Evolution of Developmental Pattern and Larval Form in the Ophiuroids of Oregon and Beyond

by

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DISSERTATION ABSTRACT

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Title: Evolution of Developmental Pattern and Larval Form in the Ophiuroids of Oregon and Beyond

Despite the ubiquity and known impacts of transitions in developmental pattern in marine invertebrates, many taxa have been insufficiently analyzed.

In Chapter II, I report the effects of larval feeding in an undescribed facultative planktotroph, *Amphiodia* sp. opaque. By culturing larvae with and without food and observing development, I found that larval feeding led to faster development times, greater percent metamorphosis, and larger juveniles able to evade starvation longer than individuals that did not receive food. We compared metrics of early life stages using a series of generalized linear models, and model fitting was determined using Akaike's Information Criterion (AIC). Scores for each model and test are included as supplementary tables.

In Chapter III, I present a summary of developmental diversity in the ophiuroids of the northeast Pacific Ocean. We used DNA barcoding to identify eighteen species from the plankton of the southern Oregon coast. We found four species with reduced plutei, one with vitellaria, three with pelagic direct development, and ten with planktotrophic ophioplutei (including one followed by a vitellaria).

This diversity of larval forms suggests multiple transitions from feeding to nonfeeding larvae in the Amphiuridae, which I tested for in Chapter IV using comparative phylogenetic

analyses. To do so, I constructed a four-gene phylogenetic hypothesis for species with known development pattern in the family Amphiuridae. Single-gene trees were made to check for congruence between loci and the multi-gene dataset and are included as supplementary figures. This analysis inferred a brooding ancestor, an instance of re-acquisition of feeding, and multiple transitions back to nonfeeding larvae. The analysis inferred multiple transitions from brooding to nonfeeding planktonic development via pelagic direct development.

Altogether, this work introduces new examples of the developmental patterns in Ophiuroidea, including an example of facultative planktotrophy, a rare developmental pattern that may represent an evolutionary intermediate between feeding and nonfeeding larvae. We show the effectiveness of DNA barcoding for identifying the early life stages of benthic marine invertebrates. Finally, I found that reconstructed ancestral states of developmental pattern are influenced by tree topology and completeness of the dataset.

This dissertation includes previously published and unpublished co-authored material.

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Many people contributed to this project by bringing us individual larvae of interest, enumerating and photographing larvae from plankton samples, conducting gene amplification, and confirming adult identifications. They include George von Dassow, MacKenna Hainey, Gordon Hendler, Chris Mah, Jenna Valley, Ella Lamont, and Craig Young.

DEDICATION

To my parents, Fran and Norm Nakata, and my partner, Tommy Ashcraft.

Because of you, I have everything.

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CHAPTER I

INTRODUCTION

The evolutionary history of developmental patterns in marine invertebrates is an important system for examining the loss and possible regain of complex and important traits. In most marine taxa, benthic adults spawn or release planktonic larvae that vary in size, complexity, and developmental duration based on the level of parental investment. Many marine invertebrates have elaborate feeding larvae that develop from very many small eggs; others, sometimes even closely related species, have simple nonfeeding larvae that develop from moderately sized eggs. In some taxa, both planktonic and benthic brooding strategies exist (Hendler, 1991; Strathmann & Strathmann, 1982).

Evolutionary transitions from planktonic feeding to nonfeeding larvae are persistent and widespread across marine invertebrates, and have consequences for species distributions and population connectivity (Strathmann, 1985). Many groups of marine invertebrates contain a diversity of developmental strategies, sometimes even within families or genera, suggesting multiple transitions in mode of development (Allen & Podolsky, 2007; Collin, 2004; Krug et al., 2015). Some groups, such as the brittle stars (Echinodermata: Ophiuroidea) have great potential to study evolutionary transitions in development but have historically lacked sufficient data for analysis.

In the following chapters I investigate the evolution of development in brittle stars. Three categories are accepted (Hendler, 1975): obligate feeding (planktotrophy) in ophioplutei, abbreviated development in vitellaria, reduced plutei, and pelagic direct developers, and direct

development by brooding. These developmental patterns occur throughout ophiuroid taxa, suggesting transitions between divergent patterns. However, developmental pattern of many ophiuroid species have yet to be characterized. Unlike their echinoid and asteroid relatives, ophiuroids lack reliable means to obtain mature gametes in the lab (Selvakumaraswamy & Byrne, 2000). Instead, I collected wild larvae from the plankton to study development patterns and taxonomic diversity of ophiuroids in the northeast Pacific.

First, in Chapter II, I describe a facultatively feeding larva with a reduced pluteus morphology that may feed but can also form a juvenile in the absence of food. This is a rarely documented developmental strategy that has been described in just a handful of marine invertebrate groups, including two urchins and another brittle star (Allen & Pernet, 2007; Emlet, 1986; Hart, 1996). I hypothesized that larval feeding would provide benefits to the larva and carry over post metamorphosis. Chapter II was published in the journal *Ecology and Evolution* with co-author Dr. Richard Emlet, who provided editorial and manuscript preparation assistance (Nakata & Emlet, 2023).

The species from Chapter II is only known in its larval form. We collected all experimental material from the plankton as part of a decade-long project to identify brittle star larvae by DNA barcoding. DNA barcoding has been used to identify marine invertebrate larvae in many taxa, can reveal the presence of rare or difficult to collect species, and greatly increases estimates of regional species diversity (Collin et al., 2020a, 2021; Heimeier et al., 2010; Maslakova et al., 2022; Shanks et al., 2020). In Chapter III, I use DNA barcoding and records collected by R. Emlet and students to investigate the developmental diversity of brittle stars from the northeast Pacific Ocean. I ask if development mode and species identity can be determined

from larval form. Chapter III is unpublished but will include Dr. Richard Emlet as a co-author, as he contributed all data collected prior to 2018 and will assist with the final manuscript preparation.

Brittle stars display a diversity of developmental strategies and larval forms. As such, they have great potential as a system in which to study the evolutionary loss and gain of complex larval morphologies and life history patterns. However, analyses have been conducted in few taxa (Allen & Podolsky, 2007; O'Hara et al., 2019a). Brittle stars display many developmental patterns including obligately feeding (planktotrophic) ophioplutei, facultatively feeding reduced plutei, nonfeeding vitellaria, pelagic direct developers, and brooding (Allen & Podolsky, 2007; Byrne et al., 2008; Hendler, 1991; O'Hara et al., 2019a). In Chapter IV, I built a dataset of developmental pattern and larval form based on data from Chapter III, unpublished data from R. Emlet, and from accounts from the literature. I built a 39-spp. phylogenetic hypothesis to ask if the family Amphiuridae had an ancestor with a feeding larva. Feeding larvae have complex morphologies that aid in swimming and feeding. It may be difficult to re-evolve complex larval structures once lost (Strathmann, 1978b).

In Chapter III we observed three planktonic forms from the family Amphiuridae that are classified as abbreviated developers: nonfeeding vitellaria, pelagic direct developers, and reduced plutei. I hypothesized that abbreviated development evolved multiples times in the family and arose as distinct larval forms. To investigate this and other hypotheses on the evolution of development in Amphiuridae I estimated ancestral states for developmental pattern and larval form. I estimated transition rates between states by fitting a series of models to my

tree. Chapter IV is unpublished but will include Dr. Richard Emlet as a co-author, and he will assist with the final manuscript preparation.

Despite their potential use for the study of evolutionary patterns in development, ophiuroids have thus far received little attention due to the lack of molecular and developmental data across taxa. We present our own data for 18 species from the northeast Pacific, with additional data for 13 species from Panama and Australia from co-author R. Emlet. We use this data to characterize the developmental diversity of ophiuroids with planktonic development, and investigate hypotheses of evolution of development in the family Amphiuridae.

CHAPTER II

HAVING CAKE AND EATING TOO: THE BENEFITS OF AN INTERMEDIATE LARVAL FORM IN A BRITTLE STAR *AMPHIODIA* SP. OPAQUE (OPHIUROIDEA)

From Nakata, N. N., & Emlet, R. B. (2023). Having cake and eating too: The benefits of an intermediate larval form in a brittle star *Amphiodia* sp. opaque (Ophiuroidea). *Ecology and Evolution*, *13*(7), e10298. https://doi.org/10.1002/ece3.10298. Published open access under Creative Commons by Attribution License. Minor editorial changes have been made for dissertation consistency.

1. INTRODUCTION

The evolutionary transition in developmental mode from feeding (planktotrophic) to non-feeding (lecithotrophic) larvae is widespread across marine invertebrate taxa, and significantly impacts number and size of offspring (Collin & Moran, 2018; Marshall et al., 2012; Strathmann, 1978b, 1985). Lineages with feeding larvae have given rise to those with nonfeeding larvae in many marine taxa, including in closely related species (e.g., Byrne, 2006; Collin, 2004; Jeffery et al., 2003; Keever & Hart, 2008; Krug et al., 2015; Pappalardo et al., 2014; Waeschenbach et al., 2012), sometimes with great frequency, e.g., at least 15 times in living echinoids (Emlet, 1990). These contrasting patterns signify an evolutionary trade-off between parental investment per offspring and fecundity: feeding larvae develop from smaller eggs that are produced in far

greater numbers than those of related species with nonfeeding development (Strathmann, 1985). The limited provisions of small eggs require planktotrophic larvae to acquire materials through exogenous food to complete metamorphosis. The larger, more lipid-rich eggs of lecithotrophic larvae contain sufficient material for larvae to create a juvenile without feeding.

An intermediate pattern known as facultative planktotrophy (Chia, 1974; Emlet, 1986; Vance, 1973a), where larvae are capable of feeding in the plankton but can also complete metamorphosis without food, is a rare but persistent mode of development that has been observed at least eight times across several marine invertebrate taxa (Table 1; see also Allen & Pernet 2007). Facultative planktotrophy is often considered an intermediate mode of development in the evolutionary transition between feeding and nonfeeding larvae, but whether or not intermediate modes can maximize reproductive success over evolutionary timescales is still up for debate (Christiansen & Fenchel, 1979; Levitan, 2000; McEdward, 1997; Vance, 1973a, 1973b). Larvae with facultative planktotrophy provide an opportunity to test the effects of larval feeding on several aspects of early life history, including planktonic duration, percent metamorphosis, juvenile size, and energetic reserves in juveniles.

Table 2.1. Effects of larval feeding on development of facultative planktotrophs.
Larval and juvenile metrics are represented as increasing (+), decreasing (-), equal (=), or not measured (NM) because of larval feeding.

Taxa	Develop-ment time	Larval survivor-ship	Juvenile size	References
Echinodermata: Echinoidea				
Clypeaster rosaceus	=	=	+	Allen et al., 2006; Emlet, 1986
Brisaster latifrons	NM	NM	+	Hart, 1996
Echinodermata: Ophiuroidea				
***Amphiodia* sp. opaque**	**-**	**+**	**+**	**This study**
Macrophiothrix rhabdota	-	+	+	Allen & Podolsky, 2007
Mollusca: Gastropoda				
Adalaria proxima	-	=	NM	Kempf & Todd, 1989
Conus pennaceus	=	=	NM	Perron, 1981
Phestilla sibogae	=	+	+	Kempf & Hadfield, 1985; Miller, 1993
Annelida: Polychaeta				
Streblospio benedicti	NM	NM	NM	Pernet & McArthur, 2006
Arthropoda: Copepoda				
Tisbe sp.	-	+	+	Gangur & Marshall, 2020

Planktonic duration, the time interval between introduction of embryos or larvae to the water column and metamorphosis in the benthos, varies widely across developmental modes of planktonic larvae and affects the composition and distribution of benthic adult populations (Becker et al., 2007; Shanks, 2009; Strathmann, 1985). For feeding larvae, the accumulation of materials necessary for metamorphosis can take weeks to months, whereas nonfeeding larvae can settle in the benthos in hours to days after release (Strathmann, 1987). The larval phase is the dominant dispersal stage for benthic marine invertebrates, and larvae that spend weeks in the plankton tend to disperse further than those that spend only hours in the water column before

settlement (Shanks, 2009). Realized dispersal distances are modulated by a complex set of temporal, physical, and behavioral factors, but greater interchange of genetic propagules via feeding larvae tends to lead to greater genetic connectivity between benthic adult populations (reviewed in Cowen & Sponaugle, 2009; Shanks, 2009). The increased dispersal potential of feeding larvae influences biogeography: in cone snails, echinoids and cowries, species with feeding planktonic larvae have larger geographic ranges when compared to species with nonfeeding development (Kohn & Perron, 1994; Emlet, 1995; Paulay & Meyer, 2006); and in fossil gastropods, there is evidence that planktotrophic development can positively influence geographical distribution and species longevity (Hansen, 1978, 1980; Jablonski, 1986).

Planktonic duration can be mediated by factors both environmental and taxon-specific, but extended planktonic duration generally increases the likelihood of larval mortality prior to metamorphosis (Rumrill, 1990). Low food availability (Miner et al., 2005; Rendleman et al., 2018; Sewell et al., 2004; Strathmann et al., 1992) and low temperature (O'Connor et al., 2007) can slow larval development in echinoids, as well as facultative planktotrophs from some taxa (Allen & Podolsky, 2007; Paulay et al., 1985). Larvae with longer development times relative to conspecifics experienced lower survivorship in two fishes (Hare & Cowen, 1997; Meekan & Fortier, 1996) and increased mortality from predation in a number of invertebrate species (Cowen & Sponaugle, 2009; Rumrill, 1990). Long planktonic durations also expose larvae to the risk of advection away from suitable habitat for settlement (Pineda et al., 2010), although long-lived larvae in coastal habitats may not always disperse widely (Shanks, 2009). In these ways, planktonic duration can be tied to the percentage of larvae in a cohort that survive through metamorphosis, hereafter referred to as percent metamorphosis.

Metamorphosis does not promise a new beginning: embryonic and larval experiences can be expressed latently in juvenile and adult quality (Emlet & Sadro, 2006; Pechenik, 2006). Food limitation and prolonged planktonic duration have been shown to negatively influence juvenile size, growth, and survival (reviewed in Pechenik, 2018). In facultative planktotrophs, larval feeding resulted in larger juveniles in echinoderms (Allen & Podolsky, 2007; Emlet, 1986; Hart, 1996) and gastropods (Kempf & Hadfield, 1985; Miller, 1993). Juvenile size is an important life history characteristic because larger juveniles tend to exhibit higher survival, growth, reproduction and longevity across several species (Marshall et al. 2018).

Here we present evidence for a facultatively planktotrophic larva of a brittle star, *Amphiodia* sp. opaque (Echinodermata: Ophiuroidea), a previously unknown species that occurs in the northeastern Pacific from Oregon to British Columbia. There is limited documentation of developmental mode within the Ophiuroidea (ca. 12% of ophiuroid species, N. Nakata unpubl. data), but the following developmental modes are known: planktotrophy via an eight-armed ophiopluteus, facultative planktotrophy, lecithotrophy via a reduced pluteus or vitellaria larva that can be pelagic or demersal, and brooding (Hendler 1991; Byrne and Selvakumaraswamy 2002). There are many unresolved relationships within families, but widespread occurrence of developmental diversity suggests frequent transitions from feeding to nonfeeding larvae (Allen & Podolsky, 2007; Lessios & Hendler, 2022; O'Hara et al., 2019a). This study utilizes larvae of intermediate mode of development to assess the effect of larval feeding on planktonic duration, percent metamorphosis, juvenile size, and juvenile energetic reserves. Because our experimental individuals were collected as embryos from the plankton and the adults remain unknown (in

Oregon), we used DNA barcoding to identify our animals and to reveal their relationship to other ophiuroids in the northeast Pacific.

2. MATERIALS AND METHODS

2.1. Molecular identification of embryos and larvae

To investigate species identity of the embryonic morphotypes, we froze one embryo from each year's cohort of larvae collected for experimentation (2019-2021, see section 2.2) at -20°C in a small volume of seawater. We compared the resulting sequences with those of approximately a dozen larvae of the same morphotype collected over the last decade, as well as with sequences for adults and larvae of other amphiurids from the northeast Pacific. Genomic DNA was extracted with the chelex-based InstaGene™ Matrix (Bio-Rad) and fragments of *cytochrome c oxidase subunit I* (*COI*) were amplified and sequenced using the primers jgLCO1490 and jgHCO2198 (Geller et al., 2013). PCR amplification reactions were performed in a 20 µL total reaction volume that included 11.4 µL nuclease-free water, 4 µL 5X Green Buffer, 0.4 µL dNTP 10 mM, 0.2 µL GoTaq Polymerase (Promega), and 1 µL each of forward and reverse primers. PCR conditions were as follows: initial step 95°C for 2 min, followed by 34 cycles of denaturation at 95°C for 40 sec, annealing at 45 or 48°C for 40 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 2 min. PCR products were cleaned up using the Wizard SV Gel and PCR Clean up System (Promega) prior to Sanger sequencing (Sequetech, Mountain View, CA). Barcode sequences were compared to GenBank (www.ncbi.nlm.nih.gov/genbank/), BOLD (Ratnasingham & Hebert, 2007), and our unpublished

dataset of ophiuroid sequences using the BLAST function in Geneious Prime (https://www.geneious.com). We aligned *COI* sequences of amphiurid spp. of the northeast Pacific (Table 2.2) using the Geneious MAFFT plug-in (Katoh & Standley, 2013) created a maximum likelihood tree using the PhyML plug-in with 100 bootstraps and based on the HKY85 model (Guindon et al., 2010; Hasegawa et al., 1985).

Table 2.2. Collection and accession data for specimens included in tree (Fig. 2). Accession numbers refer to records in BOLD (Ratnasingham & Hebert, 2007), unless marked by an asterisk (*), indicating that a record is from GenBank (Benson et al., 2017).

Species ID	Specimen code	Life stage	Collection location, State/Province	Accession Number
Amphichondrius granulatus	Agran1	Adult	Catalina Is., CA	OOPH005-18
	Agran2	Adult	Catalina Is., CA	OOPH006-18
Amphipholis pugetana	Ampu1	Adult	Cape Arago, OR	OOPH003-18
	Ampu2	Adult	Cape Arago, OR	OOPH004-18
	Or43-12913	Larva	Charleston, OR	OLAB015-22
	Or811-12115	Larva	Charleston, OR	OLAB016-22
Amphiodia occidentalis	Amoc2	Adult	Charleston, OR	OOPH002-18
	AoMc1a	Adult	Charleston, OR	OOPH020-22
	Amoc1	Adult	Charleston, OR	OOPH001-18
	AoMc2a	Adult	Charleston, OR	OOPH022-22
Amphiodia sp.	FHLAmphioegg1	Egg	Orcas Island, WA	OLAB045-22
sensu Emlet 2006	OrR7	Larva	Charleston, OR	OLAB031-22
	Or8P-121812	Larva	Charleston, OR	OLAB033-22
	MMB17	Adult	Charleston, OR	OOPH032-22
***Amphiodia* sp. opaque**	BFHL 4167	Adult	Boundary Bay, WA	BBPS564-19
	Orop3a	Larva	Charleston, OR	OLAB001-22
	Opq4	Larva	Charleston, OR	OLAB006-22
	Oop1	Larva	Charleston, OR	OLAB007-22

	QHAK-00565	Larva	Quadra Island, BC	QHAK711-21
	Opq2	Larva	Charleston, OR	OLAB004-22
	opaque 11-7-18	Larva	Charleston, OR	OOPH029-22
	Opq1 FHL	Larva	Friday Harbor, WA	OLAB047-22
Amphiodia urtica	Or988	Juvenile	Charleston, OR	OLAB028-22
	A.urt adt 4-23-19	Adult	Cape Arago, OR	OOPH026-22
	DISA835-19	Adult	Los Angeles, CA	DISA835-19
	HM542069	Adult	Bamfield, BC	*HM542069
	urtica 11-5-18	Larva	Charleston, OR	OOPH027-22
	CCDB-31778 B01	Adult	Dana Point, CA	ECHCA108-18
	KU495782	Adult	Queen Charlotte Is., BC	*KU495782

2.2. Larval cultures & data collection

Adults of *Amphiodia* sp. opaque have not yet been found in Oregon. We obtained late blastulae, hatched gastrulae, and larvae of *Amphiodia* sp. from plankton tows collected with a 130 μm mesh net in the Coos Bay estuary (Oregon) approximately 3 km from the entrance to the Pacific Ocean (Charleston Marina: 43°21.2'N, 124°20'W). We collected plankton daily, approximately one hour before high tide and examined our catch within two hours using a stereomicroscope. On several occasions the bright orange embryos characteristic of *Amphiodia* sp. opaque (Fig. 2.1A, B) were abundant enough to obtain sufficient material for experimentation. In 2020, we collected approximately 120 blastulae or gastrulae, ~1 day post spawn (dps), on February 23 and 100 more of similar stage on February 24. Because of their similar stages of development, embryos from each day represented separate spawning events one day apart. In 2021, we collected 240 embryos of similar stage on March 7. In 2019, no embryos

were found, but we collected approximately 80 ophioplutei on January 28 and 29. These larvae had either formed juvenile rudiments or did so within a week of capture and were analyzed for juvenile size and starvation time as a 'wild' treatment. The ophiuroid hydrocoel rudiment wraps around the larval esophagus and marks the cessation of larval feeding; therefore, we considered larvae with rudiments to have completed or nearly completed larval feeding in their natural environment prior to their collection and monitoring in the lab. Wild ophioplutei and rudiment-stage larvae were kept in filtered sea water (FSW) for the remainder of their development. We did not manipulate microalgal food or estimate planktonic duration for larvae collected in 2019 because we could not determine their prior history in the plankton.

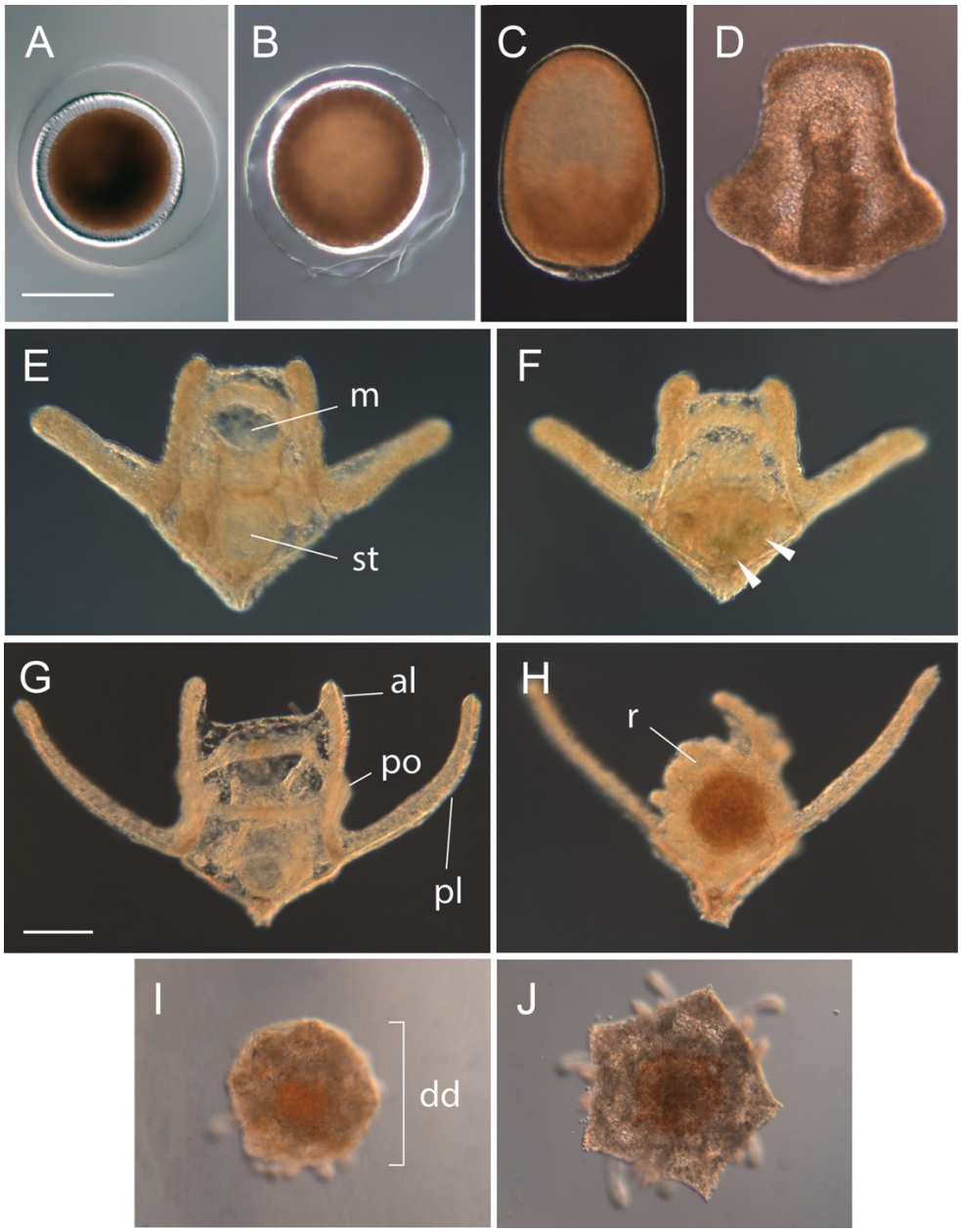


Figure 2.1. *Amphiodia* sp. opaque (A) fertilized egg, (B) unhatched blastula, (C) gastrula, (D) early pluteus 3 days post spawn (dps), (E) reduced pluteus 6 dps from no-food treatment, with mouth (m) and empty stomach (st), (F) reduced pluteus 6 dps from food treatment, with algal food visible in the stomach (arrowheads), (G) pluteus at 10 dps from no-food treatment, with three pairs of arms: anterolateral (al), postoral (po), and posterolateral (pl), (H) pluteus 10 dps from food treatment, with juvenile rudiment (r), (I) juvenile from no-food treatment with disc diameter (dd), and (J) juvenile from food treatment. Scale bars are 100 µm; same scale for A-F and for G-J.

2.3. Feeding experiments

For experimental manipulations of microalgal food, we kept embryos (collected in 2020 or 2021) in FSW until just before the formation of the mouth, approximately two days after collection. At this point we haphazardly divided early plutei into replicate finger bowls each with 10 larvae per 30 ml (2020) or 17 larvae per 51 ml (2021) for a standard density of 0.3 larva ml^{-1}. We randomly assigned bowls to 'food' or 'no-food' treatments and kept them in an incubator at 15°C. We fed larvae in the food treatment a tripartite microalgal diet composed of 2 parts by volume of *Rhodomonas lens,* to one part each of *Dunaliella tertiolecta* and *Isochrysis galbana* at a combined concentration of 5,000 cells ml^{-1}. Larvae in the no-food treatment were kept in FSW alone. Small flakes of cetyl alcohol were added to all cultures to prevent larvae from perishing in the air-water interface.

We collected data on stages of larval development every two or three days when FSW and microalgal food were refreshed. We categorized larvae visually as (1) ophiopluteus, (2) rudiment-stage larva, or (3) juvenile (Fig. 2.1). Developmental stages were defined as follows: ophioplutei had up to six larval arms and an open mouth; rudiment-stage larvae had a pair of posterolateral arms, the right anterolateral arm and a well-developed rudiment that occluded the larval mouth; and juveniles lacked larval arms including the larval skeleton and ciliary band, and all locomotion was by their podia. Planktonic duration was defined as the number of days from hatching – estimated as one day prior to the blastula stage in which they were collected – until they were scored as juveniles. We defined percent metamorphosis as the total number of juveniles produced relative to the initial number of larvae from a given bowl. When we found new juveniles, we removed them from the larval culture bowl and put each individual in its own

35 mm petri dish with FSW. We kept juveniles in FSW without food at 15°C, observing them every two to three days when FSW was changed. A juvenile was considered dead when podia did not move, even following water agitation and the juvenile did not hold onto the dish bottom.

2.4. Analysis

We conducted all statistical analyses in the R environment v3.6.0 (R Core Team, 2022), and visualized plots with the package 'ggplot2' (Wickham, 2016).

For each statistical test to follow, apart from the survival analysis of juveniles, we considered the finger bowl to be the experimental unit. We calculated the percent of larvae that successfully metamorphosed into juveniles (hereafter percent metamorphosis), and mean values for planktonic duration, juvenile size, and juvenile starvation time for each bowl. We calculated survival statistics in two ways: by bowl (Fig. 2.6B) or pooled within treatments and years in survival analysis (Fig. 2.7).

To quantify the effect of larval food on percent metamorphosis, we compared between treatments, keeping years separate. As values for percent metamorphosis were not normally distributed and did not have equal variances (Shapiro-Wilk, $p = 0.015$; Levene's test, $p = 0.004$), we used a non-parametric Kruskal-Wallis test, followed by a Dunn's test for pairwise comparisons.

We used aboral surface area of the disc as our value for juvenile size. We measured disc diameter (dd, Fig. 2.1I), the length from one arm tip through the center of the disc to the opposite interradius, using the ocular micrometer on the dissecting microscope (dh, Fig. 2.1I), measured to the nearest 20 microns. Using the disc diameter as the height of a regular pentagon (*h*,

Equation 1), we solved for the length of a single side (a) in order to calculate the area of the pentagonal juvenile disc (A, Equation 2).

$$(1) \qquad h = a * \sqrt{5 + 2\sqrt{5}} / 2$$

$$(2) \qquad A = a^2 * \sqrt{25 + 10\sqrt{5}}/4$$

The data for juvenile aboral surface area did not satisfy the assumption of normality (Shapiro-Wilk, 2020 $p = 0.001$, 2021 $p = 0.006$) but variances were not significantly different (Levene's test, 2020 $p = 0.97$, 2021 $p = 0.09$). To determine if the presence of microalgal food had an effect on juvenile size, we compared aboral surface area across treatments using a non-parametric Kruskal-Wallis test, followed by a *post hoc* Dunn's test.

To determine whether larvae that developed more quickly also produced larger juveniles, we modeled the association between planktonic duration and juvenile aboral surface area using a set of generalized linear models (GLMs) with a Gaussian family, including combinations of potential covariates including treatment and year.

To examine the relationship between juvenile size and survival, we modeled the association between juvenile aboral surface area and time to starvation using a set of GLMs and combinations of covariates treatment, year, and their interaction. As we predict time to juvenile starvation represents a waiting time, we used GLMs with a gamma distribution. We assessed model fit using Akaike's and Bayesian Information Criteria (Table S4), with the lowest value indicating the best fit.

To assess differences in juvenile survival time between treatments and years, we used survival analysis. The survival time data violated the assumptions of parametric statistics, rendering traditional tests inappropriate for their analysis. Such data can be analyzed using non-

parametric survival analysis (Kleinbaum & Klein, 2012; Moore, 2016), a collection of statistical procedures for which the outcome variable of interest is time until an event occurs. Juvenile starvation times were analyzed using survival analysis in the R package 'survival' (Therneau, 2020) and visualized with the package 'survminer' (Kassambara et al., 2020).

Most survival analyses must contend with censoring, which occurs when we have some information about individual survival time, but we don't know the survival time exactly. Our data on time to juvenile starvation are 'interval-censored', because counts were done at intervals when water changes happened every two or three days. The exact time of metamorphosis or death is unknown and can only be placed within a specified window of time. Censoring can also occur when an individual withdraws from the study prior to the time of the event. In our analysis of juvenile starvation, the event of interest was death, and no juveniles were censored as all were followed until the time interval of the event. Survival curves for juvenile death probabilities were compared using a non-parametric log-rank test.

3. RESULTS

3.1. Species identity and occurrence of larvae

The opaque orange larvae studied here belong to an undescribed species of *Amphiodia* that occurs at least from southern Oregon to British Columbia. On many occasions since March 2003, we have collected eggs, unhatched and hatched embryos, and larvae from Oregon plankton that we assigned, by color and morphology to *A.* sp. opaque. These samples were collected

between January and April, but on one occasion embryos were collected in November (2018). We also collected these larvae in the Salish Sea (Friday Harbor, Washington) in August of 2019 and September of 2020. We have generated COI- barcodes for more than 15 specimens collected from 2013 to 2019 that we assigned to *Amphiodia* sp. opaque, and their sequences are 98+ % similar (Fig. 2.2). During the times we collected these embryos and larvae we never found similar orange embryos or larvae that had different barcodes and were other species.

The sequences of our larvae are a close match (>98% pairwise nucleotide similarity) to that of a juvenile amphiurid specimen (2.5 mm disk diameter) collected from Boundary Bay WA (BOLD specimen record: BBPS564-19). The juvenile is clearly a member of the genus *Amphiodia,* because it has the appropriate pattern of 3 similarly sized oral papillae per jaw. In BOLD we found another close match (>99%) to a larva with the same morphology from Hyacinthe Bay, Quadra Island, British Columbia (BOLD specimen record: QHAK711-21, Fig. 2.2).

A neighbor-joining tree of COI sequences from larvae and adults of other amphiurid species known from the NE Pacific shows *A.* sp. opaque as the sister species to *A. urtica* which has obligately feeding larvae (Schiff & Bergen 1996, N. Nakata, unpubl. data; Fig. 2.2). Together these clades are sister to a species complex of *A. occidentalis* (R. Emlet, unpubl. data)

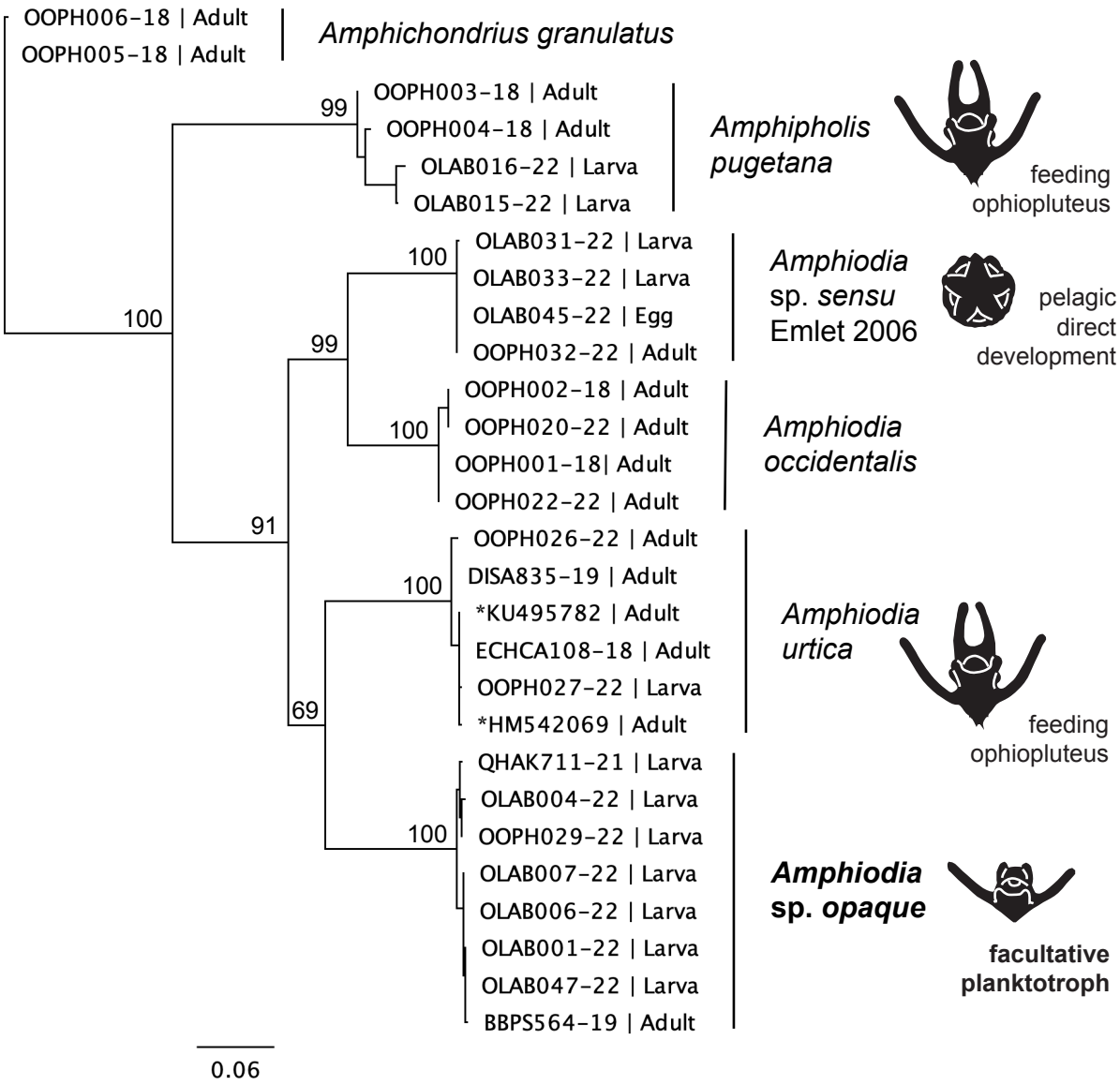


Figure 2.2. Maximum likelihood tree of *COI* sequences from adult and larval amphiurid spp. in the northeastern Pacific, constructed using the PhyML plug-in in Geneious. Sequences from GenBank (*) and BOLD are labeled with accession number (and see Table 2). Development modes are indicated on the right, with characteristic larvae depicted. Bootstrap values are shown next to nodes.

3.2. Larval developmental mode

Larvae of *Amphiodia* sp. opaque develop from eggs of moderate size (140 μm) and are facultative planktotrophs (Fig. 2.1): larvae can feed but do not require food for metamorphosis

into juveniles. While percent metamorphosis did differ between years, some larvae developed into juveniles in the absence of food. We observed larvae from unfed culture with empty stomachs, and those from fed cultures with stomachs full of microalgal food (Fig. 2.1E and F). Furthermore, aspects of larval and juvenile performance differed according to larval food treatment (*Table 2.3*).

3.3. Effects of larval food on developmental timing

Fed larvae reached metamorphosis more quickly than unfed larvae (Fig. 2.3). Planktonic duration differed between years but was about a week shorter for fed larvae than for larvae raised in FSW (2020 median 17 days vs 23.5 days; 2021 median 13 days vs 22 days; see Table 2.3).

Table 2.3. Summary of traits for larval and juvenile *Amphiodia* sp. opaque. Larvae were raised with and without microalgal food in 2020 and 2021 and collected as late-stage plutei in 2019. Planktonic duration is given for all larvae that completed metamorphosis. Planktonic duration and time to juvenile starvation are time data and are listed as minimum, median, and maximum. Percent metamorphosis and juvenile aboral surface area are given as means ± standard error for replicate bowls and for individuals, respectively.

Year	Treatment	Total larvae / # replicate bowls	Planktonic duration (days)	Juv. n	Percent meta-morphosis	Juv. aboral surface area (mm^2)	Time to starvation (days)
2019	Wild	80 / 7	-	46	-	0.032 ± 0.001	0–6–38
2020	Food	110 / 11	15–17–21	88	80 ± 3	0.046 ± 0.001	2–59–104
	No-food	110 / 11	20–23.5–30	16	14 ± 6	0.030 ± 0.001	0–48.5–76
2021	Food	120 / 7	13–13–19	54	45 ± 4	0.047 ± 0.001	2–23–65
	No-food	120 / 7	15–22–33	64	53 ± 3	0.032 ± 0.001	2–18.5–54

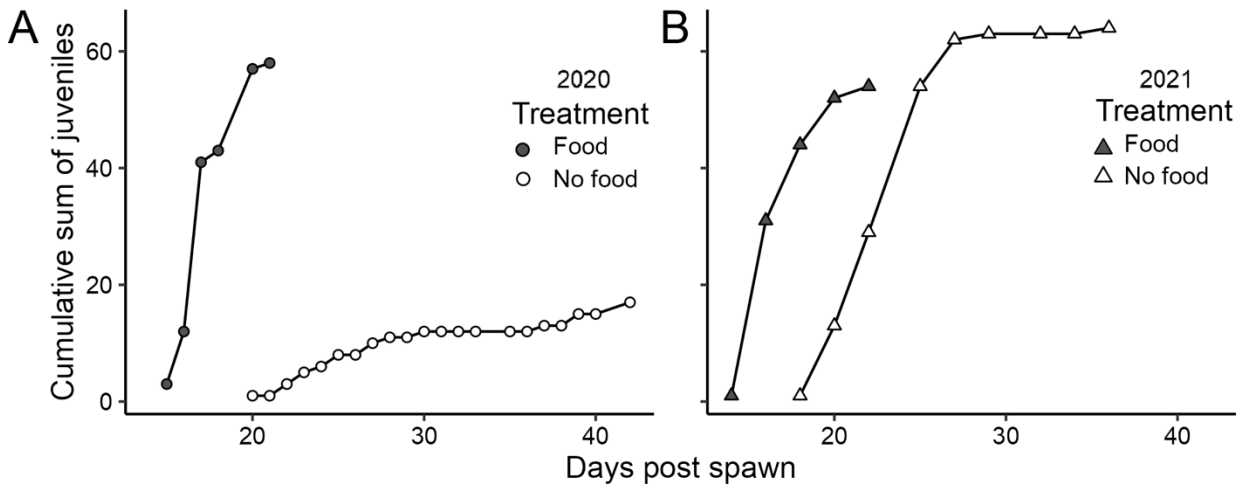


Figure 2.3. Cumulative sum of juveniles over time by treatment and year: (A) 2020, (B) 2021. Each year had the same initial number of larvae in each treatment (2020 n=110 per treatment, 2021 n=120). Final time points represent date of discovery of the last juvenile in that treatment.

