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Brittle stars in the plankton: Descriptions of larvae and developing juveniles of the northeastern Pacific Ocean, with a key for their identification

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Abstract

Here we describe 19 species of ophiuroid larvae from the southern coast of Oregon, showing a broad range of planktonic forms including feeding ophioplutei, reduced plutei, vitellaria, and pelagic direct developers. We report molecular, developmental, morphological data for identifying the planktonic stages of brittle stars from the northeastern Pacific Ocean. We identified embryos, larvae, and juveniles from plankton by using mitochondrial *COI* and *16S* compared with public databases and our own adult ophiuroid sequences. The 19 species include many types of development: 11 planktotrophic ophioplutei from four families, from the genera *Ophiothrix* (1 sp.), *Ophiopholis* (2 spp.), *Amphiodia* (2 spp.), *Amphipholis* (1 sp.), *Ophiacantha* (1 sp.), *Ophiopteris* (1 sp.), *Ophiocten* (1 sp.), and *Ophiura* (2 spp.); eight species with abbreviated development, including four reduced plutei from *Amphiodia* (3 spp.) and *Ophiura*, a vitellaria from *Amphioplus*, and three pelagic direct developers from *Amphiodia*, *Amphiura*, and *Gorgonocephalus*. Using identifications made by DNA barcoding, we describe morphological characters useful for the identification of each species. We include descriptions, photos, and a key for the identification of ophiuroid plutei, nonfeeding larvae, and planktonic juveniles. In addition, we report plankton collection records that show brittle stars in the region spawn primarily in winter months (January–March).

KEYWORDS

DNA barcoding, Echinodermata, Ophiuroidea, ophiopluteus, plankton, vitellaria

1 | INTRODUCTION

With ca. 2,100 described species, the brittle stars (Echinodermata: Ophiuroidea) are the most diverse class of echinoderms and can dominate benthic environments across all ocean depths (O'Hara, Hugall, Woolley, et al., 2019; Stöhr et al., 2012). Ophiuroids have a wide variety of ecological roles, and, when abundant, are significant contributors to benthic communities (Blicher & Sejr, 2011; Gerald et al., 2017; Packer et al., 1994; Stöhr et al., 2012; Summers & Nybakken, 2000). Brittle stars exhibit diverse reproductive patterns,

including planktonic feeding and nonfeeding larvae, brooding, and fissiparity (Hendler, 1975; McEdward & Miner, 2001). These variations in development are widely distributed across Ophiuroidea, and closely related species can have very different ontogenies (Allen & Podolsky, 2007; Byrne, Cisternas, O'Hara, & Selvakumaraswamy, 2024; Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Byrne, et al., 2025; Byrne & Selvakumaraswamy, 2002; Emlet, 2006; Hender, 1991; O'Hara, Hugall, Cisternas, et al., 2019; Selvakumaraswamy & Byrne, 2023).

Widespread variations in development and associated differences in offspring number and parental investment per offspring make brittle stars ideal for the comparative study of life history evolution, which requires an understanding of phylogenetic relationships and knowledge of developmental patterns at the species level. Currently, phylogenetic data are available for less than 10% of species and developmental patterns are known for about 15% of species (Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Hendler, 1991, Nakata, unpublished). Of the brittle stars for which development is known or inferred from egg size (approx. 275 species), about a third brood their young; the remainder have planktonic larvae or directly developing juveniles (Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Hendler, 1991; Nakata & Emlet, unpublished data).

Over several decades of sampling the plankton on the Oregon coast we found many larval and juvenile stages of ophiuroid. Since 2011 we have used DNA barcoding to identify these stages and place them within a phylogeny. Ophiuroid development has proven difficult to study in a laboratory setting because methods to induce spawning do not work for many taxa and so raising larvae of known species is not readily accomplished (Allen & Podolsky, 2007; Hodin et al., 2019; Kitazawa et al., 2015; Selvakumaraswamy & Byrne, 2000; Strathmann, 1987). Additionally, barcoding of all life stages can lead to higher estimates of regional species diversity (Maslakova et al., 2022).

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 (Figure 1B); and lecithotrophs, with nonfeeding larvae that develop from large eggs produced in smaller quantities (Byrne, Cisternas, O'Hara, Sewell, et al., 2024; McEdward & Miner, 2001). The high variability in larval phenotypes in ophiuroids is not accommodated by these simple categorizations, requiring subdivisions of increasing number as more developmental patterns are described (Byrne & Selvakumaraswamy, 2002; Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Cisternas et al., 2004; Selvakumaraswamy & Byrne, 2004). Most notably, intermediate forms such as reduced plutei that exhibit facultative planktotrophy (Allen & Podolsky, 2007; Nakata & Emlet, 2023) – which we will show to be more common in the Ophiuroidea than previously documented – do not fit in this dichotomy; therefore, we prefer Hendler's (1975, 1991) taxonomically and developmentally diverse but ecologically meaningful grouping of 'abbreviated' developers in lieu of 'lecithotrophs'. Abbreviated forms develop from moderate numbers of yolk eggs and can take a variety of phenotypes, including reduced plutei, vitellaria, and pelagic or benthic direct developers (Hendler, 1975, 1991).

Brittle stars also show variation in their patterns of metamorphosis, spurring discussion of the ancestral pattern of development for the class (Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Byrne & Selvakumaraswamy, 2002; Cisternas et al., 2004; Hendler, 1975, McEdward & Miner, 2001; Selvakumaraswamy & Byrne, 2004, 2023). Development patterns have been classified by the relationship between the feeding pattern of the initial larva and the number of larval stages. Type I development has one larval stage that metamorphoses into a juvenile; Type II development has a larval stage that forms a

second, nonfeeding, vitellaria larva before metamorphosing into a juvenile. Examples where Type I and II have evolved nonfeeding primary larvae are also known. An "intermediate metamorphosis" has been added recently to the observed patterns (see Figure 6 in Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Selvakumaraswamy & Byrne, 2023). We present data here for previously unknown developmental patterns in the families Amphiuridae, Ophiacanthidae, and Ophiuridae that suggest additional pathways from planktotrophy to lecithotrophy.

In this study we describe the planktonic forms for nineteen species of ophiuroids found in the northeastern Pacific Ocean. Our primary sampling location was the entrance to Coos Bay, Oregon, but we have also collected several of the same larvae described here in the San Juan Islands, Washington (Salish Sea). As many as 66 species of ophiuroids are reported 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 12 species, nine of which occur throughout the California-Alaska range. Adults of many of the species we identified from the plankton occur nearshore, but a few are from deeper water (> 100 m). We observed four species that occur in California but are not listed for Oregon: *Ophiothrix spiculata*, *Amphiodia* aff. *digitata*, *Amphiodia* sp. tan, and *Amphiura arcystata*. We found three additional species of larvae with molecular matches to adults collected in British Columbia, Canada: *Amphiodia* sp. opaque, *Amphiodia* sp. orange belly, and *Amphiplus* sp. vitellaria.

