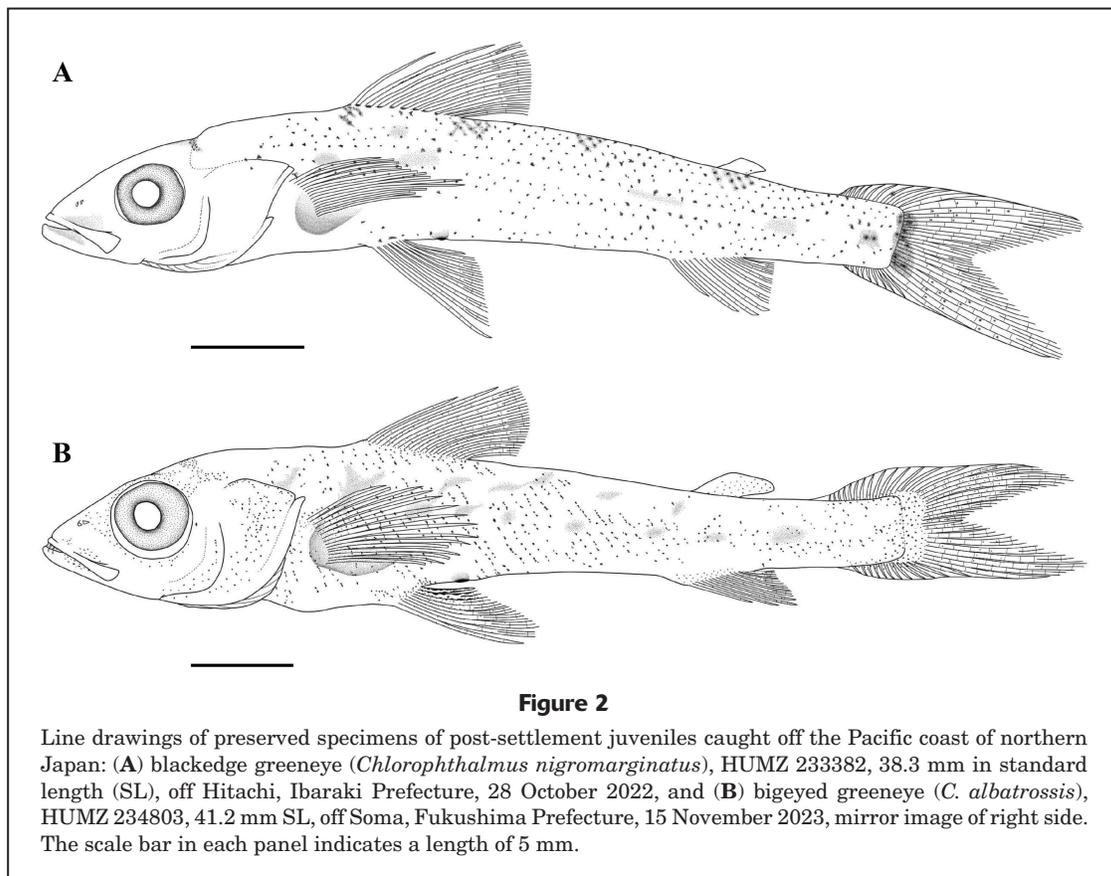


Table 2

Measurements and counts of post-settlement juveniles of blackedge greeneye (*Chlorophthalmus nigromarginatus*), humpback greeneye (*C. acutifrons*), and bigeyed greeneye (*C. albatrossis*) caught off the Pacific coast of northern Japan from late September through late November in 2022 and 2023. Measurements are given in percentage of standard length (SL). All specimens of each species were examined for each measurement, unless the number of specimens examined is given in parentheses after a measurement range for a species. Asterisks (*) indicate that values are approximate because of the badly damaged condition of a single specimen (HUMZ 234650). *n*=number of specimens.

Characteristic	<i>C. nigromarginatus</i> <i>n</i> =5	<i>C. acutifrons</i> <i>n</i> =2	<i>C. albatrossis</i> <i>n</i> =9
SL (mm)	38.3–44.3	42.8*–43.9	41.2–49.5
Measurements (% SL)			
Head length	28.2–30.7	28.2–31.3*	27.4–31.1
Head depth	13.5–15.2 (4)	13.9–14.7*	14.3–16.0
Post-orbital length	11.7–12.6	11.6–12.9*	10.6–12.2
Body depth at dorsal-fin origin	13.5–19.6	13.1*–16.4	14.0–18.6
Body depth at anal-fin origin	10.5–13.1	9.3*–10.9	9.6–11.8
Body width	12.4–13.2	11.2–11.7*	11.7–13.4
Snout length	8.1–9.4	8.2–9.1*	7.3–8.7
Upper-jaw length	9.7–11.3	10.5–12.6*	10.0–12.9
Maxillary depth	2.3–2.6	2.3–2.6*	2.0–2.9
Horizontal orbit diameter	8.4–9.6	8.2–10.0*	9.2–11.3
Vertical orbit diameter	7.7–8.2	7.3–9.3*	7.7–9.8 (8)
Horizontal pupil diameter	3.4–4.2	3.2–4.4*	3.3–5.1
Anterior interorbital width	9.3–10.1	8.7–9.3*	9.5–11.1
Posterior interorbital width	12.4–13.8	11.6–12.9*	13.3–14.6
Least interorbital width	4.0–7.6	3.3*–5.5	3.1–8.3 (8)
Caudal-peduncle length	17.0–19.1	17.3*–19.8	16.0–19.5
Caudal-peduncle depth	7.7–8.5 (4)	7.5*–8.0	6.8–7.9
Anus-to-anal-fin-origin length	26.4–29.1	27.3–27.8*	26.0–29.0
Pre-adipose-fin length	77.3–79.8	77.4–81.1*	75.0–78.3
Dorsal-to-adipose-fin length	40.5–44.2	43.3–45.3*	37.1–42.5
Pre-dorsal-fin length	36.3–39.2	34.6–37.9*	36.7–38.8
Dorsal-caudal length	63.4–65.9	63.8*–66.1	63.1–66.3
Dorsal-fin base length	11.9–13.6	11.9*–12.1	11.5–13.4
Longest dorsal-fin ray length	17.5–19.3 (3)	17.8–20.8*	17.2–20.9 (7)
Pre-anal-fin length	74.4–75.4	74.0–75.9*	74.7–77.3
Anal-caudal length	24.2–26.7	25.7*–27.1	24.5–27.3
Anal-fin base length	7.5–7.9	8.4–8.9*	7.5–9.0
Longest anal-fin ray length	9.5–11.1 (4)	9.8–11.4*	12.0–14.2
Pre-pectoral-fin length	27.5–30.0	27.6–30.1*	27.4–30.9
Pectoral-fin length	18.0–21.9 (4)	19.8 (1)	21.1–26.4
Pre-pelvic-fin length	37.4–39.2	37.8–39.3*	38.4–41.8
Pelvic-fin length	17.0–18.8	17.3 (1)	18.7–22.2 (6)
Pelvic-fin base length	3.6–4.2	3.7*–3.9	3.5–4.2
Pectoral-to-pelvic-fin length	11.9–13.5	11.0*–11.8	12.6–14.1
Pelvic-to-anal-fin length	34.5–38.9	36.7*–36.9	35.0–38.4
Counts			
Dorsal-fin rays	11	11	10–11
Anal-fin rays	9	9	8–10
Pectoral-fin rays	14–16	15–16	15–16
Pelvic-fin rays	9	9	9
Principal caudal-fin rays	19	19	19
Upper caudal procurrent rays	10–11 (4)	10–12	13–14
Lower caudal procurrent rays	9–10 (4)	9–10	12–13
Gill rakers	3 + 16–17 =19–20	2–3 + 15–16 =17–19	2–3 + 16–18 =18–21

Table 3

Frequency distributions for the number of pectoral-fin rays and gill rakers on juvenile and adult specimens of blackedge greeneye (*Chlorophthalmus nigromarginatus*) and humpback greeneye (*C. acutifrons*) examined in this study. Juveniles were collected during bottom-trawl surveys off the Pacific coast of northern Japan from late September through late November in 2022 and 2023. Adults were collected from waters of Kochi and Shizuoka, Japan, or the Okinawa Trough during 1972–2020 or from waters of Indonesia in 2005. *n*=number of specimens.

