

Early life history of oxudercine goby *Pseudapocryptes elongatus* in the Mekong Delta, Vietnam

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Pseudapocryptes elongatus is one of the oxudercine gobies, which show varying degrees of amphibious behaviour and capacities to breathe air. There is little information on the early life history of *P. elongatus*, particularly of their morphology and larval habitat and duration. This study focused on the life history of larval and juvenile stages of *P. elongatus* investigated by genetic species identification, morphological observation and otolith analyses using specimens collected in June and October 2012 from estuaries in Bac Liêu Province, southern Vietnam (09°14'N 105°43'E). Genetically identified juvenile *P. elongatus* were characterized by (1) a slender body form, (2) the anterior edges of both the anal and second dorsal fins located at the midpoint along the body axis, (3) scarce chromatophores over the body surface, (4) melanophores in the parietal region between the eyes, and (5) a single row of melanophores along the base of the anal fin. Mean age at recruitment to estuaries was 38.0 ± 4.1 days, and otolith Sr/Ca ratios ranged from 8.9 to 9.9 mmol mol^{-1} , suggesting that larval migration from their spawning sites requires more than 1 month in saline environments.

Keywords: Daily ring, mtDNA COI sequence, otolith, species identification

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INTRODUCTION

Pseudapocryptes elongatus (Cuvier, 1816) is a goby (Gobiidae *sensu* Gill & Mooi, 2012 or Gobionellidae *sensu* Thacker, 2009) in the subfamily Oxudercinae. Oxudercine gobies include highly amphibious genera known as mudskippers (genera *Boleophthalmus*, *Periophthalmodon*, *Periophthalmus* and *Scartelaos*), but the others also show varying degrees of amphibious behaviour. The taxonomy of the Oxudercinae was reviewed by Murdy (1989) and the taxonomic status of *P. elongatus* was described by Ferraris (1995). According to Murdy (1989), *P. elongatus* has the capacity to breathe air through its gills, by modified buccopharyngeal and opercular epithelia, and through its skin, but lacks the ability to survive for short periods out of water. However, we observed that this fish not only gulped air while in shallow water but also made

short excursions on the surface of mudflats (authors' personal observation). This fish is highly euryhaline, showing no mortality during 96-h exposure to salinities up to 50 (Bucholtz *et al.*, 2009). Our field records showed that the salinity of adult habitats ranged from 10 to 29.

Pseudapocryptes elongatus is one of the most important aquaculture fish species in southern Vietnam and recruiting juveniles are intensively captured for use in aquaculture. Aquaculture of *P. elongatus* in the Mekong Delta of Vietnam has developed rapidly during the past decade due to relatively high demand and high market value (Bucholtz *et al.*, 2009). However, increasing juvenile catch has raised concerns about a potential decline of wild populations. Therefore, it is crucial to understand the early life history of *P. elongatus*, such as larval duration and migration, and to develop rearing techniques that will enable sustainable fisheries and aquaculture management.

There is little information on the life history of *P. elongatus*, particularly of their spawning grounds, larval stage, movements and migrations. Previous studies of *P. elongatus* have addressed the morphology and systematics of adults (Das,

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1933, 1934; Murdy, 1989; Yadav & Singh, 1989; Ojha & Haque, 1994), and basic biological traits such as body size, condition or early maturation status of sub-adults (Ambak *et al.*, 2007; Bucholtz *et al.*, 2009). No morphological descriptions of juveniles have been published, probably due to technical problems of species identification among oxudercine gobies during larval and juvenile stages.

Thus, the aim of this study was to describe diagnostic morphological traits and obtain information on the larval and juvenile phases of *P. elongatus*, with the goal of forming a scientific basis for further ecological studies. We describe the morphology of juvenile *P. elongatus* based on genetically confirmed specimens, and report results on larval duration and migratory history of *P. elongatus* as inferred from daily rings and Sr:Ca ratios using otolith microstructural and chemical analyses.

MATERIALS AND METHODS

Study site and fish collection

Recruiting juvenile oxudercine gobies were purchased in June (wet season) and October (beginning of dry season) 2012 from fishermen catching them with a bag net in canal estuaries in Bac Liêu Province, Vietnam (09°15'N 105°45'E, Figure 1). The climate of the Mekong Delta region is governed by monsoons-steady winds, and the wet season begins in May and continues until late September (Hoanh *et al.*, 2003). The specimens were immediately preserved either in 95% ethanol for genetic species identification and otolith analysis (see below) or in 10% formalin for description of chromatophores on the body surface. To determine the diagnostic characters that discriminate *P. elongatus* at the juvenile stage (Figure 2A, B for *P. elongatus*), we measured standard length (SL), total length (TL), head length (HL) and pre-anal length (PAL) to the nearest millimetre using digital calipers. We estimated caudal fin length (CFL) as the difference between TL and SL. The number of anal fin rays, one of the key characteristics to the species identification in Murdy (1989), was also counted for oxudercine juveniles (N = 10) after precisely identifying individuals by DNA nucleotide sequencing. Otoliths were extracted from a total of 26 randomly chosen juvenile *P. elongatus* from each sampling month, identified on the basis of the morphological traits determined in this study (see Results section).