3.4. Effects of larval food on percent metamorphosis

The effect of treatment on the percent of larvae that completed metamorphosis differed significantly in 2020 but not in 2021 (across all treatments and years: Kruskal-Wallis test: χ^2 = 29.25, df = 3, $p < 0.001$; Fig. 2.4). Pairwise comparisons show a significant difference between treatments in 2020 (Dunn's test: adj. $p < 0.001$, Table S2.1), when food had a strong effect: 80% of fed larvae created a juvenile whereas only 15% of unfed larvae were able to do so (Fig. 2.4). By contrast, in 2021 slightly more juveniles resulted from cultures without larval food (food: 45%, no-food: 53%), and percent metamorphosis was not statistically different between treatments (p = 0.44).

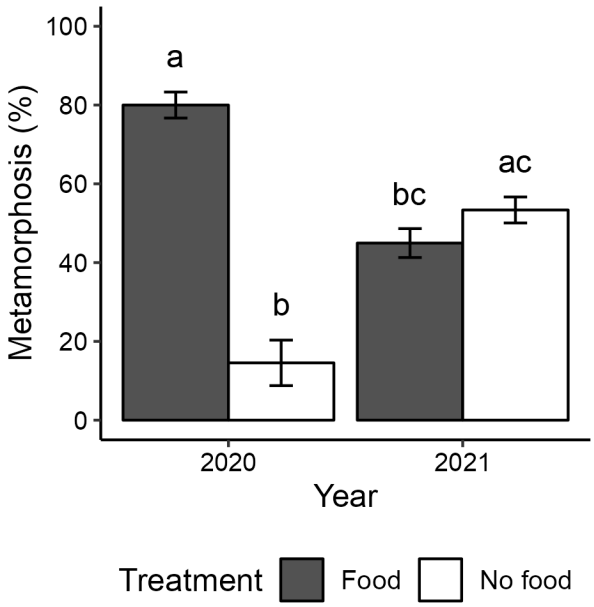


Figure 2.4. Bar plots of percent metamorphosis (= # juveniles / initial # larvae) averaged across experimental bowls for years 2020 (initial larvae n=220/22 bowls) and 2021 (n=240/14 bowls). Error bars represent standard error (SE). Lowercase letters above bars represent significant differences in pairwise comparisons.

3.5. Effects of larval food on juvenile size

Juvenile sizes measured as aboral surface area were significantly different between treatments in both years (Kruskal-Wallis test: $\chi^2 = 26.149$, $df = 4$, $p < 0.0001$). Pairwise comparisons showed that juveniles from fed larvae were larger than juveniles from unfed larvae in both years. Juveniles from wild larvae were not significantly different in size from juveniles from the no-food treatment (Dunn's test, Table S2.2; Fig. 2.5).

We modeled the association between planktonic duration and juvenile aboral surface area (Fig. 2.6A) using a series of GLMs and found that the best fit model included only treatment as a covariate (AIC = -288.3, Table S2.3).

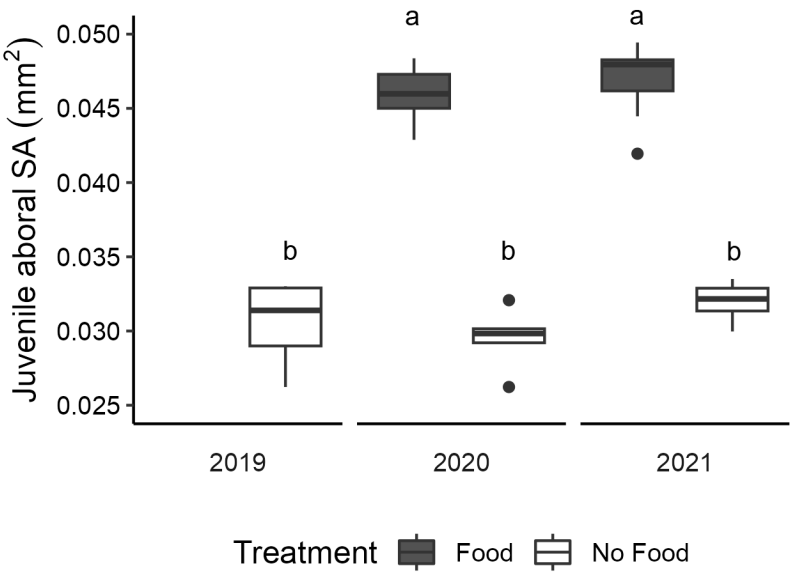


Figure 2.5. Boxplot of juvenile aboral surface area (SA) at metamorphosis by food treatment, pooled across years. Lowercase letters above boxplots represent significant differences in pairwise comparisons.

3.6. Effects of larval food on juvenile survival

The survival time in juveniles that received food as larvae was longer compared to those that did not receive food, but survival times varied between years (2020 median 59 days (n=88 vs 48.5 days, n=16; 2021 median 23 days, n=54 vs 18.5 days, n=64, Table 2.3). Juveniles that resulted from wild-caught larvae had relatively short survival times under starvation conditions (median 6 days, n=80).

Bigger juveniles tended to survive longer, and juveniles from fed larvae had greater surface areas than those from larvae that did not receive food (Fig. 2.6B). Juvenile aboral surface area was positively associated with days to juvenile starvation and the best-fit model included an

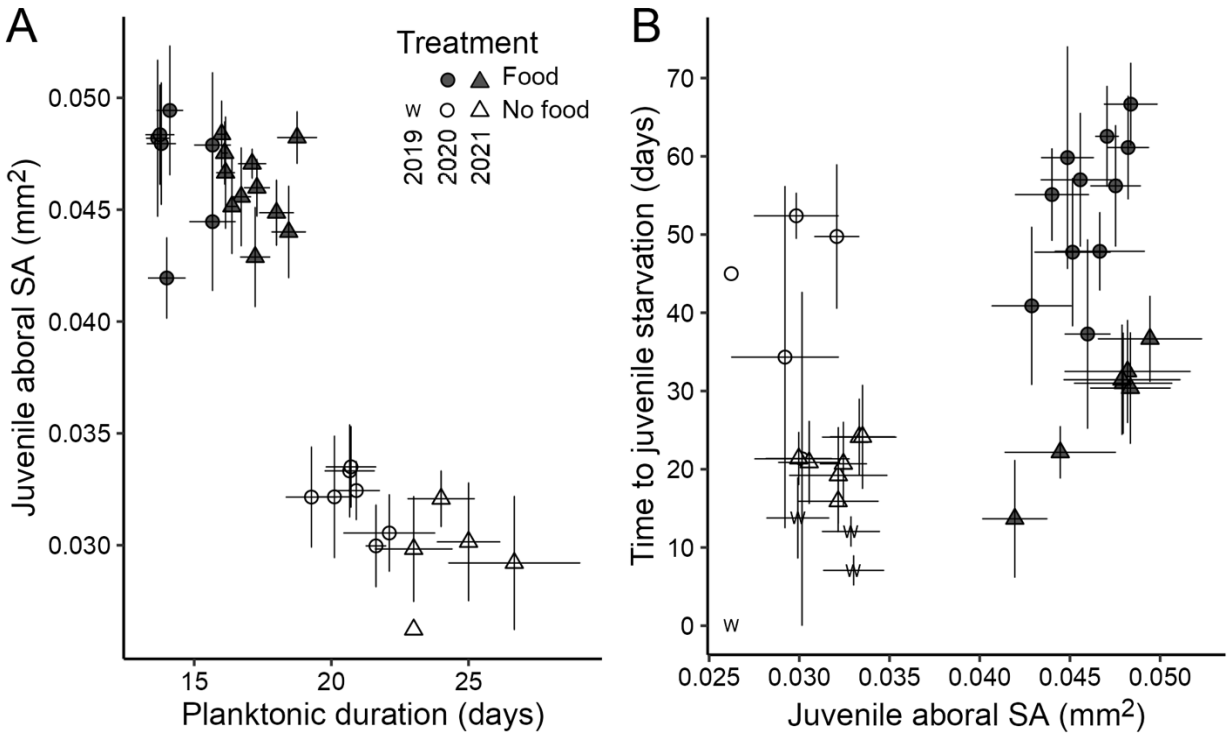


Figure 2.6. Scatterplots of (A) planktonic duration by juvenile size, and (B) juvenile aboral surface area (SA) by time to juvenile starvation. Points are mean values for replicate finger bowls (larval culture containers) and bars are standard error. Treatment is coded by color (food: dark gray, no-food: white, wild: 'w') and experimental year by shape (2020: circle, 2021: triangle, wild: 'w').

interaction between treatment and year (GLM, AIC 1672.1; Table S2.4). Treatment (food, no-food) and experimental year (2020, 2021) were both significant covariates ($p = 0.02$ and $p < 0.001$, respectively).

The survival curves for each treatment and year were significantly different from each other (log-rank test, $p < 0.0001$) and were significantly different in each pairwise comparison ($p < 0.0001$. Fig. 2.7).

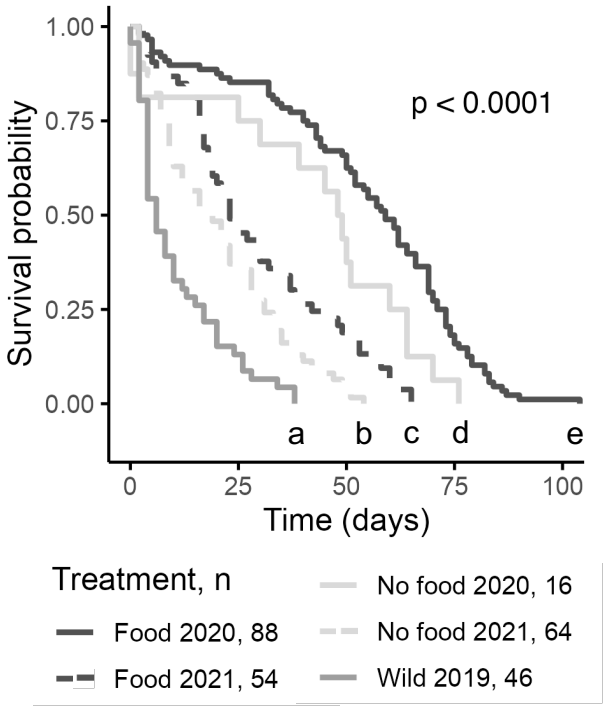


Figure 2.7. Kaplan-Meier survival curves for *Amphiodia* juveniles according to larval food treatment and year. No juveniles were censored as they were followed until the time of the event, death. Lowercase letters below survival curves indicate significantly different pairwise comparisons.

4. DISCUSSION

4.1. Species identity

Through DNA barcoding of the COI gene, we found a single species-level match (>98% pairwise nucleotide similarity) to our *Amphiodia* larvae in a juvenile specimen from Boundary Bay, WA (BBPS564-19, provided by G. Paulay), which was difficult to assign to species from its morphology. The specimen was small (2.5 mm disk diameter) and the adult characteristics necessary for identification may have been absent or reduced, as they often are in small

specimens (Stöhr, 2005). The specimen had three oral papillae of equal size and spacing, as is characteristic of *Amphiodia*. Its oral shields are pentagonal in shape, and the arm spines are tapered to a point like in *A. urtica*, but the radial shields are approximately 2 times as long as they are wide, the dorsal arm plates are oblong and the ventral arms plates are squarish like in *A. occidentalis* (Lambert & Austin, 2007). Furthermore, molecular data for *COI* delineates this specimen from adults of *A. occidentalis* or *A. urtica* (Fig. 2). Rather than signifying a new species, the lack of a molecular match with morphological identification may be due to the limited genetic sampling of the genus *Amphiodia* (8 of 34 species, N. Nakata unpubl. data) and of amphiurids of the northeast Pacific (5 of 12 species, N. Nakata unpubl. data).

4.2. Larval morphology and developmental mode

Using feeding assays, we confirmed that *Amphiodia* sp. opaque is a facultative planktotroph. *Amphiodia* sp. opaque has eggs of moderate size (140 μm), which is consistent with other ophiuroids with abbreviated development (Hendler, 1991). Larvae of this species developed into juveniles in the absence of microalgal food but were still capable of planktonic feeding. Percent metamorphosis varied across treatments and study years, but 15 to 53% of larvae given no microalgal food successfully completed metamorphosis, supporting our diagnosis of facultative planktotrophy.

This developmental mode cannot be diagnosed from larval morphology alone because larvae retain the structures necessary for feeding. Larvae must be cultured in the presence and absence of microalgal food to determine developmental mode. We did suspect facultative planktotrophy of this larva because it was opaque orange in color and has a reduced pluteus

morphology (Hendler, 1975), as determined by comparison with a sympatric congener *A. urtica*, which has a transparent, planktotrophic larva with 8-arms. The posterolateral arms are reduced in length relative to those of *A. urtica*, and the posterodorsal arms are highly reduced or absent, resulting in 6 larval arms instead of 8. Larvae of *Amphiodia* sp. opaque have other features that are associated with evolutionary transitions in developmental pattern, including an egg of intermediate size (ca.140 μm) and intermediate planktonic duration.

Facultative planktotrophy has been observed in six other marine invertebrates (Table 2.1). *Amphiodia* sp. opaque is the second facultative planktotroph to be described from the Ophiuroidea and the first from the family Amphiuridae. Only one ophiuroid was previously known to have this developmental pattern: *Macrophiothrix rhabdota* (Ophiotrichidae) develops via an eight-arm pluteus. The family Ophiotrichidae diverged from Amphiuridae approximately 200 Mya (O'Hara et al., 2017), meaning *Amphiodia* sp. opaque represents an independent evolution of the facultative planktotroph phenotype. Food-limited growth is common for larvae of benthic invertebrates (Paulay et al., 1985), and facultative planktotrophy may have evolved as a bet-hedging strategy to increase the number of resulting juveniles in regions or spawning times when planktonic food availability is low.

4.3. Development time

Fed larvae developed more quickly than larvae that did not receive food. Larvae in the food treatment completed metamorphosis in fewer days than their starved peers (Fig. 2.2). This is consistent with echinoids with feeding larvae, which are known to take longer to form their

rudiment in no- and low-food conditions (Miner et al., 2005; Sewell et al., 2004; Strathmann et al., 1992).

Compared to congenerics of contrasting development modes, *Amphiodia* sp. opaque has an intermediate planktonic duration (medians of 13 to 17 days when fed). The planktotroph *Amphiodia urtica* has a median planktonic duration of 20 days (n=10 larvae collected at different times, N. Nakata, unpublished data) and a congeneric, pelagic, direct-developer may be planktonic for 8 days at 15°C (Emlet, 2006).

The availability of food for larvae does not always decrease time to metamorphosis in facultative planktotrophs, For example, there was no difference between fed and unfed treatments in the echinoid *Clypeaster rosaceus* or the gastropods *Conus pennaceus* and *Phestilla sibogae* (Emlet, 1986; Kempf & Hadfield, 1985; Perron, 1981). In the ophiuroid *Macrophiothtrix rhabdota* planktonic duration was decreased in the presence of food (Allen & Podolsky, 2007). Shorter planktonic interval may limit mortality from predation in the plankton (Rumrill, 1990) and may reduce capacity for dispersal in species with shorter developmental times (Hendler, 1991; Shanks, 2009).

We interpret larval developmental times as dependent on the accumulation of energetic reserves necessary for metamorphosis and juvenile life, but other factors may have contributed to planktonic durations observed in this study. Little is known about cues for competency or settlement in ophiuroids (Hendler, 1991; Hodin et al., 2015). Furthermore, ophiuroids are known to be capable of undergoing metamorphosis in the plankton and continuing to ride ocean currents as juveniles (Hendler et al., 1999), indicating that some species may not require benthic cues to begin metamorphosis once sufficient food has been consumed in the plankton.

4.4. Percent metamorphosis

The effect of larval feeding on the proportion of larvae that completed metamorphosis was different across years for *Amphiodia* sp. opaque. In 2020, food had a strong effect on the proportion of larvae able to complete metamorphosis, as was true for the other facultatively planktotrophic ophiuroid (Allen & Podolsky, 2007). In 2021, percent metamorphosis was not significantly different between treatments and slightly more juveniles resulted from the no-food treatment (Figs. 2.2, 2.3). Amongst facultative planktotrophs, larval feeding did not affect percent metamorphosis (referred to as larval survival) in an echinoid (Emlet, 1986) and two gastropods (Kempf & Todd, 1989; Perron, 1981).

It is possible that the observed variation between cohorts resulted from differences in culture conditions between years, or from intrapopulation or interannual variation in parental investment and developmental regimes. As all embryos originated from the plankton, we are unable to determine if differences between cohorts reflect variation in maternal provisioning due to nutrition or genetic variation between populations.

4.5. Juvenile size

We observed that fed larvae of *Amphiodia* sp. opaque produced juveniles that were ca. 50% larger in surface area than those of starved larvae (Table 2.3; Fig. 2.5). Increased juvenile size as a consequence of larval feeding has been observed in other facultative planktotrophs

(Allen & Podolsky, 2007; Emlet, 1986; Hart, 1996; Kempf & Hadfield, 1985; Miller, 1993). Juveniles of *Amphiodia* sp. opaque from the fed treatment were slightly smaller than those of a sympatric congener with feeding larvae, *A. urtica* (juvenile aboral surface area 0.047 mm^2, n = 18; N. Nakata unpubl. data).

Advanced larvae collected from wild plankton (2019) produced juveniles that were not significantly different in size from those from the experimental no-food treatment. This suggests that these larvae had limited opportunity to feed in their natural environment (and they were not fed in the lab). Even low-food conditions can lead to smaller juveniles in a calyptraeid gastropod (Chiu et al., 2007, 2008). Evidence from a barnacle has shown that even food deprivation during a portion of the larval life can lead to smaller juveniles with the early stages being the most important (Emlet & Sadro, 2006).

4.6. Juvenile survival

Juveniles of fed larvae lived longer than those from larvae raised without food, suggesting that they gained greater energetic reserves due to larval feeding. However, juvenile survival times differed among treatments and experimental years (Fig. 2.7). Juveniles from both treatments in 2021 had shorter survival times than juveniles from either treatment in 2020. Interestingly, the juveniles that resulted from wild-caught larvae from 2019 fared the poorest of all. They were no different in size from juveniles of lab-reared larvae that received no food (Fig. 2.5), but they died from starvation more quickly (Fig 2.6B, 2.7). This may have been the result of variation in egg composition or size, but whether that was due to intraspecific genetic variation,

local environmental deficiencies that lowered maternal condition, or some combination was not determined.

5. CONCLUSION

In this study, we tested the influence of larval feeding in an unidentified facultatively planktotrophic larva, *Amphiodia* sp. opaque. We know this animal only in its larval form, the adults have not been collected. We utilized phylogenetic analysis in an attempt to determine species identity and to compare it with closely related ophiuroids from the NE Pacific. Nevertheless, we were able to conduct a series of experiments that showed clear benefits of larval feeding across multiple life history characters: larvae that received food developed more quickly, experienced higher rates of metamorphosis, and produced larger juveniles that evaded starvation conditions for longer than those of larvae that received no food.

BRIDGE

In Chapter II, I described a facultative planktotroph from the ophiuroids of the northeast Pacific that can feed but can also complete metamorphosis without food. The larva took the form of a reduced pluteus, a relatively rare larval form amongst the brittle stars that may represent an intermediate larval form between obligately feeding ophioplutei and nonfeeding larvae. In the next chapter I built upon Chapter II's results by describing the early life stages of brittle stars from Oregon, which included several more reduced plutei.

CHAPTER III

BRITTLE STAR LARVAE OF THE NORTHEAST PACIFIC

This work includes coauthor Dr. Richard Emlet as principal investigator and contributor to the final manuscript preparation. It is written in the journal style of *Invertebrate Biology*.

1. INTRODUCTION

With ca. 2100 described species, the brittle stars (Ophiuroidea) are the most diverse class of echinoderms and dominate benthic environments across all ocean depths (O'Hara et al., 2019b; Stöhr et al., 2012). Ophiuroids have a wide variety of ecological roles, and when abundant are significant contributors to benthic communities. Brittle stars exhibit diverse reproductive patterns including planktonic feeding and nonfeeding larvae, brooding, and fissiparity. These variations in development are distributed across Ophiuroidea, and closely related species can have very different ontogenies (Allen & Podolsky, 2007; Hendler, 1991; O'Hara et al., 2019a).

Brittle stars have great potential for the study of life history evolution, but such evaluations are limited by the available data on phylogenetic relationships (9% of spp.) and data on developmental patterns (15% of spp., Nakata, unpublished). Of the brittle stars for which development is known (approx. 275 species), about half brood their young; the remainder have planktonic larvae.

We used DNA barcoding to identify brittle star larvae collected from plankton. Barcoding of all life stages can lead to higher estimates of regional species diversity (Maslakova

et al., 2022). Here, barcoding allowed us to identify or place within a phylogeny planktonic larvae for a group of organisms that has proven difficult to study in a laboratory setting. Unlike echinoids, ophiuroids do not have reliable means for inducing spawning in the laboratory so raising larvae of known species is not readily accomplished.

Brittle star larvae are diverse in form but are generally divided into two categories: planktotrophs, with an obligately feeding and slow-growing ophiopluteus that comes from small eggs produced in large quantities; and abbreviated developers, who develop from moderate numbers of yolky eggs and can take a variety of planktonic forms, including reduced plutei, vitellaria, and pelagic direct developers (Hendler, 1991). We used morphological and developmental characters (naming scheme after Mortensen, 1921) to describe ophiuroid larvae identified by DNA barcoding.

The characteristic feeding larva of ophiuroids is the ophiopluteus, a bilaterally symmetrical larva that usually has eight arms bearing a ciliated band and supported by calcite rods (Fig 3.1A). The longest and most prominent pair of arms are the posterolateral arms. The anterolateral arms extend anteriorly and support the mouth and adjacent ciliary band. The postoral arms extend ventrally to hold up a ciliary band in below of the mouth. Finally, the posterodorsal arms originate from the posterior portion of the anterolateral arm rods and project dorsally. In many species the postoral and posterodorsal arms are mirror imaged across the frontal plane, making it difficult to see both pairs at once. The larval arms are supported by skeletal rods composed of calcite, and whose shape, proportions, and adornments can be used to distinguish between species (Mortensen, 1921). Though branching into multiple rods to give the distinctive shape of the larval arms, there are only two major calcite spicules arranged in mirror

image (across the sagittal plane) in the ophiopluteus. A third larval spicule is reported in rare cases, see (Mortensen, 1921). Each spicule has rods that support 4 arms on its side of the larva. The posterolateral, anterolateral, and postoral rods join at two posterior junctions on either side of the stomach, and are connected to the body rods, which extend posteriorly and support the bottom half of the larva. Posterior to the stomach, the body rods each bear dorsal and ventral transverse rods, which extend to the larval midline and contact along the larval midline with their counterparts from the opposite spicules. Some ophioplutei have pairs of recurrent rods that connect the postoral and ventral transverse rods on the ventral side, and the anterolateral and dorsal transverse rods on the dorsal side of the larva. Posterior to the transverse rods, the body terminates in end rods. The transverse rods may bear one more median process(es). We refer to the collection of calcite rods at the posterior of the larva as the posterior girdle (Fig. 3.1A)

Ophiuroids with abbreviated development usually develop to juvenile more quickly than species with feeding plutei and usually have small post-larvae produced from moderate numbers of yolky eggs. Abbreviated developers are a developmentally heterogenous group taking the form of reduced plutei, vitellaria, demersal larvae, or modified larvae that undergo metamorphosis in an attached fertilization envelope (Hendler, 1975). We have also observed several pelagic direct developers (Emlet, 2006). Reduced plutei have a modified or reduced morphology, and may have two, four or six arms and opaque coloration (Allen & Podolsky, 2007; Fenaux, 1963; Mladenov, 1979). Reduced plutei are often lecithotrophic, and lack digestive structures, but some may be facultative planktotrophs (Allen & Podolsky, 2007; Nakata & Emlet, 2023).

The typical nonfeeding larva of ophiuroids is the vitellaria (Fig. 3.1C), though some authors include doliolaria (McEdward & Miner, 2001). We distinguish doliolaria as being barrel-shaped and having a series of transverse ciliary rings, while vitellaria are dorsoventrally compressed and may have complete ciliary rings at the anterior and posterior, but the mediary ciliary bands are disjunct (Fig. 2.1 C). The vitellaria lacks a mouth, stomach, and anus as it does not feed. Vitellaria larvae have been observed across many ophiuroid families (Hendler, 1991), and are believed to be derived from an ophiopluteus, which is supported by the presence of bilaterally paired larval spicules in some vitellaria (Hendler, 1982, 1991; Mortensen, 1921; Selvakumaraswamy & Byrne, 2023). Furthermore, in one species a feeding ophiopluteus transforms into a vitellaria prior to metamorphosis (Mladenov, 1985b).

In ophiuroids there are several variations of direct development, in which the embryo develops into a pentaradial juvenile without first developing a larval body such as an ophiopluteus or vitellaria. The most common form of direct development in brittle stars is brooding, in which embryos and young juveniles develop in the disc of the adult until they crawl away as advanced juveniles. Another, rarer type is pelagic direct development, in which embryos and early juvenile stages are planktonic (Emlet, 2006; Hendler, 1973; Patent, 1970).

In this study we describe the planktonic forms for eighteen species of ophiuroids found in the northeastern Pacific. Our primary sampling location was Coos Bay, Oregon, but we have also observed several of the same larvae described here in the San Juan Islands, Washington. As many as 66 species of ophiuroids are known to occur from California to Alaska (Astrahantseff & Alton, 1965; Hendler, 1996; Kyte, 1969; Lambert & Austin, 2007), of which 25 have records from Oregon. Of those, we observed 11 species, nine of which occur throughout the range. Many

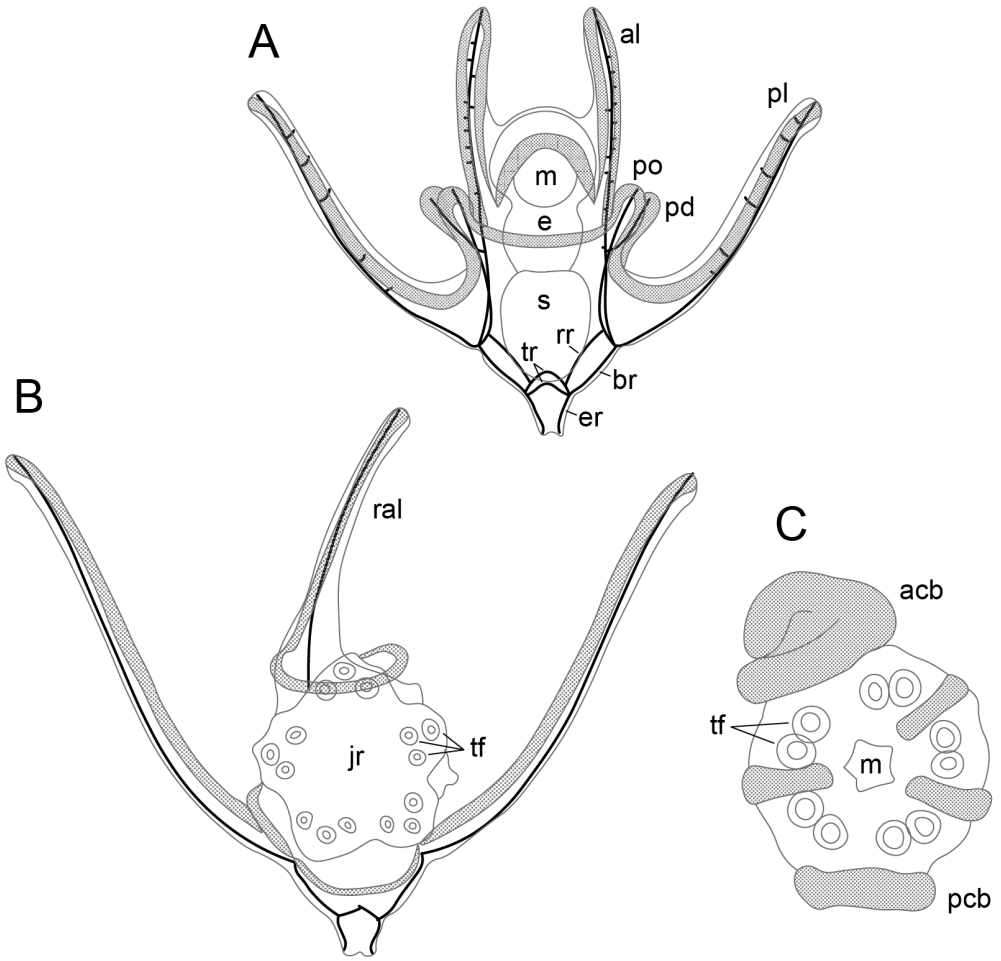


Figure 3.1. Developmental stages of the Ophiuroidea. (A) Eight-armed ophiopluteus, oral view, with posterolateral (pl), anterolateral (al), postoral (po), and posterodorsal (pd) arm pairs, which support ciliary bands (stippled regions) and mirror each other across the midline. These arms are supported by skeletal rods (solid black lines). The pl, al, and po arm rods on each side grow from a single calcite spicule that extends posteriorly as a body rod (br) and in some species a recurrent rod (rr), terminating as an end rod (er). The left and right spicules (with their arm rods) are in contact at the posterior end where short transverse rods (tr) meet along the midline. The feeding larva has a mouth (m), esophagus (e), and stomach (s), intestine, and anus. (B) Rudiment-stage pluteus, oral view with right and left posterolateral arms and the right anterolateral arm (ral). The larva has a pentagonal juvenile rudiment (jr) with buds for tube feet (tf). (C) The nonfeeding vitellaria, oral view, has several disjunct ciliary bands and complete bands at the anterior (acb) and posterior (pcb).

of the species we identified from the plankton occur nearshore. We observed three species that are not listed to occur in Oregon: *Amphiura arcystata, Ophiothrix spiculata,* and *Ophiocten*

hastatum. The species that are listed to occur in the region but that we didn't observe in the plankton have subtidal depth distributions from 50 to 3000 m. Two notable species absent from this study are *Amphipholis squamata* and *Amphiodia occidentalis*. The former is a brooder, which we find intertidally at Cape Arago, and the latter is a well-known species from the west coast of North America that is part of a species complex (see Section 3.4.3.6). Finally, four additional larvae lack molecular matches to adults.

2. METHODS

2.1. Collection of larvae and adults

We collected embryos, larvae, and juveniles of local ophiuroids from plankton tows in years 2018-2021. We collected samples in these years with 130 μm mesh net in the Coos Bay estuary (Oregon) approximately 3 km from the entrance to the Pacific Ocean (Charleston Marina: 43°21.2'N, 124°20'W). We collected plankton nearly daily, especially in the winter months, approximately one hour before high tide and examined our catch within two hours using a stereomicroscope. As many planktonic stages of brittle stars are negatively buoyant and poor swimmers, we most often found our quarry at the bottom of a given plankton sample. All brittle star material derived from the plankton was sorted into phenotypes and select individuals were preserved and analyzed using DNA barcoding. To determine species identities of planktonic life stages we compared our sequences with those available from public databases. However, we found that many of our local brittle star taxa were absent from such databases, and thus we had

to collect our own adults. Ultimately, we barcoded 102 embryos, larvae, and juveniles, and 53 adult brittle stars.

When possible, larvae were kept alive and observed through development into a juvenile. We maintained larvae in glass finger bowls at 15°C in an incubator. Feeding ophioplutei were given a tripartite microalgal diet composed of *Rhodomonas lens, Dunaliella tertiolecta,* and *Isochrysis galbana* at a combined concentration of 5,000 cells ml^{-1}. Nonfeeding larvae were kept in filtered sea water (FSW) that was changed every 2 to 3 days. Small flakes of cetyl alcohol were added to all cultures to prevent larvae from perishing in the air-water interface. Embryos, larvae, and juveniles were photographed across development using a Zeiss Universal (Carl Zeiss, Inc., Thornwood, NY) coupled with a digital camera (Flir Grasshopper Express GX-FW-28S5C-C) and software (Astro IIDC) to capture images.

For the purposes of describing the various developmental stages, we have divided development into the following stages: the early pluteus is from the first sign of bilateral symmetry, usually accompanied by paired calcite spicules in the posterior of the larva, up through the four- and six-armed stages (i.e., posterodorsal arms are absent). Reduced plutei also fit this description but can be distinguished from planktotrophic plutei by their opaque pigmentation and that they do not ever develop the eighth pair of (posterodorsal) arms. Late plutei are recognized by their eight arms (or six in reduced plutei) and visible coeloms, especially the left coelom, which develops five lobes that are the precursor to the water vascular system. We consider larvae as ‘rudiment-stage’ when the left coelom has wrapped around the larval esophagus, a pentaradial juvenile (or parts of its skeleton) are apparent, and one or more of the larval arms has been resorbed. Finally, juveniles have pentaradial symmetry, are free of any

remnant of the larval body such as skeletal rods or ciliated bands, and locomote via their tube feet.

We also reviewed records of daily samples taken at high tide from January to March during each of years 2014, 2015 and 2016 with a diaphragm pump (Emlet, Shanks, and Sutherland, unpublished data; Shanks et al., 2020). In 2014 this sampling coincided with the warm water blob (Bond et al., 2015) that resulted in northward shifts in species ranges from central and northern California to Oregon and beyond (Sanford et al., 2019).

We conducted all data validation and plot visualization in the R environment v4.2.2 using the packages 'ggplot2' and 'ggtree' (R Core Team, 2022; Wickham, 2016).

2.2. Molecular identification of embryos and larvae

To verify species identity of planktonic early life stages, we compared our sequences with those available for ophiuroids of the northeast Pacific from public databases, such as GenBank and BOLD (Benson et al., 2017; Ratnasingham & Hebert, 2007) as well as those derived from adults we collected locally. Adult material originated from several sources, including collection by hand in the intertidal and dredging soft-bottomed subtidal habitats. Adults were relaxed in a 50/50 solution of magnesium sulfate or magnesium chloride and FSW for morphological examination, relying on regional guides (Hendler, 1996; Lambert & Austin, 2007). A few podia or an arm tip were removed with fine forceps for DNA extraction.

We barcoded select individuals of each larval morphotype and compared sequences to those for adult specimens. Larvae were usually frozen individually in a small amount of seawater at -20°C, until DNA extraction and PCR. We extracted genomic DNA with the Chelex-based

InstaGene™ Matrix (Bio-Rad) after rinsing each larva in nuclease-free water. We amplified fragments of *cytochrome c oxidase subunit I* (*COI*) and *16S* rDNA using a variety of primers and thermocycler conditions (Table 3.1). PCR amplification reactions were performed in a 20 µL total reaction volume that included 11.4 µL nuclease-free water, 4 µL 5X Green Buffer, 0.4 µL dNTP 10 mM, 0.2 µL GoTaq Polymerase (Promega), and 1 µL each of forward and reverse 10 µM primers. PCR conditions were as follows: initial step 95°C for 2 min, followed by 34 cycles of denaturation at 95°C for 40 sec, annealing at 45 or 48°C for 40 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 2 min. PCR products were cleaned up using the Wizard SV Gel and PCR Clean up System (Promega) prior to Sanger sequencing (Sequetech, Mountain View, CA). Barcode sequences were compared to GenBank (www.ncbi.nlm.nih.gov/genbank/), BOLD (Ratnasingham & Hebert, 2007), and our unpublished dataset of ophiuroid sequences using the BLAST function in Geneious Prime (https://www.geneious.com). We aligned sequences using the Geneious MAFFT plug-in

Table 3.1. PCR primers and reaction conditions for molecular identification of ophiuroid larvae.

Locus	Primer Name	Primer Sequence	Reference
COI	jgLCO1490	TITCIACIAAYCAYAARGAYATTGG	(Geller et al., 2013)
	jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA	
	EchinoF1	TTTCAACTAATCATAAGGACATTGG	(Ward et al., 2008)
	EchinoR1	CTTCAGGGTGTCCAAAAAATCA	
	COIcef	ACTGCCCACGCCCTAGTAATGATATTTTTTATGGTNATGCC	(Hoareau & Boissin, 2010)
	COIcer	TCGTGTGTCTACGTCCATTCCTACTGTRAACATRTG	
16S	16SARL	CGCCTGTTTATCAAAAACAT	(Palumbi, 1996)
	16SBRH	CCGGTCTGAACTCAGATCACGT	
	16Sar	GCCTGTTTACCAAAAACAWCG	(Kirby & Lindley, 2005)
	16Sbr	GATCCAACATCTAGGTCGC	

(Katoh & Standley, 2013) created family- or superfamily-level maximum likelihood trees using the PhyML Geneious plug-in or online execution (Guindon et al., 2010), with 100 bootstraps and based on the HKY85 model of sequence evolution. Family names are after O'Hara et al. (2017).

For species delimitation, we used the online execution for Assemble Species by Automatic Partitioning (ASAP) to determine the barcoding gap for *COI* of ophiuroid species (Puillandre et al., 2021).

3. RESULTS and DISCUSSION

3.1. Taxonomic Diversity

We collected, raised larvae, and generated barcodes for 18 species from seven families of ophiuroids (Table 3.2). The best represented families were Amphiuridae with eight species, and Ophiuridae with four species. We determined species identity of larvae by creating phylogenetic trees of our larval sequences amongst barcodes for adult specimens or available from public databases. We considered sequence pairs to be molecular matches if they had less than an 8.5% difference in pairwise identity at the *COI* locus, a threshold we determined using ASAP analysis. See each section for the tree and specimen information therein.

3.2. Developmental Diversity

We observed embryos of only a few species, indicating that their natal populations may be close to shore: *Amphiodia periercta?*, *Amphiodia* sp. opaque, *Amphiodia* sp. orange belly,

Table 3.2. Ophiuroid species of southern Oregon with planktonic development. Species marked with an asterisk (*) are not regularly found in the waters near Coos Bay, OR. Larval forms are planktotrophic (=feeding) ophiopluteus (P), reduced pluteus (RP), pelagic direct (D), and indirect development via a vitellaria (V).

Taxonomy	Larva	Spawning season	Planktonic duration (days)
Ophiotrichidae			
*Ophiothrix spiculata**	P	December	?
Ophiopholidae			
Ophiopholis kennerlyi	P	Sept-Nov; Mar-Apr	83-216
Ophiopholis bakeri	P	Oct-Jan	?
Amphiuridae			
Amphiodia urtica	P	Oct-May	20
Amphipholis pugetana	P	Oct-Mar	?
Amphiodia sp. opaque	RP	Aug-May, especially Jan-Mar	13-33
Amphiodia sp. orange belly	RP	Jan-Mar	8 (n=1)
Amphiodia sp. tan*	RP	Sept	?
Amphiodia periercta?	D	Oct-Mar	8
Amphioplus sp. vitellaria	V	Nov, Jan	~7
Amphiura arcystata	D	Nov-Feb	6 (n=1)
Ophiacanthidae			
Ophiacantha diplasia	P	Jan-Apr	82 (n=1)
Ophiopteridae			
Ophiopteris papillosa	P, V	Nov-Apr	65 (n=1)
Ophiuridae			
*Ophiocten hastatum**	P	Jan	?
*Ophiura leptoctenia**	P	May	?
*Ophiura sarsii**	P	Mar-Jun[1]	?
Ophiura luetkenii	RP	Nov, Feb-Apr	5
Gorgonocephalidae			
Gorgonocephalus eucnemis	D	Jan-Mar	5

Amphiura arcystata, *Gorgonocephalus eucnemis*, and *Ophiura luetkenii*. The fertilized eggs and developing embryos of ophiuroids often have a thick hyaline layer coating the egg, enclosing the cleavage and embryonic stages, and surrounding the blastula and gastrula (Emlet, 2006; Olsen,

1942; Yamashita, 1984). Embryos of brittle stars could be reliably identified by their thick hyaline layers, which were even visible between blastomeres in the two- to eight-celled stages, especially for *A.* sp. opaque and *A. periercta?* (Sections 3.4.3.1 and 3.4.3.3).

We observed feeding larvae and different forms of nonfeeding developmental stages in the plankton of southern Oregon. We follow Hendler's (1975) classification of ophiuroid development into three functional categories: planktotrophy with a feeding ophiopluteus; abbreviated development via a reduced pluteus, vitellaria, or pelagic direct developer; and brooding. We observed ten species with a planktotrophic ophiopluteus. We observed abbreviated development in the form of four reduced plutei, one vitellaria, and three species with pelagic direct development (Table 3.2).