Species	<i>n</i>	Pectoral-fin rays				Upper gill rakers			Lower gill rakers					Total gill rakers						
		14	15	16	17	2	3	4	14	15	16	17	18	19	17	18	19	20	21	22
<i>C. nigromarginatus</i>	18	1	29	5	1	1	12	5	0	0	4	8	5	1	0	1	3	5	6	3
<i>C. acutifrons</i>	15	0	6	24	0	1	14	0	2	6	7	0	0	0	3	5	7	0	0	0

Late metamorphosing: HUMZ 234792, 44.3 mm SL, off Tomioka, Fukushima Prefecture, 37°21'7"N, 141°37'30"E–37°22'17"N, 141°37'18"E, 250–255 m depth, bottom temperature 7.2°C, stn. FG250, 20 November 2023.

Metamorphosed: HUMZ 234649, 43.0 mm SL, off Soma, Fukushima Prefecture, 37°35'43"N, 141°35'33"E–37°37'34"N, 141°35'58"E, 210–213 m depth, bottom temperature 8.3°C, stn. F210, 23 October 2023; HUMZ 234783, 42.5 mm SL, off Hitachi, Ibaraki Prefecture, 36°30'55"N, 140°58'55"E–36°29'36"N, 140°58'3.6"E, 210–213 m depth, bottom temperature 13.1°C, stn. H210, 11 November 2023.

Description

Proportional measurements and counts presented in Table 2. Body slender and cylindrical (Figs. 2A and 3); depth largest between pectoral-fin base and dorsal-fin origin. Tip of snout pointed. Anterior and posterior nostrils located near midway between snout tip and anterior margin of orbit. Eye large; horizontal orbit diameter almost equal to or slightly greater than snout length. Aphakic space on anterior portion of pupil weakly developed in metamorphosed specimens.

Mouth subterminal; tip of lower jaw slightly protruding anteriorly. Posterior margin of maxilla reaching beyond vertical through anterior margin of orbit and not reaching vertical through anterior margin of pupil. In early metamorphosing specimen (HUMZ 234796), several small, conical teeth on premaxilla (damaged on right side), and slender, curved, conical teeth in single row on dentary, with 1 tooth on anterior portion of dentary; in another early metamorphosing specimen (HUMZ 233382) and in late metamorphosing specimens, jaws edentate or with several small, conical teeth; in metamorphosed specimens, premaxilla almost edentate with several small, conical teeth, and dentary with small, conical teeth arranged in single row. Vomer with 1 tooth on each side of anterolateral margin, except for metamorphosed specimen (HUMZ 234649) with 2–3 teeth on each side. Palatine edentate or with 1 conical tooth on anterior portion, except for

metamorphosed specimen (HUMZ 234649) with several conical teeth arranged in single row. Basihyal with 3 large, posteriorly curved, fang-like teeth on each side of anterior margin in early metamorphosing specimen (HUMZ 234796); 2 fang-like teeth on right side in other early metamorphosing specimen (HUMZ 233382), 1 fang-like tooth on left side in late metamorphosing specimen, and 1 small, conical tooth on each side (HUMZ 234783) or left side (HUMZ 234649) of anterior portion of basihyal in metamorphosed specimens.

Posterior and lower margins of opercular bones smooth. Gill rakers moderately developed. Pseudobranch present. Anus located slightly posterior to pelvic-fin base. Perianal light organ present. Scales on body developed but mostly lost; few scales remaining around anus and pectoral-fin base. Dorsal fin on anterior half of body; its origin slightly anterior to vertical through pelvic-fin origin; 3rd ray longest; 1st and 2nd rays unbranched, other rays branched. Anal fin on posterior half of body; 3rd or 4th ray longest; anterior 3 or 4 rays unbranched, other rays branched. Adipose fin small; its origin above base of 3rd, 4th, 5th, or 6th anal-fin ray. Pectoral fin long; its tip hardly reaching or reaching slightly beyond vertical through posterior end of dorsal-fin base. Pelvic fin long and pointed; its origin below base of 2nd or 3rd dorsal-fin ray. Caudal fin deeply forked.

Coloration

When fresh (Fig. 3), head and body white; posterior part of head to pectoral-fin base with orange tinge. In metamorphosed specimens, silver coloration appears on opercular region, belly, and mid-lateral portion of trunk (Fig. 3, D and E). Sparse pigment on lachrymal, anterior portion of lower jaw and on occipital region in early metamorphosing specimen, becoming more concentrated with growth in late metamorphosing and metamorphosed specimens. Pigment on opercular region and dorsal surface of head appears in late metamorphosing specimen and becomes more concentrated in metamorphosed specimens. Black dots arranged in slanting rows on almost entire body except lateral line. Grayish blotches composed of fine melanophores weakly developed on dorsal and lateral surfaces of body in early