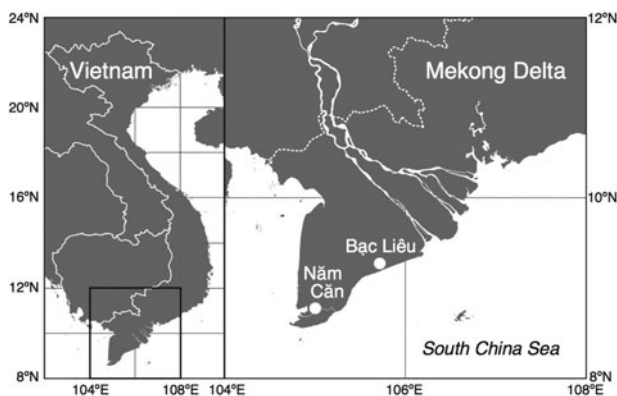


Fig. 1. Locations of fish collection in the Mekong Delta, southern Vietnam.



Fig. 2. Photographs of juvenile *Pseudapocryptes elongatus* at an unpigmented stage (A), an early stage of pigmentation (B), and a well-pigmented stage (C). Juveniles (A) and (B) were collected with a bag net in Bac Liêu, and (C) was collected in a mangrove swamp.

To obtain the reference DNA nucleotide sequence for genetic identification of juveniles, adult *P. elongatus* were collected upstream of the same canal in October 2012 (N = 5). Adult individuals of another oxudercine goby, *Parapocryptes serperaster* (Richardson, 1846), were also obtained from a bag-net catch in Năm Căn, Cà Mau Province, Vietnam (08°45'N 105°00'E) in August 2014 (N = 8) for genetic identification of some juveniles, because there were no available DNA nucleotide sequences of this genus in the DNA database for the similar body shape as *P. elongatus* in the subfamily Oxudercinae of this region. In addition, one juvenile *P. elongatus* at a well-pigmented stage was collected in a mangrove swamp using a hand net (Figure 2C) and preserved in 10% formalin for morphological description.

Species identification

Genetic species identification was used to confirm the species of juvenile fish from bag net samples. Total genomic DNA was extracted from the muscle of adult *P. elongatus* (N = 5) and caudal tissue of oxudercine juveniles (N = 10) using a QIAGEN DNeasy blood & Tissue Kit (Qiagen) following the manufacturer's instructions. A partial fragment of the cytochrome oxidase I subunit gene (COI) (about 600 bp) was amplified by PCR using fish versatile primers L5956 (5'-CAC AAA GAC ATT GGC ACC CT-3') and H6558 (5'-CCK CCW GCK GGG TCA AAG AA-3'). PCR was performed using Model 9700 (Applied Biosystems) or iCycler (Bio-Rad) thermal cyclers in a reaction volume of 10 µl containing 1.0 µl of KOD plus ver.2 Buffer (TOYOBO), 0.2 mM dNTP, 1.5 mM MgSO₄, 0.3 µM each of forward and reverse primers, 0.3 U KOD plus v.2 polymerase (TOYOBO) and 1 µl of template DNA. The thermal cycling profile was as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 64°C for 30 s, extension at 68°C for 40 s, and an additional extension at 68°C for 7 min. Double stranded PCR products were purified using LaboPassTM PCR (Cosmo Genetech) or ExoSap-IT (Affymetrics), and were subsequently used for direct cycle sequencing with the BigDyeTM Terminator v3.1

Ready Reaction Cycle Sequencing Kit (Applied Biosystems). DNA nucleotide sequences at the 3'-end were determined using the L5956 primer. Labelled fragments were analysed on an ABI PRISM model 3730 or 3130xl genetic analyser (Applied Biosystems). DNA nucleotide sequences were edited with 4Peaks (Griekspoor and Groothuis, mekentsoj.com) or Geneious (Biomatters) software. All sequences were deposited in DDBJ/EMBL/GenBank with accession nos. LCO10470–LCO10479 (oxudercine-juvenile_01-10) and LCO10480–LCO10484 (*P.elongatus*-adult_01-05). In addition, the DNA nucleotide sequences were determined for adult *Pa. serperaster* (LCO10485–LCO10492; *Pa.serperaster*-adult_12-19), which were morphologically identified according to Murdy (1989) and Tran *et al.* (2013).