The ophiopluteus was the most common larval form, present in fourteen species (Table 3.2, Fig. 3.2). Ten of these ophioplutei are planktotrophic, whereas the remaining four were reduced plutei (see below). The planktotrophic plutei we observed were spread across six families. Ophiotrichidae (1 species), Ophiopholidae (2 species), Ophiopteridae (1 species) and Ophiacanthidae (1 species) contained only feeding plutei. Reduced plutei occurred in Amphiuridae (2 species), and Ophiuridae (1 species). The opacity and color of the larval tissues was correlated with development mode. Planktotrophic plutei generally had transparent larval tissues, while abbreviated forms were translucent or opaque in varying shades of tan to pink. We found the shape and proportions of the larval skeletal rods to be the definitive way to distinguish between plutei of different species (Mortensen, 1921), which could be viewed using cross-polarized light (Fig. 3.2).

Abbreviated modes of development were present in four of seven families in this study. Reduced plutei were observed in two families, Amphiuridae and Ophiuridae: *Amphiodia* sp. opaque, *Amphiodia* sp. orange belly, and *Amphiodia* sp. tan, and *Ophiura luetkenii*. Reduced plutei were recognized by their relatively short larval arms, particularly the posterolateral arms, well-developed left coelom, opaque coloration, and short development times. We obtained sufficient material to test for facultative planktotrophy in *A.* sp. opaque (Nakata & Emlet, 2023), and suspect the remaining reduced plutei to have this developmental mode as well.

Another notable form of abbreviated development is the vitellaria, of which we observed in two species: *Amphioplus* sp. vitellaria and as the secondary larval form (following the planktotrophic ophiopluteus) in *Ophiopteris papillosa*. The vitellaria may have evolved multiple times as a modification of the pluteus (Hendler, 1991; Mortensen, 1921). We describe the first vitellaria from the Amphiuridae and the second example of an ophiopluteus developing into a vitellaria prior to metamorphosis (Cisternas & Byrne, 2005).

3.3. Patterns in Spawning Phenology

We observed embryos, larvae, and juveniles of brittle stars over the course of the years 2014–2016 and 2018–2021. Plankton observations were made daily in the months of January, February, and March of years 2014 (79 days), 2015 (73 days), and 2016 (74 days). In years 2018–2021, sampling occurred almost daily in winter months and approximately weekly from

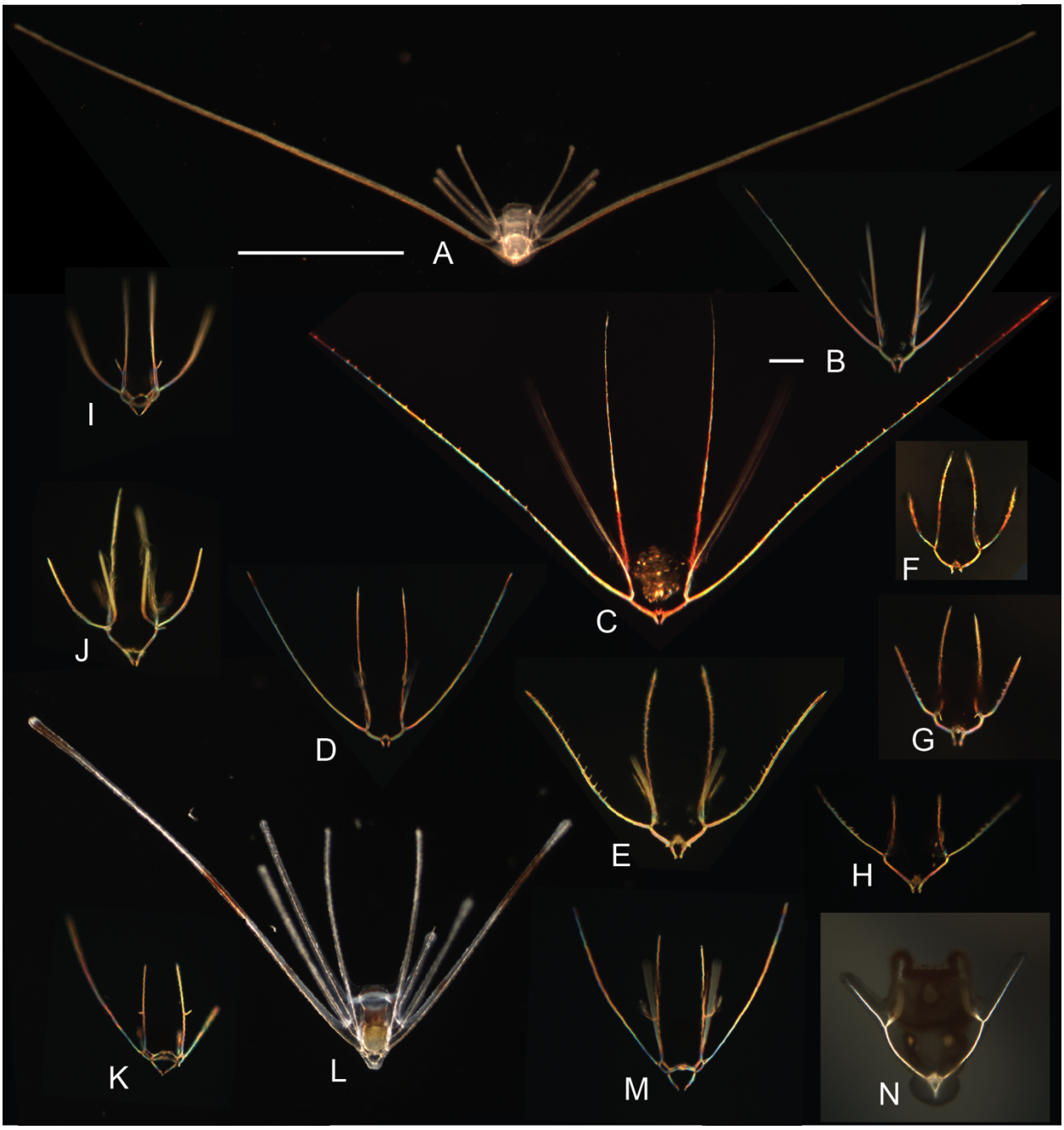


Figure 3.2. Ophioplutei of the northeast Pacific, viewed in dark field (A,L) and cross-polarized light (B–K,M,N). (A) *Ophiothrix spiculata*, scale bar 1 mm, (B) *Ophiopholis kennerlyi*, scale bar 100 µm, scale same for X-X, (C) another individual. (D) *Amphipholis pugetana,* (E) *Amphiodia urtica*, (F) *A.* sp. tan, (G) *A.* sp. orange belly, and (H) *A.* sp. opaque. (I) *Ophiacantha diplasia* and (J) *Ophiopteris papillosa*. (K) *Ophiura sarsii*, (L) *Ophiocten hastatum*, (M) *Ophiura leptoctenia*, and (N) *O. luetkenii*.

April to December. We examined plankton twice weekly during summer of 2018, but we limited our sampling to a few times a month from May to September during subsequent years due to the absence of ophiuroid larvae and high diatom abundance during those months. Because samples were collected by hand, we did not sample on days in which the high tide occurred between 8 pm and 7 am.

We observed strong seasonal patterns in presence of ophiuroid developmental stages. All but three species (*Amphiodia* sp. tan, *Ophiura leptoctenia, Ophiura sarsii,* each observed just once; see Fig. 3.4) were observed most frequently in the plankton during the fall and winter months (November to March). During ENSO years we observed rare larvae, perhaps because of altered current patterns. Spawning may have been suppressed in local populations by heat waves (Shanks et al., 2020).

We observed several patterns in our spawning records according to developmental mode. Nonfeeding larvae (*Amphiura arcystata*, *Amphioplus* sp. vitellaria, *Gorgonocephalus eucnemis*, *Ophiura luetkenii*) were observed in the winter months Jan-Mar. One exception is the pelagic direct developer, *Amphiodia periercta?* (*sensu* Emlet 2006), whose larvae were observed from October to March. Obligately and facultatively planktotrophic ophioplutei (*A.* sp. opaque, *A. urtica*, *A. pugetana*, and *Ophiopholis* spp.) occurred in the plankton from Oct to May in Coos Bay, Oregon.

Larvae of a few species were observed very rarely, and only in the El Niño years of 2015 and 2019: *Ophiothrix spiculata, Ophiura leptoctenia, Ophiura sarsii*, and *Ophiocten hastatum.* We interpret these rare occurrences as larvae advected away from their benthic adult populations during warm-water events.

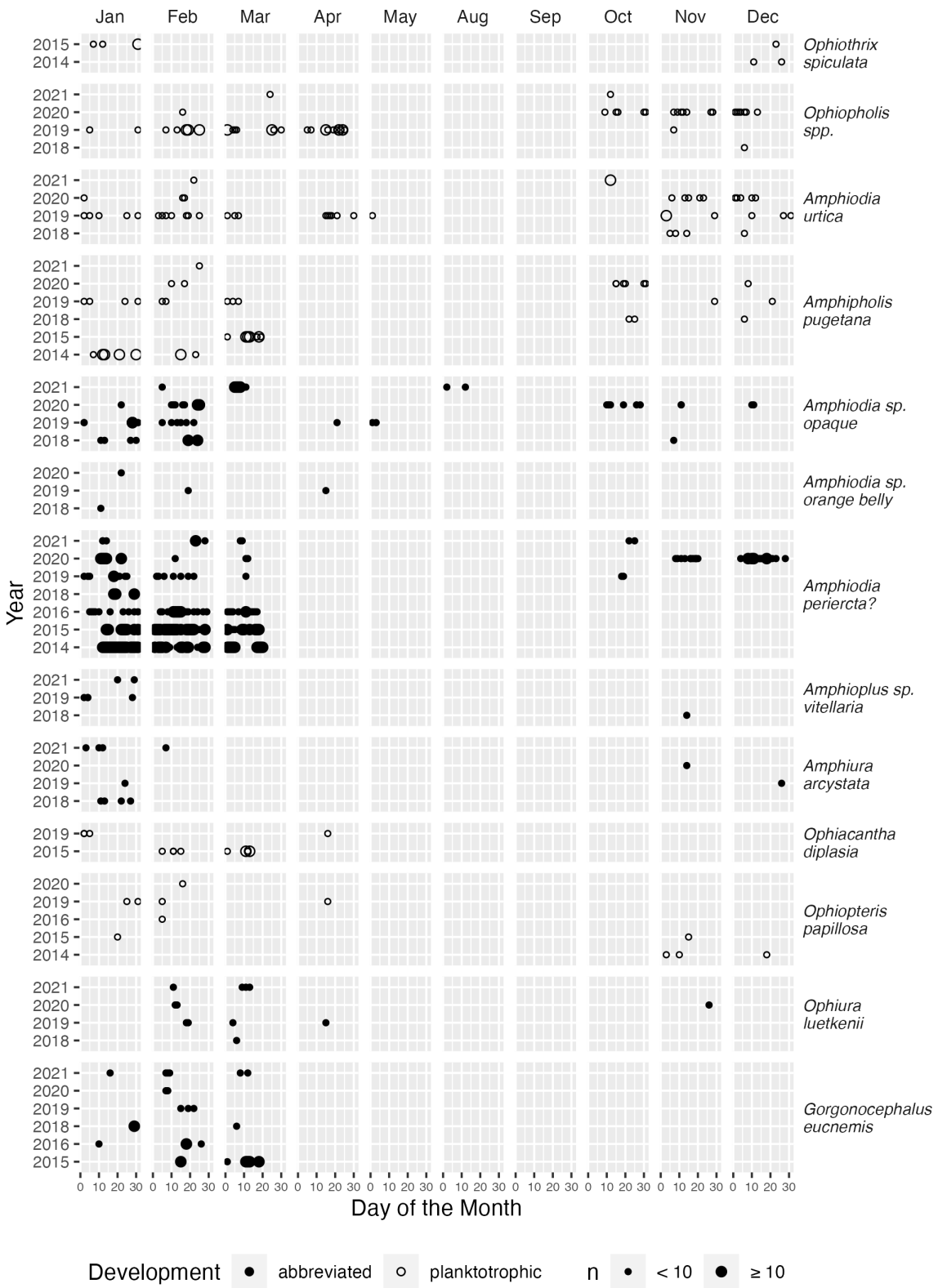

Jan
Feb
Mar
Apr
May
Aug
Sep
Oct
Nov
Dec
Ophiothrix spiculata
Ophiopholis spp.
Amphiodia urtica
Amphipholis pugetana
Amphiodia sp. opaque
Amphiodia sp. orange belly
Amphiodia periercta?
Amphioplus sp. vitellaria
Amphiura arcystata
Ophiacantha diplasia
Ophiopteris papillosa
Ophiura luetkenii
Gorgonocephalus eucnemis
Year
Day of the Month
Development
abbreviated
planktotrophic
n
< 10
≥ 10

Figure 3.3. Spawning phenology for 14 planktonic ophiuroid spp. of the southern Oregon coast. Days of the month are marked on the x-axis, with each gray box representing five days' time. The months of June, July, and September are omitted because no ophiuroid larvae were observed in plankton samples from those months. Occurrence data is represented by dots: filled for species with abbreviated development and open for species with planktotrophic development. Abundance of larvae (n) on each collection day is indicated by the size of the dot, with the smallest dot representing collections of larvae fewer than ten, and the largest representing collections of ten or more. Note that *Ophiopholis kennerlyi* and *O. bakeri* are combined in a single plot because the ophioplutei of these congeners are morphologically indistinguishable. Larvae with abbreviated development are marked by filled circles and those with planktotrophic development with empty circles.

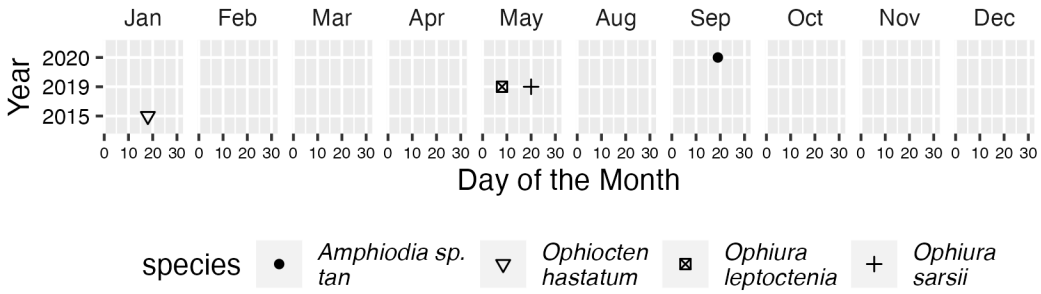


Figure 3.4. Spawning phenology for 4 additional ophiuroid spp. that were each observed on a single occasion. *Amphiodia* sp. tan has a reduced pluteus and *Ophiocten hastatum, Ophiura leptoctenia,* and *Ophiura sarsii* have planktotrophic ophioplutei.

3.4. Species accounts

3.4.1. Ophiotrichidae LJUNGMAN 1867

3.4.1.1. *Ophiothrix spiculata* LE CONTE 1851

Species Identity. DNA barcoding of a single larva and several juveniles revealed close matches to the species *Ophiothrix spiculata.* As sequences for *O. spiculata* were absent from databases at the time, R. Emlet collected adults off Catalina Island, CA, to which our larvae and juveniles were less than 4% different at the *COI* locus (Fig. 3.5, Table 3.3). Two distinct sequences attributed to *O. spiculata* from the Caribbean Sea (Bribiesca-Contreras et al., 2013)

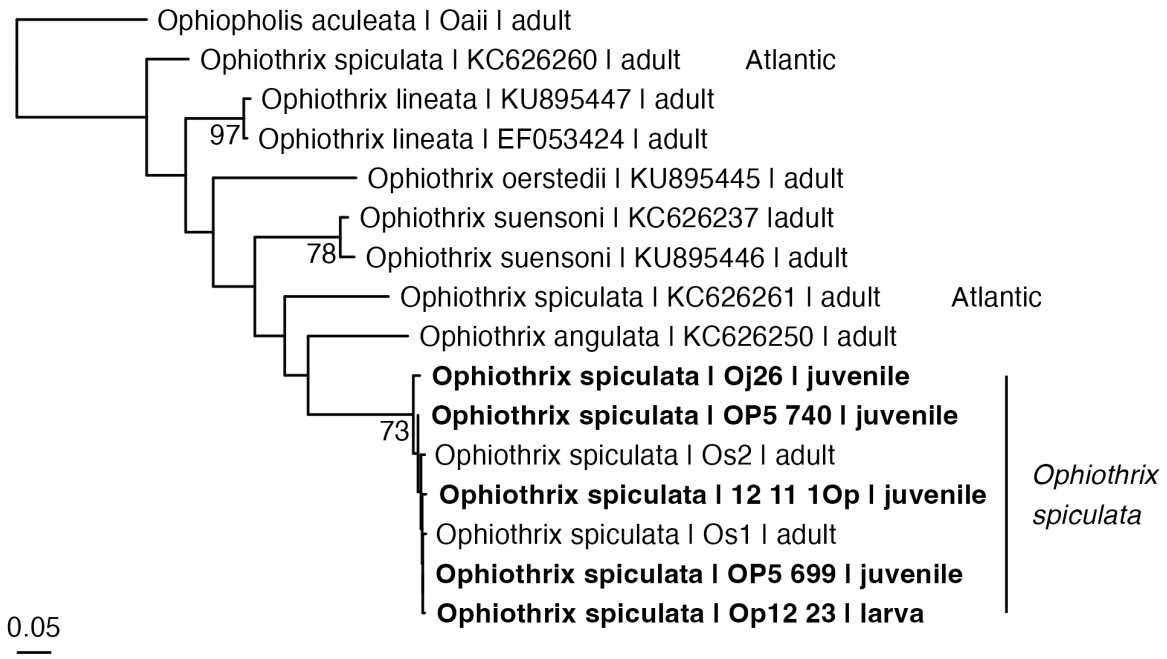


Figure 3.5. Maximum likelihood tree of *16S* and *COI* of *Ophiothrix* spp. from North America. Bootstrap support values >70 for major nodes are shown to the left of the node. Larvae identified in this study are in bold. Specimen information is listed in Table 3.3. Branch lengths are shown in scale.

Atlantic that did not form a clade with the Pacific *O. spiculata* (Fig. 3.5) and were over 18% different from our specimens at the *COI* locus. Based on these data and our species-level threshold of 8.5% determined by ASAP analysis, we treat Pacific and Atlantic *O. spiculata* as different species. Furthermore, the species was described from Pacific, which suggests that animals in the Pacific, while morphologically similar to those in the Atlantic, should be raised to species.

Distribution and Local Sites. *Ophiothrix spiculata* has not been listed as part of the benthic fauna of the southern Oregon coast and is known only from California to Chile. Its Specimen information for *Ophiothrix* spp. included in Figure 3.5. Additional information for specimens collected in this study can be accessed using the BOLD Project IDs below.

Table 3.3. Specimen information for *Ophiothrix* spp. included in Fig. 3.5. Additional information for specimens collected in this study can be accessed using the BOLD Project IDs below.

Species	Specimen Code	Locus	BOLD[B] or GenBank[G]	Collection Locality	Reference
Ophiothrix angulata	KC626250	*COI*	KC626250[G]	Cozumel Island, Mexico	(Bribiesca-Contreras et al., 2013)
Ophiothrix lineata	EF053424	*COI*	EF053424[G]	Florida	(Richards et al., 2007)
Ophiothrix lineata	KU895447	*COI*	KU895447[G]	USA	(Hugall et al., 2016)
Ophiothrix oerstedii	KU895445	*COI*	KU895445[G]	USA	(Hugall et al., 2016)
Ophiothrix spiculata	12-11-1Op	*16S, COI*	OLAB089-23[B]	Charleston, OR	This study
Ophiothrix spiculata	KC626260	*COI*	KC626260[G]	Cozumel Island, Mexico	(Bribiesca-Contreras et al., 2013)
Ophiothrix spiculata	KC626261	*COI*	KC626261[G]	Cozumel Island, Mexico	(Bribiesca-Contreras et al., 2013)
Ophiothrix spiculata	Oj26	*16S, COI*	OLAB090-23[B]	Charleston, OR	This study
Ophiothrix spiculata	Op12-23	*16S, COI*	OLAB091-23[B]	Charleston, OR	This study
Ophiothrix spiculata	OP5-699	*16S*	OLAB092-23[B]	Charleston, OR	This study
Ophiothrix spiculata	OP5-740	*16S*	OLAB093-23[B]	Charleston, OR	This study
Ophiothrix spiculata	Os1	*16S, COI*	OOPH012-18[B]	Catalina, CA	This study
Ophiothrix spiculata	Os2	*16S, COI*	OOPH013-18[B]	Catalina, CA	This study
Ophiothrix suensoni	KC626237	*COI*	KC626237[G]	Cozumel Island, Mexico	(Bribiesca-Contreras et al., 2013)
Ophiothrix suensoni	KU895446	*COI*	KU895446[G]	Cozumel Island, Mexico	(Bribiesca-Contreras et al., 2013)

previous northern limit was Moss Beach, San Mateo County, California (Austin & Hadfield, 1980; Lambert & Austin, 2007), which was expanded northward to Patrick's Point State Park, Humboldt County, CA based on iNaturalist records (Sanford et al., 2019). A small number of juveniles were observed in Victoria, BC but surveys conducted in the region since 2001 have not documented any more *O. spiculata* (Lambert & Austin, 2007; Sanford et al., 2019).

Embryonic Description. We did not observe the embryos of this species.

Larval Description. *Ophiothrix spiculata* develops via an eight-armed ophiopluteus, and it is one of the largest of all the plutei we observed (Fig. 3.2A). The epidermis is transparent, and

the larva develops very long, straight, and wideset posterolateral arms that may be over five times the length of the other arms (Fig. 3.2A). The postoral and posterdorsal arms are approximately the same length, and slightly shorter than the anterolateral arms. The body rods are short and in line with the posterolateral arms (Fig. 3.6J). The left transverse rod may bear a median process, as reported for ophioplutei of this genus (Mortensen, 1921), but cannot be clearly seen in our photos (Fig. 3.6J).

Similar Species. We observed *O. spiculata* only as eight-armed ophiopluteus and juvenile, both of which are similar to that of *Ophiopholis* spp. (see Section 3.4.1.1 below). In both species, the posterolateral arms are straight, long, and wideset; the remaining arms are similar in length to one another but markedly shorter than the posterolateral arms. Plutei of *O. spiculata* have posterolateral arms up to five times the length of their other arms, whilst the posterolateral arms of *Ophiopholis* spp. are up to three times the length of the other arms. This comparison is most useful in advanced larvae, as the ultimate length of the arms is accumulated across time as the larva eats and grows.

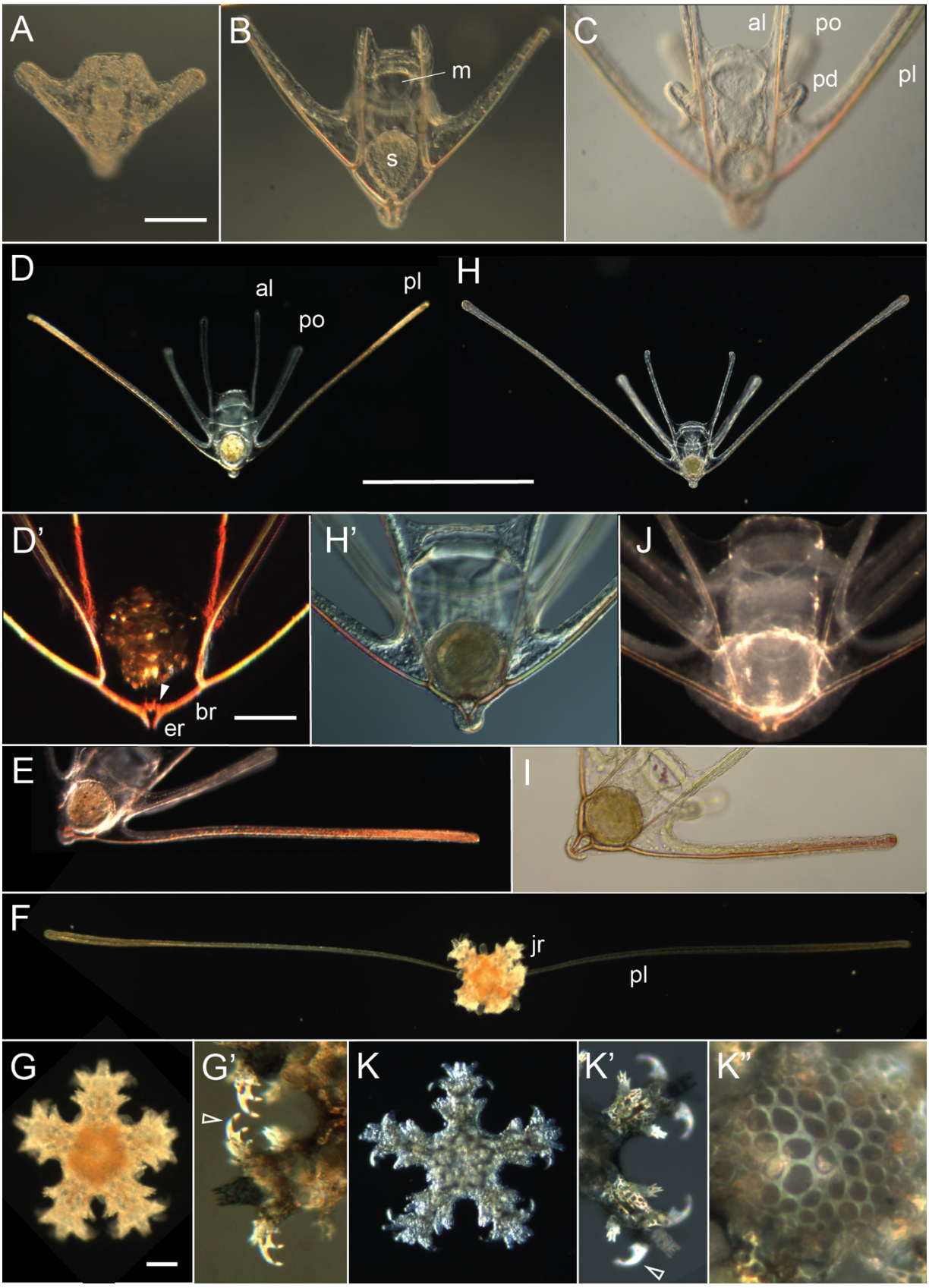
Development Mode. *Ophiothrix spiculata* has an obligately feeding, 8-armed ophiopluteus, as expected from a small egg of diameter 110 μm (Pearse, 1994). Feeding larvae are the norm in *Ophiothrix* (Guille, 1964; Hendler, 2005, 1995; Hendler & Littman, 1986; Kitazawa et al., 2015; Mladenov, 1983, 1985a; Morgan & Jangoux, 2005; Mortensen, 1938; Selvakumaraswamy & Byrne, 2000, 2006), although nonfeeding planktonic larvae (Mladenov, 1979; Richards et al., 2007) and one brooder (Schoppe, 1996) are also known. Another genus in the same family, *Macrophiothrix*, has feeding ophioplutei and several reduced plutei (Allen & Podolsky, 2007).

Juvenile Description. The juvenile is large and motile at metamorphosis, with three arm segments, the proximal two of which bear hooked arm spines (Fig. 3.6K') that can be used to secure the individual in rock crevices or algal holdfasts. The hooked arm spines can be used to distinguish the juveniles of *O. spiculata* and *Ophiopholis* spp., as *O. spiculata* has a single hook, and *Ophiopholis* spp. have a double hook. The juvenile arms are often folded under the disk while still attached to the larval arms, and this condition may persist following metamorphosis. The stereom of the juvenile disk is shown in Fig. 3.6K".

Reproductive Timing. This larva is not usually observed in the plankton of Charleston, OR, but five larvae or recently metamorphosed juveniles were collected from plankton samples during the marine heatwave known as The Blob from December 2014 and January 2015 (Fig. 3.3). Spawning has been observed from late winter to July in central California (Lambert & Austin, 2007; Rumrill, 1982).

Planktonic Duration. We were not able to observe the planktonic duration for *O. spiculata,* but we suspect it to be a month or longer given the planktotrophic development and large size of the ophiopluteus (see Fig. 3.2A).

Figure 3.6. Larvae and juveniles of (A-G) *Ophiopholis kennerlyi*, (H,I) *O. bakeri*, and (J,K) *Ophiothrix spiculata*. Secondary and tertiary views of the same individual are marked with an apostrophe (') and quotation mark ("), respectively. (A) Aboral view of the early pluteus of *O. kennerlyi*, (B) oral view of six-armed pluteus, and (C) aboral view of early eight-armed pluteus, with anterolateral (al), postoral (po), posterodorsal (pd), and posterolateral (pl) arms. B and C are same scale as A, scale bar is 100 μm. (D) Oral view of a more advanced eight-armed pluteus and (D') closeup of the posterior girdle in cross-polarized light with end rods (er), body rods (br) and transverse rods (arrowhead). Scale bars are 1 mm in D and 100 μm in D'. In both D and D' the posterodorsal arms are largely behind the postoral arms. (E) Another eight-armed pluteus, showing the reddish coloration of the skeleton, especially the posterolateral, body, and end rods, as well as the accumulation of red pigmentation in the distal portion of the posterolateral arms. (F) A rudiment-stage larva with a juvenile rudiment (jr) carried by the posterolateral arms, same scale as D. (G) Juvenile collected from the plankton, and (G') a closeup of the hooked spines with two teeth on the terminal segment of the arm (open arrowhead). Scale bar in G is 100 μm. (H) Eight-armed pluteus of *O. bakeri*, oral view at same scale as D and (H') closeup of the posterior girdle, same scale as D'. (I) Aboral view of another individual, showing the red-orange coloration of the posterolateral, body, and end rods. (J) Closeup of the posterior girdle of *O. spiculata*, same individual as Fig. 3.2.2A and same scale as G'. (K) Juvenile collected from the plankton, aboral view in transmitted light, and (K') closeups of the hooked spines on the terminal segments of the arms, and (K") of the disc stereom in partially cross-polarized light.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3.4.2. Ophiopholidae O'HARA, STÖHR, HUGALL, THUY & MARTYNOV 2018

Ophiopholidae was recently raised to family status (O'Hara et al., 2018) and contains a single genus, *Ophiopholis*, which is comprised of eight species. Five of these occur in the northeastern Pacific: *aculeata, bakeri, japonica, kennerlyi,* and *longispina* (Lambert & Austin, 2007). We observed two species, *Ophiopholis kennerlyi* and *O. bakeri*, whose larvae were not distinguishable based on morphology (see Fig. 3.6D, H) but barcoding showed to be distinct (Fig 7, Table 3.4). Therefore, we will give a description of both species, although we suspect that the early pluteus material is of *O. kennerlyi* due to its intertidal to shallow subtidal distribution compared to that of *O. bakeri*, which occurs subtidally at depths of up to 1200 m (Lambert & Austin, 2007).

3.4.2.1. *Ophiopholis kennerlyi* LYMAN 1860 and *O. bakeri* MCCLENDON 1909

Species Identity. Although there are characters listed in the literature that distinguish *O. kennerlyi* from *O. aculeata*, (Lambert & Austin, 2007) state that adult specimens of *O. aculeata* occur in the Bering Sea and into British Columbia but that positively identified specimens of *O. aculeata* have not been collected in waters of Oregon and Washington. We have not collected *O. aculeata* in our samples, nor do any of our molecular sequences match those of *O. aculeata* from the Atlantic (Fig. 3.7).

The deep-water species *O. bakeri* and O. *longispina* are recognized as separate species and are distinguished by presence or absence of spines on their radial shields, and length of arm spines (Lambert and Austin 2007). They are also acknowledged to be like one another as

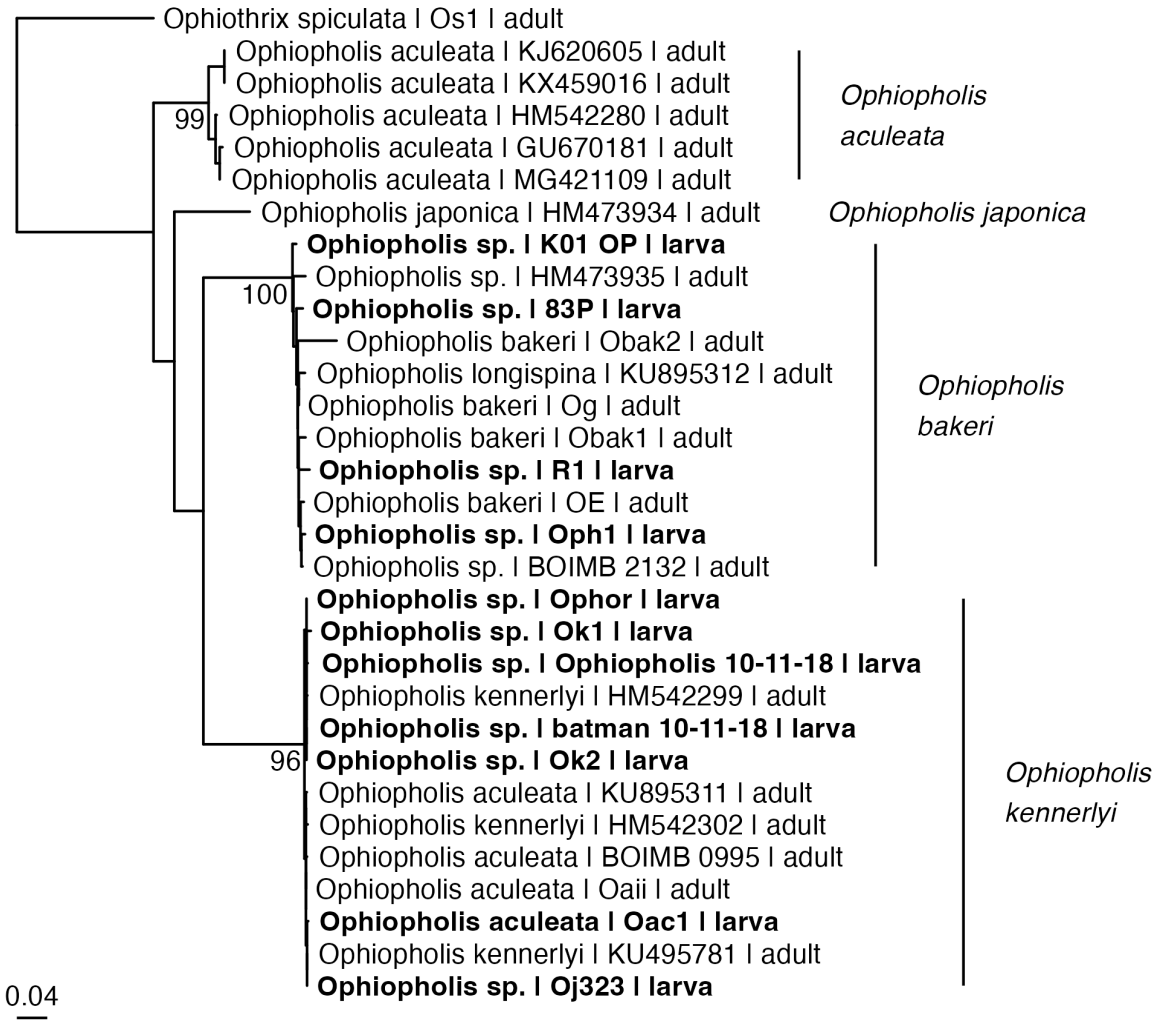

Figure 3.7. Maximum likelihood tree of *COI* sequences for *Ophiopholis* spp. Barcodes of larvae identified in this study are in bold.

Table 3.4. Specimen information for *Ophiopholis* spp. included in Fig. 3.7. Additional information for specimens collected in this study can be accessed using the BOLD Project IDs below.

Species	Specimen Code	Locus	BOLD[B] or GenBank[G]	Collection Locality	Reference
Ophiopholis aculeata	GU670181	*COI*	GU670181[G]	Manitoba, Canada	(Corstorphine, 2011)
Ophiopholis aculeata	HM542280	*COI*	HM542280[G]	New Brunswick, Canada	(Corstorphine, 2011)
Ophiopholis aculeata	KJ620605	*COI*	KJ620605[G]	Iceland	(Khodami et al., 2014)

Ophiopholis aculeata	KU895311	*COI*	KU895311[G]	USA	(Hugall et al., 2016)
Ophiopholis aculeata	KX459016	*COI*	KX459016[G]	North Sea	(Laakmann et al., 2017)
Ophiopholis aculeata	MG421109	*COI*	MG421109[G]	Newfoundland, Canada	Dewaard, unpublished
Ophiopholis bakeri	83P	*COI*	OLAB073-23[B]	Charleston, OR	This study
Ophiopholis bakeri	BOIMB-2132	*COI*		Cape Arago, OR	G. Paulay, pers. comm.
Ophiopholis bakeri	HM473935	*COI*	HM473935[G]	British Columbia, Canada	
Ophiopholis bakeri	K01-OP	*COI*	OLAB074-23[B]	Charleston, OR	This study
Ophiopholis bakeri	KU895312	*COI*	KU895312[G]	USA	
Ophiopholis bakeri	Obak1	*COI*	OOPH047-23[B]	Florence, OR	This study
Ophiopholis bakeri	Obak2	*COI*	OOPH048-23[B]	Florence, OR	This study
Ophiopholis bakeri	Oph1	*COI*	OLAB075-23[B]	Charleston, OR	This study
Ophiopholis bakeri	R1	*COI*	OLAB076-23[B]	Charleston, OR	This study
Ophiopholis japonica	HM473934	*COI*	HM473934[G]	British Columbia, Canada	(Corstorphine, 2011)
Ophiopholis kennerlyi	Batman 10-11-18	*COI*	OLAB077-23[B]	Charleston, OR	This study
Ophiopholis kennerlyi	BOIMB-0995	*COI*			G, Paulay, pers. comm.
Ophiopholis kennerlyi	HM542299	*COI*	HM542299[G]	British Columbia, Canada	(Corstorphine, 2011)
Ophiopholis kennerlyi	HM542302	*COI*	HM542302[G]	British Columbia, Canada	(Corstorphine, 2011)
Ophiopholis kennerlyi	KU495781	*COI*	KU495781[G]	British Columbia, Canada	(Corstorphine, 2011)
Ophiopholis kennerlyi	Oac1	*COI*	OLAB097-23[B]	Cape Arago, OR	This study
Ophiopholis kennerlyi	Oaii	*COI*	OOPH007-18[B]	Cape Arago, OR	This study
Ophiopholis kennerlyi	Oj323	*COI*	OLAB078-23[B]	Charleston, OR	This study
Ophiopholis kennerlyi	Ok1	*COI*	OLAB079-23[B]	Charleston, OR	This study
Ophiopholis kennerlyi	Ok2	*COI*	OLAB080-23[B]	Charleston, OR	This study
Ophiopholis kennerlyi	Ophiopholis 10-11-18	*COI*	OLAB081-23[B]	Charleston, OR	This study
Ophiopholis kennerlyi	Ophor	*COI*	OLAB082-23[B]	Charleston, OR	This study
Ophiopholis longispina	OE/Oplo1	*COI*	OOPH017-18[B]	Cape Arago Shelf, OR	This study
Ophiopholis longispina	Og/Oplo2	*COI*	OOPH019-18[B]	Cape Arago Shelf, OR	This study

Clark (1911, p123) states that “the line of separation between the two forms is very narrow”. Molecular data we collected and obtained from public databases does not show *bakeri* and *longispina* to form reciprocally monophyletic groups, but the data for *longispina* is limited (Fig. 3.7). Our larvae group most closely with an adult collected and identified by us as *O. bakeri*. As such, we will refer to the larvae by that name in this manuscript.

Distribution and Local Sites. *Ophiopholis kennerlyi* and *O. bakeri* occur across similar ranges, from British Columbia to southern California (Lambert & Austin, 2007). They are separated by depth: *O. kennerlyi* occurs intertidally to 366 m and *O. bakeri* occurs from 37 to 1,204 m depth (Lambert & Austin, 2007). We encounter adult *O. kennerlyi* intertidally and from dredges offshore, where we have also collected *O. bakeri* (Table 3.4).

Embryonic Description. We did not observe the embryos of these species, but the embryology of *O.kennerlyi* (misidentified as *O. aculeata*) is documented (Primus, 2005; Strathmann, 1987; Strathmann et al., 2020).

Larval Description. *Ophiopholis* spp. develop via an eight-armed ophiopluteus. The larva of *O. bakeri* was previously unknown, but *O. kennerlyi* (identified as *O. aculeata*) has been studied extensively in the laboratory, including the feeding mechanism of the ophiopluteus (Strathmann, 1971, 1978b), and larval cloning (Balser, 1998).

