



El Colegio de la Frontera Sur

Estableciendo la identidad y abundancia de los huevos de peces en el Sureste de la Península de Yucatán mediante los códigos de barras de ADN

TESIS

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por

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DEDICATORIA

A la familia Leyva Cruz porque todo lo que soy se lo debo a ellos.

A mi pequeña familia, Gualberto y Armando, los amo con toda mi alma.

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INTRODUCCIÓN

Un problema muy común al que se enfrentan los ictiólogos, es establecer la identidad taxonómica de los estadios tempranos del ciclo de vida de los peces. Esta situación se debe a que en las etapas tempranas los organismos se encuentran poco diferenciados y los caracteres que se toman en cuenta para la identificación son limitados. Por lo tanto, determinar a qué especie pertenecen los huevos y larvas de los peces no es una tarea sencilla. En el caso de los huevos, la problemática es aún más compleja debido a que el número de caracteres morfológicos que se toman en cuenta para identificarlos es de 8 a 12, si el huevo está fecundado (Ahlstrom y Moser, 1980). Si el huevo no está fecundado su identificación es prácticamente imposible. Además, esta etapa del ciclo de vida solo dura de 24 a 48 horas, para la mayoría de las especies (Richards, 2006).

En el mundo se han llevado a cabo esfuerzos para describir la mayor cantidad de especies en las primeras etapas de su ciclo de vida (Russel, 1976; Leis y Rennis, 1983; Moser et al., 1984; Ozawa, 1986; Leis y Trnski, 1989; Kellerman, 1989; Aboussouan, 1989; Matarese et al., 1989; Olivar y Fortuño, 1991; Neira et al., 1998; Munk y Nielsen, 2005; Richards, 2006; Fahay, 2007) mediante las características morfológicas que presentan o llevando huevos y larvas hasta etapas de crecimiento identificables.

A partir del primer gran esfuerzo de compilación, realizado por Moser y colaboradores en 1984, sobre ontogenia y sistemática de peces, Richards (1985) estimó que se conocían solo el 9% de las larvas y el 3.5% de los huevos a nivel de especie en el mundo. Una revisión posterior sobre los avances en taxonomía y

sistemática de estadios tempranos de peces mostró cifras similares, 10% de las larvas y 4% de los huevos, contaban con la descripción de al menos una fase de su desarrollo ontogenético a nivel de especie (Kendall y Matarese, 1994; Fahay, 2007).

Richards (2006) publicó una edición con los huevos y larvas de peces de las especies del Atlántico Noroccidental Central, que es el área que incluye al Golfo de México y el Mar Caribe (área 31 según la FAO). En este libro se registran 2235 especies para la zona, de las cuales 901 tienen descrita alguna etapa larval y tan solo 206 tienen descrita la etapa de huevo.

En la actualidad hace falta una revisión detallada del avance en la descripción de las primeras etapas del desarrollo de los peces. Sin embargo estas cifras nos proporcionan una perspectiva del estado del conocimiento del ictioplancton y la necesidad de implementar estrategias que permitan llenar los vacíos en el reconocimiento de las especies en sus primeras etapas de desarrollo.

Recientemente la utilización de herramientas genéticas empleando ADN para la separación de especies ha sido particularmente efectiva. Un método utilizado en la identificación de huevos y larvas de peces es la secuenciación de fragmentos de ADN de genes específicos como el citocromo b (Wen-Feng et al., 2005), la región 16S (Saitoh et al., 2008; Kawakami et al., 2010) y la NADH 4 deshidrogenasa (Carreón-Martínez et al., 2010).

Por su parte, Paul Hebert (2003) propuso a la Citocromo Oxidasa subunidad I (COI, código de barras de la vida o barcode en inglés) como un marcador genético universal. El COI es un gen del ADN mitocondrial de aproximadamente 1200 pares de

bases. Sin embargo como marcador solo se utiliza la primera mitad (648 pb aproximadamente).

En base a esta técnica surgió la iniciativa del proyecto internacional denominado Códigos de Barras de la Vida (BOLD por sus siglas en inglés), cuyo principal objetivo es obtener las secuencias de todas las especies animales, vegetales y de hongos existentes en el planeta. Para almacenar toda la información recabada a partir de esta iniciativa, se construyó una plataforma informática que almacena los códigos de todos los individuos obtenidos hasta la actualidad. A través de esta biblioteca se pueden contrastar las nuevas secuencias contra las secuencias almacenadas en esta plataforma y de esta manera identificar rápido y eficazmente los organismos (Ratnasingham y Hebert, 2007).

Desde el surgimiento de la iniciativa los códigos de barras de la vida se han empleado en la identificación de peces. Existen más de 200 trabajos (Ward et al., 2005; Victor, 2007; Hubert et al., 2008; Rock et al., 2008; Valdez-Moreno et al., 2009; Lara et al., 2010; Valdez-Moreno et al., 2010; Oliveira-Ribeiro et al., 2012) los cuales corroboran el éxito que ha tenido esta técnica.

A partir de los buenos resultados obtenidos con los peces adultos, surgió la idea de utilizar este método para identificar los estadios tempranos del ciclo de vida de estos organismos (Pegg et al., 2006) y así poder describir todas las etapas del desarrollo de las especies.

Ya han sido reportados trabajos utilizando los códigos de barras para identificar huevos de peces. Gleason y Burton (2012) identificaron huevos de 23 especies de peces provenientes de cruceros oceanográficos llevados a cabo en las costas de California en Estados Unidos Americanos. Lin y colaboradores (2013) colectaron 8,933 huevos en el Mar Este de China. Los ejemplares fueron divididos en 87 morfotipos, posteriormente identificaron 73 morfotipos que pertenecían a 32 especies. Los morfotipos restantes no pudieron ser identificados a nivel de especie. Burghart y asociados (2014) de 843 huevos, identificaron 13 especies y un género en la bahía de Terra Ceia, Florida, Estados Unidos Americanos.

Sin embargo, uno de los primeros trabajos realizados con este enfoque fue realizado por Valdez-Moreno y colaboradores (2010) con peces marinos de la Península de Yucatán. En esta investigación identificaron y secuenciaron 1392 especímenes de los cuales 610 eran adultos o juveniles, 757 larvas y 25 huevos. En el caso de los huevos se identificaron 5 especies entre las que destaca el boquinete (*Lachnolaimus maximus*) por su importancia como recurso pesquero. Leyva-Cruz (2010) también utilizó el COI para establecer la identidad de 94 huevos colectados en 2007, de los cuales, se obtuvieron 69 secuencias exitosas de 18 especies.

La identificación de huevos y larvas de peces permite saber que especies están desovando, cuando y donde. También permite conocer las zonas que utilizan como refugio y sus posibles rutas de migración. Esta información es importante para el monitoreo ecológico, el manejo de los recursos y establecimiento de áreas marinas protegidas (Gleason y Burton, 2012; Ko et al., 2013; Burghart, 2014). La información de distribución y abundancia del ictioplancton provee un medio para medir el futuro

reclutamiento, analizar y diferenciar los efectos naturales y los causados por el hombre en las poblaciones de peces, así como la importancia de las especies y su rol en el ecosistema como depredador, presa o herbívoro (Richards, 1985; Kawakami, 2010).

Por lo tanto el objetivo principal de este trabajo es identificar a que especies pertenecen los huevos de peces encontrados en aguas del Sureste de la Península de Yucatán y una vez identificados realizar descripciones morfológicas de los mismos, así como determinar la abundancia y distribución de los huevos en la zona de estudio.

ARTÍCULO

Who laid the egg? Establishing the identity and abundance of fish eggs in the southeast of the Yucatan Peninsula with DNA barcodes (April, 2011)

Sometido a Plos One

Who laid the egg? Establishing the Identity and Abundance of Fish Eggs in the Southeast Yucatan Peninsula with DNA Barcodes (April, 2011)

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Competing Interests

The authors have declared that no competing interests exist.

Author Contributions

Conceived and designed the experiments: MVM, ELC, LVY. Performed the experiments: ELC.

Analyzed the data: ELC, MVM, LVY, LC. Contributed reagents/materials/analysis tools: MVM,

LVY, ELC. Wrote the paper: ELC, MVM, LVY, LC.

Abstract

Along the Mesoamerican reef system, there are many spawning and nursery areas for various types of fish. However, identifying eggs to determine which species the eggs belong to is currently almost impossible because of the lack of morphological characters. A good alternative to solve this problem is the use of DNA barcodes, which have been effective in connecting the early developmental stages of fish with the adults. Although DNA barcoding has become popular, it has rarely been used to identify fish eggs. The aim of this work is to recognize which species of fish spawn in waters southeast of the Yucatan Peninsula using DNA barcoding and morphological characters of the eggs to establish the time and date of spawning for the species. The samples were collected in 2011, during an oceanographic survey supported by the National Oceanic and Atmospheric Administration (NOAA), El Colegio de la Frontera Sur (ECOSUR), and the University of Miami. In total, 1391 fish eggs were sorted, and 94 morphotypes were identified. Three hundred eggs were photographed, described and the COI gene was amplified and sequenced. We obtained 140 sequences that were compared with the Barcode of Life Database; after, we identified 42 species, 34 genera and 23 families. The most common species were *Nesiarchus nasutus*, *Diplospinus multistriatus* and *Regalecus glesne*. The most economically important were *Auxis thazard*, *Caranx hippos*, *Coryphaena hippurus*, *Istiophorus platypterus*, *Kajikia albida*, *Katsuwonus pelamis*, *Thunnus atlanticus* and *Xiphias gladius*. The sampling stations near Banco Chinchorro had the highest abundance of eggs, dominated by *K. pelamis*. The eggs of 20 species and the spawning areas and seasons for 16 species were described for the first time. With this basic information, more effective conservation measures can be developed for sustainable fisheries and protection of the second largest reef formation of the world.

Key words: Early fish stages; Mesoamerican Reef; conservation; spawning areas; fish eggs description.

Introduction

The overexploitation of fisheries and the decline of marine resources is a global problem that requires the constant evaluation of the population dynamics of fish [1,2]. There are several processes that influence the increase or decrease of fish populations, such as recruitment at different stages of the life cycle, dispersal and retention of organisms [3,4]. For a detailed and reliable study of these phenomena, the species must be accurately identifiable at all stages of their life cycle [5].

Accurate identification of organisms is the main key to providing fundamental knowledge of spawning areas and times as well as possible variations in the abundance of the different species in different seasons [3]. All of this information, together with knowledge of the local current regimen, will be the best source of primary information to design better conservation and fisheries management strategies.

The morphological features used to recognize adult fish species are not present in the early stages of development, making them much more difficult to identify. Nevertheless, efforts have been made to obtain descriptions and produce identification guides to the early developmental stages of fish around the world [6-12]. Some authors [13-14] suggest that in the Northeast Pacific region, from 592 registered species, only 14% have descriptions for the egg stage. From 131 species in the Northeast Atlantic, 70% of the eggs are already described, and for the Southeast Atlantic, of 239 reported species, 20% have descriptions for the egg stage. From

the Northwest Pacific, Antarctic, Indo-Pacific and the Mediterranean, only a few larvae have been described.

In the Western Central North Atlantic (WCNA, FAO area 31), which includes the Yucatan Peninsula, there are approximately 2235 known species and only 9% of them have an identifiable egg stage [11].

Generally, it is estimated that only 10% of the world's fish species are known from the early stages [13,14], and in most cases, they are incomplete. For example, not all stages of larval development are described, or a description of the egg stage is missing.

For ichthyologist, it is almost impossible to identify eggs using only morphology because they have few morphological features that can be used to recognize them. Among the most important characters are the shape, the size, the perivitelline space, the oil globules, the inner membrane, the chorion, the embryo, the yolk shape, and the pigment pattern [15,16]. Other important features that hinder identification are ontogenetic changes [17] because some eggs are fertilized and others are not [11,16].

The use of molecular characters, such as DNA barcodes (cytochrome oxidase subunit I, or COI), has been successful to identify most animals to species [18]. In fish, there are 106,276 barcodes, and of these, 10,779 species can be found in the Fish Barcode of Life (www.fishbol.org, consulted in 12 February, 2015).

After the first paper demonstrating the value of barcodes for fish identification [19], many publications have confirmed the value in different regions of the world [20-25]. Other markers have been used with the same purpose, such as cytochrome b [26], 16S [27] and the NADH 4 [28], but they were only used for a small number of species.

The idea to identify the early stages of the life cycle of fish with DNA barcoding was first proposed approximately 8 years ago [29]. After, Victor [30] conducted one of the first studies using barcodes to identify early developmental stages, describing the larvae of a new species of goby, *Coryphopterus kuna*, in the WCNA. Some previous egg studies identified a limited number of species; for example, in California waters, 23 species were reported [3]. From 8,933 analyzed eggs from the East China Sea, only 32 species were recognized [31]; in Florida, 13 species and one genus are known [32] from Terra Ceia Bay. The first study in the Yucatan Peninsula reported 1392 specimens, of which 25 were eggs belonging to 5 species [24].

However, the Mexican Caribbean lies in the Mesoamerican Barrier Reef System (MBRS), the second largest reef in the world [33]. This area provides suitable habitat for species such as jacks, snappers and groupers, all of them of commercial [34] and game interest [35,36]. Moreover, the main touristic attractions of the region are associated with the coral reef formations [37]. The Mexican Caribbean is under strong pressure because of modification of the coast. Actually, mangroves and seagrasses have been removed, altering the habitat of many organisms, including fish. If overfishing and global threats, such as climate change and ocean acidification, are added to the pressures, we find a vulnerable area with huge management potential because the economy depends on these species [38].

The aim of this study is to provide basic information on the identification of fish eggs from an ample region in the south central part of the Mesoamerican reef using morphology and DNA barcodes with abundance and spatial distribution data. These data and additional information will provide a strong basis for environmentalists and developers to establish sustainable development of this important region.

Materials and methods

Ethics statement

We collected at several oceanic stations in the Mexican Caribbean and from some stations in Belize waters. The marine zooplankton that included eggs and fish larvae are not protected under any Mexican laws; however, we also have permits for this type of field study. The authorities that issued the permits were the Comisión Nacional de Acuacultura y Pesca (CONAPESCA), through the Secretaria de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA, Mexico), and the Fisheries Department through the Ministry of Foreign Affairs and Foreign Trade in Belize.

Sampling

The samples were collected during an oceanographic campaign supported by a collaborative effort between El Colegio de la Frontera Sur (ECOSUR), the National Oceanic and Atmospheric Administration (NOAA) and Miami University on board the Research Vessel Gordon Gunter. Sampling was conducted from 20-22 April 2011. Samples were collected from 17 stations in waters surrounding the largest pseudo-atoll in the Caribbean, the Biosphere Reserve Banco Chinchorro (17°45' -19°30' N 85°40' -88°00' W) [38]. All stations were different distances from the coast. According to geographic coordinates, stations 82 and 83 were in Belize jurisdiction waters, and the others were in Quintana Roo State, Yucatan Peninsula, Mexico (Fig. 1).

Fig. 1. Sample stations. Location of the sample stations.

Two types of nets were used: Neuston (0.947 μ) and Spanish Neuston (0.505 μ) to collect the zooplankton. Tows lasted 10 min and were performed at 0.5 and 10 m deep. Each sample was fixed with 96% ethanol, replacing the sea water.

Laboratory analyses

From the plankton sample, eggs were extracted, counted and sorted according to recognizable morphotypes. The main characteristics considered for morphological descriptions were size, shape (spherical, ellipsoidal), width of the perivitelline space (narrow, wide, medium), presence or absence of oil globules, presence or absence of an inner membrane, pigmentation patterns and yolk characteristics (color, segmentation), chorion (ornamentation, texture) and embryo (formation, pigments) [11,15,16]. After, 1 to 5 eggs of each morphotype were photographed with Nikon SMZ745T and Canon EOS Rebel T31 cameras, both attached to a stereomicroscope. One hundred to 250 photographs were stacked into one image with Helicon Focus 6.2.0 software [39] to obtain full focus of the entire surface. Once photographed, the complete egg was placed in a 96-well EppendorfTM plate for molecular analysis. To prevent contamination, all tools were flame sterilized before and after use with each sample.

