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Reproductive Parasitism between Distant Phyla: Molecular Identification of Snailfish (Liparidae) Egg Masses in the Gill Cavities of King Crabs (Lithodidae)

Jennifer R. Gardner[1], James W. Orr[2], Duane E. Stevenson[2], Ingrid Spies[3], and David A. Somerton[2]

Snailfishes of the genus *Careproctus* deposit egg masses inside the gill cavity of lithodid crabs. Previous attempts to identify the species depositing the eggs have been inconclusive because of the difficulties in identifying snailfish eggs and larvae. For this study, samples were collected from egg masses found in crabs in the commercial fishery and during benthic trawl survey work. Egg masses were found in *Lithodes aequispinus* (Golden King Crab) and, for the first time, in *L. couesi* (Scarlet King Crab). Mitochondrial DNA from the cytochrome c oxidase subunit I (COI) region was amplified from embryos and sequenced. Comparison of COI sequences from eggs to COI sequences from positively identified adult voucher specimens yielded identifications of 75 egg masses to species. A total of 38 egg masses were identified as *Careproctus melanurus;* 29 as *C. colletti;* seven as *C. furcellus*; and one as *C. simus*. Each egg mass contained eggs from only one species, and there was no indication of multiple maternal parentage. These results are the first positive identification of the snailfish species responsible for depositing egg masses in lithodid crabs. Identification of species involved may be useful in understanding the nature of this unique relationship between snailfishes and crabs.

FISHES exhibit some of the most bizarre reproductive behaviors among vertebrates (Breder and Rosen, 1966). These behaviors include elaborate sexual associations, such as sexual parasitism in deep-sea anglerfishes (Pietsch, 2009), and a variety of egg deposition strategies, such as mouth brooding and other elaborate behaviors among cichlids (Fryer and Iles, 1972), forehead brooding in nursery fishes (Kurtidae: Berra, 2003), and brooding within modified fins and body elements in pipefishes and seahorses (Syngnatha: Wilson and Orr, 2011). Eggs are also deposited on sessile biological substrates (living and dead), such as sponges (hemitripterid sculpins: Busby et al., 2012), corals (snailfishes of the genus *Allocareproctus*: Busby et al., 2006), polychaete worm tubes (hemitripterids: Munehara, 1992), ascidians (Aulorhynchidae: Uchida, 1934), or in nearly immobile bivalves (bitterlings, acheilognathine cyprinids: Smith et al., 2004). Only among snailfishes of the genus *Careproctus*, however, are eggs deposited in, and essentially brooded by, another highly mobile animal—adult king crabs of the family Lithodidae (Karplus, 2014). The lithodids involved (Table 1) are large species of anomuran crabs, which, unlike brachyurans or "true crabs," have an asymmetrical abdomen, and gaps between the carapace and abdomen that provide space for the insertion of an ovipositor and eggs.

The selective advantage of this mode of reproduction is likely related to several factors. Eggs deposited within crabs are protected by the hard spiny carapace of the crab and are situated on top of the crab's gill filaments, where the eggs are aerated as the crab circulates water through its branchial chamber (perhaps incidentally aided by a fifth pair of pereiopods that are used by the crab for cleaning the gills; Pohle, 1989). This association provides long-term protection of large yolk-filled eggs, from which large, well-developed larvae hatch, without the need for additional parental investment. The crab also provides protected habitat for developing larvae and small juveniles, which have been found commonly within crab carapaces (Love and Shirley, 1993; Somerton and Donaldson, 1998; Poltev and Mukhametov, 2009; this study).

The relationship between crabs and the snailfishes that deposit their eggs inside them is clearly a parasitic one. While snailfishes derive the benefit of protection and aeration of the eggs, crabs are actually or potentially can be harmed by the compression of gill filaments by egg masses, and no known benefits accrue to the crabs (Somerton and Donaldson, 1998). However, adult king crabs make an ideal site for protecting eggs because they are rarely preyed upon, except through fishing activities, and are vulnerable to non-anthropogenic predation only when newly molted. Although large fishes prey on juvenile and newly molted king crabs, sea otters (*Enhydra lutris*) are the only known predators of post-molt adult king crabs. As Stevens and Jewett (2014:153) noted, "Once a king crab reaches maturity, it gains a refuge in size from which few predators (except humans) can dislodge it."

The snailfish family Liparidae is distributed in cold and temperate waters worldwide (Chernova et al., 2004; Nelson, 2006; Orr, 2012; Chernova, 2014a, 2014b). While some members of the family (species of *Liparis* and related genera) live in the intertidal and other shallow waters, the highest diversity of the family is found in deep water and includes the deepest-dwelling vertebrates (*Pseudoliparis amblystomopsis*, Fujii et al., 2010; an undescribed liparid, Daily Telegraph, 20 Dec 2014). Currently more than 350 species in 30 genera are recognized (Chernova et al., 2004), with more than 50 species known from the North Pacific (Imamura and Noetsu, 2002; Orr and Maslenikov, 2007; Kai et al., 2011; Machi et al., 2012; Orr, 2012; Orr et al., 2015). Many undescribed species

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are known to exist and more continue to be discovered (Stein, 2006; Orr, 2012). Contributing to this high diversity are the varied life history characteristics of the family. While species of the relatively depauperate shallow-water genus *Liparis* produce demersal eggs and pelagic larvae, species of the speciose and primarily deep-water genus *Careproctus* produce eggs and larvae that are both demersal.

Although tentatively noted as early as 1915 (Nakazawa, 1915), members of the genus *Careproctus* were first confirmed to deposit agglutinating egg masses within the gill cavities of lithodid crabs in 1950, when Rass (1950) and Vinogradov (1950) documented finding eggs and embryos in *Paralithodes camtschaticus* (Red King Crab). Since then, several authors have reported eggs of *Careproctus* in other genera and species of lithodid crabs in other regions of the world, including the North and South Pacific, and South Atlantic (Table 1). However, because identifying eggs and larvae of liparids is difficult (Matarese et al., 1989), the species responsible for laying the eggs have remained uncertain. In the eastern North Pacific, early attempts to identify species using meristic characters informed by species abundance generally concluded that *Careproctus melanurus* was responsible for laying the eggs (Parrish, 1972; Peden and Corbett, 1973), despite the fact that *C. melanurus* was not distinguishable from several other species by meristic characters alone.

Only one attempt, conducted in the eastern North Pacific region, has been made to use genetic techniques to identify egg masses (Somerton and Donaldson, 1998). This early study was limited by its use of restriction fragment length polymorphism (RFLP) methods applied to only three reference species of the 50 species of *Careproctus* known in the North Pacific. Eggs of *Careproctus furcellus* were identified among clusters, based on comparisons with a few species associated with crabs in crab pots: *C. furcellus*, *C. rastrinus* (=*C. scottae*; Orr et al., 2015), and *C. cypselurus* (Somerton and Donaldson, 1998). More recently, *C. furcellus* and *C. cypselurus* have also been identified as likely candidates in the western North Pacific based on snailfish species caught with infested crabs and comparisons of ovarian egg diameters with those found inside crabs (Poltev and Mukhametov, 2009; Poltev, 2013).