The species that are listed to occur in the region but that we did not observe in the plankton have subtidal depth distributions from 50 to 3000 m. Two notable coastal 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 3.4.3.7).

We used DNA barcoding of the mitochondrial COI and 16S genes to identify the eggs, embryos, larvae, and juveniles of 19 species of ophiuroids, collected from the plankton of southern Oregon over 14 years. By reviewing the corresponding dataset of larval photographs, we described phenotypic characters to recognize individual species, using a naming scheme after Mortensen (1921; Figure 1) and summarized in a key at the end of this paper. First, we give descriptions accompanied by photographs for each species.

2 | METHODS

2.1 | Collection of planktonic stages

We collected embryos, larvae, and juveniles of local ophiuroids from plankton in years 2011–2013, 2014–2016, 2018–2021, and 2022–2024. Plankton sampling was done with varying levels of effort across seasons and years. The most intensive sampling was conducted in the winter months when many local taxa are known to spawn (Emlet, Shanks, and Sutherland, unpublished data; Shanks et al., 2020), and less intensive sampling occurred during the rest of the year. Plankton observations were made daily in the months of January, February, and March in years 2014 (79 days), 2015 (73 days), and 2016 (74 days);

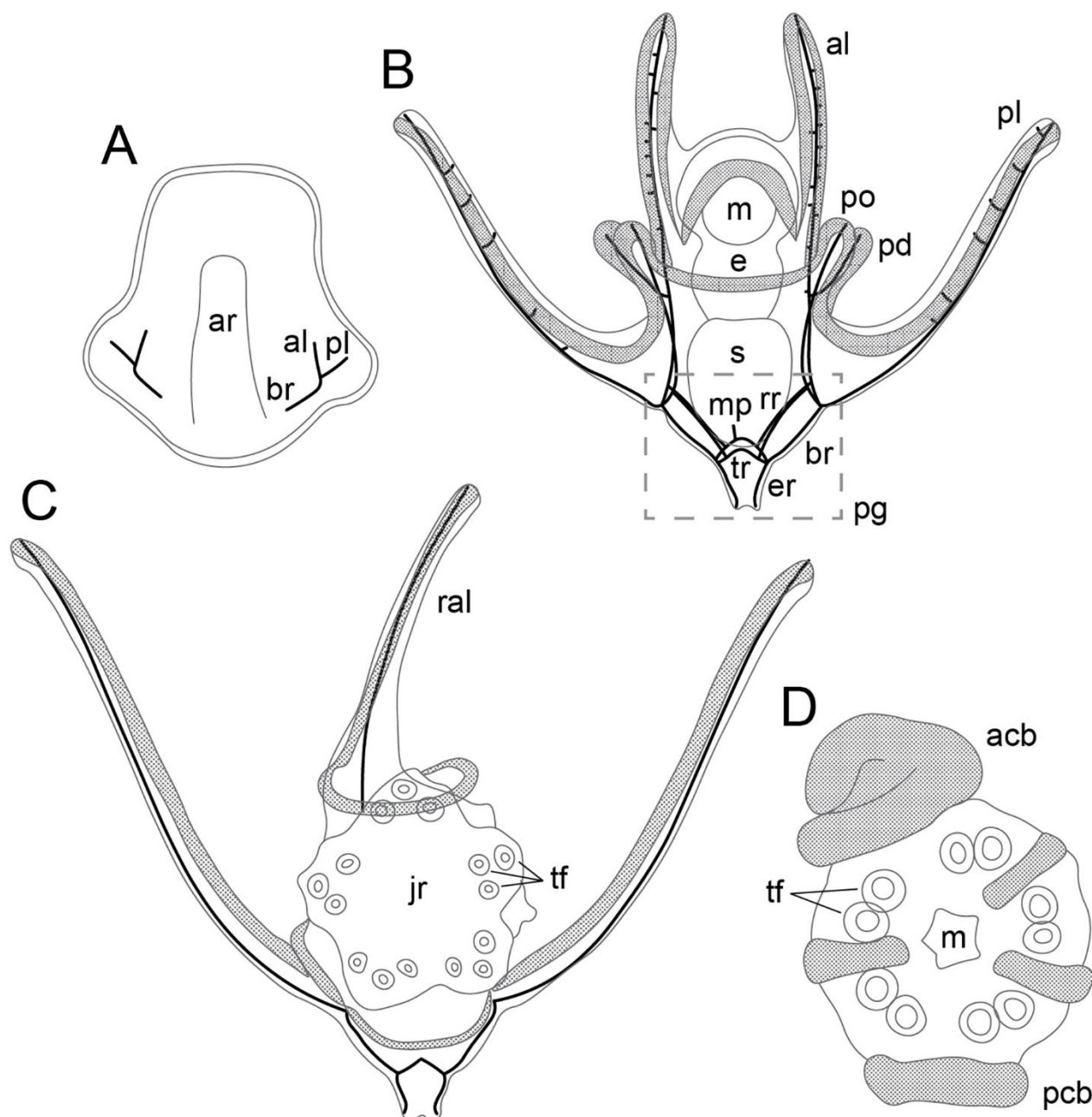


FIGURE 1 Developmental stages of the Ophiuroidea. **A.** Simplified early pluteus, aboral view, with an archenteron (ar) and the two calcite spicules of the skeleton (solid black lines), mirrored across the sagittal plane. The rods of the spicules are the beginnings of the anterolateral (al), posterolateral (pl), and body (br) rods. **B.** Eight-armed ophiopluteus, oral view, with anterolateral, posterolateral, postoral (by mouth), and posterodorsal (pd) arm pair, which support ciliary bands (stippled regions) and mirror each other across the midline. The skeleton extends posteriorly with a pair of body rods (br) connected by dorsal and ventral transverse rods (tr) and terminates in end rods (er). Some larvae also have recurrent rods (rr) in the body region. The transverse rods may bear one or more median processes (mp) or thorns. We refer to the posterior portion of the ophiopluteus skeleton (delineated by the dashed line box) as the posterior girdle (pg). The feeding larva has a mouth (m), esophagus (e), and stomach (s), intestine, and anus. **C.** 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). **D.** The nonfeeding vitellaria, oral view, has several disjunct ciliary bands and complete bands at the anterior (acb) and posterior (pcb).

almost daily in the months of October to April in years 2018–2021; and occasionally in years 2011–2013 and 2022–2024. Plankton samples were collected twice weekly during the months of June to September in 2018 to verify that ophiuroids spawn rarely during the summer season; we made only opportunistic sampling during the summers of the other years in this dataset. Sampling in the San Juan Islands, WA occurred in August 2019 and September 2020.