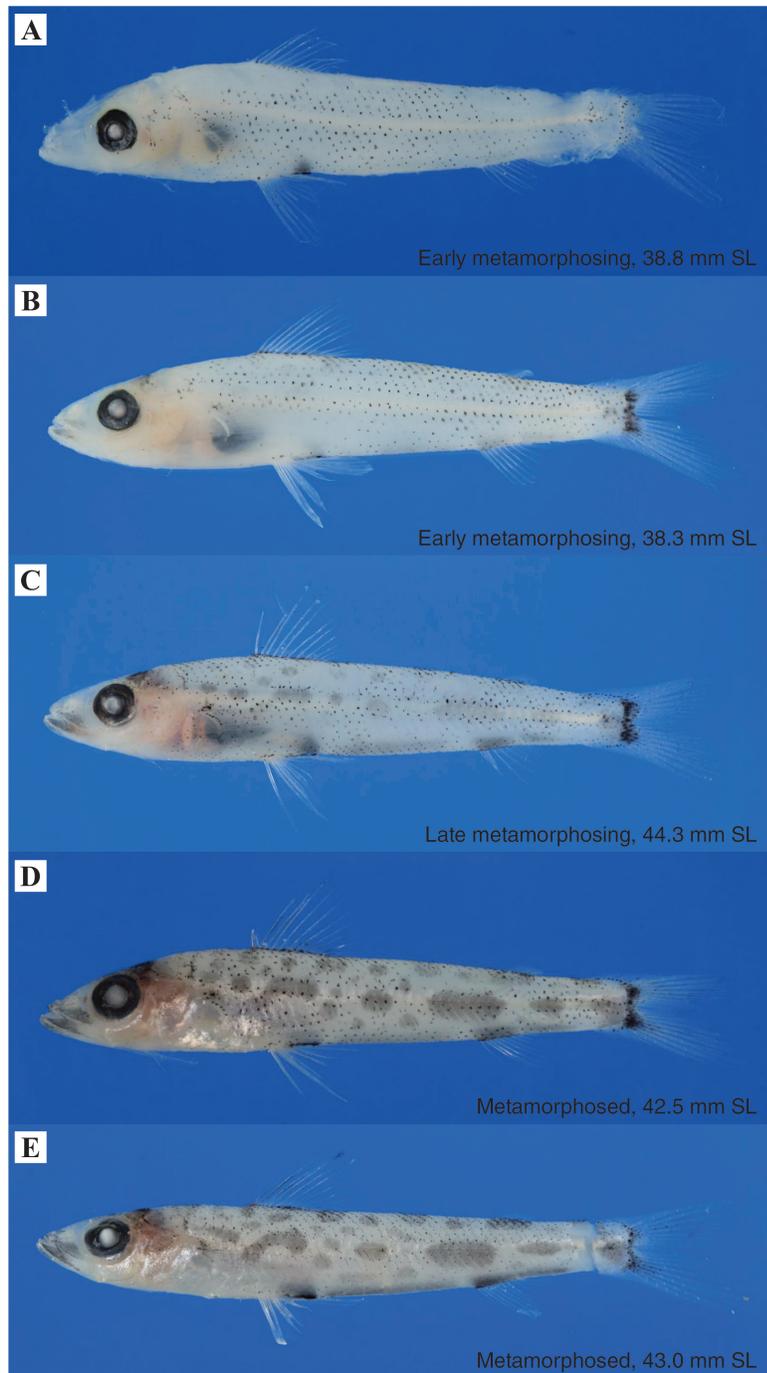


Figure 3

Fresh specimens of post-settlement juvenile blackedge greeneye (*Chlorophthalmus nigromarginatus*) from northern Japan: (A) HUMZ 234796, 38.8 mm in standard length (SL), off Minamisanriku, Miyagi Prefecture, 16 November 2023; (B) HUMZ 233382, 38.3 mm SL, off Hitachi, Ibaraki Prefecture, 28 October 2022; (C) HUMZ 234792, 44.3 mm SL, off Tomioka, Fukushima Prefecture, 20 November 2023; (D) HUMZ 234783, 42.5 mm SL, off Hitachi, Ibaraki Prefecture, 11 November 2023, mirror image of right side; and (E) HUMZ 234649, 43.0 mm SL, off Soma, Fukushima Prefecture, 23 October 2023. The developmental stage of each specimen—early metamorphosing, late metamorphosing, or metamorphosed—is provided.

metamorphosing specimens (Fig. 3, A and B), more distinct in late metamorphosing specimens (Fig. 3C), and well developed in fully metamorphosed specimens (Fig. 3, D and E); shape of blotches mostly round or elliptical, without transverse bands; single large blotch below middle of dorsal-fin base oblong and merged with anteroventral blotch.

Single peritoneal pigment section near pectoral-fin base visible externally in metamorphosing specimens (Fig. 3, A–C) but not in metamorphosed specimens. Perianal light organ black. Iris black; dorsal portion of iris with yellowish tinge in metamorphosed specimen (HUMZ 234649; Fig. 3E). Single black blotch on caudal-fin base (Fig. 3, B–E) except for early metamorphosing specimen (HUMZ 234796; Fig. 3A). Dorsal, pectoral, pelvic, and caudal fins sparsely pigmented. Anal and adipose fins almost completely unpigmented. In preservation, head and body white or yellowish white; orangish, yellowish, and silver coloration lost. Other coloration almost same as when fresh.

Remarks

Although Chiu (1999) described larvae and juveniles of the genus collected around Taiwan as *C. nigromarginatus*, including illustrations of a 7.4-mm larva and 30.7-mm juvenile, descriptions fail to clearly identify the specimens. The illustrated juvenile, at least, is unlikely to be *C. nigromarginatus* and more closely resembles *C. albatrossis* because it has approximately 13 upper and 12 lower procurrent caudal-fin rays (see the “Morphological comparisons” section in the “Discussion” section). Fricke et al. (2019) reported a single small specimen (50.5 mm in total length) of *C. nigromarginatus* from New Ireland of Papua New Guinea, but they provided neither a morphological description nor a figure of the specimen. Adults or young of the species have been previously reported from southern Japan, Taiwan, the South China Sea, Indonesia, New Ireland, the Northern Territory in Australia, and Western Australia (Randall and Lim, 2000; Hutchins, 2001; Larson et al., 2013; Nakabo and Kai, 2013; Fricke et al., 2019; Teramura et al., 2019; Koeda, 2020; Ho et al., 2021). Because the previous northernmost record of the species was a

specimen from Suruga Bay in Japan (Teramura et al., 2019), the juvenile specimens recently collected off the Pacific coast of Tohoku District extend the northern distributional limit of the species, with a single specimen (HUMZ 234796) from off Minamisanriku, Miyagi Prefecture, representing the northernmost record of the species.

Chlorophthalmus acutifrons Hiyama, 1940

Humpback greeneye

Standard Japanese name: Tomo-mehikari

Figure 4, Tables 2 and 3

Materials examined

Late metamorphosing: HUMZ 234793, 43.9 mm SL, off Tomioka, Fukushima Prefecture, 37°21'7"N, 141°37'30"E–37°22'17"N, 141°37'18"E, 250–255 m depth, bottom temperature 7.2°C, stn. FG250, 20 November 2023.

Metamorphosed: HUMZ 234650, ca. 42.8 mm SL (fractured in front of anal-fin origin), off Soma, Fukushima Prefecture, 37°35'43"N, 141°35'33"E–37°37'34"N, 141°35'58"E, 210–213 m depth, bottom temperature 8.3°C, stn. F210, 23 October 2023.

Description

Proportional measurements and counts presented in Table 2. Body depth largest at pectoral-fin base (Fig. 4).

Horizontal orbit diameter equal to or slightly greater than snout length. Aphakic space on anterior portion of pupil weakly developed. Posterior margin of maxilla reaching beyond vertical through anterior margin of orbit and not reaching vertical through anterior margin of pupil. In late metamorphosing specimen, jaws almost edentate, with 1 small, conical tooth on anterior portion of premaxilla and dentary; in metamorphosed specimen, conical teeth arranged in 1–2 rows on premaxilla and dentary, with 3 teeth on anterior portion of dentary. Vomer with 1 tooth (in late metamorphosing) or 2–3 teeth (in metamorphosed) on each side of anterolateral margin. Palatine edentate in late metamorphosing specimen; conical teeth arranged in single row in metamorphosed specimen. Basihyal with 1 large, posteriorly curved, fang-like tooth on right side in late metamorphosing specimen; 1 small, conical tooth on each side in metamorphosed specimen. Third anal-fin ray longest; anterior 4 anal-fin rays unbranched, other rays branched. Adipose-fin origin above base of 5th or 6th anal-fin ray. Tip of pectoral fin reaching slightly beyond vertical through posterior end of dorsal-fin base. Pelvic-fin origin below base of 3rd dorsal-fin ray.

Coloration

When fresh (Fig. 4), head and body white. In metamorphosed specimen, silver coloration present on opercular region, belly, and mid-lateral portion of trunk (Fig. 4B). Grayish blotches on body mostly round or elliptical, without transverse bands; single large blotch below middle of dorsal-fin base round and not merged with other blotches. Single peritoneal pigment section near pectoral-fin base visible externally in late metamorphosing specimen (Fig. 4A) and invisible in metamorphosed specimen (Fig. 4B). Dorsal portion of iris with yellowish tinge in metamorphosed specimen (Fig. 4B). Single black blotch on caudal-fin base. Dorsal, pectoral, pelvic, and caudal fins sparsely pigmented. Anal and adipose fins almost completely unpigmented.