Sequencing the "barcoding" gene cytochrome c oxidase subunit I (COI) has become a useful method of species identification, particularly when traditional morphological approaches fail, and has been successfully implemented with North Pacific fishes (Spies et al., 2006; Steinke et al., 2009). Because of the widespread use of the genetic barcoding method, a wealth of COI sequence data has been published, and large numbers of comparative sequences are available in online data depositories such as GenBank, maintained by The National Center for Biotechnology and Information (NCBI) and the Barcode of Life Database (BOLD; Ratnasingham and Hebert, 2007). However, because of difficulties associated with identification of adult snailfishes, many of the sequences in these and other databases are based on misidentified specimens (JWO, pers. obs.). Our objective was to identify egg masses and larvae found in crabs by comparing COI sequences of the eggs and larvae with sequences from properly identified voucher specimens.

Table 1. Summary of all previously published reports of liparid eggs or larvae in crabs, including identifications, geographic regions, and methods used to identify liparids. RFLP = Restriction Fragment Length Polymorphism.

Citation	Crab	Snailfish	Geographic region	Identification method
Nakazawa, 1915	Lithodidae	Liparidae or Cyclopteridae?	Southern Kuril Islands	Egg size, morphology
Rass, 1950	*Paralithodes camtschatica*	"*Careproctus sinensis*" = *C. furcellus*	Sea of Okhotsk	Not stated
Vinogradov, 1950	*P. camtschatica*	"*Careproctus sinensis*" = *C. furcellus*	Kamchatka	Not stated
Hunter, 1969	*P. camtschatica*	*Careproctus* sp.	Central Aleutian Islands	Not stated
Parrish, 1972	*Lopholithodes foraminatus*	*C. melanurus*	Monterey Bay, California	Not stated
Peden and Corbett, 1973	*L. foraminatus*	*C. melanurus*	British Columbia	Counts
Anderson and Cailliet, 1974	*P. californiensis*	*Careproctus* sp.	Monterey Bay, California	Counts
Campodónico and Guzmán, 1977	"*Lithodes antarctica*" = *L. santolla*	*Careproctus* sp.	Strait of Magellan	Morphology, distribution
Balbontín et al., 1979	*Paralomis granulosa*, "*L. antarctica*" = *L. santolla*	*Careproctus* sp., *C. falklandicus*	Strait of Magellan	Counts, morphology
Melville-Smith and Louw, 1987	"*L. tropicalis*" = *L. ferox*	"*C. griseldea*" = *C. albescens*	Namibia	Association with crabs in fishing gear; distribution
Love and Shirley, 1993	*L. aequispinus*	*Careproctus* sp.	Southeastern Alaska	Counts, morphology
Somerton and Donaldson, 1998	*L. aequispinus*	*Careproctus* sp., *C. furcellus*	Eastern Bering Sea	Genetics (RFLP), association with crabs in fishing gear
Poltev and Mukhametov, 2009	*L. aequispinus*, *Paralomis verrilli*, *P. camtschatica*	*C. furcellus*, *C. cypselurus*	Northern Kuril Islands	Association with crabs in fishing gear; egg size and shape
Poltev, 2013	*L. aequispinus*, *P. multispina*, *P. verrilli*	*C. furcellus*, *C. cypselurus*	Sea of Okhotsk	Association with crabs in fishing gear; egg size and shape

MATERIALS AND METHODS

Adult snailfishes were collected during bottom trawl surveys in the Bering Sea, Aleutian Islands, and Gulf of Alaska, conducted by NOAA's National Marine Fisheries Service,

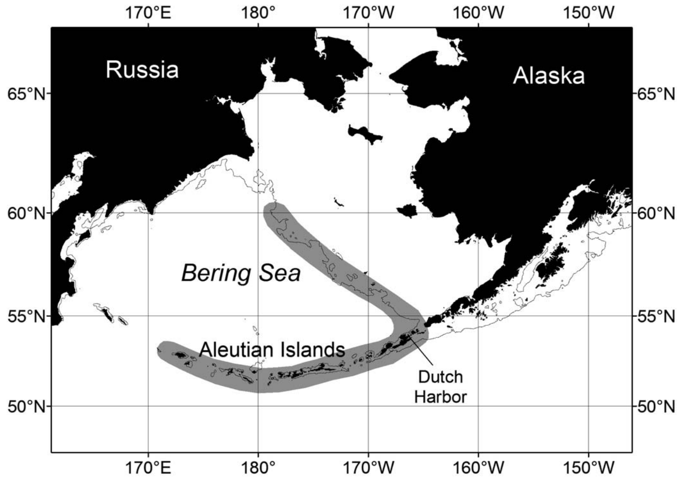

Fig. 1. Region in which snailfish egg masses were collected (shaded area).

Alaska Fisheries Science Center (AFSC), Resource Assessment and Conservation Engineering (RACE) Division (Fig. 1). Vouchered adults with available tissues were selected to represent some of the most common species of snailfishes in Alaska and to include as many genera as possible. DNA was extracted from vouchered adults of nine genera and 23 species (see Material Examined). Fin clips were taken from adult specimens and stored in 95% ethanol, and whole specimens were then fixed in formalin and preserved in 70% ethanol. Whole specimens and fin clips were archived in the University of Washington Fish Collection (UWFC).

Over 100 snailfish egg masses were collected and preserved in 95% ethanol while processing crabs taken in a commercial fishery for *Lithodes aequispinus* in the Aleutian Islands in August 2008 (Fig. 1). This fishery is limited to Aleutian Islands king crab Registration Area O, which extends from the western end of Unimak Island in the eastern Aleutians west to the U.S.-Russian border (Baechler, 2012). From three to ten eggs containing embryos were sampled from 68 egg masses. In addition, to address the possibility of variation within egg masses, 60 embryos were sampled from three of these masses: 20 from one end of the mass, 20 from the middle of the mass after the mass had been cut in half, and 20 from the other end of the mass.

An additional 12 egg masses and two groups of hatched larvae were collected from 11 crabs captured during trawl surveys of the Aleutian Islands and Bering Sea upper continental slope in 2012. Four randomly selected embryos from each of these masses were sampled.

All embryos observed were eyed. Because yolk sac and chorion tended to clog the filters used for extracting DNA, these embryos were removed from the chorion and yolk sac and used whenever possible. However, when embryos were unavailable, DNA was extracted from half the yolk sac with the chorion removed. DNA was extracted from tissue samples using Qiagen DNeasy tissue kits (Qiagen Inc., Valencia, CA). Primers HCO 2198 and LCO 2190 (Folmer et al., 1994) were used to amplify a 710 bp fragment of COI through the polymerase chain reaction (PCR). Reactions were run in 25 μl volumes containing 1 μl template DNA, 1 mM dNTPs, 1.5 mM $MgCl_2$, 0.4 μM of each primer, 10X Bioline Buffer, 1.25 U Bioline *Taq* polymerase (Bioline USA, Inc., Boston, MA). The thermal cycle profile consisted of 1 min at 95°C; 37 cycles of 95°C for 40 sec, 42°C for 40 sec, 72°C for 1 min; and a final cycle of 4°C for 5 min. The size of PCR products was checked using gel electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized with a UV light box. PCR products were cleaned using ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced in both directions using Sanger sequencing and the same primers as used in the PCR. Sequencing was performed by the High Throughput Sequencing Center of the Department of Genome Sciences at the University of Washington.