We used two methods for plankton collection. In the years 2014–2016 plankton was primarily collected during predawn or daytime high tide using a diaphragm pump (sample volumes: 3–12 m³, mesh size 125 µm; Shanks et al., 2020, 2026) and augmented with hand tows, which we used exclusively in years before 2014 and in 2018–2024. Hand tows were collected approximately one hour before high tide with 130 µm mesh net in the Coos Bay estuary approximately 3 km from the entrance to the Pacific Ocean (Charleston Marina, Oregon: 43°21.2'N, 124°20'W). Collections with a plankton net were taken at the outer ends of the floating docks in the Charleston marina; we usually let the plankton net filter water horizontally for 10 minutes during the flood tide, within one meter of the water's surface. We did not take hand tows on days in which the high tide occurred between 8 pm and 7 am. With either collection method, samples were examined within two hours of collection with a stereomicroscope and ophiuroid larvae were picked out by hand. As many planktonic stages of brittle stars are negatively buoyant and poor swimmers, we most often found them at the bottom of a given plankton sample. All brittle star material derived from the plankton was sorted into phenotypes and select individuals were frozen individually in a small amount of seawater at -20°C for DNA barcoding.

When possible, larvae were kept alive and observed through development into juveniles. 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 with Zeiss Universal or Olympus SZH10 stereo-microscopes 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 three stages: 1) 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; Figure 1). Reduced plutei also fit this description but can be distinguished from planktotrophic plutei by their opaque pigmentation and that they do not develop the eighth pair of (posterodorsal) arms. 2) Late plutei are recognized by their eight arms (or four to six arms in reduced plutei) and visible coeloms, especially the left hydrocoel, which develops five lobes that are the precursor to the water vascular system

(Figure 1B). 3) 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 (Figure 1C). 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 conducted all data validation and plot visualization in the R environment v4.2.2 using the package 'ggplot2' (R Core Team, 2022; Wickham, 2016).

2.2 | Molecular identification

Many local brittle star taxa were absent from public sequence databases such as GenBank and BOLD (Benson et al., 2017; Ratnasingham & Hebert, 2007), thus we collected sequence data from adult specimens representative of the region. Adult material originated from several sources, including collection by hand in the intertidal, dredging soft-bottomed subtidal habitats, and from preserved specimens of the Natural History Museum of Los Angeles County. New adult material has been deposited in museums (see BOLD records, SupplementaryTable S1). See species sections below for further details. We relaxed live adults in a 50/50 solution of isotonic magnesium sulfate or magnesium chloride and FSW for morphological examination, and relied on regional guides for identification (Hendler, 1996; Lambert & Austin, 2007). We removed a few podia or an arm tip with fine forceps and samples preserved in ethanol were rinsed in nuclease-free water prior to DNA extraction.

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 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), 1 µL each of forward and reverse 10 µM primers, and 2 µL of template DNA. PCR conditions were as follows: initial step 95°C for 2 min, followed by 34 cycles of denaturation at 95°C for 40 s, annealing at 45 or 48°C for 40 s, 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 and Eurofins Genomics, Louisville, KY). Ultimately, we barcoded 80 embryos, larvae, and juveniles, and 48 adult brittle stars. Larval and adult barcodes were uploaded to BOLD and are available as a dataset: DS-OPHIO24 or in Genbank as accession numbers: COI - PX584168-PX584282 and 16S - PX555359-PX555439.

Barcode sequences for larvae and juveniles for each morphotype were compared to GenBank (www.ncbi.nlm.nih.gov/genbank/), BOLD (Ratnasingham & Hebert, 2007), and our unpublished dataset of adult ophiuroid sequences with the BLAST function in BOLD or Geneious Prime (<https://www.geneious.com>). For trees of larvae with many

TABLE 1 PCR primers for molecular identification of ophiuroid larvae.

Locus	Primer name	Primer sequence	Reference
COI	jgLCO1490	TITCIACIAAYCAYAARGAYATTGG	(Geller et al., 2013)
	jgHCO2198	TAIACYTCIGGRTGICCRARAAYCA	
	EchinoF1	TTTCAACTAATCATAAGGACATTGG	
	EchinoR1	CTTCAGGGTGTCCAAAAAATCA	(Ward et al., 2008)
	COlcef	ACTGCCACGCCCTAGTAATGATATTTTTATGGTNATGCC	
	COlcer	TCGTGTGTCTACGTCCATTCTACTGTRAACATRTG	(Hoareau & Boissin, 2010)
16S	LCO1490	GGTCAACAAATCATAAAGATATTGG	(Folmer et al., 1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	
	16SARL	CGCCTGTTTATCAAAAACAT	
	16SBRH	CCGGTCTGAACTCAGATCACGT	(Palumbi, 1996)
	16Sar	GCCTGTTTACCAAAAAACAWCG	
	16Sbr	GATCCAACATCTAGGTCGC	(Kirby & Lindley, 2005)

sequences of closely related taxa we included BLAST matches within 90% and mostly specimens from the northern Pacific and Atlantic Oceans, and we chose representative specimens from publications that uploaded similar sequences for many individuals. For larvae with very few closely related taxa we included specimens up to 80% similar at COI from around the world in addition to members of the same family from North America (see captions for Supplementary Table S1 and Figures S1–S11 for more details on each tree). We aligned sequences by using the Geneious MAFFT plug-in (Kato & Standley, 2013) and then created genus-level or family-level maximum likelihood trees by using the PhyML 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).

All trees (Supplementary Figures S1–11) were visualized and annotated with the R package 'ggtree' (Xu et al., 2022). For species delimitation based on COI sequences, we used BOLD Bin assignment (barcode index numbers generated by an algorithm of Boldsystems.org) that groups sequences that "show high concordance with species". We also used the online execution for Assemble Species by Automatic Partitioning (ASAP) to determine multiple different groupings (partitions) that are ranked by a combination probability of panmixia and relative gap width metrics (Puillandre et al., 2020). Based on our total data set (Table S1) the partition scheme with the lowest ASAP score had 40 partitions and the second lowest had 70 partitions. The former was associated with an interspecific barcode gap of ca. 9% and the later with ca. 4% genetic distance, respectively. (We excluded the sequence of *Ophiothrix rudis* from this analysis due to limited or no overlap several other sequences when aligned). The ASAP scheme with 40 partitions was very conservative compared to assignments of BOLD Bins and does not distinguish species (or populations) that some published datasets support (e.g., Li et al., 2020; O'Hara & Thuy, 2022). The ASAP scheme with 70 partitions was still conservative but more closely approximates the BOLD Bins.

3 | RESULTS AND DISCUSSION

3.1 | Taxonomic diversity

We collected plankton, raised larvae, and generated barcodes for 19 species from seven families of ophiuroids (Table 2). The best represented families were Amphiridae with nine 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 (Supplementary Figures S1–S11).

