Identification of ichthyoplankton in the East China Sea off the Coast of Zhoushan Archipelago using an integrated strategy of morphology and DNA barcoding

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Abstract

The East China Sea (ECS) off the Coast of Zhoushan Archipelago, Zhejiang (ECS-CZA) is home to abundant fishery resources and an important spawning, feeding, and nursing ground for a variety of fish species. Due to long-term overfishing, the ichthyoplankton structure has been dramatically altered. Understanding the species composition and distribution of fish eggs and larvae is one of the most essential tasks to accurately regulate fishery resources and formulate effective management policies; however, little is known about the ichthyoplankton in this region. In this study, an integrated strategy of morphology identification (MI) and mitochondrial COI DNA barcoding were used to identify species of fish eggs and larvae collected from the ECS-CZA. MI revealed 15 fish egg species belonging to 12 families and 12 fish larva species belonging to 12 families; in contrast, DNA barcoding altogether identified 30 species, including 18 fish egg species and 13 fish larva species. One species was shared between the egg and larva samples. Our study offers useful tools and critical scientific information for understanding the recruitment, distribution, and conservation management of various fish species in this marine environment.

Running Title

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Abstract

The East China Sea (ECS) off the Coast of Zhoushan Archipelago, Zhejiang (ECS-CZA) is home to abundant fishery resources and an important spawning, feeding, and nursing ground for a variety of fish species. Due to long-term overfishing, the ichthyoplankton structure has been dramatically altered. Understanding the species composition and distribution of fish eggs and larvae is one of the most essential tasks to accurately regulate fishery resources and formulate effective management policies; however, little is known about the ichthyoplankton in this region. In this study, an integrated strategy of morphology identification (MI) and mitochondrial COI DNA barcoding were used to identify species of fish eggs and larvae collected from the ECS-CZA. MI revealed 15 fish egg species belonging to 12 families and 12 fish larva species belonging to 12 families; in contrast, DNA barcoding altogether identified 30 species, including 18 fish egg species and 13 fish larva species. One species was shared between the egg and larva samples. Our study offers useful tools and critical scientific information for understanding the recruitment, distribution, and conservation management of various fish species in this marine environment.

Keywords : Zhoushan Archipelago; ichthyoplankton; morphology; DNA barcoding; recruitment; conservation management

Ichthyoplankton are characterized by species of fish eggs and larvae during the early developmental stages of fish life history (Borja et al. 2018), and are the basis for the sustainable development of marine resources and study of fish population dynamics. Understanding the species composition and distribution of fish eggs and larvae is one of the most essential efforts to accurately regulate fishery resources and formulate effective fishery and conservation management policies (Ziober et al. 2012). Though ichthyoplankton offers a precise evaluation of species-specific spawning characteristics, egg and larva collection, particularly the former is greatly neglected due to the limitations of morphology-based identification.

Due to the insignificant morphological differences in fish eggs and larvae, the power of morphological identification (MI) of ichthyoplankton to the species level in terms of resolution is limited to the life stage and quality of specimens. Recently, the over-reliance on traditional morphology has lifted with more accurate and specific criteria for variation (Lewis et al. 2016). New approaches developed in the classification and identification of fish eggs and larvae include allozyme (Turan et al. 2005), nuclear DNA (Imsiridou et al. 2007) and mitochondrial DNA (Fraga et al. 2007). Especially, the DNA-based molecular tool is efficient in terms of detection, resolution, and coverage compared to morphology since fish eggs and larvae are cryptic and difficult to identify through morphological diagnostic features.

DNA barcoding is a molecular technology that has broadly been used in species identification and determination over the past decade. This method allows for species identification by taking advantage of a gene fragment shared by organisms with interspecies differences, e.g., mitochondrial cytochrome c oxidase subunit I, COI (Hebert and Gregory 2005; Overdyk et al. 2016). The COI gene is generally regarded as a species barcode and used for identification in animals having recorded many successes (Aquino et al. 2011; Zhang and Hanner 2011). For example, a previous study barcode 26 fish species from 70 larvae samples from Dongsha Islands, South China Sea (Chen et al. 2013).