Forward and reverse sequences were obtained from 440 eggs and embryos. Sequence contigs were assembled using Sequencher v. 5.0 (Gene Codes Corp., Ann Arbor, MI). A 492 bp fragment was chosen for analyses based on sequence overlap and quality. Aligned consensus sequences were created using BioEdit v. 7.1 (Hall, 1999). The final fragment used for analysis was trimmed to the size of the shortest readable sequence used in the analysis. Species identification was determined using a local BLAST query of each egg or embryo sample against the sequences from all adult voucher sequences and confirmed with phylogenetic analysis. Catalog numbers and GenBank sequence numbers are listed in Material Examined and Appendix 1.

Phylogenetic analysis was performed under both maximum parsimony and Bayesian optimality criteria. Several candidate species of *Careproctus* were included in the analysis, as well as outgroup species from several other liparid genera (*Acantholiparis*, *Allocareproctus*, *Crystallichthys*, *Elassodiscus*, *Liparis, Lipariscus*, *Nectoliparis, Paraliparis*, and *Rhinoliparis*). The final data matrix included 128 sequences, 492 base pairs in length. Trees were rooted with *Liparis gibbus* (UW 119133). Parsimony analysis, along with bootstrap and Bremer support, was conducted with the Willi Hennig Society edition of TNT (Goloboff et al., 2003, 2008). The initial parsimony consensus tree was calculated using the Traditional Search option with the tree bisection reconnection (TBR) swapping algorithm. Bootstrap support was determined using 1000 standard bootstrap replicates of the matrix and the Traditional Search option. Bremer support was calculated using TBR of all trees within 50 steps of the shortest tree.

For the Bayesian analysis, a test of 24 different nucleotide substitution models was run in MEGA6 (Tamura et al., 2013). The model with the greatest log likelihood score was a generalized time reversible (GTR) model with gamma-distributed rate variation across sites and invariant sites ($GTR + I + \Gamma$). This model was chosen for the Bayesian analysis, conducted with MrBayes v3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Posterior probability distributions were generated by running four Markov chains, under the default of three heated chains and one cold. After 2 million generations, minimum ESS values were greater than 200 for all parameters, and PSRF scores were either 1.000 or 1.001 for all parameters. Sampling frequency was 100 generations. The initial 5000 samples were discarded as burn-in, and the remaining 15,000 samples were used to estimate tree topology and posterior probabilities.

In order to examine potential interspecific differences in egg size, a random sample of 20 eggs was measured from each sequenced egg mass. Some of the eggs showed signs of crenation, presumably due to dehydration in ethanol, so only eggs without visible distortion were chosen for measurement. For selected eggs, maximum diameter was recorded to the nearest 0.01 mm using digital calipers and a dissecting microscope. A total of 1460 eggs were measured

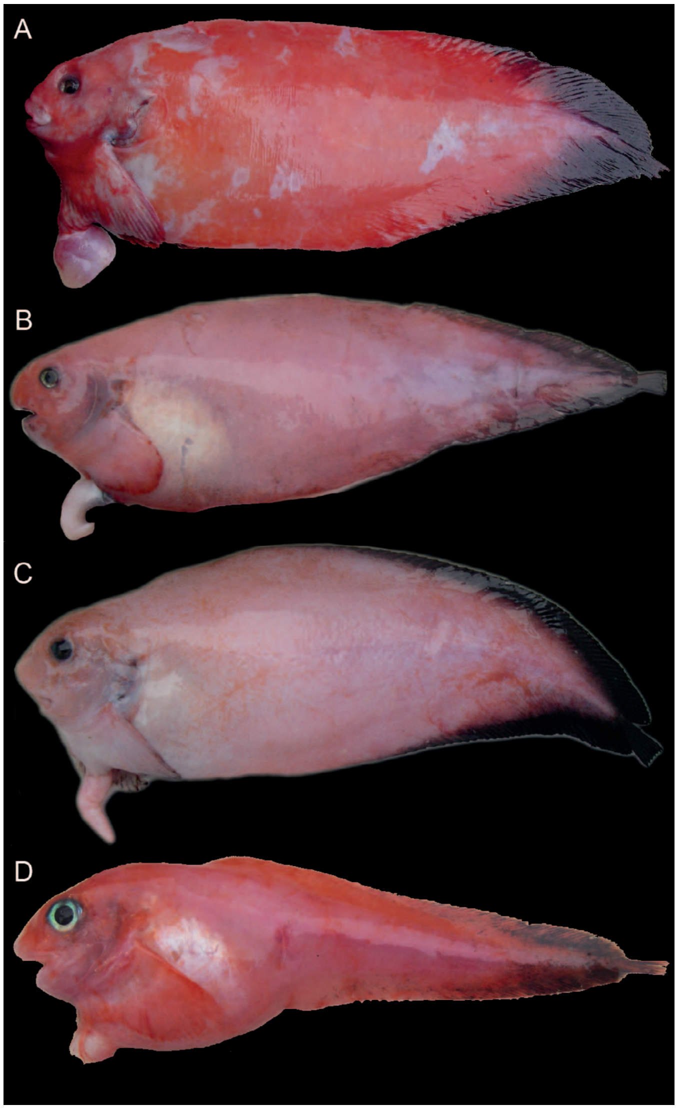

Fig. 2. Ripe females of species of *Careproctus* identified in egg masses found in lithodid crabs of Alaska: (A) *C. melanurus*, UW 116382, 370 mm, Aleutian Islands; (B) *C. colletti*, UW 119446, 270 mm, Bering Sea; (C) *C. furcellus*, UW 119295, ca. 350 mm, Bering Sea, tissue only; (D) *C. simus*, UW 150897, 140 mm, Bering Sea.

from 73 egg masses (29 *C. colletti*, 37 *C. melanurus*, and 7 *C. furcellus*). The only mass identified as *C. simus* consisted entirely of newly hatched larvae, so egg measurement was not possible. Cluster means were calculated as the simple average of 20 egg diameters for each egg mass, and the means were compared among species using a Student's *t*-test.

RESULTS

Four species of snailfishes were identified among egg masses found in lithodid crabs from the Aleutian Islands and Bering Sea: *Careproctus melanurus*, *C. colletti*, *C. furcellus*, and *C. simus* (Figs. 2, 3). Eggs from a total of 80 masses were sequenced, but five of those masses had too many ambiguous base pairs to be included in analyses. Among the remaining 75 egg masses, 440 eggs and embryos were identified to species. Within each egg mass, all eggs sampled had a single haplotype. Three of the 11 crabs collected during trawl surveys harbored more than one egg mass. In two crabs, the masses were from different species. In both cases, one mass was identified as *C. colletti* and the other was *C. furcellus* (Fig. 3D). In the third crab with two masses, both were identified as *C. colletti*.

