# Mating Season, Egg Laying Season, and Internal Gametic Association in Sympatrically Occurring the Fluffy Sculpin (*Oligocottus snyderi*) and Rosy Sculpin (*O. rubellio*)

Running head: Reproductive Strategies in two marine sculpin species

Takeshi Ito[1,2], Breana N. Goldman[3], Satoshi Awata[2], and Karen D. Crow[3]

Correspondence

[1]Maizuru Fisheries Research Station, Field Science Education and Research Center, Kyoto University, Nagahama, Maizuru 625-0086, Japan.

Email: takeshi.itos12d418e@gmail.com

ORCID: 0000-0001-8064-6722

[2]Department of Biology, Graduate School of Science, Osaka Metropolitan University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585, Japan.

[3] Department of Biology, College of Science and Engineering, San Francisco State University, 1600 Holloway Avenue, San Francisco, CA 94132

# Abstract

Some marine sculpins (Psychrolutidae) exhibit an unusual reproductive mode called internal gametic association (IGA), in which sperm transfer between the sexes occurs during copulation, but fertilization is delayed until the eggs are released in seawater. IGA is suggested in many internally inseminating marine sculpins, but experimental evidence of IGA is limited to a few species. The Fluffy Sculpin (*Oligocottus snyderi*) and sister species Rosy Sculpin (*O. rubellio*), occur in sympatry in intertidal zones along the central California coast. Although these species likely exhibit internal insemination, their reproductive strategy is not well understood. Here, we investigate reproductive mode, mating season, egg-laying season, and sperm morphology and activity in Fluffy and Rosy Sculpin near Pillar Point, CA. Embryonic development was observed for the eggs of the Rosy Sculpin exposed to seawater, indicating IGA in this species. We were unable to demonstrate IGA by initiation of development in the Fluffy Sculpin because we were unable to collect females with ovulated oocytes. Nevertheless, we found that sperm morphology with elongated head and high motility in isotonic solution while immotile in seawater in both species represent characteristics associated with IGA. Seasonal changes in gonadosomatic index (GSI) of both sexes revealed asynchronous gonadal maturation between the sexes in the Fluffy Sculpin, and suggests the similar pattern in the Rosy Sculpin, however small sample size in the latter was not definitive. These patterns indicate that males copulate with females before egg maturation, and females store sperm for several months. Our study supports the generality of IGA across marine sculpins and shows their association with the annual pattern in GSI. Further, while Fluffy and Rosy Sculpins are similar in body morphology, habitat, and reproductive mode, the slight difference in mating season (pre-mating isolation) and sperm head and flagella length

(post-mating isolation) may have contributed to divergence in sympatry with reduced probability of hybridization.

# INTRODUCTION

While most oviparous fishes are broadcast spawners, in which eggs and sperm are released into the water column followed by external fertilization, some exhibit internal insemination with copulatory behavior. Fishes with internal insemination either exhibit true internal fertilization or an unusual reproductive mode called internal gametic association (IGA, Munehara et al., 1989). In species with IGA, sperm transfer occurs during copulation, where a spermatozoon associates with the oocyte micropyle in the ovary (Koya et al., 2002). However, fertilization is delayed until the eggs are released in seawater (Munehara et al., 1989, 1991; Petersen et al., 2005; Koya et al., 2015). Therefore, while IGA species exhibit copulation, they do not exhibit internal fertilization. IGA was first confirmed in the elkhorn sculpin (*Alcichthys alcicornis*, superfamily Cottoidea: Munehara et al., 1989), and this reproductive mode was later found in several species of Cottoidea (e.g., Abe and Munehara, 2009 for review; Koya et al., 2015). Further, circumstantial evidence suggests that several species in other families may also exhibit IGA-like reproductive mode. Histological studies indicate that ovaries contain spermatozoa, but neither developmental oocytes nor early cleavage stages are found in the ovary (e.g., family Auchenipteridae: Burns et al., 2002; Parreira et al., 2009, family Characidae: Burns et al., 1997). Akagawa et al., (2008) also suggest that the Japanese tubesnout, *Aulichthys japonicus* (Aulorhynchidae, Gasterosteiformes), is likely to exhibit IGA from behavioral observations and a previous report (Okiyama et al., 1993, but this statement was cited from a symposium and the content could not be confirmed). Although the actual timing of fertilization has not been studied in these species, they probably exhibit a reproductive pattern similar to that of IGA. Such IGA and IGA-like reproductive mode evolved convergently in multiple lineages and therefore represent a good model for the evolutionary strategy of copulation (Abe and Munehara, 2009).

The superfamily Cottoidea previously contained seven families (Nelson 2006; Nelson et al., 2016). However, according to Smith and Busby (2014), based on phylogenetic analysis using both morphological and molecular data, the revised taxonomy of Cottoidea recognizes six families, one of which (Psychrolutidae) includes the former Bathylutichthyidae. In this revised taxonomy, nearly all former marine cottid genera (marine sculpins) and the former Psychrolutidae is included in Psychrolutidae, and the Cottidae is assigned to the majority of freshwater cottid genera (i.e. freshwater sculpins). Although the grouping of the revised taxonomy is appropriate, considering that 190 species of the traditional marine Cottidae phylogenetically include 35 Psychrolutidae, it would be more appropriate to group them together as Cottidae, or to give them a new family name, or to make marine Cottidae and freshwater Cottidae as a subfamily of Cottidae. We suggest renaming Cottidae and Psychrolutidae to other family name but followed revised taxonomy of Smith and Busby (2014), and marine sculpins are treated as Psychrolutidae.

Of the six families in superfamily Cottoidea, three families (Agonidae, Cottidae, and Psychrolutidae) include both internal insemination and/or true external fertilization (Abe and Munehara, 2009). In Cottidae (freshwater sculpin), most species exhibit true external fertilization but two species of the genus *Comephorus* have acquired internal fertilization and viviparity (e.g., Jakubowski et al, 2003, Ito et al. 2021). Psychrolutidae (marine sculpin), which refer to traditional marine Cottidae and Psychrolutidae, exhibit both true external fertilization and internal insemination with copulation. At least to date, no true external fertilization species have been found in Agonidae, all being internally inseminating species. In Psychrolutidae and Agonidae, previous studies have shown that all of the internally inseminating species examined experimentally exhibit IGA (Koya et al., 1993, 2015; Abe and Munehara, 2005, 2009). Species with IGA share several characteristics with true internal fertilizers. For example, most IGA species have a well-developed genital papilla for mating (Evans and Meisner, 2009) and have sperm with elongated head, in comparison with external fertilizing species that lack copulatory anatomy and exhibit round sperm head morphology (Koya et al., 2011; Buser et al., 2017; Ito and Awata, 2019; Ito et al., 2022). While IGA is interesting in the evolution of reproductive strategy, it is currently unknown how widespread this strategy is within Agonidae and Psychrolutidae. At present, 24 genera of Agonidae and Psychrolutidae are known to perform internal insemination spanning multiple independent clades (Agonidae: *Agonomalus*, *Blepsias*, *Brachyopsis*, *Hemitripterus*, *Ocella*, *Pallasina*, *Podothecus*; Psychrolutidae: *Alcichthys*, *Artedius*, *Astrocottus*, *Bero*, *Chitonotus*, *Clinocottus*, *Enophrys*, *Furcina*, *Icelus*, *Oligocottus*, *Orthonopias*, *Pseudoblennius*, *Psychrolutes*, *Radulinopsis*, *Synchirus*, *Vellitor*. See [Abe and Munehara, 2009; Munehara, 2011; Koya et al., 2015; Momota and Munehara, 2017; Awata et al, 2019, 2022]). However, only seven species have been experimentally demonstrated to exhibit IGA by observation of initiation of development via exposing the eggs to seawater (*Al. alcicornis*, Munehara et al., 1989; *Ar. harringtoni*, Petersen et al., 2005; *Bl. cirrhosus*, Munehara et al., 1991; *H. villosus*, Munehara et al., 1997; *Pa. barbata*, Momota and Munehara, 2017; *Po. Sachi*, Munehara 1997; *V. centropomus*, Koya et al., 2015).

Marine sculpins in the subfamily Oligocottinae (Psychrolutidae) are represented by 16 species including both external fertilizers and internal inseminators (Buser et al., 2017). They are all found in shallow waters of the west coast of North America and possess a suite of traits that enable them to exploit intertidal habitats (Ramon and Knope, 2008; Buser and López, 2015). Within the Oligocottinae, species of *Oligocottus* are dominant in shallow tidepools (Green, 1971; Miller and Lea, 1972; Nakamura, 1976). Historically, the Fluffy Sculpin (*Oligocottus snyder*i) was reported to exhibit internal fertilization in 1956 (Morris, 1956), and several studies have focused on the reproduction of the Fluffy Sculpin (Morris, 1956; Nakamura, 1976; Grossman and DeVlaming, 1984; Freeman et al., 1985). However, to our knowledge, neither IGA nor variation in gonadsomatic index (GSI), which is important to understand reproductive strategies, has been investigated. There are also no studies on reproduction/reproductive mode in the Rosy Sculpin (*O. rubellio*), which is a sister species to the Fluffy Sculpin and occurs in sympatry with the Fluffy Sculpin (Miller and Lea, 1972). In this study, we focused on reproductive strategies of these two species, such as IGA and seasonal variation in gonad maturation (i.e. GSI) for both sexes. Evaluating reproductive strategies in a comparative context between these two sister species informs both the evolution of complex reproductive modes as well as mechanisms of reproductive isolation and speciation.

Seasonal variation in gonadosomatic index (GSI) can be used to infer mating season (i.e., when male gonads are ripe as indicated by max GSI) and egg-laying season (i.e., max GSI in females indicating the presence of mature oocytes) to reveal aspects of reproductive mode and strategy. In external fertilizers such as seasonal iteroparity, peak GSI is synchronized between males and females (e.g., Valdés et al., 2004; Martyniuk et al., 2009; Awata et al., 2010; Ochi et al., 2017; Kunishima et al., 2021; Samejima et al., 2021). Conversely, internally fertilizing viviparous fishes show offset peak GSI between the sexes, as females develop offspring after copulation (Yokogawa and Iguchi, 1992a; Izumiyama et al., 2020a, 2020b). Reproduction of female Fluffy Sculpins is characterized at one site (Dillon Beach, CA, USA) based on seasonal growth and oocyte maturation (Grossman and DeVlaming, 1984), indicating that egg laying occurs in winter and spring and is correlated with day length. However, seasonality of reproduction in male Fluffy and, male and female Rosy Sculpins, have not been characterized.

Therefore, it is currently unknown whether mating season and egg-laying season are synchronized between the sexes, nor if there is disparity in mating season between these sympatric sister species.

Here, we investigate the seasonal variation in male and female GSIs in Fluffy and Rosy Sculpins at Pillar Point, Half Moon Bay, CA to estimate their mating and egg-laying seasons. Second, we verified species identity of Rosy and Fluffy Sculpins based on morphological characteristics and *CO1* and *Cytb* gene trees. Third, we experimentally investigated IGA in the two species to inform whether IGA is a common strategy across copulatory sculpins. Finally, we characterized sperm morphology and motility of both species to evaluate whether sperm morphology and kinematics are associated with the IGA strategy.

# MATERIALS AND METHODS

*Sampling*

Fluffy and Rosy Sculpins reach sexual maturity at approximately 40 mm standard length (SL, Grossman and DeVlaming, 1984; Freeman et al., 1985). Based on this, we collected 338 sexually mature sculpins of mixed species larger than about 40 mm SL during tractable tidal heights (-0.3 ft to +0.5 ft) in March 2018 and almost every month from October 2019 to May 2022 in the intertidal zone of rocky shores near Mavericks Beach at Pillar Point, Half Moon Bay, San Mateo County, CA, USA (37°29'42.30"N, 122°29'53.03"W). One Smoothhead Sculpin (*Artedius lateralis*) and ten Wooly Sculpin (*Clinocottus analis*) were also collected at the same site for genetic analyses.

*Species identification*

The external morphology of Fluffy and Rosy Sculpins is very similar, especially in females (Nakamura, 1976), making species field identification challenging. Therefore, it was necessary to confirm species identity by DNA sequencing for the specimens with less certainty. Genomic DNA was extracted from fin clips taken from each individual using Qiagen DNEasy blood and tissue kit (Qiagen Inc., Valencia, CA), and the *Cytb* and *CO1* loci were sequenced to confirm species identity in a phylogenetic context. Gene trees were constructed using 561 bp of *CO1* ($n$ = 209 fish of mixed species, including one Smoothhead and ten Wooly Sculpins) and 1051 bp of *Cytb* ($n$ = 38 fish). Primer sequences (Table1) were adopted from Ward et al. (2005) or Schmidt and Gold (1993). *CO1* amplifications were accomplished following Buser and López (2015). PCR products were purified using Qiagen PCR purification kit and sequencing reactions were performed by ElimBio Pharmaceuticals (Hayward, CA). PCR amplification and sequencing of the *Cytb* locus were accomplished following Awata et al. (2019, 2022).

Gene tree topologies were constructed using MEGA version X (Stecher et al., 2020), with the maximum likelihood method with GTR+G+I model and 1,000 bootstrap replicates. For species identification, 11 *CO1* sequences from ten species and 19 *Cytb* sequences from ten species were obtained from NCBI (Tables S1 and S2; Hastings and Burton, 2008; Buser and López 2015; Turanov and Kartavtsev, 2021; Awata et al., 2022). For both trees, *Scorpaenichthys marmoratus* was set as the outgroup.

*Embryonic development and evaluation of IGA*

We used the onset of development to indicate IGA in Rosy Sculpin and characterized the first 21 days of embryonic development, following the methods of Munehara et al. (1989, 1991), Petersen et al. (2005), and Koya et al. (2015). After euthanization of a gravid female of Rosy Sculpin ($n$ = 1) with overdose of MS-222 (200 mg/L) followed by cervical transection, eggs were stripped from the female by pushing on the abdomen with no contamination from seawater, blood, or urine. The egg clutch was split into two portions, and each was placed into experimental and control petri dishes with covers. Seawater was added to the experimental dish but not to the control dish. Control dish contained eggs with ovarian fluid and moistened Kimwipes. The dishes were floated on the top of seawater to regulate the temperature (13–15 ˚C) and humidity. Early developmental progress was checked daily before egg-hatching. We also attempted to assess IGA and egg development of five females of Fluffy Sculpin but did not find any females with ovulated oocytes in the ovaries (see Results).

*Characterization of sperm motility and morphology*

To evaluate the association between sperm characteristics and IGA, sperm motility and morphology were evaluated using males of Fluffy ($n$ = 8) and Rosy ($n$ = 3) sculpins. Testes were dissected from the euthanized male, and semen was collected by cutting the posterior region of the testis and was diluted in seawater or isotonic solution (150 mM NaCl, 10 mM HEPES, pH 8.0, following Ito and Awata [2019] and Koya et al. [1993] in a 1:30 ratio to mimic ovarian fluid) on glass slides coated with 1% bovine serum albumin. Sperm motility was recorded using phase-contrast microscopy (DSM-III-104, Daiko Science, Japan), equipped with a digital CCD camera (HD 212, AmScope, USA). Sperm swimming speed (measured as curvilinear velocity) was calculated using cell motility analysis software (BohBoh version 4.51J, BohBohSoft, Japan). A small amount of semen was preserved in a fixative of 2.5% glutaraldehyde, 0.45 M glucose, and 60 mM HEPES, following Ito and Awata (2019) for characterization of sperm morphology.

We photographed sperm using a differential interference microscope (BX53, Olympus, Japan), equipped with a digital color CCD camera (DP73, Olympus, Japan) and CellSens Standard software ver. 1.9 (Olympus, Japan). We measured the morphology of the sperm components in the images using ImageJ ver. 1.50i. Fine structure of sperm of Fluffy Sculpin ($n$ = 1) was also photographed using a scanning transmission electron microscope (Zeiss Ultra 55, GEMINI technology, England).

*Calculation of gonadosomatic index (GSI)*

Total body mass, gut mass, and gonad mass (to, 0.001 g) were recorded for each euthanized individual to examine seasonal variation in gonadosomatic index (GSI, $Mass_{gonad}$ / [$Mass_{body}$-$Mass_{gut}$] × 100) of Fluffy and Rosy Sculpins. GSI was calculated as gonad mass divided by total body mass minus gut mass to remove variation associated with feeding. Male and female GSI were plotted for each month to infer mating- and egg-laying seasonality, regardless of sampling year. The monthly average water temperature at the study site was obtained from the website (https://seatemperature.net/current/united-states/half-moon-bay-sea-temperature) to evaluate effects of temperature on mating or egg laying seasons.

*Statistical analyses*

As the sperm morphological characteristics, we measured total sperm length (μm), flagellum length (μm), head length and width (μm), and midpiece length and width (μm) and also calculated head length / head width, head length / total sperm length, head length / flagellum length, and midpiece length / midpiece width. We compared these 11 variables and sperm swimming speed between Fluffy and Rosy Sculpins. In these comparisons, linear mixed models

(LMMs) were fitted considering species as fixed effects and individual ID as a random effect, given the measurement of each characteristic per male from multiple sperms. Likelihood ratio tests were performed to determine the statistical significance ($P < 0.05$) of the fixed effects. Statistical analyses were conducted by R software (R Core Team, 2016).

# RESULTS

*Species identification*

As has been described in Morris (1956), male Fluffy and Rosy Sculpins exhibit an anal fin modification, where the first two anal soft rays are distinct and elongated for optimization of copulation, with slight morphological variation between species (Fig. S1). Buser et al. (2017) identified morphological variation between the species, such as body depth, eye size, and shape, but these morphological characteristics are difficult to discern in the field, especially in females. Therefore, we verified species identification by using the reconstructed phylogeny of the specimens. Gene trees of *CO1* and *Cytb* were diagnostic for the two species (Fig. S2 and Fig. S3). In the *CO1* phylogenetic trees (Fig. S2), 138 samples were assigned to the Fluffy Sculpin, including two samples (1.5 %) being misidentified as the Rosy Sculpin during field morphological identification. Such a misidentification rate was higher for the Rosy Sculpin (Fig. S2); of 60 genetically assigned to the Rosy Sculpin, 21 (35.0 %) were morphologically assigned as the Fluffy Sculpin in the field. In the *Cytb* phylogenetic trees (Fig. S3), 21 and 17 samples were assigned as the Fluffy and Rosy Sculpin, respectively. We also found that the *CO1* sequence of the Fluffy Sculpin registered in GenBank (KP827314: Buser and López 2015) was misidentified as the Woolly Sculpin. The *Cytb* sequences of Rosy Sculpins were deposited in

GenBank ($n$ = 5 fish; Table S2). In total, 90 males and 150 females of Fluffy Sculpin, 36 males and 62 females of Rosy Sculpin were verified from the morphological and genetic analyses.