The East China Sea (ECS) located in the West Pacific Ocean is surrounded by China, South Korea, and Japan, and composed of one of the world ocean's widest continental shelves (Xu and Oda 1999), and provides habitats to more than 440 fishes, including many ecologically and economically important fishery species for spawning, feeding, and nursery (Cheng et al. 2009). The ECS off the Coast of Zhoushan Archipelago (ECS-CZA) is located at the openings of Yangtze, Qianjiang and Yongjiang River and influenced by several ocean currents, including the coastal current of Jiangsu-Zhejiang, cold water mass of the Yellow Sea, and warm current of Taiwan. These factors contribute abundant nutrition and support the most of marine fisheries in the ECS (Li et al. 2007). However, long-term overfishing has harmed the coastal marine ecosystem in the

past decades, and the ichthyoplankton structure has been drastically altered (Liu 2013).

Despite the importance, little is known about the structure of ichthyoplankton in the area. To better understand the status of ichthyoplankton in the ECS-CZA, we used an integrated strategy of morphology and DNA barcoding to assess the ichthyoplankton species across the sea. Our findings will have immediate and practical implications for the value of the study area, in terms of being spawning, nursing, and feeding grounds for commercial fish species, and for managing and protecting the ecosystem and its functionality for supporting the ichthyoplankton community and regional fisheries.

Materials and Methods

Sample collection

Samples of fish eggs and larvae were collected from 30 stations between 121.5°~124°E and 29.5°~31°N (Fig. 1) based on the Technical Regulations of Marine Biological Ecological Investigation in the ECS-CZA in April and November of 2018, and April and November of 2019. Sample collections used horizontal opening trawls (opening diameter in 80 cm; net opening area of $0.5m^2$; net length of 280 cm). Trawling used a constant speed of 2km for about 10 minutes. All the sample collection locations were shown in Fig 1. The planktonic samples were sieved through smaller sized meshes, washed with seawater, and then fish eggs and larvae were sorted and separated from the plankton samples and placed into different jars with 100% ethanol according to sampling stations and seasons.

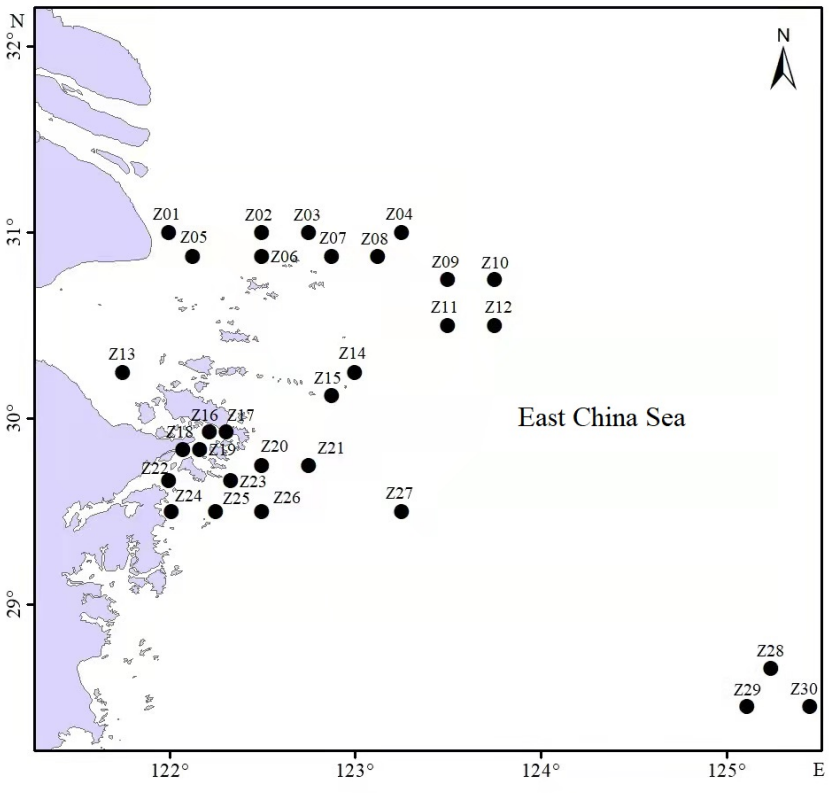

Fig. 1 Map showing locations where the fish eggs and larvae were sampled. Each dark circle represents a specific site in the East China Sea off the Coast of Zhoushan Archipelago.