We attributed larval morphotypes to species when there was a close molecular match to an adult sequence (usually < 3% difference) at the COI locus with a positively identified adult specimen. This level of genetic distance is based on consideration of BOLD Bin assignments and our ASAP partition scheme with 70 partitions (see Methods); it is also in agreement with the up to 3% intraspecific divergence reported for 51 species of echinoderms (Table 2 in Ward et al., 2008). Almost all our larvae were < 1–3% divergent from adult specimens, except those of an amphirid vitellaria, which was 5.7–9% different at the COI locus from adult specimens identified to species. In this case, we conservatively refrained from assigning our larva to its closest named match, *Amphioplus strongyloplax*, and instead gave it a provisional name of *Amphioplus* sp. vitellaria. See each section for the tree and specimen information therein.

3.2 | Developmental diversity

We observed eggs or embryos up to gastrulae of only a few species, indicating that their natal populations may occur subtidally near the coast: *Amphiodia* sp. opaque, *Amphiodia* sp. orange belly, *Amphiodia* aff. *periercta*, *Amphiura arcystata*, *Ophiura luetkenii*, and *Gorgonocephalus*

TABLE 2 Nineteen ophiuroid species found in Oregon waters and included in this study. Two species with new adult records from Oregon are marked with superscript 1. Three species marked with superscript 2 are known only as larvae in our study, but adults are known in the Canadian Salish Sea. Species marked with superscript 3 have adults known from California. Larval forms are planktotrophic (= feeding) ophiopluteus (P), reduced pluteus (RP), pelagic direct (D), and indirect development via a vitellaria (V). When planktonic duration is based on a single specimen we denote (n = 1).

Taxonomy	Egg (μm)	Larva	Days to metamorphosis	In Plankton (month)	Species depth (m)
Ophiotrichidae					
<i>Ophiothrix spiculata</i> ¹	110	P	?	Dec–Jan	1–2,059
Ophiophilidae					
<i>Ophiophilis kennerlyi</i>	105	P	83–216	Oct–Apr	0–366
<i>Ophiophilis bakeri</i>	?	P	?	Oct–Apr	37–1204
Amphiuridae					
<i>Amphiodia</i> aff. <i>digitata</i> ¹	160	P	?	Dec–Jan	18–280
<i>Amphiodia urtica</i>	120	P	20	Oct–Apr	0–370
<i>Amphipholis pugetana</i>	?	P	?	Oct–Mar	0–1204
<i>Amphiodia</i> sp. <i>opaque</i> ²	135	RP	13–33	Oct–Apr	5–23.5
<i>Amphiodia</i> sp. <i>orange belly</i> ²	?	RP	8 (n = 1)	Jan–Apr	
<i>Amphiodia</i> sp. <i>tan</i> ³	?	RP	12 (n = 1)	Sep	
<i>Amphiodia</i> aff. <i>periercta</i>	190	D	8	Oct–Mar	0–92
<i>Amphioplus</i> sp. <i>vitellaria</i> ²	?	V	~7	Nov–Feb	
<i>Amphiura arcystata</i> ³	?	D	6 (n = 1)	Nov–Feb	15–844
Ophiacanthidae					
<i>Ophiacantha diplasia</i>	?	P	82 (n = 1)	Jan–Apr	71–1178
Ophiopteridae					
<i>Ophiopteris papillosa</i>	102	P, V	65 (n = 1)	Nov–Feb	1–140
Ophiuridae					
<i>Ophiocten hastatum</i>	?	P	?	Jan–Feb	916–2877
<i>Ophiura leptoctenia</i>	?	P	?	Feb, May	25–3239
<i>Ophiura luetkenii</i>	?	RP	5	Nov–Apr	9–1265
<i>Ophiura sarsii</i>	120	P	?	May	0–1460
Gorgonocephalidae					
<i>Gorgonocephalus eucnemis</i>	220	D	5	Jan–Mar	8–1850

eucnemis. 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. aff. periercta* (Sections 3.4.3.4 and 3.4.3.7).

The ophiopluteus was the most common larval form, present in fifteen species (Table 2). Eleven of these ophioplutei are plank-

totrophic, whereas the remaining four were reduced plutei (see below). The planktotrophic plutei we observed were spread across six families: Ophiotrichidae (1 species), Ophiophilidae (2 species), Ophiopteridae (1 species), Ophiacanthidae (1 species), Amphiuridae (3 species), and Ophiuridae (3 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 which could be viewed using

cross-polarized light (Figure 2) to be the definitive way to distinguish between plutei of different species (Mortensen, 1921).

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, *A. sp.* orange belly, *A. sp.* tan, and *Ophiura luetkenii*. Reduced plutei were recognized by their opaque coloration; relatively short larval arms, particularly the posterolateral arms; rapidly developing left coeloms; and short development times. All four reduced plutei described in this study did not develop a pair of posterodorsal arms and had a total of six larval arms. We obtained sufficient material to test for facultative planktotrophy in *A. sp.* opaque (Nakata & Emlet, 2023), and suspect the remaining reduced plutei may feed facultatively as well.

We observed two species with vitellaria larvae, each the first from their families. One has abbreviated development (*Amphioplus* sp. vitellaria; Amphiuridae) while the other forms a feeding pluteus before transforming into a vitellaria (*Ophiopteris papillosa*; Ophiopteridae). The latter is the first example in which an ophiopluteus develops into a vitellaria prior to metamorphosis, outside of the Ophiocomidae (Byrne, Cisternas, O'Hara, & Selvakumaraswamy, 2024; Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Mladenov, 1985). The vitellaria is known from families Ophionereididae, Hemieuryalidae, Ophiolepididae, Clarkcomidae, Ophiodermatidae, and Ophiocomidae and may have evolved multiple times as a modification of the pluteus (Byrne, Cisternas, O'Hara, & Selvakumaraswamy, 2024; Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Byrne, et al., 2025; Cisternas & Byrne, 2005; Falkner et al., 2013; Grave & Brooks, 1900; Hendler, 1979, 1982, 1991; Komatsu & Shosaku, 1993; Mladenov, 1985; Mortensen, 1921 & 1938; Selvakumaraswamy & Byrne, 2023; Stanczyk, 1973; Weber et al., 2014).

We observed three species with direct development in the plankton: *Amphiodia* aff. *periercta*, *Amphiura arcystata*, and *Gorgonocephalus eucnemis*. An additional species, *Amphipholis squamata*, has direct development by brooding but was not included in this study.

Following identification by barcoding, we reviewed photographic records to determine unique characters that can be used to identify each larva morphologically. In addition to larval form (feeding pluteus, reduced pluteus, vitellaria, or pelagic direct developer), we used skeletal elements and their proportions to delineate between species. We describe these characters in the species accounts below and organized them into a dichotomous key (Section 5).