*IGA and embryonic development*

Mean ± SD of ovulated oocyte diameter was 1.33 ±1.01 mm for Rosy Sculpin ($n$ = 11 eggs from one female). After exposing a portion of an egg mass to seawater (experimental dish), the 2-cell stage of the embryo was observed within 10 h (Fig. 1A). The blastula stage and early gastrula stage were observed after 24 and 48 h, respectively (Fig. 1B, 1C). Optic vesicles were developed by 6 days post fertilization (dpf), and eye pigmentation and otoliths were observed at 11 dpf (Fig. 1D, 1E). Well-developed embryos with lateral line pigmentation hatched at approximately 22 dpf (Fig. 1F). Development within the clutch was uniform, suggesting a single fertilization event for the entire clutch (with no inference on number of sperm donors). No developmental eggs were observed in the control dish even after 24h. We were unable to observe embryonic development in the Fluffy Sculpin in five different experimental trials with five females due to the lack of ovulated oocytes in the females at the time of collection for the IGA experiment. The ovaries of these five individuals contained eggs of several size classes, but none were free from the ovarian matrix. Attempts were made to expose these eggs to seawater, but no development was observed in any of the five individuals.

*Sperm characteristics*

Sperm morphology in both Fluffy and Rosy Sculpins was similar in appearance (Fig. 2A, 2B). The morphology of the head was elongated, not spherical, and had a ‘butter knife’ shape. In the Fluffy Sculpin, fine structure imaging revealed that the flagellum was covered by midpiece and recessed into the head (Fig. 2C). Although there was a high degree of similarity in sperm morphology between species, there were significant differences in five morphometric components (Table 2). Total sperm length and flagella length of Rosy Sculpin were significantly longer than those of Fluffy Sculpin. Sperm head length and head width in Rosy Sculpins were also significantly longer than those in Fluffy Sculpins, but the head shape (head length / head width) did not differ between the species. Midpiece length, width, and their ratio were not significantly different between the species. The ratio of head length to total sperm and to flagella length also differed slightly between the species. In both Fluffy and Rosy Sculpins, sperm was motile in isotonic solution but completely immotile in seawater. Sperm swimming speed was not significantly different between the two species (Table 2).

*Seasonal changes in GSI*

The monthly average sample size used for the GSI analyses was 8 males and 13 females in Fluffy Sculpins. There was seasonal variation in male and female GSIs of Fluffy sculpins (Fig. 3). We define mating season by elevated GSI that was > c.a. 1.5% of reproductively active males (> 40 mm SL). According to this criterion, we estimated that the mating season for Fluffy Sculpin is protracted spanning a 6-month period from September to February (Fig. 3A). GSI values for males shows that peak mating occurs from October to December.

As expected from the reproductive mode in the Fluffy Sculpins, asynchronous gonadal maturation between the sexes was observed. GSI values for female Fluffy Sculpin were elevated from November to May with peak egg laying occurring season occurring from December to March (with average GSI ranging from 8.4 to 9.1%, Fig. 3B). Unfortunately, the five female Fluffy Sculpins we examined for IGA development trials did not include any individuals with ovulated oocytes. Females exhibited lower GSI values from June to October. Note that females were much less abundant in the intertidal site during this time, suggesting that they may have died after laying eggs, or migrated away from the intertidal, suggesting the intertidal is used as a nursery habitat for these species.

Although the sample size of Rosy Sculpins was too small (3 males and 5 females on monthly average) to make strong inferences due to their low abundance at our study site, seasonal variation in GSI values were also observed for both male and female Rosy sculpins. The GSI values of males increased from November to May (i.e. mating season, Fig. 3C). Egg-laying season of Rosy Sculpins, indicated by the increase in female GSI occurred from January to June (Fig. 3D). The water temperature ranged from 11 to 16 °C in 2019–2022, and female GSI of both species had an inverse relationship with water temperature (Fig. 3B and D).

# DISCUSSION

Embryonic development was observed in eggs of the Rosy Sculpin exposed to seawater but not for those in the only ovarian fluid, indicating that the Rosy Sculpins exhibit the IGA reproductive strategy. We also described, for the first time, aspects of embryonic development in Rosy Sculpin, which hatched at approximately 23 days after contact with seawater. Unfortunately, we failed to obtain mature female Fluffy Sculpins with ovulated oocytes for the IGA experiments. However, we also did not find any fertilized eggs and eggs of early cleavage stages in the ovaries of the Fluffy Sculpin, while the ovaries contained spermatozoa, indicating that copulation has taken place but fertilization has not occurred. Further, the sperm morphology of Fluffy sculpin is characterized as having an elongated head and motility in isotonic solution but immotile in seawater, similar to the sperm characteristics of the Rosy Sculpins and represented characteristics associated with IGA species (Abe and Muneahra, 2009; Koya et al., 2011; Ito and Awata, 2019; Ito et al., 2022). Therefore, although Morris (1956) has noted that the Fluffy Sculpins are internal fertilizers, we infer that their reproductive mode is IGA, as we observed in Rosy Sculpins.

Previous studies have shown that seven internally inseminating Cottoidea species have an IGA reproductive mode (Munehara 1997; Munehara et al., 1989, 1991, 1997; Petersen et al., 2005; Koya et al., 2015; Momota and Munehara, 2017) and have suggested that other copulatory species are also likely to exhibit IGA (Abe and Munehara, 2009; Koya et al., 2015; Awata et al, 2019, 2022). The present results support this assumption and indicates that IGA evolved multiple times independently in multiple clades of the Cottoidea (Cottidae, Agonidae, and Psychrolutidae). In addition, although the majority are external fertilization, IGA or IGA-like fertilization modes are recognized in a few species in Perciformes (Cottoidea) as well as in Characiformes (at least 27 species in Glandulocaudinae and Stevardiinae of Characidae: Burns et al., 1995; 1997), Gasterosteiformes (one species in Aulorhynchidae: Akagawa et al., 2008; Okiyama et al., 1993), and Siluriformes (at least five species in Auchenipteridae: Meisner et al., 2000; Burns et al., 2002; Parreira et al., 2009). The presence of IGA in a wide range of taxa suggests that IGA may have evolved independently across fishes of different families of multiple orders. In terms of life history strategy, species with IGA share several characteristics with both oviparous and viviparous fish. First, oviparous fish invest more in egg number than egg size (Sargent et al., 1987), resulting in higher larval mortality compared to viviparous fish (Roff 1992; Smith and Fretwell, 1974). This theory is also predicted to apply species with IGA because the females spawn egg mass into seawater and eggs are exposed to various extraneous factors.

Second, females of IGA species do not need to care for fertilized eggs and larvae in the ovary. This characteristic is also similar to oviparous fish with spawning eggs in seawater, and reproductive costs in the ovary are predicted to be lower than those in viviparous fishes (Abe and Munehara, 2009), although the total reproductive cost, including parental care, is unknown. Third, females can lay eggs at the appropriate timing of spawning and do not need to synchronize their spawning timing with males, especially for females that produce multiple clutches in a reproductive season (Abe and Munehara 2009). In terms of asynchrony between copulation and spawning, this character is similar to asynchrony between copulation and birth in viviparous fish. Evolution of IGA may allow females to have decoupled nest site selection from mating, giving for deciding timing and spatial site selection for egg laying, without the cost of rearing larvae and juveniles in the ovary. Although it is unclear what factors separate the evolutionary pathways from external fertilization to IGA or true internal fertilization in fish, accumulating evidence of IGA in different clades of fish could contribute to our understanding of the underlying mechanisms and potential implications of this reproductive strategy.

Aspects of sperm morphology, including head length, ratio, and motility, were similar between Rosy and Fluffy Sculpins; both species had sperm with an elongated head and were motile in the isotonic solution but not in seawater. These characteristics contrast with those in external fertilizing species, which have round-head sperm and sperm that are motile in their fertilization environment such as seawater (Koya et al., 1993, 2011; Petersen et al., 2005; Abe and Munehara, 2007; Ito and Awata, 2019; Ito et al., 2022) and freshwater (Ito et al., 2021). Sperm with an elongated and slender head characterized in IGA species is thought to be advantageous for swimming in a viscous environment such as ovarian fluid (Javonillo et al., 2009). Interestingly, Humphries et al (2008) proposed that sperm with a longer head (ellipsoidal shape) exhibit increased the drag force, resulting in decreased sperm velocity. Revealing the detailed structure, not just the simple head-to-flagellum ratio, is important for clarifying the physical function of sperm; the thickness might also be associated with sperm swimming speed and adaptation to a viscous environment.

Mating and spawning periods in Fluffy Sculpins were not synchronized, as shown by divergence in peak GSI between males (September to December) and females (December to March). These results suggest that females store sperm for over three months before laying their first clutch of the season. In the absence of verified monogamy, it is reasonable to assume that females mate with multiple males resulting in clutch polyandry as the null model. Morris (1956) noted that, in captivity, copulation of the Fluffy Sculpin is frequently observed. Although polyandry in Fluffy Sculpin is remains untested, females store sperm (from one or more males), and it is currently unknown when exactly fertilization takes place. Every female examined had oocytes at multiple stages of development at any one time (Grossman and DeVlaming, 1984), indicating that they lay multiple egg clutches per season. Therefore, it is not surprising that the mating season is protracted in both species. Asynchrony in peak GSI is observed in several sculpin species with IGA (Shinomiya, 1985; Abe and Munehara, 2005, 2007). Fluffy and Rosy Sculpins are sister taxa (Ramon and Knope, 2008; Buser and López, 2015; Buser et al., 2017) and overlap in geographic range and habitat, occupying the same pools in the intertidal (this study). Although our sample size for Rosy Sculpin was too small to reliably infer mating season, the possibility of divergence in mating season suggests a mechanism for reproductive isolation and divergence in sympatry.

In marine sculpins (Psychrolutidae), copulatory IGA species are classified into three egg care patterns; paternal care, maternal care, and no care with egg masses deposited in invertebrates (Abe and Munehara, 2009; Awater et al., 2019; 2022). Such egg care patterns may be related to synchronization of mating season and egg-laying season (Munehara 2011). For example, the timing of copulation and spawning are close together in IGA species with paternal care (*Alcichthys alcicornis*, Munehara, 1988; *Artedius harringtoni,* Ragland and Fischer, 1987). Indeed, *Al. alcicornis* are likely to exhibit synchrony in peak GSI between the sexes (Koya et al., 1994; Munehara and Murahana, 2010). Alternatively, IGA species with maternal care (*Radulinopsis taranetzi*: Abe and Munehara, 2005) and those with no care (five species of *Pseudoblennius*, two species of *Furcina*, and one species of *Vellitor*: Koya et al., 2015; Awata et al., 2019; *Orthonopias triacis*: Awata et al., 2022) exhibit delayed timing of spawning from timing of mating. Males of *R. taranetzi* copulate with females that do not yet have mature oocytes about three months before the egg-laying season (Abe and Munehara, 2005, 2007). *Vellitor centropomus* females also copulate while still immature (Koya et al., 2015), suggesting that sperm can be stored for several months. Viable sperm is retained for two weeks in *O. triacis*, demonstrating sperm storage capacity (Bolin, 1941). Although egg care patterns in the Fluffy and Rosy Sculpins are unknown in the field (Awata et al., 2022), the asynchrony in peak GSI between the sexes implies that these species do not exhibit paternal care behaviors. In fact, none of the male collected in this study had protected eggs. Female egg-care is known only in species that inhabit sandy and gravel areas (Abe and Munehara, 2005, 2007). Many parts of the intertidal area have pebble and are not unlike gravel. Therefore, we cannot rule out the possibility of maternal care, but as with the males, none of the females we collected were protecting their eggs.

Asynchrony in mating season and egg-laying season in marine sculpin is also suggested to be related to sperm competition (Munehara 2011), which is the competitive process for fertilization between sperm from different males (Parker, 1970). Males can have a capacity of copulating earlier than when eggs are mature, thereby reducing the chance of fertilization by other males. Indeed, females of *R. taranetzi* mate with multiple partners, resulting in intense sperm competition in the ovary (Abe and Munehara, 2005, 2007). Although little information is available on parental egg care and variation in GSI for both males and females in marine sculpins, the asynchrony of the GSI may be the result of male-male competition because males that mate earlier may be able to fertilize more eggs or the first mature oocyte of multiple clutch (Abe and Munehara 2009; Munehara et al., 1994; Munehara 2011).

Asynchrony in peak GSI is also observed in internally inseminating catfishes (Freitas et al., 2011) and characid fishes (Azevedo et al., 2000), internally fertilizing surfperches (Izumiyama et al., 2020b) and rockfishes (Yokogawa and Iguchi, 1992a, 1992b). While GSI is protracted and there is some overlap between the sexes in IGA species including Fluffy and Rosy Sculpins, GSI between the sexes are less overlapped in true internal fertilizers (i. e., viviparous fish). The protracted seasonality and disparity in GSI peaks between the sexes may be driven by the number of clutches laid per season. Viviparous fish with seasonal iteroparity exhibit single brood per year. Surfperch females copulate with multiple males, but fertilization and early embryo development are initiated after mating season ends (Izumiyama et al., 2020a, 2020b), thus, asynchrony is obvious. Different from the surfperches, female Fluffy and Rosy Sculpins exhibit multiple size classes of oocytes within the ovary between October and May (Grossman and DeVlaming, 1984), indicating multiple clutches are laid per season. Therefore, since males of the Fluffy and Rosy Sculpins have multiple chances to fertilize eggs due to the multiple spawning by an individual female, the GSI peaks of both sexes would be prolonged and have slight overlap each other.

In summary, we demonstrate seasonal variation in GSI between males and females of Fluffy and Rosy Sculpins. We also demonstrated IGA in Rosy Sculpin directly and inferred IGA in Fluffy Sculpin indirectly based on sperm morphology, asynchrony of GSI between the sexes, and lack of embryonic development in the ovary. This reproductive strategy of IGA together with protracted and asynchronous mating season could be associated with male-male competition, sperm storage, multiple spawning, and optimal nest site selection by females. Furthermore, we propose that the slight difference in mating season (pre-mating isolation) and sperm characteristics (post-mating isolation) may prevent hybridization between the two species, while they are sympatric and share the similarities in morphology and reproductive mode. Our study supports the generality of IGA across marine sculpins and demonstrates diagnostic aspects patterns for diagnosing the IGA reproductive strategy.

## ACKNOWLEDGMENTS

We are grateful to the members of Fish Lab, San Francisco State University for fish sampling in the field. We also thank an associate editor, Thaddaeus Buser, and an anonymous reviewer for their helpful comments on an earlier version of the manuscript. This work was financed by the Japan Society for the Promotion of Science (JSPS) KAKENHI (Grant No. JP19J11278 to TI, and JP16H04841 and JP17K19518 to SA), JSPS Overseas Challenge Program for Young Researchers (Grant No. 201980182 to TI), Sasakawa Scientific Research Grant from the Japan Science Society (Grant No. 29-541 to TI), and the Osaka City University (OCU) Strategic Research Grant 2020 (to SA). All field collections were conducted under the California Department of Fish and Wildlife scientific collection permit (Permit 2018 and 2020 to K.D.C.,

SC-13856 to S.A., and SC-192190004 to T.I.). All procedures were conducted under an approved IACUC protocol at San Francisco State University (SFSU_A19_05).

## DATA ACCESSIBILITY

Supplemental material is available at https://www.ichthyologyandherpetology.org/XXX.

## LITERATURE CITED

**Abe, T., and H. Munehara**. 2005. Spawning and maternal-care behaviours of a copulating sculpin, *Radulinopsis taranetzi*. Journal of Fish Biology 67:201–212. doi: 10.1111/j.0022-1112.2005.00728.x

**Abe, T., and H. Munehara**. 2007. Histological structure of the male reproductive organs and spermatogenesis in a copulating sculpin, *Radulinopsis taranetzi* (Scorpaeniformes: Cottidae). Ichthyological Research 54:137–144. doi: 10.1007/s10228-006-0385-8

**Abe, T., and H. Munehara**. 2009. Adaptation and evolution of reproductive mode in copulating cottoid species. *In*: Reproductive biology and phylogeny in fishes (agnathans and bony fishes), volume 8B. B. G. M. Jamieson (ed.). Science Publishers, Enfield, New Hampshire, pp. 221–246.

**Akagawa, I., M. Hara, and T. Iwamoto**. 2008. Egg concealment in ascidians by females of the Japanese tubesnout, *Aulichthys japonicus* (Gasterosteiformes), and its subsequent copulation. Ichthyological Research 55:85–89.

**Awata, S., T. Ito, K. D. Crow, Y. Koya, and H. Munehara**. 2022. The first record of egg masses in tunicates deposited by the snubnose sculpin, *Orthonopias triacis* , from the Northeastern Pacific: evidence for convergent evolution of an unusual reproductive strategy. Journal of Fish Biology 100:82–91. doi: 10.1111/jfb.14920

**Awata, S., S. Miura, S. Seki, T. Sagawa, N. Sato, and K. Sakai**. 2010. Seasonal changes in reproductive and physical condition, sexual dimorphism, and male mating tactics in the jewelled blenny *Salarias fasciatus*. Ichthyological Research 57:161–168. doi: 10.1007/s10228-009-0142-x

**Awata, S., H. Sasaki, T. Goto, Y. Koya, H. Takeshima, A. Yamazaki, and H. Munehara**. 2019. Host selection and ovipositor length in eight sympatric species of sculpins that deposit their eggs into tunicates or sponges. Marine Biology 166:1–13. doi: 10.1007/s00227-019-3506-4

**Azevedo, M. A., L. R. Malabarba, and C. B. Fialho**. 2000. Reproductive biology of the inseminating Glandulocaudine *Diapoma speculiferum* Cope (Teleostei: Characidae). Copeia 2000:983–989.

**Bolin, R**. 1941. Embryonic and early larval stages of the cottid fish *Orthonopias triacis* Starks and Mann. Stanford Ichthyol Bull 2:73–82.

**Burns, J. R., A. D. Meisner, S. H. Weitzman, and L. R. Malabarba**. 2002. Sperm and Spermatozeugma Ultrastructure in the Inseminating Catfish, *Trachelyopterus lucenai* (Ostariophysi: Siluriformes: Auchenipteridae). Copeia 2002:173–179.

**Burns, J. R., S. H. Weitzman, H. J. Grier, and N. A. Menezes**. 1995. Internal fertilization, testis and sperm morphology in glandulocaudinae fishes (Teleostei: Characidae: Glandulocaudinae). Journal of Morphology, 224:131–145.

**Burns, J. R., S. H. Weitzman, and L. R. Malabarba**. 1997. Insemination in Eight Species of Cheirodontine Fishes (Teleostei: Characidae: Cheirodontinae). Copeia 1997:433. doi: 10.2307/1447767

**Buser, T. J., and J. A. López**. 2015. Molecular phylogenetics of sculpins of the subfamily Oligocottinae (Cottidae). Molecular Phylogenetics and Evolution 86:64–74. doi: 10.1016/J.YMPEV.2015.03.006

**Buser, T. J., M. D. Burns, and J. A. López**. 2017. Littorally adaptive? Testing the link between habitat, morphology, and reproduction in the intertidal sculpin subfamily Oligocottinae (Pisces: Cottoidea). PeerJ 2017:1–30. doi: 10.7717/peerj.3634

**Evans, J. P., and A. D. Meisner**. 2009. Copulatory structures: taxonomic overview and the potential for sexual selection. *In*: Reproductive biology and phylogeny of fishes (agnathans and bony fishes), volume 8B. B. G. M. Jamieson (ed.). Science Publishers, Enfield, New Hampshire, pp. 138–180.