Morphological identification

MI of fish eggs and larvae was conducted in the Laboratory of Marine Fisheries Research Institute of Zhejiang. A dissecting microscope attached with a camera (Nikon smz 800-Japan) was used for MI. In the MI procedure for fish eggs, diagnostic characters chosen for observation included (1) the size of egg, (2) oil ball, and (3) pigment in the egg shown in Table 1 (Zhang et al. 1985). In the MI procedure for fish larvae, diagnostic characters observed included (1) dorsal fin, (2) anal fin, and (3) melanin/pigment in the larva shown in Table 2 (Zhang et al. 1985).

Table 1. Morphological identification of species for fish egg samples collected from different stations (see station codes on Fig. 1) in the East China Sea off the Coast of Zhoushan Archipelago (ECS-CZA) in the years of 2018 and 2019. N/I – Not Identified. +Some small oil balls nearby a large yellow oil ball.

Species	Family	Egg diameter (mm)	Oil ball diameter (mm)	Collection station (Mont
Acanthopagrus schlegelii	Sparidae	0.98-1.20	0.20-0.23	Z22(04-2018)-E001
Auxis rochei	Scombridae	0.95		Z28(05-2019)-E002, Z30-
Chrysochir aureus	Sciaenidae	0.70-0.79	0.20	Z21(11-2018)-E004, Z15(
Coryphaena hippurus	Coryphaenidae	1.35-1.46	0.26-0.36	Z29(05-2018)-E006, Z28(
Cynoglossus abbreviatus	Cynoglossidae			Z12(04-2018)-E009
Dinematichthys iluocoeteoides	Bythitidae	0.80	0.11	Z23(11-2018)-E010
Konosirus punctatus	Clupeidae	1.28-1.43		Z18(04-2018)-E011, Z24(
Larimichthys crocea	Sciaenidae	1.70-1.21	0.31+	Z15(11-2018)-E014
Lateolabrax japonicus	Lateolabracidae	1.26-1.33		Z22(11-2018)-E015, Z25(
Minous monodactylus	Synanceiidae			Z09(05-2018)-E017, Z04(
Minous sp. FOAL810-10	Synanceiidae			Z30(05-2019)-E019
Pampus argenteus	Stromateidae	1.39-1.41	0.39	Z06(04-2018)-E020, Z16(
Pseudaesopia japonica	Poaceae	1.84-2.03		Z04(04-2018)-E023, Z08(
Saurida macrolepis	Synodontidae	0.90-0.97		Z30(05-2019)-E026
Scomber japonicus	Scombridae	1.02-1.38	0.33	Z10(05-2018)-E027
Scomberomorus niphonius	Scombridae	1.60-1.70	0.40	Z24(04-2018)-E028, Z24(
Sillago japonica	Sillaginidae	0.63-0.70	0.15-0.18	Z11(05-2018)-E031
Trichiurus lepturus	Trichiuridae	1.90-2.00	0.48	Z09(05-2019)-E032, Z30(

Table 2. Morphological identification of species for fish larva samples collected from different stations (see station codes on Fig. 1) in the East China Sea off the Coast of Zhoushan Archipelago (ECS-CZA) in the years of 2018 and 2019. N/I – Not Identified.