Extraction, amplification and sequencing of the COI gene

For extraction, amplification and sequencing, we followed the protocols of Valdez-Moreno and coworkers [24]. PCR amplification was performed in the barcode laboratory at ECOSUR (Chetumal). The fish primers used were Fish F1, Fish R1, Fish F2 and Fish R2 [19]. Sequencing was performed using a M13-tailed fish primer-cocktail [40]. Sequences were aligned using Codon Code 5.0.1 software. Primer details, trace files and sequences are available in the

project code MXFEC, Fish Eggs of the Caribbean of the Barcode of Life Database (BOLD, www.boldsystems.org) and GenBank (<http://www.ncbi.nlm.nih.gov>).

Data analysis

The sequences obtained were compared with sequences in the Barcode of Life Database (BOLD) using the identification tool provided in the system [41]. The criteria to assign identification to species level and when a sequence match was not found in the DNA barcode reference library followed Valdez Moreno et al. [42]. Sequence divergence was calculated using the Kimura two parameter distance model (K2P) [43] provided in BOLD. An ID tree is provided for a graphic representation of the identifications.

Abundance values were obtained by the counts per station. Maps and plots were made with abundance values, and the distribution of more representative species was prepared with ArcView GIS^R 3.2 software.

Results and Discussion

Species identification

In total, 1391 eggs were collected. Morphologically they were recognized as 94 different types, but only 39 eggs were identified, representing 10 species: *Auxis thazard*, *Cheilopogon exsiliens*, *Gempylus serpens*, *Lachnolaimus maximus*, *Oxyporhamphus micropterus*, *Sparisoma viride*, *Diplospinus multistriatus*, *Lactoprhys trigonus*, *Synodus synodus* and *Xiphias gladius*.

Three hundred eggs were processed for barcoding. From these, a third (117 eggs) were unfertilized, and the remainder had embryos in different developmental stages.

In total, 140 good sequences were obtained; this represented 46.6% success in sequencing. These results were consistent with those of other authors [31, 32] and our own previous results that demonstrated additional difficulties when attempting to obtain amplicons for eggs rather than adults or larvae. The difficulty may be caused by various factors, such as poor fixation, storage time, egg handling, or the presence of oil globules that interfere with polymerase chain reaction success. The exact cause still requires some investigation.

The read length of the sequences ranged from 188 to 665 base pairs (bp). Short sequences were reliable for identification using mini-barcodes that have been shown to be highly efficient for species level identification [44]. No insertions, deletions or stop codons were observed. The average K2P distance among conspecific individuals was 0.59%, whereas congeneric species averaged 13.3%. All species, except *Priacanthus arenatus* (451 bp) and *Peristedion sp.* (265 bp), were assigned a BIN number within the BOLD system (S1 Fig.).

Of 140 sequences, 82.3% matched sequences in the BOLD reference library with greater than 98% similarity, allowing species recognition. The remaining 17.7% could be identified only to genus, family or order.

With DNA barcodes seven orders, 23 families, 34 genera and 42 species (32 with a binomial name) were identified. The families with the most species were Gempylidae, Stomiidae (4), followed by Carangidae, Scombridae, Synodontidae (3), Bramidae, Coryphaenidae, Istiophoridae (2) and Acropomatidae, Diodontidae, Echeneidae, Exocoetidae, Lampridae, Lophotidae, Ostraciidae, Peristediidae, Priacanthidae, Regalecidae, Sparidae, Trachipteridae, Trichiuridae and Xiphiidae (1) (Fig. 2).

Figure 2. Neighbor-joining tree. The tree is based on genetic distances (K2P) of the COI gene. The base of the triangle represents the quantity of specimens sequenced.

Of 32 species, nine had not previously been recorded in the Mexican Caribbean: *Chauliodus danae*, *C. sloani*, *D. multistriatus*, *Lampris guttatus*, *Lophotus lacepede*, *Prognichthys occidentalis*, *Promethichthys prometheus*, *Remora osteochir* and *Synagrops bellus* [24,45-47]. The economically important species that we found were: *A. thazard*, *C. hippos*, *C. hippurus*, *I. platypterus*, *K. albida*, *K. pelamis*, *T. atlanticus* and *X. gladius* [35, 36].

Sixteen eggs were identified only to genus: *Benthodesmus*, *Brama*, *Chauliodus*, *Decapterus*, *Leptostomias*, *Peristedion* and *Trachipterus*; one egg was identified as belonging to the family Exocoetidae and five were identified as belonging to the order Perciformes. The BLAST analyses in GenBank produced similar results to BOLD; therefore, both databases remain incomplete.

Concerning specimens identified as *Benthodesmus*, they matched 100% with *B. simonyi*, *B. elongatus* and *B. tenuis*. The latter was the only species previously recorded in the Western Central North Atlantic (WCNA), but recently, *B. simonyi* was also recorded from Panama, Nicaragua, Portugal and one larva was collected near Punta Allen, Quintana Roo, as shown by sequences in BOLD. The taxonomic confusion among these three species requires more detailed analysis of the entire genus.

A similar situation exists with *Brama* eggs that matched two different species (98.89% similarity or more), *B. orcini* and *B. dussumieri*; the latter is distributed in the study area, but the identification of the individuals represented by sequences in BOLD is not totally reliable because they are from larvae and not adults.

The eggs identified as *Decapterus* matched the species *D. macarellus* (99.54-100%) and *D. tabl* (99.54-99.69%). It is necessary to review all the specimens of both species in more detail to establish their correct taxonomic identity.

Three specimens matched the genus *Leptostomias*. Currently, there are 12 *Leptostomias* species reported worldwide [48]; in BOLD, there are sequences of *L. gladiator*, *L. longibarba* and three unidentified species. In the WCNA, only six species are known: the two previously mentioned and *L. analis*, *L. bermudensis*, *L. bilobatus* and *L. haplocaulus* [49]; thus, our specimens most likely belong to one of the latter four.

Another similar case occurred with *Peristedion*. Currently, 23 species have been described [48], 11 are distributed in the WCNA [49], and from these, seven are in BOLD. The remaining four, *P. antillarum*, *P. brevirostre*, *P. longispathus* and *P. unicuspis*, remain missing from the database; therefore, our sequence could belong to one of these species.

The *Trachipterus* egg matched (99.8-98.4% similarity) sequences of *T. arcticus* and *T. jacksonensi*. The latter is distributed in the WCNA [49], Western Indian Ocean, southern Australia, Southwest Pacific and Southwest Atlantic: Brazil and Argentina [12,48,50], and the former is distributed in the Northeast Atlantic. Fish Base mentions that the Western Atlantic populations are most likely a separate species [48,50]. Because of this information, the identification was made only to genus.

Concerning *Chauliodus*, we found six eggs. After barcode analysis, they were split in two groups with greater than 3% divergence. The first group comprised five eggs (MXFEC52, MXFEC95, MXFEC183, MXFEC110 and MXFEC201) that were identified as *C. sloani*. The second group, represented by a single specimen (MXFEC022), was identified as *Chauliodus* sp. The species *C. sloani* is broadly distributed, and the BOLD data from *C. sloani* indicate more than one species; therefore, all these specimens require major revision.

Compared to all other studies, we have identified the most species from eggs based on COI gene DNA identifications (1391 eggs, 42 species) [31,32]. Using other genes, the number of species found is smaller; for example, 16 species were identified from 2698 eggs from the Mariana Islands using 16S [17]. In another study, 5 species were recognized from 52 eggs using a shorter version of 16S [27]. Other studies focused on the identification of certain species from a family; for example, 5 species were recognized from the istiophorid and xiphiid billfish, coryphaenid, dolphinfish and wahoo [26] and 5 species from the family Scianidae [28].

Considering the five species in the egg stage (*Carangoides bartholomaei*, *Haemulon plumieri*, *L. maximus*, *Mugil cephalus* and *Acanthurus bahianus*) that were previously identified in MBRS [24], plus the 42 species reported here, in total, 47 species are confirmed to spawn in the Mexican Caribbean and surrounding waters.

Finally, if we compare the results obtained in the egg identification using barcodes (42 taxa) versus morphological characters (10 species), the difference is significant. Only four species (*D. multistriatus*, *L. trigonus*, *S. synodus* and *X. gladius*) could be confirmed with both morphology and barcodes. The remaining specimens previously identified morphologically were not successfully sequenced, or the identification was incorrect. These results indicate a necessity to find a better method of fixing fish eggs (even larvae) to obtain greater success with COI sequences. Additionally, the results to confirm the use of barcodes as a good method of identification for early developmental stages, as long as a good reference fish database is available.

Morphological features of the eggs

Egg shape was spherical in all species, but in some cases, the morphology was damaged during net collecting and storage. The egg diameter ranged from 0.4-2.1 mm, approximating ranges registered for marine fish [15,16]. The smallest eggs were from *I. platypterus*. The largest eggs were from *R. glesne* and *Chauliodus* sp.

Most eggs were smaller than reported in the literature [11,12]. However, the diameter increased slightly when introducing the eggs in water, so the diameter difference is because the eggs were in alcohol, not formaldehyde.

For 20 species, we provide morphological descriptions of the egg stage for the first time. All the characters are summarized in Table 1, and the images of each eggs are shown in Fig. 3-9 (*Calamus calamus* (Fig. 3a, b, c), *C. hippos* (Fig. 3d), *C. danae* (Fig. 3e), *Decapterus punctatus* (Fig. 3f), *D. multistriatus* (Fig. 4a, b, c), *G. serpens* (Fig. 4d), *K. albida* (Fig. 4e, f), *L. trigonus* (Fig. 5a, b, c), *N. nasutus* (Fig. 5d, e, f), *P. arenatus* (Fig. 6a, b), *P. occidentalis* (Fig. 6c), *P. prometheus* (Fig. 6d, e, f), *Pterycombus brama* (Fig. 7a, b, c), *R. osteochir* (Fig. 7d, e), *Saurida normani* (Fig. 8a), *Scombrolabrax heterolepis* (Fig. 8b), *S. bellus* (Fig. 8c), *S. synodus* (Fig. 8d, e, f), *T. atlanticus* (Fig. 9a) and *Trachinotus falcatus* (Fig. 9b, c)).

Table 1. Morphological features of the studied eggs for 20 species

Species	Shape	Diam (mm)	Chorion	Perivitelline space	Yolk	Oil globule	Pigmentation	Embryo	Observations
<i>Calamus calamus</i>	Spherical	1	Smooth and transparent	Narrow	Homogeneous	Absent	Black spots in the head of the embryo	Large, with a wide head	Fertilized eggs, with an intussusception in the yolk sac
<i>Caranx hippos</i>	Spherical	0.5	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	Unfertilized egg

<i>Chauliodus danae</i>	Spherical	1.7	Smooth and transparent	Undefined	Undefined	Absent	None	None	None	Unfertilized egg
<i>Decapterus punctatus</i>	Spherical	0.5	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	None	Unfertilized egg
<i>Diplospinus multistriatus</i>	Spherical	1	Smooth and transparent	Narrow	Homogeneous	Some	Stains in the head and some around. 1 spot more in the tail	Slim, in some cases with caudal fin forming		Fertilized eggs, with an intussusception in the yolk sac with pigments inside
<i>Gempylus serpens</i>	Spherical	0.8	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	None	Unfertilized eggs
<i>Kajikia albida</i>	Spherical	0.6-1.1	Smooth and transparent	Narrow	Homogeneous	Some	In the dorsal part of the embryo and on the head	Short, head and eyes forming		Fertilized eggs with an intussusception in the yolk sac, with pigments inside
<i>Lactophrys trigonus</i>	Spherical	1.3	Smooth and transparent	Narrow	Homogeneous with black spots	Absent	Pigments brown color in the embryo and the yolk	Wide head with eyes forming		Fertilized egg, with an intussusception in the yolk sac
<i>Nesiarchus nasutus</i>	Spherical	0.5-0.7	Transparent and wrinkled	Narrow	Homogeneous	Absent	None	Embryo short and with the same thickness along of the body		Fertilized and unfertilized eggs, with an intussusception in the yolk sac
<i>Priacanthus arenatus</i>	Spherical	0.6	Transparent and wrinkled	Medium	Homogeneous	Absent	Black spots in the dorsal part of the embryo	Newly formed, is not enough to distinguish the head		Fertilized egg, with an intussusception in the yolk sac with pigments inside. There is a circle in the chorion with black spots

<i>Prognichthys occidentalis</i>	Spherical	1.8-2	Transparent, with short cilia scattered	Narrow	Homogeneous	Absent	None	Wide and big head with eyes forming	Fertilized and unfertilized eggs
<i>Promethichthys prometheus</i>	Spherical	0.8	Transparent and wrinkled	Medium	Homogeneous	Absent	Some in the head of embryo	Slim, with the head forming	Fertilized eggs, Apparently with a double membrane
<i>Pterycombus brama</i>	Spherical	1	Clear and wrinkled	Narrow	Homogeneous	Some	None	Slim, with the head forming	Fertilized eggs
<i>Remora osteochir</i>	Spherical	1.2-1.3	Smooth and clear	Narrow	Homogeneous	Some	Black spots in the dorsal part of the embryo and the yolk sac	Large, the head and tail almost meet. The head and eyes forming	Fertilized eggs, with an intussusception in the yolk sac, with black spots inside
<i>Saurida normani</i>	Spherical	1.2	Smooth and whitish	Narrow	Homogeneous	Absent	None	Thin, just forming	Fertilized egg
<i>Scombrobrax heterolepis</i>	Spherical	0.8	Clear and wrinkled	Narrow	Homogeneous	Absent	None	None	Unfertilized egg
<i>Synagrops bellus</i>	Spherical	0.7	Smooth and transparent	Narrow	Homogeneous	Absent	None	None	Unfertilized egg
<i>Synodus synodus</i>	Spherical	0.8-0.9	Ornate with hexagon shaped figures	Narrow	Homogeneous	Absent	None	Frontal part (head and neck) thickness and the rest of embryo thin	Fertilized eggs, Embryo similar to <i>S. intermedius</i>
<i>Thunnus atlanticus</i>	Spherical	0.7-1.5	Smooth and unclear	Narrow	Homogeneous	Some	None	Embryo just forming	Mostly unfertilized eggs, only 1 fertilized egg

<i>Trachinotus falcatus</i>	Spherical	1	Smooth and unclear	Narrow	Homogeneous	Absent	Faint pigments brown color in the dorsal part of the embryo	Eyes and head forming, body and head with the same thickness	Fertilized egg, with an intussusception in the yolk sac, with brown, faint spots inside
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Fig. 3a-f. New morphological descriptions. *Calamus calamus* (a, b, c) *Caranx hippos* (d) *Chauliodus danae* (e), *Decapterus punctatus* (f). Under 4x magnification, the scale bar is equal to 0.95 mm, and under 10x magnification, the scale bar is equal to 0.37 mm. The main morphological characteristics are highlighted in each photo.

Fig. 4a-f. New morphological descriptions. *Diplospinus multistriatus* (a, b, c), *Gempylus serpens* (d), *Kajikia albida* (e, f). Increase 4x, measuring slide equals 0.95 mm and increase 10x equals 0.37 mm. The main morphological characteristics are highlighted in each photo.

Fig. 5a-f. New morphological descriptions. *Lactophrys trigonus* (a, b, c), *Nesiarchus nasutus* (d, e, f). Increase 4x, measuring slide equals 0.95 mm and increase 10x equals 0.37 mm. The main morphological characteristics are highlighted in each photo.