**Freeman, M. C., N. Neally, and G. D. Grossman**. 1985. Aspects of the life history of the fluffy sculpin, *Oligocottus snyderi*. Fishery Bulletin 83:645–655.

**Freitas, T. M. da S., V. H. da C. Almeida, L. F. de A. Montag, R. M. da Rocha, and N. F. Fontoura**. 2011. Seasonal changes in the gonadossomatic index, allometric condition factor and sex ratio of an auchenipterid catfish from eastern Amazonia. Neotropical Ichthyology 9:839–847. doi: 10.1590/S1679-62252011005000044

**Green, J. M**. 1971. Local distribution of *Oligocottus maculosus* Girard and other tidepool cottids of the west coast of Vancouver Island, British Columbia. Canadian Journal of Zoology 49:1111–1128. doi: 10.1139/z71-172

**Grossman, D. G., and V. DeVlaming**. 1984. Reproductive Ecology of Female *Oligocottus snyderi* Greeley: a North American intertidal sculpin. Journal of Fish Biology 25:231–240. doi: 10.1002/ajp.22084

**Hastings, P. A., and R. S. Burton**. 2008. Establishing a DNA sequence database for the marine fish Fauna of California. California Sea Grant College Program. Retrieved from https://escholarship.org/uc/item/1ck9b3qs

**Humphries, S., J. P. Evans, and L. W. Simmons**. 2008. Sperm competition: linking form to function. BMC Evolutionary Biology 8:1–11. doi: 10.1186/1471-2148-8-319

**Ito, T., and S. Awata**. 2019. Optimal methods to fix fish sperm for optical microscopic observation: comparisons among different fixative solutions using sperms of copulatory and non-copulatory marine fishes. Ichthyological Research 66:307–315. doi: 10.1007/s10228-018-0672-1

**Ito, T., I. Kinoshita, D. Tahara, A. Goto, S. Tojima, V. G. Sideleva, A. B. Kupchinsky, and S. Awata**. 2021. Fertilization modes drive the evolution of sperm traits in Baikal sculpins. Journal of Zoology, 314:20–30. doi: 10.1111/jzo.12867

**Ito, T., M. Morita, S. Okuno, K. Inaba, K. Shiba, H. Munehara, Y. Koya, M. Homma, and S. Awata. 2022**. Fertilization modes and the evolution of sperm characteristics in marine fishes: Paired comparisons of externally and internally fertilizing species. Ecology and Evolution, 12:1–18. doi: 10.1002/ece3.9562.

**Izumiyama, M., S. Awata, and K. Crow**. 2020a. Evaluating reproductive strategies and female bateman gradients in *Ditrema temminckii*: Is the number of fathers a good approximation for the number of mates? Copeia 108:532–537. doi: 10.1643/CE-19-271

**Izumiyama, M., M. F. Westphal, and K. D. Crow**. 2020b. In the surf zone: Reproductive strategy of the calico surfperch (*Amphistichus koelzi*) in a comparative context. Journal of Fish Biology 96:939–949. doi: 10.1111/jfb.14283

**Jakubowski, M., P. Y., Tugarina, P. and K. Żuwała**. 2003. Pectoral fin development in the Baikalian viviparous golomyankas (Comephoridae; Cottoidei), with a remark on eggs and embryos of *Comephorus baicalensis* (Pallas). Journal of anatomy, 203:317–322. doi: 10.1046/j.1469-7580.2003.00221.x

**Javonillo, R., J. R. Burns, and S. H. Weitzman. 2009**. Sperm modifications related to insemination, with examples from the Ostariophysi. *In*: Reproductive biology and phylogeny of fishes (agnathans and bony fishes), volume 8A. B. G. M. Jamieson (ed.). Science Publishers, Enfield, New Hampshire, pp. 723–763.

**Koya, Y., Y. Hayakawa, A. Markevich, and H. Munehara**. 2011. Comparative studies of testicular structure and sperm morphology among copulatory and non-copulatory sculpins (Cottidae: Scorpaeniformes: Teleostei). Ichthyological Research 58:109–125. doi: 10.1007/s10228-010-0196-9

**Koya, Y., H. Munehara, and K. Takano**. 1994. Reproductive cycle and spawning ecology in elkhorn sculpin, *Alcichthys alcicornis*. Japanese Journal of Ichthyology 41:39–45.

**Koya, Y., H. Munehara, and K. Takano**. 2002. Sperm storage and motility in the ovary of the marine sculpin *Alcichthys alcicornis* (Teleostei: Scorpaeniformes), with internal gametic association. Journal of Experimental Zoology 292:145–155. doi: 10.1002/jez.1150

**Koya, Y., H. Munehara, K. Takano, and H. Takahashi**. 1993. Effects of extracellular environments on the motility of spermatozoa in several marine sculpins with internal gametic association. Comparative Biochemistry and Physiology Part A: Physiology 106:25–29. doi: 10.1016/0300-9629(93)90033-Z

**Koya, Y., M. Naoya, S. Awata, T. Ito, and H. Munehara**. 2015. Identification of the reproductive mode for internal gamete association in *Vellitor centropomus* (Cottidae). Japanese Journal of Ichthyology 62:121–131.

**Kunishima, T., S. Higuchi, Y. Kawabata, K. Furumitsu, I. Nakamura, A. Yamaguchi, K. Tachihara, M. Tokeshi, and S. Arakaki**. 2021. Age, growth, and reproductive biology of the blackfin seabass *Lateolabrax latus*, a major predator in rocky coastal ecosystems of southwestern Japan. Regional Studies in Marine Science 41:101597. doi: 10.1016/j.rsma.2020.101597

**Martyniuk, C. J., K. J. Kroll, W. F. Porak, C. Steward, H. J. Grier, and N. D. Denslow**. 2009. Seasonal relationship between gonadotropin, growth hormone, and estrogen receptor mRNA expression in the pituitary gland of largemouth bass. General and Comparative Endocrinology 163:306–317. doi: 10.1016/j.ygcen.2009.04.028

**Meisner, A. D., J. R. Burns, S. H. Weitzman, and L. R. Malabarba**. 2000. Morphology and histology of the male reproductive system in two species of internally inseminating South American catfishes, *Trachelyopterus lucenai* and *T. galeatus* (Teleostei: Auchenipteridae). Journal of Morphology, 246:131–141.

**Miller, D. J., and R. N. Lea**. 1972. Guide To The Coastal Marine Fishes of California. Fish Bulletin 157:1–235.

**Momota, K., and H. Munehara**. 2017. Reproductive ecology and morphological changes during the early life stages of *pallasina barbata* (Steindachner,1876). Bulletin of Fisheries Sciences, Hokkaido University 67:7–12.

**Morris, R. W**. 1956. Clasping Mechanism of the Cottid Fish *Oligocottus snyderi* Greeley. Pacific Science 10:314–317.

**Munehara, H**. 1988. Spawning and subsequent copulating behavior of the elkhorn sculpin *Alcichthys alcicornis* in an aquarium. Japanese Journal of Ichthyology 35:358–364. doi: 10.1007/bf02938434

**Munehara, H**. 1997. The reproductive biology and early life stage of *Podothecus sachi* (Pisces: Agonidae). Fishery Bulletin 95:612–619

**Munehara, H**. 2011. Evolutionary process of adaptive diversity of the cottoid fishes discussed from ecological aspects. *In*: Diversity of cottoid fishes: adaptation and evolution. H. Munehara, A. Goto and M. Yabe (eds.). Tokai University Press, Kanagawa, pp. 85–119.

**Munehara, H., and H. Murahana**. 2010. Sperm allocation pattern during a reproductive season in the copulating marine cottoid species, *Alcichthys alcicornis*. Environmental Biology of Fishes 88:323–331. doi: 10.1007/s10641-010-9644-7

**Munehara, H., K. Takano, and Y. Koya**. 1989. Internal gametic association and external fertilization in the elkhorn sculpin, *Alcichthys alcicornis*. Copeia 1989:673–678.

**Munehara, H., K. Takano, and Y. Koya**. 1991. The little dragon sculpin *Blepsias cirrhosus*, another case of internal gametic association and external fertilization. Japanese Journal of Ichthyology 37:391–394.

**Munehara, H., A. Takenaka and O. Takenaka**. 1994. Alloparental care in the marine sculpin *Alcichthys alcicornis* (Pisces: Cottidae): copulating in conjunction with paternal care. Journal of Ethology, 12:115–120.

**Munehara, H., Y. Koya, Y. Hayakawa, and K. Takano**. 1997. Extracellular environments for the initiation of external fertilization and micropylar plug formation in a cottid species, *Hemitripterus villosus* (Pallas)(Scorpaeniformes) with internal insemination. Journal of Experimental Marine Biology and Ecology 211: 279–289.

**Nakamura, R**. 1976. Experimental assessment of factors influencing microhabitat selection by the two tidepool fishes *Oligocottus maculosus* and *O. snyderi*. Marine Biology 37:97–104. doi: 10.1007/BF00386784

**Nelson, J. S**. 2006. Fishes of the World, 4th Edition. In.: John Wiley & Sons, Inc, Hoboken, NJ, USA.

**Nelson, J. S., T. C. Grande, and M. V. H. Wilson**. 2016. Fishes of the World, 5th Edition. In.: John Wiley & Sons, Inc, Hoboken, NJ, USA. doi: 10.1002/9781119174844

**Ochi, Y., Y. Fukui, Y. Sakai, and H. Hashimoto**. 2017. Age, growth and reproduction of the Asian sheephead wrasse *Semicossyphus reticulatus* in the western Seto Inland Sea, Japan. Ichthyological Research 64:415–422. doi: 10.1007/s10228-017-0575-6

**Okiyama M., M. Hara, and I. Akagawa**. 1993. Fertilization mechanism of a marine gastetosteoid fish, Aulichthys japonicus, referable to ‘‘internal gametic association.’’ *In*: The first international symposium on the molecular and cell biology of egg- and embryo-coats, Yokohama, Japan

**Parker, G. A**. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Reviews 45:525–567. doi: 10.1111/j.1469-185X.1970.tb01176.x

**Parreira, G. G., H. Chiarini-Garcia, R. C. Melo, F. O.Vieira, and H. P. Godinho**. 2009. Spermatozoon and its relationship with the ovarian lamellae in the internally inseminating catfish *Trachelyopterus galeatus*. Microscopy Research and Technique 72:889–897.

**Petersen, C. W., C. Mazzoldi, K. A. Zarrella, and R. E. Hale**. 2005. Fertilization mode, sperm characteristics, mate choice and parental care patterns in *Artedius* spp. (Cottidae). Journal of Fish Biology 67:239–254. doi: 10.1111/j.0022-1112.2005.00732.x

**R Core Team**. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing.

**Ragland, H. C., and E. A. Fischer**. 1987. Internal fertilization and male parental care in the scalyhead sculpin, *Artedius harringtoni*. Copeia 1987:1059–1062.

**Ramon, M. L., and M. L. Knope**. 2008. Molecular support for marine sculpin (Cottidae; Oligocottinae) diversification during the transition from the subtidal to intertidal habitat in the Northeastern Pacific Ocean. Molecular Phylogenetics and Evolution 46:475–483. doi: 10.1016/j.ympev.2007.11.005

**Roff, D**. 1993. *Evolution of life histories: theory and analysis*. Chapman and Hall, NewYork

**Samejima, S., M. Uehara, and K. Tachihara**. 2021. Life-history traits of a widely distributed coral reef fish, *Mulloidichthys flavolineatus* (Mullidae), Okinawa Island, southern Japan. Environmental Biology of Fishes 104:1559–1574. doi: 10.1007/s10641-021-01168-7

**Sargent, R. C., P.D., Taylor, and M. R. Gross**. 1987. Parental care and the evolution of egg size in fishes. The American Naturalist, 129:32–46.

**Schmidt, T. R., and J. R. Gold**. 1993. Complete Sequence of the Mitochondrial Cytochrome b Gene in the Cherryfin Shiner, *Lythrurus roseipinnis* (Teleostei: Cyprinidae). Copeia 1993:880. doi: 10.2307/1447258

**Shinomiya, A**. 1985. Studies on the reproductive ecology and physiology of the three species of marine sculpin. *Hokkaido University Doctoral Dissertation*.

**Smith, W. L., and M. S. Busby**. 2014. Phylogeny and taxonomy of sculpins, sandfishes, and snailfishes (Perciformes: Cottoidei) with comments on the phylogenetic significance of their early-life-history specializations. Molecular Phylogenetics and Evolution, 79:332–352. doi: 10.1016/j.ympev.2014.06.028

**Smith, C. C., and S. D. Fretwell**. 1974. The optimal balance between size and number of offspring. The American Naturalist, 108:499–506

**Stecher, G., K. Tamura, and S. Kumar**. 2020. Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution*. [Online.] Available at https://doi.org/10.1093/molbev/msz312.

**Turanov, S. V., and Y. P. Kartavtsev**. 2021. A complement to DNA barcoding reference library for identification of fish from the Northeast Pacific. Genome, 64:927–936. doi: 10.1139/gen-2020-0192

**Valdés, P., A. García-Alcázar, I. Abdel, M. Arizcun, C. Suárez, and E. Abellán**. 2004. Seasonal changes on gonadosomatic index and maturation stages in common pandora *Pagellus erythrinus* (L.). Aquaculture International 12:333–343. doi: 10.1023/B:AQUI.0000042136.91952.9e

**Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last, and P. D. N. Hebert**. 2005. DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society B: Biological Sciences 360:1847–1857. doi: 10.1098/rstb.2005.1716

**Yokogawa, K., and M. Iguchi**. 1992a. Food habit and maturation of black rockfish, *Sebastes inermis* in southern coastal waters of the Harima Sea. Suisanzoshoku 40:139–144.

Fig. captions

**Fig. 1.** Embryonic development of the Rosy Sculpin (*Oligocottus rubellio*). (A) Eggs were immersed in seawater (Fertilization). (B) Blastula stage (24 hours post fertilization, hpf). (C) Early gastrula stage (48 hpf). (D) Phylotypic stage (6 days post fertilization, dpf). Optic and otic vesicles were visible. (E) Embryonic stage (11 dpf). Eye pigmentation and otoliths were clearly visible. (F) Embryonic stage just before hatching (21 dpf). Lateral line pigmentation was visible. Water temperature was maintained at 13–15 ˚C.

**Fig. 2.** Sperm of (A) the Fluffy Sculpin (*Oligocottus snyderi*) and (B) the Rosy Sculpin (*O. rubellio*) observed under a differential interference microscope. (C) The fine structure of sperm head in the Fluffy Sculpin observed under a scanning transmission electron microscope. mi: midpiece, fl: flagellum. The left and right white arrows indicate the boundary between head and anterior end of the midpiece and between anterior and posterior ends of the midpiece, respectively.

**Fig. 3.** Seasonal changes of gonadosomatic index (GSI) in males and females of the Fluffy Sculpin (*Oligocottus snyderi*) and the Rosy Sculpin (*O. rubellio*) and monthly average water temperature at study site in Half Moon Bay, San Mateo County, CA, USA. (A) Male ($n$= 90) and (B) female ($n$= 150) Fluffy Sculpin. (C) Male ($n$= 36) and (D) female ($n$= 62) Rosy Sculpin. Each dot represents an individual. In boxplot, box show median, interquartile range, and whiskers show the lowest/highest value within 1.5 times the interquartile range, with black dots of outlier. Boxplots were illustrated only if the number of individuals collected per month was

four or more. Solid lines indicate the loess regressions, and shadings indicate 95% confidence intervals. The number below the month denotes the number of individuals.

Sup. Fig. captions

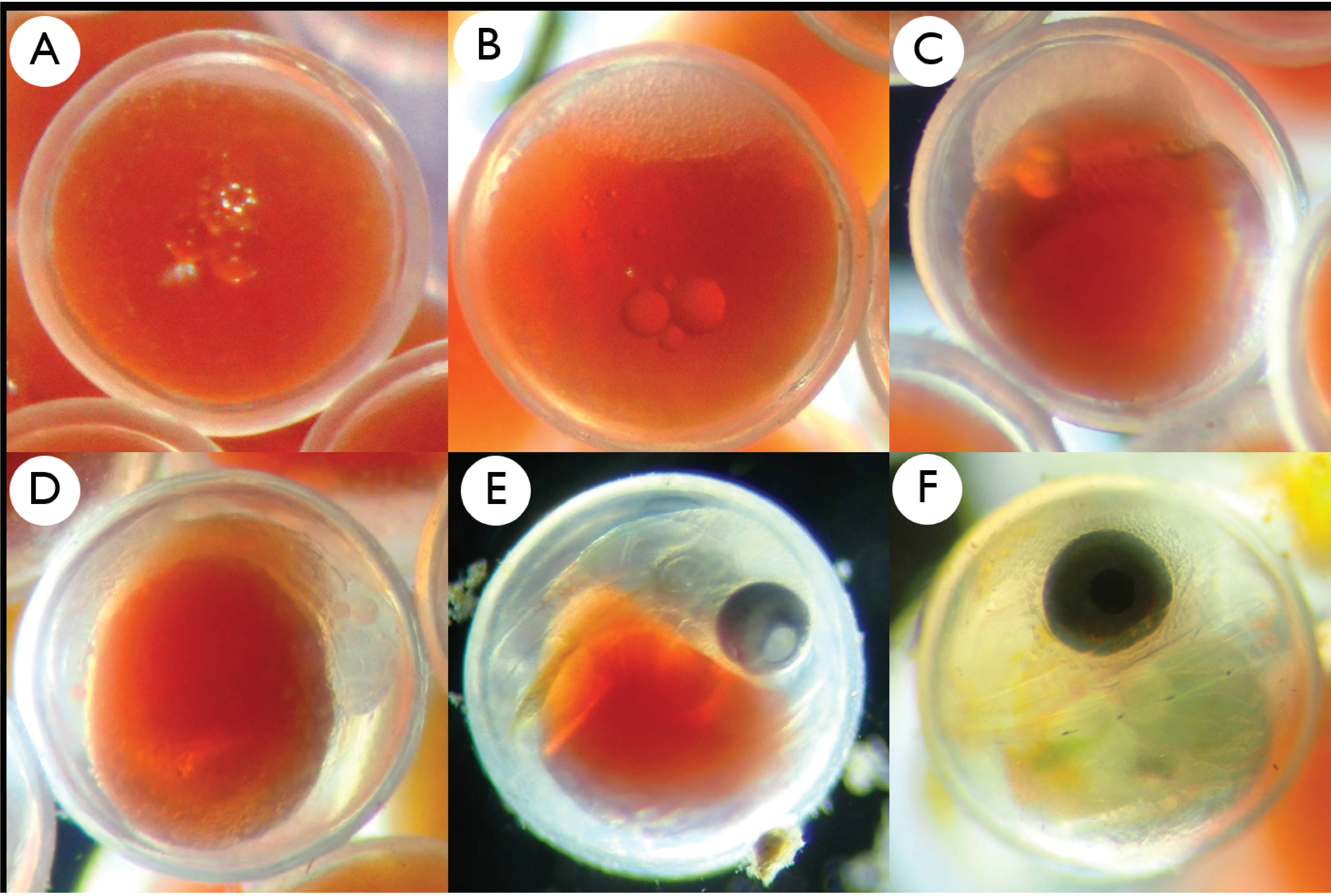
Fig. S1. Comparison of anal fin modification between Fluffy Sculpin (*Oligocottus snyderi*) and Rosy Sculpin (*O. rubellio*). (A) Male Fluffy Sculpin, with fin membrane thickening concentrated in first anal ray to the tip. (B) Male Rosy Sculpin, with fin membrane thickening concentrated in first and second (partial) anal ray to the tip. (C) Female Fluffy Sculpin, with no elongated anal fin. (D) Female Rosy Sculpin, with no elongated anal fin.