We observed variations in the pattern of metamorphosis across the six families containing ophioplutei (*Gorgonocephalus* is restricted to direct development). The number of larval arms retained during rudiment formation varied from two posterolateral arms in *Ophiothrix* and *Ophiopholis*, to all eight in *Ophiacantha diplasia*. Both the planktotrophic and reduced plutei of Amphiuridae resorb five arms, leaving both posterolateral arms and the right anterolateral arm (Figure 1C). The same is true for the planktotrophic *Ophiura sarsii*, but its congener with abbreviated development, *Ophiura luetkenii*, retains both anterolateral arms during rudiment development. Finally, the ophiopluteus of *Ophiopteris papillosa* transforms into a vitellaria prior to metamorphosis. We did not observe the rudiment-stage larva of *Ophiura leptoctenia*. Selvakumaraswamy and Byrne (2023) discuss

other variation in the number of arms and pattern of arm resorption during metamorphosis in ophiuroids.

3.3 | Patterns in spawning phenology

We observed strong seasonal patterns in the presence of ophiuroid developmental stages. Most species were observed primarily in the fall and winter months (November to March; Figure 3). The most abundant and frequently encountered larvae were those of *Ophiopholis* spp., *Amphiodia* aff. *periercta*, *Amphiodia urtica*, and *Amphipholis pugetana*.

Five species were observed just one to three times: *Amphiodia* aff. *digitata*, *Amphiodia* sp. tan, *Ophiocten hastatum*, *Ophiura leptoctenia*, and *Ophiura sarsii* (see Figure 4). Some of these rarely observed species were collected during the El Niño years of 2015, 2019, and 2024, perhaps because of altered current patterns. Spawning may have been suppressed in local populations by heat waves, such as the warm water blob that coincided with our 2014 sampling (Bond et al., 2015; Shanks et al., 2020) that resulted in northward shifts in species ranges from central and northern California to Oregon and beyond (Sanford et al., 2019).

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 (January–March). One exception is the pelagic direct developer, *Amphiodia* aff. *periercta* (see 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 October to May in Coos Bay, Oregon.

3.4 | Species accounts

3.4.1 | Ophiotrichidae LJUNGMAN 1867

The Ophiotrichidae is a large family that includes 13 genera, including the polyphyletic *Ophiothrix* (O'Hara et al., 2017, 2018), which currently contains 94 species that occur across the world (Stöhr et al., 2024). We observed larvae of one species, *Ophiothrix spiculata*, rarely in our plankton samples (Figure 3).

Ophiothrix spiculata LE CONTE 1851

Species identity. A survey of adult specimens identified as *Ophiothrix spiculata* in BOLD and GenBank revealed at least two distinct clades and a third specimen that groups with Caribbean species *O. stri*, *O. cimar*, and *O. lineata* (Figure S1). One clade occurs off Pacific Central America and includes a specimen from the species type locality, the Bay of Panama (Figure S1, Table S1; BOLD: ACC6919, BOLD: ACC6920). The second clade occurs off Oregon and California and is the one to which all our larvae and juveniles belonged. This clade consists of four BOLD bins: AFP8107, ADR9573, ADR9572, and AAW9067; Table S1; Figure S1,). Two larvae and one juvenile were < 2.1% different from one another and in BOLD bin AAW9067 with several adults from southern California. A second juvenile (OLAB090-23, BOLD bin AFP8107) was 4% different from our other planktonic specimens

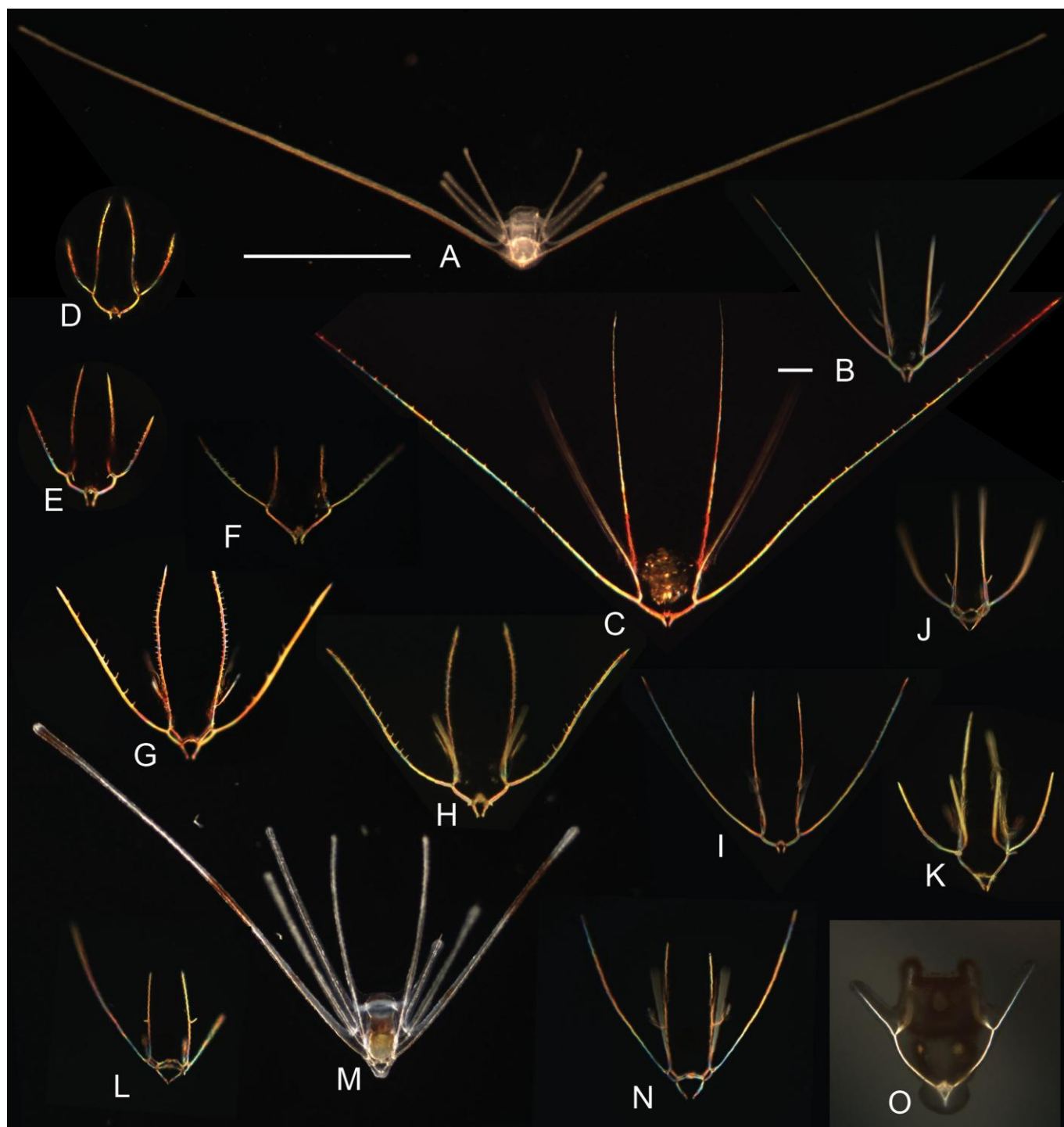


FIGURE 2 Ophioplutei of the northeastern Pacific Ocean, viewed in dark field (A,M) and cross-polarized light (B–L,N,O). **A.** *Ophiothrix spiculata*, scale bar 1 mm, scale same for **M.** **B.** *Ophiopholis kennerlyi*, scale bar 100 μ m, scale same for **B–L,N,O**; **C.** another individual. **D.** *Amphiodia* sp. tan, **E.** *Amphiodia* sp. orange belly, **F.** *Amphiodia* sp. opaque, **G.** *Amphiodia* aff. *digitata*, **H.** *Amphiodia* *urtica*, **I.** *Amphipholis pugetana*, **J.** *Ophiacantha diplasia*, and **K.** *Ophiopteris papillosa*. **L.** *Ophiura sarsii*, **M.** *Ophiocten hastatum*, **N.** *Ophiura leptoctenia*, and **O.** *Ophiura luetkenii*.