The early pluteus of *Ophiopholis* is transparent, has four arms, which extend anteriorly and terminate at approximately the same height (Fig. 3.6A). The posterolateral arms grow in length and become increasingly wideset as the larva develops. These first two pairs are followed quickly by the postoral arms, which support a ciliary band in front of the mouth (Fig. 3.6B). The posterodorsal rods bud from the anterolateral rods (Fig. 3.6) and eventually grow to be of equal

anterior height with the postoral arms (Fig. 3.6D). The posterolateral arms of the eight-armed pluteus are greater in height than the rest of the arms, which are similar in length to one another and may be more wideset in *O. bakeri*, although this was not consistent across all specimens (Fig. 3.6D, H). As the larva develops, the posterolateral, body, and end rods may take on an orange-red hue (Fig. 3.5E, I), and pigmentation that is yellow to red in color may accumulate in the distal portions of the posterolateral arms (Fig. 3.6D, E, I). This appears to be more common and more extreme in *kennerlyi*, but the number of specimens of *bakeri* were limited. Furthermore, larvae cultured in the laboratory with microalgal food offered *ad libitum* develop coloration on the skeletal rods and in the posterolateral tissues to far greater effect than any wild-caught larvae we have observed (N. Nakata, personal observation).

Similar species. Early plutei of *Ophiopholis* spp. larvae can be difficult to distinguish from those of *Amphiodia urtica* and *Amphipholis pugetana.* While the plutei of all three species have transparent epidermises, *A. urtica* and *A. pugetana* also have red pigmentation at the distal tips of their posterolateral arms. The skeletal rods of *Ophiopholis* are generally straight, whereas those of other taxa described here are usually curved (Fig. 3.2). Posterolateral arm rods of *Ophiopholis* larvae may bear very small thorns on their inner side, that are well-spaced (Fig. 3.6, compare with amphiurids in Fig. 3.10).

The late ophiopluteus of *Ophiopholis* spp. is most like that of *Ophiothrix spiculata*, which occurs rarely in southern Oregon. From the small number of *O. spiculata* larvae we observed, we determined a few characters in the advanced ophioplutei that distinguish the species: first, posterolateral arms of *Ophiopholis* spp. never exceed three times the length of the other arms, while posterolateral arms of *O. spiculata* can be five times as long; second, the

posterolateral arms of the ophiopluteus (before the juvenile is formed) become increasingly wideset in both species, but in *Ophiopholis* spp. their angle does not exceed 120°, while in *O. spiculata* the angle of the posterolateral arms can exceed 120°. Finally, many ophioplutei of *Ophiothrix* have a medial process on the transverse rod (Mortensen, 1921), this process is absent in early plutei of *Ophiopholis*, and if present there are two medial processes.

Development Mode. Both *O. kennerlyi* and *O. bakeri* have a planktotrophic ophiopluteus. This is well known from the literature for *O. kennerlyi* as it is often called *O. aculeata*, but we consider to be *O. kennerlyi* in the Pacific (Austin & Hadfield, 1980; Balser, 1998; Strathmann, 1987).

Juvenile Description. Plutei of *Ophiopholis* undergo a metamorphosis, in which the anterolateral, postoral and posterodorsal arms are all resorbed in the creation of the juvenile rudiment. The juvenile has 2-3 arm segments; the arms develop folded in on the juvenile oral surface. Juveniles have hook-shaped arm spines with two hooks, distinguishing *Ophiopholis* spp. from *O. spiculata*, which has arm spines with single hooks (Fig. 3.6G').

Reproductive Timing/Spawning Phenology. Larvae of *Ophiopholis* spp. occurred in the plankton from October to April, although the timing of their appearance varied from year to year (Fig. 3.3). *Ophiopholis kennerlyi* is known to spawn throughout the year in the laboratory (Strathmann, 1987).

Planktonic Duration. *Ophiopholis kennerlyi* has a long-lived ophiopluteus, with a planktonic duration of 83-216 days (Austin & Hadfield, 1980; Strathmann, 1978a).

3.4.3. Amphiuridae LJUNGMAN 1867

We observed eight species of amphiurids in the plankton and were able to match five types of larvae to adult specimens via DNA barcoding: *Amphiodia urtica, A. periercta*?*, and A.* sp. opaque*, Amphipholis pugetana* and *Amphiura arcystata*. The remaining three species are 'orphan larvae' without molecular matches to adult specimens. Two of these 'orphans' group with other species of *Amphiodia* (sp. orange belly and sp. tan), and the last with *Amphioplus* (sp. vitellaria). See Fig. 3.8, Table 3.5.

We observed several developmental patterns amongst the amphiurids of the NE Pacific. The only brittle star known to brood its young in the NE Pacific is the cosmopolitan species *Amphipholis squamata* (Hugall et al., 2023; Strathmann, 1987), which was not described here because it lacks a planktonic form. Two of the eight plankton

ic stages of amphiurids had planktotrophic development via an ophiopluteus, *Amphipholis pugetana* and *Amphiodia urtica*. Remarkably, we observed six amphiurids with abbreviated development, including three species that develop via a reduced pluteus (*Amphiodia* spp. opaque, orange belly, and tan), one via a nonfeeding vitellaria (*Amphioplus* sp. vitellaria), and the two others that develop directly into juveniles in the plankton (*Amphiodia periercta*?, *Amphiura arcystata*). Facultative planktotrophy has been confirmed for *A.* sp. opaque (Nakata & Emlet, 2023), and the other two reduced plutei may also be facultative planktotrophs because they form complete guts, have ciliated bands and larval arms. Abbreviated forms of development are not unknown from the family Amphiuridae, *Amphioplus abditus* (Hendler, 1973) and *Amphiura chiajei* (Hendler, 1975), with several more species inferred to have abbreviated development based on egg size (Hendler, 1995; see Nakata Chapter IV).

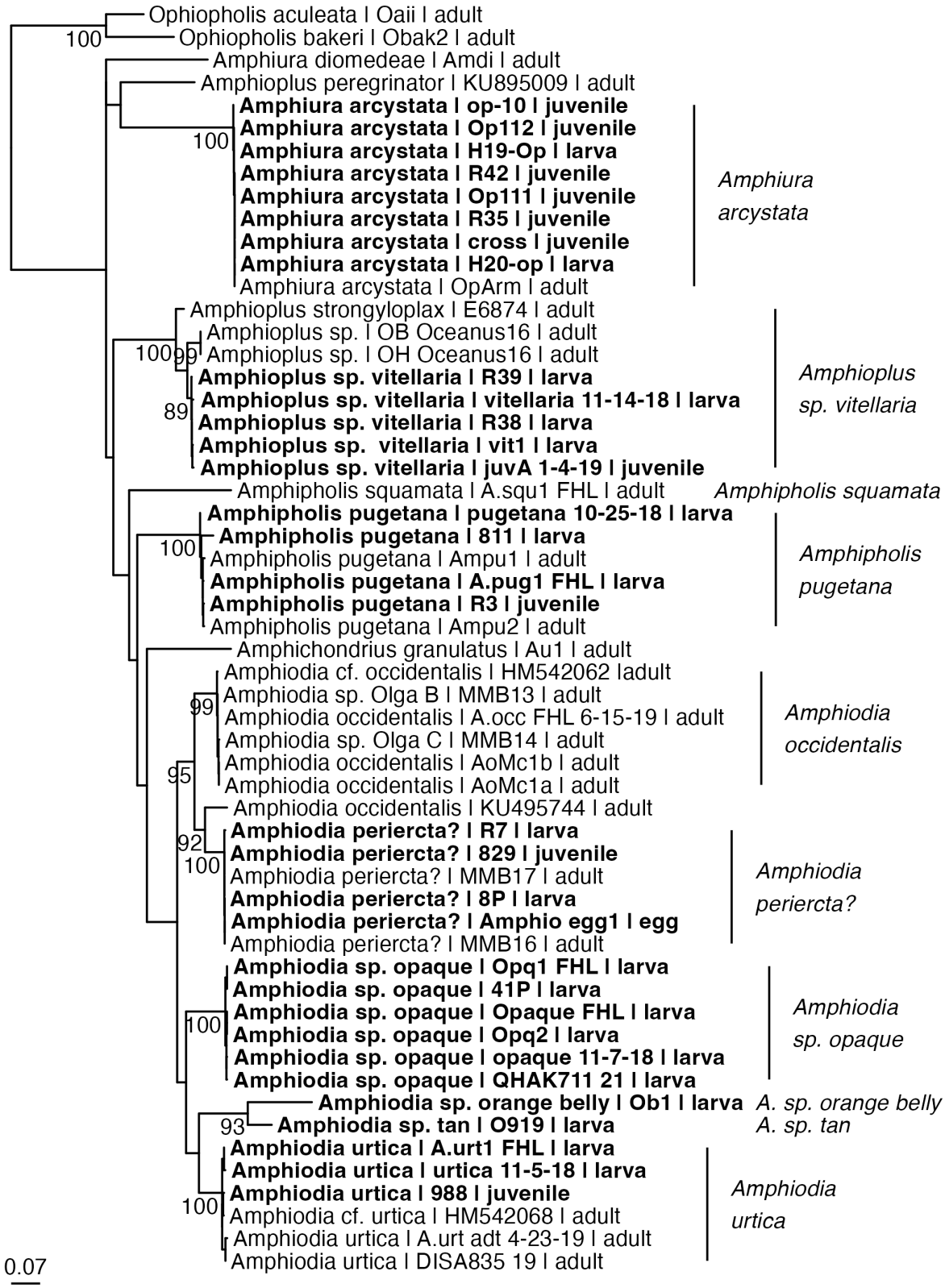
Ophiopholis aculeata I Oaii I adult
100
Ophiopholis bakeri I Obak2 I adult
Amphiura diomedeae I Amdi I adult
Amphioplus peregrinator I KU895009 I adult
Amphiura arcystata I op-10 I juvenile
Amphiura arcystata I Op112 I juvenile
100
Amphiura arcystata I H19-Op I larva
Amphiura arcystata I R42 I juvenile
Amphiura arcystata I Op111 I juvenile
Amphiura arcystata I R35 I juvenile
Amphiura arcystata I cross I juvenile
Amphiura arcystata I H20-op I larva
Amphiura arcystata I OpArm I adult
Amphiura arcystata
Amphioplus strongyloplax I E6874 I adult
Amphioplus sp. I OB Oceanus16 I adult
100
99
Amphioplus sp. I OH Oceanus16 I adult
Amphioplus sp. vitellaria I R39 I larva
Amphioplus sp. vitellaria I vitellaria 11-14-18 I larva
89
Amphioplus sp. vitellaria I R38 I larva
Amphioplus sp. vitellaria I vit1 I larva
Amphioplus sp. vitellaria I juvA 1-4-19 I juvenile
Amphioplus sp. vitellaria
Amphipholis squamata I A.squ1 FHL I adult
Amphipholis squamata
Amphipholis pugetana I pugetana 10-25-18 I larva
Amphipholis pugetana I 811 I larva
100
Amphipholis pugetana I Ampu1 I adult
Amphipholis pugetana I A.pug1 FHL I larva
Amphipholis pugetana I R3 I juvenile
Amphipholis pugetana I Ampu2 I adult
Amphipholis pugetana
Amphichondrius granulatus I Au1 I adult
Amphiodia cf. occidentalis I HM542062 Iadult
Amphiodia sp. Olga B I MMB13 I adult
99
Amphiodia occidentalis I A.occ FHL 6-15-19 I adult
Amphiodia sp. Olga C I MMB14 I adult
Amphiodia occidentalis I AoMc1b I adult
95
Amphiodia occidentalis I AoMc1a I adult
Amphiodia occidentalis
Amphiodia occidentalis I KU495744 I adult
Amphiodia periercta? I R7 I larva
92
Amphiodia periercta? I 829 I juvenile
100
Amphiodia periercta? I MMB17 I adult
Amphiodia periercta? I 8P I larva
Amphiodia periercta? I Amphio egg1 I egg
Amphiodia periercta? I MMB16 I adult
Amphiodia periercta?
Amphiodia sp. opaque I Opq1 FHL I larva
Amphiodia sp. opaque I 41P I larva
Amphiodia sp. opaque I Opaque FHL I larva
100
Amphiodia sp. opaque I Opq2 I larva
Amphiodia sp. opaque I opaque 11-7-18 I larva
Amphiodia sp. opaque I QHAK711 21 I larva
Amphiodia sp. opaque
Amphiodia sp. orange belly I Ob1 I larva
A. sp. orange belly
93
Amphiodia sp. tan I O919 I larva
A. sp. tan
Amphiodia urtica I A.urt1 FHL I larva
Amphiodia urtica I urtica 11-5-18 I larva
Amphiodia urtica I 988 I juvenile
100
Amphiodia cf. urtica I HM542068 I adult
Amphiodia urtica I A.urt adt 4-23-19 I adult
Amphiodia urtica I DISA835 19 I adult
Amphiodia urtica
0.07

Figure 3.8. Maximum likelihood tree of *16S* and *COI* sequences for Amphiuridae species from the northeast Pacific. See Table 3.5 for specimen and locus data. Branch lengths denote patristic distances according to the legend in the bottom left. Support values for major nodes are marked when >70.

Table 3.5. Specimen information for Amphiuridae spp. included in Fig. 3.8. Additional information for specimens collected in this study can be accessed using the BOLD Project IDs below.

Species	Specimen Code	Locus	BOLD[B] or GenBank[G]	Collection Locality	Reference
Amphichondrius granulatus	Au1	*16S, COI*	OOPH005-18[B]	Catalina Island, CA	This study
Amphiodia cf. *occidentalis*	HM542062	*COI*	HM542062[2]	British Columbia, Canada	(Corstorphine, 2011)
Amphiodia occidentalis	A.occ FHL 6-15-19	*16S, COI*	OOPH034-23[B]	Friday Harbor, WA	This study
Amphiodia occidentalis	AoMc1a	*16S, COI*	OOPH020-22[B]	Charleston, OR	This study
Amphiodia occidentalis	AoMc1b	*16S, COI*	OOPH021-22[B]	Charleston, OR	This study
Amphiodia occidentalis	KU495744	*COI*	KU495744[G]	British Columbia, Canada	(Corstorphine, 2011)
Amphiodia sp. Dome House	MMB17	*16S, COI*	OOPH032-22[B]	Charleston, OR	(Nakata & Emlet, 2023)
Amphiodia sp. Olga B	MMB13	*16S, COI*	OOPH039-23[B]	Olga, WA	This study
Amphiodia sp. Olga C	MMB14	*16S, COI*	OOPH040-23[B]	Olga, WA	This study
Amphiodia sp. opaque	41P	*COI*	OLAB099-23[B]	Charleston, OR	This study
Amphiodia sp. opaque	Opaque 11-7-18	*COI*	OOPH029-22[B]	Charleston, OR	(Nakata & Emlet, 2023)
Amphiodia sp. opaque	Opaque FHL	*16S*	OLAB048-23[B]	Friday Harbor, WA	This study
Amphiodia sp. opaque	Opq1 FHL	*16S, COI*	OLAB047-22[B]	Friday Harbor, WA	(Nakata & Emlet, 2023)
Amphiodia sp. opaque	Opq2	*16S, COI*	OLAB004-22[B]	Charleston, OR	(Nakata & Emlet, 2023)
Amphiodia sp. opaque	QHAK711 21	*COI*	QHAK711-21[B]	British Columbia, Canada	Hakai Inst., unpublished
Amphiodia sp. orange belly	Ob1	*16S, COI*	OLAB050-23[B]	Charleston, OR	This study
Amphiodia periercta?	829	*COI*	OLAB032-22[B]	Charleston, OR	This study
Amphiodia periercta?	8P	*COI*	OLAB033-22[B]	Charleston, OR	This study
Amphiodia periercta?	Amphio egg1	*COI*	OLAB045-22[B]	Charleston, OR	(Nakata & Emlet, 2023)
Amphiodia periercta?	R7	*16S*	OLAB031-22[B]	Charleston, OR	This study

Amphiodia sp. Portside	MMB16	*16S, COI*	OOPH031-22[B]	Charleston, OR	(Nakata & Emlet, 2023)
Amphiodia sp. tan	O919	*COI*	OLAB051-23[B]	Charleston, OR	This study
Amphiodia urtica	988	*COI*	OLAB028-22[B]	Charleston, OR	This study
Amphiodia urtica	A.urt adt 4-23-19	*COI, 16S*	OOPH056-23[B]	Cape Arago Shelf, OR	This study
Amphiodia urtica	A.urt1 FHL	*COI*	OLAB053-23[B]	Friday Harbor, WA	This study
Amphiodia urtica	DISA835	*COI*	DISA835-19[B]	Los Angeles, CA	DISCO MBC LACM, unpublished
Amphiodia urtica	HM542068	*COI*	HM542068[G]	British Columbia, Canada	(Corstorphine, 2011)
Amphiodia urtica	urtica 11-5-18	*COI*	OOPH027-22[B]	Charleston, OR	(Nakata & Emlet, 2023)
Amphioplus peregrinator	KU895009	*COI*	KU895009[G]	Antarctica	(Hugall et al., 2016)
Amphioplus sp.	OB	*16S*	OOPH046-23[B]	Newport Valley, OR	This study
Amphioplus sp.	OH	*16S, COI*	OOPH049-23[B]	Newport Valley, OR	This study
Amphioplus sp. vitellaria	juvA 1-4-19	*COI*	OLAB055-23[B]	Charleston, OR	This study
Amphioplus sp. vitellaria	R38	*16S, COI*	OLAB056-23[B]	Charleston, OR	This study
Amphioplus sp. vitellaria	R39	*16S, COI*	OLAB057-23[B]	Charleston, OR	This study
Amphioplus sp. vitellaria	vit1	*16S, COI*	OLAB054-23[B]	Charleston, OR	This study
Amphioplus sp. vitellaria	Vitellaria 11-14-18	*16S, COI*	OLAB058-23[B]	Charleston, OR	This study
Amphioplus strongyloplax	E6874	*COI*	NA	Northeast Pacific	T. O'Hara, pers. comm.
Amphipholis pugetana	811	*COI*	OLAB016-22[B]	Charleston, OR	This study
Amphipholis pugetana	A.pug1 FHL	*16S, COI*	OLAB059-23[B]	Friday Harbor, WA	This study
Amphipholis pugetana	Ampu1	*16S, COI*	OOPH003-18[B]	Cape Arago, OR	This study
Amphipholis pugetana	Ampu2	*16S, COI*	OOPH004-18[B]	Cape Arago, OR	This study
Amphipholis pugetana	pugetana 10-25-18	*COI*	OOPH028-22[B]	Charleston, OR	This study
Amphipholis pugetana	R3	*16S, COI*	OLAB020-22[B]	Charleston, OR	This study
Amphipholis squamata	A.squ1 FHL	*COI*	OOPH035-23[B]	Friday Harbor, WA	This study

Amphiura arcystata	OpArm	*16S, COI*	OOPH050-23[B]	Charleston, OR	This study
Amphiura diomedeae	Amdi	*COI*	OOPH015-18[B]	Newport, OR	This study
Amphiura arcystata	Cross	*16S, COI*	OLAB106-23[B]	Charleston, OR	This study
Amphiura arcystata	H19-Op	*16S, COI*	OLAB060-23[B]	Charleston, OR	This study
Amphiura arcystata	H20-op	*16S, COI*	OLAB061-23[B]	Charleston, OR	This study
Amphiura arcystata	op-10	*COI*	OLAB103-23[B]	Charleston, OR	This study
Amphiura arcystata	Op111	*COI*	OLAB062-23[B]	Charleston, OR	This study
Amphiura arcystata	Op112	*COI*	OLAB063-23[B]	Charleston, OR	This study
Amphiura arcystata	R35	*16S*	OLAB064-23[B]	Charleston, OR	This study
Amphiura arcystata	R42	*16S, COI*	OLAB065-23[B]	Charleston, OR	This study

3.4.3.1. *Amphiodia urtica* (LYMAN 1860)

Species Identity. We collected larvae from Charleston, OR and Friday Harbor, Washington whose *COI* sequences were 2% different adults from southern CA, British Columbia, Canada, and the Cape Arago Shelf, OR (Fig. 3.8, Table 3.5), which falls within the 8.5% threshold determined by ASAP analysis. In our tree of amphiurids from the northeast Pacific, *Amphiodia urtica* is sister to the clade made up of *A.* sp. orange belly and *A.* sp. tan, two species of orphan larvae.

Distribution and Local Sites. Benthic populations of *A. urtica* are known from Alaska to California, from intertidal to 370 m depth (Austin & Hadfield, 1980; Hendler, 1996; Kyte, 1969; Lambert & Austin, 2007). We have collected a few juvenile individuals by dredge at 75–100 m off Cape Arago, Oregon.

Embryonic Description. We did not observe the embryos of these species.

Larval Description. *Amphiodia urtica* develops via an eight-armed planktotrophic ophiopluteus. Planktotrophic development was predicted by small egg size (<100 μm) (Schiff &

Bergen, 1996). The early pluteus is small, with four larval arms and red pigmentation at the tips of the posterolateral pair (Fig. 3.9A). The postoral arms emerge next, supporting a portion of the ciliated band ventral to the mouth (Fig. 3.9B). Lastly the posterodorsal arms grow out from the bases of the anterolateral arms to make an eight-armed ophiopluteus (Fig. 3.9C). As the larva grows the posterolateral arms become curved and bear thorns (Fig. 3.9C'); anterolateral and postoral arms may also bear thorns, but these tend to be shorter than those on the posterolateral arms. Red pigmentation is often visible at the distal ends of the posterolateral arms, but never spans the length of the arm. The posterolateral and body rods may be orange red in color. The postoral and posterodorsal arms are relatively short, and their distal tips rarely exceed the height of the larval mouth. The body rods curve anteriorly to meet the anterolateral and posterolateral rods and descend posteriorly toward each other at an approximately 45° angle. The body rods are connected by thorny transverse rods and the end rods are pointed but not touching (Fig. 3.9C").

The juvenile rudiment develops to the left of the digestive system. The lobes of the left coelom are visible (Fig. 3.9C). The rudiment-stage larva has three arms - a pair of posterolateral arms and one remaining (right) anterolateral arm - with the pentagonal juvenile rudiment bearing tube feet centered among the arms (Fig. 3.9D).

Similar Species. As an early pluteus, *A. urtica* is most easily confused with *Amphipholis pugetana*. Both larvae have red-orange skeletons covered in transparent epidermis, with an accumulation of red pigmentation at the distal ends of the posterolateral arms. The epidermis of *A. urtica* adheres closely to the larval skeleton and follows the curvature of the arms. In *A. pugetana*, the epidermis is separated from the skeleton and has an inflated appearance, creating a club shape at the distal ends of the anterolateral and posterolateral arms. Finally, the pluteus of *A.*

urtica bears many thorns, especially later in development, and thorns are absent or nearly imperceivable in *A. pugetana*. The skeleton of *A. urtica* is most like that of *A.* sp. opaque and *A.* sp. orange belly, with its curved posterolateral arms and the presence of spines. The pluteus of *A. urtica* can be distinguished from its congeners by the transparency of its epidermis, which is opaque pink to salmon in *A.* sp. opaque and orange in the body and white on the arms in *A.* sp. orange belly.

Development Mode. *Amphiodia urtica* develops as an obligately feeding ophiopluteus (N. Nakata, unpublished). Prior reports for the life history of this species are limited to egg size, approximately 0.12 mm diameter, indicating development as a feeding ophiopluteus (Hendler, 1996).

Juvenile Description. Juvenile is star shaped, comprised of a pentagonal disc and triangle-shaped arm plates (Fig 9F).

Reproductive Timing. In our data, larvae were present in the Oregon plankton from November to April, with rare observations in October and May. We also have collected these larvae in August and September in the San Juan Islands, WA.

In Southern California, where this species is very abundant on the shelf, *A. urtica* has been observed spawning in November, and in October in British Columbia (Austin & Hadfield, 1980).

Planktonic Duration. Planktonic duration for *A. urtica* is approximately 20 days (Nakata & Emlet, 2023), which is relatively short for an obligate planktotroph.

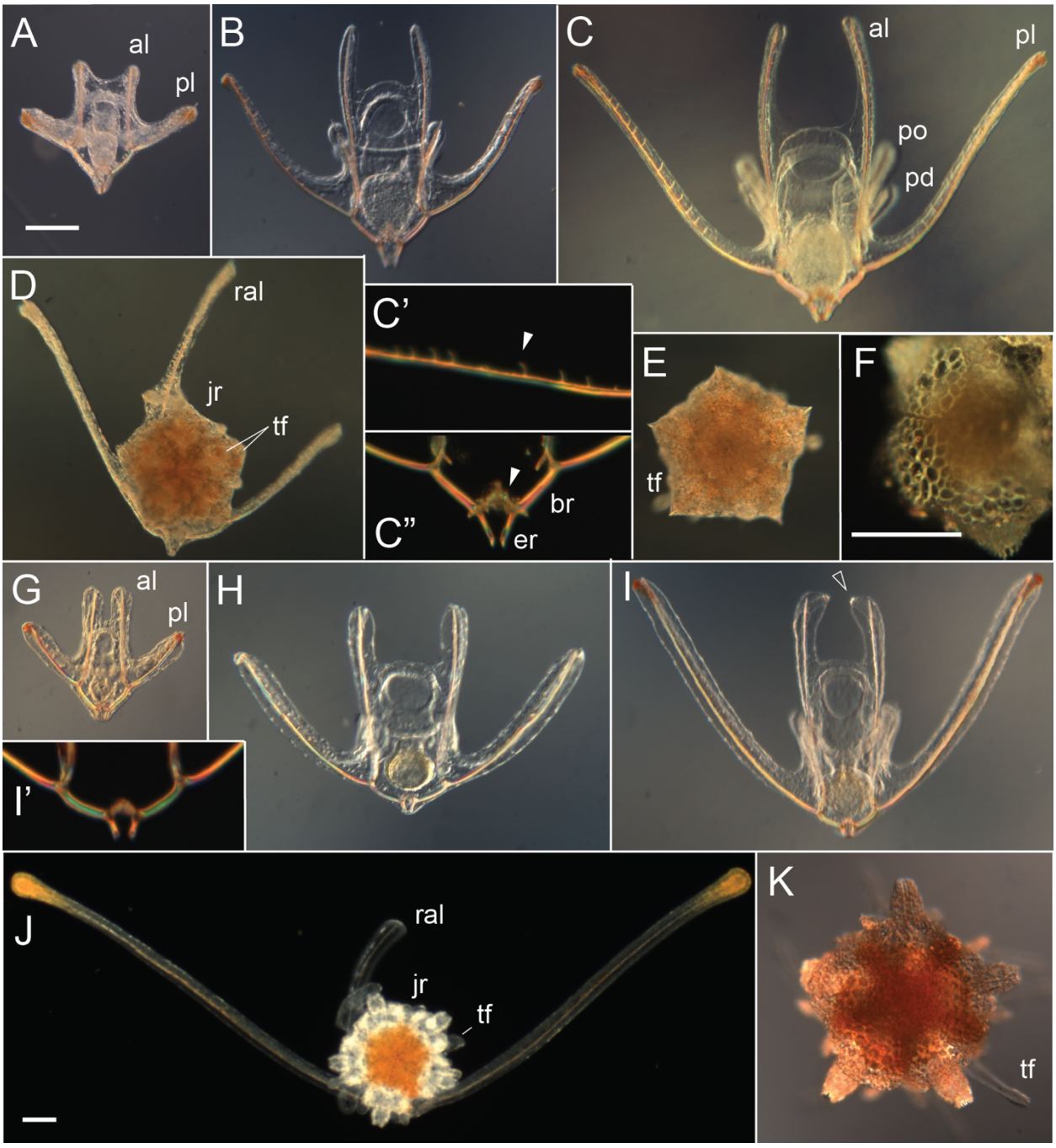

Figure 3.9. Planktotrophic plutei of Amphiuridae: *Amphiodia urtica* (A–F) and *Amphipholis pugetana* (G–K). Secondary and tertiary views of the same individual are marked with an apostrophe (’), or a quotation mark (”), respectively. Scale bar in A is 100 µm and the same for A-E, G-I, K. Scale bars in F and J are 100 µm. (A) Oral view of the early pluteus of A. urtica with anterolateral (al) and posterolateral (pl) arms, the latter with red pigment accumulated at the distal tips. (B) Early eight-armed (oral view), and (C) advanced eight-arm pluteus (oral view) with anterolateral, posterolateral, postoral (po), and posterodorsal (pd) arm pairs. (C’) Closeups of thorns on posterolateral arms, and (C”) posterior girdle, respectively, in cross-polarized light. The posterior girdle is composed

of the transverse rods (filled arrowhead), body rods (br) and end rods (er). (D) Oral view of a rudiment-stage larva with right anterolateral arm (ral) and juvenile rudiment (jr) bearing tube foot buds (tf). (E) The juvenile of the same individual, one day later. (F) A closeup of the stereom of another individual in cross-polarized light. (G) Early pluteus of Amphipholis pugetana with red pigmentation at the distal tip of the posterolateral arms. (H, I) Six- and eight-armed plutei, respectively, of A. pugetana. The portion of the ciliated band on the distal end of the anterolateral arms often bends medially (open arrowhead). (J) Rudiment-stage larva with right anterolateral arm (ral), juvenile rudiment (jr) with tube feet (tf), scale 100 μm. (K) Juvenile in partially cross-polarized light highlights the stereom of the disk plates and spine tips.

3.4.3.2. *Amphipholis pugetana* (LYMAN 1860)

Species Identity. There are two species of *Amphipholis* that occur in the northeast Pacific (26 *Amphipholis* spp. total): *A. pugetana* and the cosmopolitan brooder *A. squamata.* Our specimens of *A. pugetana* and *A. squamata* do not group together, and the genus *Amphipholis* is probably polyphyletic (Fig. 3.8, Table 3.5)(O'Hara et al., 2018). The placement of *Amphipholis* relative to *Amphiodia* and *Amphiura* is uncertain and the family Amphiuridae probably requires revision (O'Hara et al., 2018).

Distribution and Local Sites. *Amphipholis pugetana* occurs from the Gulf of Alaska to southern California, 0-1204 m (Lambert & Austin, 2007). We have collected adults by dredge at approximately 50 m depth off Cape Arago, Oregon.

Embryonic Description. We did not observe the embryos of these species.

Larval Description. *Amphipholis pugetana* develops via a planktotrophic eight-armed pluteus, which is recognized by its inflated epidermis that is well separated from the larval arm rods. The epidermis is transparent, often with an accumulation of red pigmentation at the distal ends of the posterolateral arms. This feature is present even in the early pluteus (Fig. 3.9G).

The posterolateral arms are curved, and any thorns are very small, and triangle shaped, if they are present at all. The anterolateral rods are straight but may become slightly curved proximally and distally in the advanced pluteus (Fig. 3.2D); the anterolateral rods taper to a sharp point, and never bear thorns, unlike plutei from the genus *Amphiodia* (*e.g., Amphiodia urtica, A.* sp. opaque, *A.* sp. orange belly, and *A.* sp. tan). The ciliated band on the tips of anterolateral arms may extend medially, giving this larva a 'bunny-eared' appearance (Fig. 3.9I). The body rods are relatively short, curved, and nearly horizontal. The transverse rods are unadorned. The end rods are short and run parallel to one another, not touching at their distal ends. The postoral and posterodorsal arms remain short, with the postoral arms terminating just above the mouth at their longest, and the posterodorsal arms never terminating above the larval mouth.

Similar Species. As an early pluteus, *A. pugetana* is most likely to be confused with *A. urtica* or *Ophiopholis* spp. *Amphipholis* and *A. urtica* often have an accumulation of red pigmentation at the distal ends of the posterolateral arms (Fig. 3.9G, I, J). *Amphipholis pugetana* has thicker arms than *A. urtica* due to its inflated epidermis, which becomes more apparent as the larva develops. Thorns are present on the skeletal rods of *A. urtica* but arm rods of *A. pugetana* lacks thorns. Plutei of *Ophiopholis* may have red pigmentation on the posterolateral arms and have straight posterolateral and body rods (Fig. 3.6); while these are curved in *A. pugetana* (Figs. 2D, 9I). *Ophiura leptoctenia* also has an inflated epidermis, but it is widest at the base of the posterolateral arms and tapers to a point at their distal ends. Furthermore, the pluteus of *O. leptoctenia* has recurrent rods, which are absent in *A. pugetana*.

Development Mode. *Amphipholis pugetana* broadcast spawns its gametes (Lambert & Austin, 2007) and develops as a planktotrophic ophiopluteus (Shanks, 2001). As the juvenile rudiment develops. The postoral, posterodorsal and left anterolateral are resorbed, leaving a three-armed larva (Fig. 3.9J).

Juvenile Description. The juvenile has a roughly pentagonal disc with five arm tips that end bluntly (Fig. 3.9K). The shape of the arm tips and the distinct shape of the red pigmentation often visible through the disc are characteristic for this species. There are several amphiurid juveniles with light pink to cream-colored bodies, often with reddened gut. Only the juveniles of *A. pugetana* juveniles have extensions of the red gut into the interradii. Juveniles were found in the plankton.

Reproductive Timing. The ophioplutei and juveniles of *A. pugetana* occur in the Charleston plankton from October to March. We also have collected them in August and September in the San Juan Islands. WA.

Planktonic Duration. Unknown.

3.4.3.3. *Amphiodia* sp. opaque

Species Identity. The species identity of this larva remains unresolved, but *COI* and *16S* sequences group with species in the genus *Amphiodia*.

Distribution and Local Sites. The location of adult populations in Oregon are not known. The embryos and larvae of this species have been collected off the Oregon coast (see also Nakata and Emlet 2023), at least 10 juvenile and adult stages that have been barcoded have been collected in the Salish Sea (private data BOLD)

Development Mode. *A.* sp. opaque is a facultative planktotroph, as determined by feeding experiments. The larva can complete metamorphosis into a juvenile even under starvation conditions, but has quicker development, higher metamorphic success, and larger juveniles when given food (Nakata & Emlet, 2023).

Embryonic Description. Embryos of this species are orange to salmon in color and have a thick hyaline layer (Fig. 3.10A). This hyaline layer can be seen between blastomeres at the 2-8 cell stages, like *A. periercta?* (Emlet, 2006). Embryos usually hatch as blastulae. Gastrulae are shaped like a chicken's egg, wider toward the posterior and more pointed in the anterior., and uniformly ciliated. The first spicules develop as a pair of spicules in the posterior of the gastrula (Fig. 3.10A').

Larval Description. *Amphiodia* sp. opaque develops via a six-armed reduced pluteus with opaque epidermis. The early pluteus is dorsoventrally compressed but has the beginnings of the posterolateral and anterolateral arms, supported by paired triradiate spicules (Fig. 3.10B'). The postoral ciliated band is present, stretching across the ventral side of the larva from the tips of the posterolateral arms.

The ophiopluteus can be recognized by an epidermis that is light pink to salmon in color, and semi- to fully opaque so that internal structures are not easily viewed. The posterolateral arms are often straight but may be curved or become curved during rudiment formation. The posterolateral and anterolateral rods often bear thorns (Fig. 3.10D'). The anterolateral arms can be straight or curved and bear short thorns (Fig 10C', D'). The body rods are mostly straight, curving to connect with the posterolateral and end rods, which are of moderate length, connected by thorny transverse rods. The end rods do not touch at their distal ends.

Juvenile Description. The juvenile is pentagonal juvenile (Fig. 3.10E), very similar to *A.* sp. orange belly (Fig. 3.10L).

Similar Species. Due to their morphology as reduced plutei, their opaqueness and coloration in shades of orange, salmon, pink, and tan the early plutei of *A.* sp. opaque, *A.* sp. orange belly, *A.* sp. tan, and *Ophiura luetkenii* can be confused with one another. In *A.* sp. opaque and *A*. sp. tan the color of the epidermis is uniform across the larval body and arms, light pink to salmon in the former (Fig. 3.10C), tan in the latter (Fig 10O). In *A.* sp. orange belly and *O. luetkenii* the larval body is orange, and the posterolateral and anterolateral arms are white (Fig. 3.10H).

Reproductive Timing. Embryos and larvae of *A.* sp. opaque can be found in the Charleston plankton most reliably and in greatest density in the winter, particularly the month of February. Larvae have also been observed in fall (October, November). In Friday Harbor, WA, larvae were collected in late summer (July, August), but this was the only season we were able sample at that location. Larvae of various developmental stages (early pluteus, pluteus, and rudiment-stage larva) were observed on February 24, 2023, just outside of Newport, OR, suggesting that adult populations may be nearby.

Planktonic duration. Planktonic duration in this species has been shown to vary according to food availability during the larval stage. Median planktonic durations (PD) for fed larvae were 17 and 13 days in two study years, with a maximum PDs of 21 and 19 days, respectively (Nakata & Emlet, 2023).

3.4.3.4. *Amphiodia* sp. orange belly

Species Identity. We know *Amphiodia* sp. orange belly only from its larva, which we collected only four times (Fig. 3.3). Adult populations of *A.* sp. orange belly have not been identified near Charleston, Oregon. DNA barcoding and larval morphology places the larva within the genus *Amphiodia*, sister to another orphan larval species, *A.* sp. tan (Fig. 3.8, Table 3.5). There are at least two described *Amphiodia* species known to occur in the NE Pacific that are lacking developmental information: *digitata* and *psara* (Lambert & Austin, 2007), but we lack sequences from adults to connect these species with our larval morphotypes.

Distribution and Local Sites. Adult populations in Oregon are not known.

Embryonic Description. We did not observe the embryos of this species.

Larval Description. Orange belly develops via a six-armed pluteus. The early pluteus is similar to that of *A.* sp. opaque, with a well-developed left coelom (Fig. 3.10F) The reduced pluteus is small (Fig. 3.10G, H) and has shortened posterolateral arms (compare with *A. urtica*) and postoral arms are lacking. It also has a deep orange stomach and left coelom and salmon to white colored larval arms (Fig 10G–K). The pluteus has an open mouth and hollow stomach and intestine.

The posterolateral arms are somewhat straight, and bear thorns, as do the anterolateral arms (Fig. 3.10K'). The body rods are curved and similar in shape to those of *Amphipholis pugetana*. The end rods are of moderate length and united by thorny transverse rods.

Similar Species. The reduced pluteus of *A.* sp. opaque is most similar in size and shape to its congener, *A.* sp. opaque, but differs in color by having an orange body with white posterolateral arms. It also has posterolateral arms that are more strongly curved than those

usually found on opaque. The curvature and spination call to mind that of *Amphiodia urtica*. *Amphiodia* sp. orange belly has similar coloration to *Ophiura luetkenii*.

Development Mode. We suspect that this larva is a facultative planktotroph, like *A.* sp. opaque, but have lacked the material to conduct feeding experiments. We have observed larvae metamorphose after raised in FSW without algal cells, but larvae appear to have a complete larval digestive system and may be capable of eating and benefitting from larval feeding.

Juvenile Description. Pentagonal juvenile, very similar to that of other Amphiodia spp. (Fig. 3.10L).

Reproductive Timing. We have observed the larvae of *A.* sp. orange belly four times in the Oregon plankton. Collections were made in January and February, and just once in April (Fig. 3.3).

Planktonic Duration. The planktonic duration of *A.* sp. orange belly is about 8 days (n=1).

3.4.3.5. *Amphiodia* sp. tan

Species Identity. We know this species from a single larva that groups with *Amphiodia* orange belly but is genetically and morphologically distinct (Fig. 3.8, Table 3.5). We have no adult match.

Distribution and Local Sites. Local adult populations remain unknown.

Embryonic Description. The single specimen was collected in September 2020, as a tan blastula and recognized as an ophiuroid embryo from its thick hyaline coat. When first collected

it, we assumed incorrectly that it was *A*. sp. opaque; but the color was not orange or salmon typical of *A*. sp. opaque.

Larval Description. *Amphiodia* sp. tan develops via a six-armed ophiopluteus with a semi-opaque epidermis tan in color. The early pluteus is dorsoventrally compressed with short posterolateral and anterolateral arms (Fig. 3.10M).

The final ophiopluteus has curved posterolateral and anterolateral arms, that bear small thorns (Fig. 3.10O'). The larva has a mouth and stomach, but it is not clear if the larva feeds. The body rods are curved, and the end rods are short and parallel to one another. (Fig. 3.2F)

The rudiment stage larva is distinct from the other amphiurid reduced plutei. The rudiment-stage larva retains part of the left anterolateral rod as well as the right anterolateral rod (Fig. 3.10P).

Similar Species. The reduced pluteus of *A.* sp. tan is most like those of *A.* sp. opaque and *A.* sp. orange belly. The tan coloration of the epidermis is what distinguishes it, as does the manner of arm resorption.

Development Mode. We suspect that *A.* sp. tan is a facultative planktotroph due to its reduced pluteus morphology and the presence of a mouth and stomach. We did not attempt to give the single individual food, but it successfully made a juvenile without any food.

Juvenile Description. The juvenile is small and pentagonal like other *Amphiodia* spp.

Reproductive Timing. We collected one early pluteus on September 19, 2022 (Fig. 3.4).