Species	Family	Dorsal fin	Anal fin	Collection station (Month-Year)-Sample code	P
Acanthogobius hasta	Oxudercidae	VIII, 18	I-16	Z16(04-2019)-L001	A
Benthosema pterotum	Myctophidae	12	17	Z23(11-2018)-L002	O
Callionymus enneactis	Callionymidae	IV-8	7	Z21(11-2018)-L003	St
Chaeturichthys stigmatias	Gobiidae	VII,22	19-20	Z03(04-2018)-L004	St
Chelidonichthys spinosus	Triglidae	IX-16	15	Z14(04-2018)-L005	St
Dinematichthys iluocoeteoides	Bythitidae	80	59	Z27(11-2018)-L006	P
Engraulis japonicus	Engraulidae	14-16	15-18	Z04(05-2018)-L007	St
Harpadon nehereus	Synodontidae	11-13-Nov	13-15	Z20(11-2018)-L008	Se
Larimichthys polyactis	Sciaenidae	X,31-36	II,9	Z07(04-2018)-L009, Z07(04-2018)-L010	B
Lophiogobius ocellicauda	Gobiidae			Z01(04-2018)-L011, Z05(04-2018)-L012	N
Salanx ariakensis	Salangidae	12	25-27	Z05(11-2018)-L013, Z13(11-2018)-L014	P
Sebastiscus marmoratus	Sebastidae	XII,11	III,5	Z02(04-2018)-L015	D
Takifugu xanthopterus	Tetraodontidae	16-18	14-16	Z17(04-2019)-L016	D

DNA barcoding analysis

After photographing, the samples preliminarily identified to be different species were preceded with DNA barcoding. Total DNA was isolated from a small piece of muscle tissue of fish larvae or a single fish egg using standard Wizard® Genomic DNA Purification Kit and following the manufacturer's instructions (Promega Inc, U.S.A.), rehydrated in ddH_2O and examined through OD260/OD280 spectrometry and agarose gel electrophoresis, and then kept at 4 °C for subsequent PCR amplification.

Amplification of the COI DNA fragment via polymerase chain reaction (PCR) used the universal primers of FishF1 and FishR1 (Ward et al. 2005) and referred PCR conditions as described by Hebert and Gregory (2005), Becker et al. (2015), and Ivanova et al. (2007). PCR products were verified via gel electrophoresis using 1.5% agarose gel stained with SybrGreen fluorescent dye. Quality amplicons were sequenced at Sangon Biotech Cooperation Ltd (Shanghai, China).

DNA sequences were confirmed, edited and assembled using Sequencher v5.4 (Gene Codes) and then aligned in Clustal X2 (Chenna et al. 2003). Additional sequences were retrieved from the GenBank database (http://www. ncbi.nlm.nih.gov/) via the Basic Local Alignment Search Tool (BLAST) for identifying specimens when the resulting sequences were at a minimum of 98% similarity. K-2-P distances (Kimura 1980) were used to calculate pairwise differences of the sequences in MEGA v.7.0 (Kumar et al. 2016). In addition, a clustering analysis of neighbor-joining (NJ) based on K-2-P distances and 10,000 bootstrap replications was performed to provide a graphic representation of relationship of taxa using MEGA v.7.0.

Results

Species identification by morphology

A total of 15 species were identified from fish eggs, belonging to 12 families; 12 species were identified from fish larvae, belonging to 12 families. One species, *Dinematichthys iluocoeteoides* was shared between the fish egg and larva samples. Four egg samples (E009, E017, E018, and E019) could not be identified due to partial damage (i.e., missing the key feature for identification); two larva samples (L011 and L012) were unable to be identified due to the missing key feature for identification.