Fig. 6a-f. New morphological descriptions. *Priacanthus arenatus* (a, b), *Prognichthys occidentalis* (c), *Promethichthys prometheus* (d, e, f). Increase 4x, measuring slide equals 0.95 mm. The main morphological characteristics are highlighted in each photo.

Fig. 7a-e. New morphological descriptions. *Pterycombus brama* (a, b, c), *Remora osteochir* (d, e). Increase 4x, measuring slide equals 0.95 mm. The main morphological characteristics are highlighted in each photo.

Fig. 8a-f. New morphological descriptions. *Saurida normani* (a), *Scombrobrax heterolepis* (b), *Synagrops bellus* (c) and *Synodus synodus* (d, e, f). Increase 4x, measuring slide equals 0.95 mm and increase 10x equals 0.37 mm. The main morphological characteristics are highlighted in each photo.

Fig. 9a-c New morphological descriptions. *Thunnus atlanticus* (a), and *Trachinotus falcatus* (b, c). Increase 4x, measuring slide equals 0.95 mm. The main morphological characteristics are highlighted in each photo.

The remaining 12 species reported here were known from previous descriptions [11,12], but we found some differences: *A. thazard* (a), *C. equiselis* (b), *C. hippurus* (c), *I. platypterus* (d) and *K. pelamis* (e, f) had no oil globules, but most likely the alcohol and storage time dissolved them. An intussusception in the chorion was observed in *I. platypterus* and *K. pelamis* (see summary of dissimilarities in Fig. 10).

Fig. 10a-f Differences from previous fish eggs descriptions. *Auxis thazard* (a), *Coryphaena equiselis* (b), *Coryphaena hippurus* (c), *Istiophorus platypterus* (d), and *Katsuwonus pelamis* (e, f), without oil globules and the two last species with an intussusceptions in the yolk sac.

C. sloani (a) lacked the double membrane and the narrow perivitelline space previously reported [13]; indeed, it was reported as having a wide perivitelline space [15,16]. No tiny spines were observed in eggs of *L. lacepede* (b). Oil globules and pigments were not observed in *R. glesne* (c, d), but the eggs appeared unfertilized; by contrast, we observed small projections in the chorion. *Synodus intermedius* (e, f) did not have a sculptured chorion, and we observed pigments in the embryo head (see Fig. 11).

Fig. 11a-f. Differences from previous fish eggs descriptions. *Chauliodus sloani* (a) with wide previtelline space and without double membrane. *Lophotus lacepede* (b) without spines. *Regalecus glesne* (c, d) with small projections and without oil globules and pigments. *Synodus intermedius* (e, f) with pigments in the head and without an ornate chorion.

All original descriptions consulted did not indicate the magnification of the observations. Here we used 4x, 5x and 10x magnifications, allowing us to see some characters that were not previously observed

Egg abundance and richness

The number of eggs collected varied between the different sampling stations. Station 93, in northeast Banco Chinchorro, had the highest abundance (279), with the most common species being *K. pelamis* (124) and *N. nasutus* (101). Station 83, with 271 eggs was next, dominated by *N. nasutus* (235). The third most abundant area was station 90 (262), with specimens that were identified only as the *Decapterus* sp. (234). The stations with the least abundance were 84 and 85, with only 15 eggs found at each (Fig. 12 and Table 2).

Fig. 12. Total abundance of eggs by station. The size of the circle represents the number of eggs per station.

Table 2. Abundance of eggs by station and species.

Taxa/Station	78	79	80	81	82	83	84	85	86	87	88	89	90	91	93	94	95
<i>Auxis thazard</i>	0	0	0	0	0	10	0	0	69	0	6	0	0	0	33	0	0
<i>Benthodesmus</i> sp.	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	4	1
<i>Brama</i> sp.	0	6	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1
<i>Calamus calamus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0
<i>Chauliodus danae</i>	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
<i>Chauliodus sloani</i>	0	0	0	0	0	0	3	0	0	0	2	1	0	0	0	0	0
<i>Chauliodus</i> sp.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caranx hippos</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
<i>Coryphaena equiselis</i>	0	0	2	0	0	3	0	0	0	0	2	1	0	0	2	0	0
<i>Coryphaena hippurus</i>	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0
<i>Decapterus punctatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0
<i>Decapterus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	234	62	0	0	0
<i>Diplospinus multistriatus</i>	9	0	0	3	20	5	5	0	0	3	0	4	0	0	5	0	0
<i>Diodon holocanthus</i>	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Exocoetidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Gempylus serpens</i>	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0

<i>Istiophorus platypterus</i>	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0
<i>Kajikia albida</i>	3	2	1	0	0	0	0	0	0	0	3	0	0	0	0	0	0
<i>Katsuwonus pelamis</i>	0	1	0	0	5	0	0	0	0	0	0	0	0	0	124	0	1
<i>Lactophrys trigonus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
<i>Lampris guttatus</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leptostomias sp.</i>	0	0	0	9	0	0	0	1	0	0	0	12	0	0	0	0	0
<i>Lophotus lacepede</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0
<i>Nesiarchus nasutus</i>	41	68	0	0	0	235	2	4	0	0	0	0	0	0	101	0	0
Perciformes I	0	0	0	0	0	0	0	4	0	0	0	0	0	3	0	0	0
Perciformes II	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Peristedion sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Priacanthus arenatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Prognichthys occidentalis</i>	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Promethichthys prometheus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
<i>Pterycombus brama</i>	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
<i>Regalecus glesne</i>	0	0	0	0	0	9	0	0	1	11	2	6	4	0	3	0	7
<i>Remora osteochir</i>	0	0	0	0	0	3	1	0	0	0	0	0	1	0	0	0	1
<i>Saurida normani</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Scombrolabrax heterolepis</i>	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synagrops bellus</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

<i>Synodus intermedius</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
<i>Synodus synodus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	34	0	0	0
<i>Thunnus atlanticus</i>	0	0	0	0	0	0	0	0	0	3	5	0	0	5	0	0	0
<i>Trachinotus falcatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Trachipterus sp.</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Xiphias gladius</i>	0	3	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0

The richest station was 91, with ten taxa, followed by stations 88 and 90, both with nine taxa. The southern stations 78, 81 and 82 were the least rich, with only three taxa present (Fig. 13 and Table 2).

Fig. 13. Eggs richness by station. The size of the circle indicates the number of taxa per station.

The most common species was *N. nasutus*, represented by 451 eggs, distributed in six stations. *D. multistriatus* and *R. glesne*, distributed in eight stations, are considered the most broadly distributed (Table 2).

No pattern of distribution was found for any species. However, *N. nasutus* was more common in the southernmost stations. *D. multistriatus*, one of two widely distributed species, was present in the south and central stations, and *R. glesne* was recorded at the north and central stations (Fig. 14).

Fig. 14a-c. Species distribution. Distribution of fish eggs from the more abundant and more common species, a) *N. nasutus*, b) *D. multistriatus* and c) *R. glesne*

New information about spawning locality and time was obtained from egg records for *C. calamus*, *C. danae*, *C. sloani*, *D. multistriatus*, *G. serpens*, *L. trigonus*, *L. guttatus*, *L. lacepede*, *N. nasutus*, *P. arenatus*, *P. occidentalis*, *P. prometheus*, *P. brama*, *R. osteochir*, *S. normani* and *S. synodus*. These data are critical to protect nurseries and spawning areas, especially for species of economical or game interest [51].

When the eggs were collected, some (258) were unfertilized, the rest were fertilized and the majority were more or less in the same developmental stage, except in *K. albida* (Fig. 15a, b, c, d), *K. pelamis* (Fig. 16a, b, c, d, e, f) and *R. osteochir* (Fig. 17a, b, c, d, e, f). In these species, we observed differences in the embryo morphology and the tone and distribution of the pigments between the eggs.

Fig. 15a-d. Changes in the morphology of *Kajikia albida*. Photographs of eggs from the same species, collected in different stations, with diverse morphological characteristics.

Fig. 16a-f. Changes in the morphology of *Katsuwonus pelamis*. Photographs of eggs from the same species, collected in different stations, with diverse morphological characteristics.

Fig. 17a-f. Changes in the morphology of *Remora osteochir*. Photographs of eggs from the same species, collected in different stations, with diverse morphological characteristics.

Generally, when we analyzed the developmental stage of these three species, the day and the station in which the eggs were collected, we observed that the less developed stages were found in the southern stations (78, 79, 82 and 83), whereas the more developed stages were found in northern stations (90, 93 and 95) during the three sampling days. With these data, we can make

some inferences: most likely, spawning was performed in Belizean waters, and eggs were advected northward according to observed currents in this area [52,53]. Another possibility is that the spawning zones were in the coastal area of Xcalak and the vicinity of Banco Chinchorro, where spawning aggregations sites have been documented [54-56]. In both cases, egg hatching most likely would occur in the northern part of the Yucatan Peninsula. Timing of egg release and the likely trajectories caused by the currents is out of the scope of the present work; however, it is an interesting issue to be investigated.

Generally, it has been reported that the duration of egg incubation is inversely proportional to the water temperature [57]. In the Caribbean, the surface water is warm, and specifically at the sampling stations, the temperature averaged approximately 27°C.

The sampling area is influenced by the Caribbean circulation system [52,53], and it has been suggested that there may be connectivity among all the MBRS [53,58]. Therefore, the presence of eggs of species that were not previously recorded may be caused by the advection from the currents, or the adults might migrate to these places only to spawn. However, it requires deeper and systematic oceanographic studies to determine the path of the eggs.

Conclusion

Our results add fundamental evidence for fisheries management, allowing focus on the conservation efforts in breeding areas. The information on egg distribution and possible spawning time provides a fundamental basis for studies that will support the sustainable use of the Mesoamerican Reef System. This information is of utmost importance because these species could be more vulnerable in their early developmental stages than in other stages. This assertion must be tested with more fieldwork and continuous research in the area.

This work is the first study in the MBRS to describe fish eggs relying on barcodes for identification. The importance of databases, such as BOLD, which allows connecting specimens by their geographical origin and sequences for future identification, is demonstrated. Thus, these types of studies will reduce the complexity of laboratory work, making it unnecessary to reproduce natural conditions to hatch the eggs and rear the larvae to identifiable stages, as required in the past. Finally, obtaining accurate identifications of fish eggs will be important to assess fish stocks in this large Mesoamerican region

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Supporting Information

S1 Fig. Full neighbor-joining tree. The tree is based on genetic distances (K2P) from the COI gene. Specimens found in this work by taxa, with their respective BIN.

Figures

Figure 1. Sample stations. Geographical location of the sample stations

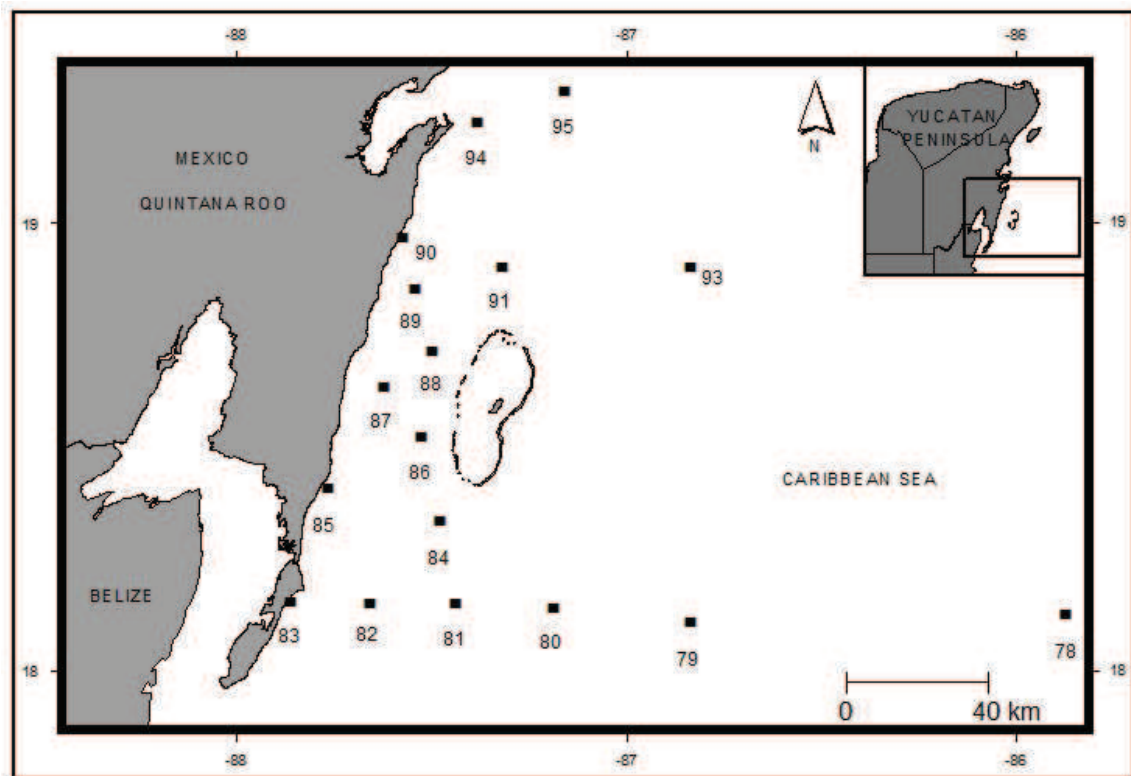
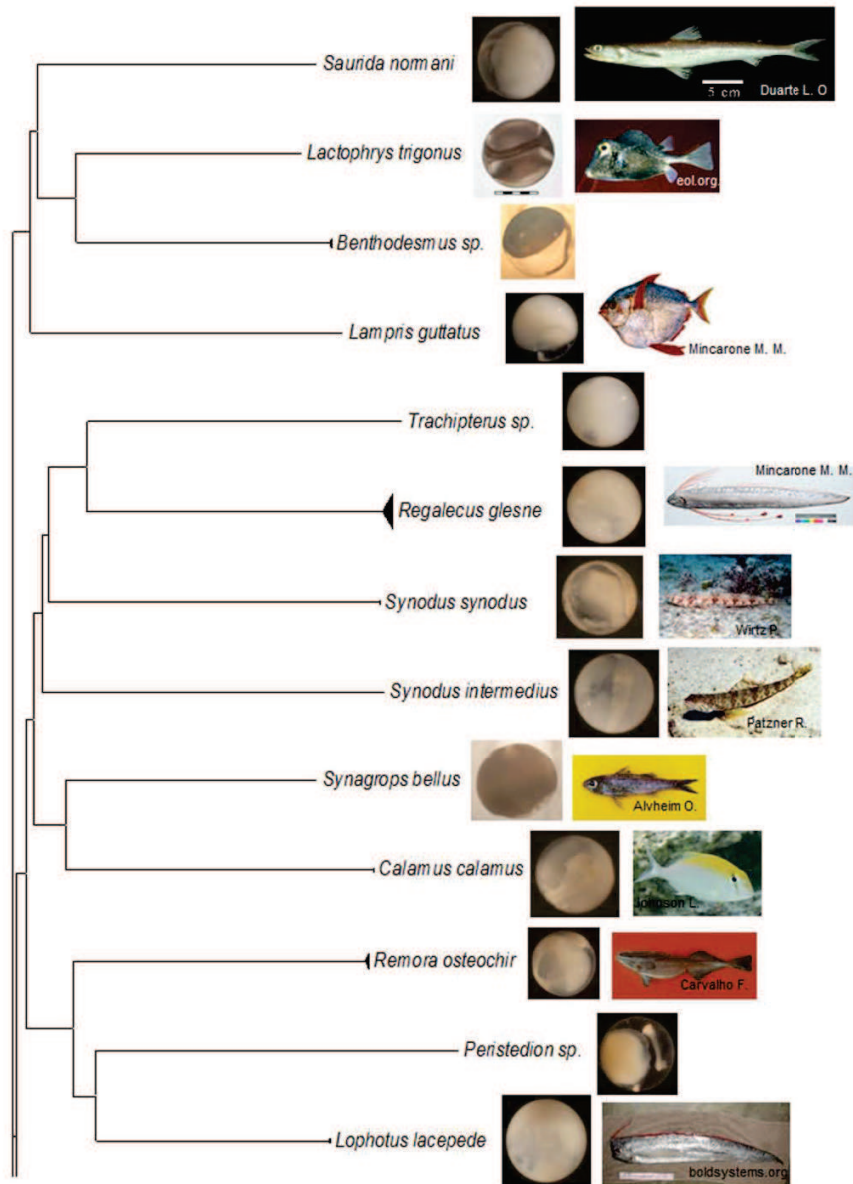
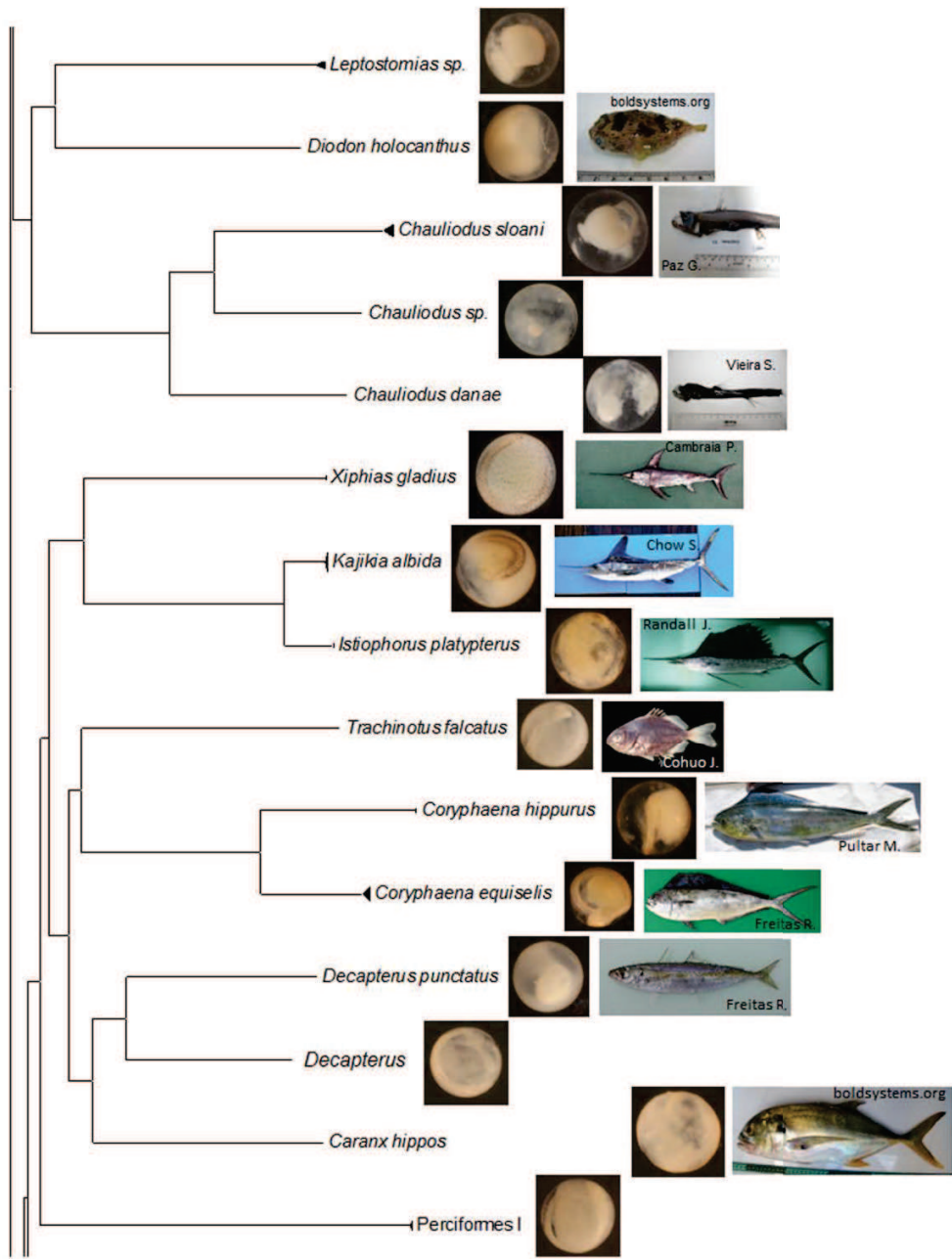