Fig. S2. Confirmation of species identity using a gene tree constructed from 561 bp *CO1* sequences of ten species including marine sculpins (Psychrolutidae). Gene tree topologies were constructed using MEGA version X, with the maximum likelihood method with GTR+G+I model and 1,000 bootstrap replicates. The branch labels indicate bootstrap values. The samples collected in the present study are shown using the code Ala, Can, Oru, and Osn for fish identified as Smoothhead Sculpin (*Artedius lateralis*), Wooly Sculpin (*Clinocottus analis*), Fluffy Sculpin (*Oligocottus snyderi*), and Rosy Sculpin (*O. rubellio*), respectively. Scientific names with accession numbers were downloaded from NCBI, and *Scorpaenichthys marmoratus* (EU403072) was used as the outgroup in this tree. The * denotes the species that were misidentified in the field, while the ** represents the samples that had been misidentified and registered in GenBank.

Fig. S3. Confirmation of species identity using a gene tree constructed from 1051 bp *Cytb* sequences of ten species including marine sculpins (Psychrolutidae). Gene tree topologies were constructed using MEGA version X, with the maximum likelihood method with GTR+G+I model and 1,000 bootstrap replicates. The branch labels indicate bootstrap values. The samples collected in the present study are shown using the code Osn and Oru for fish identified as Fluffy Sculpin (*Oligocottus snyderi*) and Rosy Sculpin (*O. rubellio*), respectively. Scientific names with accession numbers were downloaded from NCBI, and *Scorpaenichthys marmoratus* was used as the outgroup in this tree.

**Table 1. Primers used in the analysis of the Cottidae mitochondrial genome.**

| Primer | mtDNA Region | Sequence 5’ -> 3’ | Ref. |
|---|---|---|---|
| H15915/L14724 | *Cytb* | H15915- 5'-CAACGATCTCCGGTTT-3'<br>L14724- 5'-GTGACTTGAAAAACCA-3' | Schmidt and Gold (1993) |
| FISH_F1/FISH_R1 | *CO1* | FISH_F1 TCAACCAACCACAAAGACATTGGCAC<br>FISH_R1 TAGACTTCTGGGTGGCCAAAGAATCA | Ward *et al.* (2005) |


A
B
C
D
E
F

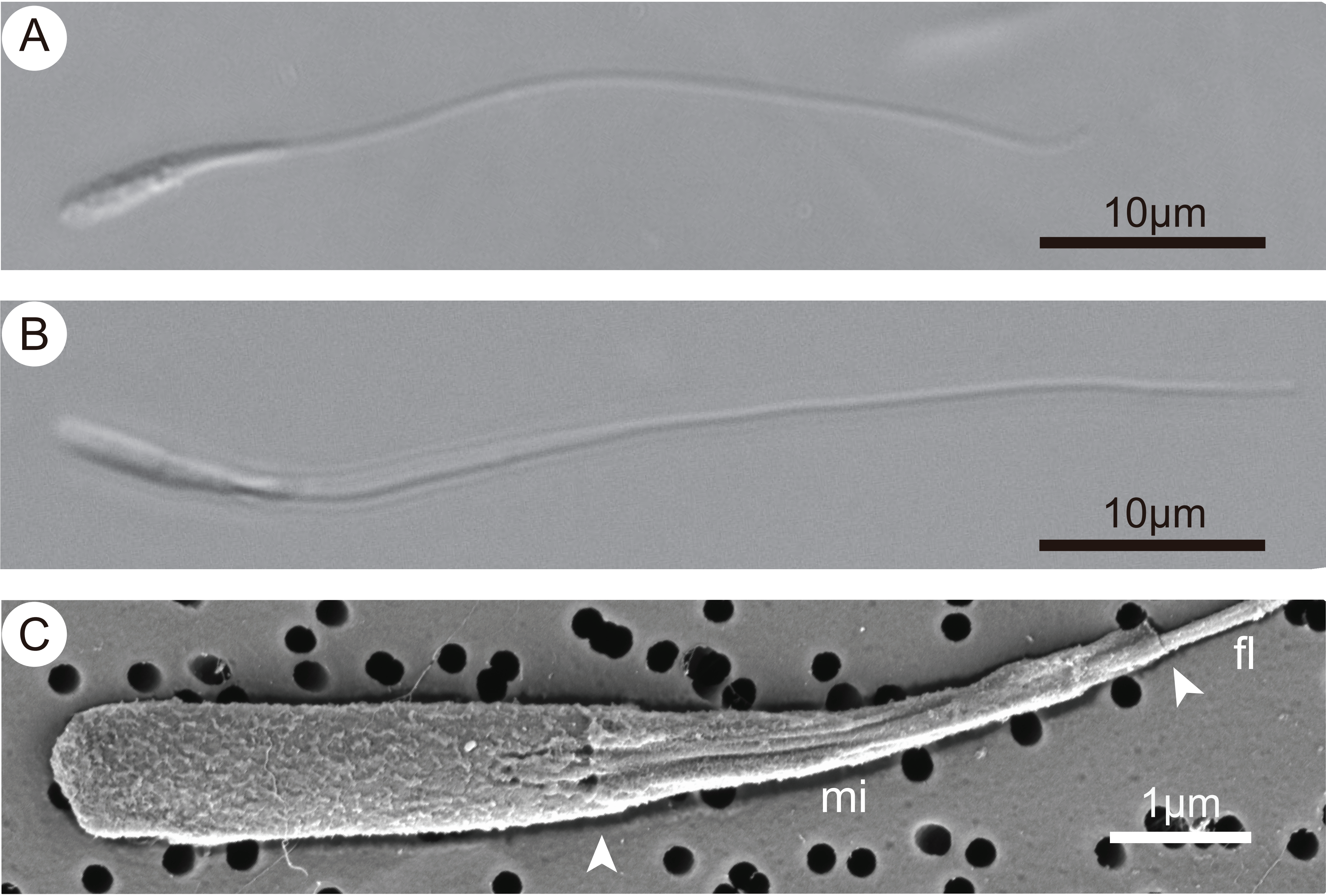
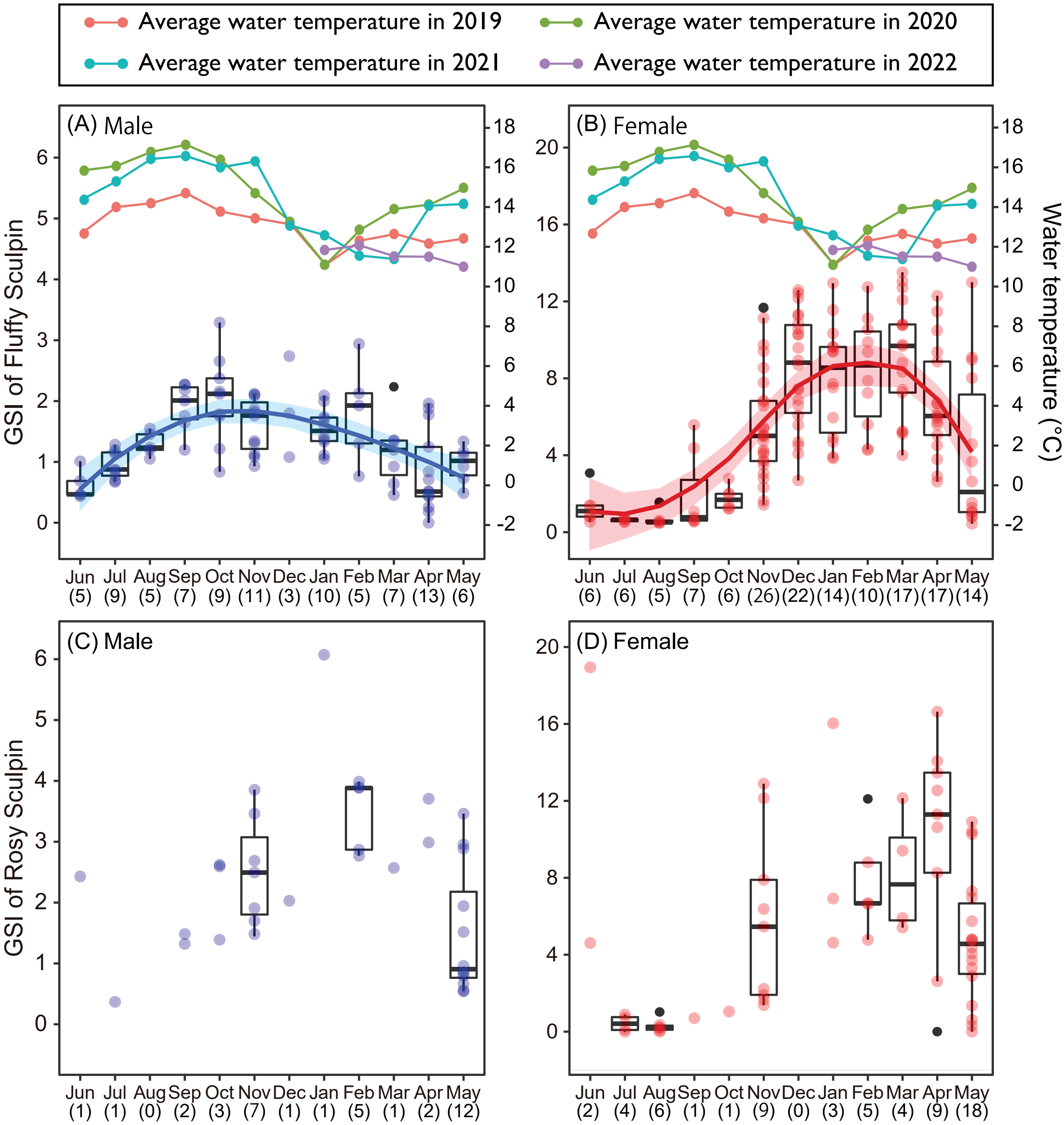

A
10μm
B
10μm
C
fl
mi
1μm

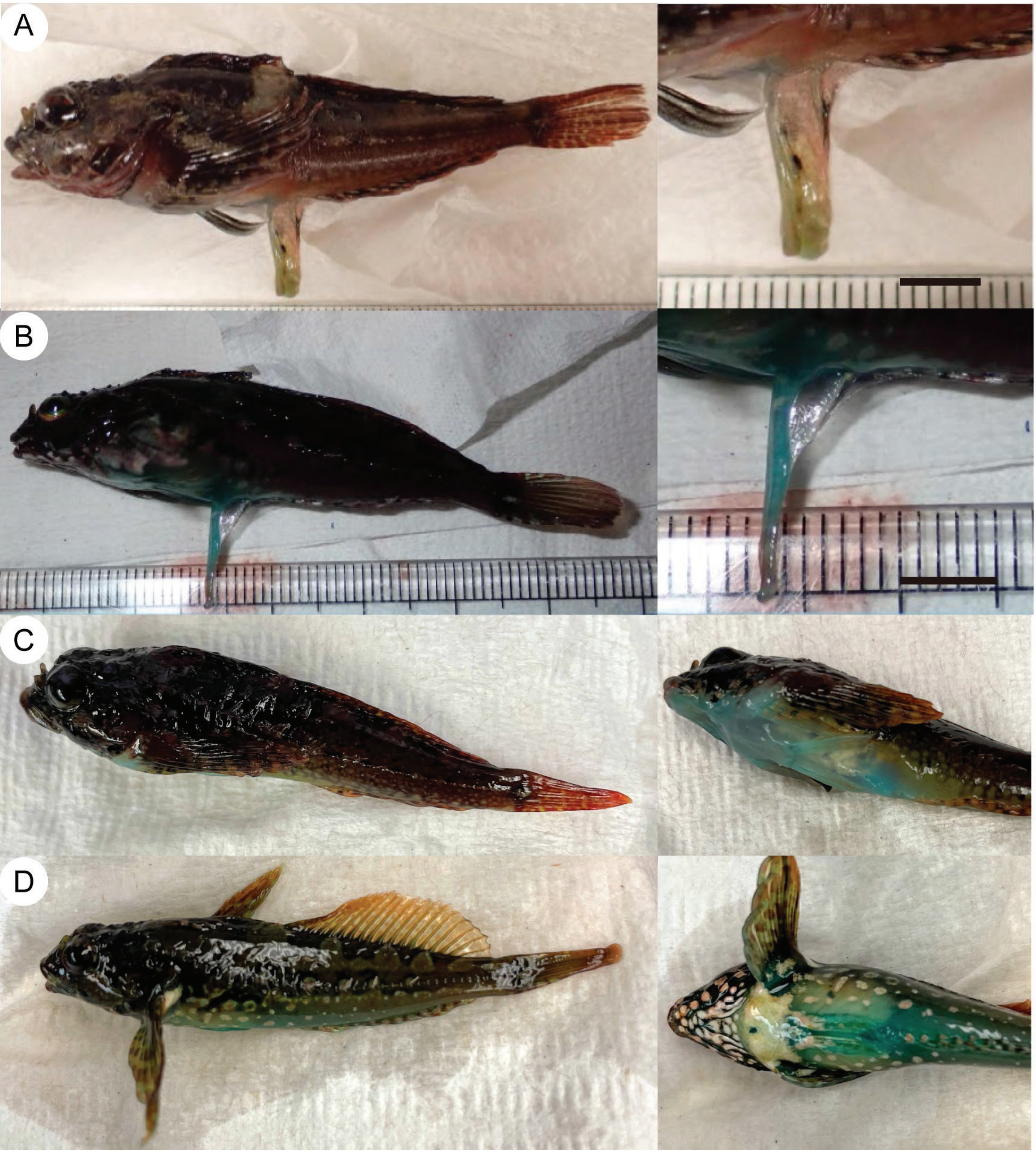
**Table 2. Sperm morphological characteristics and swimming speed in Fluffy Sculpin (*Oligocottus snyderi*) and Rosy Sculpin (*O. rubellio*).**

| Sperm characteristics | *O. snydri* | *O. rubellio* | Statistics | |
|---|---|---|---|---|
| | | | $\chi^2$ | P |
| Total sperm length (µm) | 44.36 ± 1.29 (9, 70) | 52.99 ± 1.19 (3, 23) | 30.00 | <0.0001 |
| Head length (µm) | 5.2 ± 0.32 (8, 53) | 5.6 ± 0.3 (3, 23) | 4.63 | 0.031 |
| Head width (µm) | 1.36 ± 0.17 (7, 51) | 1.81 ± 0.36 (3, 23) | 6.71 | 0.009 |
| Midpiece length (µm) | 4.42 ± 0.49 (8, 52) | 4.55 ± 0.77 (3, 23) | 0.49 | 0.49 |
| Midpiece width (µm) | 0.99 ± 0.14 (7, 50) | 1.08 ± 0.06 (3, 23) | 2.55 | 0.11 |
| Flagella length (µm) | 39.57 ± 1.67 (8, 52) | 47.39 ± 1.47 (3, 23) | 21.34 | <0.0001 |
| Head length / head width | 3.91 ± 0.67 (7, 51) | 3.35 ± 0.37 (3, 23) | 1.93 | 0.17 |
| Midpiece length / midpiece width | 4.76 ± 0.54 (7, 50) | 4.25 ± 0.96 (3, 23) | 0.79 | 0.38 |
| Head length / total sperm length | 0.12 ± 0.01 (8, 52) | 0.11 ± 0.01 (3, 23) | 4.06 | 0.044 |
| Head length / flagella length | 0.13 ± 0.01 (8, 52) | 0.12 ± 0.01 (3, 23) | 3.79 | 0.052 |
| Sperm swimming speed (µm/s) | 75.61 ± 19.41 (6, 118) | 91.81 ± 2.23 (2, 25) | 1.48 | 0.22 |

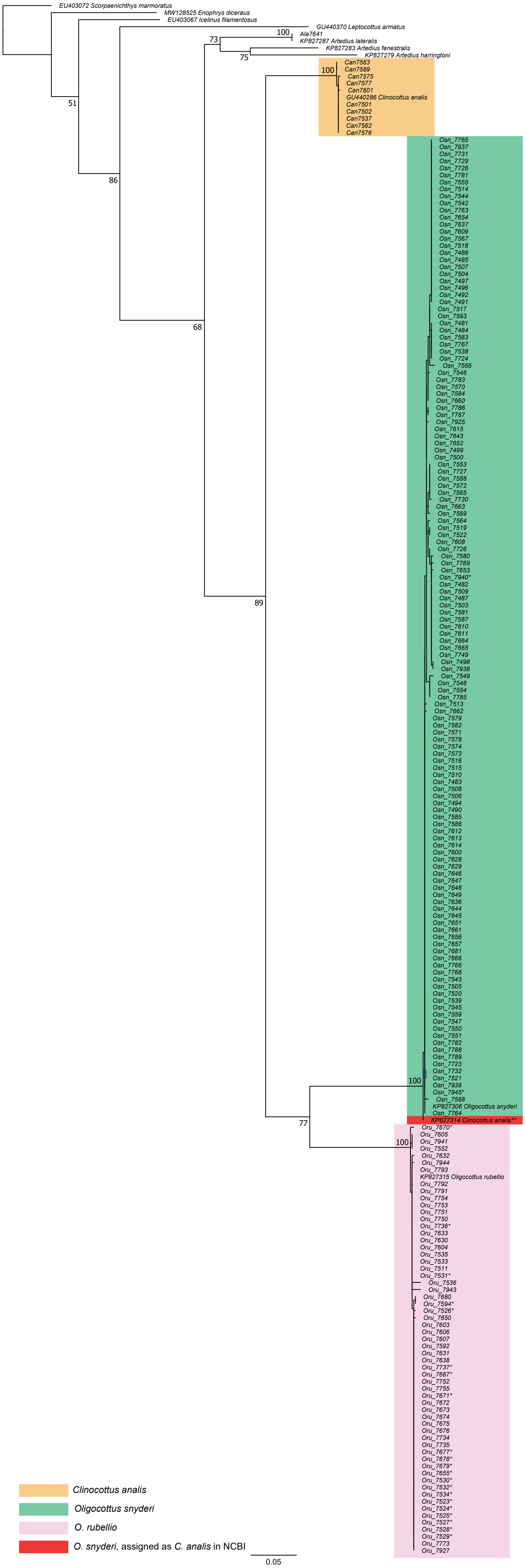
All values represent mean ± SD. Brackets show the number of individuals (left) and sperm cells (right) used for the analyses. Linear mixed models were performed, setting individual ID as a random effect.