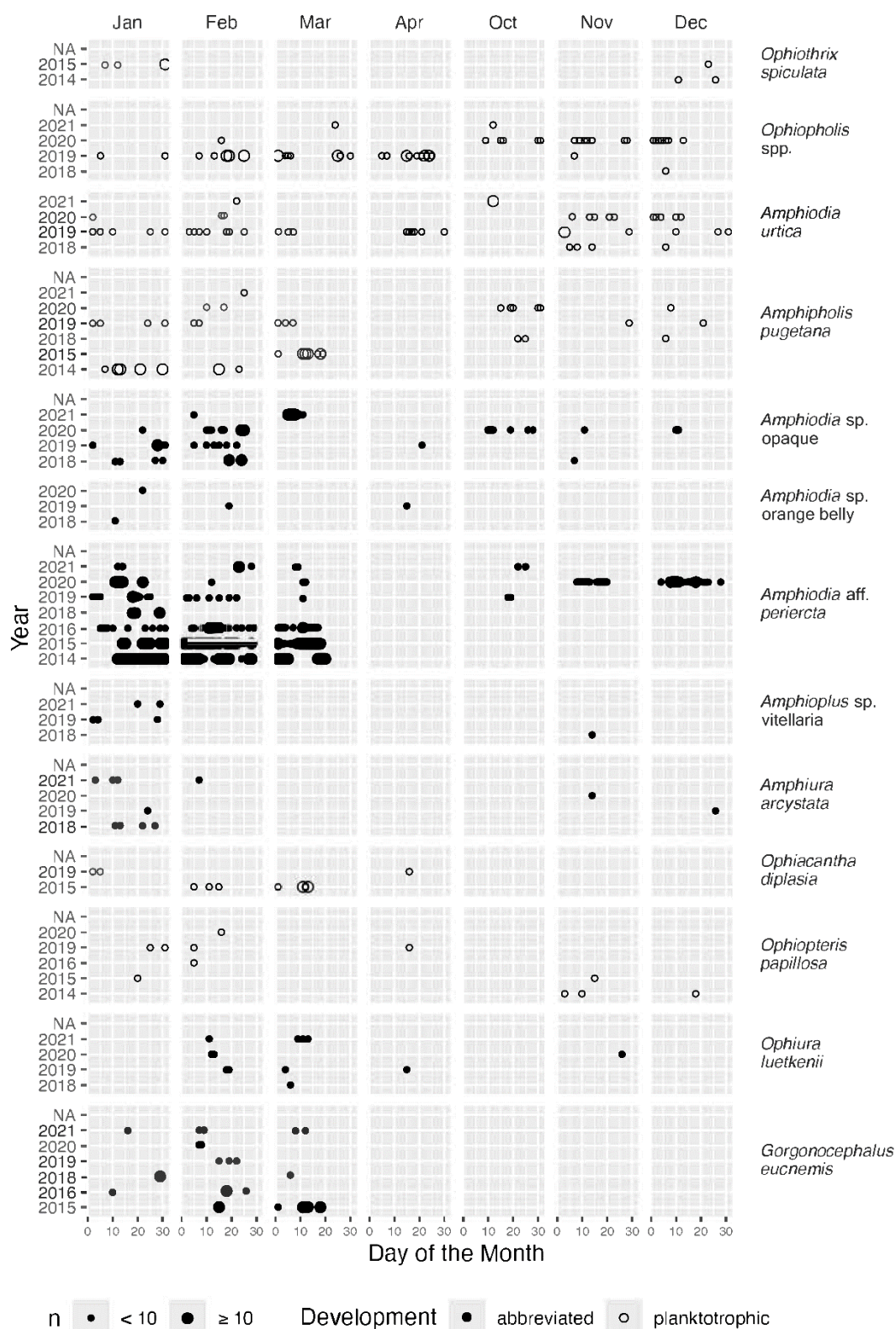


FIGURE 3 Spawning phenology for 14 planktonic ophiuroid species of the southern Oregon coast. Days of the month are marked on the x-axis, with each gray box representing five days' time. The months of May to September are omitted because we rarely made observations in those months. Occurrence data are 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. Four observations of *Amphiodia sp. opaque* are omitted from May 2019 (n = 2) and August 2021 (n = 2).

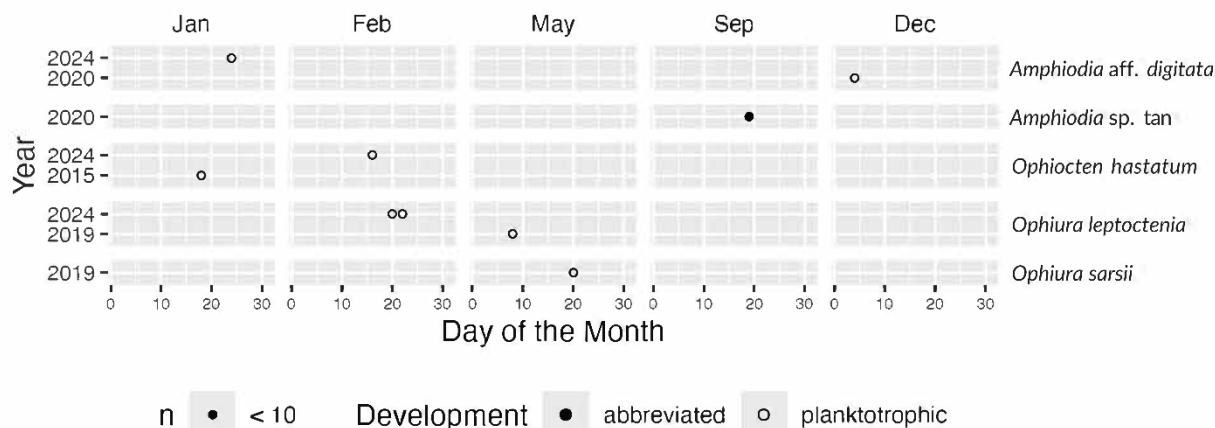


FIGURE 4 Spawning phenology for 5 additional ophiroid species that were observed rarely, i.e., on three or fewer occasions across the ten-year span. Note that we include only the months in which these species were observed: January, February, May, September, and December. Occurrence data are represented by dots: filled for species with abbreviated development and open for species with planktotrophic development. Each dot is one larva.

and was most closely related (96–98%) to other adults from California and Oregon (in BOLD bins ADR9572 and ADR9573). The COI sequences of the two clades of *O. spiculata* were > 18% different and are almost certainly separate species. To our knowledge this is the first report of sequence comparisons within samples assigned to *O. spiculata* and further work is necessary to determine if more than one species occurs in the California-Oregon clade and how adults in this clade differ from those in the eastern Pacific of Central America (see Hendler, 1996). The genus *Ophiothrix* contains other species complexes (Hernández-Díaz et al., 2023; O'Hara et al., 2014; Taboada & Pérez-Portela, 2016).