A morphological analysis apparently revealed 15 species from the fish egg samples, of which 7 were identified using the characters of egg diameter, oil ball, and pigmentation in the egg, and 8 were identified using the features of egg diameter and/or oil ball. Based on our observations, eggs of *Pampus argenteus* (E020, E021, and E022) are 1.39-1.41mm in diameter with an oil ball of 0.39mm and pigments evenly distributed on the back of embryo and spread to the caudal stalk; there are also pigments inside the oil ball. Eggs of *Konosirus punctatus* (E011, E012, and E013) are 1.28-1.43mm in diameter with dotted pigments evenly distributed on the back of the embryo. *Trichiurus lepturus* eggs (E032 and E033) are 1.90-2.00mm in diameter with an oil ball of 0.48mm and a pair of pigments accumulated on the head; there are a pile of pigments on the pectoral fin and the ventral surface of tail. *Pseudaesopia japonica* eggs (E023, E024, and E025) are 1.84-2.03mm in diameter with the absence of oil ball and many star-shaped pigments on the embryo and yolk. The *Scomber japonicus* egg (E027) is 1.02-1.38mm in diameter with an egg ball of 0.33mm and two parallel lines of pigments on the back of embryo. The*Dinemathichthys iluocoeteoides* egg (E010) is 0.80mm in diameter with an oil ball of 0.11mm and a dendritic pigment on the embryo.*Larimichthys crocea* egg (E014) is 1.70-1.21mm in diameter with some small oil balls nearby a large yellow oil ball of 0.31mm.*Scomberomorus niphonius* eggs (E028, E029, and E030) are 1.60-1.70mm in diameter with an oil ball of 0.4mm. *Acanthopagrus schlegelii* egg (E001) is 0.98-1.20mm in diameter with an oil ball of 0.20-0.23mm. *Chrysochir aureus* eggs (E004 and E005) are 070-0.79mm in diameter with an oil ball of 0.2mm. *Coryphaena hippurus* eggs (E006, E007, and E008) are 1.35-1.46mm in diameter with an oil ball of 0.26-0.36mm. The *Sillago japonica* egg (E031) is 0.63-0.70m in diameter with an oil ball of 0.15-0.18mm oil ball. *Lateolabrax japonicus* eggs (E015 and E016) are 1.26-1.33mm in diameter with no oil ball. *Auxis rochei* eggs (E002 and E003) are 0.95 mm in diameter with no oil ball. *Saurida macrolepis*(E026) is 0.90-0.97mm in diameter and has no oil ball.

A morphological analysis revealed 12 species of fish larvae that belong to 12 families. Eleven species were

identified by the characters of dorsal fin, anal fin and pigmentation; one species was identified by dorsal fin and anal fin characters. We observed that the*Chaeturichthys stigmatias* larva (L004) has a dorsal fin of VII-22 rays, an anal fin of 19-20 rays, and star-shaped pigments under the chest cavity and above and below the anus; also there is a large black star-shaped pigment at the end of anal fin and buttock.*Sebastiscus marmoratus* larva (L015) has a dorsal fin of XII-11 rays and an anal fin of III-5 rays; there are dense pigments above the chest cavity and 7-8 star-shaped pigment dots evenly arranged from the anus to the caudal stalk. Larvae of *Larimichthys polyactis* (L009 and L010) each have a dorsal fin of X-31-36 rays, an anal fin of II-9 rays and one little pigment on the abdomen and another on the buttock below the chest. *Chelidonichthys spinosus* larva (L005) has a dorsal fin of IX-16 rays, an anal fin of 16 rays, and star-shaped pigments on the head, chest cavity, abdomen, ventral fin and first dorsal fin. *Acanthogobius hasta* larva (L001) has a dorsal fin of VIII-18 rays, an anal fin of I-16 rays, and a large star-shaped pigment on the base of ventral fin; there are star-shaped pigments above the bladder, above and at the end of digestive tract and on the base of anal fin and the base of caudal fin. *Engraulis japonicus* larva (L007) has a dorsal fin of 14-16 rays, an anal fin of 15-18 rays, and star-shaped pigments on the head, anterior gill cover and above the digestive tract; there is a little pigmentation in the lower caudal fin.*Callionymus enneactis* larva (L003) has a dorsal fin of IV-8 rays, an anal fin of 7 rays, and star-shaped pigments arranged in parallel on the back of body, on the midline of body side and from the anus to the caudal stalk as well as a dendritic melanin on the abdomen.*Benthosema pterotum* larva (L002) has a dorsal fin of 12 rays, an anal fin of 17 rays, and one large chrysanthemum-like pigment in the front of abdominal sac and another at the end of digestive tract. The larva (L006) of *D. iluocoeteoides* has a dorsal fin of 80 rays, an anal fin of 59 rays, and pigments evenly distributed on the top of fish head, on the gill caps behind the eyes, on the back from the anus of abdomen to the caudal stem, and on the end of body spine. *Harpadon nehereus* larva (L008) has a dorsal fin of 11-13 rays, an anal fin of 13-15 rays, and seven large melanin plaques in the chest. *Salanx ariakensis* larvae (L013 and L014) have a dorsal fin of 12 rays, an anal fin of 25-27 rays, and pigments evenly distributed on the upper part of digestive tract and the base of fin membrane of abdomen. *Takifugu xanthopterus* larva (L016) has a dorsal fin of 16-18 rays, an anal fin of 14-16 rays, and dense pigments at the end of snout, on the back of body, and above and below the chest cavity.