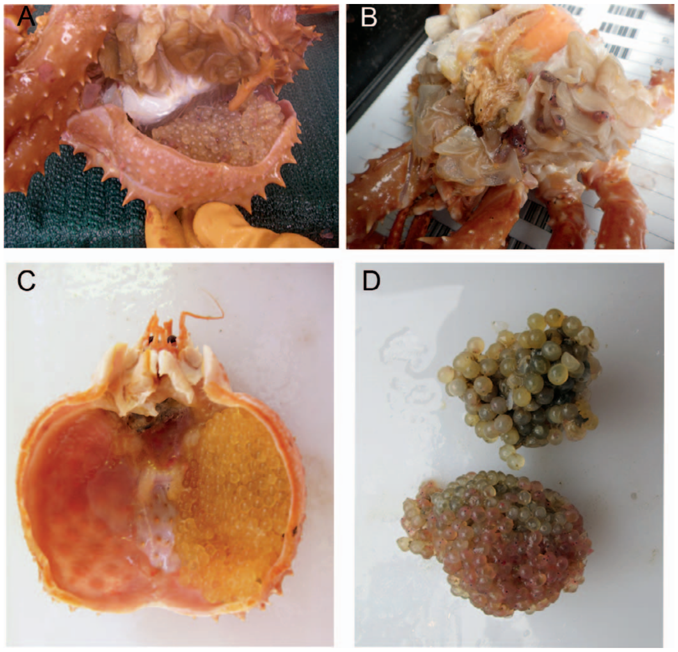

Fig. 3. Egg masses in lithodid crabs of Alaska. (A) Egg mass of *Careproctus furcellus* (UW 162749) beneath carapace of *Lithodes aequispinus*; (B) juveniles of *C. simus* (UW 162760) among gills of *L. aequispinus*; (C) egg mass of *C. melanurus* (UW 162759) in carapace of *L. aequispinus*; (D) egg masses of *C. furcellus* (UW 162753, top) and *C. colletti* (UW 162754, bottom) taken from beneath carapace of *L. couesi*.

The most common species identified among egg masses was *C. melanurus*. One batch of juveniles and 37 egg masses clustered with four adult samples (Fig. 4). Of these, the larvae and 35 egg masses matched the adult sequences exactly. The other two egg masses differed from the adult sequences and each other by a single base pair, giving a total of three haplotypes found among eggs of *C. melanurus* (Table 2), with maximum sequence divergence of 0.2% (1 bp).

The next most common species identified was *C. colletti*, in which 29 egg masses clustered with ten adult voucher sequences (Fig. 4). Six haplotypes were found among vouchers and ten among egg masses, including two identical haplotypes found in both vouchers and eggs (Table 2). Eighteen egg sequences and five adult sequences matched exactly, and the remaining sequences differed from each other by either one or two base pairs (0.2–0.4%). The adult sequence most similar to *C. colletti* was *C. furcellus*, which differed from *C. colletti* by 29 base pairs (5.8%).

Sequences from six egg masses were identical to the sequence of the voucher specimen of *C. furcellus* (Fig. 4). The sequence from one cluster of newly hatched larvae found inside a crab carapace (UW 162760) was identical to that of the sequence of the voucher adult specimen of *C. simus* (Fig. 4).

A phylogenetic hypothesis of the relationships of snailfishes is beyond the scope and taxon sampling of this project, but almost all putative species of *Careproctus* exhibited clearly distinct COI haplotypes with interspecific distances ranging from 0.8% (*C. gilberti*–*C. ostentum*) to 19% (*C. bowersianus*–*C. colletti*). Haplotypes of the two specimens identified as *C. comus* and *C. faunus* could not be distinguished from each other, although the sequence

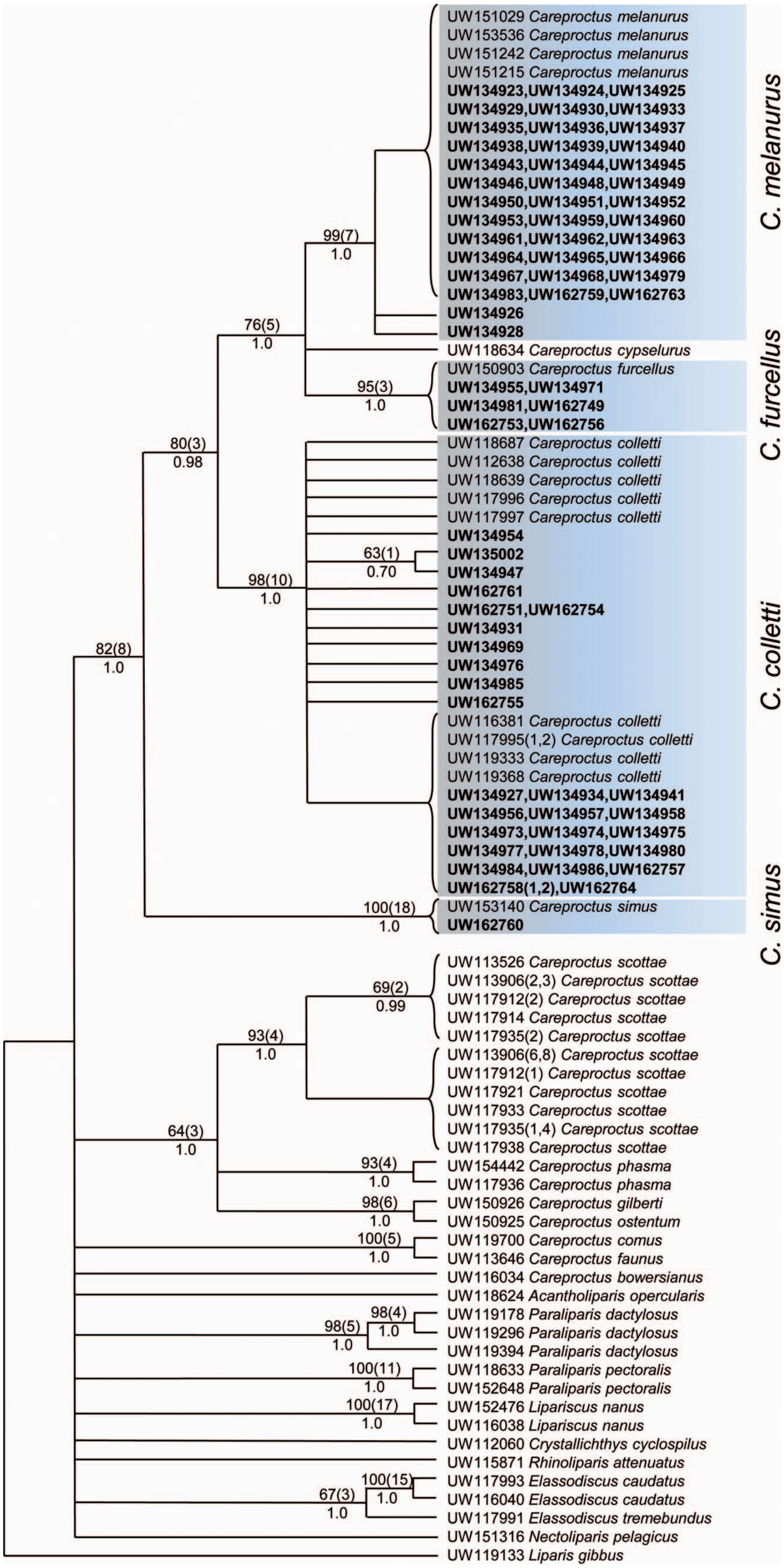

Fig. 4. Consensus tree relating snailfish specimens sequenced for this study. Bracketed nodes represent identical sequences and catalog numbers in boldface represent egg clusters. Numbers above branches are percent bootstrap scores (1000 replicates), with Bremer support in parentheses. Numbers below branches are Bayesian posterior probabilities. Nodes with less than 50% bootstrap support collapsed.