Planktonic Duration. The larva we observed went from early pluteus to juvenile in approximately 11 days.

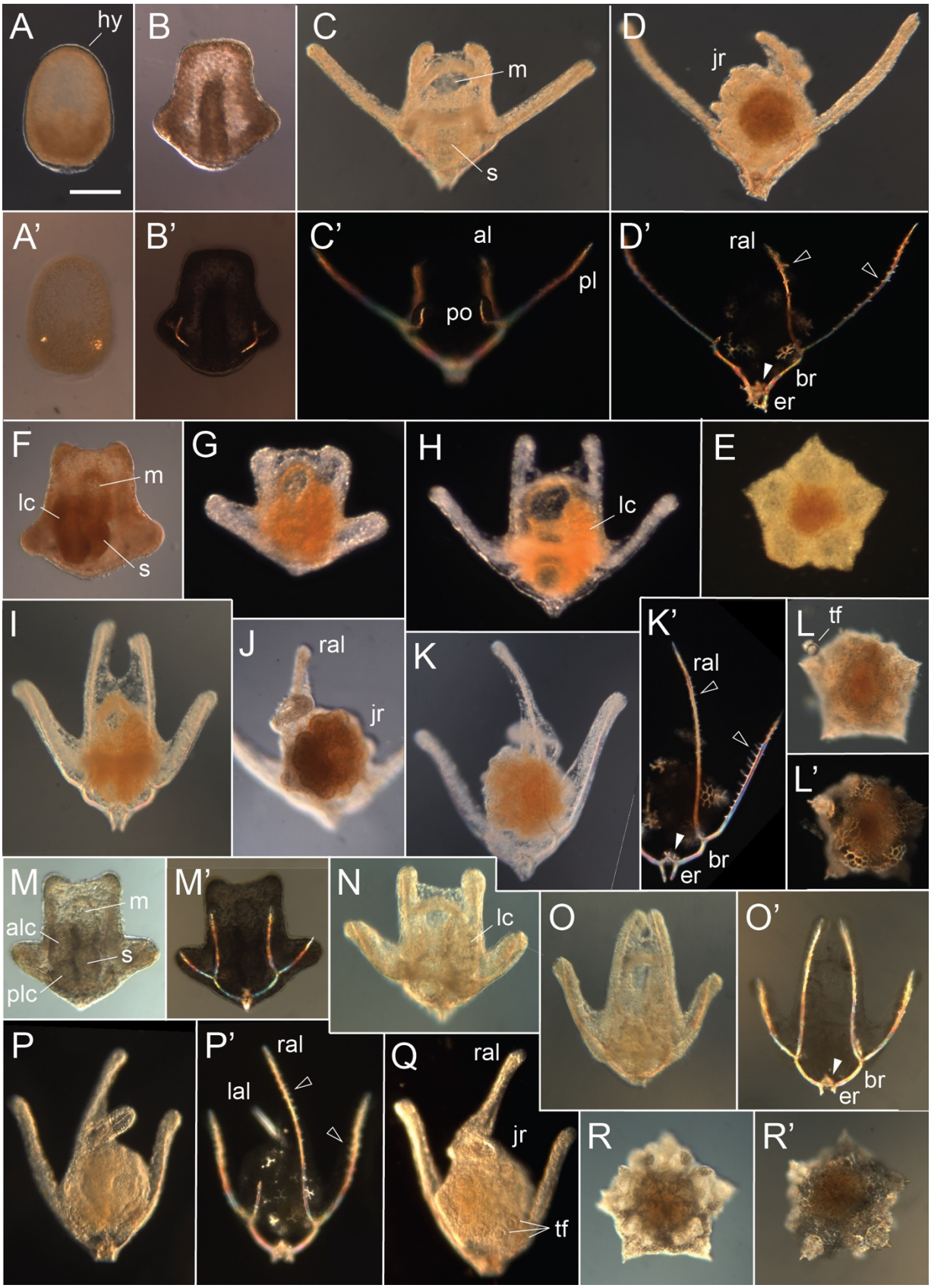
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Figure 3.10. Reduced plutei of three species of *Amphiodia*: (A–E) *Amphiodia* sp. opaque, (F-L) *Amphiodia* sp. orange belly, and (M–R) *Amphiodia* sp. tan. Secondary views of the same individual are marked with an apostrophe (’). Scale bar in A is 100 μm and applies to all images on the plate. (A) Gastrula of *A.* sp. opaque with thick hyaline layer (hy). (A’) The newly forming pair of spicules are visible at the posterior in partially cross-polarized light. (B) Aboral view of the early pluteus, the gut is medial and not yet complete; (B’) The paired spicules have branched, forming rods that will support larval arms. (C) Oral view of the six-armed pluteus. (C’) In cross-polarized light, the anterolateral (al), postoral (po), and posterolateral (pl) arm rods are visible. The anterolateral and posterolateral arms may be straight or curved. (D) In the rudiment-stage pluteus, the juvenile rudiment (jr) forms around the larval mouth and resorbs all but the right anterolateral arm (ral) and the paired posterolateral arms (aboral view). (D’) The longer arm rods are adorned with thorns (open arrowheads) as the pluteus grows and develops. The body rods (br) and end rods (er) are straight, and the transverse rods (filled arrowhead) are thorny. (E) The juvenile (aboral view) is pentagonal, often with a red-pink coloration at the center of the disk. (F,G) The early pluteus of *A.* sp. orange belly (F, aboral view, G oral view) has a large left coelom (lc), mouth (m) and stomach (s). (H, I) The left coelom has developed lobes by the time the pluteus develops six arms (H oral view, I oral view of another individual). (J,K) The rudiment-stage pluteus (J oral view, K aboral view another individual) has both posterolateral arms and the right anterolateral arm, which are covered in thorns (K’). (L,L’) The juvenile in aboral view, of the same individual as K and photographed six days later, is very similar to juveniles of other *Amphiodia* spp. The tube feet (tf) at this stage are active. F-H, J are the same individual, photographed daily over four days; I, K, L are the same individual, photographed on two consecutive days (I, K) and then again six days later (L). (M) An aboral view of an early pluteus of *A.* sp. tan with anterior and posterior left coeloms (alc, plc). (M’) Same larva under cross-polarized light reveals the larval rods. (N) One day later and in oral view, the pluteus developed the postoral arms (out of focal plane) and the left coelomic lobes. (O,O’) The anterolateral and posterolateral arms continue to grow in length (aboral view) and bear very small thorns. (P) The rudiment-stage pluteus at first retains both right and left anterolateral arms (oral view, P’ aboral view). (Q) Eventually the rudiment-stage pluteus has only the right anterolateral arm (oral view) and tube feet are visible on the oral side of the juvenile rudiment. (R, R’) The pentagonal juvenile, photographed five days after Q, in oral (R) and aboral views (R’).

3.4.3.6. *Amphiodia periercta*? H.L. Clark 1911

Species Identity. This larva was initially described as *Amphiodia occidentalis* (Emlet, 2006), but molecular evidence suggests otherwise. Within NE Pacific *Amphiodia,* there is a species complex containing at least three species: *occidentalis*, *periercta?*, and a population from the Queen Charlotte Islands, BC (Fig. 3.8, Table 3.5).

The original discovery of this pelagic direct developer (1991) was the result of a spontaneous spawning in a bucket with many adults collected near Olga on Orcas Island, WA. Most of the adults were identified as *A. occidentalis* by R. Emlet using the key in Kozloff (Kozloff, 1987); and material collected later from Olga later in the 1990’s was confirmed as *A.*

occidentalis by G. Hendler (Los Angeles County Museum of Natural History). The same phenotype was found in the plankton in Charleston Oregon from 1994 onward and assumed to be *A. occidentalis* (Emlet, 2006). In 2011, R. Emlet barcoded specimens of this phenotype from Charleston, OR only to find that they were not a species-level match with adult specimens of *A. occidentalis* from our region or the *A. occidentalis* collected subsequently from the original site at Olga, WA. (Fig. 3.8, Table 3.5). In 2019, BioBlitz researchers (G. Paulay, personal communication) and Nakata collected sub-adults in eelgrass beds in the low intertidal at several sites in Coos Bay estuary and these specimens were a molecular match to this planktonic form (Fig. 3.8, Table 3.5). Based on comparison of this adult material with species descriptions and with material from the USNM (E13853), we have provisionally identified the adult and its larval phenotype as *A. periercta*? (G. Hendler, personal communication).

Distribution and Local Sites. *Amphiodia periercta* occurs from the intertidal to 92 m depth from Unalaska (Aleutian) Island, Alaska to central California, (Lambert & Austin, 2007). We have collected small, infaunal adults in eelgrass beds at -0.5 m tidal height at sites in the marine dominated part of the Coos Bay estuary.

Embryonic Description. The eggs are light pink to salmon colored and 190um in diameter, suggestive of their abbreviated development. See Emlet (2006) for a full description.

Larval Description. Embryos and unhatched juveniles of *A. periercta*? and are easily recognized by the thick hyaline layer surrounding their cells as eggs and embryos (Fig. 3.11A, B). The embryo is also encased in a large, robust, and somewhat sticky fertilization envelope. Later in development, but still prior to hatching, the developing embryo is light pink, flattened along the (juvenile) oral-aboral axis and has characteristic peripheral ring of spicules (Fig.

3.11D, D'). After hatching, the scallop-shaped juvenile has a ring of skeletal elements supporting its periphery, and a stomodeal invagination (Fig. 3.11E).

Similar Species. The unusual developmental pattern of *A. periercta*? means that it is not mistaken for any other ophiuroids whose development has been described. There is one other directly developing amphiurid in the region, *Amphiura arcystata*. *Amphiodia periercta*? is distinct from *A. arcystata* in the presence of a peripheral skeletal ring, and the absence of the interradial bulges that occur in *A. arcystata*.

Development Mode. *Amphiodia periercta*? is a pelagic direct developer. It may represent the only of its kind, and was described in detail (Emlet, 2006).

Juvenile Description. Development into a juvenile with moving podia and jaws is achieved in 7-8 days (Emlet, 2006) and juveniles at this stage are encountered in plankton tows during the winter months. Ophiuroids are known to occur as plankton even after they metamorphose (Hendler et al., 1999). Motile juveniles of *A. periercta*? are initially pentagonal but their terminal arms plates develop relatively rapidly into pointed rays (Fig. 3.11F)

Reproductive Timing. All stages, from eggs, to embryos and hatched juveniles of *A. periercta* may occur in the plankton of Charleston, Oregon starting in mid-October, but especially from January to March after storms accompanied by large waves. Our daily plankton records from 2014-2016 suggests that *A. periercta*? spawns frequently throughout the winter (Fig. 3.3). This species is reproductive in the San Juan Islands of Washington in summer months, June to August (Emlet 2006 and 2019 unpublished observations).

Planktonic Duration. Planktonic duration is approximately eight days (Emlet, 2006). Cohorts of similarly aged stages were often discernible in our samples, though we could not determine if new cohorts represented a subsequent spawning event by the same adults.

Other Remarks. As noted by Emlet (2006), the discovery of a pelagic directly developing larva disagreed with prior – albeit sparsely evidenced – accounts for developmental mode in *A. occidentalis*. Previous accounts for *A. occidentalis* list a yellow green egg 90-106 μm in diameter, which presumably developed into an obligately planktotrophic pluteus (Rumrill, 1982; Strathmann, 1987).

At present we do not know the development of *A. occidentalis*, but it does have eggs similar in size and color to those of *A. periercta*? (R. Emlet, personal communication). We have collected adults often and never found them brooding. Despite continued attempts to induce spawning in gravid *A. occidentalis* and the extensive planktonic collection and sequencing of ophiuroid larvae presented here, we have never encountered larvae of *A. occidentalis*. We wonder if *A. occidentalis* develops in benthic capsules similar to *Amphioplus abditus* (Hendler, 1977).

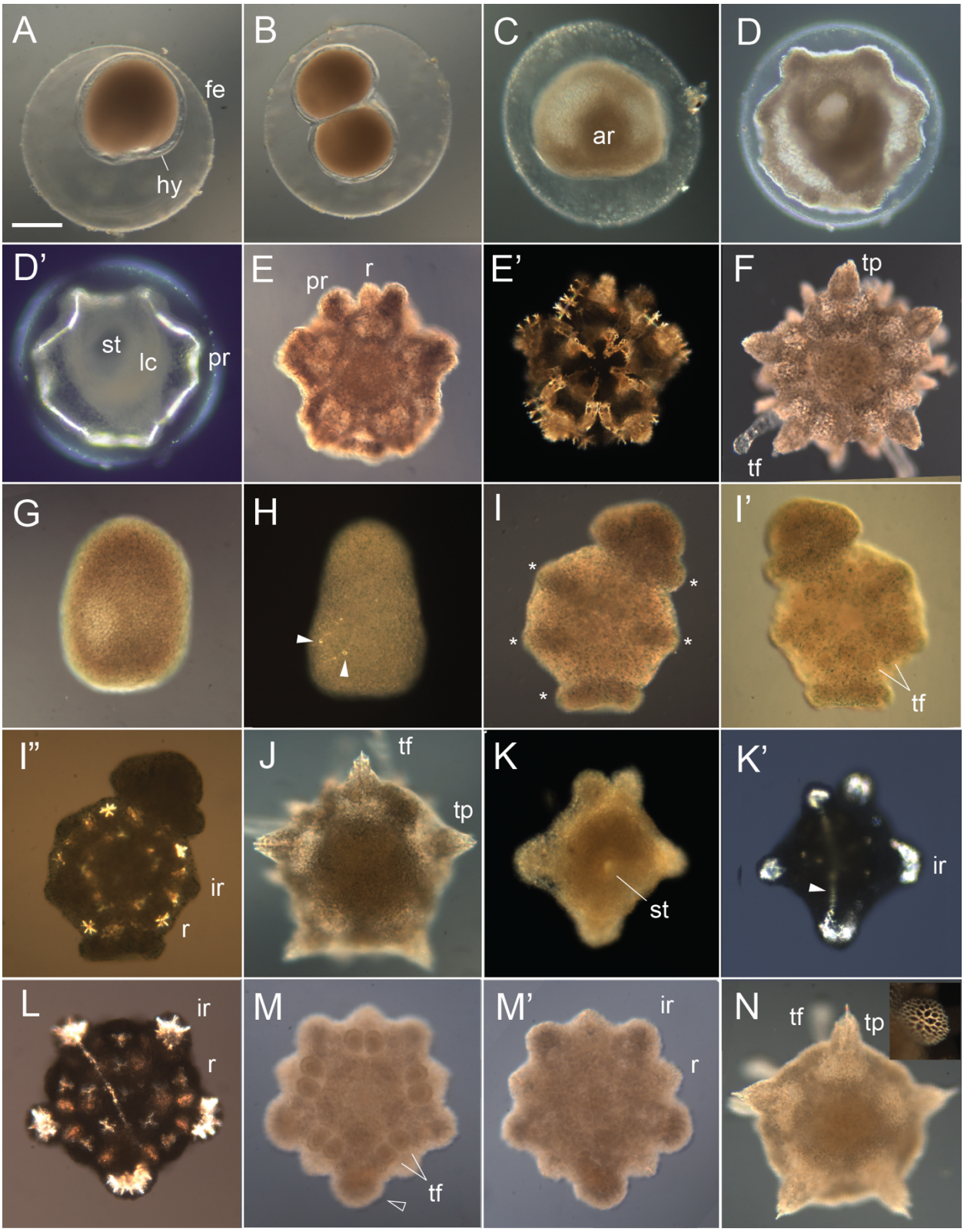
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Figure 3.11. Three nonfeeding planktonic forms of Amphiuridae: (A–F) *Amphiodia periercta?*, (G–J) *Amphioplus* sp. vitellaria, and (K–N) *Amphiura arcystata*. Secondary and tertiary views of the same individual are marked with an apostrophe (') or quotations marks ("), respectively. (A) The fertilized egg of *Amphiodia periercta?* has a thick layer of hyaline (hy). The fertilization envelope (fe) is robust and sticky. (B) The two-cell stage. (C) The gastrula has a visible archenteron (ar). (D) Oral view of an unhatched *A. periercta?*. and (D') in cross-polarized light. The embryo has a stomadeal invagination (st), left coelom (lc) and peripheral rods (pr) of skeleton. (E) After hatching the early juvenile has elaborate peripheral rods as the radials (r) develop. (F) The juvenile moves using its tube feet (tf) and resorbs the peripheral rods. Lateral views of (G) early and (H) late gastrula of *Amphioplus* sp. vitellaria, taken of the same individual one day apart. Note the pebbled exterior appearance due to many oil droplets interlaced with green pigmentation that are characteristic of this larva and the first spicules visible in partial cross-polarized light (solid arrowheads). (I) Aboral view of the vitellaria in transmitted light with the ciliary bands (cb) marked by asterisks (*). (I') The same larva in oral view, with visible tube foot buds. (I") The position of the juvenile radii (r) and interradii (ir) can be determined by identifying the arm spines under partial cross-polarized light. (J) Aboral view of the vitellaria juvenile. (K) The early juvenile of *A. arcystata* has a stomadeal invagination, and (K') five calcified projections that become interradii of the juvenile disk, two of which are connected by ephemeral skeletal elements (closed arrowhead). (L) Another individual, viewed approximately one day later, has assumed pentaradial symmetry and the cross piece skeletal elements are visible from the aboral side under partial cross-polarized light. This stage is marked by rounded interradii that exceed the radii in size initially but that quickly recede in comparison to the growing arm rays. (M) Oral and (M') aboral views of an early juvenile with buds of tube foot buds, pointed radii, and rounded interradii, one of which is larger and redder in color (open arrowhead). (N) The juvenile has a rounded disc with triangle-shaped terminal arm plates. The disc stereom under cross-polarized light is shown in the inset.

3.4.3.7. *Amphioplus* sp. vitellaria

Species Identity. The species identity of this larva is uncertain, but it groups with other *Amphioplus* specimens from the northeast Pacific (Fig 8, Table 3.5). The sequences from our larvae are approximately 5% different from adults identified as *Amphioplus strongyloplax* (E6874, T. O'Hara, personal communication). Larval sequences are 4% different from two adult specimens collected from 450 m depth on the Oregon Shelf (OH, OB), that could not be identified to species because they consisted of a disk only and a young adult that lacked the characters necessary identification (G. Hendler, personal communication). All the members of our *Amphioplus* clade could be given the identification of *A. strongyloplax* based on the threshold of 8.5% from our ASAP analysis, but we gave this larva the provisional name of

Amphioplus sp. vitellaria due to our uncertainty on this identification. The genus is polyphyletic and requires revision (O'Hara et al., 2017) and we have few adult specimens of species from this region for comparison.

Distribution and Local Sites. Two species of *Amphioplus* are known from this region, *A. strongyloplax* and *A. macraspis*. *Amphioplus macraspis* is widespread in the north Pacific, in Asia and from the Queen Charlotte Islands to Washington in the eastern Pacific, 1–876 meters depth. *Amphioplus strongyloplax* has a greater range in the eastern Pacific, ranging from the Gulf of Alaska to the Mexican border, 40–623 meters (Lambert & Austin, 2007). Adults of both species have been reported from benthic surveys off of Coos Bay, OR (Henkel & Gilbane, 2020).

Embryonic Description. We did not observe the embryos of these species.

Larval Description. The embryos and larvae of this species can be recognized at any stage by the presence of lipid droplets and dark green speckled pigmentation, visible through the tan epidermis. The vitellaria has disjunct ciliated bands that wrap laterally around the interradii, as well as anterior and posterior ciliated bands.

Similar Species. The only other species to have a vitellaria stage in this region was *Ophiopteris papillosa*, whose vitellaria is preceded by an ophiopluteus. Furthermore, the vitellaria of *O. papillosa* has remnants of the larval arms and skeletal rods at the anterior that *A.* sp. vitellaria lacks, Finally, the vitellaria of *O. papillosa* has a white body with a deep red interior and yellow ciliated bands (Figure 3.14), whereas *A.* sp. vitellaria is light pink to tan, with ciliated bands slightly darker in color.

Development Mode. The larva of *A.* sp. vitellaria is the first vitellaria larva to be described for the family Amphiuridae (Hendler, 1991). Nonfeeding, planktonic vitellaria have

evolved many time in the brittle stars, but appear to be restricted to the Ophintegrida O'HARA, HUGALL, THUY, STÖHR & MARTYNOV, 2017, in the genera *Ophionereis, Ophioplocus, Ophiolepis*, and *Ophiacantha* (Brogger et al., 2013; Hendler, 1979, 1982, 1995; Komatsu & Shosaku, 1993; Mortensen, 1938; Selvakumaraswamy & Byrne, 2000). At least two species, *Ophiopeza spinosa* and *Ophioplocus esmarki* are known to brood vitellaria larvae (Byrne et al., 2008; Sweet et al., 2019).

Juvenile Description. The juvenile is tan in color and has a round disc with pointed terminal arm plates (Fig 11J).

Reproductive Timing. *Amphioplus* sp. vitellaria occurs rarely in December and January in the Charleston plankton (Fig. 3.3). We usually find them as single individuals or pairs, not in high abundance.

Planktonic Duration. Development of *A.* sp. vitellaria proceeds from a bullet-shaped gastrula to a nonfeeding vitellaria in 4-5 days. Metamorphosis into a motile juvenile takes another 2-3 days.

3.4.3.8. *Amphiura arcystata* H.L. CLARK 1911

Species Identity. We have barcoded eight of these larvae since 2012, and sequences generated for *COI* match to a single young adult identified as *Amphiura arcystata* (Fig. 3.8, Table 3.5). This may represent a range expansion for this species, which is unexpected due to its abbreviated development and likely limited dispersal.

Distribution and Local Sites. *Amphiura arcystata* is known from Mexico to Monterey Bay, California (Hendler, 1996). The presence of the short-lived larvae suggests a possible range expansion, but as we have not yet discovered adult populations locally.

Embryonic Description. We have not observed the embryos of these species.

Larval Description. We usually find this species as a pentaradial larva with a line of skeletal elements crossing the aboral surface and only visible under polarized light (Fig. 3.11K', L). Similar skeletal elements have been observed in an undescribed direct developer from Australia (Emlet, unpublished data), and may represent ectopic vestiges of ophiopluteus skeleton.

The developing juvenile of *A. arcystata* has five distal bulges that are the interradii: the juvenile arms soon develop between them (see arm stereom under polarized light in Fig. 3.11N). One of these bulges tends to be pink to orange red in color, in contrast to the mottled tan of the rest of the larva. The buds of the tube feet are often visible at this developmental stage, though they are not mobile or capable of holding on to substrate.

Similar Species. The directly developing juvenile of *A. arcystata* could be mistaken for that of *Amphiodia periercta?*, which also develops directly in the plankton (see discussion above). However, the larva of *A. periercta?* has a peripheral ring of spicules while the larva of *A. arcystata* has a line of skeletal elements that cross the larval body. *Amphiura arcystata* could also be mistaken for *Amphioplus* sp. vitellaria, especially at developmental stages where the symmetry is unclear. Both share a tan coloration, but *A.* sp. vitellaria has a more pebbled exterior appearance.

Development Mode. We know this animal mainly from its pelagic, directly developing larva, which appears rarely in the Charleston plankton from November to January. The genus *Amphiura* is known to contain brooding species (n=17), pelagic lecithotrophs (3), and at least one planktotroph (*A. filiformis*) [citations].

Juvenile Description. The juvenile of this species is similar to other amphiurids, but thicker at the center of the disc (Fig. 3.11N).

Reproductive Timing. The larvae and juveniles of *A. arcystata* occur rarely in the Charleston plankton from November to January.

Planktonic Duration. 6 days (n=1).

3.4.4. Ophiacanthina O'HARA, HUGALL, THUY, STÖHR & MARTYNOV 2017

Species Identity. We found two species, *Ophiacantha diplasia* and *Ophiopteris papillosa*, that were the sole representatives of the superfamily Ophiacanthina (517 spp.; O'Hara et al. 2017). Despite not being very closely related (Fig. 3.12, Table 3.6), the ophioplutei of these species greatly resemble one another, but may be delineated by features of the skeleton and style of metamorphosis.

Development Mode. *Ophiacantha diplasia* and *Ophiopteris papillosa* both develop as eight-armed ophioplutei and give rise to an advanced and motile juvenile with multi-segmented arms. However, *O. papillosa* transforms from a feeding ophiopluteus into a vitellaria before metamorphosing into a juvenile. These two species are the first of their kind to be described for their families. *Ophiacantha diplasia* is the first planktotroph from the family Ophiacanthidae

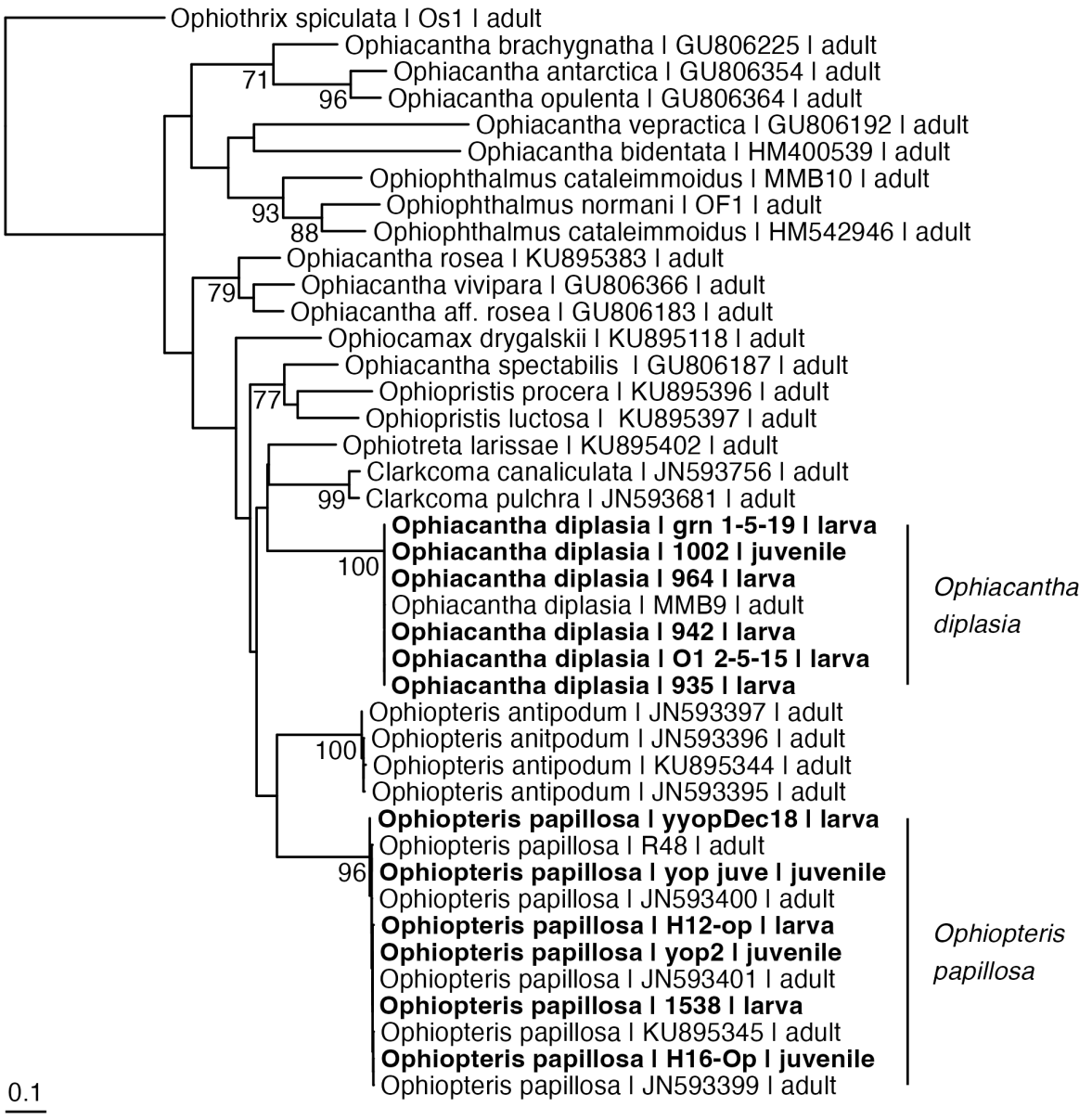


Figure 3.12. Maximum likelihood tree for a concatenated dataset of *16S* and *COI* sequences for superfamily Ophiacanthina from the Pacific Ocean. Barcodes from larvae identified in this study are in bold. Bootstrap values of 70 and higher are shown beside nodes.

Table 3.6. Specimen information for species of superfamily Ophiacanthina from the Pacific included in Fig. 3.12. New sequence data from this study is in bolded text. Additional information for specimens collected in this study can be accessed using the BOLD Project IDs below.

Species	Specimen Code	Locus	BOLD[B] or GenBank[G]	Collection Locality	Reference
Clarkcoma canaliculata	JN593756	*COI*	JN593756[G]	Australia	Naughton et al., unpublished
Clarkcoma pulchra	JN593681	*COI*	JN593681[G]	Australia	Naughton et al., unpublished
Ophiacantha aff. *rosea*	GU806183	*COI*	GU806183[G]	Australia	iBOL[1], unpublished
Ophiacantha antarctica	GU806354	*COI*	GU806354[G]	Antarctica	iBOL[1], unpublished
Ophiacantha bidentata	HM400539	*COI*	HM400539[G]	Nunavut, Canada	(Corstorphine, 2011)
Ophiacantha brachygnatha	GU806225	*COI*	GU806225[G]	Antarctica	iBOL[1], unpublished
Ophiacantha diplasia	1002	*COI*	OLAB069-23[B]	Charleston, OR	This study
Ophiacantha diplasia	935	*16S, COI*	OLAB107-23[B]	Charleston, OR	This study
Ophiacantha diplasia	942	*16S, COI*	OLAB108-23[B]	Charleston, OR	This study
Ophiacantha diplasia	964	*COI*	OLAB068-23[B]	Charleston, OR	This study
Ophiacantha diplasia	grn 1-5-19	*16S*	OLAB070-23[B]	Charleston, OR	This study
Ophiacantha diplasia	MMB9	*16S, COI*	OOPH045-23[B]	Stonewall Bank, OR	This study
Ophiacantha diplasia	O1 2-5-15	*16S, COI*	OLAB071-23[B]	Charleston, OR	This study
Ophiacantha opulenta	GU806364	*COI*	GU806364[G]	Antarctica	iBOL[1], unpublished
Ophiacantha rosea	KU895383	*COI*	KU895383[G]	Australia	(Hugall et al., 2016)
Ophiacantha spectabilis	GU806187	*COI*	GU806187[G]	Australia	iBOL[1], unpublished
Ophiacantha vepractica	GU806192	*COI*	GU806192[G]	Australia	iBOL[1], unpublished
Ophiacantha vivipara	GU806366	*COI*	GU806366[G]	Antarctica	iBOL[1], unpublished
Ophiocamax drygalskii	KU895118	*COI*	KU895118[G]	Antarctica	(Hugall et al., 2016)
Ophiophthalmus cataleimmoidus	MMB10	*16S, COI*	OOPH041-23[B]	Newport, OR	This study
Ophiophthalmus cataleimmoidus	HM542946	*COI*	HM542946[G]	BC, Canada	(Corstorphine, 2011)
Ophiophthalmus normani	OF	*COI*	OOPH018-18[B]	Newport, OR	This study
Ophiopristis luctosa	KU895397	*COI*	KU895397[G]	Australia	(Hugall et al., 2016)
Ophiopristis procera	KU895396	*COI*	KU895396[G]	Papua New Guinea	(Hugall et al., 2016)
Ophiopteris antipodum	JN593395	*COI*	JN593395[G]	Australia	Naughton et al., unpublished
Ophiopteris antipodum	JN593396	*COI*	JN593396[G]	Australia	Naughton et al., unpublished

Ophiopteris antipodum	JN593397	*COI*	JN593397[G]	Australia	Naughton et al., unpublished
Ophiopteris antipodum	KU895344	*COI*	KU895344[G]	New Zealand	(Hugall et al., 2016)
Ophiopteris papillosa	1538	*COI*	OLAB083-23[B]	Charleston, OR	This study
Ophiopteris papillosa	H12-op	*COI*	OLAB084-23[B]	Charleston, OR	This study
Ophiopteris papillosa	H16-Op	*16S*	OLAB085-23[B]	Charleston, OR	This study
Ophiopteris papillosa	JN593399	*COI*	JN593399[G]	Australia	Naughton et al., unpublished
Ophiopteris papillosa	JN593400	*COI*	JN593400[G]	Australia	Naughton et al., unpublished
Ophiopteris papillosa	JN593401	*COI*	JN593401[G]	Australia	Naughton et al., unpublished
Ophiopteris papillosa	KU895345	*COI*	KU895345[G]	USA	(Hugall et al., 2016)
Ophiopteris papillosa	R48	*16S*	OOPH054-23[B]	Castle Rock, CA	This study[2]
Ophiopteris papillosa	yop juve	*16S*	OLAB086-23[B]	Charleston, OR	This study
Ophiopteris papillosa	yop2	*16S*	OLAB087-23[B]	Charleston, OR	This study
Ophiopteris papillosa	yyopDec18	*16S*	OLAB088-23[B]	Charleston, OR	This study
Ophiotreta larissae	KU895402	*COI*	KU895402[G]	Papua New Guinea	(Hugall et al., 2016)

[1]International Barcode of Life, [2]Specimen borrowed from the Natural History Museum of Los Angeles County (NHMLAC).

LJUNGMAN 1867, and *Ophiopteris papillosa* is the first vitellaria from Ophiopteridae O'HARA, STÖHR, HUGALL, THUY & MARTYNOV 2018.

3.4.4.1. *Ophiacantha diplasia* H. L. CLARK 1911

Species Identity. We encountered several larvae of various developmental stages in 2015 and 2019 (Fig. 3.3). Barcoding revealed these larvae to be a match to a specimen we collected on the Stonewall Bank off the coast of Newport, Oregon that we identified as *Ophiacantha diplasia* (Figure 12, Table 3.6).

Distribution and Local Sites. *Ophiacantha diplasia* occurs from Haida Gwaii to Southern California at depths of 71 to 1178 meters on silty sand or hard rock (Astrahantseff & Alton, 1965; Lambert & Austin, 2007). We have encountered the adult animal only once, on Stonewall Bank, 307 m (MMB9, Table 3.6).

Embryonic Description. We did not observe embryos of this species.

Larval Description. *Ophiacantha diplasia* develops via an eight-armed feeding ophiopluteus. The epidermis is transparent, with yellow-green pigmentation at the distal ends of the larval arms. The posterolateral arms are curved near the posterior girdle, seen in the early eight-arm stage (Fig. 3.13B), and straight as they lengthen in the advanced ophiopluteus (Fig. 3.13C, D). The anterolateral arms are straight and project anteriorly beyond other arms at all stages of development. The postoral and posterodorsal arms are long, with their distal tips of an equal height with the posterolateral arms. The posterior is supported by the body rods and recurrent rods. The right dorsal recurrent rod may bear a median process, but we have also observed individuals that lack a median process. The skeletal rods lack thorns. The eight-armed pluteus has vibratile lobes, prominent enlargements of the ciliary band at the bases of the posterolateral arms. Vibratile lobes are known also from the ophioplutei from the genus *Ophiocoma* L. AGASSIZ 1836 (Hendler, 1991). Like the epaulettes of echinoplutei, the ophiopluteus vibratile lobes may act in locomotion (Mortensen, 1921; Strathmann, 1975) The juvenile rudiment is star shaped, and all or most larval arms are retained until metamorphosis (Fig. 3.13E).

Similar Species. In the northeast Pacific, the pluteus of *O. diplasia* is most likely to be confused with that of *Ophiopteris papillosa*. The larval epidermis of these two species is

transparent and accented with touches of yellow green at the distal tips of the arms. Their posterolateral arms are not markedly longer than other arms. The advanced larvae of both species have vibratile lobes, at first giving the impression of an echinopluteus. However, careful examination of the placement of the pluteus arms shows them to be ophioplutei. If one has access to a microscope with cross-polarized light, the posterior girdles of the two species can be used to differentiate them at all stages: *O. diplasia* has recurrent rods, even as a young pluteus, while *O. papillosa* lacks these. The manner of metamorphosis also distinguishes between the species: *O. diplasia* forms a juvenile rudiment as a pluteus, while *O. papillosa* transforms into a vitellaria before metamorphosis.

Development Mode. *Ophiacantha diplasia* has a feeding ophiopluteus larva. The bright red coloration of the stomach in wild-caught specimens is due their algal diet (Fig. 3.13B-D).

Juvenile Description. At metamorphosis the juvenile has arms with up to three segments and is highly motile. Juvenile arm tips are a yellow-green color, a feature that is absent from arms of juvenile *O. papillosa*.

Reproductive Timing. Larvae were observed infrequently in the Charleston plankton from January to April.

Planktonic Duration. We estimated planktonic duration as 82 days for a single larva collected from the plankton as a young ophiopluteus and raised in the laboratory on a mixed microalgal diet.

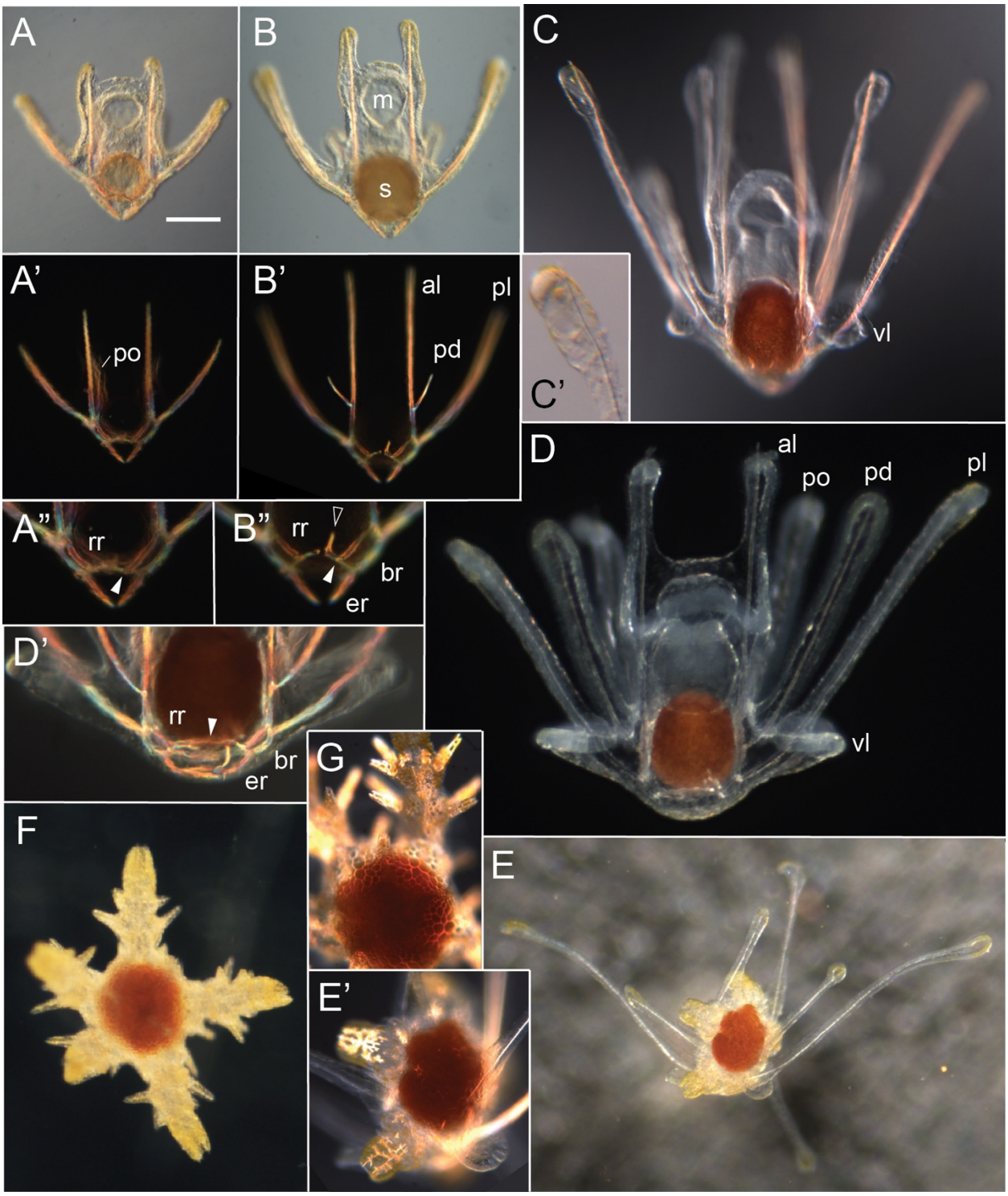


Figure 3.13. Ophioplutei and juveniles of *Ophiacantha diplasia*, all in aboral view. Secondary and tertiary views of the same individual are marked with an apostrophe (') and quotation mark ("), respectively. (A, B) Aboral views of six- and eight-armed ophioplutei, respectively (postoral arms cannot be seen). The arms tips are yellow green in

color. (A', B') In cross-polarized light the skeletal rods that support the posterolateral (pl), anterolateral (al), posterodorsal (pd), and postoral (po) arms are visible. (A", B") Closeups of the posterior girdles with recurrent rod (rr), transverse rod (filled arrowhead), median process (open arrowhead), body rod (br), and end rod (er) visible. (C) An eight-armed pluteus, with modest vibratile lobes (vl). (C") Detail of yellow-green pigmentation at the distal end of the arms. (D) Eight-armed pluteus with well-formed vibratile lobes (vl), and (po) arms in hind ground. (D') Closeup of the posterior girdle in partially cross-polarized light. (E) A rudiment-stage larva, note larval arms are present and still long. (E') Closeup of the developing juvenile arms in partially cross-polarized light. (F) A juvenile with 3 arm segments. (G) Detail of the disk and arm stereom of a juvenile in partially cross-polarized light. Scale in A is 100 μm and applies for B, D, and E. No scale for C, F, G.