Figure 2. Neighbour joining tree for taxa found in this work. Tree is based on genetic distances (K2P) for the COI gene. The base of triangle represents the quantity of specimens sequenced. Images of eggs and adults are included for species.





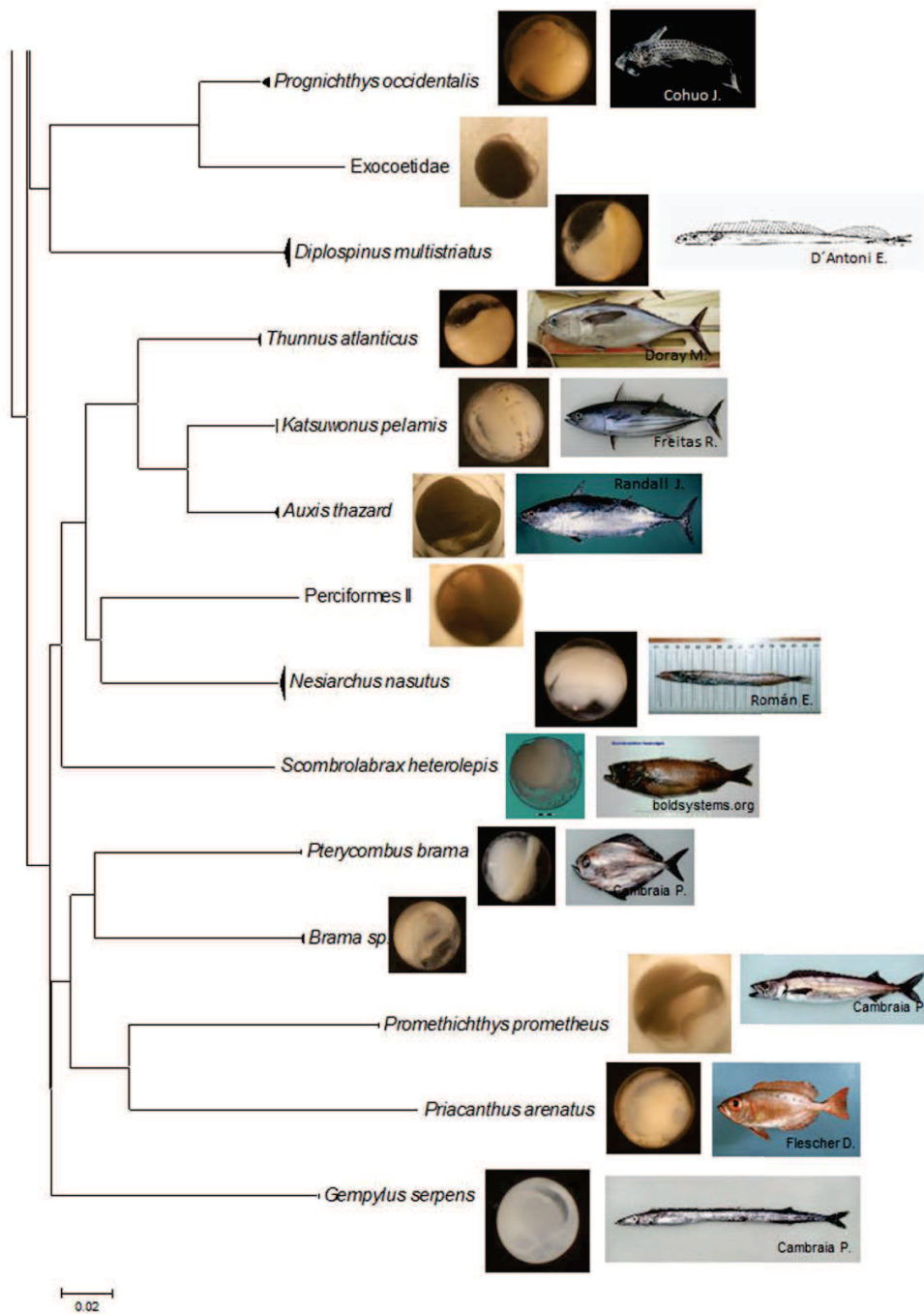


Figure 3a-f. New morphological descriptions. *Calamus calamus* (a, b, c) *Caranx hippos* (d) *Chauliodus danae* (e), *Decapterus punctatus* (f). Increase 4x, measuring slide equals 0.95 mm and increase 10x equals 0.37 mm. Main morphological characteristics are highlighted in each photo.

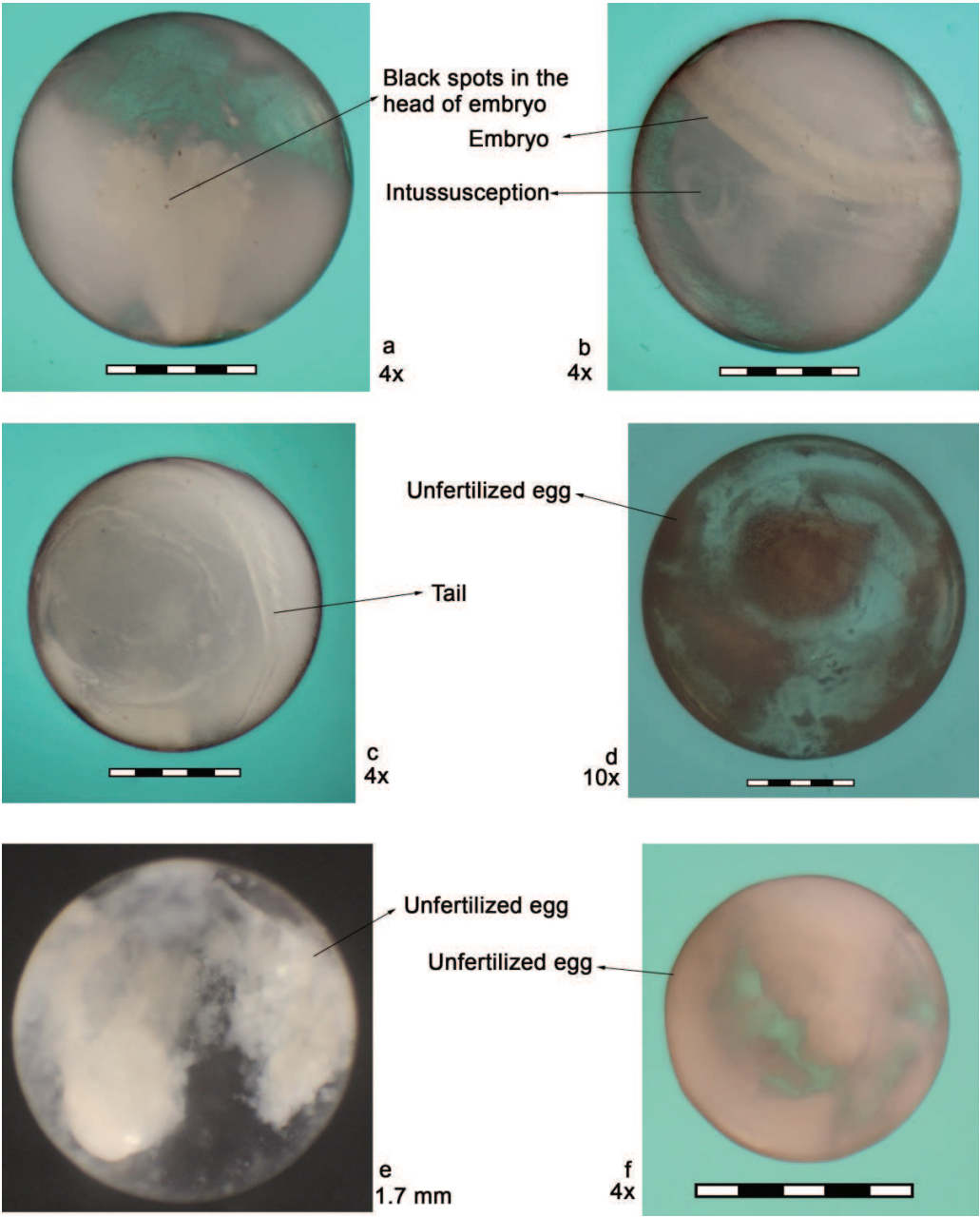


Fig. 4a-f. New morphological descriptions. *Diplospinus multistriatus* (a, b, c), *Gempylus serpens* (d), *Kajikia albida* (e, f). Increase 4x, measuring slide equals 0.95 mm and increase 10x equals 0.37 mm. Main morphological characteristics are highlighted in each photo.

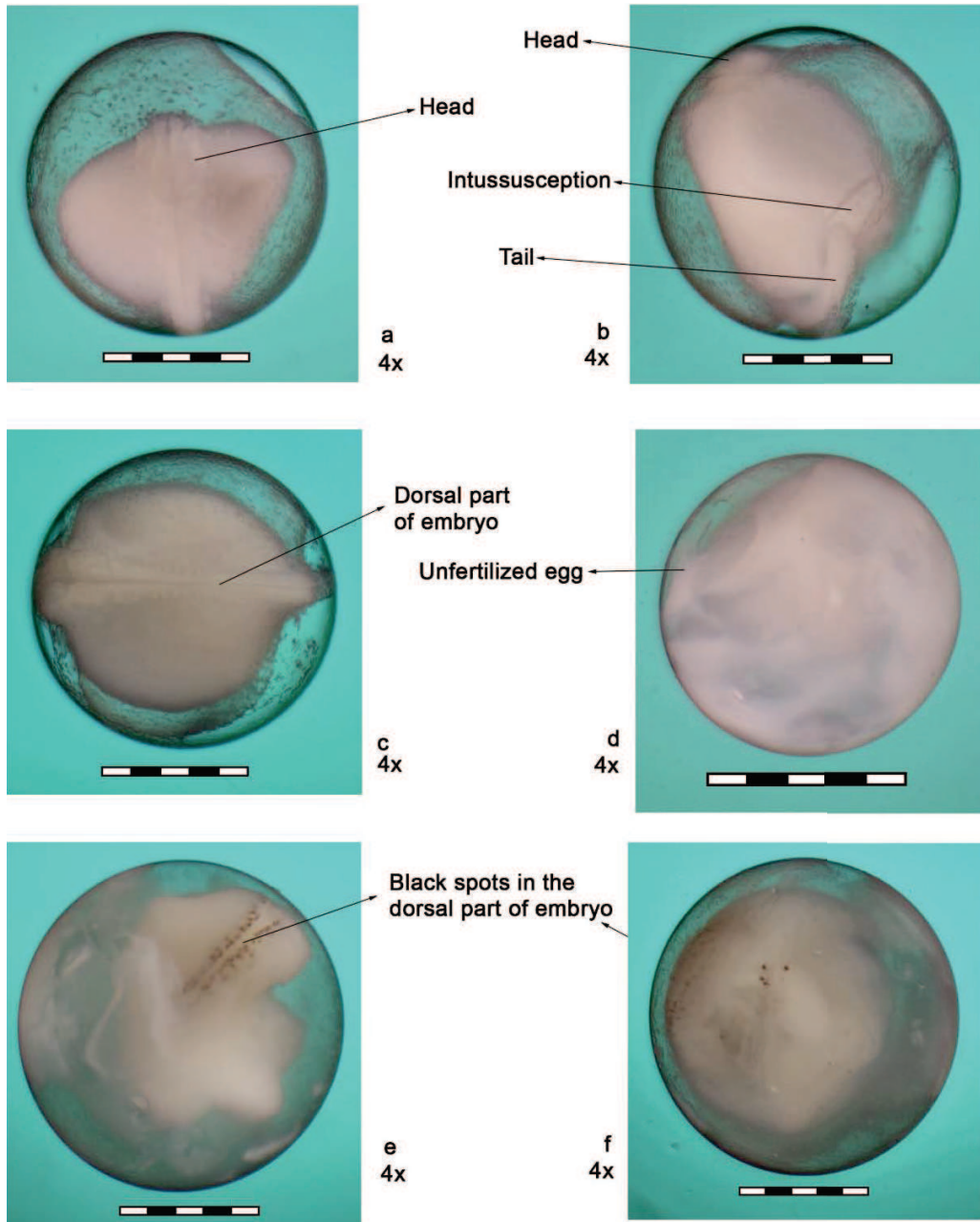


Fig. 5a-f. New morphological descriptions. *Lactophrys trigonus* (a, b, c), *Nesiarchus nasutus* (d, e, f). Increase 4x, measuring slide equals 0.95 mm and increase 10x equals 0.37 mm. Main morphological characteristics are highlighted in each photo