for *C. faunus* was of relatively poor quality and contained several ambiguous base pairs. Some samples likely amplified poorly and presented short sequence reads due to tissue quality. Many of the adult samples were collected out at sea, and it is possible the sample was not preserved until many hours after it was caught and thus degraded.

Egg masses were found in two species of king crabs taken during commercial and survey operations: Golden King Crabs (*Lithodes aequispinus*) and Scarlet King Crabs (*L. couesi*).

Table 2. Identification results of egg masses and haplotypes.

Species	Egg masses	Haplotypes
Careproctus melanurus	38	3
C. colletti	29	10
C. furcellus	6	1
C. simus	1	1

Only *L. aequispinus* was sampled from the commercial fishery. Egg masses were found in both species on the Bering Sea upper continental slope during survey operations. Specimens of the other species of large lithodids encountered during the survey, *Paralomis multispina* and *P. verrilli*, were also examined, but none contained egg masses.

Eggs of all species were identified from both the Aleutian Islands and Bering Sea, with the exception of *C. simus* in which a single batch of juveniles was taken in the Aleutian Islands. For *C. melanurus*, only a single egg mass and one mass of juveniles were identified from the Bering Sea; all others were taken in the Aleutian Islands. All masses identified as *C. furcellus* or *C. colletti* collected from the Aleutian Islands were found in the carapaces of *L. aequispinus*, while those collected in the Bering Sea were found in *L. couesi*. Mean egg size ranged from 2.97 to 4.87 mm in the 73 clusters measured (Fig. 5, Table 3). Cluster mean egg sizes of *C. colletti* were significantly smaller than those of *C. melanurus* and *C. furcellus* ($t(64) = 9.06$, $P < 0.0001$, and $t(34) = 6.20$, $P < 0.0001$, respectively), while egg size in *C. furcellus* was not significantly different from that of *C. melanurus* ($t(42) = 1.92$, $P = 0.062$). For *C. colletti*, the only species in which >3 egg masses were collected from each region, eggs from the Aleutian Islands were significantly larger than those from the Bering Sea ($t(27) = 6.75$, $P < 0.0001$).

DISCUSSION

More than one species of snailfish is responsible for depositing egg masses inside the gill cavities of lithodid crabs. Each individual mass is deposited by a single species and likely by a single female because masses consist of a single adhesive cluster of eggs. Further work could examine the number of individuals (male and female) contributing to each egg mass if other maker types such as single nucleotide polymorphisms (SNPs) or microsatellites are used (Canino et al., 2010). However, a single crab can have within it multiple egg masses deposited by different species. We found two egg masses in several crabs, and up to four masses (or egg masses and larval clusters) have been recorded in *L. aequispinus* (Somerton and Donaldson, 1998).

Table 3. Summary of egg size (maximum diameter) for three species of *Careproctus* measured for this study. Cluster means represent an average of 20 randomly selected eggs measured from each cluster.

	Count of clusters	Range of cluster means (mm)	Average of cluster means (mm)
C. furcellus	7	4.10–4.79	4.54
Aleutian Is.	4	4.59–4.79	4.67
Bering Sea	3	4.10–4.56	4.37
C. melanurus	37	3.82–4.87	4.36
C. colletti	29	2.97–4.26	3.72
Aleutian Is.	22	3.30–4.26	3.86
Bering Sea	7	2.97–3.46	3.26

The eggs of the four species of snailfishes identified here are all members of the genus *Careproctus*, as we expected from previous work (Table 1). However, these four are unlikely to be the only species of *Careproctus* in the eastern North Pacific that deposit eggs inside crabs. Although all egg masses clustered with voucher specimens in phylogenetic analyses (Fig. 4), 13 clusters matched no vouchers exactly, all differing from vouchers by less than 0.4%. These differences are likely intraspecific, but known interspecific sequence differences may be as low as 0.8% (e.g., *C. gilberti* and *C. ostentum* in this study). Thus, these clusters may represent species of *Careproctus* that were not sampled for this study. Although these sequences do not match any previously published snailfish sequence (based on a BLAST search of GenBank), many species in the Alaska region have not been sampled or sequenced and others are yet to be described.

All egg masses were collected in June through August either in the commercial crab fishery or during trawl surveys. Species of *Careproctus* and those of closely related genera are thought to be continuous or prolonged periodic spawners that deposit eggs either throughout the year or during a prolonged spawning season (Stein, 1980). The date of deposition and the duration of development is unknown for the eggs examined in this study, but running ripe females, with large extended ovipositors and easily expressed eggs, of all four species were observed during surveys in June and July conducted on the upper continental slope of the Bering Sea, in the Aleutian Islands, and in the Gulf of Alaska (Fig. 2).

Whether egg deposition in crabs is obligatory for species of *Careproctus* remains uncertain, but it is likely that the relationship is facultative or limited to only some species of

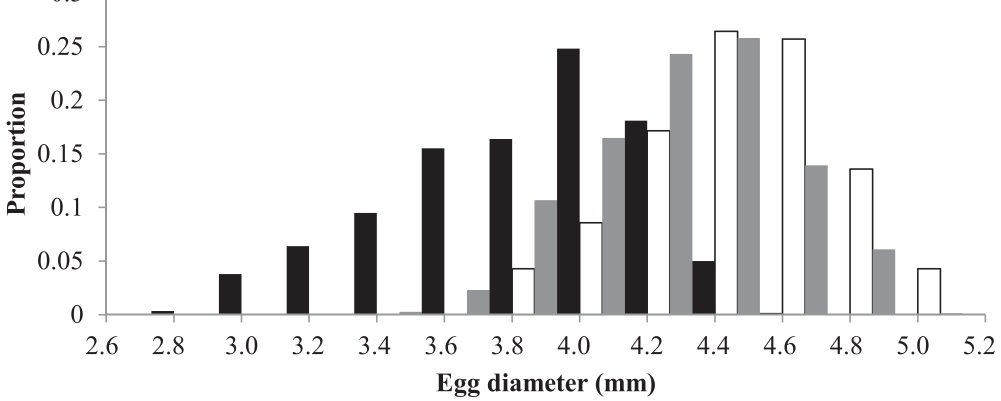

Fig. 5. Distribution of individual egg diameter measurements for egg masses identified as *Careproctus colletti* (black bars), *C. melanurus* (gray bars), and *C. furcellus* (white bars).

