Pelagic larval duration, growth rate, and population genetic structure of the tidepool snake moray *Uropterygius micropterus* around the southern Ryukyu Islands, Taiwan, and the central Philippines

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The relationships between pelagic larval duration (PLD) and geographic distribution patterns or population genetic structures of fishes remain obscure and highly variable among species. To further understand the early life history of the tidepool snake moray *Uropterygius micropterus* and the potential relationship between PLD and population genetic structure of this species, otolith microstructure and population genetics based on concatenated mtDNA sequence (cytochrome *b* and cytochrome oxidase subunit I, 1,336 bp) were analyzed for 195 specimens collected from eight locations around the southern Ryukyu Islands, Taiwan, and the central Philippines. Eels with longer PLD and lower otolith growth rates were observed at relatively higher latitudes with lower water temperatures (54.6 ± 7.7 days and 1.28 ± 0.16 μm day^{-1} on Ishigaki Island, Japan, vs. 43.9 ± 4.9 days and 1.60 ± 0.19 μm day^{-1} on Badian, the Philippines), suggesting that leptocephali grew faster and had shortened pelagic periods in warmer waters. Meanwhile, the eels along the southwest coast of Taiwan had relatively longer PLD (57.9 ± 10.5 days), which might be associated with the more complex ocean current systems compared to their counterparts collected along the east coast of Taiwan (52.6 ± 8.0 days). However, the southwestern and eastern Taiwan groups had similar otolith growth rates (1.33 ± 0.19 μm day^{-1} vs. 1.36 ± 0.16 μm day^{-1}). Despite the intergroup variation in PLD, genetic analysis revealed fluent gene flow among the tidepool snake morays in the study regions, implying that intraspecies PLD variation had a weak effect on genetic structure. The leptocephalus stage might have ensured the widespread gene flow among the study areas and leptocephalus growth was likely influenced by regional water temperature.

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ABSTRACT

The relationships between pelagic larval duration (PLD) and geographic distribution patterns or population genetic structures of fishes remain obscure and highly variable among species. To further understand the early life history of the tidepool snake moray *Uropterygius micropterus* and the potential relationship between PLD and population genetic structure of this species, otolith microstructure and population genetics based on concatenated mtDNA sequence (cytochrome *b* and cytochrome oxidase subunit I, 1,336 bp) were analyzed for 195 specimens collected from eight locations around the southern Ryukyu Islands, Taiwan, and the central Philippines. Eels with longer PLD and lower otolith growth rates were observed at relatively higher latitudes with lower water temperatures (54.6 ± 7.7 days and 1.28 ± 0.16 μm day^{-1} on Ishigaki Island, Japan, vs. 43.9 ± 4.9 days and 1.60 ± 0.19 μm day^{-1} on Badian, the Philippines), suggesting that leptocephali grew faster and had shortened pelagic periods in warmer waters. Meanwhile, the eels along the southwest coast of Taiwan had relatively longer PLD (57.9 ± 10.5 days), which might be associated with the more complex ocean current systems compared to their counterparts collected along the east coast of Taiwan (52.6 ± 8.0 days). However, the southwestern and eastern Taiwan groups had similar otolith growth rates (1.33 ± 0.19 μm day^{-1} vs. 1.36 ± 0.16 μm day^{-1}). Despite the intergroup variation in PLD, genetic analysis revealed fluent gene flow among the tidepool snake morays in the study regions, implying that intraspecies PLD variation had a weak effect on genetic structure. The leptocephalus stage might have ensured the widespread gene flow among the study areas and leptocephalus growth was likely influenced by regional water temperature.

41 # INTRODUCTION

42 The population structure of fishes with pelagic larvae is influenced by biological and
43 environmental factors (Leis *et al.*, 2013; Nanninga *et al*., 2014). However, many of these factors
44 are difficult to parameterize (Nanninga *et al*., 2014) and pelagic larval duration (PLD) is used as
45 a direct predictor of dispersal potential and population connectivity, especially for site-attached
46 coral reef fishes that do not display migratory behaviors during their juvenile and adult stages
47 (Bowen *et al*., 2006; Macpherson & Raventos, 2006). Although PLD has been found to strongly
48 influence population genetic structure only in extreme cases with very short or very long PLD
49 (Thresher *et al*., 1989; Bowen *et al*., 2001; Weersing & Toonen, 2009), some studies have still
50 suggested that PLD may be a strong determinant for evaluating larval dispersal and population
51 connectivity (e.g., Faurby & Barber, 2012). Variation in PLD may be affected by numerous
52 factors at inter- and intraspecific levels, including genotypes (Tsukamoto *et al*., 2002),
53 physiological conditions of larvae (Reveillac *et al*., 2008; Han *et al*., 2010) and environmental
54 changes (Searcy & Sponaugle, 2000; Sponaugle & Pinkard, 2004; Bergenius *et al*., 2005).
55 Therefore, larval fishes that experience different environmental conditions could have varied
56 early life history traits, leading to specific PLDs among populations (Bay *et al*., 2006). However,
57 the effects of variable PLDs on population genetics remain unclear and infrequently evaluated
58 for many fish.
59 Most true eels (Anguilliformes) are demersal fish with limited migration during the juvenile
60 to adult stages (Bassett & Montgomery, 2011; Correia *et al.*, 2012), except for temperate
61 anguillids and some congrids that have offshore spawning areas (Tsukamoto, 2006; Kurogi *et al*.,
62 2012). The long pelagic larval stage of leptocephalus may play an important role in their
63 distribution and population genetic structures (Miller & McCleave, 2007; Kuroki *et al.*, 2009;

Reece *et al.*, 2011). Moreover, variations in the intraspecific PLD of anguillids have been observed within and among geographic regions without genetic divergence. These variations in PLD are likely influenced by nutrition status among individuals (Reveillac *et al*., 2008; Han *et al*., 2010). Few studies have been conducted on intraspecific variation in PLDs in marine eels. Kimura *et al*. (2004) found various PLDs and growth rates for *Conger myriaster* leptocephali along the east coast of central Japan. Despite the various PLDs, *C. myriaster* breed in specific spawning areas (Kurogi *et al*., 2012), which may counteract the influence of PLD variation on their genetic structure. For other marine eel taxa with near-shore spawning strategies, the correlations between variable PLD and genetic structure among geographic areas have yet to be explored thoroughly.

The family Muraenidae, known as moray eels, is the second largest family after the Ophichthidae in the Anguilliformes, with approximately 200 species in 15 genera and two subfamilies (Smith, 2012). Moray eels are broadly distributed in tropical and temperate oceans. Most muraenids inhabit rocky ledges and coral reefs from the intertidal zone to depths of over 300 m, and some species are occasionally found in sandy or freshwater habitats (Tsukamoto *et al*., 2014). Moray eels have high fidelity to their habitats (Bassett & Montgomery, 2011) and spawn without migrations (Moyer & Zaiser, 1982). Therefore, moray eels are solely dispersed in the pelagic leptocephalus stage, providing an effective means of evaluating the effects of PLD on genetic divergence for marine eels with a local spawning strategy. For example, the tidepool snake moray *Uropterygius micropterus* (Bleeker 1852) usually resides in the rocky intertidal zone at depths shallower than 3 m (Chen, 1997), leading to fragmented habitat use across the Indo–Pacific oceans from East Africa to Samoa, north to southern Japan, and south to Australia (Froese & Pauly, 2016). *Uropterygius micropterus* is a small species measuring less than 40 cm in length (Loh *et al*., 2011) that has a local spawning strategy. *Uropterygius*

micropterus were observed to reproduce in the rocky intertidal zone of Taitung, Taiwan during summer. Numerous males followed and entwined with a female, snapping at the female's head and trunk. Afterward, a cloud of sperm and transparent, buoyant eggs were discharged into the water (Chen, H.M., unpublished data). Due to its small size, local spawning strategy, and specific habitat use, *U. micropterus* would be a favorable candidate for evaluating larval dispersal and its effects on genetic structure. The present study aimed to (1) examine the otolith microstructure of *U. micropterus* to evaluate variation in PLD among sampling sites and (2) to test the relationship of differences in PLD to genetic structure.