Species identification by DNA barcoding

A total of 49 PCR products were sequenced successfully from 49 samples, of which 33 sequences were related to fish eggs and 16 sequences were related to fish larvae. Aligned sequences in a consensus length of 540bp yielded 212 polymorphic sites. The 49 DNA sequences were deposited to GenBank (see sequence accessions MZ461989-MZ462037 and their corresponding sample codes on Fig. 2).

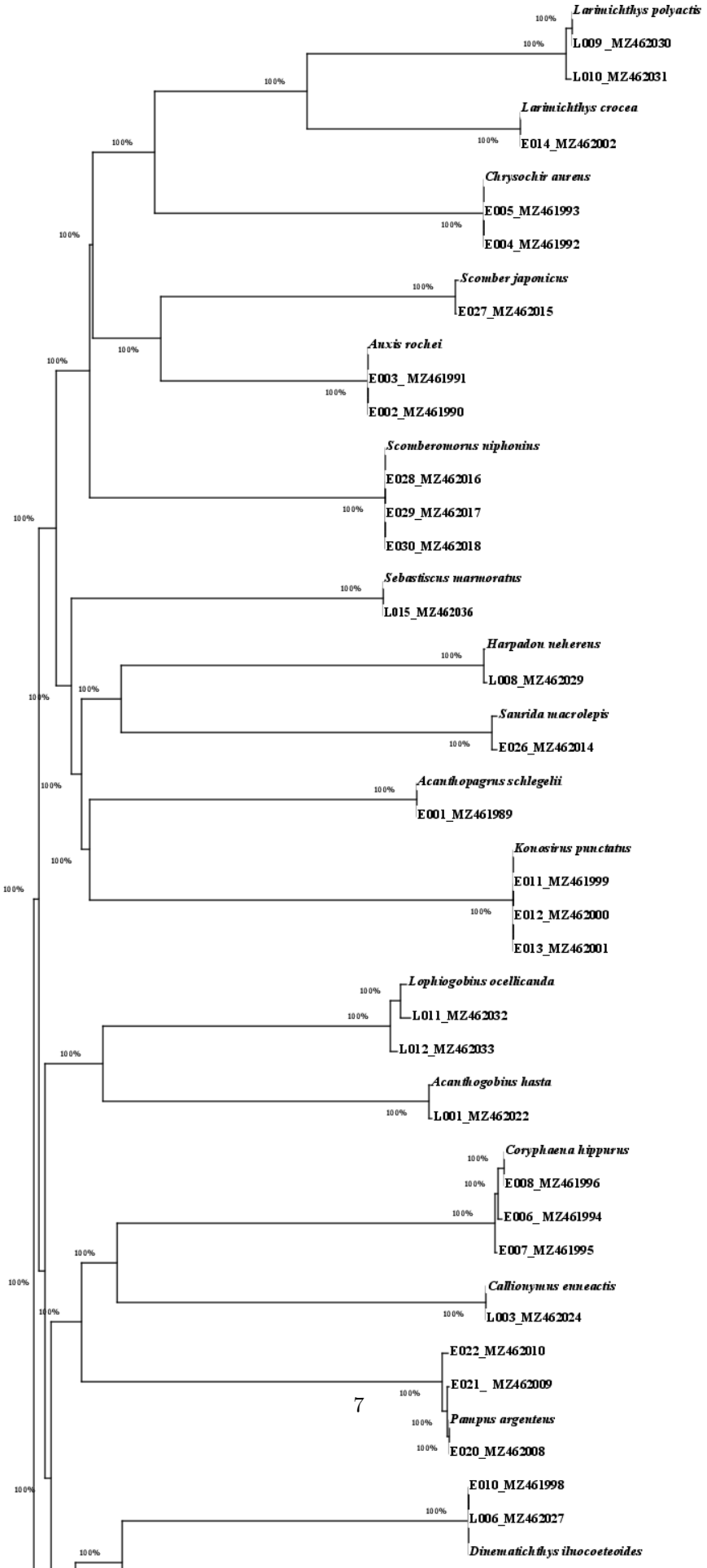

Fig. 2 Neighbor-joining tree for the COI DNA sequences of fish eggs and larvae collected from the East China Sea off the Coast of Zhoushan Archipelago. On the tree, the percentages ([?] 99%) shown next to the nodes represent the statistical supports derived from 10,000 bootstrapping permutations; each taxon is indicated with a sample code and its corresponding GenBank accession.

On the NJ tree (Fig. 2), all the sequences of 49 samples and 30 references were classified into a total of 30 smallest monophyletic clades (i.e., SMC1-30) and all sequences of the same species converged together. Each SMC had strong bootstrapping supports (value = 100%), joined by a reference sequence (ref) and sample sequences (see codes in Table 1 and Table 2). SMC1 comprised two samples (L009 and L010) and one ref of *L. polyactis* . SMC2 consisted of one sample (E014) and one ref of *L. crocea.* SMC3 consisted of two samples (E004 and E005) and one ref of *C. aureus.* SMC4 was composed of one sample (E027) and one ref of *S. japonicus.* SMC5 included two samples (E002 and E003) and one ref of *A. rochei.* SMC6 included three samples (E028, E029, and E030) and one ref of *S. niphonicus.