Remarks

Saruwatari et al. (2006) reported a larva (9.9 mm SL) of *C. acutifrons* from eastern water off Taiwan on the basis of molecular identification using a 16S rRNA sequence, but they provided no morphological description of the specimen. Shinohara et al. (1996) reported 4 small specimens (50–54 mm SL) of the species, collected during bottom-trawl surveys off Fukushima Prefecture in Japan, without any descriptions and figures. Therefore, the juvenile morphology

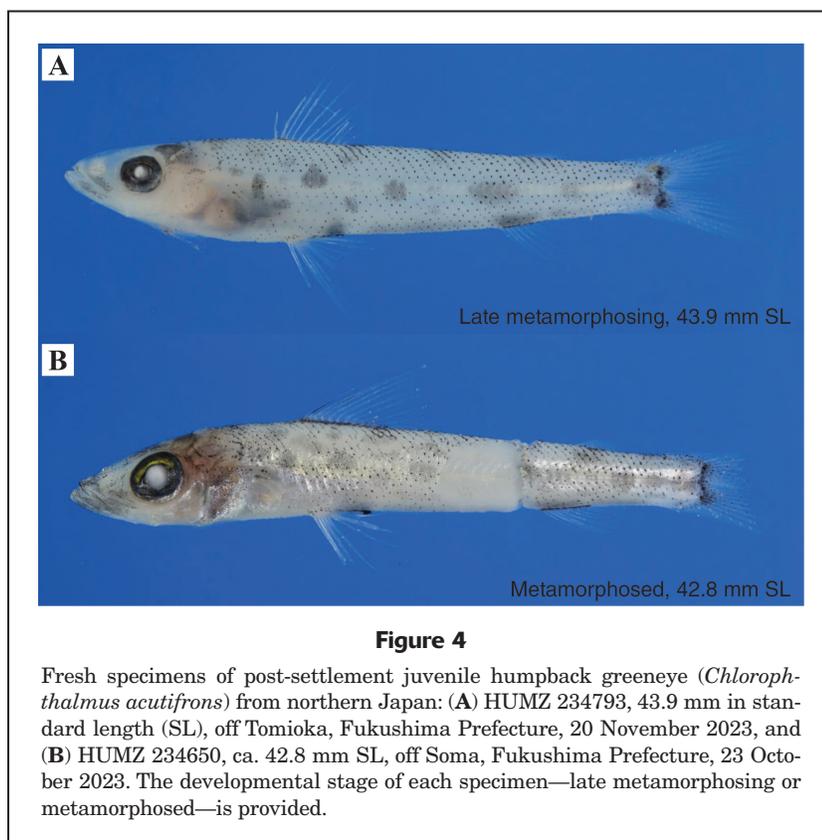


Figure 4

Fresh specimens of post-settlement juvenile humpback greeneye (*Chlorophthalmus acutifrons*) from northern Japan: (A) HUMZ 234793, 43.9 mm in standard length (SL), off Tomioka, Fukushima Prefecture, 20 November 2023, and (B) HUMZ 234650, ca. 42.8 mm SL, off Soma, Fukushima Prefecture, 23 October 2023. The developmental stage of each specimen—late metamorphosing or metamorphosed—is provided.

of the species is described for the first time herein on the basis of the results of our study.

Chlorophthalmus albatrossis Jordan and Starks, 1904

Bigeyed greeneye

Standard Japanese name: Aome-eso

Figures 2B, 5, and 6; Table 2

Materials examined

Early metamorphosing: HUMZ 234803, 41.2 mm SL, off Soma, Fukushima Prefecture, 37°49'35"N, 141°58'22"E–37°48'4"N, 141°58'37"E, 481–484 m depth, bottom temperature 4.3°C, stn. F490, 15 November 2023; HUMZ 234806, 42.4 mm SL, off Soma, Fukushima Prefecture, 37°44'56"N, 141°53'32"E–37°43'50"N, 141°53'47"E, 409–410 m depth, bottom temperature 4.8°C, stn. F410, 15 November 2023.

Late metamorphosing: HUMZ 234780, 45.2 mm SL, off Iwaki, Fukushima Prefecture, 36°57'55"N, 141°31'28"E–36°56'7"N, 141°30'49"E, 354–372 m depth, bottom temperature 6.3°C, stn. G350, 12 November 2023.

Metamorphosed: HUMZ 234534, 49.2 mm SL, off Rikuzentakata, Iwate Prefecture, 38°54'28"N, 142°5'30"E–38°56'15"N, 142°5'41"E, 354–348 m depth, bottom temperature 3.1°C, stn. D350, 28 September 2023; HUMZ 234787–234789, 3 specimens, 42.7–45.1 mm SL, off Iwaki, Fukushima Prefecture, 36°54'53"N, 141°26'34"E–36°56'15"N, 141°26'55"E, 299–311 m depth, bottom temperature 6.3°C, stn. G310, 8 November 2023; HUMZ 234804–234805, 2 specimens, 45.0–49.5 mm SL, off Soma, Fukushima Prefecture, 37°49'35"N, 141°58'22"E–37°48'4"N, 141°58'37"E, 481–484 m depth, bottom temperature 4.3°C, stn. F490, 15 November 2023.

Description

Proportional measurements and counts presented in Table 2. Body depth largest between pectoral-fin base and dorsal-fin origin (Figs. 2B and 5). Horizontal orbit diameter slightly greater than snout