MATERIALS AND METHODS

SAMPLE COLLECTION

One hundred and ninety-five juvenile and adult *U. micropterus* were collected by dip net and hand-lining at rocky intertidal zones along Ishigaki Island, Japan (n = 14), six Taiwanese sites (n = 142), and Badian, the Philippines (n = 39) during 2014–2016 (Table 1; Fig. 1). Shitiping (n = 36), Jihui (n = 32), and Green Island (n = 20) are located on the east coast of Taiwan and are influenced by the strong, constant Kuroshio Current that flows northward year-round. Checheng (n = 31), Wanlitong (n = 11), and Liuqiu (n = 12) are located on the southwest coast of Taiwan, in a more complex ocean environment that is affected by numerous water masses on a seasonal basis (Shaw, 1991; Farris & Wimbush, 1996; Hu *et al*., 2010). Ishigaki is also affected by the Kuroshio Current, and Badian is a relatively closed environment located on Cebu in the central Philippines.

OTOLITH PREPARATION AND ANALYSIS

Left sagittal otoliths were extracted under stereo microscope, cleaned with deionized water, dried at 55 °C overnight, embedded in Epofix resin, and fixed on microscope slides. The

111 prepared otoliths were grounded along the sagittal plane with 2000 and 2400 grit sandpapers
112 until the core was revealed on the surface, and then polished until smooth with 0.05 μm alumina
113 powder. The ground otoliths were photographed and the growth increments were counted under
114 a compound light microscope (BX-51 Olympus, Japan) from the first feeding check (FFC) to the
115 growth check (GC) (Fig. 2). The otolith growth increments during the leptocephalus stage of *U.*
116 *micropterus* were distinguishable and the narrowest rings (approx. 1 μm) were larger than the
117 resolution limitation of the compound light microscope. The GC was defined by Ling *et al*.
118 (2005) as the prominent check at which the growth increments change from a circular to a
119 radiating pattern, accompanied by a lowered Sr/Ca ratio. This prominent check has been
120 assumed to be associated with the beginning of metamorphosis in leptocephalus and has been
121 used in several studies of marine eel species (Ling *et al*., 2005; Lee *et al.*, 2008). Otolith growth
122 increments from the first feeding ring to the GC were expressed as T_{GC} (i.e., PLD). Meanwhile,
123 the radius of GC was measured along the longest axis and divided by T_{GC} to calculate the mean
124 otolith growth rate (μm increment^{-1}, as G_{GC}). Some sub-increments between the wide increments
125 before GC could be ignored, and the blurred rings before FFC were excluded from the count,
126 which may have led to a slight underestimation of the actual number of increments. Fourteen
127 otoliths were randomly chosen for the Sr/Ca ratio analysis to assist in the judgment of GC.
128 Polished otoliths were coated with a layer of carbon and analyzed by an electron probe
129 microanalyzer (EPMA, JEOL JXA-8900R). The Sr/Ca ratios were measured from the core to the
130 edge of each otolith under electron beam conditions of 15 kV and 3 nA, beam size 5 × 4 μm, and
131 10 μm of spot intervals. Since the otolith growth increments of anguillid species are typically
132 deposited daily in the early leptocephalus and glass eel stages (Sugeha *et al*., 2001; Shinoda *et*
133 *al*., 2004), the otolith increments counted in this study were assumed to be deposited daily (i.e.,
134 T_{GC} = days and G_{GC} = μm day^{-1}).

The daily ages and otolith growth rates of *U. micropterus* at different latitudes were divided into three groups (Ishigaki, Taiwan, and Badian) for statistical analysis. The Taiwanese sampling sites were additionally divided into eastern and southwestern groups, representing different oceanic current conditions at similar latitudes, to test whether ocean currents affected the early life characteristics of *U. micropterus* despite the small geographic scale. The statistical differences in daily ages and mean daily otolith growth rates between groups were tested by analysis of variance (ANOVA) and the post hoc Tukey HSD test in R (R Development Core Team, 2013). The mean increment widths of sagittal otoliths for intervals of every three rings were also estimated in ImageJ (Abràmoff *et al*., 2004) along the longest axis. The percentages of daily ages for five-day intervals are shown in bar charts.

POPULATION GENETIC ANALYSIS

DNA was extracted from muscle tissue using a Qiagen DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. Polymerase chain reactions (PCRs) were run in a total volume of 50 μL, including 6 μL of TaKaRa (www.clontech.com) 10 × buffer, 4 μL of 2.5 mM dNTPs, 4 μL of 10 μM of each primer, 0.25 μL of TaKaRa *Ex Taq* DNA polymerase, 6 μL of template DNA at 50 ng/μL, and 25.75 μL of deionized water. The fragments of cytochrome *b* (cyt *b*) 680-bp and cytochrome oxidase subunit I (*COI*) 656-bp were respectively amplified using the primers cyt *b*: L14725 (5'-GTG ACT TGA AAA ACC ACC GTT G-3') (Song *et al*., 1998) and H15573 (5'-AAT AGG AAG TAT CAT TCG GGT TTG ATG-3') (Taberlet *et al*., 1992); and *COI*: FishF2 (5'-TCG ACT AAT CAT AAA GAT ATC GGC AC-3') and FishR2 (5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-3') (Ward *et al*., 2005). The annealing temperatures of cyt *b* and *COI* were 47 °C and 50 °C, respectively. The thermal profiles of PCR were 94 °C for 5 min, followed by 37 cycles of 94 °C for 1 min, annealing temperature for 45 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The quality

of PCR products was checked by electrophoresis with 1.5% agarose gel and then purified using a Macherey-Nagel purification kit (www.mn-net.com) according to the manufacturer's protocols. DNA sequences were generated by an ABI 3730 automated sequencer at the Center for Biotechnology, National Taiwan University. Sequences were assembled and edited manually and aligned using MEGA version 6.0 (Tamura *et al*., 2013).