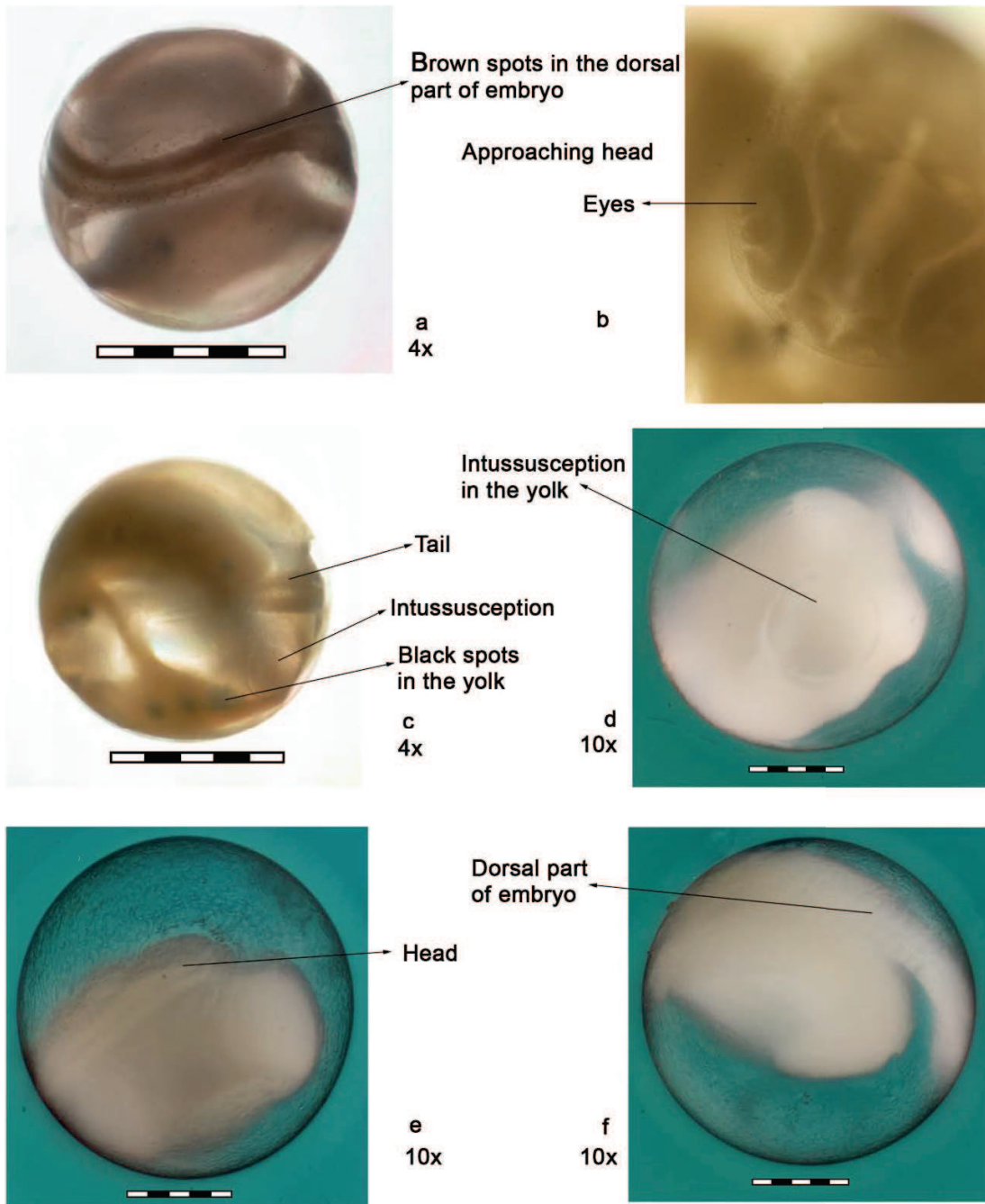


Fig. 6a-f. New morphological descriptions. *Priacanthus arenatus* (a, b), *Prognichthys occidentalis* (c), *Promethichthys prometheus* (d, e, f). Increase 4x, measuring slide equals 0.95 mm. Main morphological characteristics are highlighted in each photo.

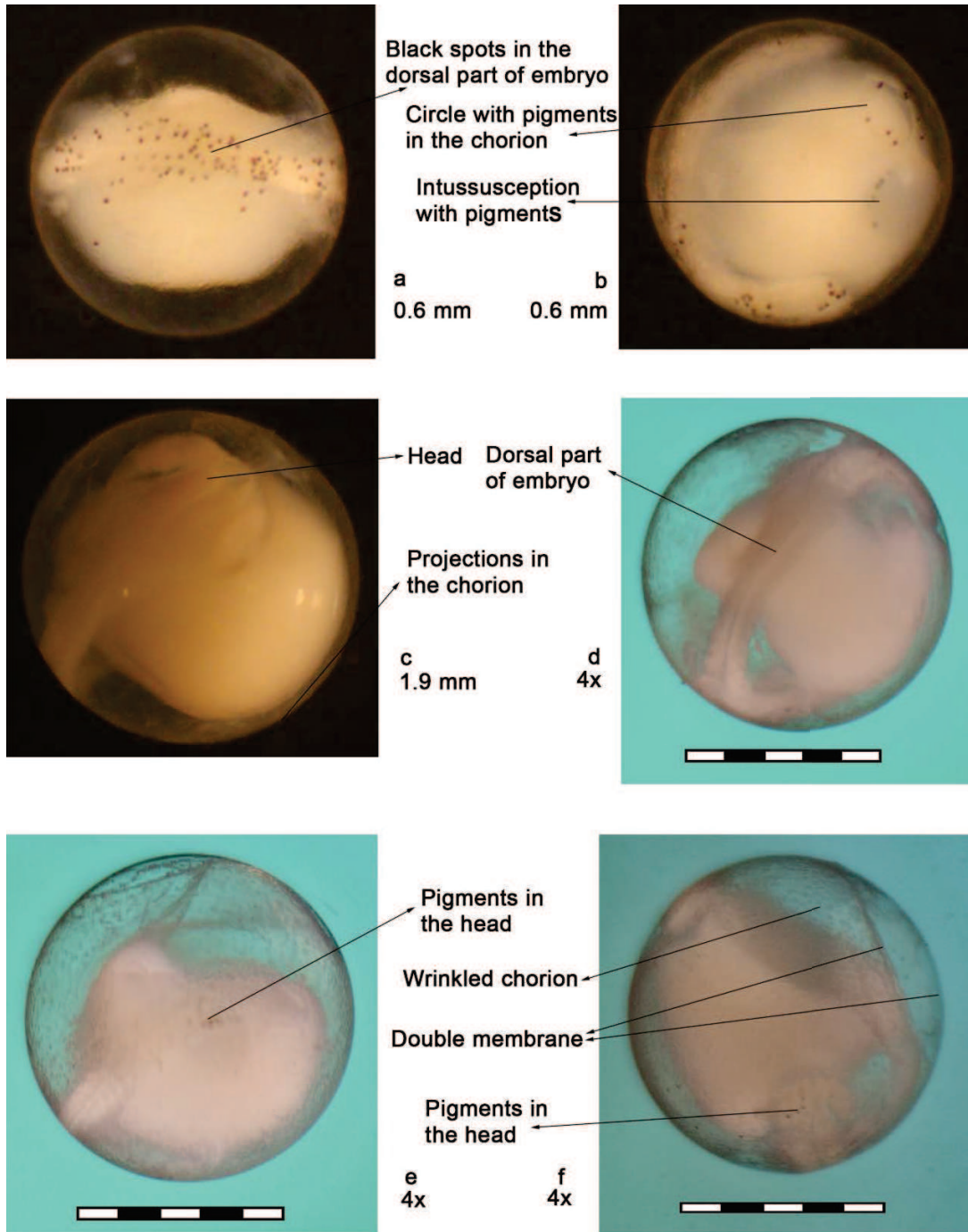


Fig. 7a-e. New morphological descriptions. *Pterycombus brama* (a, b, c), *Remora osteochir* (d, e). Increase 4x, measuring slide equals 0.95 mm. Main morphological characteristics are highlighted in each photo.

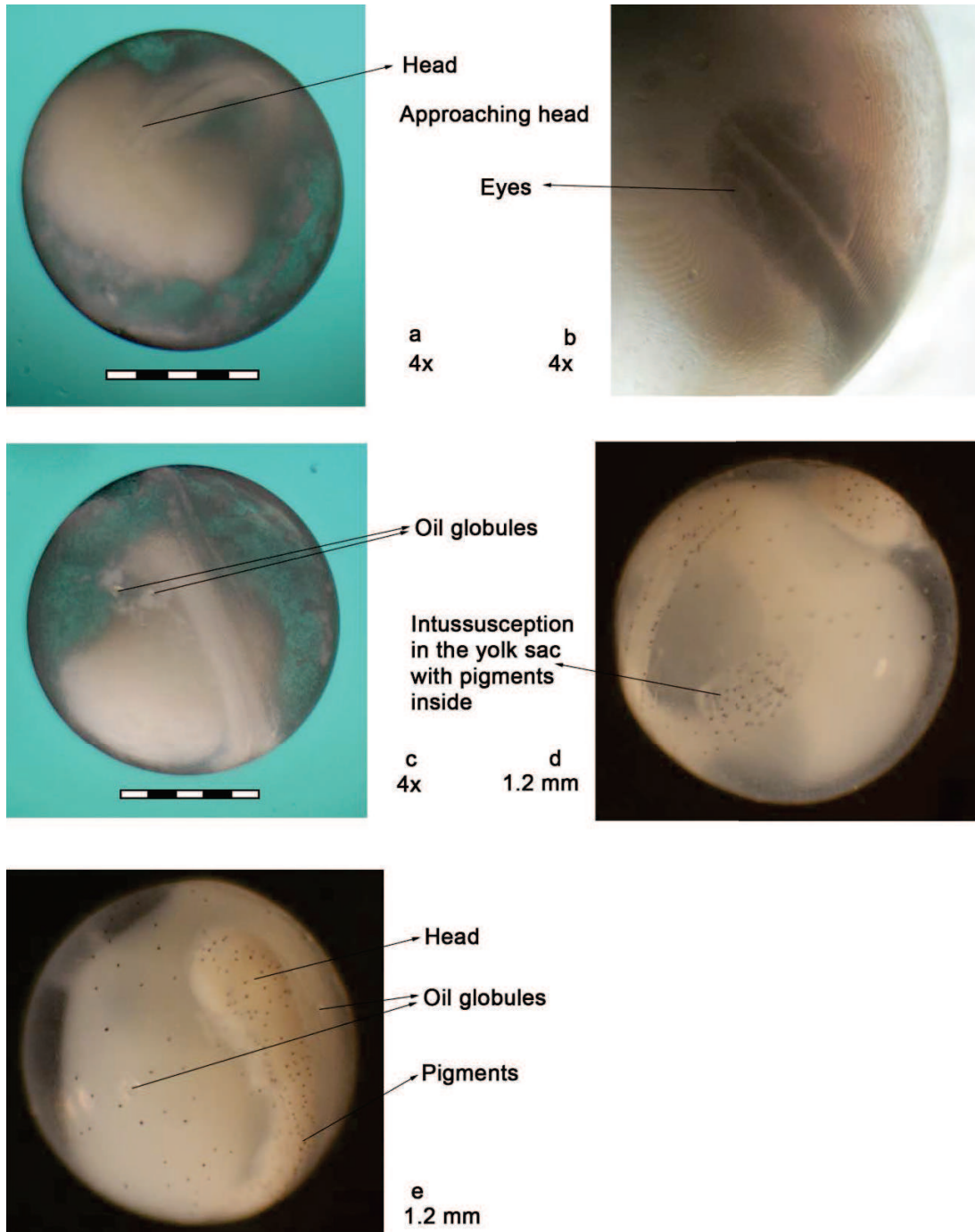


Fig. 8a-f. New morphological descriptions. *Saurida normani* (a), *Scombrolabrax heterolepis* (b), *Synagrops bellus* (c) and *Synodus synodus* (d, e, f). Increase 4x, measuring slide equals 0.95 mm and increase 10x equals 0.37 mm. Main morphological characteristics are highlighted in each photo

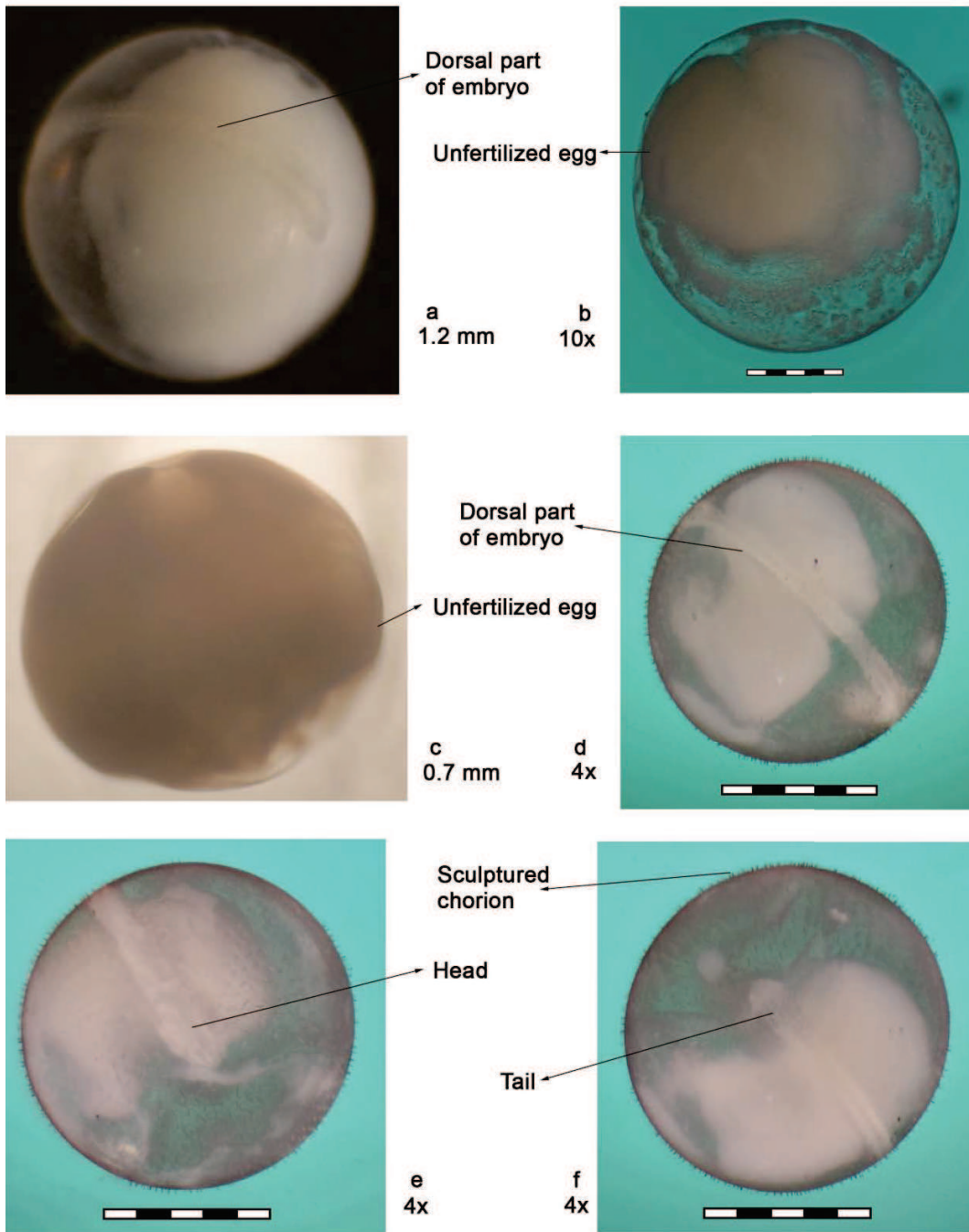


Fig. 9a-c New morphological descriptions. *Thunnus atlanticus* (a), and *Trachinotus falcatus* (b, c). Increase 4x, measuring slide equals 0.95 mm. Main morphological characteristics are highlighted in each photo