Careproctus. An instance of *C. reinhardti* depositing eggs on an aquarium wall has been recorded (Chernova, 1992). Snailfishes (likely a species of *Careproctus*; see Chernova, 2014b) have also been hatched from egg clusters found in trawled sponges (Knipovich, 1902), and egg clusters assumed to be those of *Careproctus* have been found in trawled sponges (Chernova, 2014b) and outside of lithodid crabs in other trawl catches (Poltev and Mukhametov, 2009). While these authors identified eggs by unknown methods, Overdick et al. (2014) used meristic and general morphological characters to identify embryos of "*Careproctus* sp." (p. 139: "likely to be *C. colletti"*) from eggs recovered outside of crabs in a trawl, although the authors noted that the eggs may have been dislodged from damaged crabs. Poltev and Mukhametov (2009) estimated that the biomass of mature females of *Careproctus* was much higher than that of lithodid crabs off the Kuril Islands and concluded that snailfishes must be using other egg deposition sites. However, many factors, including catchability and seasonality, may affect these estimates of biomass and their resulting conclusions.

Other species of *Careproctus* may use other substrates to deposit their eggs as species of other deep-water snailfish genera have been confirmed to deposit their eggs on structure, either biological or artificial. *Allocareproctus unangas*, a member of a closely related genus, lays its eggs in the branches of primnoid corals (Busby et al., 2006). Eggs of *Squaloliparis dentatus* have been collected from crabpot lines (Poltev and Steksova, 2010) and those of *Paraliparis rosaceus* have been taken from a mooring cable (Overdick et al., 2014).

Although the size of ripe ovarian eggs is known for several species of deep-water snailfishes (Stein, 1980; Able et al., 1984), sizes of deposited eggs have not been reported for most species of *Careproctus* and preservation methods of these eggs differ widely. This study suggests that deposited eggs of *C. melanurus* and *C. furcellus* in Alaska waters are 4–5 mm in diameter, which generally agrees with previous reports of egg size in *C. melanurus* from British Columbia (Peden and Corbett, 1973), and in *C. furcellus* and *C. melanurus* from the Aleutian Islands and Bering Sea (Somerton and Donaldson, 1998), but is larger than *C. melanurus* from off California (3.2–3.7 mm; Ambrose, 1996; Overdick et al., 2014). This study also suggests that eggs deposited by *C. colletti* are smaller than the other two species, at approximately 3–4 mm in diameter, which also agrees with a previous report of egg size in *C. colletti* from the Bering Sea (Overdick et al., 2014).

The relationship between snailfishes and lithodid crabs is a parasitic one, with snailfishes benefitting from the protection of their eggs by the crab carapace and crabs being afflicted with damaged gills (Somerton and Donaldson, 1998). However, unlike documented examples of brood parasitism (Royle et al., 2012), lithodid hosts do not actively brood snailfish eggs among their own eggs, nor do they use the same brooding mechanism as for their own eggs, which are clustered on the external surface of the female's abdomen rather than inside its carapace. This example of reproductive parasitism is most similar to the interphyletic symbioses between the aulorhynchid *Aulichthys japonicus* and its tunicate host (Uchida, 1934) and acheilognathin cyprinids (bitterlings: *Rhodeus*) and their bivalve hosts (Smith et al., 2004). In each of these cases, the fish deposits its eggs inside the body cavity of the sessile or nearly sessile invertebrate host, which then aerates the eggs by a natural flow of water while feeding and respiring.

Because egg masses and snailfishes for this study were collected only during the summer, further research should emphasize collections of egg masses from lithodids during other seasons. Other large lithodids, such as *Paralithodes platypus* (Blue King Crab), should also be examined for snailfish eggs. Although *P. platypus* is a relatively shallow-water lithodid, at least one species of *Careproctus* (*C. phasma*) is also common in similar depths (Orr et al., 2015). In addition, egg masses of snailfishes have been reported in *P. camtschaticus* in the western Pacific but have not been documented in the eastern Pacific, despite the high abundance and commercial significance of the species in the Bering Sea and eastern Pacific.

The symbiotic relationship between snailfishes and lithodid crabs is complex and not fully understood. However, this study demonstrates conclusively that multiple species of snailfishes and crabs are involved in these relationships, and that, unlike many parasitic symbioses, the pairings are not species-specific. The total taxonomic breadth of these symbiotic interactions, the degree to which they are facultative or obligate for the snailfishes, and the degree to which they are harmful to the lithodid hosts, are just a few of the many remaining questions.

MATERIAL EXAMINED

Adults

Acantholiparis opercularis (GenBank KU053762): Bering Sea: UW 118624, 75–80 mm, 58.5257°N, 174.8659°W, 997 m, 27 June 2008.

Careproctus bowersianus (GenBank KU053755): Bering Sea: UW 116034, 107 mm, 56.202°N, 169.2373°W, 539 m, 20 July 2004.

Careproctus colletti (GenBank KU053706–15): Bering Sea: UW 112638, 280 mm, 56.7672°N, 173.4486°W, 749 m, 25 July 2004; UW 116381, 4, 87–280 mm, 57.027°N, 173.539°W, 719 m, 24 July 2004; UW 117995, 2, 88–240 mm, 58.6659°N, 177.9734°W, 860 m, 29 June 2008; UW 117996, 8, 105–200 mm, 58.1184°N, 175.538°W, 925 m, 28 June 2008; UW 117997, 6, 190–230 mm, 58.5032°N, 177.8813°W, 1178 m, 29 June 2008; UW 118639, 240 mm, 58.7786°N, 177.9114°W, 500 m, 29 June 2008; UW 118687, 280 mm, 59.4024°N, 177.7352°W, 412 m, 1 July 2008; UW 119333, 88 mm, 60.4981°N, 178.8443°W, 286 m, 2 July 2008; UW 119368, 2, 165–190 mm, 58.5819°N, 177.7004°W, 999 m, 9 July 2008.

Careproctus comus (GenBank KU053756): Bering Sea: UW 119700, 80 mm, 57.7963°N, 174.1976°W, 331 m, 10 July 2008.

Careproctus cypselurus (GenBank KU053752): Bering Sea: UW 118634, 150 mm, 59.361°N, 178.4662°W, 1082 m, 30 June 2008.

Careproctus faunus (GenBank KU053757): Aleutian Islands: UW 113646, paratype, 90–96 mm, 52.2102°N, 172.206°W, 341 m, 23 June 2004.

Careproctus furcellus (GenBank KU053745): Bering Sea: UW 150903, 155 mm, 58.6659°N, 177.9734°W, 860 m, 29 June 2008.

Careproctus gilberti (GenBank KU053753): Bering Sea: UW 150926, 59–95 mm, 57.8157°N, 173.8598°W, 524 m, 26 June 2008.