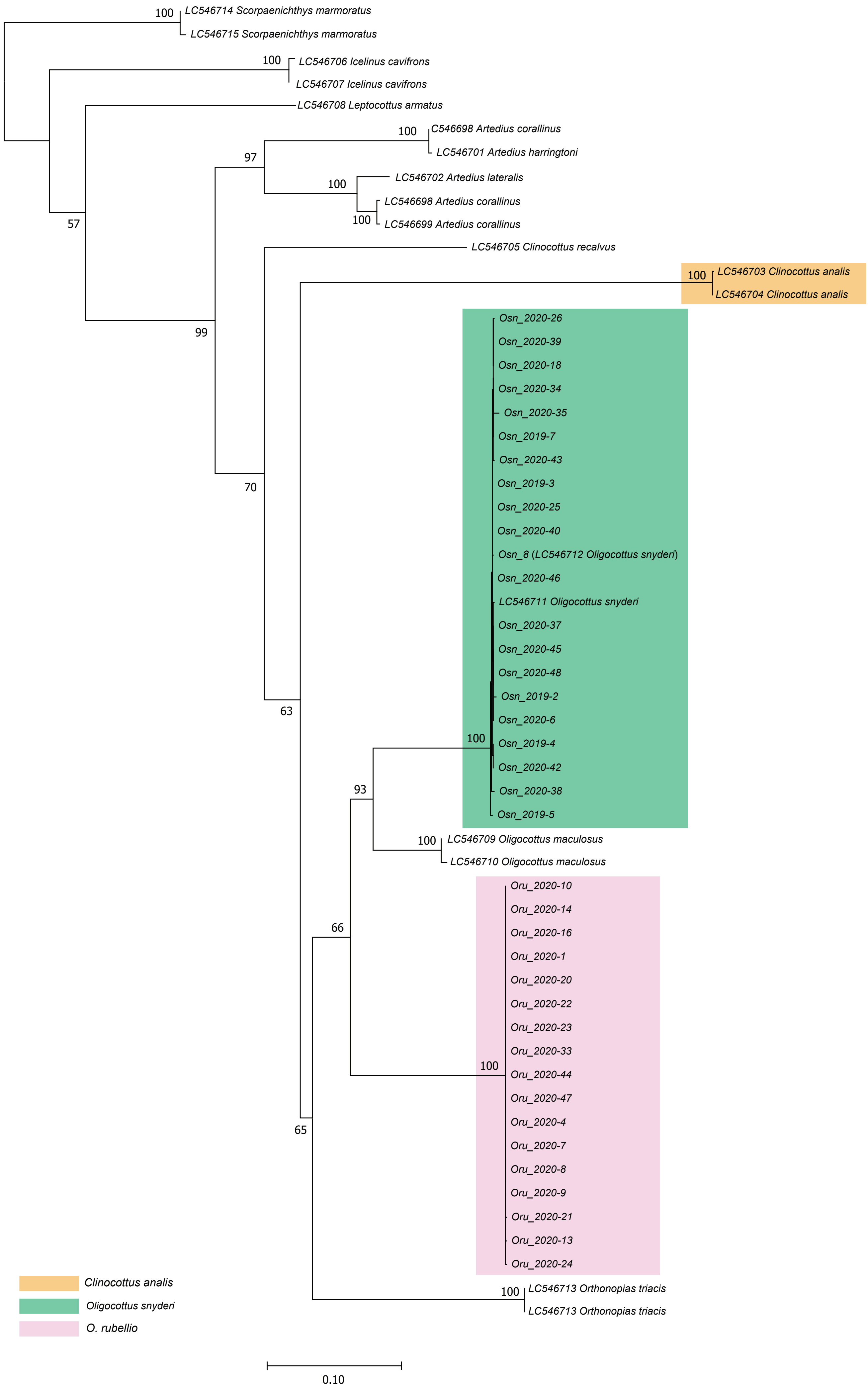


Average water temperature in 2019
Average water temperature in 2020
Average water temperature in 2021
Average water temperature in 2022
(A) Male
(B) Female
(C) Male
(D) Female
GSI of Fluffy Sculpin
GSI of Rosy Sculpin
Water temperature (°C)
Jun (5) Jul (9) Aug (5) Sep (7) Oct (9) Nov (11) Dec (3) Jan (10) Feb (5) Mar (7) Apr (13) May (6)
Jun (6) Jul (6) Aug (5) Sep (7) Oct (6) Nov (26) Dec (22) Jan (14) Feb (10) Mar (17) Apr (17) May (14)
Jun (1) Jul (1) Aug (0) Sep (2) Oct (3) Nov (7) Dec (1) Jan (1) Feb (5) Mar (1) Apr (2) May (12)
Jun (2) Jul (4) Aug (6) Sep (1) Oct (1) Nov (9) Dec (0) Jan (3) Feb (5) Mar (4) Apr (9) May (18)



A
B
C
D


EU403072 Scorpaenichthys marmoratus
MW128525 Enophrys diceraus
EU403067 Icelinus filamentosus
GU440370 Leptocottus armatus
100
Ala7641
KP827287 Artedius lateralis
73
KP827283 Artedius fenestralis
75
KP827279 Artedius harringtoni
100
Can7563
Can7589
Can7575
Can7577
Can7601
GU440286 Clinocottus analis
Can7501
Can7502
Can7537
Can7562
Can7576
51
86
68
89
Osn_7765
Osn_7937
Osn_7731
Osn_7729
Osn_7728
Osn_7781
Osn_7555
Osn_7514
Osn_7544
Osn_7542
Osn_7763
Osn_7654
Osn_7637
Osn_7609
Osn_7567
Osn_7518
Osn_7486
Osn_7485
Osn_7507
Osn_7504
Osn_7497
Osn_7496
Osn_7492
Osn_7491
Osn_7517
Osn_7593
Osn_7481
Osn_7484
Osn_7583
Osn_7767
Osn_7538
Osn_7724
Osn_7566
Osn_7546
Osn_7783
Osn_7570
Osn_7584
Osn_7660
Osn_7786
Osn_7787
Osn_7925
Osn_7615
Osn_7643
Osn_7652
Osn_7499
Osn_7500
Osn_7553
Osn_7727
Osn_7588
Osn_7572
Osn_7565
Osn_7730
Osn_7663
Osn_7569
Osn_7564
Osn_7519
Osn_7522
Osn_7608
Osn_7726
Osn_7580
Osn_7769
Osn_7653
Osn_7940*
Osn_7482
Osn_7509
Osn_7487
Osn_7503
Osn_7581
Osn_7587
Osn_7610
Osn_7611
Osn_7664
Osn_7665
Osn_7749
Osn_7498
Osn_7938
Osn_7549
Osn_7548
Osn_7554
Osn_7785
Osn_7513
Osn_7662
Osn_7579
Osn_7582
Osn_7571
Osn_7578
Osn_7574
Osn_7573
Osn_7516
Osn_7515
Osn_7510
Osn_7483
Osn_7508
Osn_7506
Osn_7494
Osn_7490
Osn_7585
Osn_7586
Osn_7612
Osn_7613
Osn_7614
Osn_7600
Osn_7628
Osn_7629
Osn_7646
Osn_7647
Osn_7648
Osn_7649
Osn_7636
Osn_7644
Osn_7645
Osn_7651
Osn_7661
Osn_7656
Osn_7657
Osn_7681
Osn_7666
Osn_7766
Osn_7768
Osn_7543
Osn_7505
Osn_7520
Osn_7539
Osn_7545
Osn_7559
Osn_7547
Osn_7550
Osn_7551
Osn_7782
Osn_7788
Osn_7789
Osn_7723
Osn_7732
100
Osn_7521
Osn_7939
Osn_7945*
Osn_7568
KP827306 Oligocottus snyderi
Osn_7764
KP827314 Clinocottus analis**
77
Oru_7670*
Oru_7605
100
Oru_7941
Oru_7552
Oru_7632
Oru_7944
Oru_7793
KP827315 Oligocottus rubellio
Oru_7792
Oru_7791
Oru_7754
Oru_7753
Oru_7751
Oru_7750
Oru_7736*
Oru_7633
Oru_7630
Oru_7604
Oru_7535
Oru_7533
Oru_7511
Oru_7531*
Oru_7536
Oru_7943
Oru_7680
Oru_7594*
Oru_7526*
Oru_7650
Oru_7603
Oru_7606
Oru_7607
Oru_7592
Oru_7631
Oru_7638
Oru_7737*
Oru_7667*
Oru_7752
Oru_7755
Oru_7671*
Oru_7672
Oru_7673
Oru_7674
Oru_7675
Oru_7676
Oru_7734
Oru_7735
Oru_7677*
Oru_7678*
Oru_7679*
Oru_7655*
Oru_7530*
Oru_7532*
Oru_7534*
Oru_7523*
Oru_7524*
Oru_7525*
Oru_7527*
Oru_7528*
Oru_7529*
Oru_7773
Oru_7927
Clinocottus analis
Oligocottus snyderi
O. rubellio
O. snyderi, assigned as C. analis in NCBI
0.05



100
LC546714 Scorpaenichthys marmoratus
LC546715 Scorpaenichthys marmoratus
100
LC546706 Icelinus cavifrons
LC546707 Icelinus cavifrons
LC546708 Leptocottus armatus
100
C546698 Artedius corallinus
LC546701 Artedius harringtoni
97
100
LC546702 Artedius lateralis
LC546698 Artedius corallinus
100
LC546699 Artedius corallinus
57
LC546705 Clinocottus recalvus
100
LC546703 Clinocottus analis
LC546704 Clinocottus analis
99
Osn_2020-26
Osn_2020-39
Osn_2020-18
Osn_2020-34
Osn_2020-35
Osn_2019-7
Osn_2020-43
70
Osn_2019-3
Osn_2020-25
Osn_2020-40
Osn_8 (LC546712 Oligocottus snyderi)
Osn_2020-46
LC546711 Oligocottus snyderi
Osn_2020-37
Osn_2020-45
Osn_2020-48
Osn_2019-2
Osn_2020-6
63
100
Osn_2019-4
Osn_2020-42
93
Osn_2020-38
Osn_2019-5
100
LC546709 Oligocottus maculosus
LC546710 Oligocottus maculosus
Oru_2020-10
Oru_2020-14
Oru_2020-16
Oru_2020-1
66
Oru_2020-20
Oru_2020-22
Oru_2020-23
Oru_2020-33
100
Oru_2020-44
Oru_2020-47
Oru_2020-4
65
Oru_2020-7
Oru_2020-8
Oru_2020-9
Oru_2020-21
Oru_2020-13
Oru_2020-24
Clinocottus analis
Oligocottus snyderi
O. rubellio
100
LC546713 Orthonopias triacis
LC546713 Orthonopias triacis
0.10