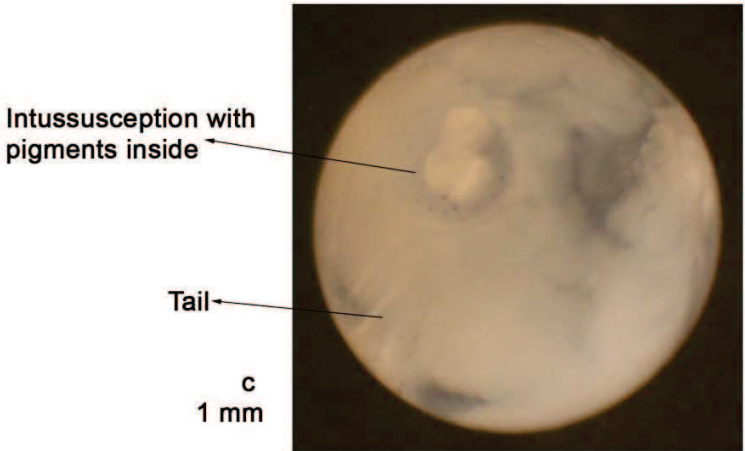
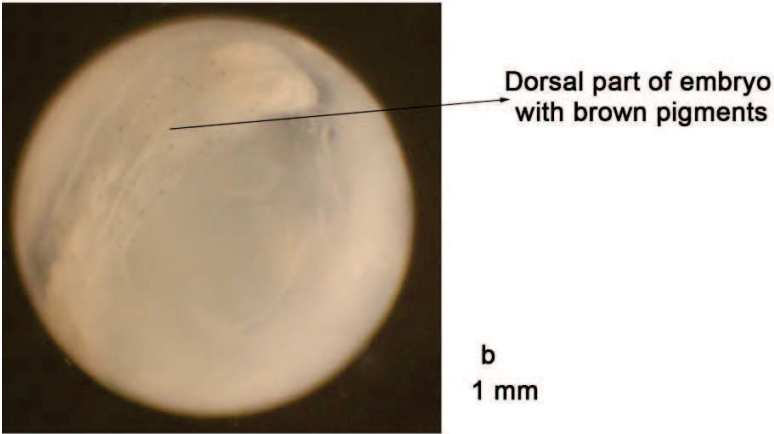
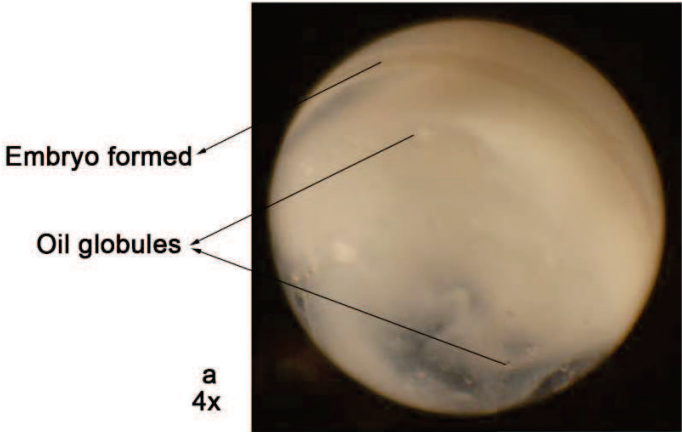


Fig. 10a-f Differences from previous fish egg descriptions. *Auxis thazard* (a) *Coryphaena equiselis* (b), *Coryphaena hippurus* (c), *Istiophorus platypterus* (d), and *Katsuwonus pelamis* (e, f) without oil globules and the two last species with an intussusceptions in the yolk sac.

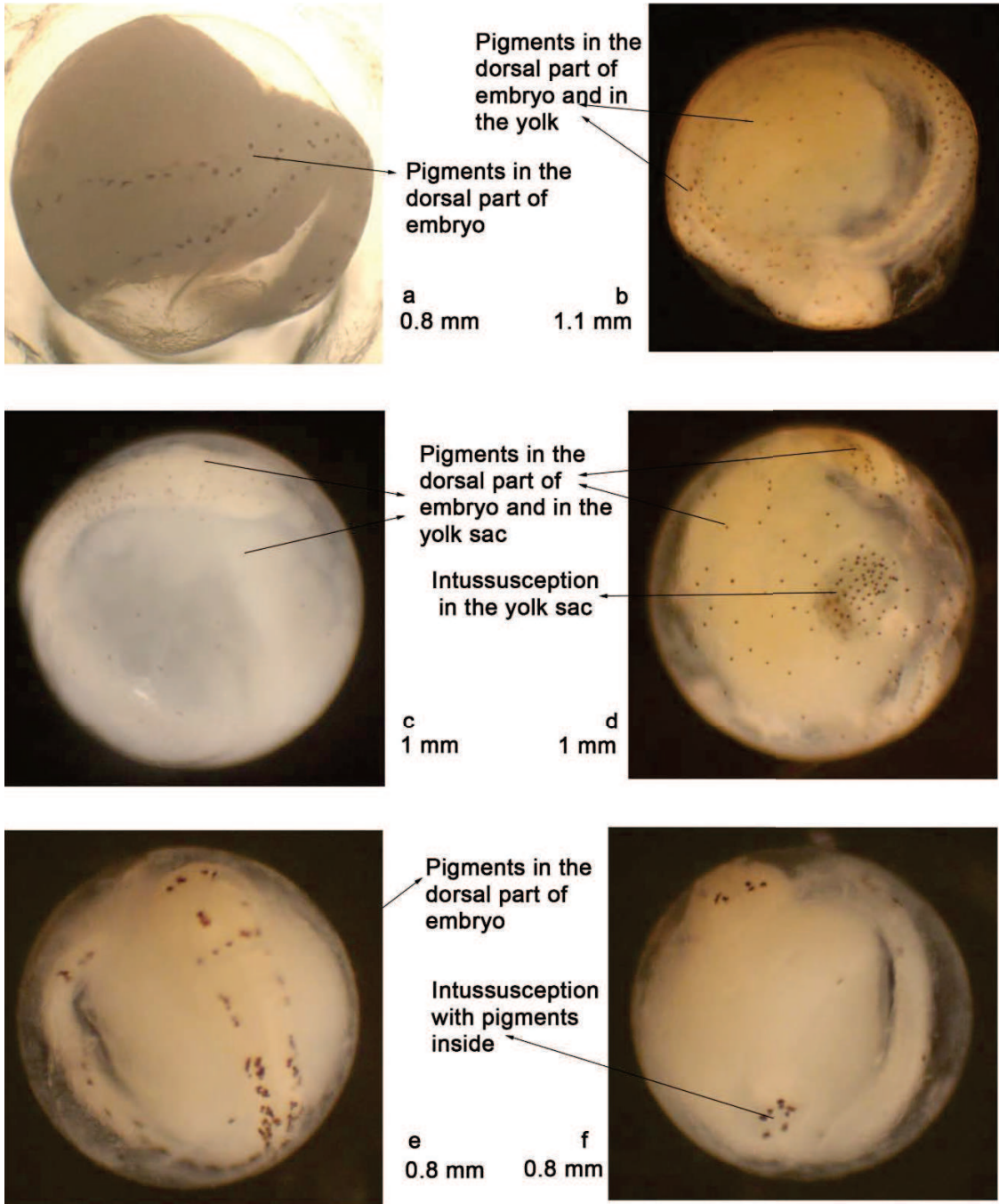


Fig. 11a-f. Differences from previous fish egg descriptions. *Chauliodus sloani* with wide perivitelline space and without double membrane (a). *Lophotus lacepede* without spines (b). *Regalecus glesne* with small projections and without oil globules and pigments(c, d). *Synodus intermedius* with pigments in the head and without ornate corion (e, f).

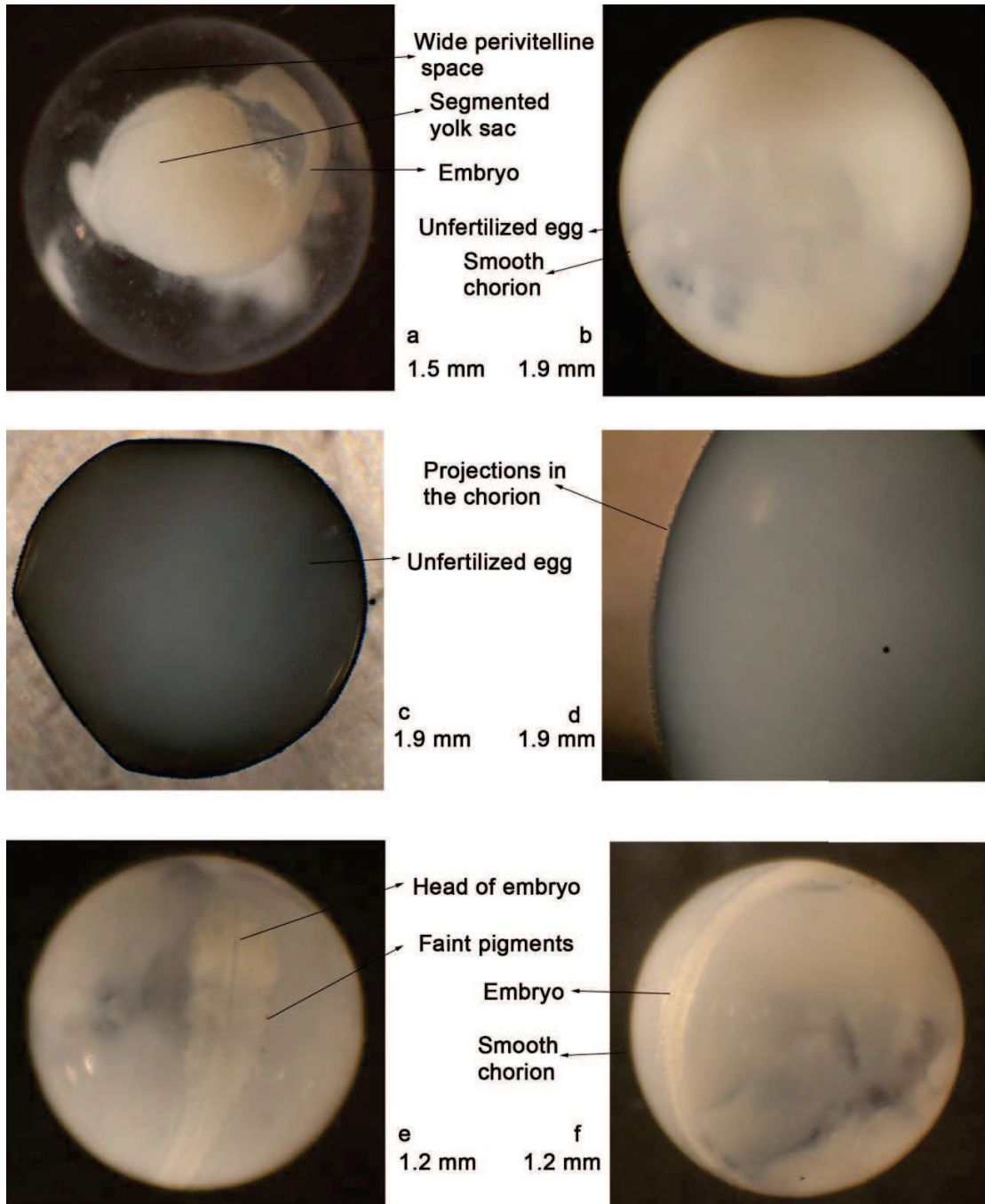


Fig. 12. Total abundance of eggs by station. The size of circle represents the number of eggs by station.

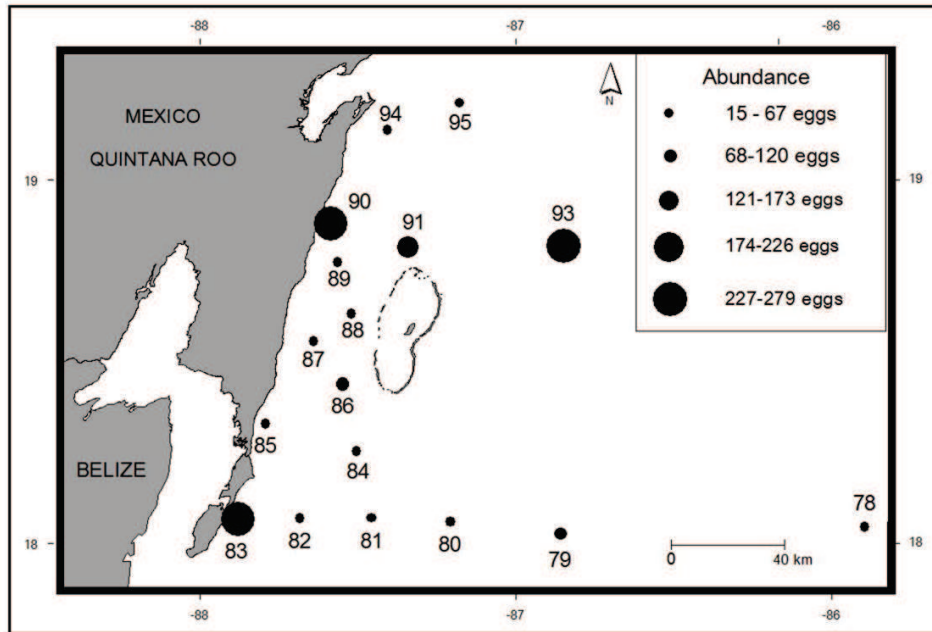


Fig. 13. Eggs richness by station. The size of circle indicates the number of taxa for station.