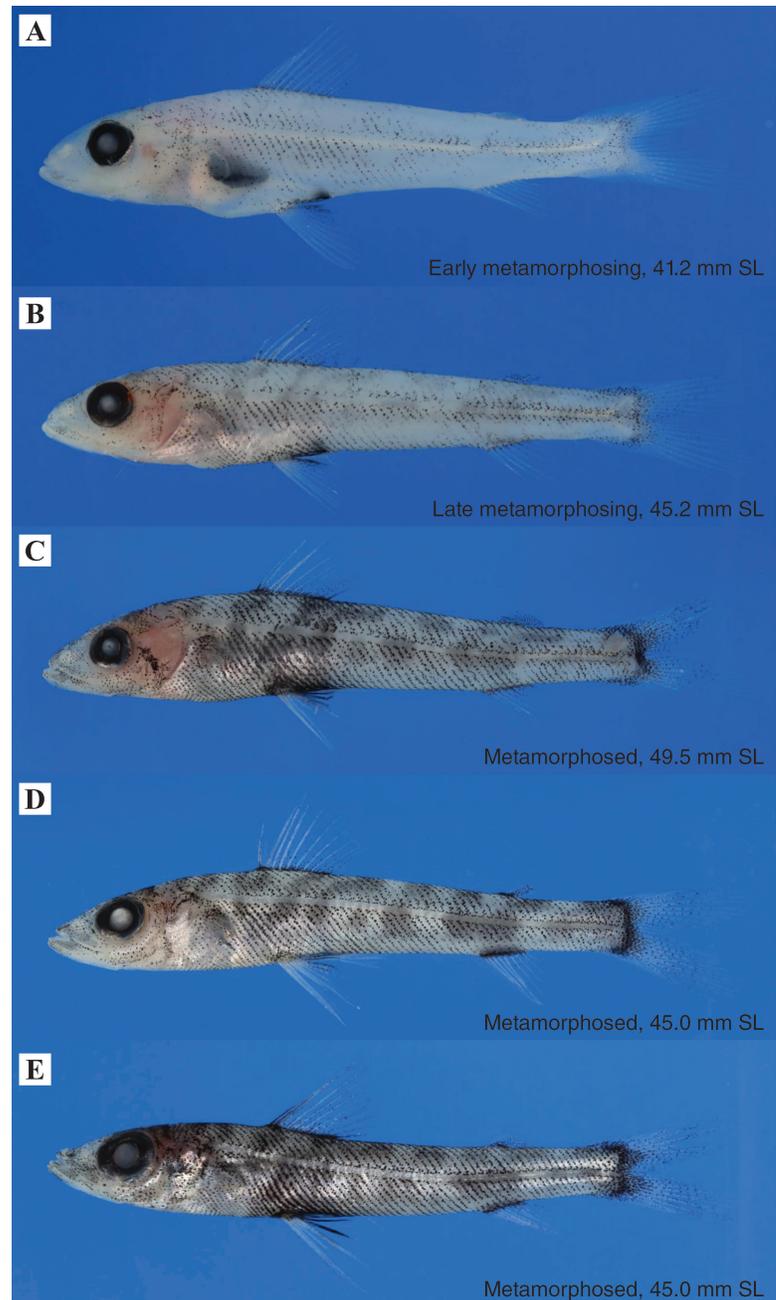
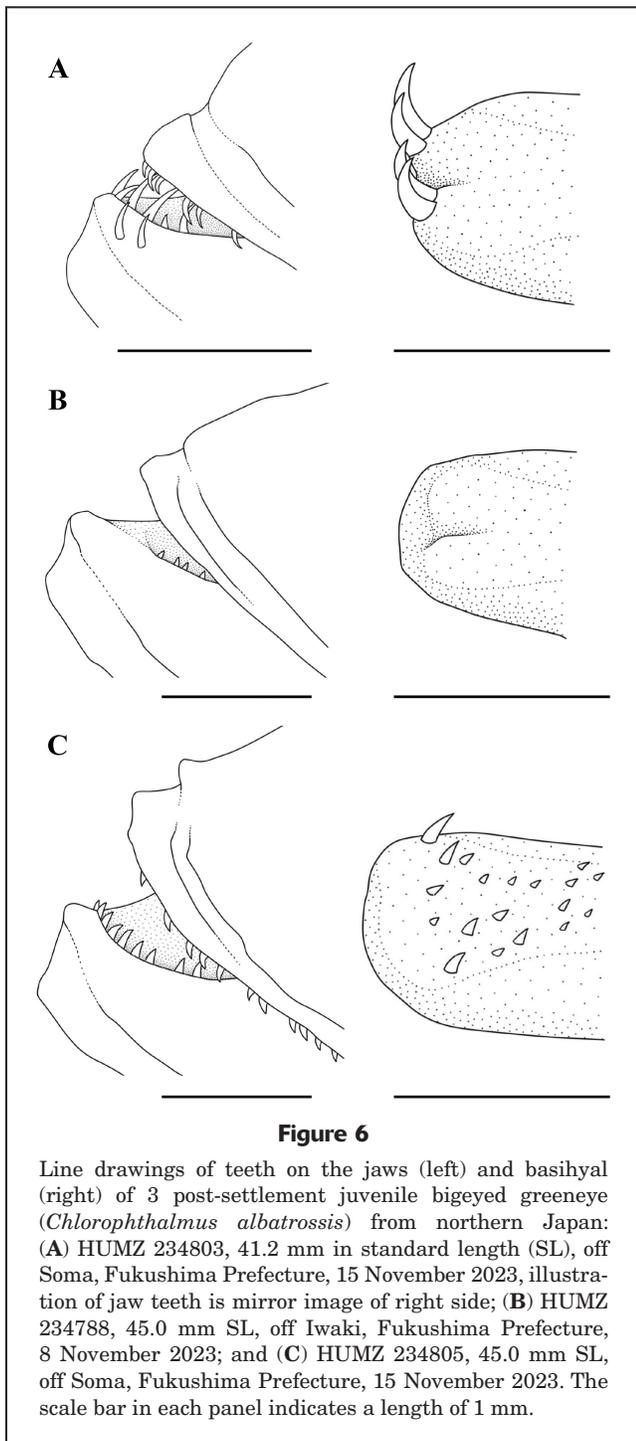


Figure 5

Fresh specimens of post-settlement juvenile bigeyed greeneye (*Chlorophthalmus albatrossis*) from northern Japan: (A) HUMZ 234803, 41.2 mm in standard length (SL), off Soma, Fukushima Prefecture, 15 November 2023, mirror image of right side; (B) HUMZ 234780, 45.2 mm SL, off Iwaki, Fukushima Prefecture, 12 November 2023; (C) HUMZ 234804, 49.5 mm SL, off Soma, Fukushima Prefecture, 15 November 2023; (D) HUMZ 234788, 45.0 mm SL, off Iwaki, Fukushima Prefecture, 8 November 2023; and (E) HUMZ 234805, 45.0 mm SL, off Soma, Fukushima Prefecture, 15 November 2023. The developmental stage of each specimen—early metamorphosing, late metamorphosing, or metamorphosed—is provided.



length. Aphakic space on anterior portion of pupil weakly developed in metamorphosed specimens except for HUMZ 234804. Posterior margin of maxilla reaching beyond vertical through anterior margin of orbit and not reaching vertical through anterior margin of pupil; in single metamorphosed specimen (HUMZ 234805), it reaches slightly beyond vertical through anterior margin of pupil. In single early metamorphosing specimen (HUMZ 234803), curved, conical teeth in single row on anterior part of premaxilla,

and slender, curved, conical teeth in single row on dentary, with 1–2 teeth on anterior portion of dentary (Fig. 6A); in other early metamorphosing (HUMZ 234806), late metamorphosing, and single metamorphosed (HUMZ 234804) specimens, jaws edentate or with few small, conical teeth; in 4 metamorphosed specimens (HUMZ 234534, HUMZ 234787–234789), premaxilla edentate or with few small, conical teeth, and dentary with small, conical teeth arranged in 1–2 rows (Fig. 6B); in single metamorphosed specimen (HUMZ 234805), premaxilla with small, conical teeth arranged in single row, and dentary with small, conical teeth arranged in 1–2 rows (Fig. 6C).

Vomer with slightly slender, curved, conical teeth arranged on anterior margin in early metamorphosing specimens; 1–2 small, conical teeth on each side of anterolateral margin in late metamorphosing and metamorphosed specimens, except for HUMZ 234534 with 3–4 small, conical teeth on each side of anterolateral margin and for HUMZ 234805 with small, conical teeth arranged on anterior margin. Palatine with slightly slender, conical teeth in single row in single early metamorphosing specimen (HUMZ 234803); edentate or with 1–2 small, conical teeth in other early metamorphosing (HUMZ 234806), late metamorphosing, and metamorphosed specimens, except for 2 specimens (HUMZ 234534 and HUMZ 234805) with about 5 small, conical teeth in single row. Basihyal with 2 large, posteriorly curved, fang-like teeth on each side of anterior margin in early metamorphosing specimens (Fig. 6A); 1 fang-like tooth on right side in late metamorphosing specimen; edentate in 2 metamorphosed specimens (HUMZ 234804 and HUMZ 234788) (Fig. 6B); small, conical teeth scattered on anterior portion of basihyal in other metamorphosed specimens (Fig. 6C). Third or 4th anal-fin ray longest; 4 anterior anal-fin rays unbranched, other rays branched. Adipose-fin origin above base of 2nd, 3rd, or 4th anal-fin ray. Tip of pectoral fin reaching slightly or well beyond vertical through posterior end of dorsal-fin base. Pelvic-fin origin below base of 3rd or 4th dorsal-fin ray.