* SMC 7, 8, 9 and 10 each had one sample (i.e. L015, L008, E026 and E001) and one ref of *S. marmoratus, H. nehereus, S. macrolepis,* and *A. schlegelii* , respectively. SMC11 had three samples (E011, E012, and E013) and one ref of *K. punctatus* . SMC12 had two samples (L011 and L012) and one ref of *L. ocellicauda,* SMC13 had one sample (L001) and one ref of *A. hasta.* SMC14 had three samples (E006, E007, and E008) and one ref of *C. hippurus.* SMC15 had one sample (L003) and one ref *C. enneactis.* SMC16 consisted of three sample (E020, E021, and E022) and one ref of *P. argenteus.* SMC17 had two samples (E010 and L006) and one ref of *D. iluocoeteoides.* SMC18 and SMC19 each had one sample (i.e. L007 and E019) and one ref of*E. japonicus* and *Minous sp* ., respectively. SMC20 had two samples (E017 and E018) and one ref of *Minous monodactylus.*SMC21, SMC22, SMC23, SMC24 and SMC25 each included one sample (i.e. L016, E009, L004, L002, and E031) and one ref of *T. xanthopterus* ,*Cynoglossus abbreviates* , *C. stigmatis, B. pterotum* and*S. japonica* , respectively. SMC26 had three samples (E023, E024, and E025) with one ref of *P. japonica.* SMC27 and SMC28 each had two samples, (E032 and E033) and (L013 and L014) and one ref of *T. lepturus* and *S. ariakensis* , respectively. SMC29 had one sample (L005) and one ref of *C. spinosus* . SMC30 included two samples (E015 and E016) and one ref of *L. japonicus.* On the COI sequence data, the K-2-P distances ranged from 0.00 to 0.03 within SMCs and fell into the range of 0.15 to 0.34 between SMCs.