| No | MeasurementDate | Month | Month_Order | Species | SpeciesIndID | Sex | TL | SL | BM | GutMass | GM | BM-GutMass | GSI | Sequence | GenBankAccessNo |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2018.03.02 | Mar | 10 | *Oligocottus snyderi* | Osn_6 | F | 67.06 | 55.78 | 4.400 | 0.300 | 0.400 | 4.100 | 9.756 | NA | |
| 2 | 2018.03.02 | Mar | 10 | *Oligocottus snyderi* | Osn_7 | F | 67.07 | 55.78 | 4.000 | 0.300 | 0.500 | 3.700 | 13.514 | NA | |
| 3 | 2018.03.02 | Mar | 10 | *Oligocottus snyderi* | Osn_8 | F | 59.88 | 49.86 | 2.600 | 0.200 | 0.200 | 2.400 | 8.333 | Cytb | LC546712 |
| 4 | 2019.10.27 | Oct | 5 | *Oligocottus snyderi* | Osn_T2 | M | NA | 43.00 | 2.980 | 0.121 | 0.051 | 2.860 | 1.784 | NA | |
| 5 | 2019.10.27 | Oct | 5 | *Oligocottus snyderi* | Osn_T4 | M | NA | 42.00 | 2.330 | 0.184 | 0.018 | 2.150 | 0.839 | NA | |
| 6 | 2019.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_2019-1 | M | 67.50 | 54.50 | 3.738 | 0.293 | 0.073 | 3.445 | 2.119 | NA | |
| 7 | 2019.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_2019-2 | F | 66.50 | 54.00 | 3.699 | 0.452 | 0.042 | 3.247 | 1.294 | Cytb | |
| 8 | 2019.11.25 | Nov | 6 | *Oligocottus snyderi* | Osn_2019-3 | F | 75.00 | 60.50 | 6.013 | 0.883 | 0.397 | 5.130 | 7.739 | Cytb | |
| 9 | 2019.11.25 | Nov | 6 | *Oligocottus snyderi* | Osn_2019-4 | F | 79.50 | 64.50 | 7.913 | 0.990 | 0.771 | 6.923 | 11.137 | Cytb | |
| 10 | 2019.11.25 | Nov | 6 | *Oligocottus snyderi* | Osn_2019-5 | F | 79.00 | 64.00 | 6.562 | 0.701 | 0.314 | 5.861 | 5.357 | Cytb | |
| 11 | 2019.12.24 | Dec | 7 | *Oligocottus snyderi* | Osn_2019-6 | M | 65.00 | 53.50 | 3.350 | 0.172 | 0.087 | 3.178 | 2.738 | NA | |
| 12 | 2019.12.24 | Dec | 7 | *Oligocottus snyderi* | Osn_2019-7 | F | 83.00 | 67.50 | 7.532 | 0.703 | 0.508 | 6.829 | 7.439 | Cytb | |
| 13 | 2020.02.07 | Feb | 9 | *Oligocottus snyderi* | Osn_2020-3 | M | 77.50 | 64.00 | 5.761 | 0.810 | 0.038 | 4.951 | 0.768 | NA | |
| 14 | 2020.04.06 | Apr | 11 | *Oligocottus snyderi* | Osn_2020-6 | M | 70.50 | 58.00 | 4.099 | 0.272 | 0.068 | 3.827 | 1.777 | Cytb | |
| 15 | 2020.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_2020-11-1 | F | 66.50 | 53.50 | 3.979 | 0.423 | 0.215 | 3.556 | 6.046 | NA | |
| 16 | 2020.04.27 | Apr | 11 | *Oligocottus snyderi* | Osn_2020-15 | M | 70.00 | 57.00 | 4.096 | 0.302 | 0.017 | 3.794 | 0.448 | NA | |
| 17 | 2020.05.09 | May | 12 | *Oligocottus snyderi* | Osn_2020-18 | F | 76.00 | 63.50 | 5.519 | 0.642 | 0.443 | 4.877 | 9.083 | Cytb | |
| 18 | 2020.06.10 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-25 | F | 78.00 | 64.00 | 6.018 | 0.876 | 0.042 | 5.142 | 0.817 | Cytb | |
| 19 | 2020.06.10 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-26 | F | 77.00 | 64.00 | 5.342 | 0.469 | 0.067 | 4.873 | 1.375 | Cytb | |
| 20 | 2020.06.10 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-27 | F | 76.50 | 63.00 | 5.633 | 0.623 | 0.154 | 5.010 | 3.074 | NA | |
| 21 | 2020.06.10 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-28 | M | 71.00 | 57.50 | 4.301 | 0.432 | 0.018 | 3.869 | 0.465 | NA | |
| 22 | 2020.06.10 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-30 | M | 81.00 | 67.50 | 7.309 | 0.600 | 0.031 | 6.709 | 0.462 | NA | |
| 23 | 2020.06.11 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-31 | M | 79.00 | 65.00 | 6.685 | 0.430 | 0.043 | 6.255 | 0.687 | NA | |
| 24 | 2020.06.11 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-32 | M | 81.00 | 66.50 | 6.956 | 0.512 | 0.028 | 6.444 | 0.435 | NA | |
| 25 | 2020.06.20 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-34 | F | 67.00 | 54.00 | 4.052 | 0.566 | 0.028 | 3.486 | 0.803 | Cytb | |
| 26 | 2020.06.20 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-35 | F | 69.50 | 56.50 | 4.193 | 0.281 | 0.021 | 3.912 | 0.537 | Cytb | |
| 27 | 2020.06.21 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-36 | M | 77.50 | 64.50 | 5.505 | 0.451 | 0.051 | 5.054 | 1.009 | NA | |
| 28 | 2020.06.21 | Jun | 1 | *Oligocottus snyderi* | Osn_2020-37 | F | 75.50 | 62.50 | 4.945 | 0.423 | 0.063 | 4.522 | 1.393 | Cytb | |
| 29 | 2020.07.06 | Jul | 2 | *Oligocottus snyderi* | Osn_2020-38 | F | 82.00 | 67.50 | 6.610 | 0.694 | 0.039 | 5.916 | 0.659 | Cytb | |
| 30 | 2020.07.06 | Jul | 2 | *Oligocottus snyderi* | Osn_2020-39 | M | 81.00 | 66.50 | 6.925 | 0.510 | 0.043 | 6.415 | 0.670 | Cytb | |
| 31 | 2020.07.06 | Jul | 2 | *Oligocottus snyderi* | Osn_2020-40 | M | 77.00 | 63.50 | 6.272 | 0.478 | 0.069 | 5.794 | 1.191 | Cytb | |
| 32 | 2020.07.06 | Jul | 2 | *Oligocottus snyderi* | Osn_2020-41 | M | 77.00 | 63.50 | 5.866 | 0.374 | 0.048 | 5.492 | 0.874 | NA | |
| 33 | 2020.07.06 | Jul | 2 | *Oligocottus snyderi* | Osn_2020-42 | F | 76.00 | 62.00 | 5.454 | 0.503 | 0.027 | 4.951 | 0.545 | Cytb | |
| 34 | 2020.07.06 | Jul | 2 | *Oligocottus snyderi* | Osn_2020-43 | M | 73.00 | 59.50 | 5.197 | 0.436 | 0.041 | 4.761 | 0.861 | Cytb | |
| 35 | 2020.09.17 | Sep | 4 | *Oligocottus snyderi* | Osn_2020-45 | M | 73.00 | 59.00 | 5.510 | 0.289 | 0.114 | 5.221 | 2.183 | Cytb | |
| 36 | 2020.09.17 | Sep | 4 | *Oligocottus snyderi* | Osn_2020-46 | M | 62.00 | 50.50 | 3.020 | 0.173 | 0.050 | 2.847 | 1.756 | Cytb | |
| 37 | 2020.09.17 | Sep | 4 | *Oligocottus snyderi* | Osn_2020-48 | F | 87.50 | 71.00 | 8.789 | 0.790 | 0.446 | 7.999 | 5.576 | Cytb | |
| 38 | 2020.09.17 | Sep | 4 | *Oligocottus snyderi* | Osn_2020-49 | F | 54.50 | 46.50 | 3.012 | 0.176 | 0.124 | 2.836 | 4.372 | NA | |
| 39 | 2020.10.16 | Oct | 5 | *Oligocottus snyderi* | Osn_7490 | F | 65.00 | 52.00 | 4.450 | 0.140 | 0.120 | 4.310 | 2.784 | COI | |
| 40 | 2020.10.16 | Oct | 5 | *Oligocottus snyderi* | Osn_7491 | M | 59.00 | 48.00 | 3.480 | 0.190 | 0.040 | 3.290 | 1.216 | COI | |
| 41 | 2020.10.16 | Oct | 5 | *Oligocottus snyderi* | Osn_7492 | M | 69.00 | 56.00 | 4.900 | 0.340 | 0.150 | 4.560 | 3.289 | COI | |
| 42 | 2020.10.16 | Oct | 5 | *Oligocottus snyderi* | Osn_7493 | M | 71.00 | 57.00 | 6.150 | 0.500 | 0.150 | 5.650 | 2.655 | NA | |
| 43 | 2020.11.03 | Nov | 6 | *Oligocottus snyderi* | Osn_7494 | F | 63.00 | 51.00 | 3.700 | 0.410 | 0.280 | 3.290 | 8.511 | COI | |
| 44 | 2020.11.03 | Nov | 6 | *Oligocottus snyderi* | Osn_7495 | F | 61.00 | 50.00 | 3.780 | 0.530 | 0.110 | 3.250 | 3.385 | NA | |
| 45 | 2020.11.03 | Nov | 6 | *Oligocottus snyderi* | Osn_7496 | F | 67.00 | 54.00 | 4.460 | 0.480 | 0.270 | 3.980 | 6.784 | COI | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 46 | 2020.11.03 | Nov | 6 | *Oligocottus snyderi* | Osn_7497 | M | 60.00 | 49.00 | 2.980 | 0.310 | 0.030 | 2.670 | 1.124 | COI |
| 47 | 2020.11.03 | Nov | 6 | *Oligocottus snyderi* | Osn_7498 | F | 61.00 | 48.00 | 3.030 | 0.430 | 0.070 | 2.600 | 2.692 | COI |
| 48 | 2020.11.03 | Nov | 6 | *Oligocottus snyderi* | Osn_7499 | M | 63.00 | 50.00 | 3.080 | 0.310 | 0.030 | 2.770 | 1.083 | COI |
| 49 | 2020.11.03 | Nov | 6 | *Oligocottus snyderi* | Osn_7500 | F | 51.00 | 40.00 | 2.400 | 0.250 | 0.090 | 2.150 | 4.186 | COI |
| 50 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7481 | F | 83.00 | 65.00 | 9.520 | 0.980 | 0.340 | 8.540 | 3.981 | COI |
| 51 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7482 | M | 63.00 | 52.00 | 4.660 | 0.380 | 0.090 | 4.280 | 2.103 | COI |
| 52 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7483 | F | 67.00 | 53.00 | 5.400 | 0.440 | 0.110 | 4.960 | 2.218 | COI |
| 53 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7484 | F | 63.00 | 51.00 | 4.640 | 0.560 | 0.120 | 4.080 | 2.941 | COI |
| 54 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7485 | F | 86.00 | 70.00 | 10.150 | 0.840 | 0.340 | 9.310 | 3.652 | COI |
| 55 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7486 | F | 63.00 | 50.00 | 4.380 | 0.380 | 0.060 | 4.000 | 1.500 | COI |
| 56 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7487 | M | 60.00 | 46.00 | 3.460 | 0.240 | 0.030 | 3.220 | 0.932 | COI |
| 57 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7503 | F | 69.00 | 55.00 | 6.530 | 0.730 | 0.390 | 5.800 | 6.724 | COI |
| 58 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7504 | F | 61.00 | 48.00 | 4.000 | 0.340 | 0.170 | 3.660 | 4.645 | COI |
| 59 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7505 | F | 59.00 | 47.00 | 3.420 | 0.350 | 0.110 | 3.070 | 3.583 | COI |
| 60 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7506 | F | 66.00 | 52.00 | 4.980 | 0.550 | 0.180 | 4.430 | 4.063 | COI |
| 61 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7507 | F | 57.00 | 46.00 | 3.690 | 0.310 | 0.220 | 3.380 | 6.509 | COI |
| 62 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7508 | F | 59.00 | 47.00 | 3.310 | 0.240 | 0.150 | 3.070 | 4.886 | COI |
| 63 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7509 | M | 56.00 | 46.00 | 2.970 | 0.240 | 0.050 | 2.730 | 1.832 | COI |
| 64 | 2020.11.13 | Nov | 6 | *Oligocottus snyderi* | Osn_7510 | M | 67.00 | 53.00 | 4.720 | 0.200 | 0.080 | 4.520 | 1.770 | COI |
| 65 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7513 | F | 66.00 | 53.00 | 5.720 | 0.470 | 0.510 | 5.250 | 9.714 | COI |
| 66 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7514 | F | 57.00 | 46.00 | 3.290 | 0.280 | 0.160 | 3.010 | 5.316 | COI |
| 67 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7515 | F | 68.00 | 55.00 | 5.900 | 0.360 | 0.520 | 5.540 | 9.386 | COI |
| 68 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7516 | F | 58.00 | 46.00 | 3.340 | 0.310 | 0.150 | 3.030 | 4.950 | COI |
| 69 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7517 | M | 66.00 | 52.00 | 4.910 | 0.340 | 0.060 | 4.570 | 1.313 | COI |
| 70 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7518 | F | 57.00 | 46.00 | 3.630 | 0.290 | 0.390 | 3.340 | 11.677 | COI |
| 71 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7519 | M | 60.00 | 48.00 | 3.970 | 0.260 | 0.050 | 3.710 | 1.348 | COI |
| 72 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7520 | F | 66.00 | 52.00 | 5.420 | 0.390 | 0.250 | 5.030 | 4.970 | COI |
| 73 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7521 | M | 64.00 | 52.00 | 4.430 | 0.210 | 0.090 | 4.220 | 2.133 | COI |
| 74 | 2020.11.30 | Nov | 6 | *Oligocottus snyderi* | Osn_7522 | M | 60.00 | 47.00 | 3.400 | 0.210 | 0.060 | 3.190 | 1.881 | COI |
| 75 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7538 | F | 82.00 | 67.00 | 9.610 | 0.350 | 0.950 | 9.260 | 10.259 | COI |
| 76 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7539 | F | 80.00 | 64.00 | 7.710 | 0.250 | 0.350 | 7.460 | 4.692 | COI |
| 77 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7540 | F | 75.00 | 60.00 | 6.400 | 0.340 | 0.640 | 6.060 | 10.561 | NA |
| 78 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7541 | F | 71.00 | 57.00 | 5.600 | 0.310 | 0.460 | 5.290 | 8.696 | NA |
| 79 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7542 | F | 69.00 | 56.00 | 4.910 | 0.320 | 0.440 | 4.590 | 9.586 | COI |
| 80 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7543 | F | 64.00 | 52.00 | 4.490 | 0.200 | 0.540 | 4.290 | 12.587 | COI |
| 81 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7544 | F | 60.00 | 47.00 | 3.610 | 0.250 | 0.380 | 3.360 | 11.310 | COI |
| 82 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7545 | F | 66.00 | 54.00 | 4.370 | 0.220 | 0.450 | 4.150 | 10.843 | COI |
| 83 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7546 | F | 58.00 | 46.00 | 3.310 | 0.190 | 0.380 | 3.120 | 12.179 | COI |
| 84 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7547 | F | 58.00 | 47.00 | 3.230 | 0.220 | 0.310 | 3.010 | 10.299 | COI |
| 85 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7548 | F | 63.00 | 50.00 | 3.530 | 0.100 | 0.140 | 3.430 | 4.082 | COI |
| 86 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7549 | F | 58.00 | 47.00 | 2.740 | 0.140 | 0.070 | 2.600 | 2.692 | COI |
| 87 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7550 | M | 57.00 | 46.00 | 2.840 | 0.060 | 0.030 | 2.780 | 1.079 | COI |
| 88 | 2020.12.10 | Dec | 7 | *Oligocottus snyderi* | Osn_7551 | M | 60.00 | 48.00 | 2.820 | 0.040 | 0.050 | 2.780 | 1.799 | COI |
| 89 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7553 | F | 57.00 | 45.00 | 3.310 | 0.260 | 0.170 | 3.050 | 5.574 | COI |
| 90 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7554 | F | 83.00 | 67.00 | 10.620 | 0.680 | 1.120 | 9.940 | 11.268 | COI |
| 91 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7555 | F | 58.00 | 47.00 | 3.840 | 0.220 | 0.450 | 3.620 | 12.431 | COI |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 92 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7556 | F | 51.00 | 41.00 | 2.430 | 0.190 | 0.200 | 2.240 | 8.929 | NA |
| 93 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7557 | F | 62.00 | 50.00 | 3.860 | 0.310 | 0.160 | 3.550 | 4.507 | NA |
| 94 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7558 | F | 81.00 | 65.00 | 9.300 | 0.570 | 0.600 | 8.730 | 6.873 | NA |
| 95 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7559 | F | 57.00 | 45.00 | 3.990 | 0.200 | 0.230 | 3.790 | 6.069 | COI |
| 96 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7560 | F | 81.00 | 60.00 | 9.280 | 0.790 | 0.560 | 8.490 | 6.596 | NA |
| 97 | 2020.12.27 | Dec | 7 | *Oligocottus snyderi* | Osn_7561 | F | 65.00 | 52.00 | 4.790 | 0.350 | 0.330 | 4.440 | 7.432 | NA |
| 98 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7564 | M | 55.00 | 44.00 | 2.970 | 0.130 | 0.040 | 2.840 | 1.408 | COI |
| 99 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7565 | M | 57.00 | 47.00 | 3.050 | 0.120 | 0.050 | 2.930 | 1.706 | COI |
| 100 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7566 | M | 58.00 | 47.00 | 2.880 | 0.170 | 0.030 | 2.710 | 1.107 | COI |
| 101 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7567 | M | 52.00 | 41.00 | 2.370 | 0.120 | 0.030 | 2.250 | 1.333 | COI |
| 102 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7568 | M | 53.00 | 42.00 | 2.090 | 0.090 | 0.040 | 2.000 | 2.000 | COI |
| 103 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7569 | F | 57.00 | 46.00 | 3.170 | 0.190 | 0.230 | 2.980 | 7.718 | COI |
| 104 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7570 | F | 60.00 | 48.00 | 3.860 | 0.280 | 0.370 | 3.580 | 10.335 | COI |
| 105 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7571 | F | 65.00 | 52.00 | 4.790 | 0.310 | 0.210 | 4.480 | 4.688 | COI |
| 106 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7572 | F | 57.00 | 46.00 | 3.040 | 0.250 | 0.210 | 2.790 | 7.527 | COI |
| 107 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7573 | F | 59.00 | 50.00 | 3.570 | 0.280 | 0.380 | 3.290 | 11.550 | COI |
| 108 | 2021.01.12 | Jan | 8 | *Oligocottus snyderi* | Osn_7574 | F | 68.00 | 55.00 | 5.960 | 0.400 | 0.720 | 5.560 | 12.950 | COI |
| 109 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7578 | F | 59.00 | 47.00 | 3.020 | 0.170 | 0.140 | 2.850 | 4.912 | COI |
| 110 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7579 | F | 68.00 | 54.00 | 5.430 | 0.340 | 0.490 | 5.090 | 9.627 | COI |
| 111 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7580 | F | 61.00 | 49.00 | 3.620 | 0.210 | 0.320 | 3.410 | 9.384 | COI |
| 112 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7581 | F | 61.00 | 50.00 | 3.260 | 0.250 | 0.290 | 3.010 | 9.635 | COI |
| 113 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7582 | F | 71.00 | 58.00 | 5.650 | 0.550 | 0.480 | 5.100 | 9.412 | COI |
| 114 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7583 | F | 63.00 | 52.00 | 3.490 | 0.300 | 0.190 | 3.190 | 5.956 | COI |
| 115 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7584 | M | 58.00 | 48.00 | 3.050 | 0.170 | 0.050 | 2.880 | 1.736 | COI |
| 116 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7585 | M | 62.00 | 50.00 | 3.820 | 0.170 | 0.050 | 3.650 | 1.370 | COI |
| 117 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7586 | M | 65.00 | 52.00 | 4.030 | 0.220 | 0.040 | 3.810 | 1.050 | COI |
| 118 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7587 | M | 68.00 | 54.00 | 4.800 | 0.450 | 0.070 | 4.350 | 1.609 | COI |
| 119 | 2021.01.29 | Jan | 8 | *Oligocottus snyderi* | Osn_7588 | M | 58.00 | 47.00 | 3.020 | 0.150 | 0.060 | 2.870 | 2.091 | COI |
| 120 | 2021.02.06 | Feb | 9 | *Oligocottus snyderi* | Osn_7593 | F | 53.00 | 42.00 | 2.110 | 0.130 | 0.220 | 1.980 | 11.111 | COI |
| 121 | 2021.02.06 | Feb | 9 | *Oligocottus snyderi* | Osn_7595 | F | 55.00 | 45.00 | 2.440 | 0.230 | 0.160 | 2.210 | 7.240 | NA |
| 122 | 2021.02.06 | Feb | 9 | *Oligocottus snyderi* | Osn_7596 | F | 57.00 | 47.00 | 2.880 | 0.210 | 0.250 | 2.670 | 9.363 | NA |
| 123 | 2021.02.06 | Feb | 9 | *Oligocottus snyderi* | Osn_7597 | F | 78.00 | 63.00 | 7.560 | 0.810 | 0.860 | 6.750 | 12.741 | NA |
| 124 | 2021.02.06 | Feb | 9 | *Oligocottus snyderi* | Osn_7598 | M | 59.00 | 49.00 | 2.460 | 0.150 | 0.030 | 2.310 | 1.299 | NA |
| 125 | 2021.02.06 | Feb | 9 | *Oligocottus snyderi* | Osn_7599 | M | 63.00 | 52.00 | 3.440 | 0.300 | 0.060 | 3.140 | 1.911 | NA |
| 126 | 2021.02.06 | Feb | 9 | *Oligocottus snyderi* | Osn_7600 | M | 58.00 | 47.00 | 2.510 | 0.140 | 0.050 | 2.370 | 2.110 | COI |
| 127 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7608 | F | 54.00 | 43.00 | 2.880 | 0.350 | 0.330 | 2.530 | 13.043 | COI |
| 128 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7609 | F | 61.00 | 48.00 | 3.190 | 0.200 | 0.380 | 2.990 | 12.709 | COI |
| 129 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7610 | F | 59.00 | 47.00 | 3.510 | 0.270 | 0.350 | 3.240 | 10.802 | COI |
| 130 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7611 | F | 67.00 | 56.00 | 4.200 | 0.360 | 0.200 | 3.840 | 5.208 | COI |
| 131 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7612 | F | 66.00 | 55.00 | 4.680 | 0.410 | 0.310 | 4.270 | 7.260 | COI |
| 132 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7613 | F | 60.00 | 47.00 | 2.980 | 0.190 | 0.270 | 2.790 | 9.677 | COI |
| 133 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7614 | F | 54.00 | 43.00 | 2.540 | 0.130 | 0.260 | 2.410 | 10.788 | COI |
| 134 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7615 | F | 62.00 | 51.00 | 2.980 | 0.240 | 0.140 | 2.740 | 5.109 | COI |
| 135 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7616 | F | 54.00 | 44.00 | 2.300 | 0.160 | 0.230 | 2.140 | 10.748 | NA |
| 136 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7617 | M | 46.00 | 36.00 | 1.180 | 0.100 | 0.010 | 1.080 | 0.926 | NA |
| 137 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7618 | M | 54.00 | 44.00 | 1.900 | 0.110 | 0.040 | 1.790 | 2.235 | NA |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 138 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7619 | M | 50.00 | 40.00 | 1.660 | 0.100 | 0.010 | 1.560 | 0.641 NA |
| 139 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7620 | M | 73.00 | 59.00 | 5.560 | 0.400 | 0.070 | 5.160 | 1.357 NA |
| 140 | 2021.03.07 | Mar | 10 | *Oligocottus snyderi* | Osn_7621 | M | 72.00 | 58.00 | 4.730 | 0.350 | 0.020 | 4.380 | 0.457 NA |
| 141 | 2021.03.26 | Mar | 10 | *Oligocottus snyderi* | Osn_7625 | F | 65.00 | 51.00 | 3.810 | 0.250 | 0.430 | 3.560 | 12.079 NA |
| 142 | 2021.03.26 | Mar | 10 | *Oligocottus snyderi* | Osn_7626 | F | 52.00 | 47.00 | 2.540 | 0.250 | 0.120 | 2.290 | 5.240 NA |
| 143 | 2021.03.26 | Mar | 10 | *Oligocottus snyderi* | Osn_7627 | F | 54.00 | 45.00 | 2.290 | 0.130 | 0.160 | 2.160 | 7.407 NA |
| 144 | 2021.03.26 | Mar | 10 | *Oligocottus snyderi* | Osn_7628 | F | 53.00 | 43.00 | 1.910 | 0.160 | 0.070 | 1.750 | 4.000 COI |
| 145 | 2021.03.26 | Mar | 10 | *Oligocottus snyderi* | Osn_7629 | F | 61.00 | 50.00 | 3.270 | 0.320 | 0.260 | 2.950 | 8.814 COI |
| 146 | 2021.03.26 | Mar | 10 | *Oligocottus snyderi* | Osn_7636 | M | 54.00 | 43.00 | 1.790 | 0.120 | 0.020 | 1.670 | 1.198 COI |
| 147 | 2021.03.26 | Mar | 10 | *Oligocottus snyderi* | Osn_7637 | M | 57.00 | 46.00 | 2.420 | 0.190 | 0.030 | 2.230 | 1.345 COI |
| 148 | 2021.04.05 | Apr | 11 | *Oligocottus snyderi* | Osn_7643 | F | 63.00 | 51.00 | 3.060 | 0.270 | 0.320 | 2.790 | 11.470 COI |
| 149 | 2021.04.05 | Apr | 11 | *Oligocottus snyderi* | Osn_7644 | F | 66.00 | 53.00 | 3.270 | 0.300 | 0.150 | 2.970 | 5.051 COI |
| 150 | 2021.04.05 | Apr | 11 | *Oligocottus snyderi* | Osn_7645 | F | 61.00 | 50.00 | 3.280 | 0.290 | 0.280 | 2.990 | 9.365 COI |
| 151 | 2021.04.05 | Apr | 11 | *Oligocottus snyderi* | Osn_7646 | F | 59.00 | 51.00 | 2.680 | 0.200 | 0.260 | 2.480 | 10.484 COI |
| 152 | 2021.04.05 | Apr | 11 | *Oligocottus snyderi* | Osn_7647 | F | 66.00 | 54.00 | 3.370 | 0.290 | 0.190 | 3.080 | 6.169 COI |
| 153 | 2021.04.05 | Apr | 11 | *Oligocottus snyderi* | Osn_7648 | F | 72.00 | 60.00 | 4.330 | 0.290 | 0.240 | 4.040 | 5.941 COI |
| 154 | 2021.04.05 | Apr | 11 | *Oligocottus snyderi* | Osn_7649 | M | 51.00 | 42.00 | 1.490 | 0.120 | 0.000 | 1.370 | 0.000 COI |
| 155 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7651 | F | 62.00 | 53.00 | 3.520 | 0.420 | 0.090 | 3.100 | 2.903 COI |
| 156 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7652 | F | 70.00 | 56.00 | 4.310 | 0.270 | 0.270 | 4.040 | 6.683 COI |
| 157 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7653 | F | 67.00 | 55.00 | 3.980 | 0.290 | 0.140 | 3.690 | 3.794 COI |
| 158 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7654 | F | 60.00 | 47.00 | 3.640 | 0.330 | 0.190 | 3.310 | 5.740 COI |
| 159 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7656 | F | 65.00 | 53.00 | 3.330 | 0.290 | 0.080 | 3.040 | 2.632 COI |
| 160 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7657 | M | 69.00 | 58.00 | 4.220 | 0.330 | 0.020 | 3.890 | 0.514 COI |
| 161 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7658 | M | 69.00 | 55.00 | 4.190 | 0.370 | 0.007 | 3.820 | 0.183 NA |
| 162 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7659 | M | 70.00 | 56.00 | 4.040 | 0.330 | 0.019 | 3.710 | 0.512 NA |
| 163 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7660 | M | 60.00 | 48.00 | 3.170 | 0.220 | 0.008 | 2.950 | 0.254 COI |
| 164 | 2021.04.23 | Apr | 11 | *Oligocottus snyderi* | Osn_7661 | M | 63.00 | 51.00 | 3.180 | 0.180 | 0.013 | 3.000 | 0.433 COI |
| 165 | 2021.05.03 | May | 12 | *Oligocottus snyderi* | Osn_7662 | F | 72.00 | 59.00 | 4.850 | 0.540 | 0.560 | 4.310 | 12.993 COI |
| 166 | 2021.05.03 | May | 12 | *Oligocottus snyderi* | Osn_7663 | F | 73.00 | 61.00 | 5.640 | 0.400 | 0.420 | 5.240 | 8.015 COI |
| 167 | 2021.05.03 | May | 12 | *Oligocottus snyderi* | Osn_7664 | F | 68.00 | 57.00 | 3.710 | 0.250 | 0.310 | 3.460 | 8.960 COI |
| 168 | 2021.05.03 | May | 12 | *Oligocottus snyderi* | Osn_7665 | M | 68.00 | 58.00 | 4.020 | 0.320 | 0.018 | 3.700 | 0.486 COI |
| 169 | 2021.05.03 | May | 12 | *Oligocottus snyderi* | Osn_7666 | F | 60.00 | 59.00 | 2.640 | 0.270 | 0.025 | 2.370 | 1.055 COI |