Distribution and local sites. From the literature, *O. spiculata* occurs in the Pacific Ocean from California to northern Chile from the low intertidal zone to a depth of 2059 m, but it has not been listed as part of the benthic fauna of the southern Oregon coast (Austin & Hadfield, 1980; Hendler, 1996; Lambert & Austin, 2007).

The previous northern limit in California was Moss Beach, San Mateo County, California (Austin & Hadfield, 1980; Hendler, 1996; 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). In August 2024, we collected a single adult specimen off Cape Arago, OR (OOPH070-24, BOLD bin ADR9572). Additional information for specimens collected in this study can be accessed by using the BOLD Process IDs (Table S1).

Larval description. *Ophiothrix spiculata* develops via an eight-armed ophiopluteus, and it was one of the largest > 6 mm of all the plutei we observed (Figure 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 in late plutei (Figure

2A). The postoral and posterodorsal 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 (Figure 5A). 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 (Figure 5A).

Similar species. We observed *O. spiculata* only as the eight-armed ophiopluteus or juveniles (likely detached from the larval arms during collection), both of which are similar to those stages of *Ophiopholis* spp. (see Section 3.4.2.1 below). In both species, the larva has posterolateral arms that are straight, long, and wideset; the remaining arms are similar in length to one another but markedly shorter than the posterolateral arms. Late plutei of *O. spiculata* have posterolateral arms up to five times the length of their other arms, whilst the posterolateral arms of late plutei of *Ophiopholis* spp. are up to three times the length of the other arms. This comparison is useful only in advanced larvae, as the length of the arms is accumulated across time as the larva grows.

Development mode. *Ophiothrix spiculata* has an obligately feeding, 8-armed ophiopluteus, as expected from a small egg of diameter 110 μ m (Pearse, 1994). Feeding larvae are the norm in *Ophiothrix* (Guille, 1964; Hendler, 1995, 2005; Hendler & Littman, 1986; Kitazawa et al., 2015; Mladenov, 1983, 1985a; Morgan & Jangoux, 2005; Mortensen, 1938; Selvakumaraswamy & Byrne, 2006 & 2000), although nonfeeding planktonic larvae (Mladenov, 1979; Richards et al., 2007) and one brooder (Schoppe, 1996) are also known. Another genus in the same family, *Macrophiathrix*, 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 that can be used to secure the individual in rock crevices or algal holdfasts. The hooked arm spines can be used to distinguish between the juveniles of *O. spiculata* and *Ophiopholis* spp.,

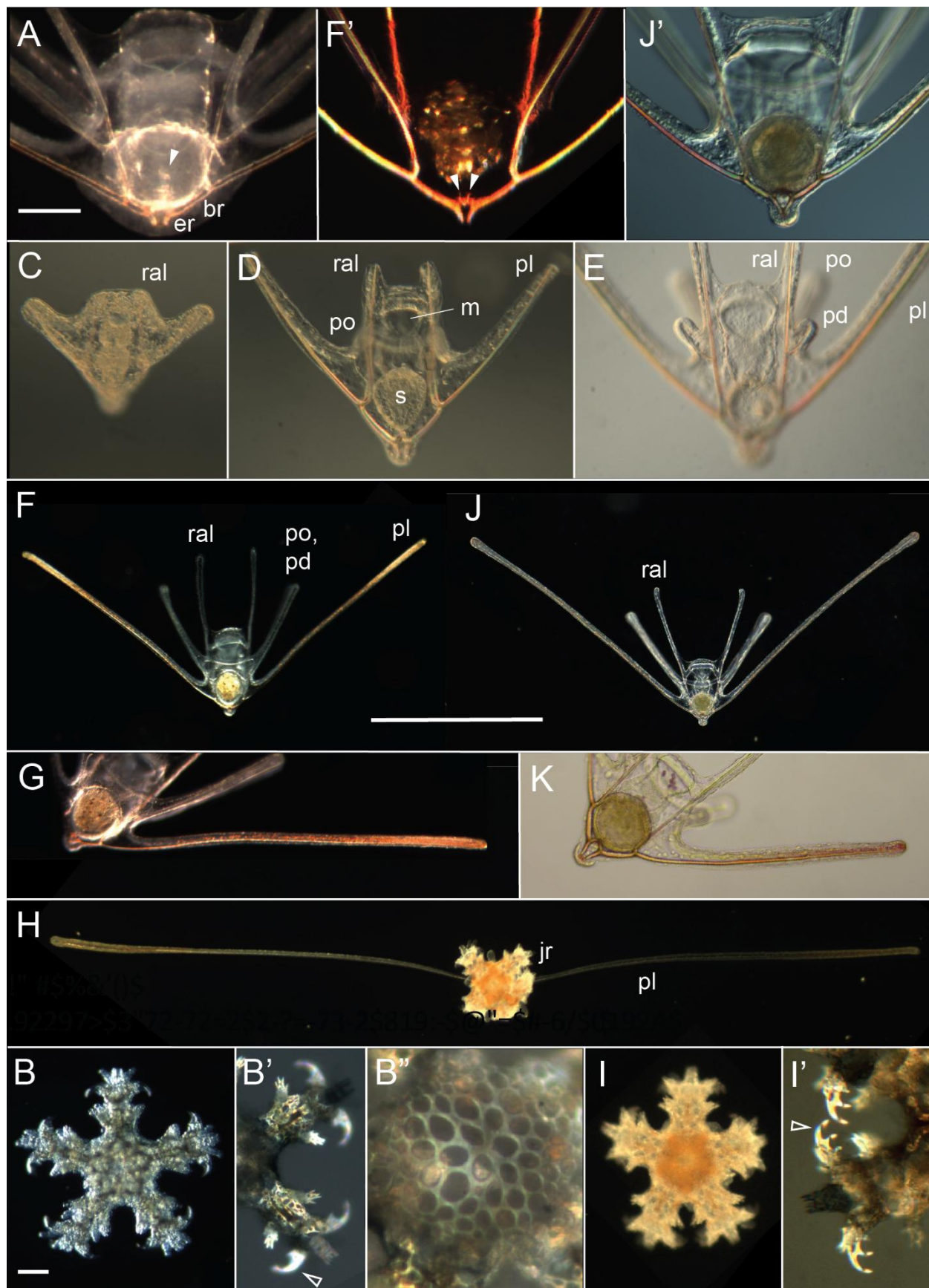


FIGURE 5 Legend on next page.