3.4.4.2. *Ophiopteris papillosa* (LYMAN 1875)

Species Identity. We were able to connect larvae at different developmental stages to the species *Ophiopteris papillosa* through DNA barcoding and comparison with sequences for adult specimens from GenBank or material borrowed from NHMLAC (Fig. 3.12, Table 3.6). The family Ophiopteridae contains just two species, *Ophiopteris papillosa* throughout the west coast of North America, and *O. antipodum* to the south (Naughton et al., 2014).

Distribution and Local Sites. Coos Bay, Oregon, is well within the range of *O. papillosa*, which occurs from Barkley Sound, British Columbia, and southern Oregon to Isla Cedros, Baja California (Austin & Hadfield, 1980; Lambert & Austin, 2007).

Previously, a planktotrophic larva was predicted by the small egg size, diameter 100 μm, of the species (Pearse, 1994).

Embryonic Description. We did not observe the embryos of these species.

Larval Description. The larval forms of *O. papillosa* have a transparent epidermis, sometimes with yellow to green pigmentation on at the distal ends of the larval arms and on the ciliary bands. As an early pluteus *O. papillosa* resembles *Ophiacantha diplasia*, but can be distinguished by its single body rods, which are double (body rod and recurrent rod) in *O.*

diplasia. In later pluteal stages the posterolateral arms are not markedly longer than the others, and postoral and posterodorsal arms shorter in height than the anterolateral arms. The larval arms may, at their distal end, bend outward except those of the anterolateral arms may point medially (Fig. 3.14).

After approximately a month, the ophiopluteus develops into a vitellaria (Fig. 3.14D). The developing juvenile body at the center is white and may have a dark red gut with five lobes projecting into the interradial spaces. The ciliated band of the pluteus reorganizes into complete or partial transverse ciliated bands that are yellow in color and distributed in four locations from anterior or posterior (Fig. 3.14D, D'). The anterior most ciliated band wraps around the distal ends of the remnants of larval arms. The juvenile arms are multisegmented and develop folded toward the center of the oral side.

Similar Species. As an ophiopluteus, *O. papillosa* resembles *Ophiacantha diplasia* (see above). There is only one other vitellaria that occurs in this region that *O. papillosa* could be confused with, that of *Amphioplus* sp. vitellaria (Section 3.4.3.6), which differs from *O. papillosa* in several ways. First, the vitellaria of *O. papillosa* has a mostly transparent epidermis with yellow-green coloration at the ciliated bands. Vestiges of the pluteus skeleton are visible as two rods bearing loops of ciliated band projecting from the anterior end of the vitellaria (Fig 14D), a feature absent in *Amphioplus* sp. vitellaria.

Development Mode. *Ophiopteris papillosa* has a feeding ophiopluteus that transforms into a nonfeeding vitellaria before metamorphosis into a juvenile. This type of development is known previously from *Ophiocomella pumila* (Cisternas & Byrne, 2005).

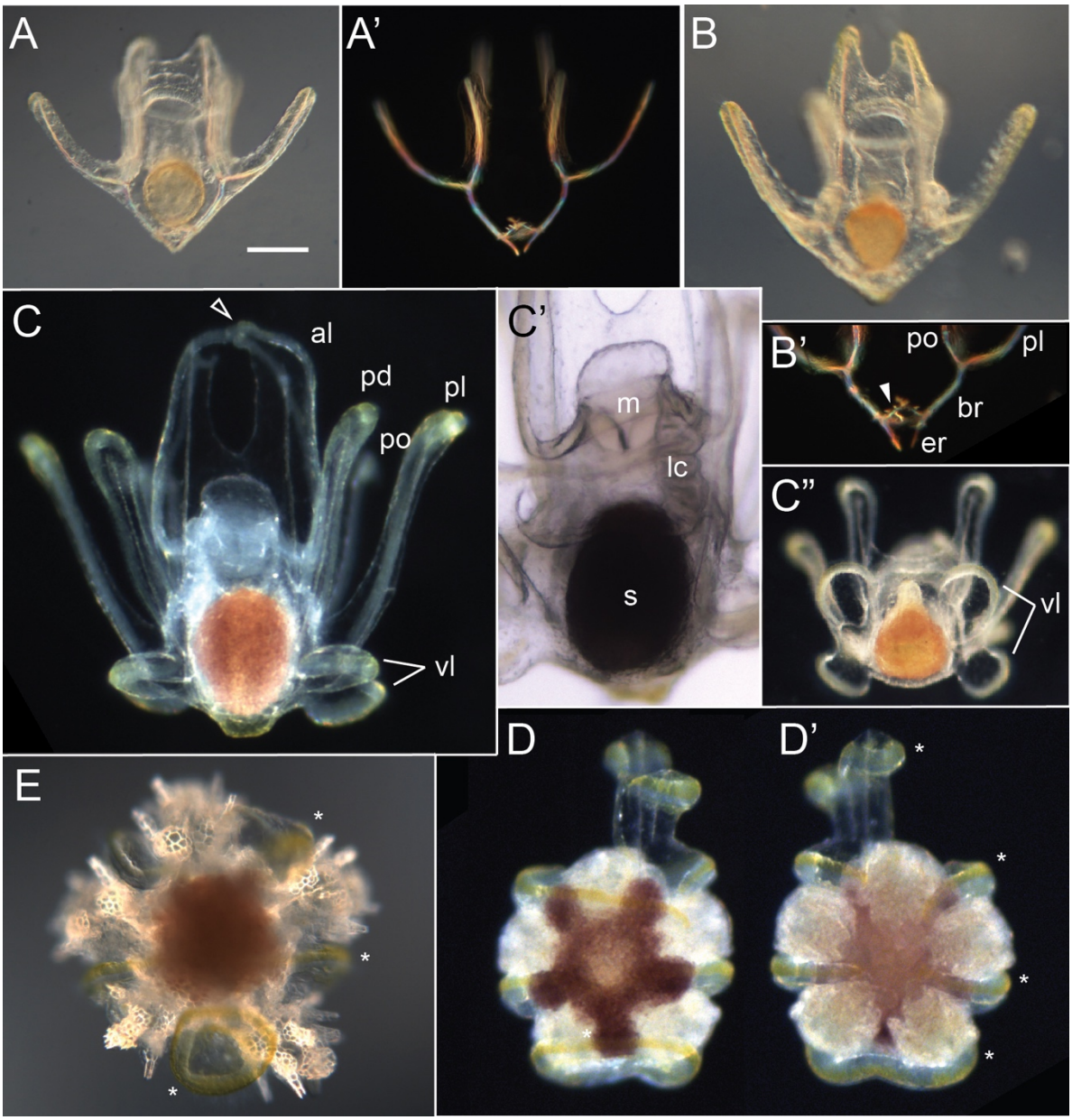


Figure 3.14. Ophioplutei, vitellaria, and juvenile of *Ophiopteris papillosa*. Secondary and tertiary views of the same individual are marked with an apostrophe (') and quotation mark ("), respectively. (A) An oral view in DIC optics and (A') partially polarized light which highlights the larval skeleton of six-armed pluteus at time of collection and (B) eight days later. (B') The posterior girdle in cross-polarized light with postoral (po) and posterolateral (pl) arm rods, thorny transverse rods (filled arrowhead), body rods (br), and end rods (er). (C) The advanced eight-arm pluteus shown in aboral view has anterolateral (al), posterodorsal, postoral, and posterolateral arms, and vibratile lobes (vl). Note the way the portion of ciliated band at the distal end of the anterolateral arm (open arrowhead) bends medially. (C') An oral view of the same individual shows the mouth (m), stomach (s), and lobed left coelom (lc). (C") The vibratile lobes are shown in a posterior view. (D) The eight-armed ophiopluteus develops into a vitellaria, shown in aboral and (D') oral view. (E) An early juvenile, still bearing remnants of the yellow ciliated bands (*). The scale bar in A is 100 μm and applies to B-E.

Ophiopteris antipodum is inferred to have planktotrophic development from egg size (Naughton et al., 2014). It is possible that *O. antipodum* also has an ophiopluteus followed by a vitellaria stage like *O. papillosa*.

Juvenile Description. The juvenile of this species develops from the vitellaria larva. The developing arms are folded between the ciliated bands of the vitellaria on the oral side. The juvenile may be motile while still bearing remnants of the ciliated bands.

Reproductive Timing. We found a small number of 8-armed ophioplutei in the winter of 2015, from November to February, and rarely in April (Fig. 3.3).

Planktonic Duration. We estimated planktonic duration as 65 days for a single larva collected from the plankton as a young ophiopluteus and raised in the laboratory on a mixed microalgal diet.

3.4.5. Ophiuridae MÜLLER & TROSCHEL 1840

We observed the ophioplutei of four species from the family Ophiuridae, all of which were identified using DNA barcoding (Fig. 3.15, Table 3.7). The family has 65 described species (O'Hara et al., 2017), and is dominated by the genus *Ophiura*, three of which are known from the northeastern Pacific and documented here: *O. leptoctenia*, *O. luetkenii* and *O. sarsii* (Lambert & Austin, 2007). The final species from the family we observed was *Ophiocten hastatum*, a deep-water species that occurs at depths of 1000 m and greater.

3.4.5.1. *Ophiocten hastatum* LYMAN 1878

Species Identity. We observed a single larva from this species, collected on January 19, 2015. The large ophiopluteus (Fig. 3.2L) was a species-level match at the *COI* locus with two adult sequences from GenBank (Fig. 3.15, Table 3.7).

Distribution and Local Sites. *Ophiocten hastatum* has a cosmopolitan distribution at bathyal to abyssal depths, occurring at over 2000 m depth in the NE Atlantic, 1408–2877 m in tropical eastern Pacific, 916–2877 m in British Columbia and Washington (Gage et al., 2004; Kyte, 1969; Lütken & Mortensen, 1899).

Embryonic Description. We did not observe the embryos of these species.

Larval Description. The larva we collected was a large, eight-armed ophiopluteus and obligately planktotrophic. The larval arms were long and bilaterally asymmetric in length, especially the posterolateral arms. This asymmetry could be due to earlier damage. Reddish brown coloration on the posterolateral arms approximately halfway up the length and at the distal tenth (of one arm), distinguish this larva from all other ophioplutei we collected (Fig. 3.2L). The posterior girdle is supported by end rods, relatively short body rods, and recurrent rods of the same length, the oral and aboral pairs of which lie largely over one another so it's difficult to see both pairs (Fig. 3.16J).

Similar Species. The advanced eight-armed ophiopluteus of *O. hastatum* is not easily confused with any of the other plutei we observed, but the presence of recurrent rods may be used to distinguish it from other large ophioplutei in this study.

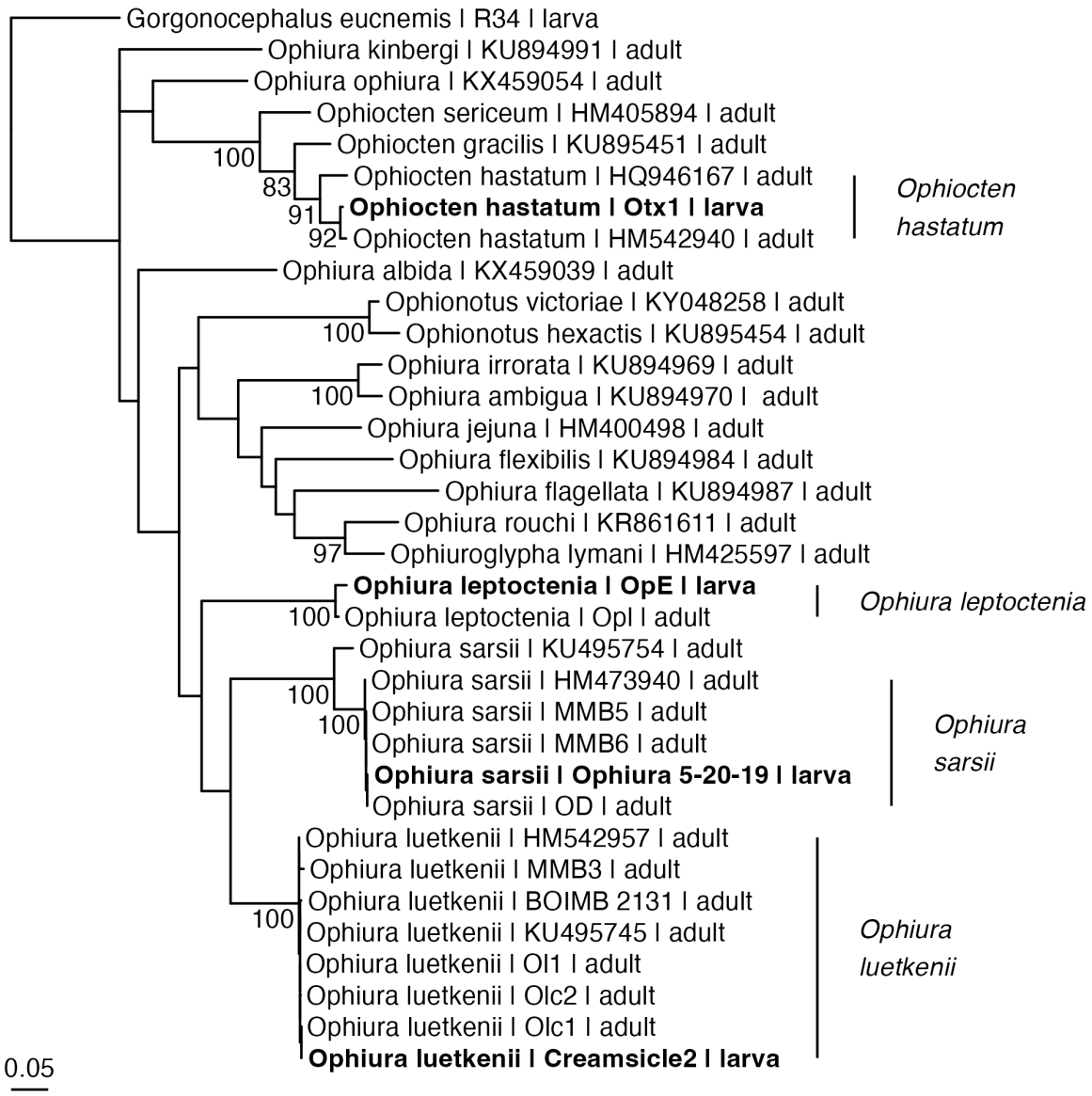


Figure 3.15. Maximum likelihood tree for a concatenated dataset of *16S* and *COI* sequences for Ophiuridae spp. Barcodes for larvae identified in this study are in bold.

Table 3.7. Specimen information for Ophiuridae spp. included in Fig. 3.15. Additional information for specimens collected in this study can be accessed using the BOLD Project IDs below.

Species	Specimen Code	Locus	BOLD[B] or GenBank[G]	Collection Locality	Reference / Field ID

Ophiocten gracilis	KU895451	*COI*	KU895451[G]	Ireland	(Hugall et al., 2016)12/20/23 3:47:00 PM
Ophiocten hastatum	HM542940	*COI*	HM542940[G]	British Columbia, Canada	(Corstorphine, 2011)
Ophiocten hastatum	HQ946167	*COI*	HQ946167[G]	Australia	iBOL[1], unpublished
Ophiocten hastatum	Otx1	*16S, COI*	OLAB072-23[B]	Charleston, OR	This study
Ophiocten sericeum	HM405894	*COI*	HM405894[G]	Prince of Wales Strait, Canada	(Corstorphine, 2011)
Ophionotus hexactis	KU895454	*COI*	KU895454[G]	Antarctica	(Hugall et al., 2016)
Ophionotus victoriae	KY048258	*16S*	KY048258[G]	Antarctica	(Galaska et al., 2017)
Ophiura albida	KX459039	*COI*	KX459039[G]	North Sea	(Laakmann et al., 2017)
Ophiura ambigua	KU894970	*COI*	KU894970[G]	Antarctica	(Hugall et al., 2016)
Ophiura flagellata	KU894987	*COI*	KU894987[G]	Australia	(Hugall et al., 2016)
Ophiura flexibilis	KU894984		KU894984[G]	Antarctica	(Hugall et al., 2016)
Ophiura irrorata	KU894969		KU894969[G]	Australia	(Hugall et al., 2016)
Ophiura leptoctenia	Opl	*16S, COI*	OOPH051-23[B]	Cape Arago Shelf, OR	This study
Ophiura leptoctenia	OpE	*16S, COI*	OLAB094-23[B]	Charleston, OR	This study
Ophiura luetkenii	BOIMB-2131	*COI*		Cape Arago Shelf, OR	G. Paulay, personal communication
Ophiura luetkenii	Creamsicle2	*16S*	OLAB095-23[B]	Charleston, OR	This study
Ophiura luetkenii	HM542957	*COI*	HM542957[G]	British Columbia, Canada	(Corstorphine, 2011)
Ophiura luetkenii	KU495745	*COI*	KU495745[G]	British Columbia, Canada	(Corstorphine, 2011)
Ophiura luetkenii	MMB3	*COI*	OOPH042-23[B]	Cape Arago Shelf, OR	This study
Ophiura luetkenii	Ol1	*16S*	OOPH008-18[B]	Cape Arago, OR	Ol1
Ophiura luetkenii	Olc1	*16S*	OOPH010-18[B]	Catalina Is., CA	Olc1
Ophiura luetkenii	Olc2	*16S*	OOPH011-18[B]	Catalina Is., CA	Olc2
Ophiura ophiura	KX459054	*COI*	KX459054[G]	North Sea	(Laakmann et al., 2017)
Ophiura rouchi	KR861611	*COI*	KR861611[G]	Antarctica	(Sands et al., 2015)
Ophiura sarsii	HM473940	*COI*	HM473940[G]	British Columbia, Canada	(Corstorphine, 2011)

Ophiura sarsii	KU495754	*COI*	KU495754[G]	Baffin Bay, Canada	(Corstorphine, 2011)
Ophiura sarsii	MMB5	*16S, COI*	OOPH043-23[B]	Cape Arago Shelf, OR	This study
Ophiura sarsii	MMB6	*16S, COI*	OOPH044-23[B]	Cape Arago Shelf, OR	This study
Ophiura sarsii	OD	*16S, COI*	OOPH016-18[B]		This study
Ophiura sarsii	Ophiura 5-20-19	*COI*	OLAB096-23[B]	Charleston, OR	This study
Ophiuroglypha lymani	HM425597	*COI*	HM425597[G]	Antarctica	iBOL[1], unpublished

[1]International Barcode of Life

Development Mode. The larva we found confirms that *Ophiocten hastatum* is planktotrophic, which was suggested by small size of oocytes seen in histological sections of the gonads of adults from the Porcupine Abyssal Plain in the north Atlantic (Gage et al., 2004). The timing of observation of this larva in the Pacific differs somewhat from spawning phenology in the Atlantic, where spawning is inferred for late winter and planktotrophic development until settlement in summer (Gage et al., 2004). Two congeners, *O. gracilis* and *O. sericeum*, also have feeding ophioplutei (Gage & Tyler, 1981; Thorson, 1934; Tyler & Gage, 1982, 1980).

Juvenile Description. We did not observe the juvenile of this species.

Reproductive Timing. We observed this larva just once, on January 18, 2015, a warm water year.

Planktonic Duration. Unknown.

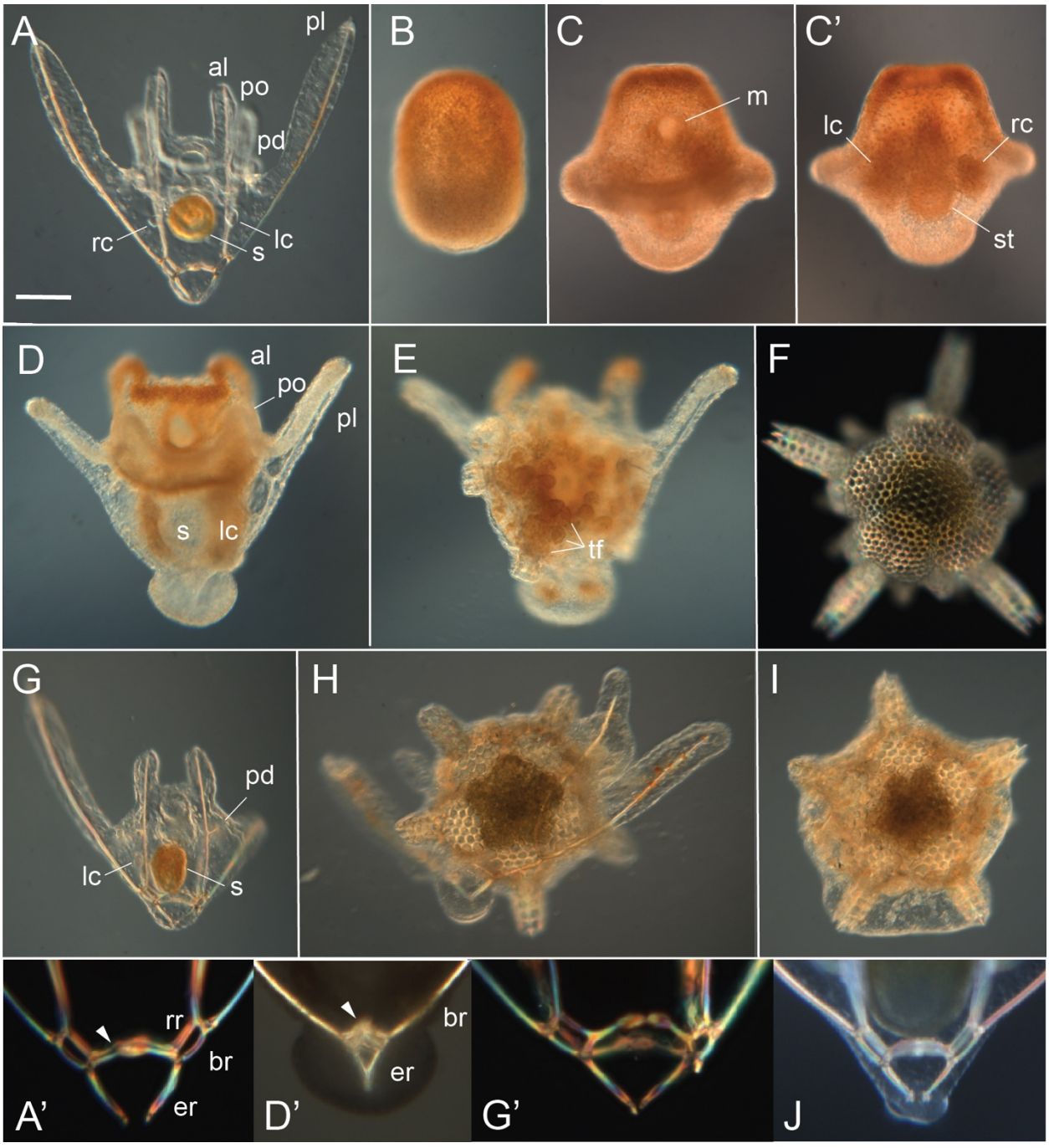


Figure 3.16. Ophioplutei of four species of Ophiuridae: (A) *Ophiura leptoctenia*, (B–F) *O. luetkenii*, (G–I) *O. sarsii*, and (J) *Ophiocten hastatum*. Secondary views of the same individual are marked with an apostrophe (’). (A) *Ophiura leptoctenia,* (B–F) *O. luetkenii*, (G–I) *O. sarsii*, and (J) *Ophiocten hastatum*. Scale bar in A is 100 µm; same scale for A–I. (A) Oral view of eight-armed ophiopluteus of *O O. leptoctenia* in transmitted light has posterodorsal arms (pd), and small right and left coeloms (rc, lc). (A’) The posterior girdle in cross-polarized light, with transverse rods (filled arrowhead), recurrent rods (rr), body rods (br) and end rods (er). (B) Oral views of *O. luetkenii* at the gastrula, (C) early pluteus, and (D) six-armed reduced pluteus stages. The reduced pluteus has

anterolateral (al), postoral (po), and posterolateral (pl) arms, a stomach (s), and left coelom (lc). (D') The posterior girdle in partial cross-polarized light shows the transverse rods (filled arrowhead), body rods (br), and end rods (er). (E) The rudiment-stage larva has a star-shaped juvenile rudiment with five tubefoot buds (tf) on each radius, with two pairs leading proximally to the mouth and a single terminal podium. (F) The juvenile of *O. luetkenii* in cross-polarized light, aboral view. Note the honeycomb pattern of the primary plates and disc plate. (G) Pluteus of *O. sarsii*, aboral view, and (H) aboral view of a rudiment-stage pluteus, and (I) it's juvenile a day later. (J) The posterior girdle of *Ophiocten hastatum* is of the same individual as Fig. 3.2L.

3.4.5.2. *Ophiura leptoctenia* H. L. CLARK 1911

Species Identity. We observed a single larva on May 8, 2019, differed by 0.9% at the *COI* locus to an adult specimen collected from the continental shelf off Oregon (Fig. 3.15; Table 3.7).

Distribution and Local Sites. *Ophiura leptoctenia* was characterized by Clark (1911) as one of the most common ophiuroid species in the north Pacific, and they occur in large numbers in off California (Astrahantseff & Alton, 1965). This species has a bathyal depth range, 122-3239 m (Hendler, 1996). We collected one adult from the Cape Arago shelf at 120 m depth.

Embryonic Description. We did not observe the embryos of these species.

Larval Description. The advanced ophiopluteus of *O. leptoctenia* has eight arms, and a transparent epidermis that appears inflated due to the distance from the skeletal rods. The epidermis tapers at the distal tip of the posterolateral arms. The skeletal rods lack thorns and curve slightly toward the midline (Fig. 3.16A). The body rods are short and there are ventral and dorsal recurrent rods (Fig. 3.16A').

Similar Species. The ophiopluteus of *O. leptoctenia* is very similar to that of *O. sarsii*. Both have eight arms, an inflated transparent epidermis, and a similarly structured posterior girdle with short body rods and recurrent rods. The pluteus of *Amphipholis pugetana* also has an

inflated epidermis, but it is of a uniform width along the length of the arm and terminates with red pigmentation at the distal ends of the posterolateral arms. The epidermis around the posterolateral arms of *O. leptoctenia* is widest at approximately halfway the length, and then tapers to a dull point at the distal end.

Development Mode. To our knowledge, this is the first report of the larva of *O. leptoctenia*. Hendler (1996) reported an egg diameter of ca. 0.2 mm and suggested the species had either abbreviated development or an ophiopluteus. The transparent epidermis, small stomach, and lack of a developing rudiment suggest to us that this species is planktotrophic.

Juvenile Description. We did not observe the juvenile of *O. leptoctenia*.

Reproductive Timing. We observed a single larva on May 8, 2019, an El Niño year.

Planktonic Duration. Unknown.

3.4.5.3. *Ophiura luetkenii* (LYMAN 1860)

Species Identity. DNA barcoding revealed that this white and orange colored larva is that of *Ophiura luetkenii*, which is closely related to *O. sarsii* (Fig. 3.15, Table 3.7).

Distribution and Local Sites. *Ophiura luetkenii* occurs in large aggregations across its range, including off the Oregon coast (Hemery et al., 2018; Henkel et al., 2014) on the continental shelf off Cape Arago. We have collected in large numbers of adults along with those of its congener *O. sarsii*.

Embryonic Description. O. luetkenii spawned in the laboratory on one occasion. After repeat flipping adults under bright lights at room temperature, three females spawned

approximately eight hours later in the early evening; we fertilized the eggs with sperm dissected from a male.

The earliest stage we have observed from the plankton is the gastrula, which is ovoid and dorsoventrally flattened. The gastrula is orange with deeper color at the anterior lightening to the posterior.

Larval Description. *Ophiura luetkenii* has a reduced pluteus. The early pluteus has posterolateral arms, a mouth, and visible left and right coeloms (Fig. 3.16C, C'). At the six-armed stage the pluteus the posterolateral arms are translucent white, and the body region is orange.

The places where the posterolateral and anterolateral arms meet are elevated towards the anterior of the larva compared to other ophioplutei. At the posterior end the epidermis is distinctively swollen around the body and end rods (Fig. 3.16D, E). The larva is large and develops into a juvenile in about seven days.

Similar Species. The orange body and white arms of the larvae of *O. luetkenii* give it a superficial resemblance to that of *Amphiodia* sp. orange belly, and even certain individuals of *Amphiodia* sp. opaque. However, the swollen posterior end of the larva of *O. luetkenii*, and the resulting juvenile should all clue the reader in to its identity.

Development Mode. *Ophiura luetkenii* has a reduced pluteus that may also be facultatively planktotrophic (Nakata and Emlet, unpublished data).

Juvenile Description. The juvenile is large and has elongate terminal arm segments, approximately one third the length of the diameter of the juvenile disc (Fig. 3.16F). Podia are papillate.

Reproductive Timing. We observed the embryos and larvae of *O. luetkenii* in February and March.

Planktonic Duration. Approximately 9 days, based on larvae raised in the laboratory at 15°C (N. Nakata, personal observation).

3.4.5.4. *Ophiura sarsii* LÜTKEN 1855

Species Identity. We identified the larva of *O. sarsii* from DNA barcoding and by comparing our sequences to GenBank and to those of adults of the Oregon coast (Fig. 3.16G, Table 3.7).

Distribution and Local Sites. *Ophiura sarsii* occurs throughout the Arctic Ocean, the northern Atlantic, and in Pacific, from the Bering Sea to California, Japan and Korea, from 0–1460 meters (Lambert & Austin, 2007). Adults occur in large aggregations on the continental shelf off the Cape Arago and Columbia River (Astrahantseff & Alton, 1965) on muddy bottoms.

Given the abundance of adults we are surprised that we have collected only two larvae of *O. sarsii.*

Embryonic Description. We did not observe the embryos of these species.

Larval Description. We collected two larvae on May 20, 2019, including a six-armed pluteus and a rudiment-stage larva of *O. sarsii* (Fig. 3.16F–H). The ophiopluteus (Fig. 3.16F) has a transparent epidermis and eight arms. The individual we observed had not fully developed the posterodorsal arms, but the skeletal rods are visible on the dorsal side.

The body rods are short and bear long end rods that are wide apart at their origin and nearly touching at their distal ends. Images of ophioplutei of *O. sarsii* prior to rudiment

formation suggest that this arrangement occurs to accommodate the juvenile rudiment and that the long end rods are closer to one another at the proximal sides in the ophiopluteus (Thorson, 1934). There may also be variation in the transverse rods.

In the rudiment-stage larva the right anterolateral and postoral arms had been resorbed and there was a developing juvenile rudiment (Fig. 3.16H). The larva bore a striking resemblance to Mortensen's (1921) *Ophiopluteus fusus*. The remnants of the larval body are transparent in color, with a small number of red to orange pigment cells occurring along the skeleton of the posterolateral arms.

Similar Species. The pluteus of *O. sarsii* is very similar to that of *O. leptoctenia* (see section above, Fig. 3.16A, G).

Development Mode. *Ophiura sarsii* develops via a small egg of 100–110 μm diameter that gives rise to a planktotrophic ophiopluteus (Dautov & Selina, 2009; Kungurtzeva & Dautov, 2001; Strathmann, 1987).

Juvenile Description. The juvenile of *O. sarsii* is very similar to that of *O. luetkenii* in skeletal structure (Fig. 3.16I, F), but was tan in color in the single specimen we observed, compared to the orange coloration of *O. luetkenii*.

Reproductive Timing. The two larvae we observed, including the rudiment-stage ophiopluteus on May 20, 2019, is in accordance with published accounts of spawning period occurring from March to June (Strathmann, 1987).

Planktonic Duration. At least 26 days (R. Emlet, unpublished data).

3.4.6. Gorgonocephalidae LJUNGMAN 1867

3.4.6.1. *Gorgonocephalus eucnemis* (MÜLLER & TROSCHEL 1842)

Species Identity. The genus *Gorgonocephalus* is comprised of ten species with an anti-tropical distribution. Five species occur in the northern hemisphere: *eucnemis* and *diomedeae* in the north Pacific; *arcticus* in the Arctic; and *lamarcki*, *caputmedusae* in the north Atlantic (D'yakonov, 1967). *Gorgonocephalus eucnemis* represents several synonymies: *G. caryi*, *japonica,* and *stimpstonii* (Clark, 1911). Our own analysis shows several species within the genus to be closely related at the *COI* locus (within 6%; Fig. 3.17, Table 3.8). The sequences from our larvae were identical to that of an adult we collected off Cape Arago and another from California. The clade formed by our specimens was divergent from another clade of *G. eucnemis* from the west coast of Canada and Asia (clades 1, 2 in Fig. 3.17).

Distribution and Local Sites. *Gorgonocephalus eucnemis* is known to occur in the North Atlantic and in the eastern Pacific from the Bering Sea south to Laguna Beach, CA at depths of 8-1850 m (Austin & Hadfield, 1980; Lambert & Austin, 2007). In the northeast Pacific *G. eucnemis* occurs from Alaska to California, from the shallow subtidal to 1240 meters (Lambert & Austin, 2007). No other basket stars occur in our region; though a congener, *G. diomedeae,* occurs in Panama.

Embryonic Description. Eggs are approximately 0.22 mm diameter and give rise to nonfeeding embryos and juveniles (Emlet, unpublished data). The development has been described previously under the synonymized name *G. caryi* (Patent, 1970). The embryos of *G. eucnemis* are pear-shaped (Fig. 3.18A) and have a thick hyaline layer with a textured appearance

like that of translucent spheres (Fig. 3.18B'). The peach to salmon colored embryo is unciliated and negatively buoyant. Embryos develop into pentaradial juveniles within a week and have five arm buds by three days post spawn, and complete pentaradial symmetry by day four.

Juvenile Description. Juveniles have five lobate arms bearing symmetrical claws at their distal ends. The pink to beige epidermis is still semi-transparent, and one can see the juvenile skeleton (Fig. 3.18E). The arms of early juveniles of *Gorgonocephalus* that have not yet begun to branch are thought to associate with the soft coral *Gersemia rubiformis* (Austin & Hadfield, 1980).

Similar Species. The early juvenile of *Amphiodia periercta?* is also pink in color, unciliated, and is dorsal-ventrally flattened. However, the early juvenile of *G. eucnemis* can be distinguished by its textured hyaline layer, which is smooth in *Amphiodia*, and the pear shape of the embryo. The juvenile can be distinguished from those of other ophiuroids in the region by the absence of stereom in the central disc.

Development Mode. Freely spawned, fertilized eggs give rise to nonfeeding embryos and juveniles. Development is unknown for other members of *Gorgonocephalus*, but *G. caputmedusae* free-spawns eggs, but it is unknown if they develop into an ophiopluteus larva (Mortensen, 1927).

Reproductive Timing. We observed embryos and juveniles of *G. eucnemis* in the Charleston plankton a few times each year from late January to late March (Fig. 3.3).

Planktonic Duration. Approximately 5 days (Nakata and Emlet, unpublished data), similar to that found by (Patent, 1970).

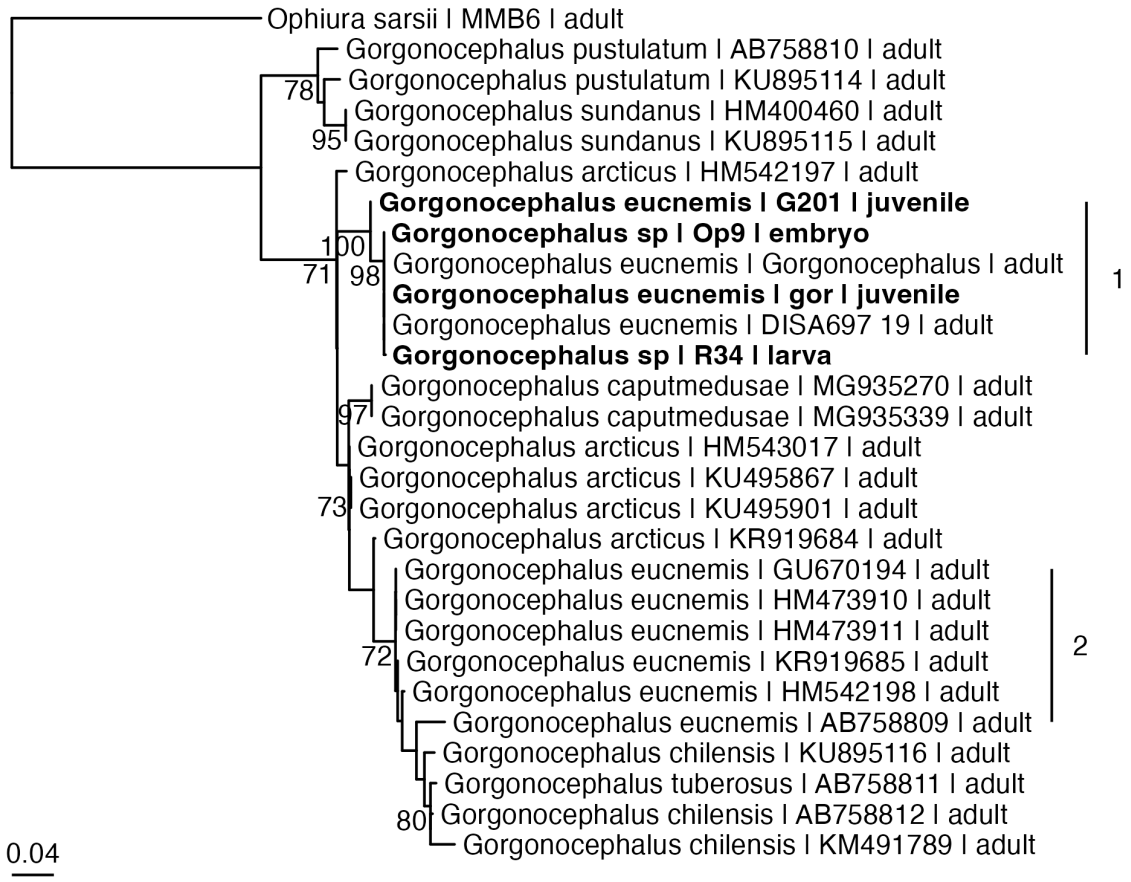


Figure 3.17. Maximum likelihood tree of *COI* for 27 spp. of *Gorgonocephalus* from the Pacific and the outgroup *Ophiura sarsii*. Barcodes of larvae identified in this study are in bold. Bootstrap values of 70 and higher are shown beside nodes.

Table 3.8. Specimen information for *Gorgonocephalus* spp. included in Fig. 3.17. Additional information for specimens collected in this study can be accessed using the BOLD Project IDs below.