Sequences of cyt *b* and *COI* were concatenated as a single genetic marker and the analyses that followed were based on this data set. The genetic diversity indexes of haplotype diversity (*h*) and nucleotide diversity (π) were calculated in DnaSP version 5.0 (Librado & Rozas, 2009) according to Nei (1987). Pairwise Φ_{ST} comparisons among sampling sites and among groups with different classes of T_{GC} were estimated in Arlequin version 3.5 (Excoffier & Lischer, 2010), and 10,000 permutations were used to estimate the departure from the null hypothesis of genetic homogeneity. The statistical significance of pairwise Φ_{ST} values was adjusted with Bonferroni correction (Rice, 1989) for multiple comparisons. The hierarchical levels of genetic diversity were tested through analysis of molecular variance (AMOVA; Excoffier *et al*., 1992), and the proportions of variations among groups (Φ_{CT}), among populations within groups (Φ_{SC}), and within populations (Φ_{ST}) were calculated in Arlequin; 10,000 permutations were used to estimate statistical significance. Two hypothetical grouping treatments were used for AMOVA: (1) based on the three different latitudinal groups of Ishigaki, Taiwan, and Badian; and (2) based on the sampling sites that were associated with three different ocean current conditions, namely the Kuroshio Current system (Ishigaki, Shitiping, Jihui, and Green Island), mixed effect by numerous water masses (Checheng, Wanlitong, and Liuqiu), and the interior current systems of the Philippine archipelago (Badian). The minimum spanning network (MSN) of haplotypes was built using Arlequin version 3.5 and HapStar version 0.7 (Teacher & Griffiths, 2011) to connect haplotypes based on the minimum differences between sequences.

RESULTS

OTOLITH MICROSTRUCTURE AND MICROCHEMISTRY

The otolith microstructure of *U. micropterus* was similar to those of other marine eels (Ling *et al*., 2005; Correia *et al*., 2004; Lee *et al.*, 2008). After polishing, the otolith core became a hole surrounded by a thick ring, referred to as a hatch check (HC; Fig. 2). The first feeding check (FFC) was assumed to form when yolks were absorbed completely and the leptocephali began to ingest external food. There were three to five blurry increments between HC and FFC in some individuals. The increments beyond FFC were circular, and the increment widths gradually increased to a peak of 1.1–2.0 μm at approximately the 10^{th} to 20^{th} increments, followed by a gradual decrease to a minimum of 0.5–0.9 μm. Then, the growth increment width abruptly increased to 1.5–4.0 μm by three to 28 increments and formed a profound growth check (GC). The growth increments after GC were wider (5–15 μm), diffused, and radiative. The Sr/Ca ratios of 14 *U. micropterus* fluctuated between 3 and 16 × 10^{-3} from the core to the GC, with no apparent pattern. The Sr/Ca ratios then dropped rapidly, accompanied by the appearance of the GC in all but one individual (Fig. 3).

GENETIC DATA

One hundred and thirty-two haplotypes from 1,336 bp concatenated mtDNA sequences from 195 *U. micropterus* were identified (GenBank accession number MF190188–MF190364). In total, 179 polymorphic sites, 112 parsimony informative sites, and 67 singleton variable sites were found. Haplotype diversity (h) and nucleotide diversity (π) ranged from 0.9921 to 1 (average = 0.9938) and 0.0060 to 0.0073 (average = 0.0065), respectively (Table 1). The minimum spanning network showed many unique haplotypes, with only 28 of the 132 haplotypes shared by more than one individual. These unique haplotypes were connected to the

206 center haplotype that occurred in Ishigaki, Shitiping, Checheng, and Badian. The most common
207 haplotype consisted of seven individuals from all locations except Liuqiu and Wanlitong.
208 Closely related haplotypes consisted of individuals from distinct regions, revealing no obvious
209 geographic pattern (Fig. 4).

210 The pairwise Φ_{ST} values among sampling sites ranged from −0.025 to 0.121. Liuqiu
211 revealed low but significant genetic variations with all sampling sites except Green Island. Only
212 Liuqiu versus Checheng was statistically significant after the most conservative Bonferroni
213 correction (Table 2). All pairwise Φ_{ST} values among groups with different classes of T_{GC}
214 remained low and insignificant (Table S1). The AMOVA results showed that over 99% of
215 variations occurred at the population level under both groupings. Only the Φ_{SC} of different ocean
216 current conditions revealed low but statistically significant structure ($\Phi_{SC} = 0.017$, $P < 0.05$)
217 (Table 3). The results of genetic analysis support the conclusion that *U. micropterus* in the study
218 areas should be considered genetically homogeneous with weak genetic structure.

219 **PELAGIC LARVAL DURATION AND GROWTH RATE**

220 The pelagic larval duration represented by the T_{GC} ranged from 33 to 98 days for the eels
221 examined (Table 4). The maximum T_{GC} of 98 days occurred in Liuqiu and the minimum of 33
222 days occurred in Badian. Liuqiu specimens had the longest and most variable T_{GC}. Ishigaki and
223 Taiwanese specimens had significantly longer T_{GC} than those from Badian (Tukey HSD, $P <$
224 0.01). The otolith growth rate from the first feeding ring to the GC represented by G_{GC} ranged
225 from 0.91 to 2.40 μm day^{-1}. The maximum G_{GC} of 2.40 μm day^{-1} occurred in Badian and the
226 minimum value of 0.91 μm day^{-1} occurred in Jihui. Significantly lower G_{GC} were observed in
227 Ishigaki and Taiwanese specimens compared with Badian (Tukey HSD, $P < 0.01$).

228 The eels were divided into three latitudinal groups according to sampling site, namely
229 Ishigaki (n = 14), Taiwan (Shitiping, Jihui, Green Island, Checheng, Wanlitong, and Liuqiu; n =

141) and Badian (n = 39). The respective mean ± SD daily age and otolith growth rate were 54.6 ± 7.7 days with 1.28 ± 0.16 μm day^{-1}, 54.6 ± 9.3 days with 1.35 ± 0.18 μm day^{-1}, and 43.9 ± 4.9 days with 1.60 ± 0.19 μm day^{-1}. Ishigaki specimens had the lowest growth rate, whereas the Badian specimens had the highest growth rate and shortest mean T_{GC} (Table 5, Fig. 5(a) & 6). There were no significant differences in T_{GC} and G_{GC} between the Ishigaki and Taiwanese specimens (Tukey HSD, $P > 0.05$), and both were significantly different from the Badian specimens (Tukey HSD, $P < 0.001$). The percentage of T_{GC} peaked at 56–60 days (29%) in the Ishigaki specimens, 46–50 days (24%) in the Taiwanese specimens, and 41–45 days (44%) in the Badian specimens [Fig. 5(a)]. *Uropterygius micropterus* tended to have longer T_{GC} and lower G_{GC} at higher latitudes.

The Taiwanese specimens were further divided into eastern (Shitiping, Jihui, and Green Island; n = 87) and southwestern groups (Checheng, Wanlitong, and Liuqiu; n = 54), which had mean ± SD days and otolith growth rates of 52.6 ± 8.0 days (1.36 ± 0.16 μm day^{-1}) and 57.9 ± 10.5 days (1.33 ± 0.19 μm day^{-1}), respectively. The specimens collected in southwestern Taiwan had significantly longer T_{GC} than those collected in eastern Taiwan (Tukey HSD, $P < 0.01$), whereas there was no significant difference in G_{GC} (Tukey HSD, $P > 0.05$) (Table 6). The percentage of T_{GC} peaked at 46–50 days (30%) for the eastern Taiwan group and 51–55 days (22%) for the southwestern Taiwan group [Fig. 5(b)]. *Uropterygius micropterus* from southwestern Taiwan had longer T_{GC}.