For phylogenetic analyses, available DNA nucleotide sequences of other oxudercine species were obtained from the database as follows: *Oxuderces dentatus* Eydoux & Souleyet, 1850 (JN631361.1), *Scartelaos histophorus* (Valenciennes, 1837) (EU595271.1–EU595281.1, FJ238025.1–FJ238032.1), *Boleophthalmus pectinirostris* (Linnaeus, 1758) (JX679027.1, JN631362.1), *Boleophthalmus boddarti* (Pallas, 1770) (KF874277.1), *Periophthalmus modestus* Cantor, 1842 (JX679050.1), *Periophthalmus barbarus* (Linnaeus, 1766) (AF391339.1), *Periophthalmus magnuspinnatus* Lee, Choi & Ryu, 1995 (JX679049.1). The sequences of two sicydiinae species, *Sicyopterus japonicus* Tanaka, 1909: JX628620.1 and *Sicyopterus lagocephalus* (Pallas, 1770): KF482068.1, were used as an out-group for the neighbour-joining tree analysis. The DNA nucleotide sequences were aligned, and the NJ tree was constructed based on a Tamura-Nei model with bootstrapping for 1000 replications using the Geneious software. Tamura-Nei distances were calculated using MEGA v.6.0.2.

Otolith analysis

Extracted otoliths from juvenile *P. elongatus* (June: N = 15, October: N = 11) were embedded in epoxy resin (EpoFix, Struers), ground to near the otolith core using a grinding machine equipped with a diamond cup-wheel (Discoplan-TS, Struers), and then polished with a colloidal silica suspension (OP-S, Struers) on an automated polishing wheel equipped with a semi-automatic specimen mover (MD-Chem and Rotopol-35 equipped with PdM-Force20, Struers). The number of rings was counted under a light microscope to estimate daily age of the juveniles. Rings of the otoliths were assumed to be deposited daily according to the previous studies of other oxudercine gobies (Mazlan & Rohaya, 2008).

Otoliths of the three specimens (TL: 16.7–17.1 mm) collected in June 2012 were further cleaned using distilled water and ethanol, and dried at 50°C in an oven prior to elemental analysis. Otoliths were Pt-Pd coated by a high-vacuum evaporator (E-1030, Hitachi). These otoliths were used for line analysis of Sr and Ca concentrations, which were measured in a line along the longest axis of each otolith from the core to the edge using a wavelength-dispersive X-ray electron microprobe analyser (JXA-8230, JEOL). CaSiO₃ and SrTiO₃ were used as assay standards. The accelerating voltage and beam current were 15 kV and 12 nA, respectively. The electron beam was focused on a point 2 µm in diameter, with measurements every 2 µm from the core to edge. All Sr:Ca ratios were calculated as molar ratios (mmol mol⁻¹).

Data analyses

Statistical analyses (linear model framework such as *t*-test for means, *F*-test for variances, ANCOVA for slopes) were performed with the programs Prism v. 6.0c (GraphPad Software, Inc., La Jolla, CA, USA) and the lm library in R v. 3.0.2 (R Core Team, 2013). Welch's correction was used for the *t*-test to adjust for small sample sizes and slightly different variances of samples. Values are shown as means ± SD, wherever possible.

RESULTS

DNA nucleotide sequences of the juvenile oxudercine gobies divided into three genetically distinct groups in the NJ tree, indicating that they were composed of three species (Figure 3). Among the specimens analysed, four juveniles were identified as *P. elongatus* because their DNA nucleotide sequences were unambiguously nested within a clade of *P. elongatus*. Similarly, five juveniles were identified as *Parapocryptes serperaster* and one as *S. histophorus* on the basis of the NJ tree. The sequences in each clade were closely matched with the reference sequences for *P. elongatus*, *Pa. serperaster* and *S. histophorus*. The genetic distances within each species (Tamura-Nei distance = 0–0.0025) were much smaller than the intraspecific distances compared with reference sequences and the interspecific distances among the oxudercine species (0.123–0.222). Divergences between genera had quite low bootstrap values, except for *Parapocryptes*, and the monophyly of genus *Periophthalmus* was not supported by the NJ tree.