| 170 | 2021.05.21 | May | 12 | *Oligocottus snyderi* | Osn_7681 | F | 53.00 | 42.00 | 2.010 | 0.180 | 0.019 | 1.830 | 1.038 COI |
| 171 | 2021.05.21 | May | 12 | *Oligocottus snyderi* | Osn_7682 | F | 56.00 | 44.00 | 2.460 | 0.190 | 0.010 | 2.270 | 0.441 NA |
| 172 | 2021.05.21 | May | 12 | *Oligocottus snyderi* | Osn_7683 | F | 54.00 | 44.00 | 2.100 | 0.170 | 0.015 | 1.930 | 0.777 NA |
| 173 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7723 | F | 80.00 | 62.00 | 5.130 | 0.410 | 0.032 | 4.720 | 0.678 COI |
| 174 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7724 | F | 70.00 | 58.00 | 4.650 | 0.480 | 0.022 | 4.170 | 0.528 COI |
| 175 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7726 | F | 79.00 | 65.00 | 6.860 | 0.590 | 0.046 | 6.270 | 0.734 COI |
| 176 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7727 | F | 74.00 | 63.00 | 4.740 | 0.390 | 0.028 | 4.350 | 0.644 COI |
| 177 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7728 | M | 84.00 | 71.00 | 7.830 | 0.650 | 0.083 | 7.180 | 1.156 COI |
| 178 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7729 | M | 71.00 | 65.00 | 6.930 | 0.390 | 0.045 | 6.540 | 0.688 COI |
| 179 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7730 | M | 82.00 | 67.00 | 7.680 | 0.510 | 0.092 | 7.170 | 1.283 COI |
| 180 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7731 | M | 67.00 | 55.00 | 3.910 | 0.290 | 0.041 | 3.620 | 1.133 COI |
| 181 | 2021.07.15 | Jul | 2 | *Oligocottus snyderi* | Osn_7732 | M | 71.00 | 64.00 | 6.900 | 0.820 | 0.047 | 6.080 | 0.773 COI |
| 182 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7739 | F | 78.00 | 63.00 | 7.170 | 0.740 | 0.033 | 6.430 | 0.513 NA |
| 183 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7740 | F | 76.00 | 62.00 | 6.720 | 0.500 | 0.097 | 6.220 | 1.559 NA |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 184 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7741 | F | 78.00 | 64.00 | 7.520 | 0.570 | 0.042 | 6.950 | 0.604 NA |
| 185 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7742 | F | 71.00 | 60.00 | 4.900 | 0.390 | 0.021 | 4.510 | 0.466 NA |
| 186 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7743 | F | 66.00 | 54.00 | 3.920 | 0.360 | 0.018 | 3.560 | 0.506 NA |
| 187 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7745 | M | 80.00 | 66.00 | 8.230 | 0.550 | 0.092 | 7.680 | 1.198 NA |
| 188 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7746 | M | 81.00 | 66.00 | 8.880 | 0.350 | 0.132 | 8.530 | 1.547 NA |
| 189 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7747 | M | 69.00 | 56.00 | 5.150 | 0.300 | 0.051 | 4.850 | 1.052 NA |
| 190 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7748 | M | 71.00 | 58.00 | 5.300 | 0.280 | 0.073 | 5.020 | 1.454 NA |
| 191 | 2021.08.12 | Aug | 3 | *Oligocottus snyderi* | Osn_7749 | M | 82.00 | 69.00 | 8.130 | 0.440 | 0.095 | 7.690 | 1.235 COI |
| 192 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7763 | F | 64.00 | 51.00 | 3.590 | 0.350 | 0.025 | 3.240 | 0.772 COI |
| 193 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7764 | F | 58.00 | 48.00 | 2.730 | 0.360 | 0.013 | 2.370 | 0.549 COI |
| 194 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7765 | F | 58.00 | 48.00 | 2.900 | 0.270 | 0.015 | 2.630 | 0.570 COI |
| 195 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7766 | F | 64.00 | 52.00 | 3.660 | 0.340 | 0.021 | 3.320 | 0.633 COI |
| 196 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7767 | F | 73.00 | 59.00 | 5.820 | 0.580 | 0.056 | 5.240 | 1.069 COI |
| 197 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7768 | M | 57.00 | 46.00 | 2.780 | 0.190 | 0.031 | 2.590 | 1.197 COI |
| 198 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7769 | M | 72.00 | 57.00 | 5.830 | 0.500 | 0.107 | 5.330 | 2.008 COI |
| 199 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7770 | M | 78.00 | 64.00 | 7.640 | 0.710 | 0.114 | 6.930 | 1.645 NA |
| 200 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7771 | M | 75.00 | 61.00 | 5.840 | 0.510 | 0.121 | 5.330 | 2.270 NA |
| 201 | 2021.09.22 | Sep | 4 | *Oligocottus snyderi* | Osn_7772 | M | 84.00 | 69.00 | 8.560 | 0.540 | 0.182 | 8.020 | 2.269 NA |
| 202 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7781 | F | 79.00 | 65.00 | 6.490 | 0.410 | 0.074 | 6.080 | 1.217 COI |
| 203 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7782 | F | 74.00 | 61.00 | 5.970 | 0.580 | 0.074 | 5.390 | 1.373 COI |
| 204 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7783 | F | 64.00 | 52.00 | 4.070 | 0.440 | 0.072 | 3.630 | 1.983 COI |
| 205 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7784 | F | 58.00 | 47.00 | 2.570 | 0.320 | 0.045 | 2.250 | 2.000 NA |
| 206 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7785 | F | 61.00 | 51.00 | 2.900 | 0.240 | 0.033 | 2.660 | 1.241 COI |
| 207 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7786 | M | 78.00 | 63.00 | 7.740 | 0.580 | 0.170 | 7.160 | 2.374 COI |
| 208 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7787 | M | 63.00 | 51.00 | 3.290 | 0.320 | 0.052 | 2.970 | 1.751 COI |
| 209 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7788 | M | 60.00 | 49.00 | 3.090 | 0.260 | 0.061 | 2.830 | 2.155 COI |
| 210 | 2021.10.21 | Oct | 5 | *Oligocottus snyderi* | Osn_7789 | M | 59.00 | 48.00 | 2.780 | 0.230 | 0.054 | 2.550 | 2.118 COI |
| 211 | 2022.01.28 | Jan | 8 | *Oligocottus snyderi* | Osn_7925 | F | 62.00 | 54.00 | 3.595 | 0.277 | 0.130 | 3.318 | 3.918 COI |
| 212 | 2022.01.28 | Jan | 8 | *Oligocottus snyderi* | Osn_7926 | F | 64.00 | 52.00 | 3.238 | 0.321 | 0.112 | 2.917 | 3.840 NA |
| 213 | 2022.02.25 | Feb | 9 | *Oligocottus snyderi* | Osn_7936 | F | 60.00 | 52.00 | 3.110 | 0.300 | 0.120 | 2.810 | 4.270 NA |
| 214 | 2022.02.25 | Feb | 9 | *Oligocottus snyderi* | Osn_7937 | F | 64.00 | 52.00 | 3.190 | 0.270 | 0.230 | 2.920 | 7.877 COI |
| 215 | 2022.02.25 | Feb | 9 | *Oligocottus snyderi* | Osn_7938 | F | 69.00 | 57.00 | 4.240 | 0.260 | 0.420 | 3.980 | 10.553 COI |
| 216 | 2022.02.25 | Feb | 9 | *Oligocottus snyderi* | Osn_7939 | F | 55.00 | 44.00 | 1.890 | 0.170 | 0.170 | 1.720 | 9.884 COI |
| 217 | 2022.02.25 | Feb | 9 | *Oligocottus snyderi* | Osn_7940 | F | 71.00 | 58.00 | 5.170 | 0.500 | 0.260 | 4.670 | 5.567 COI |
| 218 | 2022.02.25 | Feb | 9 | *Oligocottus snyderi* | Osn_7945 | M | 66.00 | 55.00 | 4.810 | 0.340 | 0.130 | 4.470 | 2.908 COI |
| 219 | 2022.02.25 | Feb | 9 | *Oligocottus snyderi* | Osn_7946 | F | 59.00 | 48.00 | 2.460 | 0.150 | 0.100 | 2.310 | 4.329 NA |
| 220 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7951 | F | 70.00 | 56.00 | 3.900 | 0.350 | 0.160 | 3.550 | 4.507 NA |
| 221 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7952 | F | 64.00 | 53.00 | 3.280 | 0.300 | 0.200 | 2.980 | 6.711 NA |
| 222 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7953 | F | 59.00 | 48.00 | 2.640 | 0.280 | 0.290 | 2.360 | 12.288 NA |
| 223 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7954 | F | 61.00 | 51.00 | 2.220 | 0.250 | 0.110 | 1.970 | 5.584 NA |
| 224 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7955 | F | 61.00 | 50.00 | 2.700 | 0.220 | 0.220 | 2.480 | 8.871 NA |
| 225 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7956 | M | 72.00 | 58.00 | 4.300 | 0.360 | 0.028 | 3.940 | 0.711 NA |
| 226 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7957 | M | 66.00 | 55.00 | 3.200 | 0.240 | 0.058 | 2.960 | 1.959 NA |
| 227 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7958 | M | 68.00 | 56.00 | 3.260 | 0.290 | 0.037 | 2.970 | 1.246 NA |
| 228 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7959 | M | 66.00 | 55.00 | 2.970 | 0.230 | 0.023 | 2.740 | 0.839 NA |
| 229 | 2022.04.07 | Apr | 11 | *Oligocottus snyderi* | Osn_7960 | M | 56.00 | 46.00 | 1.830 | 0.180 | 0.031 | 1.650 | 1.879 NA |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 230 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7983 | M | 84.00 | 71.00 | 7.477 | 0.355 | 0.083 | 7.122 | 1.165 | NA | |
| 231 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7984 | M | 74.00 | 61.00 | 5.127 | 0.520 | 0.051 | 4.607 | 1.107 | NA | |
| 232 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7985 | M | 85.00 | 69.00 | 6.433 | 0.375 | 0.081 | 6.058 | 1.337 | NA | |
| 233 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7986 | M | 67.00 | 54.00 | 3.491 | 0.343 | 0.023 | 3.148 | 0.731 | NA | |
| 234 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7987 | M | 70.00 | 59.00 | 4.071 | 0.401 | 0.034 | 3.670 | 0.926 | NA | |
| 235 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7988 | F | 66.00 | 56.00 | 3.640 | 0.338 | 0.087 | 3.302 | 2.635 | NA | |
| 236 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7989 | F | 62.00 | 51.00 | 2.714 | 0.261 | 0.031 | 2.453 | 1.264 | NA | |
| 237 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7990 | F | 65.00 | 53.00 | 3.409 | 0.274 | 0.048 | 3.135 | 1.531 | NA | |
| 238 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7991 | F | 64.00 | 54.00 | 3.060 | 0.283 | 0.023 | 2.777 | 0.828 | NA | |
| 239 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7992 | F | 66.00 | 55.00 | 4.059 | 0.335 | 0.137 | 3.724 | 3.679 | NA | |
| 240 | 2022.05.23 | May | 12 | *Oligocottus snyderi* | Osn_7993 | F | 65.00 | 55.00 | 3.287 | 0.298 | 0.137 | 2.989 | 4.583 | NA | |
| 241 | 2019.10.27 | Oct | 5 | *Oligocottus rubellio* | Oru_T1 | M | NA | 48.00 | 5.820 | 0.380 | 0.106 | 5.440 | 1.949 | NA | |
| 242 | 2020.01.10 | Jan | 8 | *Oligocottus rubellio* | Oru_2020-1 | F | 72.00 | 59.50 | 6.573 | 0.503 | 0.420 | 6.070 | 6.919 | Cytb | |
| 243 | 2020.01.10 | Jan | 8 | *Oligocottus rubellio* | Oru_2020-2 | M | 73.00 | 60.50 | 6.139 | 0.418 | 0.348 | 5.721 | 6.083 | NA | |
| 244 | 2020.04.04 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-4 | F | 71.00 | 58.50 | 5.601 | 0.530 | 0.636 | 5.071 | 12.542 | Cytb | |
| 245 | 2020.04.06 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-10 | F | 66.00 | 54.50 | 4.567 | 0.358 | 0.592 | 4.209 | 14.065 | Cytb | |
| 246 | 2020.04.06 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-7 | F | 76.00 | 62.50 | 6.409 | 0.469 | 0.671 | 5.940 | 11.296 | Cytb | LC760541 |
| 247 | 2020.04.06 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-8 | F | 61.50 | 52.00 | 3.284 | 0.264 | 0.321 | 3.020 | 10.629 | Cytb | LC760542 |
| 248 | 2020.04.06 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-9 | F | 56.50 | 47.00 | 2.622 | 0.142 | 0.334 | 2.480 | 13.468 | Cytb | |
| 249 | 2020.04.12 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-11 | M | 75.00 | 62.00 | 6.443 | 0.318 | 0.183 | 6.125 | 2.988 | NA | |
| 250 | 2020.04.12 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-12 | M | 66.00 | 54.50 | 4.284 | 0.237 | 0.150 | 4.047 | 3.706 | NA | |
| 251 | 2020.04.12 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-13 | F | 70.00 | 57.50 | 5.996 | 0.532 | 0.909 | 5.464 | 16.636 | Cytb | LC763453 |
| 252 | 2020.04.12 | Apr | 11 | *Oligocottus rubellio* | Oru_2020-14 | F | 64.50 | 53.50 | 4.157 | 0.356 | 0.314 | 3.801 | 8.261 | Cytb | |
| 253 | 2020.05.09 | May | 12 | *Oligocottus rubellio* | Oru_2020-16 | F | 73.50 | 61.50 | 6.317 | 0.727 | 0.321 | 5.590 | 5.742 | Cytb | |
| 254 | 2020.05.09 | May | 12 | *Oligocottus rubellio* | Oru_2020-17 | M | 73.00 | 59.50 | 5.804 | 0.330 | 0.158 | 5.474 | 2.886 | NA | |
| 255 | 2020.05.24 | May | 12 | *Oligocottus rubellio* | Oru_2020-19 | M | 76.00 | 63.50 | 6.058 | 0.568 | 0.190 | 5.490 | 3.461 | NA | |
| 256 | 2020.05.24 | May | 12 | *Oligocottus rubellio* | Oru_2020-20 | F | 62.50 | 52.50 | 3.667 | 0.496 | 0.346 | 3.171 | 10.911 | Cytb | |
| 257 | 2020.05.24 | May | 12 | *Oligocottus rubellio* | Oru_2020-21 | F | 58.50 | 48.50 | 2.792 | 0.336 | 0.015 | 2.456 | 0.611 | Cytb | LC763454 |
| 258 | 2020.05.24 | May | 12 | *Oligocottus rubellio* | Oru_2020-22 | F | 74.00 | 62.00 | 5.891 | 0.598 | 0.176 | 5.293 | 3.325 | Cytb | |
| 259 | 2020.05.24 | May | 12 | *Oligocottus rubellio* | Oru_2020-23 | F | 67.00 | 56.50 | 4.449 | 0.543 | 0.113 | 3.906 | 2.893 | Cytb | |
| 260 | 2020.06.10 | Jun | 1 | *Oligocottus rubellio* | Oru_2020-24 | F | 73.00 | 60.50 | 5.154 | 0.944 | 0.194 | 4.210 | 4.608 | Cytb | LC763455 |
| 261 | 2020.06.10 | Jun | 1 | *Oligocottus rubellio* | Oru_2020-29 | M | 73.50 | 61.50 | 5.023 | 0.375 | 0.113 | 4.648 | 2.431 | NA | |
| 262 | 2020.06.11 | Jun | 1 | *Oligocottus rubellio* | Oru_2020-33 | F | 67.50 | 56.00 | 5.066 | 0.373 | 0.889 | 4.693 | 18.943 | Cytb | |
| 263 | 2020.09.17 | Sep | 4 | *Oligocottus rubellio* | Oru_2020-44 | M | 60.20 | 50.00 | 4.095 | 0.320 | 0.056 | 3.775 | 1.483 | Cytb | |
| 264 | 2020.09.17 | Sep | 4 | *Oligocottus rubellio* | Oru_2020-47 | M | 57.50 | 49.00 | 3.837 | 0.204 | 0.048 | 3.633 | 1.321 | Cytb | |
| 265 | 2020.11.13 | Nov | 6 | *Oligocottus rubellio* | Oru_7511 | M | 71.00 | 58.00 | 7.520 | 0.550 | 0.270 | 6.970 | 3.874 | COI | |
| 266 | 2020.11.13 | Nov | 6 | *Oligocottus rubellio* | Oru_7512 | M | 70.00 | 56.00 | 6.730 | 0.460 | 0.170 | 6.270 | 2.711 | NA | |
| 267 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7523 | F | 55.00 | 44.00 | 3.230 | 0.280 | 0.190 | 2.950 | 6.441 | COI | |
| 268 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7524 | M | 57.00 | 46.00 | 3.550 | 0.160 | 0.050 | 3.390 | 1.475 | COI | |
| 269 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7525 | M | 65.00 | 51.00 | 4.950 | 0.190 | 0.120 | 4.760 | 2.521 | COI | |
| 270 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7526 | F | 81.00 | 64.00 | 10.860 | 0.590 | 1.330 | 10.270 | 12.950 | COI | |
| 271 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7527 | F | 54.00 | 44.00 | 2.630 | 0.170 | 0.300 | 2.460 | 12.195 | COI | |
| 272 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7528 | F | 62.00 | 50.00 | 3.910 | 0.290 | 0.200 | 3.620 | 5.525 | COI | |
| 273 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7529 | F | 61.00 | 50.00 | 3.770 | 0.250 | 0.280 | 3.520 | 7.955 | COI | |
| 274 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7530 | M | 61.00 | 49.00 | 3.280 | 0.180 | 0.060 | 3.100 | 1.935 | COI | |
| 275 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7531 | F | 60.00 | 49.00 | 4.490 | 0.340 | 0.060 | 4.150 | 1.446 | COI | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 276 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7532 | F | 63.00 | 51.00 | 4.850 | 0.230 | 0.080 | 4.620 | 1.732 | COI |
| 277 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7533 | M | 62.00 | 50.00 | 4.810 | 0.180 | 0.080 | 4.630 | 1.728 | COI |
| 278 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7534 | F | 61.00 | 49.00 | 5.380 | 0.320 | 0.100 | 5.060 | 1.976 | COI |
| 279 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7535 | F | 58.00 | 48.00 | 4.190 | 0.260 | 0.090 | 3.930 | 2.290 | COI |
| 280 | 2020.11.30 | Nov | 6 | *Oligocottus rubellio* | Oru_7536 | M | 57.00 | 46.00 | 4.150 | 0.130 | 0.140 | 4.020 | 3.483 | COI |
| 281 | 2020.12.10 | Dec | 7 | *Oligocottus rubellio* | Oru_7552 | M | 63.00 | 52.00 | 4.480 | 0.540 | 0.080 | 3.940 | 2.030 | COI |
| 282 | 2021.01.29 | Jan | 8 | *Oligocottus rubellio* | Oru_7592 | F | 61.00 | 50.00 | 4.400 | 0.290 | 0.190 | 4.110 | 4.623 | COI |
| 283 | 2021.02.06 | Feb | 9 | *Oligocottus rubellio* | Oru_7594 | F | 52.00 | 42.00 | 1.690 | 0.120 | 0.190 | 1.570 | 12.102 | COI |
| 284 | 2021.02.06 | Feb | 9 | *Oligocottus rubellio* | Oru_7603 | M | 68.00 | 56.00 | 5.510 | 0.440 | 0.140 | 5.070 | 2.761 | COI |
| 285 | 2021.02.06 | Feb | 9 | *Oligocottus rubellio* | Oru_7604 | M | 68.00 | 55.00 | 5.400 | 0.260 | 0.200 | 5.140 | 3.891 | COI |
| 286 | 2021.02.06 | Feb | 9 | *Oligocottus rubellio* | Oru_7605 | M | 71.00 | 58.00 | 5.590 | 0.350 | 0.150 | 5.240 | 2.863 | COI |
| 287 | 2021.02.06 | Feb | 9 | *Oligocottus rubellio* | Oru_7606 | M | 54.00 | 44.00 | 2.720 | 0.210 | 0.100 | 2.510 | 3.984 | COI |
| 288 | 2021.02.06 | Feb | 9 | *Oligocottus rubellio* | Oru_7607 | M | 65.00 | 52.00 | 4.600 | 0.220 | 0.170 | 4.380 | 3.881 | COI |
| 289 | 2021.03.26 | Mar | 10 | *Oligocottus rubellio* | Oru_7630 | F | 63.00 | 53.00 | 4.750 | 0.220 | 0.550 | 4.530 | 12.141 | COI |
| 290 | 2021.03.26 | Mar | 10 | *Oligocottus rubellio* | Oru_7631 | F | 81.00 | 68.00 | 8.770 | 0.480 | 0.780 | 8.290 | 9.409 | COI |
| 291 | 2021.03.26 | Mar | 10 | *Oligocottus rubellio* | Oru_7632 | F | 67.00 | 55.00 | 4.210 | 0.330 | 0.210 | 3.880 | 5.412 | COI |
| 292 | 2021.03.26 | Mar | 10 | *Oligocottus rubellio* | Oru_7633 | F | 55.00 | 44.00 | 2.450 | 0.250 | 0.130 | 2.200 | 5.909 | COI |
| 293 | 2021.03.26 | Mar | 10 | *Oligocottus rubellio* | Oru_7638 | M | 66.00 | 55.00 | 5.180 | 0.510 | 0.120 | 4.670 | 2.570 | COI |
| 294 | 2021.04.05 | Apr | 11 | *Oligocottus rubellio* | Oru_7650 | F | 48.00 | 39.00 | 1.340 | 0.100 | 0.000 | 1.240 | 0.000 | COI |
| 295 | 2021.04.23 | Apr | 11 | *Oligocottus rubellio* | Oru_7655 | F | 55.00 | 42.00 | 2.600 | 0.310 | 0.060 | 2.290 | 2.620 | COI |
| 296 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7667 | F | 64.00 | 54.00 | 2.960 | 0.240 | 0.130 | 2.720 | 4.779 | COI |
| 297 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7668 | M | 73.00 | 59.00 | 4.880 | 0.320 | 0.039 | 4.560 | 0.855 | NA |
| 298 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7669 | M | 77.00 | 64.00 | 5.740 | 0.390 | 0.029 | 5.350 | 0.542 | NA |
| 299 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7670 | M | 73.00 | 62.00 | 5.270 | 0.370 | 0.047 | 4.900 | 0.959 | COI |
| 300 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7671 | M | 89.00 | 74.00 | 7.790 | 0.540 | 0.110 | 7.250 | 1.517 | COI |
| 301 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7672 | F | 72.00 | 61.00 | 5.610 | 0.450 | 0.530 | 5.160 | 10.271 | COI |
| 302 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7673 | F | 70.00 | 58.00 | 4.420 | 0.310 | 0.180 | 4.110 | 4.380 | COI |
| 303 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7674 | F | 72.00 | 62.00 | 6.130 | 0.460 | 0.590 | 5.670 | 10.406 | COI |
| 304 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7675 | M | 68.00 | 56.00 | 4.670 | 0.270 | 0.130 | 4.400 | 2.955 | COI |
| 305 | 2021.05.03 | May | 12 | *Oligocottus rubellio* | Oru_7676 | F | 55.00 | 45.00 | 2.070 | 0.180 | 0.006 | 1.890 | 0.317 | COI |
| 306 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7677 | M | 66.00 | 53.00 | 3.300 | 0.250 | 0.026 | 3.050 | 0.852 | COI |
| 307 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7678 | M | 67.00 | 52.00 | 3.710 | 0.280 | 0.019 | 3.430 | 0.554 | COI |
| 308 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7679 | M | 67.00 | 54.00 | 4.450 | 0.240 | 0.028 | 4.210 | 0.665 | COI |
| 309 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7680 | F | 62.00 | 50.00 | 3.310 | 0.360 | 0.000 | 2.950 | 0.000 | COI |
| 310 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7684 | F | 65.00 | 53.00 | 4.080 | 0.240 | 0.280 | 3.840 | 7.292 | NA |
| 311 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7685 | F | 57.00 | 46.00 | 3.620 | 0.290 | 0.160 | 3.330 | 4.805 | NA |
| 312 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7686 | F | 62.00 | 50.00 | 3.970 | 0.270 | 0.150 | 3.700 | 4.054 | NA |
| 313 | 2021.05.21 | May | 12 | *Oligocottus rubellio* | Oru_7687 | F | 62.00 | 51.00 | 4.110 | 0.400 | 0.050 | 3.710 | 1.348 | NA |
| 314 | 2021.07.15 | Jul | 2 | *Oligocottus rubellio* | Oru_7725 | F | 76.00 | 62.00 | 6.440 | 0.680 | 0.041 | 5.760 | 0.712 | NA |
| 315 | 2021.07.15 | Jul | 2 | *Oligocottus rubellio* | Oru_7734 | F | 58.00 | 48.00 | 2.510 | 0.210 | 0.000 | 2.300 | 0.000 | COI |
| 316 | 2021.07.15 | Jul | 2 | *Oligocottus rubellio* | Oru_7735 | F | 64.00 | 52.00 | 3.730 | 0.240 | 0.004 | 3.490 | 0.115 | COI |
| 317 | 2021.07.15 | Jul | 2 | *Oligocottus rubellio* | Oru_7736 | F | 67.00 | 55.00 | 3.790 | 0.280 | 0.031 | 3.510 | 0.883 | COI |
| 318 | 2021.07.15 | Jul | 2 | *Oligocottus rubellio* | Oru_7737 | M | 70.00 | 58.00 | 4.710 | 0.360 | 0.016 | 4.350 | 0.368 | COI |
| 319 | 2021.08.12 | Aug | 3 | *Oligocottus rubellio* | Oru_7750 | F | 67.00 | 56.00 | 5.720 | 0.620 | 0.011 | 5.100 | 0.216 | COI |
| 320 | 2021.08.12 | Aug | 3 | *Oligocottus rubellio* | Oru_7751 | F | 66.00 | 53.00 | 4.250 | 0.240 | 0.041 | 4.010 | 1.022 | COI |
| 321 | 2021.08.12 | Aug | 3 | *Oligocottus rubellio* | Oru_7752 | F | 65.00 | 51.00 | 4.540 | 0.290 | 0.008 | 4.250 | 0.188 | COI |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 322 | 2021.08.12 | Aug | 3 | *Oligocottus rubellio* | Oru_7753 | F | 61.00 | 49.00 | 3.130 | 0.150 | 0.002 | 2.980 | 0.067 | COI |
| 323 | 2021.08.12 | Aug | 3 | *Oligocottus rubellio* | Oru_7754 | F | 54.00 | 44.00 | 2.930 | 0.320 | 0.009 | 2.610 | 0.345 | COI |
| 324 | 2021.08.12 | Aug | 3 | *Oligocottus rubellio* | Oru_7755 | F | 54.00 | 43.00 | 2.200 | 0.130 | 0.000 | 2.070 | 0.000 | COI |
| 325 | 2021.09.22 | Sep | 4 | *Oligocottus rubellio* | Oru_7773 | F | 61.00 | 51.00 | 3.680 | 0.250 | 0.024 | 3.430 | 0.700 | COI |
| 326 | 2021.10.21 | Oct | 5 | *Oligocottus rubellio* | Oru_7791 | F | 65.00 | 54.00 | 4.060 | 0.140 | 0.041 | 3.920 | 1.046 | COI |
| 327 | 2021.10.21 | Oct | 5 | *Oligocottus rubellio* | Oru_7792 | M | 70.00 | 57.00 | 5.320 | 0.310 | 0.130 | 5.010 | 2.595 | COI |
| 328 | 2021.10.21 | Oct | 5 | *Oligocottus rubellio* | Oru_7793 | M | 63.00 | 52.00 | 4.640 | 0.290 | 0.114 | 4.350 | 2.621 | COI |
| 329 | 2022.01.28 | Jan | 8 | *Oligocottus rubellio* | Oru_7927 | F | 66.00 | 55.00 | 5.124 | 0.478 | 0.745 | 4.646 | 16.035 | COI |
| 330 | 2022.02.25 | Feb | 9 | *Oligocottus rubellio* | Oru_7941 | F | 66.00 | 55.00 | 4.900 | 0.470 | 0.390 | 4.430 | 8.804 | COI |
| 331 | 2022.02.25 | Feb | 9 | *Oligocottus rubellio* | Oru_7942 | F | 61.00 | 50.00 | 4.120 | 0.230 | 0.260 | 3.890 | 6.684 | NA |
| 332 | 2022.02.25 | Feb | 9 | *Oligocottus rubellio* | Oru_7943 | F | 65.00 | 53.00 | 3.200 | 0.270 | 0.140 | 2.930 | 4.778 | COI |
| 333 | 2022.02.25 | Feb | 9 | *Oligocottus rubellio* | Oru_7944 | F | 55.00 | 45.00 | 2.390 | 0.130 | 0.150 | 2.260 | 6.637 | COI |
| 334 | 2022.05.23 | May | 12 | *Oligocottus rubellio* | Oru_7994 | M | 56.00 | 47.00 | 2.586 | 0.203 | 0.019 | 2.383 | 0.797 | NA |
| 335 | 2022.05.23 | May | 12 | *Oligocottus rubellio* | Oru_7995 | M | 76.00 | 59.00 | 5.722 | 0.780 | 0.096 | 4.942 | 1.943 | NA |
| 336 | 2022.05.23 | May | 12 | *Oligocottus rubellio* | Oru_7996 | F | 67.00 | 55.00 | 4.480 | 0.565 | 0.186 | 3.915 | 4.751 | NA |
| 337 | 2022.05.23 | May | 12 | *Oligocottus rubellio* | Oru_7997 | F | 71.00 | 59.00 | 4.996 | 0.266 | 0.330 | 4.730 | 6.977 | NA |
| 338 | 2022.05.23 | May | 12 | *Oligocottus rubellio* | Oru_7998 | F | 66.00 | 54.00 | 3.728 | 0.219 | 0.130 | 3.509 | 3.705 | NA |