Careproctus melanurus (GenBank KU053662–65): Aleutian Islands: UW 151029, 330 mm, 52.838°N, 172.3383°E, 307 m, 6 August 2010; Gulf of Alaska: UW 151215, 130 mm, 58.9902°N, 152.6027°W, 148 m, 7 July 2011; UW 151242, 2, 132–135 mm, 55.731°N, 154.3303°W, 625 m, 8 July 2011; UW 153536, 260 mm, 58.7803°N, 140.9751°W, 621 m, 22 July 2005.

Careproctus ostentum (GenBank KU053754): Bering Sea: UW 150925, 24 mm, 57.8157°N, 173.8598°W, 524 m, 26 June 2008.

Careproctus phasma (GenBank KU053660–61): Bering Sea: UW 117936, 5, 145–205 mm, 61.6765°N, 175.0699°W, 83 m, 16 July 2008; Gulf of Alaska: UW 154442, 107 mm, 58.2548°N, 151.4142°W, 150 m, 29 July 2005.

Careproctus scottae (GenBank KU053646–59): Bering Sea: UW 113526, 2, 155–180 mm, 56.5573°N, 172.0973°W, 272 m, 26 July 2004; UW 113906, 8, 98–160 mm, 55.1243°N, 167.5251°W, 199 m, 30 July 2004; UW 117912, 2, 97–138 mm, 55.0349°N, 167.3564°W, 201 m, 1 August 2008; UW 117914, 130 mm, 56.0423°N, 168.3796°W, 213 m, 30 July 2008; UW 117921, 102 mm, 59.6657°N, 176.5128°W, 134 m, 21 July 2008; UW 117933, 212 mm, 58.9901°N, 170.4839°W, 69 m, 30 June 2008; UW 117935, 4, 105–220 mm, 60.3397°N, 177.374°W, 145 m, 19 July 2008; UW 117938, 6, 65–195 mm, 60.6697°N, 177.5335°W, 144 m, 19 July 2008.

Careproctus simus (GenBank KU053704): Aleutian Islands: UW 153140, 113 mm, 51.4373°N, 178.4322°W, 369 m, 24 July 2010.

Crystallichthys cyclospilus (GenBank KU053758): Aleutian Islands: UW 112060, 280 mm, 52.4495°N, 171.3522°W, 83 m, 16 June 2004.

Elassodiscus caudatus (GenBank KU053760–61): Bering Sea: UW 116040, 13, 49–73 mm, 57.0704°N, 173.8373°W, 505 m, 24 July 2004; UW 117993, 147 mm, 59.3649°N, 178.2511°W, 832 m, 30 June 2008.

Elassodiscus tremebundus (GenBank KU053759): Bering Sea: UW 117991, 152 mm, 58.5255°N, 174.8224°W, 938 m, 27 June 2008.

Liparis gibbus (GenBank KU053772): Bering Sea: UW 119133, 225 mm, 57.9898°N, 170.3359°W, 72 m, 28 June 2009.

Lipariscus nanus (GenBank KU053764): Bering Sea: UW 116038, 3, 43–50 mm, 55.4198°N, 168.2659°W, 404 m, 28 July 2004; Aleutian Islands: UW 152476, 48 mm, 52.1049°N, 173.7813°E, 405 m, 31 July 2010.

Nectoliparis pelagicus (GenBank KU053771): Gulf of Alaska: UW 151316, 30–35 mm, 55.3033°N, 131.2505°W, 12 August 2011.

Paraliparis dactylosus (GenBank KU053765–67): Bering Sea: UW 119296, 135 mm, 59.3247°N, 178.3819°W, 860 m, 8 July 2008, UW 119394, 115 mm, 59.3247°N, 178.3819°W, 860 m, 8 July 2008; California: UW 119178, 3, 100–110 mm, North of Pt. Sur, California, 24 October 1997.

Paraliparis pectoralis (GenBank KU053769): Bering Sea: UW 118633, 150 mm, 59.361°N, 178.4662°W, 1082 m, 30 June 2008; UW 152648, 2, 150–160 mm, 60.1218°N, 179.4957°W, 1114 m, 21 June 2012.

Rhinoliparis attenuatus (GenBank KU053770): Bering Sea: UW 115871, 95 mm, 55.1276°N, 167.8108°W, 423 m, 29 July 2004.

Eggs and larvae

Careproctus colletti (GenBank KU053716–44): Bering Sea: UW 162751, UW 162754, UW 162755, UW 162757, *Lithodes couesi*, 59.38933°N, 178.25685°W, 764 m, 17 June 2012; UW 162758-1, UW 162758-2, *L. couesi*, 59.39858°N, 178.2134°W, 625 m, 18 June 2012; UW 162764, *L. couesi*, 60.25048°N, 179.02952°W, 704 m, 21 June 2012; Aleutian Islands: UW 162761, *L. aequispinus*, 52.46785°N, 173.5849°W, 200 m, 22 June 2012; Commercial: UW 134927, UW 134931, UW 134934, UW 134941, UW 134947, UW 134954, UW 134956, UW 134957, UW 134958, UW 134969, UW 134973, UW 134974, UW 134975, UW 134976, UW 134977, UW 134978, UW 134980, UW 134984, UW 134985, UW 134986, UW 135002, *L. aequispinus*, commercial king crab fishery, Aleutian Islands, Summer 2008.

Careproctus furcellus (GenBank KU053746–51): Bering Sea: UW 162753, UW 162756, *L. couesi*, 59.38933°N, 178.25685°W, 764 m, 17 June 2012; Aleutian Islands: UW 162749, *L. aequispinus,* 51.96393°N, 178.18657°E, 253 m, 11 July 2010; Commercial: UW 134955, UW 134971, UW 134981, *L. aequispinus*, commercial king crab fishery, Aleutian Islands, Summer 2008.

Careproctus melanurus (GenBank KU053666–703): Bering Sea: UW 162759, *L. aequispinus*, 59.29955°N, 177.69013°W, 331 m, 18 June 2012; UW 162763, larvae, *L. aequispinus*, 56.08485°N, 168.65262°W, 624 m, 11 June 2012; Commercial: UW 134923, UW 134924, UW 134925, UW 134926, UW 134928, UW 134929, UW 134930, UW 134933, UW 134935, UW 134936, UW 134937, UW 134938, UW 134939, UW 134940, UW 134943, UW 134944, UW 134945, UW 134946, UW 134948, UW 134949, UW 134950, UW 134951, UW 134952, UW 134953, UW 134959, UW 134960, UW 134961, UW 134962, UW 134963, UW 134964, UW 134965, UW 134966, UW 134967, UW 134968, UW 134979, UW 134983, *L. aequispinus*, commercial king crab fishery, Aleutian Islands, Summer 2008.

Careproctus simus (GenBank KU053705): Aleutian Islands: UW 162760, larvae, *L. aequispinus*, 51.92278°N, 178.25243°E, 239 m, 15 July 2012.

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Appendix 1. Catalog numbers and GenBank identification numbers for all CO1 sequences obtained for snailfish adults, larvae, and eggs in this study.