Morphological observations of the genetically identified juvenile oxudercine gobies revealed the several morphological features among three species of this study. Juvenile *P. elongatus* were characterized by the following five morphological features (Figures 2 & 4): (1) a slender body form, (2) the anterior edges of both the second dorsal fin and the anal fin located at the midpoint of the body axis, (3) relatively low numbers of chromatophores over the body surface, (4) melanophores in the parietal region between the eyes and (5) a single row of melanophores at the base of the anal fin (Figure 4A, B, E, F). Juvenile specimens of *Pa. serperaster* were characterized by the following morphological features (Figure 4C, G): relatively high numbers of fringe-like chromatophores over the body surface, melanophores in the parietal region not only between the eyes, and similar features to *P. elongatus*: a slender body form, the anterior edges of both the second dorsal fin and the anal fin located at the midpoint of the body axis, a single row of melanophores at the base of the anal fin. Juvenile specimen of *S. histophorus* had a slender body form like other species but clearly different morphological features to other species of this study (Figure 4D, H): high numbers of chromatophores at the base of the dorsal fin, dense melanophores in the parietal region evenly, and absence of the single row of melanophores at the base of the anal fin.

Morphological measurements of genetically identified oxudercine juvenile specimens are summarized in Table 1. The range of the morphological characteristics of the genetically identified juveniles overlapped among species, except SL and CFL between *P. elongatus* and *Pa. serperaster*. Standard lengths of the juveniles were quite similar among three species, although the SLs of *P. elongatus* (13.0 ± 0.1 mm) were slightly larger than that of *Pa. serperaster* (11.7 ± 0.5 mm) and