DISCUSSION

EARLY LIFE HISTORY TRAITS

The diameters of fertilized eggs and total lengths of newly hatched preleptocephalus larvae of *U. micropterus* were 3.0–3.5 and 10.0 mm (Chen, H.M., unpublished data). The total lengths

253 of *U. micropterus* leptocephali are approximately 60.0 mm at metamorphosis, based on
254 observations of two *Uropterygius* spp. in the early metamorphosis stage with total lengths of
255 51.1 and 56.5 mm (Okiyama, 2014). Therefore, the larval growth rates of *U. micropterus* with
256 PLD of 33–98 days are likely to be between 0.5 and 1.5 mm day^{-1}. This fast larval growth rate
257 greater than 1 mm day^{-1} was also observed in four other eel species in the eastern Gulf of
258 Mexico (*Gymnothorax saxicola*, *Ophichthus gomesii*, *Ariosoma balearicum*, and *Paraconger*
259 *caudilimbatus*) (Bishop *et al*., 2000).

260 In the present study, the commencement of leptocephalus metamorphosis was defined by
261 the GC where the Sr/Ca ratio drastically decreased and the otolith growth increment widths
262 abruptly increased, similar to findings in other muraenids and eel species (Marui *et al*., 2001;
263 Correia *et al*., 2004; Ling *et al*., 2005). Although the biological meaning of GC remains unclear,
264 the first growth increment for this check should contain most of the leptocphalus stage and
265 sufficiently represent the PLD of *U. micropterus* among sampling locations.

266 Insignificant pairwise Φ_{ST} values among groups with different PLDs (Table S1) indicate
267 that the plasticity of the PLD is likely due to individual acclimatization rather than different
268 genotypes. Although PLD variation can be explained by the different growth conditions or
269 birthplaces of the larvae, clearly characteristic PLDs were still observed among the groups in this
270 study, indicating that regional environmental factors may influence tendencies in early life
271 history traits (Searcy & Sponaugle, 2000; Sponaugle & Pinkard, 2004; Bay *et al*., 2006). Leis *et*
272 *al.* (2013) suggested three general classes of factors that might lead to differences in larval
273 dispersal, including biological (e.g. spawning mode and PLD), physical (water movement and
274 habitat fragmentation) and biophysical differences (principally temperature). In this study, *U.*
275 *micropterus* at relatively higher latitudes tend to have lower otolith growth rates and longer
276 PLDs compared with specimens at lower latitudes, potentially owing to water temperature. The

277 sea surface temperatures (SSTs) during the spawning season and pelagic leptocephalus stage of
278 *U. micropterus* (Chen, 1997) were derived from Global Sea Temperature
279 (http://www.seatemperature.org/) (Fig. S1). The SST of Itoman (Japan) is 5 °C lower than that of
280 Guihulngan (the Philippines) in May and December, indicating that the leptocephali in the
281 different study areas experienced different SSTs regardless of whether the eels were self-
282 recruited or transported from other habitats. The effects of temperature on larval development
283 were discussed in rearing experiments that demonstrated positive correlations in *Anguilla*
284 *japonica* glass eels and elvers (Fukuda *et al*., 2009). Leptocephali at lower latitudes with higher
285 SSTs may grow faster and require less time to reach the minimum size for metamorphosis
286 (Reveillac *et al*., 2008). Because the latitude of Ishigaki is similar to that of eastern Taiwan,
287 neither the mean PLDs nor the otolith growth rates were expected to be different.
288 Since the effect of SST on larval growth can be excluded from the sampling sites in
289 Taiwanese waters, the relatively longer PLD in the southwestern specimens might be attributable
290 to different current systems or recruitment routes. Larval dispersal route is considered to be
291 strongly influenced by ocean currents (Kim *et al*., 2007). For instance, newly hatched larvae of
292 *Conger myriaster* can be retained in mesoscale eddies for several months, resulting in longer
293 PLD than expected (Kurogi *et al*., 2012). The strong Kuroshio Current flows northward along
294 the east coast of Taiwan year-round (Rudnick *et al*., 2011). When leptocephali competent to
295 metamorphose drift to this area, they can instantly metamorphose and settle. However, the
296 current system in southwestern Taiwan is affected by different water masses seasonally,
297 including the intrusion of the Kuroshio branch, the substitution of South China Sea surface water
298 mass into the Taiwan Strait, and other transient oceanographic events (Shaw, 1991; Farris &
299 Wimbush, 1996; Hu *et al*., 2010). In addition, a weak anticyclonic eddy with a diameter of 100–
300 200 km was identified in this area (Fig. 1, Fig. S2). The leptocephali might entrain in the

anticyclonic eddy or complicated current system and therefore require a longer time to settle along the southwest coast of Taiwan.

POPULATION STRUCTURE AND LARVAL DISPERSAL

Previous studies have shown that the dispersal or retention of larvae may greatly influence gene flow for demersal fishes (Taylor & Hellberg, 2003). During the field collection stage, we found that *U. micropterus* inhabited shallow water close to the upper tidal zone, which was usually shallower than 1 m and was exposed to air during low tide. These habitats are usually fragmented, but each has an extremely high density of individuals, suggesting that some larvae hatched in the upper tidal zone may remain in nearshore areas and contribute to local habitats. However, the fast-flowing Kuroshio Current is frequently used by the larvae of many eel species for transportation to a wide range of areas (Miller *et al.*, 2002), which may facilitate *U. micropterus* gene flow. The sampling site at Badian is relatively isolated from the main oceanic current systems around the Philippine archipelago. Compared with the Taiwanese sites in this study, there may be fewer *U. micropterus* leptocephali drifting away from Badian per generation. It is likely that a handful of migrants have sufficiently contributed to the genetic homogenization among the study regions over timescales of tens to hundreds of thousands of years (Reece *et al.*, 2010).

Nevertheless, it seems that limited larval exchange over time is inadequate to explain the significant genetic divergence of Liuqiu from all locations but Checheng with Φ_{ST} before Bonferroni correction. Unknown environmental mechanisms that restrict larval dispersal and enhance massive self-recruitment may be a possible explanation. Previous studies have suggested that the self-recruitment of larvae is a common phenomenon for marine fishes and invertebrates, disregarding larval dispersal capabilities (Taylor & Hellberg, 2003; Teske *et al*., 2015). Self-recruitment of leptocephali is also surmised more often than dispersal in some areas

and is attributed to local current systems, semi-enclosed ocean environments, or the swimming ability of the larvae (Miller *et al*., 2011; Miller *et al*., 2016). Furthermore, the PLD of Liuqiu specimens was the longest and most variable (46 to 98 days) of all sampling sites, indicating that *U. micropterus* larvae had more complicated composition and different transportation routes to Liuqiu. The longer PLD implies that some larvae might recruit from places with cooler water or remote populations not included in this study. Further studies with larger-scale sampling among a range of habitats may provide more details on the population genetic structure of *U. micropterus*.

CONCLUSION

In the present study, intraspecific variations in PLD were found in *U. micropterus* among defined groups without obvious population genetic structure. These variations were likely acclimatization-dependent rather than genotype-dependent. Weak divergence of *U. micropterus* was observed in Liuqiu, southwestern Taiwan, most likely owing to the different recruiting routes of the leptocephali. This study suggests that the intraspecific variation in the PLDs of the eels might have resulted from different seawater temperatures and complex ocean conditions.