Coloration

When fresh (Fig. 5), head and body white in metamorphosing specimens and pale gray in metamorphosed specimens. In late metamorphosing and metamorphosed specimens, silver coloration appears on opercular region, belly, and mid-lateral portion of trunk (Fig. 5, B–E). Grayish blotches on body variegated and form transverse band from posterior end of dorsal-fin base to pelvic-fin origin. Single peritoneal pigment section near pectoral-fin base visible externally in early metamorphosing specimen (Fig. 5A) and invisible in late metamorphosing and metamorphosed specimens (Fig. 5, B–E). Dorsal portion of iris with yellowish tinge in metamorphosed specimen (HUMZ 234805) (Fig. 5E). Single black blotch on caudal-fin base absent in metamorphosing specimens (Fig. 5, A and B) and present in metamorphosed specimens (Fig. 5, C–E). Each fin sparsely pigmented; inner pelvic-fin rays heavily pigmented (Figs. 2B and 5).

Remarks

Although Fricke et al. (2019) reported 6 small specimens (37.6–65.9 mm SL) from Papua New Guinea as *C. albatrossis* without a morphological description or figure, the identification should be reassessed because no adults of the species are known from waters of Papua New Guinea and several undescribed species similar to *C. albatrossis* have been reported to occur in tropical waters of the Indo-Pacific (Gomon et al., 2014). Saruwatari et al. (2005) and Saruwatari (2008) briefly reported the morphology of pelagic larvae and juveniles (from 7.3 mm in notochord length to 33.5 mm SL) of *C. albatrossis* collected from the East China Sea, basing their identification on molecular evidence based on 16S rRNA.

Although occurrences of small post-settlement juveniles (ca. 40–60 mm SL) of the species from Japan have been documented in several fishery and life history studies (Yoshida, 2003; Sakaji et al., 2006; Hirakawa et al., 2007; Inoue and Horie, 2024), the morphology of post-settlement juveniles is provided herein for the first time. Hirakawa et al. (2007) examined otolith increments of *C. albatrossis* and estimated that the settlement season of the species in northern Japan (off Fukushima Prefecture) is winter (from October through March), whereas those in central and southern Japan are autumn and summer, respectively. The dates of the recent collection of post-settlement juveniles of the species from northern Japan (i.e., from late September through early November) mostly agree with the estimation by Hirakawa et al. (2007). On the other hand, trawl surveys conducted in Tosa Bay, in southern Japan, revealed that individuals of *C. albatrossis* measuring 40–50 mm SL (smallest individual: 31.6 mm SL) settle in the depth zone of 150–200 m from August through February and gradually migrate to greater depths with growth (Sakaji et al., 2006). Because specimens described in our study were collected at much greater depths (299–484 m), settlement depths of the species may differ between regions.

Discussion

Morphological comparisons

Among the post-settlement juveniles of the 3 species examined in this study, the specimens of *C. albatrossis* are readily distinguishable from those of *C. nigromarginatus* and *C. acutifrons* by the following characters: inner pelvic-fin rays heavily pigmented (Figs. 5 and 7E) (versus lightly pigmented or unpigmented; Figs. 3, 4, and 7, A and C); grayish blotches on body variegated and form transverse band from posterior end of dorsal-fin base to pelvic-fin origin (Figs. 5 and 7E) (versus round or elliptical, without transverse bands; Figs. 3, 4, and 7, A and C); 13–14 upper and 12–13 lower procurrent caudal-fin rays (Fig. 7F) (versus 10–12 and 9–10; Fig. 7, B and D). In addition, differences between *C. albatrossis* and the other species are evident in several proportional measurements, such as the

longest anal-fin ray, pectoral-fin, and pelvic-fin lengths (Fig. 8, A–C; Table 2). Although most pigmentation on the body is not developed in pelagic juveniles of the species (Saruwatari, 2008), the number of procurrent caudal-fin rays may be a useful character in their identification.

Similar patterns in post-settlement juvenile *C. albatrossis* have been observed not only in adults of the species (Okamura, 1982, 1984b; this study) but also in some

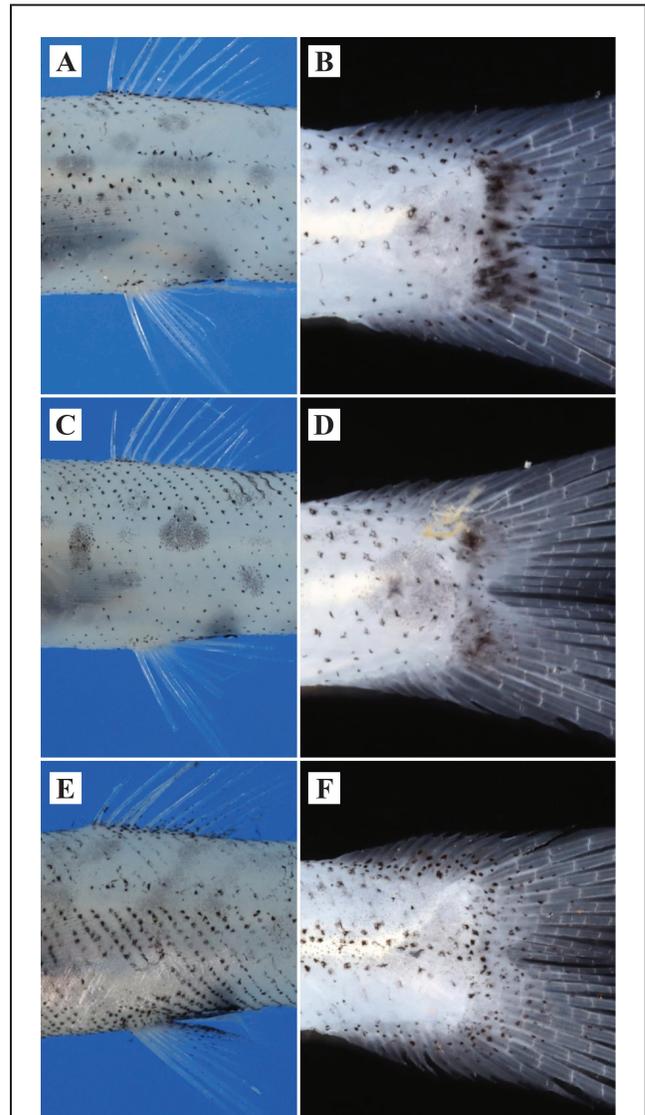


Figure 7

Body (left panels) and caudal fin (right panels) of post-settlement juveniles of 3 species of *Chlorophthalmus* collected in northern Japan: (A and B) blackedge greeneye (*C. nigromarginatus*), HUMZ 234792, 44.3 mm in standard length (SL), off Tomioka, Fukushima Prefecture, 20 November 2023; (C and D) humpback greeneye (*C. acutifrons*), HUMZ 234793, 43.9 mm SL, off Tomioka, Fukushima Prefecture, 20 November 2023; and (E and F) bigeyed greeneye (*C. albatrossis*), HUMZ 234780, 45.2 mm SL, off Iwaki, Fukushima Prefecture, 12 November 2023.