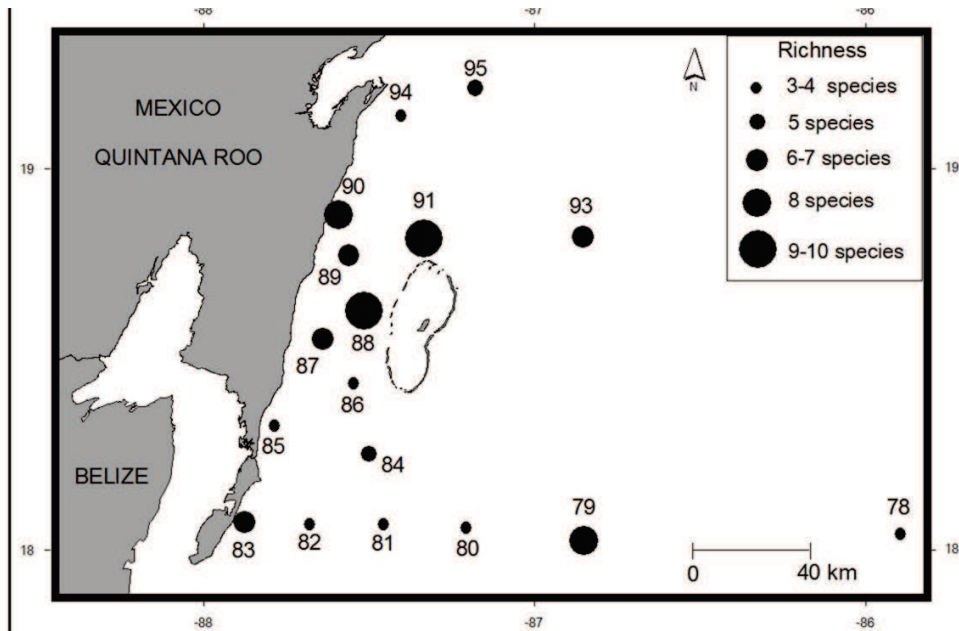


Fig. 14a-c. Species distribution. Distribution of fish eggs from the more abundant and more present species, a) *N. nasutus*, b) *D. multistriatus*, c) *R. glesne*

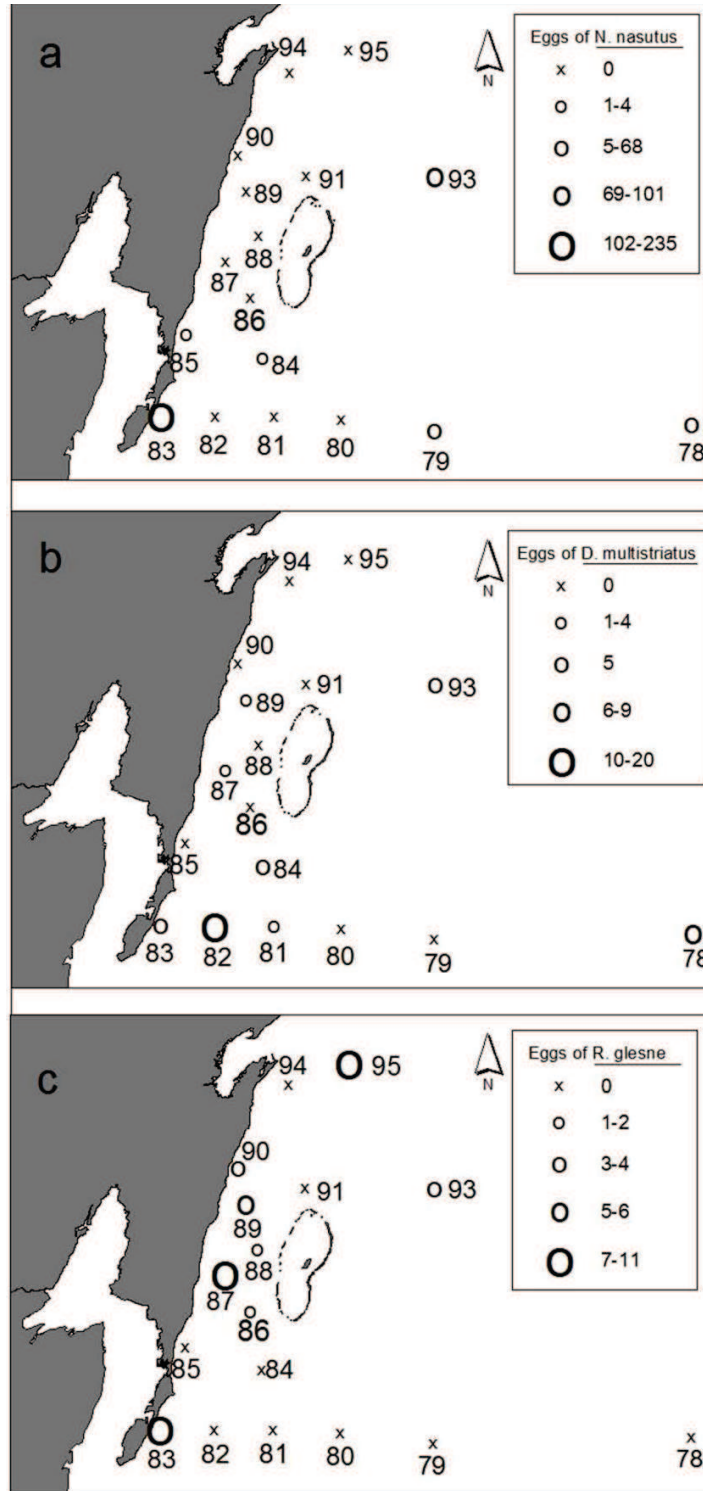


Fig. 15a-d. Changes in the morphology of *Kajikia albida*. Photographs of eggs from the same species, collected in different stations and with diverse morphological characteristic.

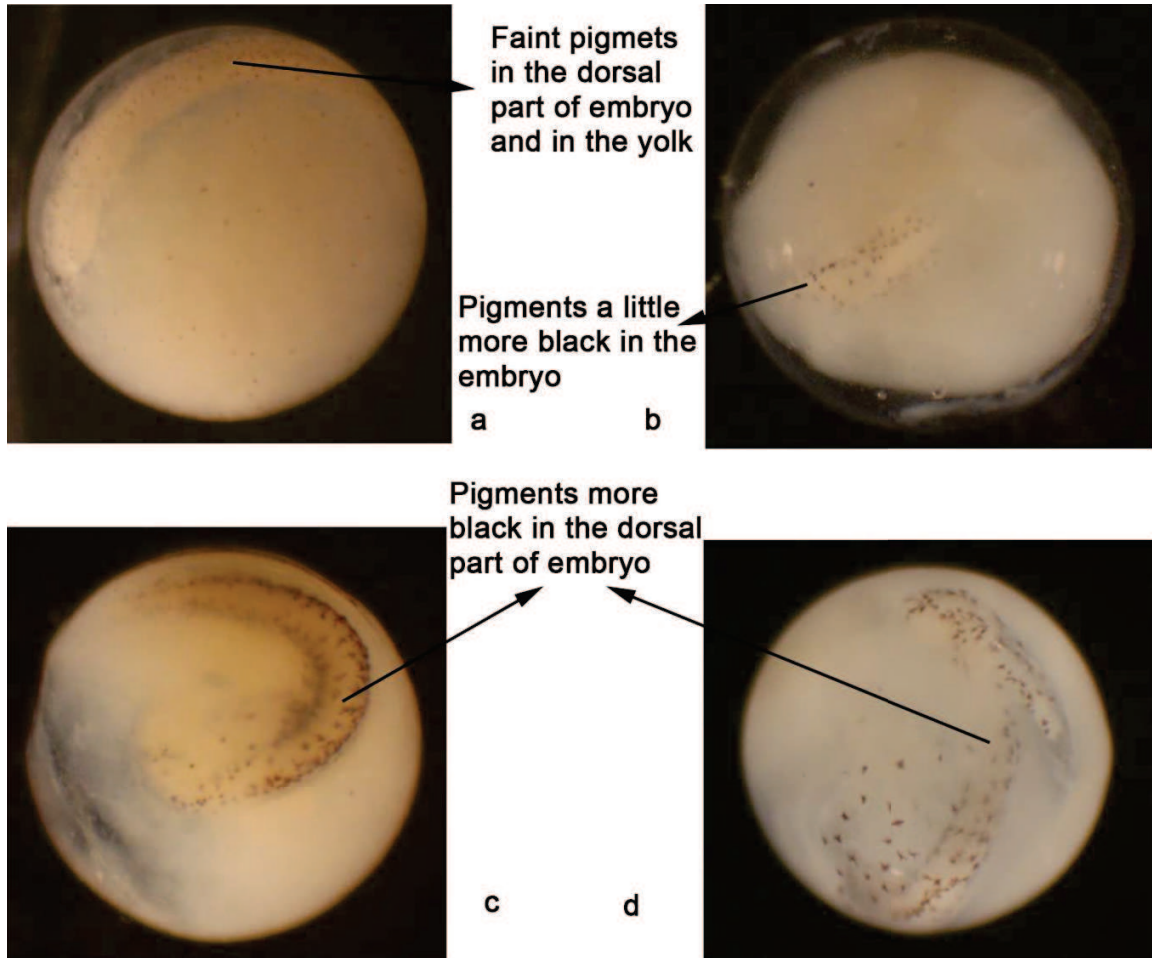


Fig. 16a-f. Changes in the morphology of *Katsuwonus pelamis*. Photographs of eggs from the same species, collected in different stations and with diverse morphological characteristic.

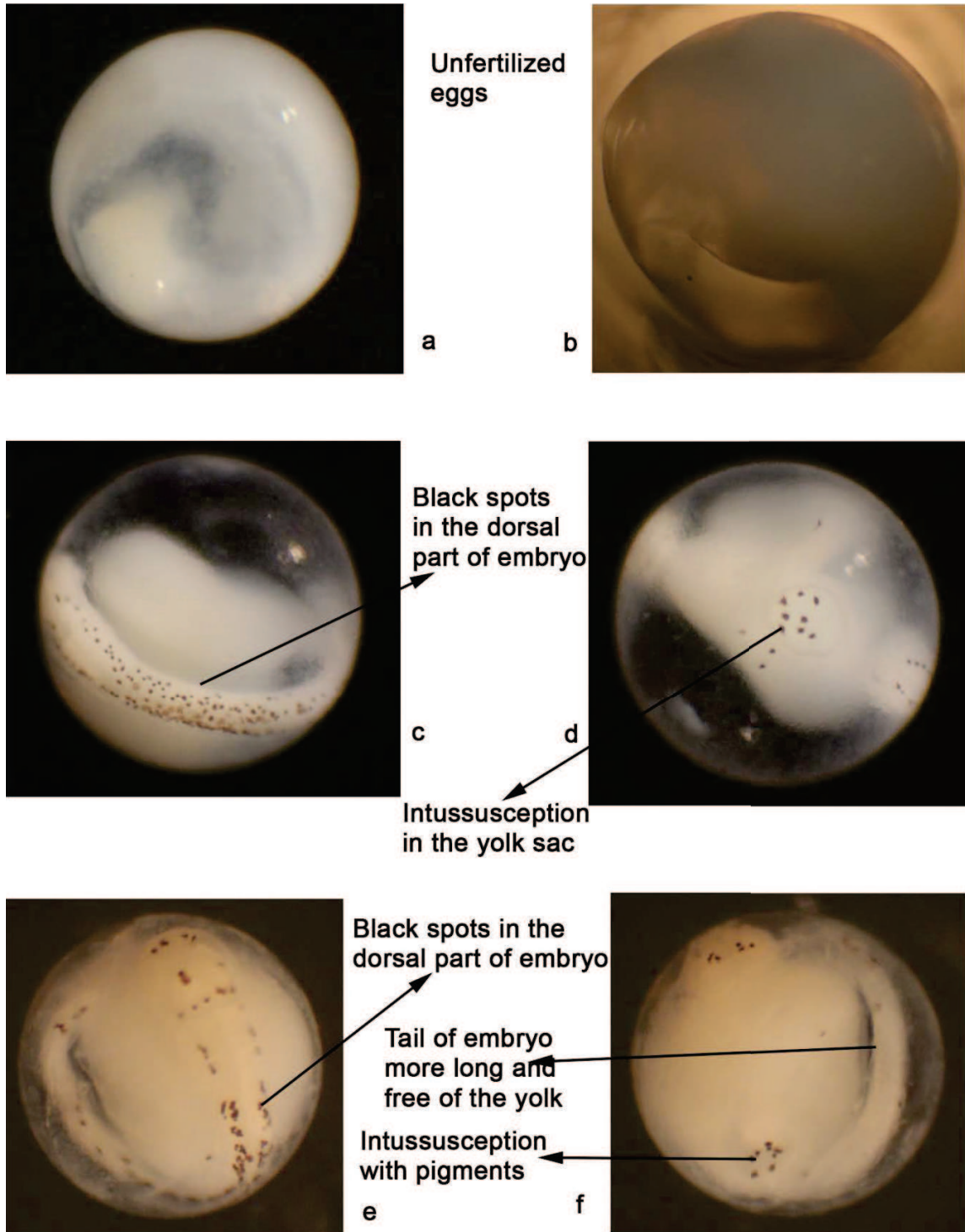
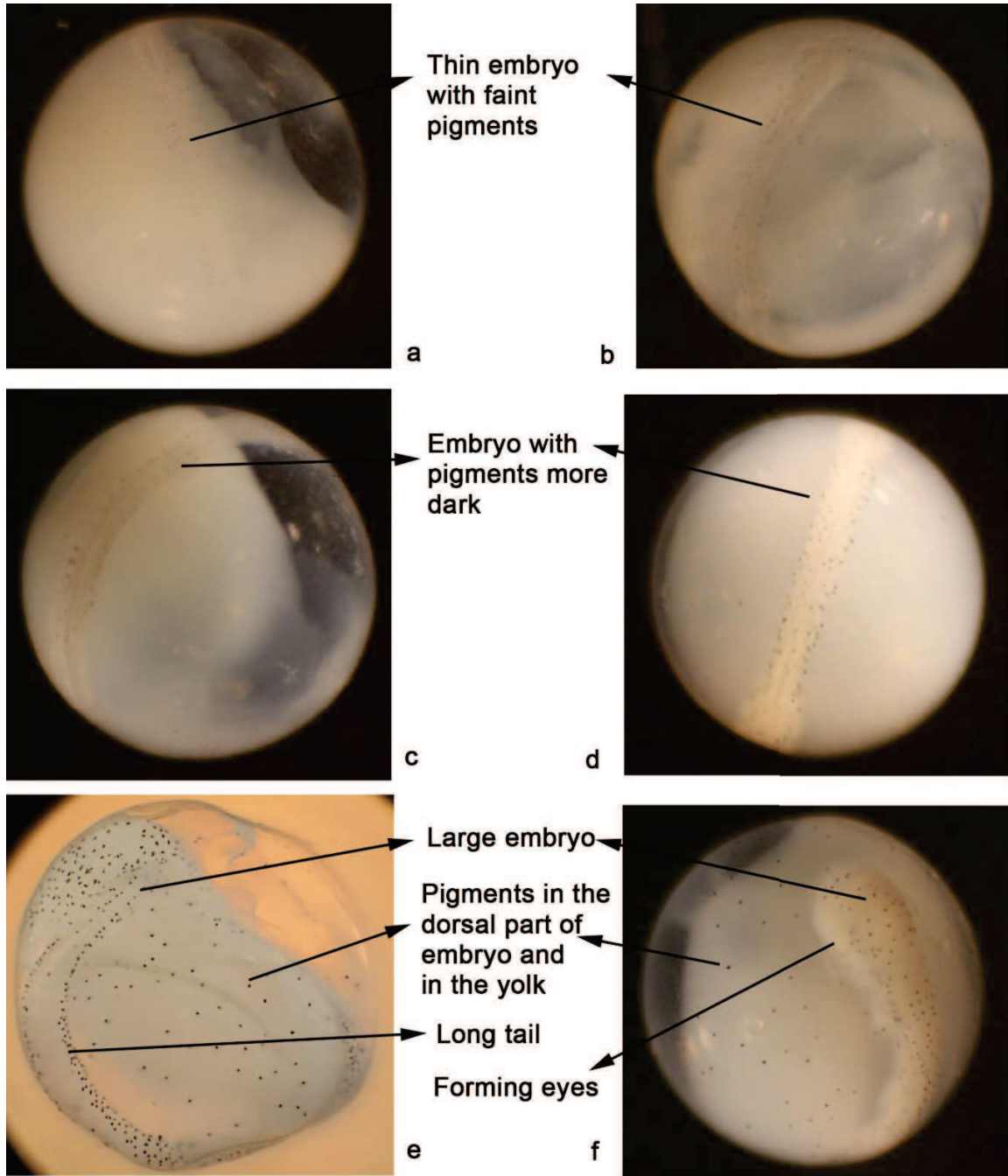