ACKNOWLEDGEMENTS

We are grateful to Dr. Yuichi Kano, Florence Evacitas, Jiunn-Shiun Chiou, Ming-Tai Chou, Ying-Ching Yuan, Li-Chi Cheng, Yun-Ting Hsieh, Zen-Wei Lin, Kai-Chiang Chang, Po-Wei Huang, Yu-Wei Liu, Chu-Feng Lu and Chang-Chun Liu for their assistance in specimen collection; Dr. Hong-Ming Chen for providing reproductive information on the eels; and Sih-Yu Chen, Han-Ting Huang, and Jhen Hsu for assistance in map drawing. We specially thank Joel Corush and Margaret Scholten for assistance in English editing.

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Table 1(on next page)

Summary of collection data and sample size for the otoliths and mtDNA analysis of *Uropterygius micropterus* used in this study.

The number of alleles, variable sites (S), haplotype diversity (*h*), and nucleotide diversity (π).

1
2

Country	Location	Code	N	N otolith	N mtDNA	No. alleles	S	*h*	π
Japan	Ishigaki	IG	14	14	14	14	44	1.0000	0.0063
Taiwan	Shitiping	ST	36	36	36	31	74	0.9921	0.0060
	Jihui	JH	32	31	32	31	88	0.9980	0.0073
	Green Island	GI	20	20	10	10	35	1.0000	0.0067
	Checheng	CC	31	31	31	30	73	0.9979	0.0061
	Wanlitong	KT	11	11	11	11	42	1.0000	0.0062
	Liuqiu	LQ	12	12	12	12	32	1.0000	0.0069
		Taiwan total	142	141	132	99	157	0.9940	0.0066
Philippines	Badian	BD	39	39	39	37	85	0.9973	0.0064
		All specimens	195	194	185	132	179	0.9938	0.0065

Table 2 (on next page)

Pairwise Φ_{ST} values between locations analyzed from the concatenated mtDNA sequence (1336 bp).

IG: Ishigaki, ST: Shitiping, JH: Jihui, GI: Green Island, CC: Checheng, KT: Wanlitong, LQ: Liuqiu, BD: Badian. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Bold, significant after Bonferroni correction.

1

	Japan	Taiwan					Philippines
	IG	ST	JH	GI	CC	KT	LQ	BD
IG								
ST	-0.015							
JH	-0.014	-0.006						
GI	0.010	-0.014	-0.016					
CC	-0.009	-0.004	-0.004	-0.005				
KT	-0.009	-0.011	-0.016	-0.009	-0.020			
LQ	0.089*	0.090**	0.044*	0.065	**0.116****	0.121**		
BD	-0.025	-0.002	-0.002	0.005	-0.003	-0.004	0.102**	

Table 3(on next page)

AMOVA results for the concatenated mtDNA sequence (1336 bp) based on two hypothetical groupings.

IG: Ishigaki, ST: Shitiping, JH: Jihui, GI: Green Island, CC: Checheng, KT: Wanlitong, LQ: Liuqiu, BD: Badian. *$P < 0.05$.

Source of variations	Degree of freedom	Sum of squares	% of variation	Fixation index
Three different latitudinal groups: Ishigaki (IG) vs. Taiwan (ST, JH, GI, CC, KT, LQ) vs. Badian (BD)				
Among groups	2	8.4	-0.97	-0.010 (Φ_{CT})
Among populations within groups	5	28.3	1.35	0.013 (Φ_{SC})
Within populations	177	781.3	99.61	0.004 (Φ_{ST})
Three different ocean current conditions: (IG, ST, JH, GI) vs. (CC, KT, LQ) vs. (BD)				
Among groups	2	7.7	-1.12	-0.011 (Φ_{CT})
Among populations within groups	5	29.1	1.67	0.017* (Φ_{SC})
Within populations	177	781.3	99.45	0.006 (Φ_{ST})

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Table 4(on next page)

Detailed data on ranges, means, and statistical analyses of otolith growth rates and increments for all locations used in this study.

Otolith increments calculated from first feeding check (FFC) to growth check (GC) are represented as T_{GC}, and otolith growth rate is represented as G_{GC}. IG: Ishigaki, ST: Shitiping, JH: Jihui, GI: Green Island, CC: Checheng, KT: Wanlitong, LQ: Liuqiu, BD: Badian. ***$P < 0.001$ (ANOVA test). Numbers with the same superscript letters (i.e., a, b, ab) are not significantly different (Tukey HSD test, $P \geq 0.05$).

		Ishigaki	Taiwan						Badian
		IG	ST	JH	GI	CC	KT	LQ	BD
T_{GC}	Range	41 – 68	42 – 69	39 – 69	44 – 74	40 – 85	41 – 66	46 – 98	33 – 56
(days)	Mean***	54.6 ± 7.7 ^{ab}	51.8 ± 7.2 ^{b}	51.7 ± 7.9 ^{b}	55.2 ± 9.1 ^{ab}	57.6 ± 9.7 ^{ab}	55.2 ± 6.9 ^{ab}	61.2 ± 14.6 ^{a}	43.9 ± 4.9 ^{c}
G_{GC}	Range	1.04 – 1.55	0.95 – 1.58	0.91 – 1.87	0.99 – 1.66	0.93 – 1.73	1.16 - 1.57	0.98 – 1.79	1.22 – 2.40
(μm day^{-1})	Mean***	1.28 ± 0.16 ^{a}	1.36 ± 0.14 ^{a}	1.39 ± 0.17 ^{a}	1.32 ± 0.18 ^{a}	1.31 ± 0.21 ^{a}	1.33 ± 0.11 ^{a}	1.38 ± 0.22 ^{a}	1.60 ± 0.19 ^{b}
	N	14	36	31	20	31	11	12	39

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Table 5(on next page)

Otolith increments and growth rates from first feeding check (FFC) to growth check (GC) based on three latitudinal groups.

IG: Ishigaki, ST: Shitiping, JH: Jihui, GI: Green Island, CC: Checheng, KT: Wanlitong, LQ: Liuqiu, BD: Badian. ***$P < 0.001$ (ANOVA test). Numbers with the same superscript letters (i.e., a, b) are not significantly different (Tukey HSD test, $P \geq 0.05$).

		Ishigaki	Taiwan	Badian
		IG	ST, JH, GI, CC, KT, LQ	BD
T_{GC}	Range	41 – 68	39 – 98	33 – 56
(days)	Mean***	54.6 ± 7.7 [a]	54.6 ± 9.3 [a]	43.9 ± 4.9 [b]
G_{GC}	Range	1.04 – 1.55	0.91 – 1.87	1.22 – 2.40
(μm day^{-1})	Mean***	1.28 ± 0.16 [a]	1.35 ± 0.18 [a]	1.60 ± 0.19 [b]
	N	14	141	39

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Table 6(on next page)

Otolith increments and growth rates from first feeding check (FFC) to growth check (GC).

Specimens from Taiwan are divided into eastern and southwestern groups to test the effect of different current conditions on early life history traits. IG: Ishigaki, ST: Shitiping, JH: Jihui, GI: Green Island, CC: Checheng, KT: Wanlitong, LQ: Liuqiu, BD: Badian. ***$P < 0.001$ (ANOVA test). Numbers with the same superscript letters (i.e., a, b, ab) are not significantly different (Tukey HSD test, $P \geq 0.05$).