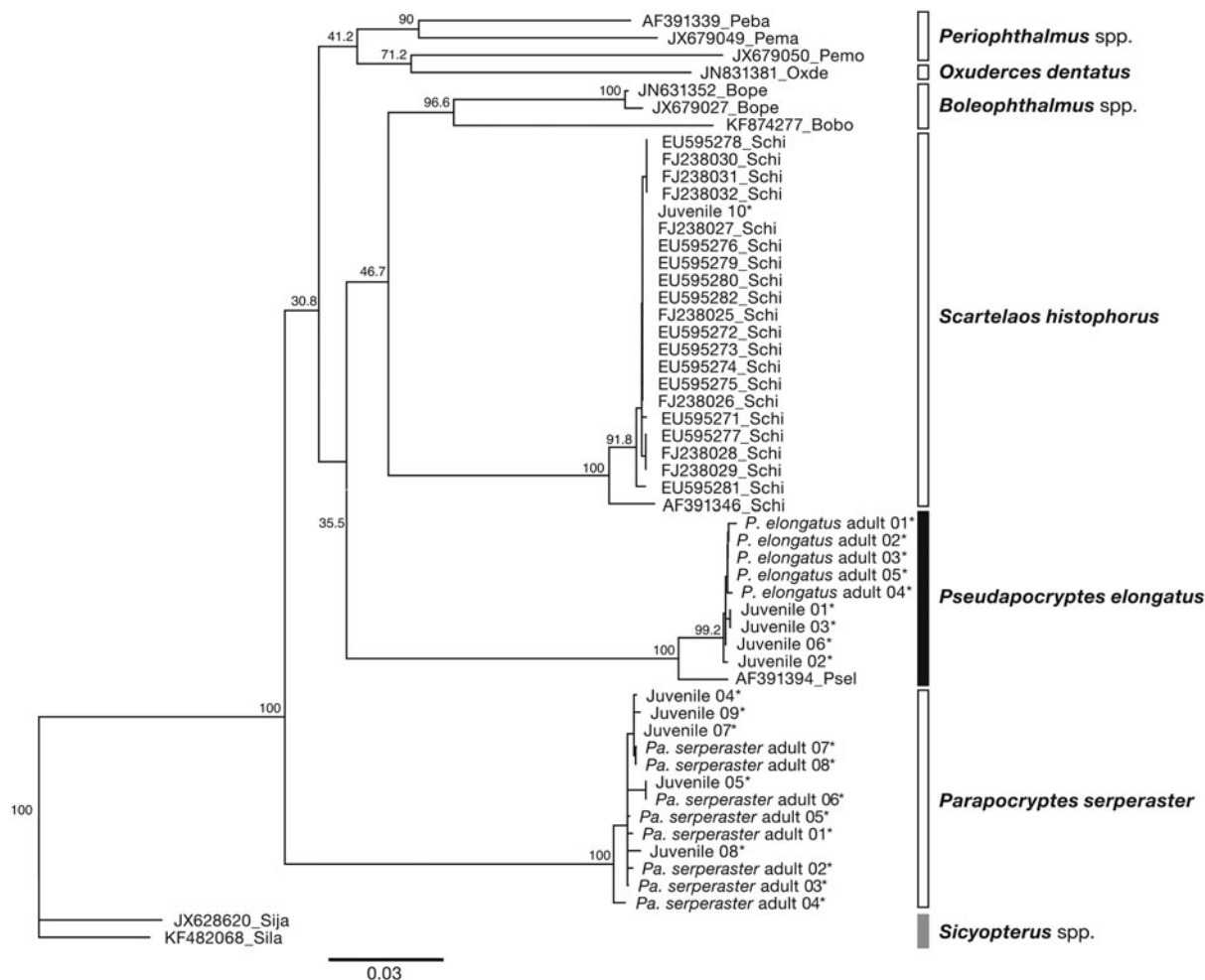


Fig. 3. Neighbour-joining tree of COI sequences of *Pseudapocryptes elongatus*, *Parapocryptes serperaster* and *Scartelaos histophorus* from the Mekong Delta, Vietnam for seven Oxudercinae species, and two Sicydiinae species (out-group). Bootstrap values and accession numbers for DDBJ/EMBL/GenBank are given. Asterisks depict newly registered sequences to DDBJ/EMBL/GenBank by this study.

S. histophorus (12.5 mm) (Table 1). Caudal fin length of *P. elongatus* (19.0%SL) was significantly smaller than that of *Pa. serperaster* (27.7%SL) (*t*-test with Welch's correction, $P < 0.01$, Table 1). In addition, juvenile *P. elongatus* can be distinguished from the *Pa. serperaster* by the absence of melanophores on ventral body surfaces, except along the bases of the anal and caudal fins. Although morphological characteristics of adult *P. elongatus* in this study were within the ranges or very close to those reported by Murdy (1989) (Table 1), relative HL and CFL of juvenile *P. elongatus* were slightly different than those of adults (Table 1).

Pseudapocryptes elongatus with the typical patterns of melanophores and relatively short caudal fins (CFL: $16.8 \pm 4.0\%$, SL: 15.8 ± 0.84 mm, $N = 26$) had an average of 38.0 ± 4.1 otolith rings (range: 31–45). Variation in the number of rings was small and not significantly different between juveniles collected in June and October (*F* test, $P = 0.97$). In contrast, there was a significant difference in the number of otolith rings between juveniles collected in June (39.7 ± 3.6 , $N = 15$) and October (35.5 ± 3.5 , $N = 11$) (*t*-test, $P < 0.01$, Figure 5). There was also a slight but significant difference in SLs between June (14.1 ± 0.4 , $N = 15$) and October (12.8 ± 0.6 , $N = 11$) (*t*-test, $P < 0.01$). However, the number of rings and SLs were not correlated (significance of the slope, $P = 0.12$

for June and $P = 0.58$ for October) and these relationships were not significantly different between June and October (ANCOVA, $P = 0.22$). Otolith Sr:Ca ratios of the three specimens of *P. elongatus* were nearly constant along the otolith transects, ranging from 8.9, 9.0, 9.9 mmol mol^{-1} on average, and 5.2, 5.6, 6.4 mmol mol^{-1} for the lowest value in each individual (Figure 6).

DISCUSSION

Species identification and morphology of juvenile *P. elongatus*

The juvenile oxudercine gobies that sympatrically recruit to estuaries of the Mekong Delta include at least three species. The genetic species identifications in this study are highly reliable because the sequences of the oxudercine juveniles nested within a single species, satisfying the strict tree-based assignment criteria described in Wilson *et al.* (2011). Although the number of specimens used for species identification of the bag-net catch was small ($N = 10$), the data indicated that the catch included at least three species of oxudercine