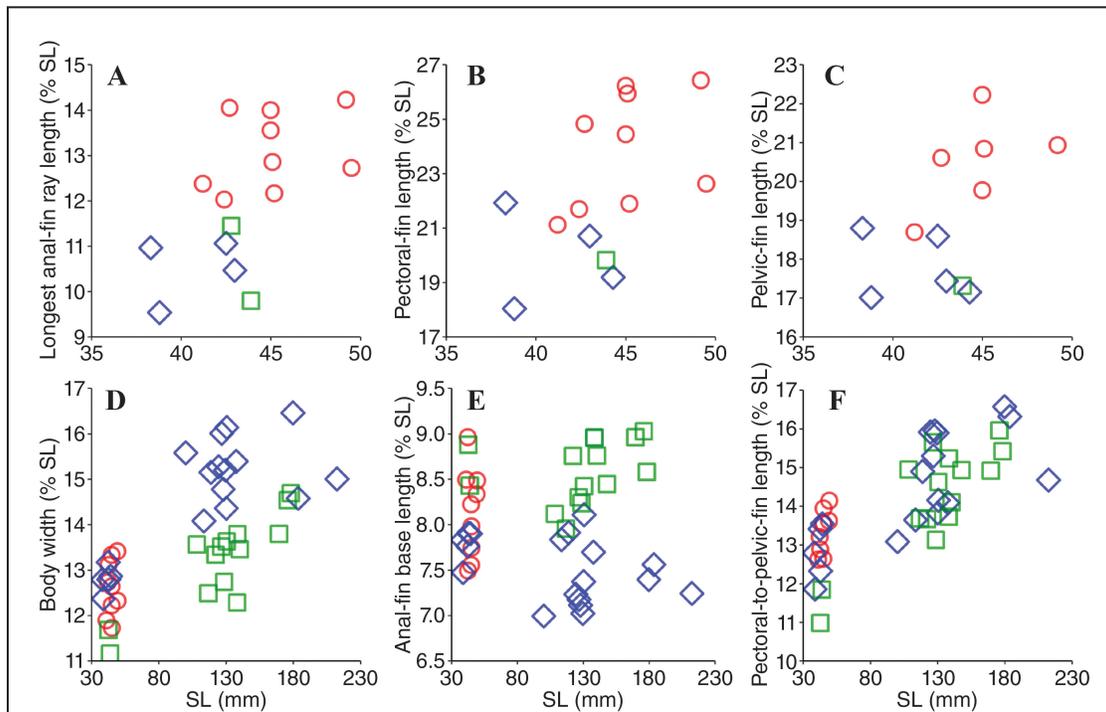


Figure 8

Relationships between standard length (SL) and (A) longest anal-fin ray length, (B) pectoral-fin length, (C) pelvic-fin length, (D) body width, (E) anal-fin base length, and (F) length between the pectoral and pelvic fins in post-settlement juvenile blackedge greeneye (*Chlorophthalmus nigromarginatus*) (diamonds), humpback greeneye (*C. acutifrons*) (squares), and bigeyed greeneye (*C. albatrossis*) (circles) caught off the Pacific coast of Tohoku District, in northern Japan, from late September through late November in 2022 and 2023. Plots in the bottom panels also include data for adults collected from waters of Kochi and Shizuoka, Japan, or the Okinawa Trough during 1972–2020 or from waters of Indonesia in 2005.

congeners, including *C. pectoralis* Okamura and Doi, 1984, *C. mascarensis* Kobylansky, 2013, and *C. imperator* Fujiwara et al., 2019 (Okamura and Doi, 1984; Kobylansky, 2013; Fujiwara et al., 2019), indicating that the pigmentation characters may not be diagnostic for post-settlement juveniles of *C. albatrossis*. Nevertheless, the aforementioned pigmentation characters are at least valid to distinguish post-settlement juveniles of *C. albatrossis* from the other 2 species described herein.

Post-settlement juveniles of *C. nigromarginatus* and *C. acutifrons* are superficially very similar to each other. Although the number of specimens of each species examined in our study was limited, we determined that post-settlement juveniles of the 2 species are separable by the following features: shape of a single large, grayish blotch below the middle of the dorsal-fin base (oblong and merged with an anteroventral blotch in *C. nigromarginatus* [Figs. 3 and 7A] versus round and not merged with other blotches in *C. acutifrons* [Figs. 4 and 7C]); body width (12.4–13.2% SL versus 11.2–11.7% SL); anal-fin base length (7.5–7.9% SL versus 8.4–8.9% SL); length between the pectoral and pelvic fins (11.9–13.5% SL versus 11.0–11.8% SL) (Fig. 8, D–F; Table 2). Adult specimens of the 2 species were examined to complement the scarcity of juvenile specimens and to evaluate interspecific differences mentioned previously

herein. As a result, a similar proportional relationship was found in the body width (14.1–16.5% SL in *C. nigromarginatus* versus 12.3–14.7% SL in *C. acutifrons*) and anal-fin base length (7.0–8.1% SL versus 8.0–9.0% SL) but not in the length between the pectoral and pelvic fins (13.1–16.6% SL versus 13.1–16.0% SL) (Fig. 8, D–F). Examinations of the adult specimens also revealed slight differences in the number of pectoral-fin rays (14–17, modally 15 in *C. nigromarginatus* versus 15–16, modally 16 in *C. acutifrons*) and lower gill rakers (16–19, modally 17 versus 14–16, modally 16) (Table 3), and these differences may help to distinguish juveniles of the 2 species with diagnostic characters that are not yet sufficiently developed.

Metamorphosis after settlement

Among post-settlement juveniles of the 3 species of *Chlorophthalmus* described herein, remarkable ontogenetic variations were observed, especially in pigmentation, tooth morphology, and eye direction. Post-settlement ontogenetic changes of these characters inferred from the specimens are discussed later in this “Discussion” section.

Pigmentation Pelagic larvae and juveniles of the genus reported in previous studies completely lack body

pigmentation, except for a single peritoneal pigment section and pigment over the hypural complex (e.g., Tåning, 1918; Ditty, 2006; Okiyama, 2014). Because other pigmentation in the recently collected post-settlement juveniles is considered to appear after settlement, for all 3 species, we inferred that the less pigmented individuals observed in our study had shorter post-settlement periods than individuals with more developed pigmentation. In post-settlement juveniles of the 3 species, body pigmentation is primarily composed of black dots arranged in slanting rows and grayish blotches. A common developmental process of body pigmentation, with black dots developing first and grayish markings appearing subsequently, was recognized in the 3 species. The peritoneal pigment section, which is characteristic of pelagic larvae and juveniles, is still clearly visible externally in early and late metamorphosing individuals of *C. nigromarginatus* (Fig. 3, A–C) and *C. acutifrons* (Fig. 4A), but it is clearly visible only in early metamorphosing individuals of *C. albatrossis* (Fig. 5A) and indistinct or invisible in subsequent stages. Marked changes were also observed in the pigmentation of the caudal-fin base, with a single black blotch appearing in early or late metamorphosing individuals of *C. nigromarginatus* (Fig. 3, B–E) and *C. acutifrons* (Fig. 4) and in only fully metamorphosed *C. albatrossis* (Fig. 5, C–E).