Species	Specimen Code	Locus	BOLD[B] or GenBank[G]	Collection Locality	Reference
Gorgonocephalus arcticus	HM542197	*COI*	HM542197[G]	New Brunswick, Canada	(Corstorphine, 2011)
Gorgonocephalus arcticus	HM543017	*COI*	HM543017[G]	Nunavut, Canada	(Corstorphine, 2011)
Gorgonocephalus arcticus	KR919684	*COI*	KR919684[G]	Korea	(Kim & Shin, 2015)
Gorgonocephalus arcticus	KU495867	*COI*	KU495867[G]	Baffin Bay, Canada	(Corstorphine, 2011)

Gorgonocephalus caputmedusae	MG935270	*COI*	MG935270[G]	Sweden	Lundin et al., unpublished
Gorgonocephalus caputmedusae	MG935339	*COI*	MG935339[G]	Sweden	Lundin et al., unpublished
Gorgonocephalus chilensis	AB758812	*COI*	AB758812[G]	Antarctica	(Okanishi & Fujita, 2013)
Gorgonocephalus chilensis	KM491789	*COI*	KM491789[G]	Antarctica	(Summers et al., 2014)
Gorgonocephalus chilensis	KU895116	*COI*	KU895116[G]	New Zealand	(Hugall et al., 2016)
Gorgonocephalus eucnemis	DISA697-19	*COI*	DISA697-19[B]	San Diego, CA	DISCO MBC LACM, unpublished
Gorgonocephalus eucnemis	G201	*COI*	OLAB104-23[B]	Charleston, OR	This study
Gorgonocephalus eucnemis	gor	*COI*	OLAB105-23[B]	Charleston, OR	This study
Gorgonocephalus eucnemis	Gorgono-cephalus	*COI*	OOPH055-23[B]	Cape Arago Shelf, OR	This study
Gorgonocephalus eucnemis	GU670194	*COI*	GU670194[G]	Queen Charlotte Is., Canada	(Corstorphine, 2011)
Gorgonocephalus eucnemis	HM473910	*COI*	HM473910[G]	Queen Charlotte Is., Canada	(Corstorphine, 2011)
Gorgonocephalus eucnemis	HM542198	*COI*	HM542198[G]	British Columbia, Canada	(Corstorphine, 2011)
Gorgonocephalus eucnemis	KR919685	*COI*	KR919685[G]	South Korea	(Kim & Shin, 2015)
Gorgonocephalus eucnemis	Op9	*COI*	OLAB066-23[B]	Charleston, OR	This study
Gorgonocephalus eucnemis	R34	*COI*	OLAB067-23[B]	Charleston, OR	This study
Gorgonocephalus pustulatum	AB758810	*COI*	AB758810[G]	New Zealand	(Okanishi & Fujita, 2013)
Gorgonocephalus pustulatum	KU895114	*COI*	KU895114[G]	New Zealand	(Hugall et al., 2016)
Gorgonocephalus sundanus	HM400460	*COI*	HM400460[G]	Australia	iBOL[1], unpublished
Gorgonocephalus sundanus	KU895115	*COI*	KU895115[G]	Australia	(Hugall et al., 2016)
Gorgonocephalus tuberosus	AB758811	*COI*	AB758811[G]	Antarctica	(Okanishi & Fujita, 2013)
Gorgonocephalus. arcticus	KU495901	*COI*	KU495901[G]	Baffin Bay, Canada	(Corstorphine, 2011)
Gorgonocephalus. eucnemis	AB758809	*COI*	AB758809[G]	Japan	(Okanishi & Fujita, 2013)
Gorgonocephalus. eucnemis	HM473911	*COI*	HM473911[G]	Queen Charlotte Is., Canada	(Corstorphine, 2011)

[1]International Barcode of Life (iBOL)

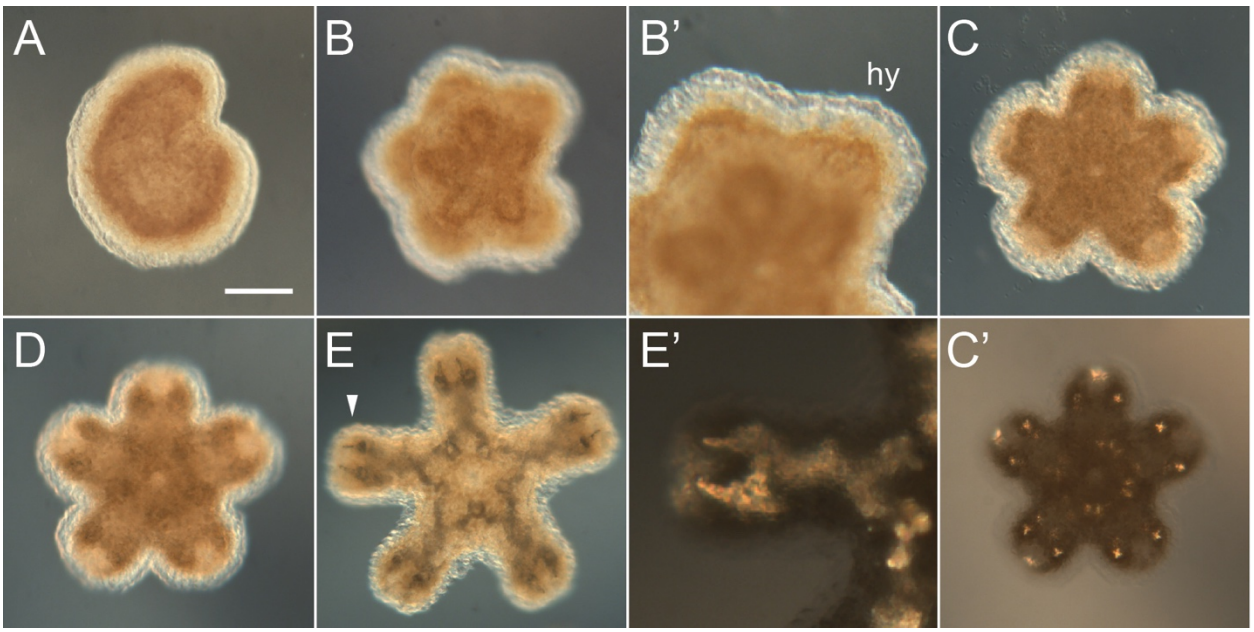


Figure 3.18. Planktonic stages of *Gorgonocephalus eucnemis.* Secondary views of the same individual are marked with an apostrophe (’). (A) Embryo, lateral view; (B) early juvenile, oral view; (C) one and (D) two days later. (B’) Closeup view of hyaline layer with bubbled appearance. (E) Another individual with mobile arms tipped with hooks (filled arrowhead), also shown in cross-polarized light. Scale bar is 100 µm, same scale for A-E.

4. CONCLUSIONS

We observed planktonic developmental stages of 18 species from seven families of brittle stars from the southern Oregon coast and identified them using DNA barcoding. The planktonic stages we observed included embryos, ophioplutei, reduced plutei, vitellaria larvae, pelagic direct developers, and juveniles; for most of these developmental stages we were able to identify morphological features that can be used to identify them to species. We include a key, species-level descriptions, and photo plates to explain how these species can be recognized.

5. KEYS TO THE OPHIUROID PLANKTONIC FORMS OF THE NORTHEAST PACIFIC OCEAN

The keys below can be used to identify the planktonic ophiuroid forms of the northeast Pacific.

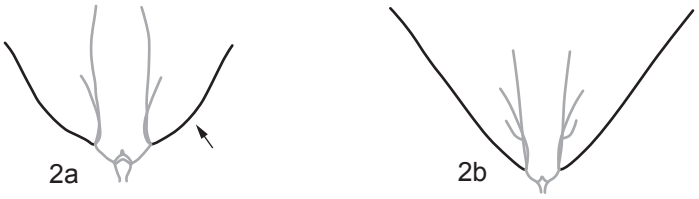
The keys are divided into ophioplutei (5.1), nonfeeding forms (5.2), and juveniles (5.3).

5.1. Key to the ophioplutei of the NE Pacific

1a. Ophiopluteus with four to eight arms; larval epidermis transparent, such that skeletal rods are visible through the epidermis under transmitted light; pigmentation is restricted to the arms, and may occur at the distal ends or along the length of the posterolateral arms … planktotrophic ophioplutei, 2

1b. Ophiopluteus with four to six arms; epidermis orange to pink in color and opaque enough to obscure the skeletal rods under transmitted light … reduced plutei, 12

2a. Posterolateral (pl) arms are curved … 3
2b. Posterolateral arms are more or less straight … 8



3a. Recurrent rods present … 4
3b. Recurrent rods absent … 6

4a. Ventral and dorsal pairs of recurrent rods are slightly offset so that both pairs are visible. Recurrent rods articulate with transverse rods at approximately half their length. Ophiopluteus has a transparent epidermis, sometimes with yellow to green pigmentation at

the distal tips of the larval arms. The pluteus has a transparent epidermis with yellow-green pigmentation at the distal tips of the arms … young *Ophiacantha diplasia*

4b. Ventral and dorsal pairs of recurrent rods are of the same length and position so that one pair obscures the other. Recurrent rods connect with the transverse rods near their connection point with the body and end rods. The pluteus has a transparent epidermis that has an inflated appearance, that is, there is an obvious gap between the epidermis and the skeletal rods, especially the posterolateral and anterolateral arms; the stomach may be colored by partially consumed microalgae … *Ophiura leptoctenia* or *O. sarsii**

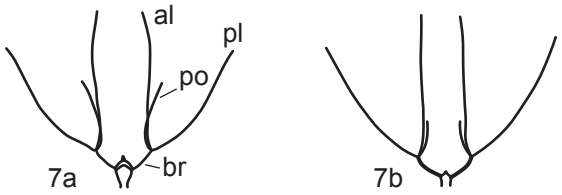
*We caution the reader that we observed each of these species from only one or two larvae. Therefore, we cannot make comparisons between species across ontogeny. I.e., young *O. hastatum* may be very similar in appearance to the specimens we observed of *O. leptoctenia* and *O. sarsii*.

6a. Ophiopluteus has a transparent epidermis with yellow-green pigmentation at the tips of the arms … young *Ophiopteris papillosa*

6b. Ophiopluteus with a mostly transparent epidermis but may have red, orange, or yellow pigmentation, especially on the posterolateral arms … 7

7a. Arm epidermis is close to the larval skeleton; skeletal rods maybe a red-orange color; anterolateral arms curved and bearing thorns; postoral arms at least twice the length of the body rods; thorns present all sets of arms, but are longest on the proximal side of the posterolateral arms; red pigmentation restricted to the distal tips of the posterolateral arms or absent … *Amphiodia urtica*

7b. Arm epidermis has an inflated appearance; skeletal rods, especially the posterolateral arms and body rods, may be red-orange in color; anterolateral arms straight to curved in advanced plutei; anterolateral arms without thorns; postoral arms approximately the same length as the body rods; red pigmentation begins at the distal tips of posterolateral arms and usually accumulates across the length of the posterolateral arms as the larva develops … *Amphipholis pugetana*



8a. Ophiopluteus is flat, with a silhouette like an inverted triangle … 9

8b. Ophiopluteus is boxy, with arms of similar heights separated by vibratile lobes. The posterolateral arms are relatively short and are held at a narrow angle, creating a rectangular silhouette like that of some echinoplutei … 11

9a. Recurrent rods absent … 10

9b. Recurrent rods present. The posterolateral arms of the advanced ophiopluteus are much longer than the other arms and may have bands of reddish-brown pigmentation … *Ophiocten hastatum**

10a. Posterolateral arms of the late pluteus are up to five times the length of other arms and are so wide that they are almost horizontal. If present, there is a single median process … *Ophiothrix spiculata*
10b. Posterolateral arms do not exceed three times the length of the other arms, and do not exceed a width of 90°. If present, there are two median processes … *Ophiopholis* spp.

11a. Recurrent rods present. Pluteus keeps most of the larval arms when forming a juvenile rudiment … late *Ophiacantha diplasia*
11b. Recurrent rods absent. The skeletal rods of the anterolateral arms are straight, but the ciliated band bends medially at the distal ends, often touching. The pluteus develops into a vitellaria prior to metamorphosis … late *Ophiopteris papillosa*

12a. Body rods long, of length equal to or greater than the short straight posterolateral arms; epidermis is orange on the body and white on the posterolateral arms; the junction where the posterolateral arms and body rods meet is at about the height of the mouth … *Ophiura luetkenii*
12b. Body rods approximately one third the length of the posterolateral arms. Coloration of the epidermis either uniformly tan to salmon or orange with white arms; posterolateral arms with curved spines, especially on their inner side … 13

13a. Epidermis uniformly tan to salmon … 14
13b. Epidermis on the body orange, white on the posterolateral arms … *Amphiodia* sp. orange belly

14a. Epidermis tan, pink, or salmon in color. Body rods and posterolateral rods are both straight (but may be curved in some larvae). Posterolateral arms are wideset, making an angle that is 90° or wider and form a continuous line with the body rods. Posterolateral and anterolateral arm rods often with thorns … *Amphiodia* sp. opaque
14b. Epidermis tan in color. Posterolateral arms are held at a narrow angle and bearing only minute thorns … *Amphiodia* sp. tan

5.2. Key to ophiuroid nonfeeding larvae

The nonfeeding larvae include pelagic direct developers and vitellaria larvae. The nonfeeding larvae are usually pink, salmon, or orange in color.

1a. Embryo with pentaradial symmetry … 2
1b. Symmetry is semi-bilateral or unclear … 4

2a. Embryo or juvenile with five arms … 3
2b. Embryo or juvenile approximately the shape of a flattened disc … 5

3a. Five arms covered with pink-orange epidermis … *Gorgonocephalus eucnemis*
3b. Five arms with multiple arm segments … *Ophiopholis* spp. or *Ophiothrix spiculata*
3c. Five arms with single segments … Amphiuridae, Ophiuridae

4a. Embryo is flattened and round … 5
4b. Embryo is ovoid and wider at one end than the other, with disjunct ciliary bands on the body and shelf-like ones on either end; center of body may have pentaradial symmetry and tube feet … vitellaria, 6

5a. Embryo with five rounded lobes, one of which may be orange to pink in color compared to tan in the others; embryo with a line of skeletal elements spanning an arc across the embryo; mouth centered … Amphiuridae sp. cross piece
5b. Embryo light pink in color, very flat with an uneven periphery; with a ring of skeletal elements around periphery of embryo; stomodeal pit not centered (until pentaradial symmetry is well established) … *Amphiodia periercta?*

6a. Vitellaria light pink in color with darkened ciliated bands. Ciliated bands five in number … *Amphioplus* sp. vitellaria
6b. Vitellaria that is colorless except for the ciliated bands which are yellow green … *Ophiopteris papillosa* (the vitellaria in this species is preceded by a feeding ophiopluteus, 6a)

5.3. Key to the juveniles

1a. Juvenile with arms not obviously segmented, covered in pink epidermis, and lacking tube feet but with a pair of distal claws … *Gorgonocephalus eucnemis*
1b. Juvenile with segmented arms … 2

2a. Juvenile arms with 2 or more segments … 3
2b. Juvenile arms with 1 segment (terminal arm plate) … 6

3a. Arm segments without hooked spines … 4
3b. A pair of hooked spines on the lateral sides of penultimate arm segment … 5

4a. Yellow pigmentation present at the distal arm segments … *Ophiacantha diplasia*
4b. Arms white throughout … *Ophiopteris papillosa*

5a. Penultimate arm segments bearing spines with one hook … *Ophiothrix spiculata*
5b. Penultimate arm segments bearing spines with two hooks … *Ophiopholis* spp.

6a. Terminal arm plates are triangular to cone shaped … Amphiuridae
6b. Terminal arm plates long and rectangular, comprised of three parallel skeletal rods bisected by regular rods that form a honeycomb pattern that echoes that of the disc … 7, Ophiuridae

7a. Larval body is orange … *Ophiura luetkenii*
7b. Larval body is transparent to tan … *Ophiura sarsii*

BRIDGE

In Chapter III, I described various planktonic stages of brittle stars in the northeast Pacific. These results beg for analysis of the evolution of development. In Chapter IV, I used comparative phylogenetic analyses to estimate the ancestral state of the Amphiuridae, a diverse family of brittle stars with planktotrophic ophioplutei, reduced plutei, vitellaria larvae, and pelagic direct developers. We derived character state data from the species accounts in Chapter III, published accounts in the literature, and unpublished data from Panama and Australia (R. Emlet, unpublished). To build a phylogenetic hypothesis we used sequence data derived from our DNA barcoding of brittle star larvae, local collections of adult specimens, and from specimens borrowed from museums to build a 39-spp. phylogenetic hypothesis of Amphiuridae.

CHAPTER IV

EVOLUTION OF LARVAL DEVELOPMENT IN THE BRITTLE STAR FAMILY AMPHIURIDAE

This work will include Dr. Richard Emlet as coauthor and contributor to the final manuscript preparation. It is written in the journal style of *Invertebrate Biology*.

1. INTRODUCTION

Developmental patterns are diverse in marine invertebrates and include production of larvae that feed in the plankton, planktonic larvae that do not feed and nonplankton development that may be brooded or develop in benthic capsules. These patterns represent differences in parental investment that result in tradeoffs in allocation to individual offspring and fecundity, the planktonic period and offspring mortality and they have consequences for dispersal. Remarkably, disparate strategies are known to occur amongst closely related species in many taxa: e.g., in sacoglossans (Krug et al., 2015), calyptraeid gastropods (Collin, 2004), barnacles (Ewers-Saucedo & Pappalardo, 2019), echinoids (Hart et al., 2011; Hoegh-Guldberg & Emlet, 1997), and ophiuroids (Hart & Podolsky, 2005). A large body of literature has sought to understand the ecological and evolutionary mechanisms that underlie transitions between these strategies (Collin & Moran, 2018; Strathmann et al., 2020). Transitions in developmental pattern from feeding to nonfeeding larvae are associated with increases in egg size, nurse eggs or extracellular materials

(Sewell & Young, 1997; Strathmann, 1978b). Comparative phylogenetic analytical methods based on molecules have been increasingly used to examine the evolutionary origin, loss, and potential regain of feeding planktonic larvae (planktotrophy). However, sequence data for marine invertebrate taxa are severely underrepresented in public databases, limiting our ability construct phylogenies to test evolutionary hypotheses even in widespread and abundant groups of macrofauna. In many groups of marine invertebrates, another limitation is lack of information on type of development.

Phylogenetic analyses are increasingly used to understand taxonomic organization and evolutionary history of marine invertebrates (e.g. Giribet & Edgecombe, 2019; O'Hara et al., 2017). Comparative phylogenetic analytical methods have been used increasingly to examine the evolutionary origin, loss, and potential regain of feeding planktonic larvae (planktotrophy) (Collin, 2004; Ewers-Saucedo & Pappalardo, 2019; Hart et al., 1997; Hart & Podolsky, 2005; Krug et al., 2015; Wray, 1996). However, sequence data for many marine invertebrate taxa are severely underrepresented in public databases, limiting our ability to test evolutionary hypotheses even in widespread groups of macrofauna.

Brittle stars (Echinodermata: Ophiuroidea, ca. 2100 spp.) are the most diverse echinoderm class and are prominent members of benthic marine environments worldwide (Stöhr et al., 2012). Their widespread distribution across ocean depths and habitats make ophiuroids a good group in which to test evolutionary hypotheses (Bribiesca-Contreras et al., 2017; O'Hara et al., 2019b). They have the potential for analysis of evolutionary patterns in reproduction to the diversity of developmental strategies exhibited by the class. Three broad developmental categories are recognized: brooding, feeding planktonic larvae, and abbreviated development. The latter group includes several larval forms including reduced plutei, vitellaria, and pelagic

direct developers (Allen & Podolsky, 2007; Hendler, 1975; O'Hara et al., 2019a; Selvakumaraswamy & Byrne, 2000). Ophiuroids have been found to have numerous instances of reduced plutei (Nakata, Ch. III) which may represent transitions from feeding to nonfeeding; understanding these occurrences in a phylogenetic context may help identify circumstances related to transitions in developmental strategy. While brooding occurs throughout echinoderm groups, it is particularly common in ophiuroids, so they are a good group in which to examine the loss (or gain) of planktonic development.

Few analyses of evolutionary patterns have been conducted for ophiuroids due to difficulties in obtaining gametes or identifying larvae to species (but see Hart & Podolsky, 2005; Lessios & Hendler, 2022; O'Hara et al., 2019a; Selvakumaraswamy & Byrne, 2004). DNA barcoding has proven to be an effective method for identifying wild-caught embryos and larvae and can greatly increase estimates of regional species and developmental diversity (Collin et al., 2019, 2020b; Hiebert & Maslakova, 2015; Maslakova et al., 2022) as well as help to test hypotheses about the evolution of development.

Here we present comparative phylogenetic analyses of developmental traits in the family Amphiuridae, a large family of brittle stars (ca. 457 spp., O'Hara et al. 2017) that burrows in soft sediments. This family has many developmental patterns, including brooding, planktotrophy via an ophiopluteus, facultative planktotrophy via a reduced pluteus, nonfeeding development via a vitellaria, and pelagic direct development (Emlet, 2006; Hendler, 1975, 1978, 1995; Hendler & Bundrick, 2001; Hendler & Littman, 1986; Mortensen, 1924; Nakata & Emlet, 2023; Stancyk, 1973). We compiled published data on larval development and added previously unpublished data on development from Oregon, Panama, and Australia. We acquired adult materia from museum collections and field collections and sequenced four loci to augment molecular sequence

data available in published databases. The family Amphiuridae needs taxonomic revision; a phylogenetic analysis based on exon-capture data found important genera in the family such as *Amphiura*, *Amphioplus*, and *Amphipholis* are polyphyletic in their current composition (O'Hara et al., 2018).

Using a 4-marker (*18S*, *28S*, *16S*, and *COI*) and 39-species phylogeny, we asked (i) what was the ancestral developmental strategy for the family, (ii) what is the direction of evolutionary transitions between developmental and larval states (and are they reversible), and (iii) did shifts in developmental mode occur with more frequency in certain clades of the tree?

2. METHODS

2.1. Egg size and developmental patterns

To infer developmental character states, we analyzed egg size for 37 spp. of Amphiuridae. We collected egg diameter data from the published literature, species accounts in Chapter III, and for seven additional species, data from collaborators (R. Emlet, unpublished data). The datasets for egg size and phylogenetic analysis differed in species composition but 18 species appear in both datasets (Table 4.1). Eggs were collected from free spawn of adults shortly after collection or were collected from plankton. We photographed eggs in transmitted light, and we measured egg diameter from the photos in Adobe Photoshop. For eggs spawned in the laboratory, a minimum of ten egg diameters were measured and the mean calculated; eggs from the plankton were usually few in number, only one to two individuals. Egg size data was used to infer developmental character states for seven species that were part of the phylogenetic analysis of developmental patterns.

Table 4.1. Egg size, developmental mode, and larval form for species included in the ancestral state reconstructions. Egg size is given as egg diameter. Development modes are as follows: brooder (Br), planktotrophic ophiopluteus (Pl), and abbreviated (Ab). In species marked with an asterisk (*) development was inferred from egg size. Entries in bold represent new developmental data presented in this study. Larva is ophiopluteus (Pl), reduced pluteus (RP), pelagic direct developer (PD), vitellaria (V), and (-) for brooders. Gene sequences used for phylogenetic analysis are marked by an X.

Species	Egg (µm)	Dev.	Larva	Source	BOLD[B] or GenBank[G]	Loci			
						18S	28S	16S	COI
Ophiopholis aculeata (LINNEAUS 1767)	105	Pl	Pl	(Strathmann, 1987)	OOPH007-18 [B]		X	X	X
Ophiothrix spiculata LE CONTE 1851	110	Pl	Pl	(Hendler, 1996)	OOPH012-18[B]		X	X	X
Amphiodia periercta?	190	Ab	PD	(Emlet, 2006)	OOPH032-22[B]	X	X	X	X
Amphiodia pulchella dark	-	Pl	Pl	Emlet, unpublished	PAOPA001-23[B]				X
Amphiodia pulchella light	85	Pl	Pl	Emlet, unpublished	PAOPA002-23[B]				X
Amphiodia sp. opaque	140	Ab	RP	(Nakata & Emlet, 2023)	OLAB004-22[B]	X	X	X	X
Amphiodia sp. orange belly	-	Ab	RP	Nakata, Ch III	OLAB050-23[B]				X
Amphiodia sp. tan	-	Ab	RP	Nakata, Ch III	OLAB051-23[B]				X
Amphiodia tabogae (NIELSEN 1932)	85	Pl*	-	Emlet, unpublished	PAOPA003-23[B]			X	X
Amphiodia urtica (LYMAN 1860)	100	Pl	Pl	Nakata, Ch III, (Schiff & Bergen, 1996)	OOPH056-23[B]		X	X	X
Amphioplus sp. vitellaria	-	Ab	V	Nakata, Ch III	OLAB058-23[B]	X	X	X	X
Amphipholis januarii LJUNGMAN 1866	150	Ab	RP	Emlet, unpublished	PAOPA004-23[B]			X	X
Amphipholis kochii LÜTKEN 1872	90	Pl	Pl	(Yamashita, 1985)	T. O'Hara, unpublished data				X

Amphipholis misera KOEHLER 1899	-	Br	-	(Mortensen, 1933)	KU895014[G]				X
Amphipholis pugetana (LYMAN 1860)	-	Pl	Pl	Nakata, Ch III	OOPH004-18[B]	X	X	X	X
Amphipholis squamata (DELLE CHIAJE 1829)	130	Br	-	(Strathmann, 1987)	OOPH035-23[B]	X	X		X
Amphipholis torelli LJUNGMAN 1872	-	Br	-	(Mortensen, 1924)	T. O'Hara, unpublished data				X
Amphiura arcystata	-	Ab	PD	Nakata, Ch III	OLAB060-23[B]	X	X	X	X
Amphiura belgicae KOEHLER 1905	-	Br	-	(Mortensen, 1936)	OOPH057-23[B]	X	X	X	X
Amphiura borealis (G.O. SARS 1872)	-	Br	-	(Stöhr, 2005)	T. O'Hara, unpublished data				X
Amphiura capensis LJUNGMAN 1867	-	Br	-	(Hendler, 1975; MacKinnon et al., 2017)	T. O'Hara, unpublished data				X
Amphiura chiajei FORBES 1843	-	Ab	RP	(Fenaux, 1963)	T. O'Hara, unpublished data				X
Amphiura constricta LYMAN 1879	-	Br	-	(O'Loughlin, 1991)	T. O'Hara, unpublished data		X		X
Amphiura deficiens KOEHLER 1922	-	Br	-	(Mortensen, 1936)	OOPH058-23[B]	X	X	X	X
Amphiura elandiformis CLARK 1966	-	Ab	PD	Emlet, unpublished	OOPH059-23[B]		X	X	X
Amphiura eugeniae LJUNGMAN 1867	-	Br	-	(Mortensen, 1936)	T. O'Hara, unpublished data		X		X
Amphiura grandisquama LYMAN 1869	-	Br	-	(Mortensen, 1933)	T. O'Hara, unpublished data				X

Amphiura magellanica LJUNGMAN 1867	-	Br	-	(Hendler, 1975)	OOPH060-23[B]	X	X	X
Amphiura ptena CLARK 1938	-	Ab	PD	Emlet, unpublished	OLAB109-23[B]		X	X
Amphiura rosea FARQUHAR 1894	80	Pl*	-	(Mortensen, 1924)	KU895019[G]			X
Amphiura spinipes MORTENSEN 1924	100	Pl*	-	(Mortensen, 1924)	KU895041[G]			X
Amphiura stictacantha H.L. CLARK 1938	-	Ab	PD	Emlet, unpublished	OLAB110-23[B]		X	X
Amphiura stimpsonii LÜTKEN 1859	-	Br	-	(Hendler, 1975)	OOPH061-23[B]	X		X
Amphiuridae sp. Au284	-	Ab	PD	Emlet, unpublished	OLAB111-23[B]		X	X
Microphiopholis geminata LE CONTE 1851	90	Pl*	-	Emlet, unpublished	PAOPA005-23[B]		X	X
Microphiopholis gracillima (STIMPSON 1854)	87	Pl	Pl	Emlet unpublished, (Hendler, 1995)	PAOPA006-23[B]	X	X	X
Ophiocnida scabriuscula (LÜTKEN 1859)	210	Ab*	-	(Hendler, 1995)	OOPH064-23[B]	X	X	
Ophiodaphne formata KOEHLER 1905	-	Pl	Pl	(Tominaga et al., 2004)	OOPH062-23[B]		X	X
Ophiophragmus filograneus (LYMAN 1875)	220	Ab*	-	(Stancyk, 1973)	KU895058[G]			X
Ophiophragmus pulcher H.L. CLARK 1918	200	Ab*	-	Emlet, unpublished	PAOPA007-23[B]			X
Ophiostigma isocanthum (SAY 1825)	187	Ab	RP	Emlet, unpublished	PAOPA008-23[B]		X	X

2.2. Sequence dataset and phylogenetic analyses

We built a 39-species database of sequences for amphiurids plus two outgroup taxa by extracting DNA from our own larval and adult collections and specimens from museums. We supplemented this dataset with sequences from public databases. We collected specimens from Oregon, Panama, and Australia

from wild plankton, intertidal and subtidal zones. Specimens for an additional 9 species were borrowed from museum collections (Table 4.1). Additional collection information and sequences for our specimens can be found in BOLD (Ratnasingham & Hebert, 2007). We extracted DNA from whole larvae and tube feet and parts of arms of adult specimens. Adults collected by R. Emlet and N. Nakata were relaxed in a 50:50 mix of $MgCl_2$ and filtered sea water (FSW) for morphological identification and photography on a Zeiss dissecting microscope. When possible, we sampled podia for DNA extraction while the specimen was still living; otherwise, podia were collected after preservation in 70-95% ethanol.

We extracted larval and adult DNA suing the Chelex-based InstaGene™ Matrix (Bio-Rad). We performed polymerase chain reaction (PCR) to amplify *COI, 16S, 28S,* and *18S* using previously published echinoderm-specific primers (Table 4.2) and thermocycler conditions (Folmer et al., 1994; Geller et al., 2013; Kerr et al., 2005; Kirby & Lindley, 2005; Littlewood, 1994; Okanishi et al., 2011; Okanishi & Fujita, 2013). In particular, we had greater success when using echinoderm- or ophiuroid-specific forward primers (Bribiesca-Contreras et al., 2013; Corstorphine, 2011; Heimeier et al., 2010) that we paired with HCO2198 or jgHCO2198. We used a PCR reaction of 20 µl total volume: 11.4 µl nuclease-free water, 4 µl 5X Green Buffer, 0.4 µl dNTP 10 mM, 0.2 µl GoTaq Polymerase (Promega), 1 µl each of forward and reverse primers, and 2 µl of template DNA. We purified the crude PCR products using the Wizard SV

Table 4.2. PCR primers used to amplify four loci for phylogenetic analysis.

Locus	Primer	Sequence (5'-3')	Reference
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	(Folmer et al., 1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
	jgLCO1490	TITCIACIAAYCAYAARGAYATTGG	(Geller et al., 2013)
	jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA	
	COIceF	ACTGCCCACGCCCTAGTAATGATATTTTTTA TGGTNATGCC	(Hoareau & Boissin, 2010)
	COIceR	TCGTGTGTCTACGTCCATTCCTACTGTRAAC ATRTG	
	EchinoF1	TTTCAACTAATCATAAGGACATTGG	(Ward et al., 2008)
	EchinoR1	CTTCAGGGTGTCCAAAAAATCA	
	Echino COI-F	TTTCYACYAAACACAAGGAYATTGG	(Heimeier et al., 2010)
	OphiF	ATAATGATAGGAGGATTTGGAAA	(Bribiesca-Contreras et al., 2013)
	LCOechlaF1	TTTTTTCTACTAAACACAAGGATATTGG	(Corstorphine, 2011)
16S	16SARL	CGCCTGTTTATCAAAAACAT	(Palumbi, 1996)
	16SBRH	CCGGTCTGAACTCAGATCACGT	
	16Sar	GCCTGTTTACCAAAAACAWCG	(Kerr et al., 2005; Kirby & Lindley, 2005)
	16Sbr	GATCCAACATCTAGGTCGC	
28S	LSU5	taggtcgACCCGCTGAAYTTAAGCA	(Littlewood, 1994)
	LSU3	tagaagctTCCTGAGGGAAACTTCGG	
	LSU001	GCTAAGGAGTGTGTAACAACTCACC	(Okanishi et al., 2011)
	LSU002	GCTTTGTTTTAATTAGACAGTCGGA	
18S	SSU001	GCTTGTCTTAAAGACTAAGCCATGC	(Okanishi et al., 2011)
	SSU002	CCGTGTTGAGTCAAATTAAGCCGC	

Gel and PCR Clean up System (Promega) and sequenced in both directions using the same primers used in PCR (Sequetech, Mountain View, CA).

We conducted all sequence validation and analysis in Geneious Prime v. 2022.1.1 (https://www.geneious.com). We trimmed and aligned individual reads to create consensus sequences and assigned bases with combined PHRED scores <20 as 'N'. We received additional sequence data for 8 species from colleagues (T. O'Hara, personal communication) and

downloaded four from GenBank (Table 4.1). We aligned sequences one gene at a time using the MAFFT plug-in (Katoh & Standley, 2013) with default parameters, and compared individual gene trees for topological similarity (Figs. S4.1–4). Altogether, we had a 1075 bp alignment of *18S* for 12 spp., a 1214 bp alignment of *28S* for 12 spp., a 513 bp alignment of *16S* for 20 spp., and a 1432 bp alignment of *COI* for 38 spp.

We created a Maximum Likelihood phylogeny using a concatenated dataset of the four loci with PhyML (Guindon et al., 2010). Model of sequence evolution was substitution model HKY85. Bootstrap analysis (100 replicates) was used to calculate clade support. For comparison, we also implemented Bayesian Inference using the HKY85 substitution model, gamma rate variation, with 1,100,000 chain length and 100,000 burn-in length in MrBayes v2.2.4 (Huelsenbeck & Ronquist, 2001). We used sequences for *Ophiothrix spiculata* and *Ophiopholis kennerlyi* as outgroup taxa.

2.3. Phylogenetic comparative analyses

We performed phylogenetic comparative analyses on a 39-taxon tree inferred by maximum likelihood with fixed topology and branch lengths. We used the *ace* function in 'ape' (Paradis & Schliep, 2019) to estimate discrete ancestral states using maximum likelihood and visualized state probabilities at nodes using standard plotting functions in 'phytools' (Revell, 2023).

We tested for asymmetry in transition rates between character states using a likelihood (ML) approach for evolution of discrete traits. To do this, we fit a series of models using the *fitMk* function in phytools, which allows the user to specify transition matrices for hypothesis testing (Lewis, 2001; Revell, 2023). We compared model fit using analysis of variance

(ANOVA). We assessed ordered ("irreversible") and unordered versions of two suites of models: three-state models of development mode (brooding, abbreviated, and planktotrophy) and five-state models of larval form (brooding, pelagic direct, vitellaria, reduced pluteus, and ophiopluteus). We used ordered models to prohibit transitions between brooding and planktotrophy states; we used the unordered models to test if allowing such trait reversals would produce a better fit for our data. We inferred development mode from egg size for nine species. As abbreviated developers have eggs of similar sizes that give rise to a variety of larval forms, we could not infer larval form from egg size alone. For the five-state larval form models we removed eight tips from the tree for which we inferred development (*Amphiura spinipes, Amphiua rosea, Amphipholis torelli, Microphiopholig geminata, Amphiodia tabogae, Ophiocnida scabriuscula, Ophiophragmus filograneus, Ophiophragmus pulcher*).

3. RESULTS

3.1. Egg size and development patterns

Egg size was a strong predictor for developmental pattern: taxa with planktotrophic development have small eggs, 50–110 μm in diameter; taxa with abbreviated development (including reduced plutei and pelagic direct developers) have moderately sized eggs, from 110-190 μm diameter; and taxa that brood had egg sizes from 120 to 700 μm diameter (Fig. 4.1).

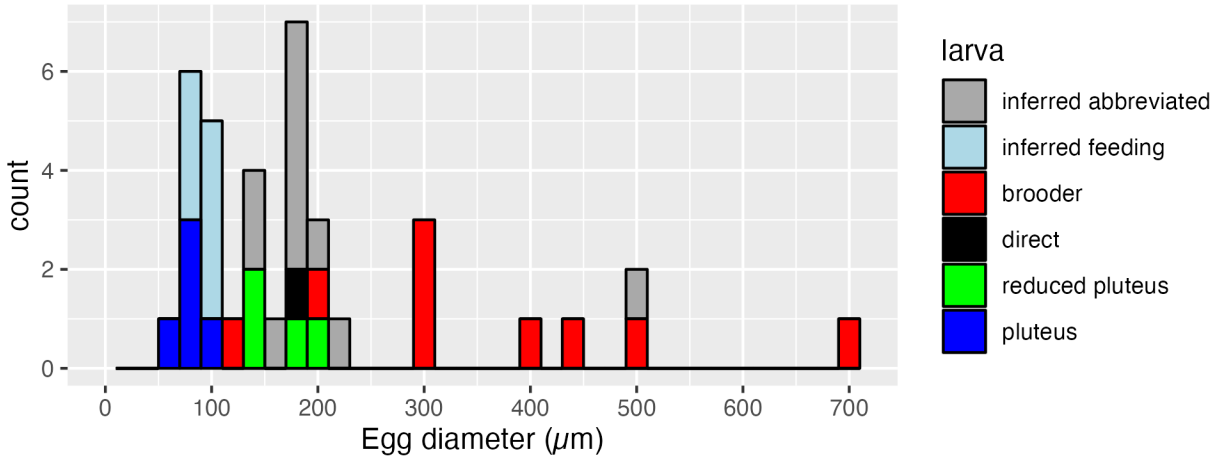


Figure 4.1. Histogram of egg diameters by development mode for 37 spp. of Amphiuridae.

We inferred planktonic development type for seven species based on egg size to include in our phylogenetic comparative analyses, including four planktotrophs (*Amphiura spinipes, Amphiura rosea, Microphiopholis geminata,* and *Amphiodia tabogae*), and three abbreviated developers (*Ophiocnida scabriuscula, Ophiophragmus filograneus,* and *Ophiophragmus pulcher*; Table 4.1, Development marked with *). We inferred development pattern for one additional species, *Amphipholis torelli* based on an early account by Mortensen (1924).

3.2. Sequence dataset and phylogenetic analyses

We obtained sequences for four loci (*COI, 16S, 28S, 18S*) for 39 species of amphiurids, including 74 loci obtained from our specimens, and 13 additional sequences from published and unpublished data (Table 4.1). We constructed phylogenetic hypotheses using maximum likelihood (Fig. 4.2, Table 4.1) and Bayesian methods (Fig. 4.3). We had data for all four loci for seven species (Table 4.1). Individual gene trees contained subsets of the species and displayed similar topologies to the four-gene tree (Figs. S4.1–4).

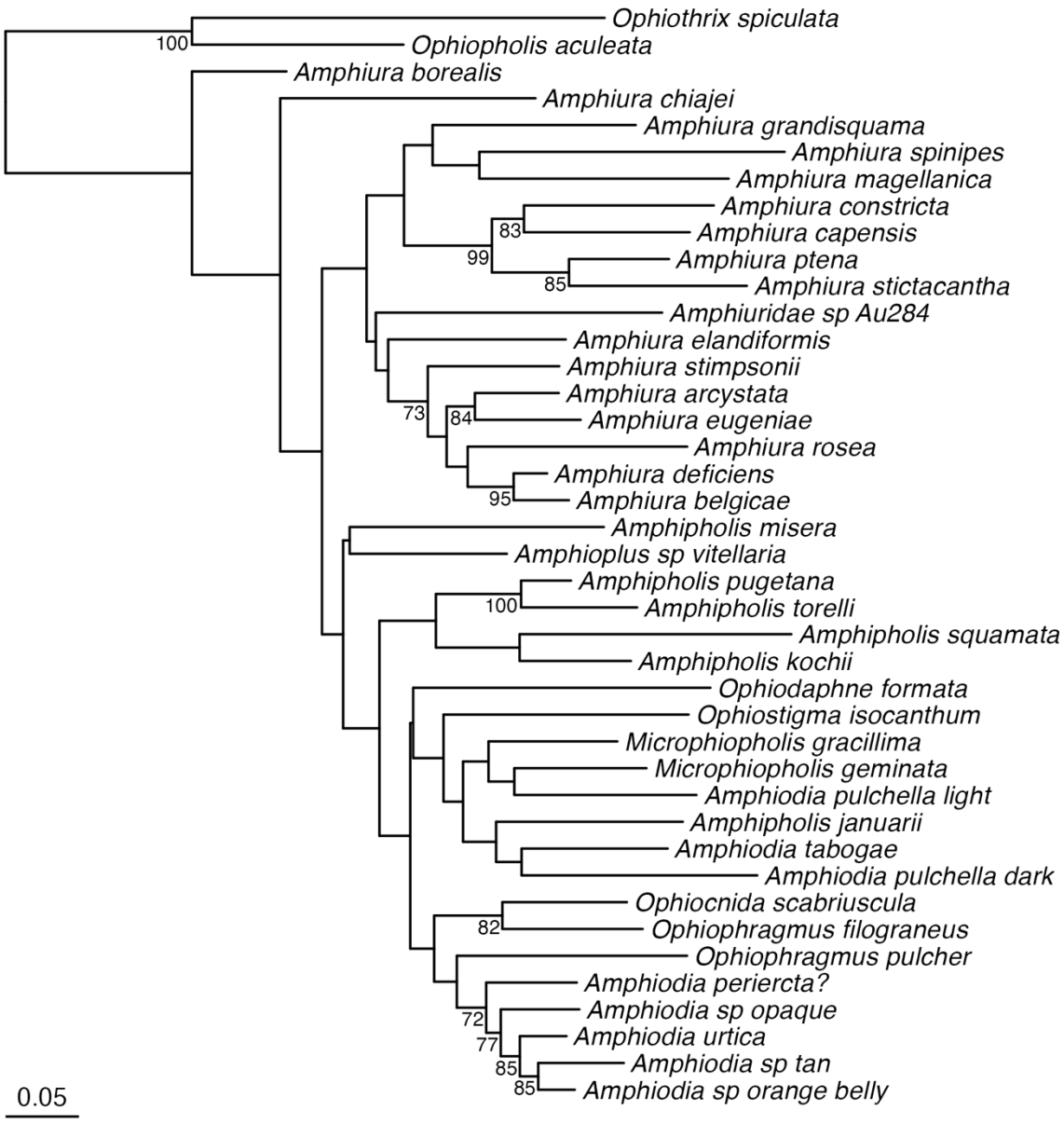


Figure 4.2. Phylogenetic hypothesis for 41 species, including 39 species from the family Amphiuridae and two outgroup taxa, *Ophiopholis aculeata* and *Ophiopthrix spiculata*. The tree was made using Maximum Likelihood analysis of a concatenated dataset (*COI*, *16S* rDNA, *18S* rDNA, and *28S* rDNA). Bootstrap support values >70 % are shown underneath the clades.