		Ishigaki IG	Southwestern Taiwan CC, KT, LQ	Eastern Taiwan ST, JH, GI	Badian BD
T_{GC}	Range	41 – 68	40 – 98	39 – 74	33 – 56
(days)	Mean***	54.6 ± 7.7 [ab]	57.9 ± 10.5 [a]	52.6 ± 8.0 [b]	43.9 ± 4.9 [c]
G_{GC}	Range	1.04 – 1.55	0.93 – 1.79	0.91 – 1.87	1.22 – 2.40
($\mu m\ day^{-1}$)	Mean***	1.28 ± 0.16 [a]	1.33 ± 0.19 [a]	1.36 ± 0.16 [a]	1.60 ± 0.19 [b]
	N	14	54	87	39

1

Figure 1

Map of sampling sites for *Uropterygius micropterus*.

Within the pie charts, colors indicate the percentages of pelagic larval duration (PLD) for each sampling site.

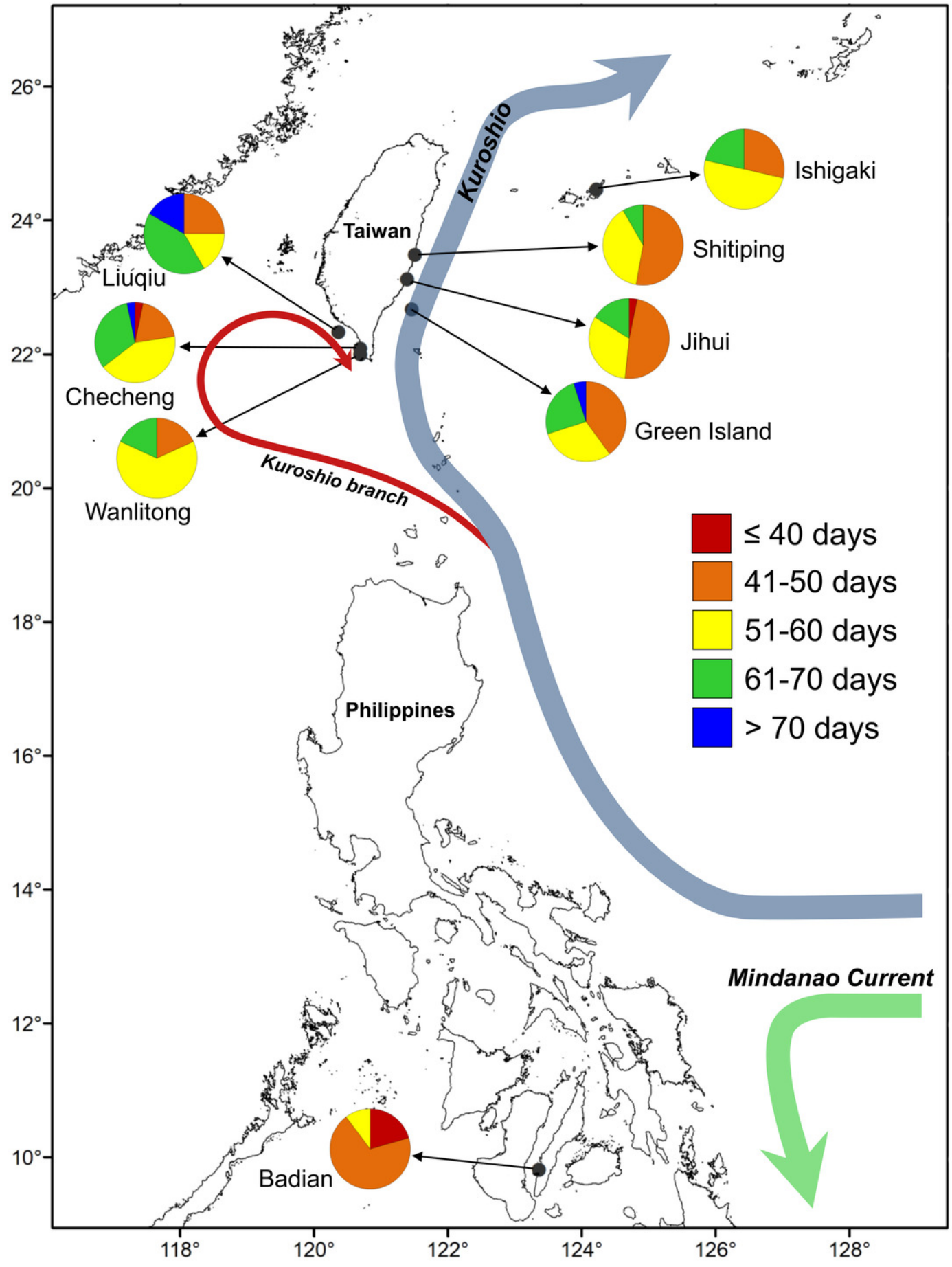
Kuroshio
Ishigaki
Taiwan
Shitiping
Liuqiu
Jihui
Checheng
Green Island
Kuroshio branch
Wanlitong
≤ 40 days
41-50 days
51-60 days
61-70 days
> 70 days
Philippines
Mindanao Current
Badian
26°
24°
22°
20°
18°
16°
14°
12°
10°
118°
120°
122°
124°
126°
128°

Figure 2

Otolith microstructure.

Otolith microstructure showing the hatch check (HC), first feeding check (FFC), and growth check (GC).