This study justified the identification of 30 fish species altogether from the sea, including 18 species from 33 eggs belonging into 12 families and 13 species from 16 larvae placed into 12 families. *D. iluocoeteoides* was identified from both the egg (E010) and the larva (L006) samples. Comparatively, DNA barcoding revealed three more egg species, *C. abbreviatus* (E009), *M. monodactylus* (E017 and E018) and *M. sp.* (E019), and one more larva species, *L .ocellicauda* (L011 and L012) than MI.

Discussion

Integration of DNA barcoding and morphology in ichthyoplankton assessment

Accurate identification of fish eggs and larvae to the species level can be difficult and time-consuming when relying solely on morphological diagnosis or comparative taxonomy (Wibowo et al. 2017). That is why, through DNA barcoding, morphologically similar fish eggs and larvae can be effectively isolated and delineated (Ko et al. 2013). This study demonstrated the convenience and power of establishing COI DNA-based identification method for fish eggs and larvae. In agreement with the morphological diagnosis, the barcoding presented recognizable genetic separation, supporting the identification of 30 ichthyoplankton species in the ECS-CZA. The intraspecific and interspecific K-2-P distances in our analysis met the proposed criteria of DNA barcoding, similar to those established in earlier COI barcoding works (Hebert et al. 2003; Ward et al. 2005; Valdez-Moreno et al. 2010).

When all taxonomic levels are used, DNA barcoding is more sensitive and accurate in ichthyoplankton assessment than MI because it does not depend on the specimen's appearance or physical quality regardless of life stage (Ko et al. 2013). Therefore, specimens that have been damaged, lack crucial diagnostic traits, or are in developmental phases and unable to be identified using morphology can be identified using barcoding (Ko et al. 2013). Hence DNA barcoding should be used for specimens that have some damage; in contrast, for

specimens that are intact, an integrated strategy of MI by an expert ichthyologist and DNA barcoding should be favorable choice of identification. There is a necessity to incorporate these two methods to have a broader view, resolve ambiguities, and improve the accuracy of species identification in particular in ichthyoplankton assessment.

Implications for fish conservation and management in the ECS-CZA

Our findings presented reliable information on fish eggs and larvae that could facilitate various marine resources management efforts and conservation strategies. Accurate identification tools must be used for environmental monitoring programs due to concerns for managing and conservating declining fish stocks. The eggs and larvae for fish species identified and characterized in the present study are all economically important and valuable species (*L. croceus* , *T. lepturus* ,*P. argenteus* , *L. japonicus* , and *S. japonicus*). They are critical sources for food and protein, commercialization, and sport fishing. For the sustainability of the adjacent ecosystems, due to over-exploitation and economic value, this information will help managers monitor and revise the current plan already in place to conserve the species in the East China Sea's coastal ecosystems.

Since the ECS-CZA hosts massive marine fish production and many resources, it is the largest and most important regional and international fishing ground. With so much ongoing shoreline and shallow sea construction and modification, uncontrolled pollution emission, and over-exploitation of fishery resources, the coastal marine life, ecosystems, and even human communities will be harmed. Conclusions about the management and preservation of fish species and the coastal marine environment inferred from our findings will be useful. Our study offers useful tools and critical scientific data that may be used to understand the taxonomic status, variety, recruitment, and distribution of fish species for eggs and larvae in coastal waters. In addition, the discovery of previously unknown species offers strong support for the importance of integrated strategy of morphology and COI DNA barcoding and ecological efforts.

Author contributions: RJ and YC contributed to the study conception and design. RY and YZ performed material preparation and data collection. MTM and ZL performed data analyses. The manuscript was written by MTM, RJ and YC. All the authors read and agreed to the final version of the manuscript.

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Data Availability Statement : COI DNA sequences: GenBank accessions MZ461989-MZ462037.

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Ethics Statement: All fish species were caught in the offshore area (not national parks, other protected areas, nor private areas, etc.), so no specific permissions were required for these locations/activities. Ethical approval was not required for this study because no endangered or protected fish species were involved. Specimen collection and maintenance were performed in strict accordance with the recommendations of Animal Care Quality Assurance in China.

Conflict of Interest : The co-authors have no conflict of interest to declare.

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