Tooth morphology Okiyama (2014) examined flexion (6.7–7.5 mm) and post-flexion (7.0–17.0 mm) larvae of *Chlorophthalmus* sp. and reported that these specimens had small, conical teeth arranged on margins of both jaws, with 3 strongly curved canine teeth on the anterolateral portion of the lower jaw. Teeth on the basihyal were illustrated by Rosen (1971, fig. 7B), and Hartel and Stiassny (1986, fig. 3C) on the basis of larvae or small juveniles (20–22 mm SL) of *Chlorophthalmus* spp. described 2 or 3 recurved, fang-like teeth arranged on each side of the anterior margin of the basihyal. Among the post-settlement juveniles examined in our study, only a single early metamorphosing specimen each of *C. nigromarginatus* (HUMZ 234796) and *C. albatrossis* (HUMZ 234803) retained this form of larval dentition (Fig. 6A); these specimens appear to have the shortest post-settlement period, judging from the poorly developed pigmentation in specimens of both species. On the other hand, the other early metamorphosing specimen of *C. albatrossis* (HUMZ 234806) has a basihyal with larval dentition and almost edentate jaws. In the other early metamorphosing specimen of *C. nigromarginatus* (HUMZ 233382) and late metamorphosing specimens of all 3 species, jaws, vomer, palatine, and basihyal were almost edentate or bearing only a few teeth. In these specimens, remaining fang-like teeth on the basihyal, if present, were loosely or barely attached to the bone. In metamorphosed specimens, these bones were still edentate or had small, conical teeth developed in various degrees (Fig. 6B); relatively well-developed dentition was observed in specimens of *C. acutifrons* (HUMZ 234650) and *C. albatrossis* (HUMZ 234805) (Fig. 6C). On the basis

of the tooth configurations observed, we assume juveniles of the genus lose larval dentition after settlement, with adult dentition developed subsequently. A similar ontogenetic loss of larval teeth in metamorphosing juveniles has also been reported for *Parasudis* Regan, 1911, the other genus of the family (Hartel and Stiassny, 1986).

Eye direction Sanzo (1915) examined larvae and juveniles (4.5–47 mm) of *C. agassizi* and noted that eyes directed sideways and slightly downwards in small larvae change direction with growth to look to the side and upwards. He suggested this metamorphosis is related to the transition from a pelagic lifestyle to a benthic lifestyle. Tåning (1918) also noted the direction of eyes in larval and juvenile *C. agassizi* and considered the movement of eyes to take place at lengths between 25 and 44 mm SL. Although the direction of eyes in the recently collected post-settlement juveniles was already dorsolateral, we observed the transition in eye direction and that it coincides with changes of the least interorbital width during developmental stages (Fig. 9). The relatively wide interorbital space in early metamorphosing specimens gradually became narrower in late metamorphosing and metamorphosed individuals, as the eye redirected more dorsally.

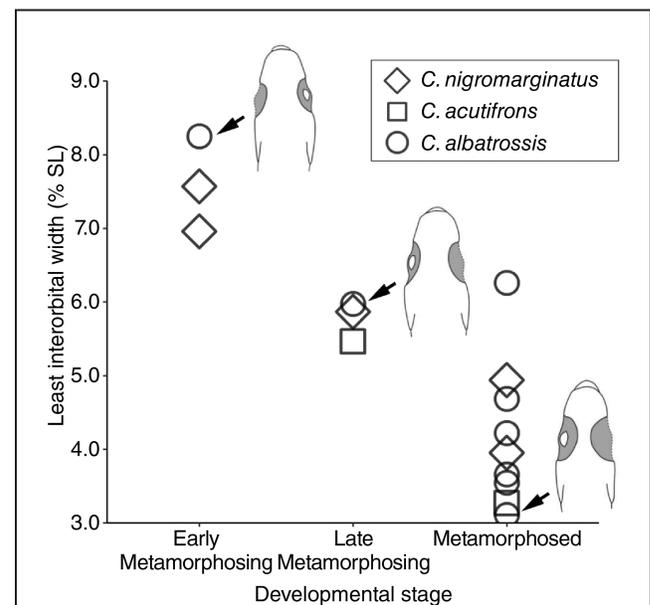


Figure 9

Relationships between least interorbital width and the 3 developmental stages in blackedge greeneye (*Chlorophthalmus nigromarginatus*), humpback greeneye (*C. acutifrons*), and bigeyed greeneye (*C. albatrossis*) collected off the Pacific coast of northern Japan from late September through late November in 2022 and 2023. Drawings of the dorsal view of the heads of specimens of *C. albatrossis* in early metamorphosing (HUMZ 234803), late metamorphosing (HUMZ 234780), and metamorphosed (HUMZ 234805) stages are provided (each arrow indicates the symbol for the specimen in that stage).

Conclusions

The remarkable morphological changes discussed herein were observed in specimens with relatively small differences in body size: 38.3–44.3 mm SL in *C. nigromarginatus*, 42.8–43.9 mm SL in *C. acutifrons*, and 41.2–49.5 mm SL in *C. albatrossis*. In addition, the degree of development for each character did not necessarily correspond to body size. In fact, individuals with the most developed characteristics for each species (Figs. 3E, 4B, and 5E) were not the largest specimens. These data indicate that juveniles of the genus undergo a very rapid metamorphosis triggered by settlement on the seafloor when the individual's lifestyle changes from pelagic to benthic. In further studies, including an analysis of otolith increments, this hypothesis will be evaluated, and more information on the process of the settlement and metamorphosis of these 3 species will be provided. Because knowledge of post-settlement juveniles of *Chlorophthalmus*, as well as pelagic larvae and juveniles, remains scarce, further sampling of larvae and juveniles and improvement in the accuracy of their identifications are essential to reveal more about the processes during the early life stages of these species.

Resumen

Se describen por primera vez el post-asentamiento de juveniles de 3 especies de ojiverdes de la familia Chlorophthalmidae, *Chlorophthalmus nigromarginatus*, *C. acutifrons* y *C. albatrossis*, a partir de 5, 2 y 9 ejemplares, respectivamente, colectados en el norte de Japón. Estas identificaciones fueron corroboradas por un análisis de las secuencias del gen mitocondrial citocromo c oxidasa I de juveniles y adultos de las 3 especies y congéneres. Entre las 3 especies, los juveniles post-asentamiento de *C. albatrossis* se distinguen fácilmente por los siguientes caracteres: radios internos de la aleta pélvica claramente pigmentados (contra ligeramente pigmentados o no pigmentados), manchas grisáceas en el cuerpo abigarradas y que forman una banda transversal desde el extremo posterior de la base de la aleta dorsal hasta el origen de la aleta pélvica (contra redondas o elípticas, que no forman bandas transversales), y 13–14 radios superiores y 12–13 inferiores de la aleta caudal (contra 10–12 y 9–10). Aunque los juveniles post-asentamiento de *C. nigromarginatus* y *C. acutifrons* son superficialmente muy similares entre sí, se pueden separar por la forma de las manchas grisáceas y varios caracteres en sus proporciones y merísticos. Al examinar los especímenes ya asentados, los juveniles del género *Chlorophthalmus* parecen sufrir una rápida metamorfosis al asentarse en el fondo marino, cuando cambian del estilo de vida pelágico a bentónico.

Acknowledgments

We are deeply grateful to the captain and crew of R/V *Wakataka-maru*, Y. Narimatsu, K. Fujiwara, Y. Suzuki,

E. Morikawa, S. Tokioka, and J. Nagao (Japan Fisheries Research and Education Agency) and M. Furusho (Hokkaido University) for collecting the specimens, T. Senda (Kyoto University) for providing literature, Y. Kai (Kyoto University) for providing comparative materials, and M. Gomon (Museums Victoria) for valuable comments on and corrections to English usage and grammar in an early draft. This research was financially supported by the Japan Fisheries Research and Education Agency and by the Fisheries Agency, Ministry of Agriculture, Forestry and Fisheries of Japan.

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