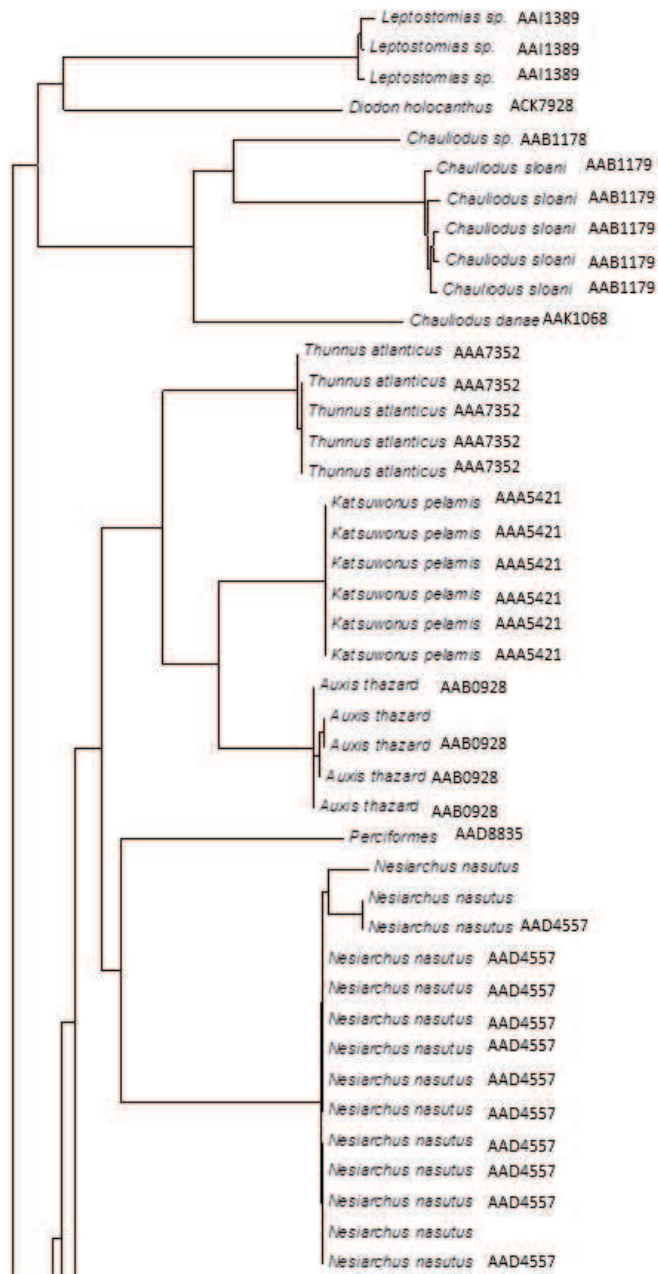
Fig. 17a-f. Changes in the morphology of *Remora osteochir*. Photographs of eggs from the same species, collected in different stations and with diverse morphological characteristic.

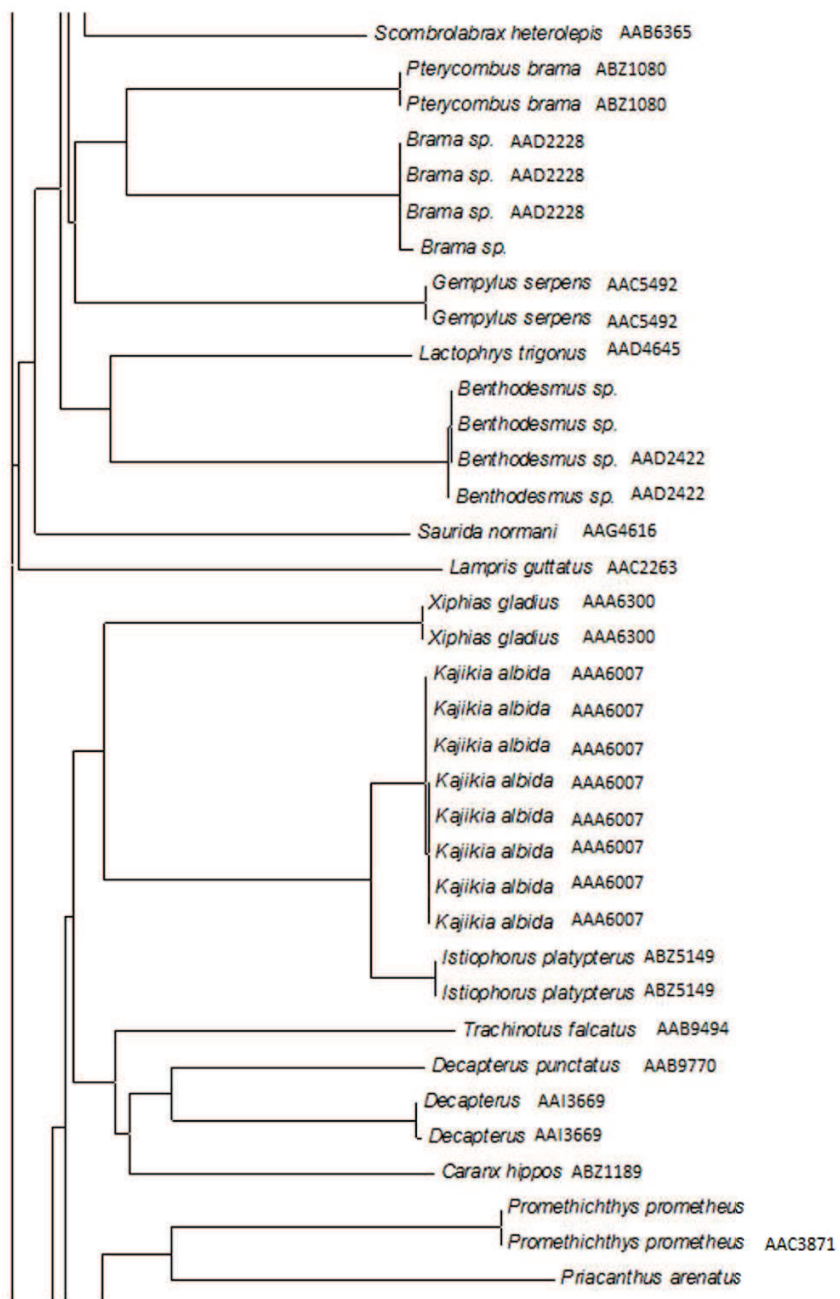


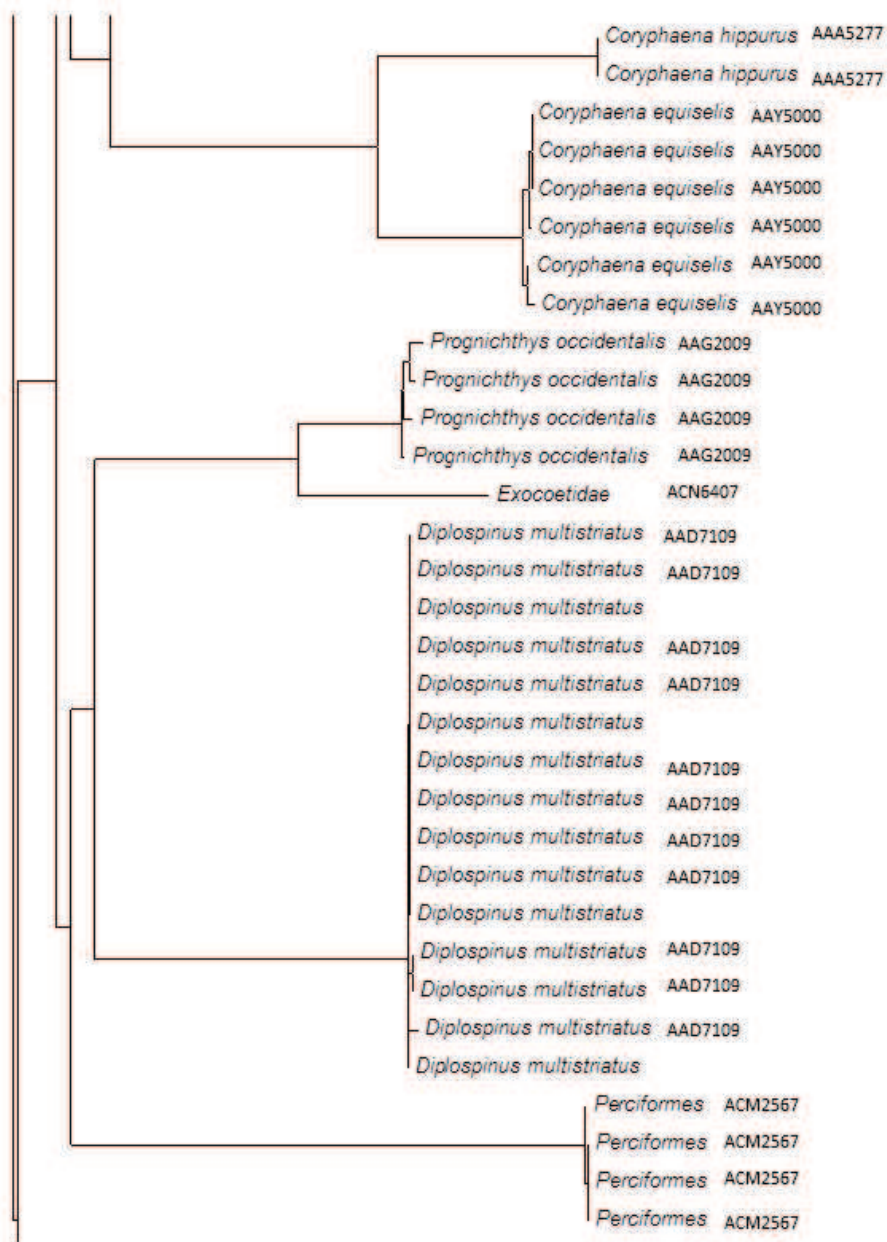
Supporting Information

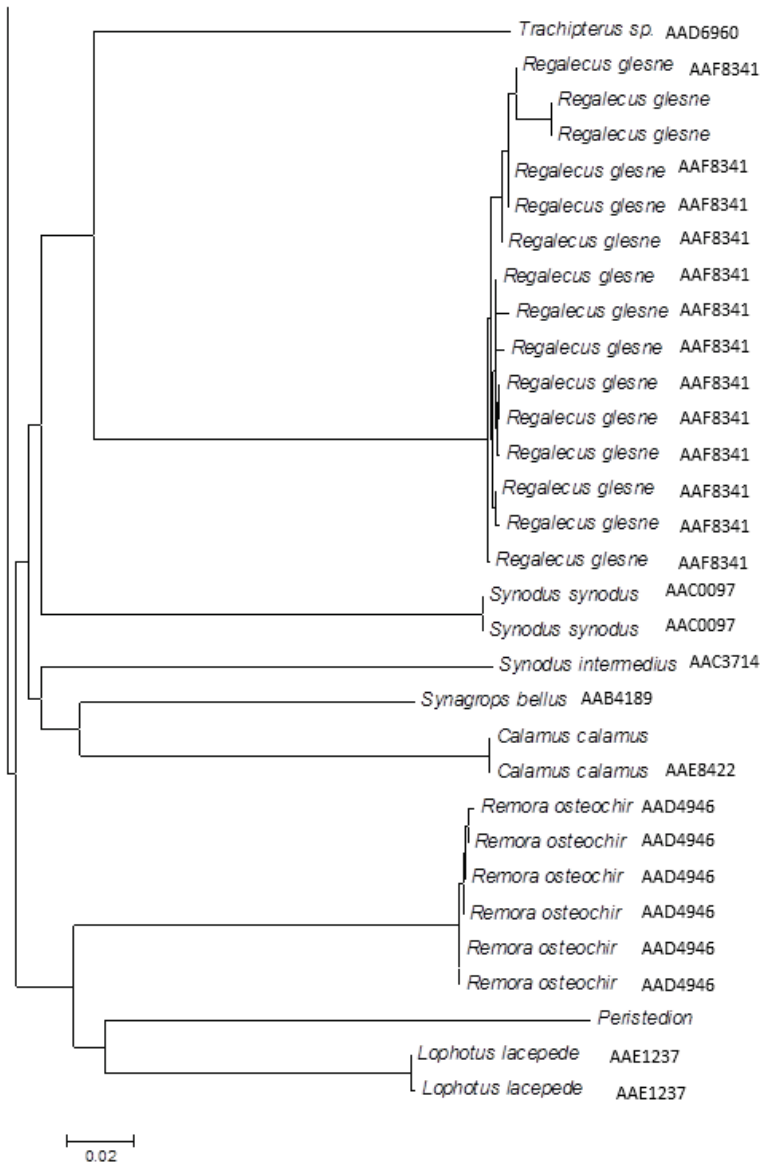
S1 Fig. Full neighbour joining tree. Tree is based on genetic distances (K2P) for the COI gene.

Specimens by taxa found in this work with their respective BIN.









CONCLUSIONES

Los códigos de barras de ADN y el uso de la base de datos de www.BOLDsystems.org han demostrado ser una muy buena herramienta para la correcta identificación de peces en estadio de huevos, en el presente trabajo se logró la identificación a nivel de especie de un buen número de taxa (42 especies). Se obtuvo una riqueza de especies alta, usando un menor número de ejemplares que los utilizados en otros trabajos.

Con lo cual se concluye que la combinación de la morfología y las herramientas moleculares (secuencias de ADN) son un complemento ideal para describir y/o confirmar la identidad de los huevos de diferentes organismos.

Se corroboraron y rectificaron algunos caracteres de 12 especies que ya presentaban descripciones previas. Por primera vez se da información de época y zona de desove para 16 especies, por lo cual el área y la temporada de muestreo del presente trabajo representan un indicio para continuar investigando sus zonas de reproducción.

No se identificó un patrón claro de distribución en las diferentes especies. Sin embargo *N. nasutus*, la especie más abundante, tuvo una mayor presencia en las estaciones ubicadas en la parte sur. *D. multistriatus*, una de las dos especies con más incidencia, se distribuyó en las estaciones del sur y centro y, *R. glesne*, la otra especie con mayor presencia, se encontró en las estaciones del norte y centro.

En este estudio se reportan nueve especies que no contaban con registro previo para la Península de Yucatán. La presencia de huevos de esas especies, podría deberse al transporte de los ejemplares por la corriente; ya que el área de muestreo se encuentra bajo la influencia del sistema de circulación de la Corriente del Caribe; o bien confirma la existencia de sitios de agregación reproductiva en los cuales los adultos migran al lugar solo para reproducirse. Sin embargo se requieren estudios más profundos y sistematizados para determinar el camino que los huevos, larvas o adultos recorren.

Nuestros resultados muestran que el área de muestreo es una zona importante de desove para varias especies. Por lo que, es importante continuar con la investigación en la zona, obtener mayor información, y así, enfocar los esfuerzos de conservación en las zonas de crianza de especies con importancia ecológica y comercial.

Este trabajo provee una base para continuar con la identificación y descripción de los huevos de peces del Caribe Mexicano, ya sea de muestras provenientes de cruceros de investigación como el Gordon Gunter o futuras colectas. La base de datos www.BOLDsystems.org ha demostrado ser de gran utilidad y confiabilidad en la conexión de las etapas tempranas del desarrollo de los peces con los adultos, permitiendo la identificación precisa de las especies.

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