Species	Catalog number	Specimen number	GenBank number
Adults			
Acantholiparis opercularis	UW 118624	1 of 2	KU053762
Careproctus bowersianus	UW 116034		KU053755
Careproctus colletti	UW 112638		KU053713
Careproctus colletti	UW 116381		KU053714
Careproctus colletti	UW 117995	1 of 2	KU053707
Careproctus colletti	UW 117995	2 of 2	KU053708
Careproctus colletti	UW 117996	1 of 8	KU053715
Careproctus colletti	UW 117997		KU053709
Careproctus colletti	UW 118639		KU053710
Careproctus colletti	UW 118687		KU053706
Careproctus colletti	UW 119333		KU053711
Careproctus colletti	UW 119368	1 of 2	KU053712
Careproctus comus	UW 119700		KU053756
Careproctus cypselurus	UW 118634		KU053752
Careproctus faunus	UW 113646		KU053757
Careproctus furcellus	UW 150903		KU053745
Careproctus gilberti	UW 150926	4 of 7	KU053753
Careproctus melanurus	UW 151029		KU053662
Careproctus melanurus	UW 151215		KU053663
Careproctus melanurus	UW 151242	1 of 2	KU053664
Careproctus melanurus	UW 153536		KU053665
Careproctus ostentum	UW 150925		KU053754
Careproctus phasma	UW 117936		KU053661
Careproctus phasma	UW 154442		KU053660
Careproctus scottae	UW 113526		KU053646
Careproctus scottae	UW 113906	2 of 8	KU053647
Careproctus scottae	UW 113906	3 of 8	KU053648
Careproctus scottae	UW 113906	6 of 8	KU053649
Careproctus scottae	UW 113906	8 of 8	KU053650
Careproctus scottae	UW 117912	1 of 2	KU053651
Careproctus scottae	UW 117912	2 of 2	KU053652
Careproctus scottae	UW 117914		KU053653
Careproctus scottae	UW 117921		KU053654
Careproctus scottae	UW 117933		KU053655
Careproctus scottae	UW 117935	1 of 4	KU053656
Careproctus scottae	UW 117935	2 of 4	KU053657
Careproctus scottae	UW 117935	4 of 4	KU053658
Careproctus scottae	UW 117938	3 of 6	KU053659
Careproctus simus	UW 153140		KU053704
Crystallichthys cyclospilus	UW 112060		KU053758
Elassodiscus caudatus	UW 116040		KU053761
Elassodiscus caudatus	UW 117993		KU053760
Elassodiscus tremebundus	UW 117991		KU053759
Liparis gibbus	UW 119133		KU053772
Lipariscus nanus	UW 116038		KU053764
Lipariscus nanus	UW 152476		KU053763
Nectoliparis pelagicus	UW 151316	1 of 5	KU053771
Paraliparis dactylosus	UW 119178	1 of 3	KU053765
Paraliparis dactylosus	UW 119296		KU053766
Paraliparis dactylosus	UW 119394		KU053767
Paraliparis pectoralis	UW 118633		KU053768
Paraliparis pectoralis	UW 152648	1 of 2	KU053769
Rhinoliparis attenuatus	UW 115871		KU053770
Eggs and larvae			
Careproctus colletti	UW 134927		KU053720
Careproctus colletti	UW 134931		KU053735
Careproctus colletti	UW 134934		KU053721
Careproctus colletti	UW 134941		KU053722
Careproctus colletti	UW 134947		KU053737
Careproctus colletti	UW 134954		KU053738
Careproctus colletti	UW 134956		KU053723

Appendix 1. Continued.

Species	Catalog number	Specimen number	GenBank number
Careproctus colletti	UW 134957		KU053724
Careproctus colletti	UW 134958		KU053725
Careproctus colletti	UW 134969		KU053739
Careproctus colletti	UW 134973		KU053726
Careproctus colletti	UW 134974		KU053740
Careproctus colletti	UW 134975		KU053741
Careproctus colletti	UW 134976		KU053742
Careproctus colletti	UW 134977		KU053727
Careproctus colletti	UW 134978		KU053728
Careproctus colletti	UW 134980		KU053743
Careproctus colletti	UW 134984		KU053729
Careproctus colletti	UW 134985		KU053744
Careproctus colletti	UW 134986		KU053730
Careproctus colletti	UW 135002		KU053736
Careproctus colletti	UW 162751		KU053734
Careproctus colletti	UW 162754		KU053719
Careproctus colletti	UW 162755		KU053732
Careproctus colletti	UW 162757		KU053731
Careproctus colletti	UW 162758	1 of 2	KU053717
Careproctus colletti	UW 162758	2 of 2	KU053718
Careproctus colletti	UW 162761		KU053733
Careproctus colletti	UW 162764		KU053716
Careproctus furcellus	UW 134955		KU053748
Careproctus furcellus	UW 134971		KU053749
Careproctus furcellus	UW 134981		KU053750
Careproctus furcellus	UW 162749		KU053751
Careproctus furcellus	UW 162753		KU053747
Careproctus furcellus	UW 162756		KU053746
Careproctus melanurus	UW 134923		KU053671
Careproctus melanurus	UW 134924		KU053672
Careproctus melanurus	UW 134925		KU053666
Careproctus melanurus	UW 134926		KU053673
Careproctus melanurus	UW 134928		KU053674
Careproctus melanurus	UW 134929		KU053675
Careproctus melanurus	UW 134930		KU053676
Careproctus melanurus	UW 134933		KU053677
Careproctus melanurus	UW 134935		KU053678
Careproctus melanurus	UW 134936		KU053679
Careproctus melanurus	UW 134937		KU053680
Careproctus melanurus	UW 134938		KU053681
Careproctus melanurus	UW 134939		KU053682
Careproctus melanurus	UW 134940		KU053683
Careproctus melanurus	UW 134943		KU053684
Careproctus melanurus	UW 134944		KU053685
Careproctus melanurus	UW 134945		KU053686
Careproctus melanurus	UW 134946		KU053687
Careproctus melanurus	UW 134948		KU053688
Careproctus melanurus	UW 134949		KU053689
Careproctus melanurus	UW 134950		KU053690
Careproctus melanurus	UW 134951		KU053691
Careproctus melanurus	UW 134952		KU053668
Careproctus melanurus	UW 134953		KU053692
Careproctus melanurus	UW 134959		KU053693
Careproctus melanurus	UW 134960		KU053694
Careproctus melanurus	UW 134961		KU053695
Careproctus melanurus	UW 134962		KU053667
Careproctus melanurus	UW 134963		KU053696
Careproctus melanurus	UW 134964		KU053697
Careproctus melanurus	UW 134965		KU053698
Careproctus melanurus	UW 134966		KU053699
Careproctus melanurus	UW 134967		KU053700
Careproctus melanurus	UW 134968		KU053701

Appendix 1. Continued.

Species	Catalog number	Specimen number	GenBank number
Careproctus melanurus	UW 134979		KU053702
Careproctus melanurus	UW 134983		KU053703
Careproctus melanurus	UW 162759		KU053670
Careproctus melanurus	UW 162763		KU053669
Careproctus simus	UW 162760		KU053705