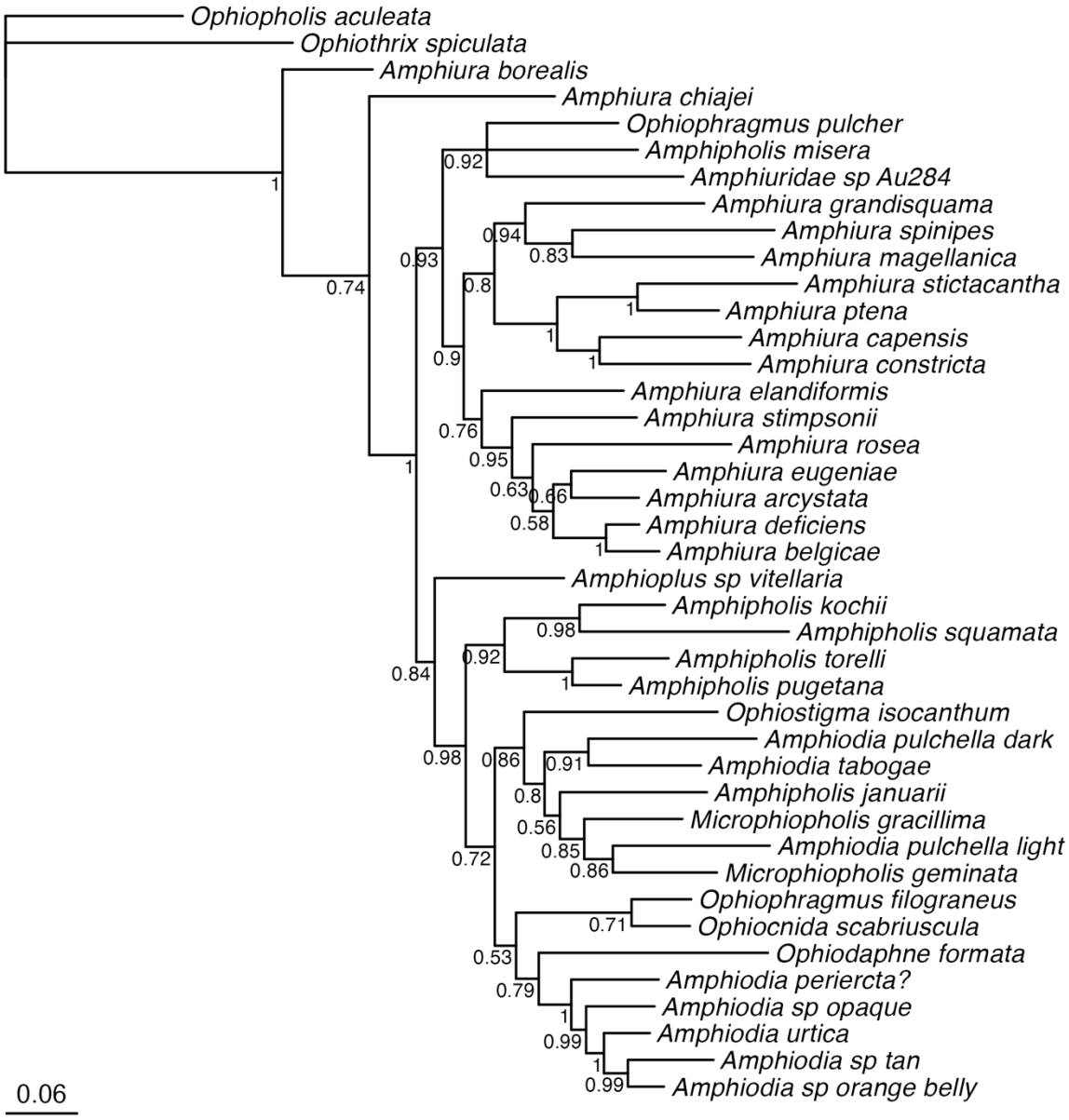


Figure 4.3. Phylogenetic hypothesis for 41 species, including 39 species from the family Amphiuridae and two outgroup taxa, *Ophiopholis aculeata* and *Ophiopthrix spiculata*. The tree was constructed by Bayesian methods using MrBayes. Posterior probabilities are shown underneath their respective clades.

Our phylogenetic hypothesis included species from nine of the 26 amphiurid genera, including important, diverse, and polyphyletic genera *Amphiura, Amphipholis,* and *Amphiodia*.

We inferred *Amphiura* as basal and paraphyletic with respect to the rest of the clade. *Amphipholis* and *Amphiodia* were polyphyletic.

We inferred that the color morphs ("dark" and "light") of *Amphiodia pulchella* represented two species; they were 19.1% different at the *COI* locus. Three of our specimens were genetically divergent from other published sequences of the same identification: *Microphiopholis gracillima, M. geminata*, and *Amphipholis januarii*. As our developmental data was associated with these adults, we used our own sequence data for those species in the tree.

3.3. Comparative phylogenetic analyses

The developmental mode character states (planktotrophy, abbreviated, brooding) were all present in the two major clades within the family. The species in the three-state models included 12 brooders, 16 abbreviated developers (three of which were inferred from egg size), and 11 planktotrophs (four of which were inferred from egg size). Most brooding species were in *Amphiura*; but there were also three brooders in *Amphipholis*. Abbreviated developers occurred throughout the tree. Feeding development, or planktotrophy, occurred in *Amphipholis*, *Amphiodia, Microphiopholis,* and *Ophiodaphne* genera, and we inferred it for two species in *Amphiura* from egg size (Mortensen, 1924).

In our five-state analysis of larval form, the 33-spp. tree included 11 brooders (nine of which were *Amphiura* spp., and two *Amphipholis* spp.), six pelagic direct developers, one vitellaria, five reduced plutei, and eight planktotrophic plutei. The pelagic direct developers occurred in *Amphiura* taxa and *Amphiodia periercta*?. Reduced plutei were present in a clade of *Amphiodia* spp. from the northeast Pacific, and one in *Amphiura chiajei*.

The ancestral state of Amphiuridae was indeterminate in both three-state and five-state ancestral estimations (Figs. 3.3, 3.4). In the three-state developmental mode model, the best-fit model had equal transition rates between all states. In the five-state larval form model, the best-fit model also had equal transition rate between all states.

For both the three-state and five-state models, the best-fit model had equal transition rates between all states (Tables 3.3, 3.4). Fitted values for the three-state model were 2.94 for state changes and -5.87. Similarly, in both cases the worst-fit models were the irreversible models that prohibited secondary gains of planktonic and feeding development.

4. DISCUSSION

4.1. Inference of development pattern from egg size

Our egg size data suggests slightly different threshold sizes between developmental patterns compared to analyses of other groups of ophiuroids. Amphiurid species with feeding planktonic development produce smaller eggs than those of other taxa (<110 µm). Species of *Macrophiothrix* H.L. CLARK, 1938 produce planktotrophic eggs up to 200 µm in diameter, and surveys across Ophiuroidea found planktotrophic eggs up to 170 µm in diameter (Allen & Podolsky, 2007; Hendler, 1991). Two species for which we inferred planktotrophy based on egg size, *Amphiura spinipes* and *A. rosea*, have reliable reports for egg size (Mortensen, 1924), but we cannot be sure if those animals are molecular matches for those represented in our tree. Amphiuridae was similar to *Macrophiothrix* in that egg size does not predict larval form of

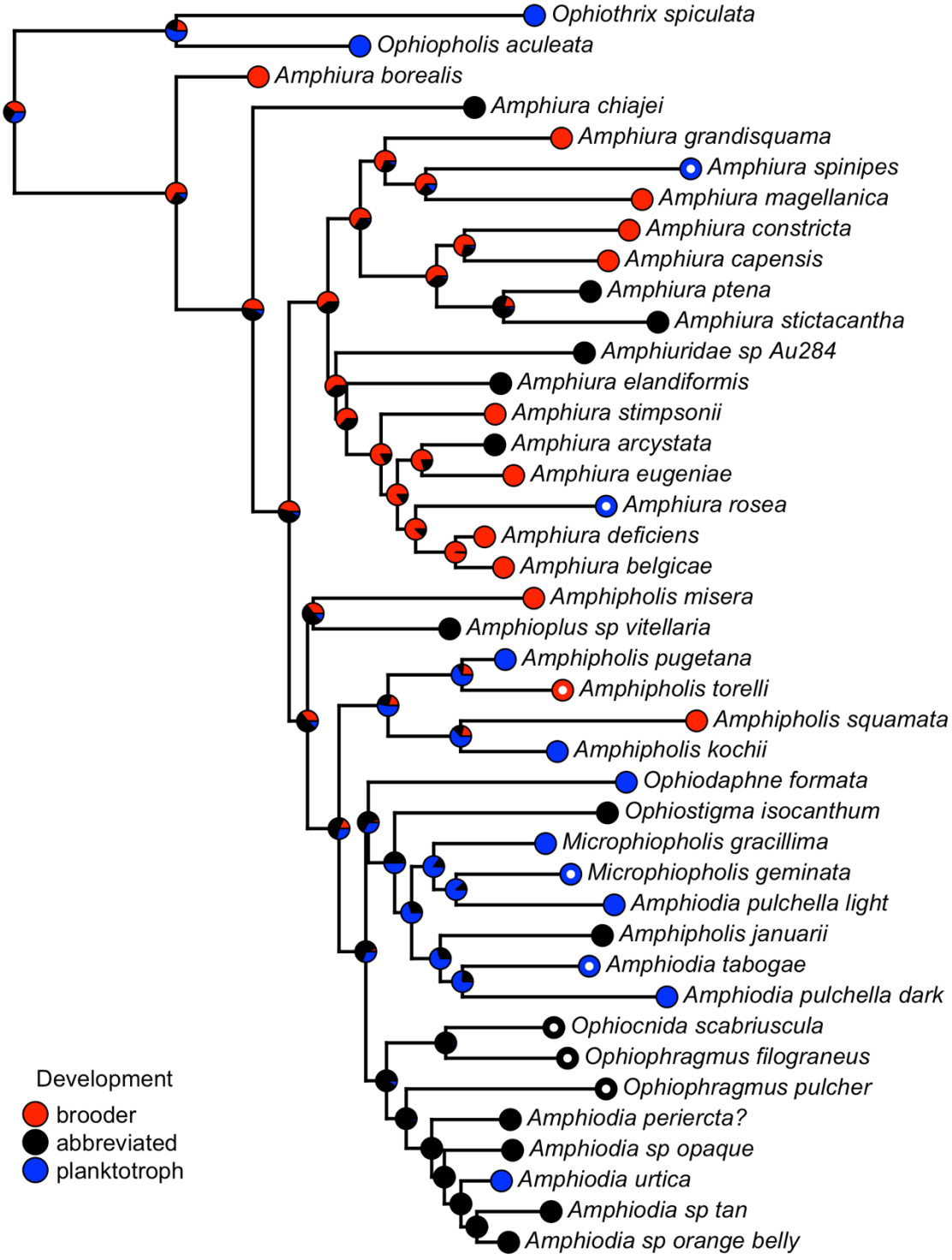

Ophiothrix spiculata
Ophiopholis aculeata
Amphiura borealis
Amphiura chiajei
Amphiura grandisquama
Amphiura spinipes
Amphiura magellanica
Amphiura constricta
Amphiura capensis
Amphiura ptena
Amphiura stictacantha
Amphiuridae sp Au284
Amphiura elandiformis
Amphiura stimpsonii
Amphiura arcystata
Amphiura eugeniae
Amphiura rosea
Amphiura deficiens
Amphiura belgicae
Amphipholis misera
Amphioplus sp vitellaria
Amphipholis pugetana
Amphipholis torelli
Amphipholis squamata
Amphipholis kochii
Ophiodaphne formata
Ophiostigma isocanthum
Microphiopholis gracillima
Microphiopholis geminata
Amphiodia pulchella light
Amphipholis januarii
Amphiodia tabogae
Amphiodia pulchella dark
Ophiocnida scabriuscula
Ophiophragmus filograneus
Ophiophragmus pulcher
Amphiodia periercta?
Amphiodia sp opaque
Amphiodia urtica
Amphiodia sp tan
Amphiodia sp orange belly
Development
brooder
abbreviated
planktotroph

Figure 4.4. Estimated ancestral character states for a three-state model of development modes mapped on a phylogeny of 39 spp. from Amphiuridae and two outgroup taxa, *Ophioholis aculeata* and *Ophiothrix spiculata*. Nodes with inferred development mode based on egg size are marked with a hollow center.

Table 4.3. Summary of the six three-state transition models tested using ML. Model types were equal rates (ER), all rates different (ARD), symmetric (SYM), and irreversible (IRR). Development modes are given as brooding (Br), abbreviated (Ab), and planktotrophy (Pl). The best fit model is in bold text.

Type	No. rates	Transition matrix				Character	Description	lnL	AIC	ΔAIC
ER	**1**		**Br**	**Ab**	**Pl**	**unordered**	**All transitions occurred at the same rate**	**-42.76**	**87.52**	**0.00**
		Br	**-**	***a***	***a***					
		Ab	***a***	**-**	***a***					
		Pl	***a***	***a***	**-**					
ARD	6		Br	Ab	Pl	unordered	All rates different	-39.47	90.94	3.42
		Br	-	*a*	*b*					
		Ab	*c*	-	*d*					
		Pl	*e*	*f*	-					
SYM	3		Br	Ab	Pl	unordered	Symmetric rates	-42.52	91.04	3.52
		Br	-	*a*	*b*					
		Ab	*a*	-	*c*					
		Pl	*b*	*c*	-					
IRR1	4		Br	Ab	Pl	ordered	Abbreviated is intermediate	-42.10	92.19	4.67
		Br	-	*a*	*0*					
		Ab	*b*	-	*c*					
		Pl	*0*	*d*	-					
IRR2	4		Br	Ab	Pl	ordered	No secondary gain of planktonic development	-46.47	100.93	13.41
		Br	-	*0*	*0*					
		Ab	*a*	-	*b*					
		Pl	*c*	*d*	-					
IRR3	3		Br	Ab	Pl	ordered	No secondary gains of feeding development	-53.67	113.34	25.82
		Br	-	*0*	*0*					
		Ab	*a*	-	*0*					
		Pl	*b*	*c*	-					

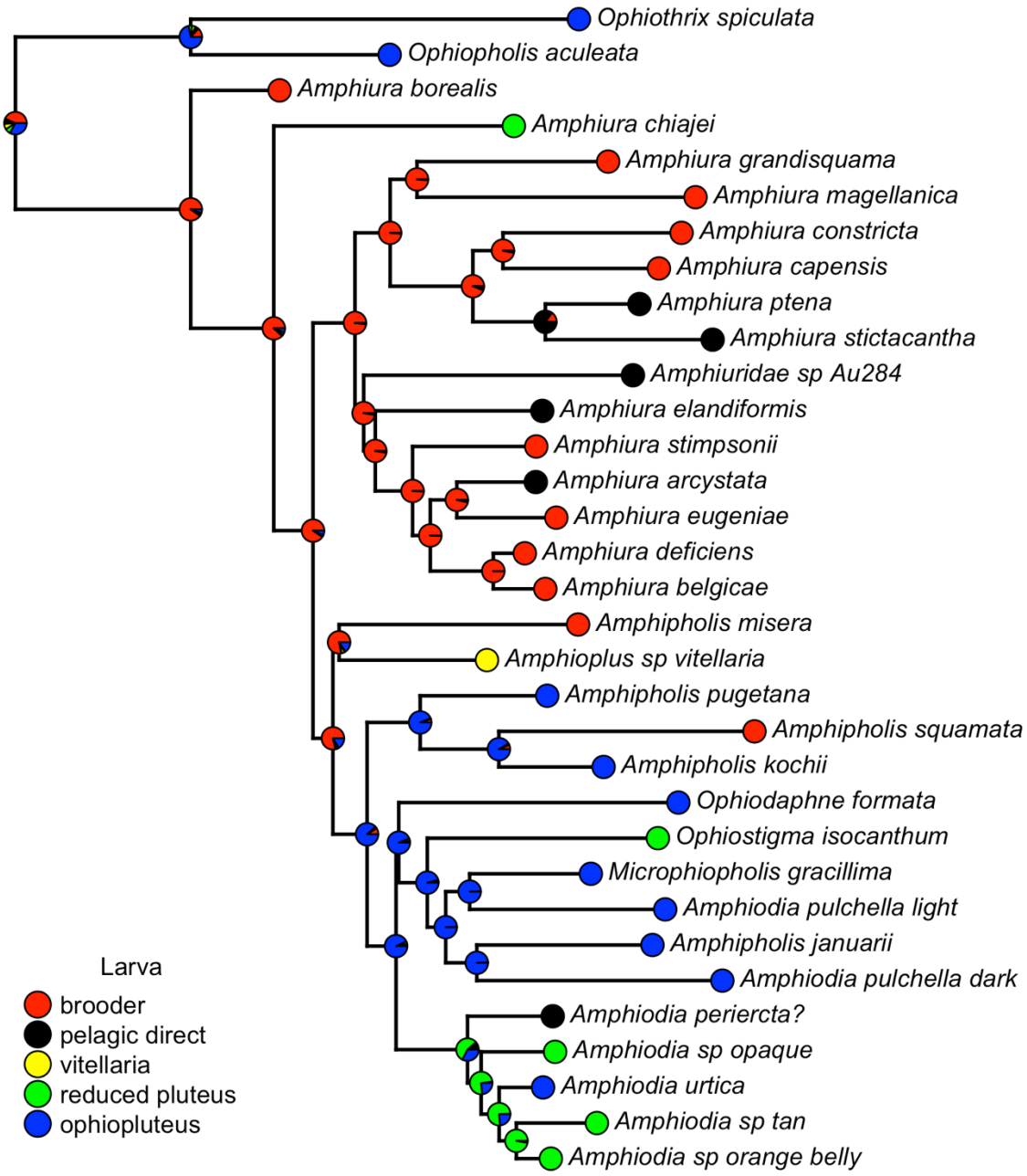


Figure 4.5. Estimated ancestral character states for a five-state model of larval form mapped on a phylogenetic hypothesis for 31 spp. of Amphiuridae.

Table 4.4. Summary of the six five-state transition models tested using ML. Model types were equal rates (ER), all rates different (ARD), symmetric (SYM), and irreversible (IRR). Larval forms are given as brooding (Br), pelagic direct developer (D), vitellaria (V), reduced pluteus (RP), and ophiopluteus (Pl). The best fit model is in bold text.

Type	No. rates	Transition matrix						Description	lnL	AIC	ΔAIC
ER	**1**		**Br**	**D**	**V**	**RP**	**Pl**	**All transitions occurred at the same rate**	**-46.68**	**95.73**	**0.00**
		Br	**-**	**a**	**a**	**a**	**a**				
		D	**a**	**-**	**a**	**a**	**a**				
		V	**a**	**a**	**-**	**a**	**a**				
		RP	**a**	**a**	**a**	**-**	**a**				
		Pl	**a**	**a**	**a**	**a**	**-**				
ARD	20		Br	D	V	RP	Pl	All rates different	-37.54	115.08	19.35
		Br	-	a	b	c	d				
		D	e	-	f	g	h				
		V	i	j	-	k	l				
		RP	m	n	o	-	p				
		Pl	q	r	s	t	-				
SYM	10		Br	D	V	RP	Pl	Symmetric rates	-41.71	103.41	7.68
		Br	-	a	b	c	d				
		D	a	-	e	f	g				
		V	b	e	-	h	i				
		RP	c	f	h	-	j				
		Pl	d	g	i	j	-				
IRR1	18		Br	D	V	RP	Pl	Abbreviated is intermediate	-45.46	126.92	31.19
		Br	-	a	b	c	0				
		D	d	-	0	0	0				
		V	h	i	-	j	k				
		RP	l	m	n	-	o				
		Pl	0	p	q	r	-				
IRR2	16		Br	D	V	RP	Pl	No secondary gain of planktonic development	-39.49	110.99	15.26
		Br	-	0	0	0	0				
		D	a	-	0	0	0				
		V	e	f	-	0	0				
		RP	0	j	k	-	l				
		Pl	m	n	o	p	-				
IRR3	13		Br	D	V	RP	Pl	No secondary gains of feeding development	-44.91	117.83	22.10
		Br	-	0	0	0	0				
		D	a	-	0	0	0				
		V	d	e	-	0	0				
		RP	g	0	i	-	0				
		Pl	j	0	0	m	-				

abbreviated developers (Allen & Podolsky, 2007). Egg sizes for brooders are similar to other analyses (Hendler, 1991).

4.2. Phylogenetic analyses

While this work presents the largest comparative phylogenetic analysis for the Amphiuridae to date, it encompasses only a small fraction of the family diversity. Our phylogenetic hypothesis represents less than 9% of the described species of Amphiuridae (O'Hara et al., 2017), as we included only species with both molecular and developmental data. Future work should focus on collecting molecular and developmental data for all species in the family. For example, although there was an 18-spp. overlap between our phylogenetic and egg size datasets, there were an additional 19 species for which developmental pattern can be inferred from egg size, but currently lack molecular data. DNA barcoding of larvae from plankton is an effective method for simultaneously capturing developmental pattern and species diversity (Collin et al., 2020a, 2021; Maslakova et al., 2022; Nakata, Ch III). Additional molecular data is also necessary to resolve taxonomic relationships. The family Amphiuridae requires revision, and contains many polyphyletic taxa (O'Hara et al., 2018). Our results show several genera – including *Amphiura, Amphipholis,* and *Amphiodia* – are polyphyletic, in agreement with a transcriptome-based analysis of 69 species (O'Hara et al., 2017, 2018).

A consequence of this analysis was the discovery of divergent sequences of specimens given the same identification based on morphology. We found two lineages of *Amphiodia pulchella* from Panama that may be distinguishable based on color. Specimens collected in Panama for *Microphiopholis gracillima, M. geminata*, and *Amphipholis januarii* were over 5% different from sequences with the same species identification in GenBank.

4.3. Ancestral state estimations

We were not able to infer the developmental pattern for the ancestor of Amphiuridae in either analysis of ancestral state estimations. This was influenced by the basal placement of *Amphiura*, a genus with over half the species brooded development. We inferred *Amphiura* to be paraphyletic to the rest of the clade. Two species, *A. borealis* and *A. chiajei* were inferred to be basal. The remaining 15 *Amphiura* spp. formed a clade that was sister to a clade formed by the other genera. The addition of more taxa may cause tree topology to change and move *Amphiura* from its basal position. In a 1484-exon dataset for 576 ophiuroid species, the two main *Amphiura* clades had a derived position, but two additional *Amphiura* species were inferred to be in the most basal clade of the family with high support (O'Hara et al, 2017, Fig. S3). This phylogenetic hypothesis included only 14 species with known development, and the three developmental patterns were distributed across the tree, suggesting multiple gains and losses of feeding development similar to our data (see below).

4.4. Repeated gains and losses of planktonic and feeding development

The family Amphiuridae is part of the suborder Gnathophiurina MATSUMOTO, 1915, which also includes families Ophiotrichidae, Ophiopholidae, Ophiactidae, and Ophiothamnidae (O'Hara et al., 2017, 2018). These taxa have mostly feeding development, with nonfeeding development inferred to be derived in the Ophiotrichidae (Allen & Podolsky, 2007; Hendler, 2005, 1995; Kitazawa et al., 2015; Mortensen, 1938; Nakata, Ch III; Pearse, 1994; Selvakumaraswamy & Byrne, 2000). This suggests a planktotrophic ancestor for Amphiuridae, as does the presence of feeding larvae in the family. Similarities of feeding larval forms across the classes of echinoderms, and essential body plan similarities among all feeding ophioplutuei

make it unlikely that feeding larvae evolved multiple times within echinoderm or with in the ophiuroids or that the ancestor of Amphiuridae was a brooder (Strathmann, 1978b; Strathmann et al., 2020).

We inferred frequent transitions in development mode in this subsample of Amphiuridae. If the ancestor was a planktotroph, in the 39-spp. amphiurid tree (Fig. 4.3), the distribution of developmental patterns we observed would require an initial loss of feeding development followed by, four secondary gains (*Amphiura spinipes, Amphiura rosea*, the ancestor to the *Amphipholis* and *Amphiodia* clade, and *Amphiodia urtica*), and five other secondary losses of feeding. If a feeding ancestor is present in all branches before it disappears completely then there are over 15 losses of feeding.

Evolutionary transitions between development modes are widespread across marine invertebrates, but they tend to be unidirectional. Transitions from nonfeeding to feeding planktonic larvae are rare (Collin, 2004; Emlet, 1995; Krug et al., 2015; Wray, 1996), probably because complex feeding structures may be difficult to re-evolve once lost (Strathmann, 1985; Strathmann et al., 2020). When feeding evolves *de novo* the morphology is likely to be different, e.g., feeding larvae of different phyla don't look or function the same. However, in our estimations of ancestral character states there are many inferred gains and reversals in developmental mode, which were supported by the best-fit models (Table 3.3, 3.4). The models in which transitions from brooding to planktonic development were prohibited were consistently the worst fits for our data.

Our data suggest several secondary gains of both nonfeeding and feeding larvae in lineages with brooding. Gains of nonfeeding larvae from brooding lineages have been inferred in barnacles (Ewers-Saucedo & Pappalardo, 2019), but secondary gains of feeding larvae are

exceedingly rare (Collin, 2004). We suspect that the gains of feeding larvae from brooding lineages inferred in this analysis may be an artifact of our limited taxonomic representation and large amount of missing data.

The transition from brooding to pelagic nonfeeding larvae appears more likely. The abbreviated larvae in *Amphiura* are pelagic direct developers (except *A. chiajei*), rather than reduced plutei or vitellaria. It is possible that these species accomplished the transition from brooding to planktonic development by release of eggs that develop into juveniles while in the plankton rather than re-evolving a ciliated larval form. In contrast, species with abbreviated development that come from inferred planktotrophic ancestors (*Amphipholis, Ophiophragmus,* and *Amphiodia*) are a mix of reduced plutei (n=4 spp.), pelagic direct developers (n=2), and vitellaria (n=1). This suggests the reduced pluteus, possibly facultatively feeding, as the intermediate larval form in the evolution of nonfeeding larvae. Future work should investigate the occurrence of reduced plutei in other ophiuroid taxa. Prior to this analysis, only one reduced pluteus was known from Amphiuridae, *Amphiura chiajei* (Fenaux, 1963); we found four new reduced plutei from the Oregon plankton using DNA barcoding, demonstrating that this larval form is more prevalent in ophiuroids than previously known.

This work shows evidence for repeated gains and losses of feeding development in a family of brittle stars or if feeding larvae only evolved one time, then there are over 10 losses of feeding among the 39 taxa examined here. However, inferring developmental transitions from such an incomplete dataset can be misleading. We include only 9% of species from the family. Future work should focus on the collection of molecular data across Amphiuridae to resolve taxonomic relationships within the family. There is also much to be done to characterize development patterns in the family. We show how DNA barcoding is a powerful tool for

identifying larvae from plankton, but this type of analysis can be expensive, time consuming, and limited to particular regions where there is regular access to wild plankton.

CHAPTER V

CONCLUSION

This work highlights the potential of brittle stars as a system to study transitions in developmental patterns in marine invertebrates. Despite their diversity of developmental patterns and evidence of repeated evolution of nonfeeding development, most groups of ophiuroids have not been analyzed for evolution of development. One reason for this is the lack of observations on development pattern and larval form across ophiuroid taxa, which is known for less than 15% of species (N. Nakata, unpublished data). A primary cause is that unlike for echinoids and asteroids, ophiuroids have no reliable means for obtaining mature gametes in the laboratory. In the research presented here, we relied instead on observations of larvae collected from plankton identified with DNA barcoding.

One type of larva, which at present could not be identified, was shown to be a facultative planktotroph, and used to test the effects of larval feeding on developmental timing, juvenile size, and survival. *Amphiodia* sp. opaque has a reduced pluteus that can make a juvenile without food but benefits from larval feeding. On average, individuals that fed as larvae had shorter development times, higher percent metamorphosis, larger juveniles, and greater capacity to avoid juvenile starvation by relying on energetic reserves accumulated during the larval period (Nakata & Emlet, 2023). Facultatively planktotrophy has only been observed eight times across marine invertebrates, including the brittle star *Macrophiothrix rhabdota*, which showed similar responses to food (Allen & Pernet, 2007; Allen & Podolsky, 2007).

In addition to *Amphiodia* sp. opaque, we found seventeen other ophiuroid species in the coastal plankton. In Chapter III we summarized over a decade's worth of observations to

describe the taxonomic, developmental, and morphological diversity of brittle star larvae. Using DNA barcoding of larvae and adults, we identified the developmental patterns of 14 described species. Four of these are currently only known in their larval form: *Amphiodia* sp. opaque, *A.* sp. orange belly, *A.* sp. tan, and *Amphioplus* sp. vitellaria. These larvae may not represent new species as many brittle stars species are missing from molecular databases for comparison, highlighting a limitation of barcoding for species identification. Still, we were able to triple the number of larval descriptions from the most recent summary of ophiuroid development in the northeast Pacific (Strathmann, 1987).

By barcoding multiple specimens of each species, we were able to connect multiple life stages – embryo, larva, rudiment-stage, and juvenile – without needing to spawn animals in the lab and culture larvae over weeks or months. After identifying individual larvae by barcoding, we used our observations and photos to describe morphological features that can be used to identify larvae found in plankton. In planktotrophic ophioplutei, we identified features of the larval skeleton that were characteristic of families and species. Abbreviated developers varied in color and shape.

One of the primary findings of Chapter III was that abbreviated development is common amongst the brittle stars of the northeast Pacific, present in nine of the eighteen species we observed. We observed four reduced plutei, two vitellaria, and three pelagic direct developers. The vitellaria were the first to be described for their respective families: *Amphioplus* sp. vitellaria, Amphiuridae, and *Ophiopteris papillosa*, Ophiopteridae. In the case of *O. papillosa*, we were also able to use barcoding to connect divergent larval forms from the same species: *Ophiopteris papillosa* has a feeding ophiopluteus that develops into a vitellaria prior to metamorphosis, only the second ophiuroid recorded to do so to date (Cisternas & Byrne, 2005).

DNA barcoding is a powerful tool for identifying larval stages of benthic invertebrates, and it can also be an important source of phylogenetic data for understudied taxa. Sampling across life stages increases estimates of biodiversity (Maslakova et al., 2022), and many brittle star species are absent from molecular databases. Molecular data increasingly inform and determine our understanding of taxonomic relationships. Molecular phylogenies have led to recent reorganization of major clades within the Ophiuroidea (O'Hara et al., 2017, 2018).

In addition to the ophiuroids of southern Oregon we described in Chapter III, R. Emlet used similar methods to collect molecular and developmental data for ophiuroids of Panama and Australia. By utilizing this dataset with data from the published literature, we built a 39-species phylogenetic hypothesis for the family Amphiuridae in Chapter IV and used it to test hypotheses of the evolution of development. We inferred frequent transitions between developmental types and larval forms, even from nonfeeding (abbreviated) to feeding development. In *Amphiodia* abbreviated development mostly took the form of reduced plutei, but a pelagic direct developer is also known (Emlet, 2006). In *Amphiura* abbreviated development was restricted to pelagic direct developers, but one reduced pluteus is known.

Due to the basal placement of *Amphiura*, a brooding ancestor was inferred in our analysis. The loss of complex evolutionary traits is regarded to be terminal, and the regain of larval feeding once lost considered to be very unlikely (Strathmann, 1978b; Strathmann et al., 2020). Therefore, we must conclude that our conclusions are an artifact of current tree topology. Future work should focus on greater resolution of taxonomic relationships and developmental patterns prior to re-analysis.

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SUPPLEMENTAL FILES

Table S2.1. Pairwise comparisons for percent metamorphosis. P-values adjusted with the Holm method. Significant values are marked with and asterisk (*).

Comparison	Z	P	P adjusted
Food 2020 – food 2021	2.97	0.003	0.015 *
Food 2020 – no food 2020	5.35	< 0.001	< 0.001 *
Food 2021 – no food 2020	1.75	0.080	0.160
Food 2020 – no food 2021	2.12	0.034	0.102
Food 2021 – no food 2021	-0.76	0.444	0.444
No food 2020 – no food 2021	-2.60	0.009	0.038 *

Table S2.2. Pairwise comparisons from Dunn's test for juvenile aboral surface area according to treatment and year: 2020 (20), 2021 (21).

Comparison	Z	P.unadj	P.adj
Food 20 – food 21	-0.558	0.577	1.000
Food 20 – no food 20	3.641	0.000	0.002 *
Food 21 – no food 20	3.814	0.000	0.001 *
Food 21 – no food 21	2.765	0.006	0.028 *
Food 21 – no food 21	3.006	0.003	0.019 *
No food 20 – no food 21	-1.071	0.284	1.000
Food 20 – wild 19	2.766	0.006	0.034 *
Food 21 – wild 19	3.007	0.003	0.021 *
No food 20 – wild 19	-0.520	0.603	1.000
No food 21 – wild 19	0.443	0.657	0.657

Table S2.3. Akaike's (AIC) and Bayesian Information Criteria (BIC) values for generalized linear models of juvenile aboral surface area (juv.size) in response to planktonic duration (p.d.). Models with the lowest AIC or BIC values are bolded.

Model	df	AIC	ΔAIC	BIC	ΔBIC
Juv. size ~ p.d.	3	-244.4	43.9	587.2	37.2
Juv. size ~ p.d. + treatment	**4**	**-288.3**	**-**	**550.0**	**-**
Juv. size ~ p.d. + year	4	-286.3	2	570.7	20.7
Juv. size ~ p.d. + treatment + year	5	-268.4	19.9	553.3	3.3

Table S2.4. Akaike's (AIC) and Bayesian Information Criteria (BIC) value for generalized linear models of juvenile starvation time (days) based on juvenile aboral surface area. AIC weights are *w*. Models with the lowest AIC or BIC values are bolded.

Model	df	AIC	ΔAIC	BIC	ΔBIC
Starve time ~ juv. size	3	2120.9	459.2	2125.4	453.2
Starve time ~ juv. size + treatment	5	1978.7	317.0	1986.2	314.0
Starve time ~ juv. size + year	5	1703.7	42.1	1711.2	39.1
Starve time ~ juv. size + treatment + year	6	1686.1	24.5	1695.1	**23.0**
Starve time ~ juv. size + treatment * year	**7**	**1661.7**	**-**	**1672.1**	-

Figure S4.1. Maximum likelihood tree for *18S* of Amphiuridae.

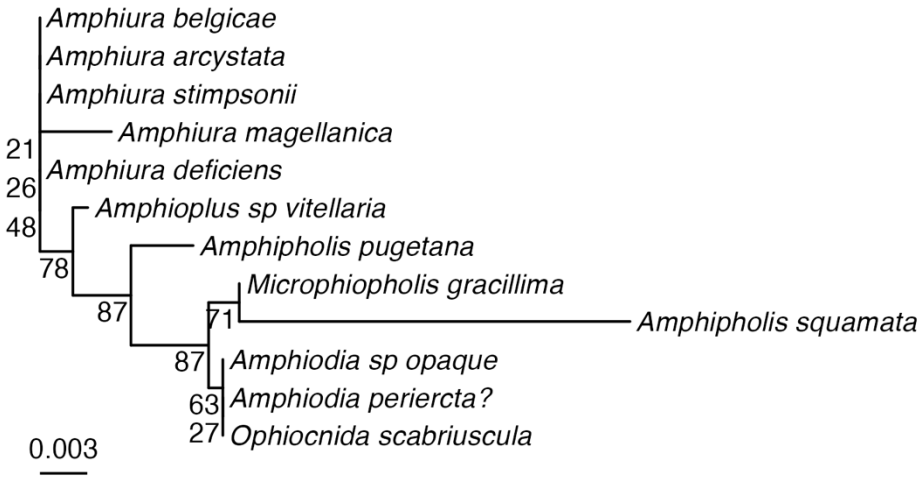

Figure S4.2. Maximum likelihood tree for *28S*.

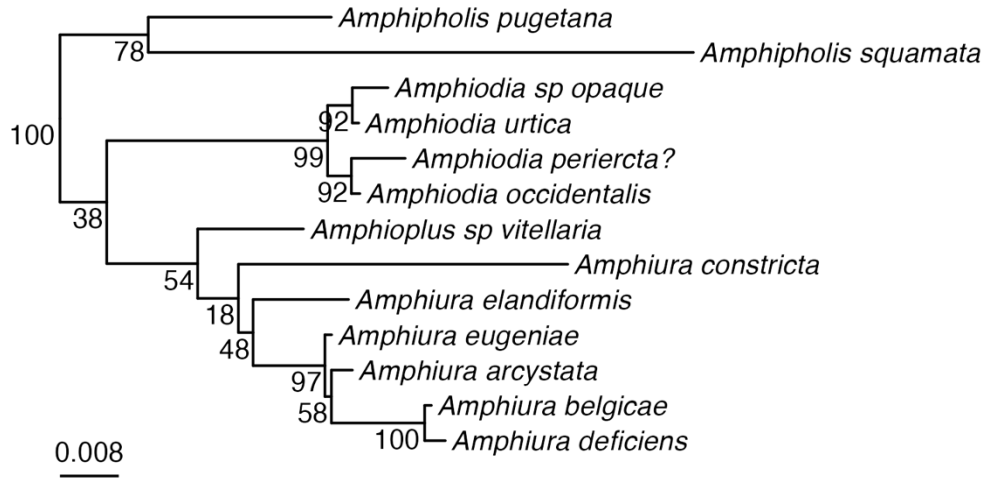

Figure S4.3. Maximum likelihood tree for *16S*.

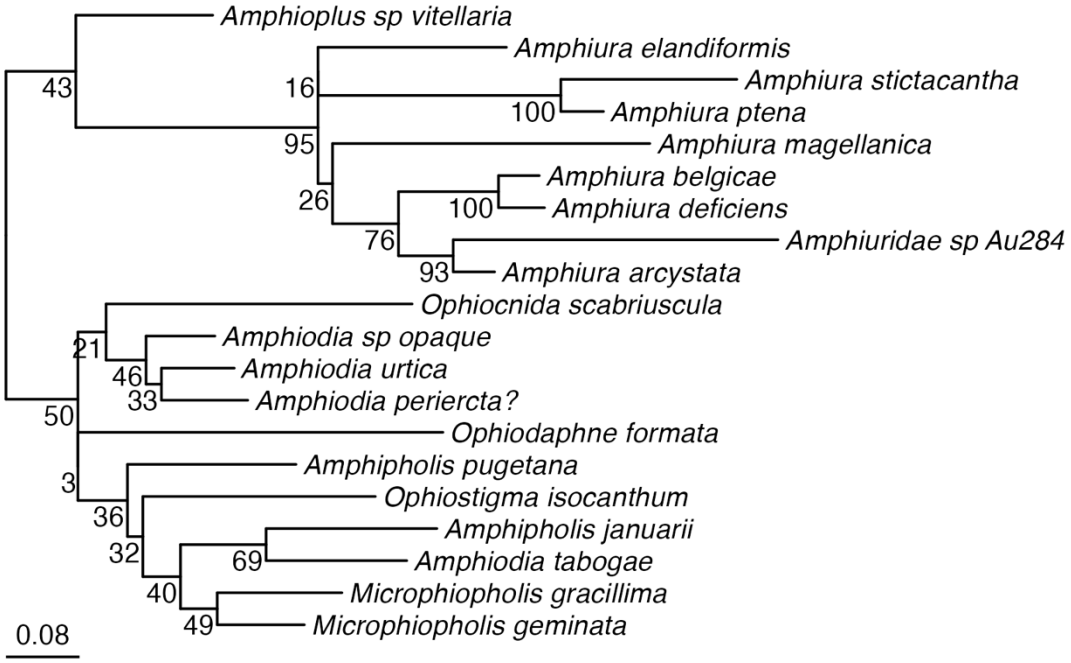

Figure S4.4. Maximum likelihood tree for 38 amphiurid spp., *COI* locus.