**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*

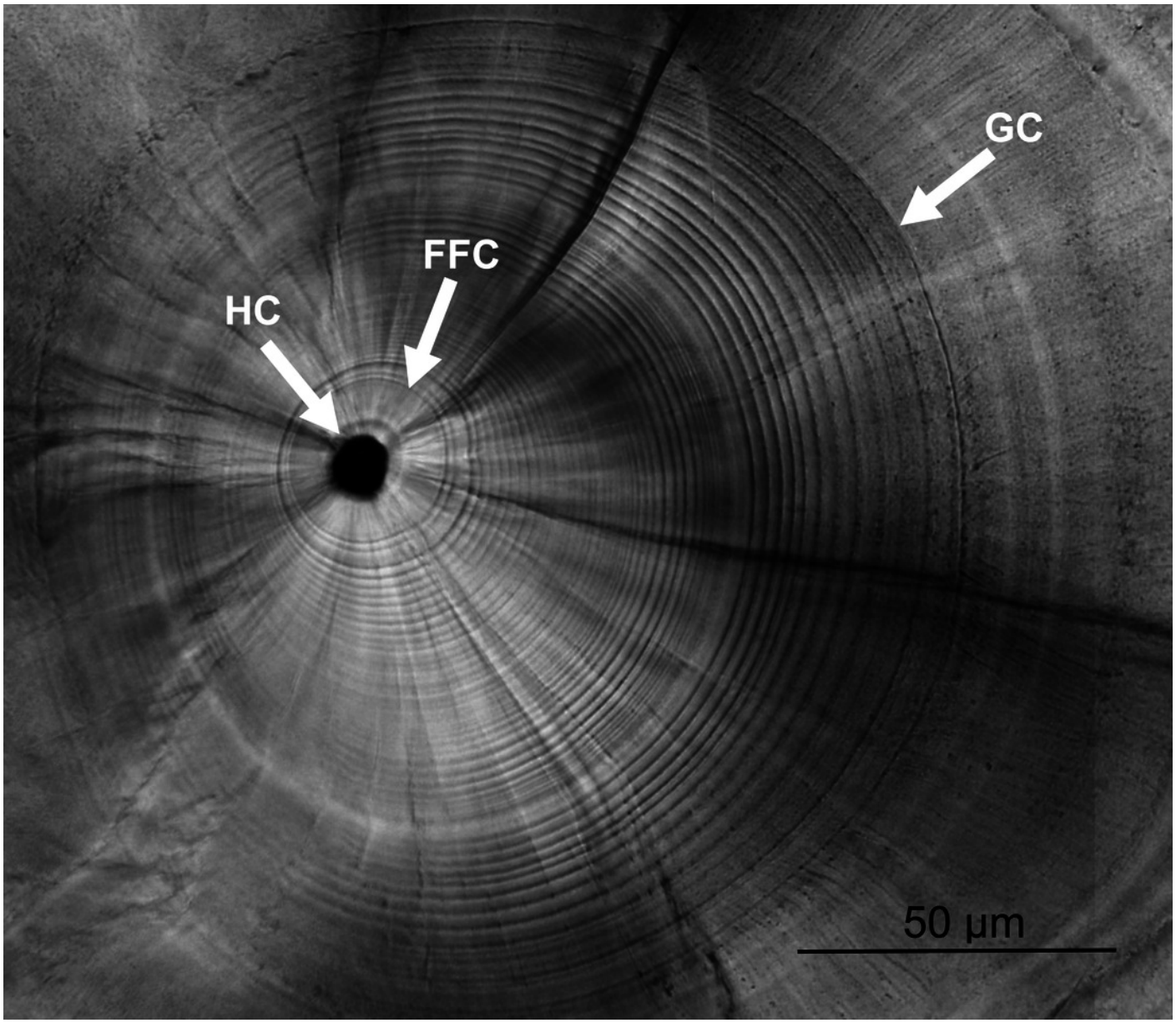

Figure 3

Patterns of otolith increment widths and Sr/Ca ratios from a Jihui specimen.

Arrows represent the position of the growth check (GC).

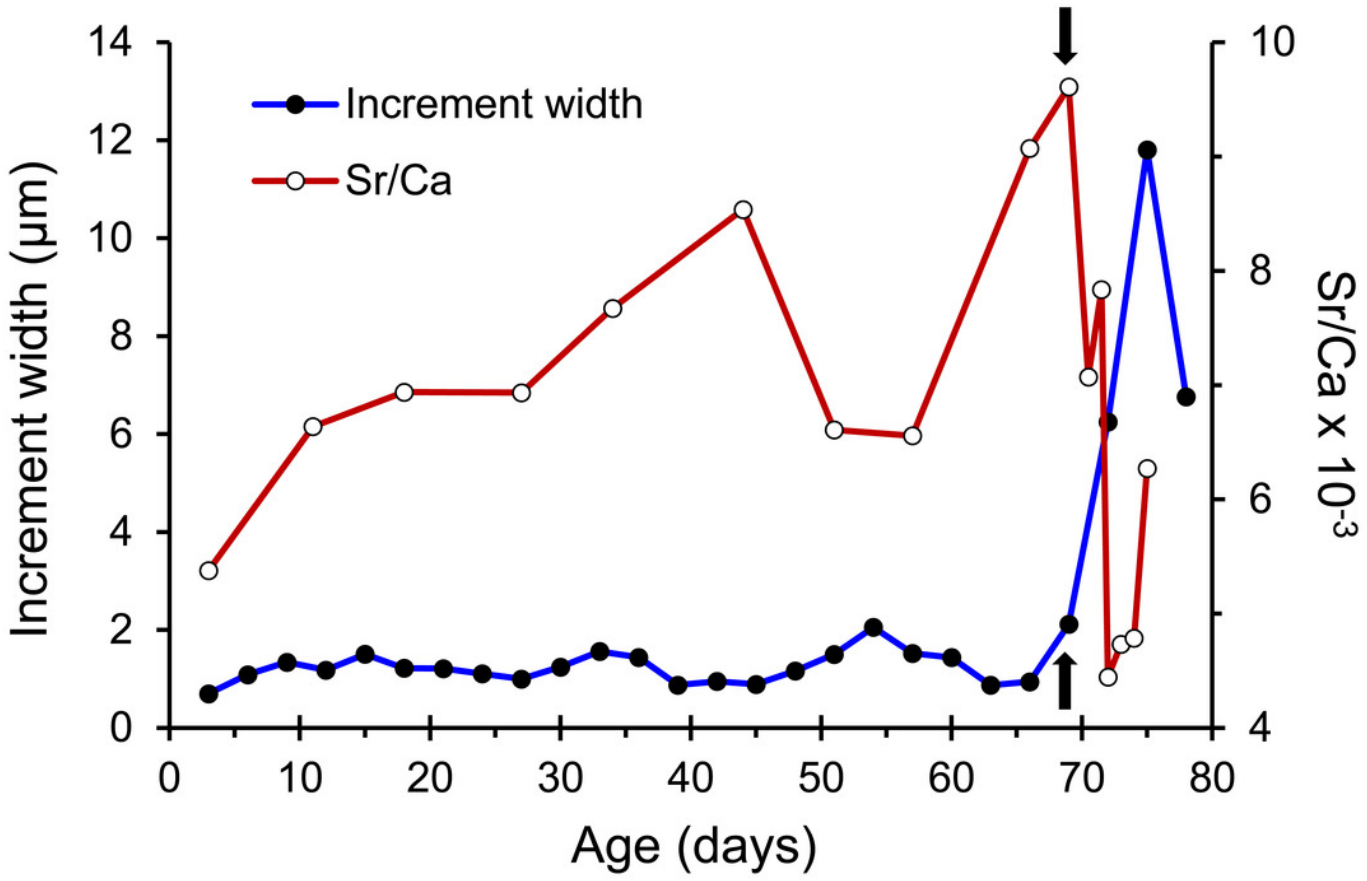

Figure 4

Minimum spanning network built from 185 concatenated mtDNA sequence (1336 bp) of *Uropterygius micropterus* with 132 haplotypes.

Colors represent correspondent sampling sites; the size of each pie chart is proportional to the number of individuals; hollow circles are haplotypes that were not collected in this study.