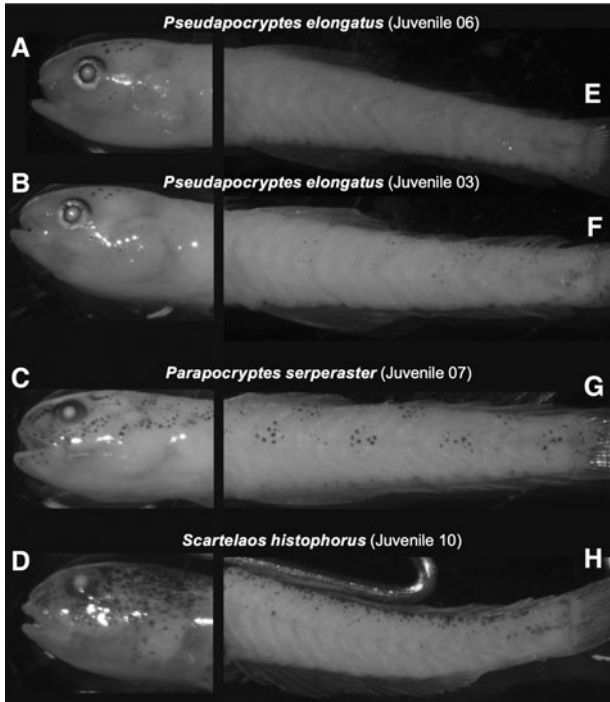


Fig. 4. Photographs of genetically identified juveniles of *Pseudapocryptes elongatus*, *Parapocryptes serperaster* and *Scartelaos histophorus* collected by bag nets in Bac Liéu, Vietnam. Head of pigmented juveniles of *P. elongatus* (A, B) (SLs: 13.0 mm), *Pa. serperaster* (C) (SL: 12.3 mm) and *S. histophorus* (D) (SL: 12.5 mm). Pigment spots at the caudal part of *P. elongatus* (E, F), *Pa. serperaster* (G) and *S. histophorus* (H).

gobies: *P. elongatus*, *Pa. serperaster* and *S. histophorus*. Fishermen suffocate the catch by leaving it in non-aerated water in order to harvest only *P. elongatus*, which can survive hypoxia exposure through aerial oxygen uptake. Currently, no data are available on the air-breathing capacities of *P. elongatus* or *Pa. serperaster*, but the latter is presumably less tolerant to hypoxia, and therefore typically dies during this procedure. Dinh *et al.* (2014) reported that *Pa. serperaster* rarely emerged from its burrow in a mudflat. Thus it is likely that these fish include several species having different tolerance to hypoxia.

The NJ tree based on the COI gene sequences of oxudercine gobies in this study was different, especially in the location of *Periophthalmus* and *Oxuderces*, from the cladogram of Murdy (1989), which was based on morphological traits. The NJ tree in this study was generated only by COI gene sequences, and this was not sufficient genetic information to determine the phylogenetic relationships among genera at the divergence between two clades – one including *Pseudapocryptes*, *Scartelaos* and *Boleophthalmus* and the other including *Periophthalmus* and *Oxuderces*. The bootstrap values for the two clades were quite low, but the two groups differ in body form. The former clade (*Pseudapocryptes*, *Scartelaos* and *Boleophthalmus*) consists of fishes with a slender body form while the latter (*Periophthalmus* and *Oxuderces*) have stouter body shapes. This may imply that the difference in body shape among oxudercine species has evolved monophyletically within the lineage. However, this study did not examine a sufficient number of DNA gene regions, and comprehensive taxon sampling would be required to determine reliable phylogenetic relationships among genera in the subfamily Oxudercinae.

Table 1. Morphological characteristics of oxudercine gobies in the Mekong delta, Vietnam in comparison with Murdy (1989).

	#	Standard length (mm)	Total length (mm)	Head length (%SL)	Pre-anal length (%SL)	Caudal fin length (%SL)	Number of anal fin rays
Juvenile							
<i>P. elongatus</i>	4	13.0 ± 0.1 (13.0–13.1)	15.5 ± 0.0 (15.5–15.5)	22.8 ± 1.6 (20.8–24.6)	50.1 ± 1.3 (48.1–50.8)	19.0 ± 0.5 (18.3–19.2)	28.5 ± 0.6 (28–29)
<i>Pa. serperaster</i>	5	11.7 ± 0.5 (11.0–12.3)	14.9 ± 0.3 (14.3–15.1)	25.1 ± 1.4 (22.8–26.7)	53.5 ± 2.6 (49.2–56.0)	27.7 ± 3.0 (22.8–30.0)	28.0 ± 0.7 (27–29)
<i>S. histophorus</i>	1	12.5	14.8	24.0	52.0	18.4	28
Adult							
<i>P. elongatus</i>	5	141.4 ± 7.9 (133.0–154.0)	–	15.5 ± 0.9 (14.6–17.0)	48.1 ± 0.8 (47.2–49.0)	26.1 ± 2.1 (24.1–29.3)	29.6 ± 0.5 (29–30)
Murdy (1989)	12	–	–	17.7 (14.8–22.0)	45.9 (40.5–48.1)	20.5 (16.1–23.4)	29.3 ± 0.8 (28–31)

Note: Values were mean ± SD (upper) and range (lower). *P.* *Pseudapocryptes*; *Pa.* *Parapocryptes*; *S.* *Scartelaos*. Species were identified by the Cytochrome Oxidase I subunit gene. See text for more details.