**Table S1** GenBank accession number, individual ID, English name, scientific name, and references of *Co1* sequencies of marine sculpins (Psychrolutidae) used for this study.

| Accession No. | Individual ID | English name | Scientific name | References |
|---|---|---|---|---|
| MW128525 | *MW128525 Enophrys diceraus* | Antlered Sculpin | *Enophrys diceraus* | Turanov and Kartavtsev (2021) |
| KP827283 | *KP827283 Artedius fenestralis* | Padded Sculpin | *Artedius fenestralis* | Buser and López (2015) |
| KP827279 | *KP827279 Artedius harringtoni* | Scalyhead Sculpin | *Artedius harringtoni* | Buser and López (2015) |
| KP827287 | *KP827287 Artedius lateralis* | Smoothhead Sculpin | *Artedius lateralis* | Buser and López (2015) |
| GU440286 | *GU440286 Clinocottus analis* | Woolly Sculpin | *Clinocottus analis* | Hastings and Burton (2008) |
| KP827314 | *KP827314 Clinocottus analis** | Woolly Sculpin* | *Clinocottus analis** | Buser and López (2015) |
| EU403067 | *EU403067 Icelinus filamentosus* | Threadfin Sculpin | *Icelinus filamentosus* | Hastings and Burton (2008) |
| GU440370 | *GU440370 Leptocottus armatus* | Pacific Staghorn Sculpin | *Leptocottus armatus* | Hastings and Burton (2008) |
| KP827315 | *KP827315 Oligocottus rubellio* | Rosy Sculpin | *Oligocottus rubellio* | Buser and López (2015) |
| KP827306 | *KP827306 Oligocottus snyderi* | Fluffy Sculpin | *Oligocottus snyderi* | Buser and López (2015) |
| EU403072 | *EU403072 Scorpaenichthys marmoratus* | Cabezon | *Scorpaenichthys marmoratus* | Hastings and Burton (2008) |

*Assigned as Fluffy Sculpin (*Oligocottus snyderi)* in this study

**Table S2** GenBank accession number, individual ID, English name, scientific name, and references of *Cytb* sequencies of marine sculpins (Psychrolutidae) used for this study.

| **Accession No.** | **Individual ID** | **English name** | **Scientific name** | **References** |
|---|---|---|---|---|
| LC546698 | *LC546698 Artedius corallinus* | Coralline Sculpin | *Artedius corallinus* | Awata et al. (2022) |
| LC546699 | *LC546699 Artedius corallinus* | Coralline Sculpin | *Artedius corallinus* | Awata et al. (2022) |
| LC546700 | *LC546700 Artedius harringtoni* | Scalyhead Sculpin | *Artedius harringtoni* | Awata et al. (2022) |
| LC546701 | *LC546701 Artedius harringtoni* | Scalyhead Sculpin | *Artedius harringtoni* | Awata et al. (2022) |
| LC546702 | *LC546702 Artedius lateralis* | Smoothhead Sculpin | *Artedius lateralis* | Awata et al. (2022) |
| LC546703 | *LC546703 Clinocottus analis* | Woolly Sculpin | *Clinocottus analis* | Awata et al. (2022) |
| LC546704 | *LC546704 Clinocottus analis* | Woolly Sculpin | *Clinocottus analis* | Awata et al. (2022) |
| LC546705 | *LC546705 Clinocottus recalvus* | Bald Sculpin | *Clinocottus recalvus* | Awata et al. (2022) |
| LC546706 | *LC546706 Icelinus cavifrons* | Pit-head Sculpin | *Icelinus cavifrons* | Awata et al. (2022) |
| LC546707 | *LC546707 Icelinus cavifrons* | Pit-head Sculpin | *Icelinus cavifrons* | Awata et al. (2022) |
| LC546708 | *LC546708 Leptocottus armatus* | Pacific Staghorn Sculpin | *Leptocottus armatus* | Awata et al. (2022) |
| LC546709 | *LC546709 Oligocottus maculosus* | Tidepool Sculpin | *Oligocottus maculosus* | Awata et al. (2022) |
| LC546710 | *LC546710 Oligocottus maculosus* | Tidepool Sculpin | *Oligocottus maculosus* | Awata et al. (2022) |
| LC760541 | *Oru_2020-7* | Rosy Sculpin | *Oligocottus rubellio* | This study |
| LC760542 | *Oru_2020-8* | Rosy Sculpin | *Oligocottus rubellio* | This study |
| LC763453 | *Oru_2020-13* | Rosy Sculpin | *Oligocottus rubellio* | This study |
| LC763454 | *Oru_2020-21* | Rosy Sculpin | *Oligocottus rubellio* | This study |
| LC763455 | *Oru_2020-24* | Rosy Sculpin | *Oligocottus rubellio* | This study |
| LC546711 | *LC546711 Oligocottus snyderi* | Fluffy Sculpin | *Oligocottus snyderi* | Awata et al. (2022) |
| LC546712 | *LC546712 Oligocottus snyderi* | Fluffy Sculpin | *Oligocottus snyderi* | Awata et al. (2022) |
| LC546713 | *LC546713 Orthonopias triacis* | Snubnose Sculpin | *Orthonopias triacis* | Awata et al. (2022) |
| LC546714 | *LC546714 Scorpaenichthys marmoratus* | Cabezon | *Scorpaenichthys marmoratus* | Awata et al. (2022) |
| LC546715 | *LC546715 Scorpaenichthys marmoratus* | Cabezon | *Scorpaenichthys marmoratus* | Awata et al. (2022) |