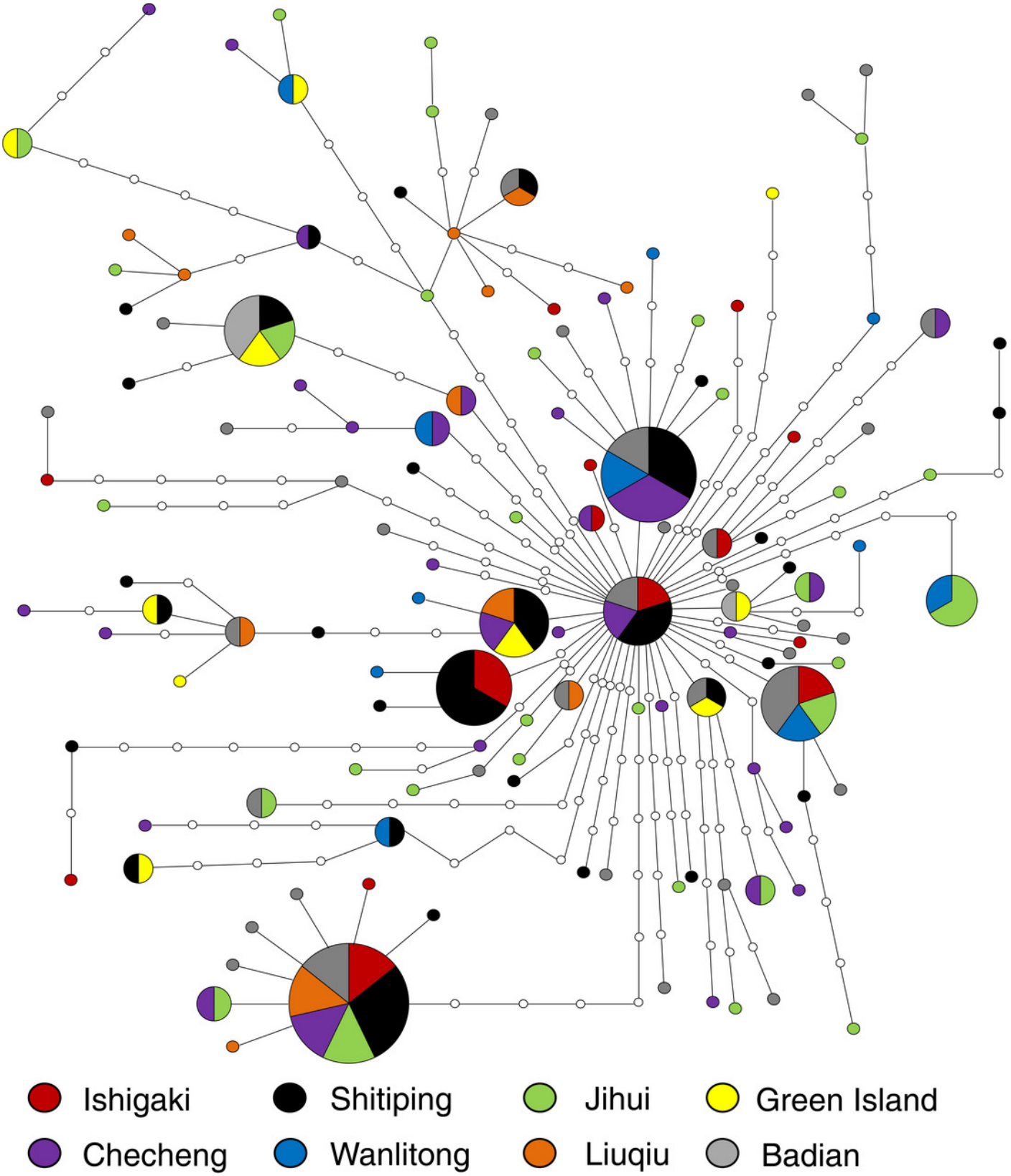
Ishigaki
Shitiping
Jihui
Green Island
Checheng
Wanlitong
Liuqiu
Badian

Figure 5

Frequency distributions of PLD.

Frequency distributions of PLD based on (a) Ishigaki (n = 14), Taiwan (n = 141), and Badian (n = 39) and (b) Eastern (Shitiping, Jihui, and Green Island, n = 87) and Southwestern Taiwan (Checheng, Wanlitong, and Liuqiu, n = 54).

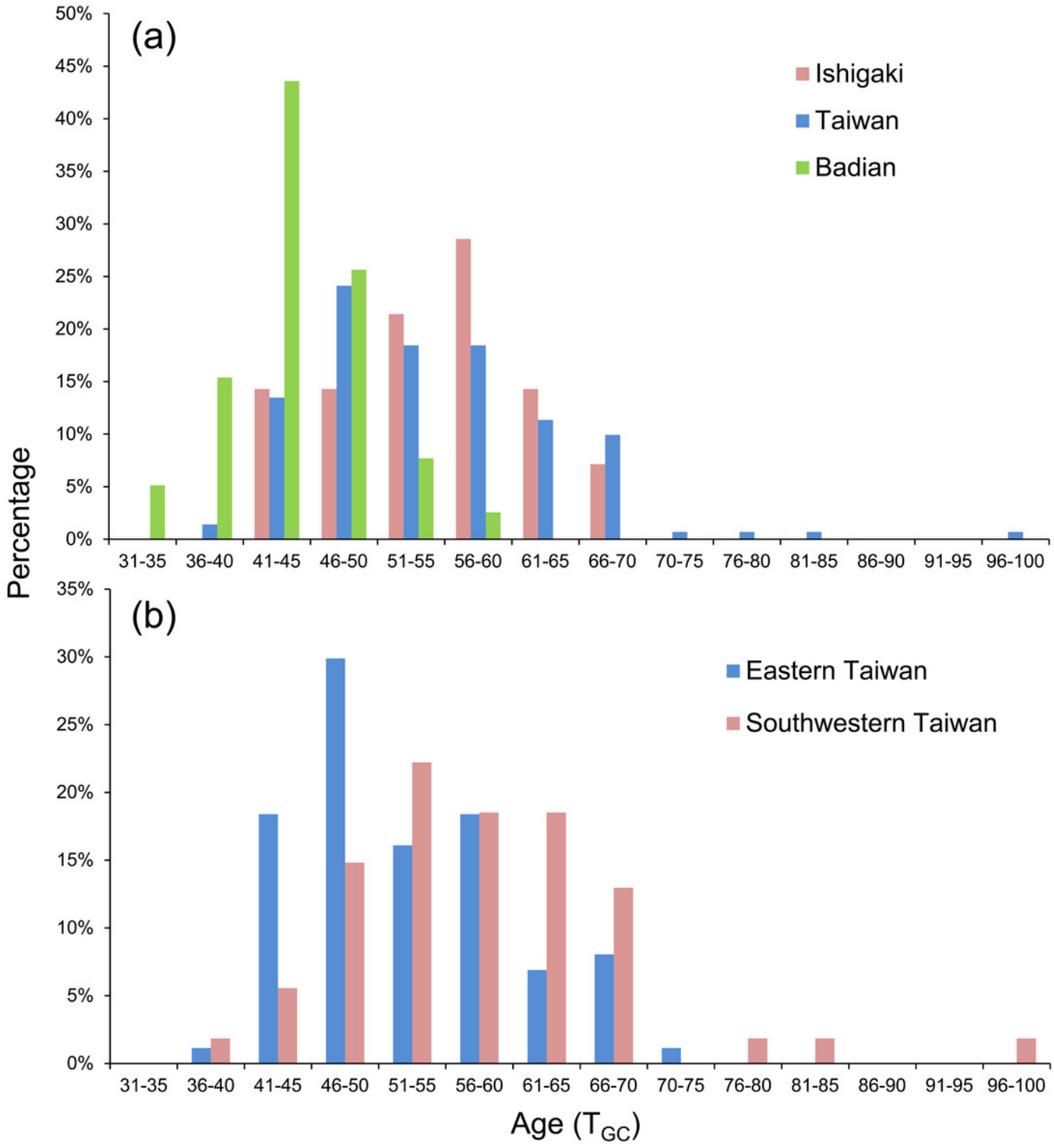
(a)
Ishigaki
Taiwan
Badian
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
Percentage
31-35
36-40
41-45
46-50
51-55
56-60
61-65
66-70
70-75
76-80
81-85
86-90
91-95
96-100
(b)
Eastern Taiwan
Southwestern Taiwan
35%
30%
25%
20%
15%
10%
5%
0%
Age (T_GC)

Figure 6

Sequential changes of increment widths from first feeding check (FFC) to growth check (GC) based on three latitudinal groups.

Ishigaki (n = 13), Taiwan (n = 126), and Badian (n = 37).