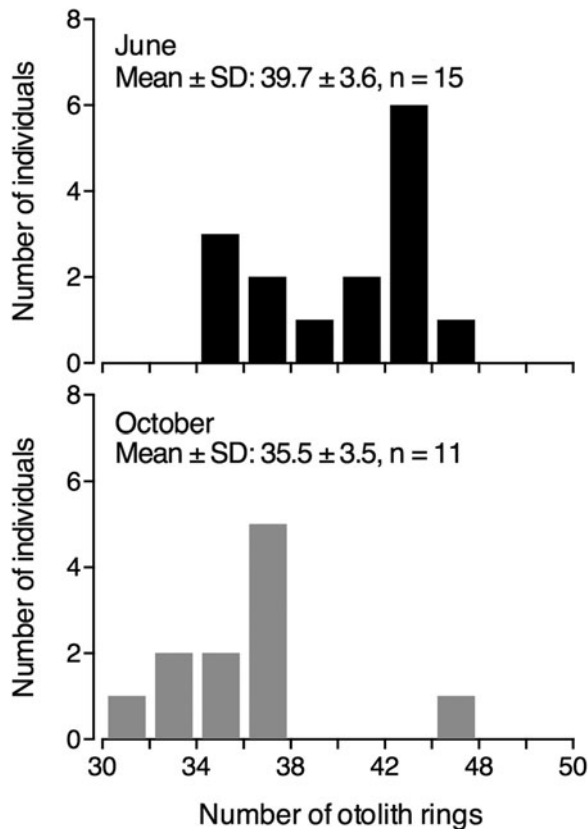


Fig. 5. Estimated daily ages of recruiting juvenile *Pseudapocryptes elongatus* collected by bag nets in Bac Liêu, southern Vietnam inferred from otolith rings.

Among the morphological characters observed in this study, patterns of the chromatophores in the parietal region and body surface and presence/absence of melanophores at the base of the anal fin would be important to distinguish among *P. elongatus*, *Pa. serperaster* and *S. histophorus* in the Mekong Delta. In addition to these characteristics, the CFL (%SL) of *P. elongatus* was significantly smaller than that of *Pa. serperaster* in juvenile specimens among various morphological measurements in this study. However, this study showed differences in CFL ratio between juvenile and adult *P. elongatus*, probably due to allometric changes during development. The CFL ratio would be used for species identification in combination with pigmentation patterns at least in

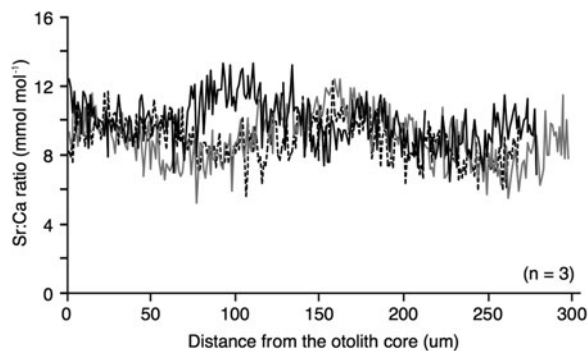


Fig. 6. Sr/Ca ratios from otolith core to the edge of otoliths of recruiting juvenile *Pseudapocryptes elongatus* collected by bag nets in Bac Liêu, southern Vietnam.

the juvenile stage. To establish a morphological species identification of oxudercine species, the CFL value needs to be considered in conjunction with development stages or life history phases.

Larval duration and migration

This study provides one of the first clues about the larval phase of *P. elongatus* such as larval duration and migration. Annual ages of mudskippers were investigated previously for *Boleophthalmus dussumieri* Valenciennes, 1837 (described as *B. dentatus*) in Gujarat, India (Soni & George, 1986) and growth zones in mudskipper otoliths were validated as annual increments in *Periophthalmus argentilineatus* Valenciennes, 1837 collected from Tanzania (Kruitwagen *et al.*, 2005). The number of rings on the sagittal otolith of recruiting juvenile *P. elongatus* showed little variance between June and October. The larval duration of *P. elongatus* was estimated as 38 days on average, which was similar to the larval duration of other oxudercine species in captivity: 39 days for *Boleophthalmus pectinirostris* (Zhang *et al.*, 1989) and 45 days for *Periophthalmus modestus* (Kobayashi *et al.*, 1972). Certainly, diurnality of otolith ring deposition in *P. elongatus* needs to be validated by further studies. Bucholtz *et al.* (2009) estimated the age of 1.5 cm post-larval *P. elongatus* as ~5 weeks in the same region, which agrees well with our data, although they gave no account of the method used.

Otolith Sr:Ca ratios of *P. elongatus* indicated that all juveniles spent their entire larval period in a marine environment. The Sr:Ca values of *P. elongatus* otoliths ranged from 8.9 to 9.9 mmol mol⁻¹, which matches typical values of seawater (8.5–8.6; de Villiers *et al.*, 1994; de Villiers, 1999). Taken together, our data on the Sr:Ca ratio and the number of rings in *P. elongatus* otoliths imply that the fish reproduce in a saline environment not far from the site of capture. In comparison of estimated age at recruitment between seasons, there was a slight but significant difference of juvenile daily age. This may be due to (1) seasonal differences at the main spawning grounds, which are at different distances from the sampling site, (2) current velocities along the Mekong Delta coast, (3) physiological and/or behavioural mechanisms that determine larval duration or (4) some combination of these factors.

Life history and spawning grounds of *P. elongatus*

Fully mature specimens of *P. elongatus* have not been reported in previous studies. Furthermore, we interviewed numerous local fishermen and officers of the Fisheries Department and none reported having seen *P. elongatus* crowding for reproduction, or even gravid females. This seems to indicate that *P. elongatus* spawn in areas not easily observable by humans. Ambak *et al.* (2007) reported that the gonadosomatic index (GSI) of *P. elongatus* collected in the Mekong Delta coast peaked between June and August, but the peak value for females was only ~2.5%. Bucholtz *et al.* (2009) also reported a very low GSI of females up to 0.29% for *P. elongatus* collected in February to May. These values are much lower than those reported for some other oxudercine species (8% in *Scartelaos gigas* Chu & Wu, 1963; Kim *et al.*, 2011; 10% in *Boleophthalmus pectinirostris*, Hong *et al.*, 2007; 13% in *Periophthalmus minutus* Eggert, 1935; Takeda *et al.*,

2011), but was similar to some other species (3.5% in *Periophthalmodon schlosseri*, Mazlan & Rohaya, 2008).

Although the air-breathing capacity of adult *P. elongatus* strongly indicates their likely occurrence in hypoxic, stagnant, inland brackish waters, the otolith Sr:Ca analyses revealed that larval hatching undoubtedly takes place in the marine environment. Inferring from the reproductive strategy of other oxudercine gobies (*Periophthalmus modestus*, Ishimatsu *et al.*, 2007; *Periophthalmus minutus*, Takeda *et al.*, 2011; *Periophthalmodon schlosseri*, Ishimatsu *et al.*, 2009; and *Boleophthalmus pectinirostris*, Hong *et al.*, 2007; Toba & Ishimatsu, 2014), it may be hypothesized that spawning of *P. elongatus* occurs in burrows excavated in soft, fine sediment in coastal mudflats.

Knowing that the larval period of *P. elongatus* is ~30–45 days in the saline environment, both oceanographic conditions affecting larval transportation by currents and active swimming of larvae relative to moving water are critically important components of their dispersal to reveal the reproductive biology and recruitment scenario of *P. elongatus* in the Mekong Delta. At the start of the pelagic larval period, fish larvae are generally poorly developed and typically drift passively with the currents, although previous studies showed that some fish larvae have better locomotory abilities, including the ability to regulate their vertical distribution (Leis, 2006). Thus, currents must be a major factor in dispersal of larvae of this stage. Determining the velocity and direction of coastal currents around the Mekong Delta over the course of the year could potentially help understand the transport from the spawning grounds. Several papers have reported surface circulation patterns of the South China Sea, including the east coast of Vietnam (Shaw & Chao, 1994; Hu *et al.*, 2000; Morimoto *et al.*, 2000). These studies showed that the current off the Mekong Delta flows from south-west to north-east in August, while the direction of the current is reversed, flowing from north-east to south-west in December. These studies indicate that early-stage larvae of *P. elongatus* are probably transported from the south-west of the main juvenile-catch locations (Bac Liêu Province and its environs) in the peak season, possibly in Cà Mau Province. At the latter end of the pelagic larval period, fish larvae are better adapted to regulating their position in the water column and in locating appropriate habitats for settlement (Leis, 2006). Therefore, investigation is needed of the behavioural traits exhibited by the various developmental stages of *P. elongatus* larvae in addition to the determination of coastal hydrodynamics in the Mekong Delta to understand their life history and to ensure the conservation of this unique fish species.

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