FIGURE 5 Larvae and juveniles of (A,B) *Ophiothrix spiculata*, (C–I) *Ophiopholis kennerlyi*, and (J,K) *Ophiopholis bakeri*. Secondary and tertiary views of the same individual are marked with an apostrophe (') and quotation mark ("), respectively. **A.** Closeup of the posterior girdle of *O. spiculata*, same individual as Figure 2A. Note the short end rods (er) attached to the body rods (br), and possible median process (arrowhead). **B.** Juvenile of *Ophiothrix spiculata* collected from the plankton, aboral view in transmitted light, and (**B'**) closeups of the hooked spines on the terminal segments of the arms, and (**B''**) of the disc stereom in partially cross-polarized light. **C.** *O. kennerlyi* early pluteus, aboral view, **D.** six-armed pluteus, oral view, and **E.** early eight-armed pluteus, aboral view, with anterolateral (al), postoral (po), posterodorsal (pd), and posterolateral (pl) arms. **F.** Oral view of a more advanced eight-armed pluteus and (**F'**) closeup of the posterior girdle in cross-polarized light with transverse rods bearing two medial processes (arrowheads). In both **F** and **F'** the posterodorsal arms are largely behind the postoral arms. **G.** 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. **H.** A rudiment-stage larva with a juvenile rudiment (jr) carried by the wideset posterolateral arms, same scale as **E**. **I.** Juvenile collected from the plankton, and (**I'**) a closeup of the hooked spines with two teeth on the terminal segment of the arm (open arrowhead). **J.** Eight-armed pluteus of *O. bakeri*, oral view at same scale as **E** and (**J'**) closeup of the posterior girdle, same scale as **A**. **K.** Aboral view of another individual, showing the red-orange coloration of the posterolateral, body, and end rods. Same scale as **H**. Scale bar in **A** is 100 μ m, same scale for **A**, **C–E**, **F'**, and **J'**. Scale in **F** is 1 mm, same scale for **H**, **J**. Scale in **B** is 100 μ m, same scale for **I**.

as *O. spiculata* has a single hook (Figure 5B), and *Ophiopholis* spp. have a double hook (Figure 5I). The juvenile arms are often folded under the disk while still attached to the larval arms, and this condition may persist following metamorphosis.

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 (Peterson et al., 2017); another larva was collected during El Niño, February 2024. Spawning has been observed from latewinter 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 Figure 2A).

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 composed 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 readily distinguishable based on morphology (see Figure 5F,J) but barcoding showed to be distinct (Supplementary Table S1; Figure S2). Therefore, we provide 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 18 m to 1200 m (Lambert & Austin, 2007) and > 90 m off Coos Bay, Oregon.

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

Species identity. Although there are characters listed in the literature that distinguish adult *O. kennerlyi* from *O. aculeata*, Lambert and 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 (Supplementary Figure S2). Our larvae identified as *O. kennerlyi* were < 1% different from adults from Oregon, Washington, and British Columbia, and larvae and juveniles of similar phenotypes from British Columbia (Supplementary Table S1; Figure S2).

The deep-water species *O. bakeri* and *O. longispina* are recognized as separate species distinguished by presence or absence of spines on their radial shields, and length of arm spines (Lambert & Austin, 2007). They are also acknowledged to be very similar to one another as 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 did not show *bakeri* and *longispina* to form reciprocally monophyletic groups, but the data for *longispina* was limited (Figure S2). 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 here. Our larvae are 97% to 100% similar at COI compared to all adults in the *bakeri/longispina* clade (Supplementary Table S1; Figure S2).

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* at 100 m depth (Supplementary Table S1).

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

Larval description. *Ophiopholis* spp. develop via an eight-armed ophiopluteus. We recognized the ophioplutei of *Ophiopholis* spp. by their long, straight posterolateral arms and transparent epidermis. 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 and has four arms which extend anteriorly and terminate at approximately the same 'height'; that is, a line connecting the distal tips of the posterolateral

arms would touch the tips of the anterolateral arms (see Key 5a; contrast Figures 5F,J with 2B,C). The posterolateral arms grow in length and become increasingly laterally directed as the larva develops, such that the anterolateral arms are 'shorter' than the posterolateral arms (Figures 5F,J).

These first two arm pairs are followed quickly by the postoral arms, which support a ciliary band in front of the mouth (Figure 5D). The posterodorsal rods bud from the anterolateral rods (Figure 5E) and eventually grow to be of equal height with the postoral arms (Figure 5F). The posterodorsal arms are mirror-imaged across the frontal plane, such that it is difficult to view both pairs at once.

As the larva develops, the posterolateral, body, and end rods may take on an orange-red hue (Figures 5G,K), and pigmentation that is yellow to red in color may accumulate in the distal portions of the posterolateral arms (Figures 5F,J,H). This appears to be more common and more extreme in *kennerlyi*, but the number of specimens of *bakeri* that we found 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 and R Emlet, personal observations).

Similar species. Early plutei of *Ophiopholis* spp. larvae are most like those of *Amphiodia urtica* and *Amphipholis pugetana*. The plutei of all three species have a transparent epidermis often with red pigmentation on the posterolateral arms. In *Ophiopholis* spp. pigmentation is absent in the four- and six-armed plutei and may accumulate in the posterolateral arms over development. In amphiuroid planktotrophic plutei red pigmentation is present even in the early pluteus but is restricted to the very tips of the posterolateral arms.

The skeleton can also be used to distinguish larvae from the families Ophiopholidae and Amphiuroidae. At the four- and six-armed stages the posterolateral arms have not grown to sufficient length to show the shapes that will distinguish between them. The posterolateral arms are straight and moderately wided set in *Ophiopholis* spp. and curved in the amphiuroids. In the early pluteus, the anterolateral arms in *Ophiopholis* spp. terminate at the same height as the posterolateral arms, whereas in amphiuroids the anterolateral arms exceed the height of the posterolateral arms (see Key 5b). Posterolateral arm rods of *Ophiopholis* spp. larvae may bear very small thorns on their inner side, that are well-spaced, much unlike the numerous, conspicuous thorns of *Amphiodia* ophioplutei.

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 laterally directed in both species, their angle is approximately 90° in *Ophiopholis* spp., while in *O. spiculata* the angle of the posterolateral arms can greatly exceed 90° (Key 7a, b). 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 later in development there are two short medial processes (Figure 5F').

Development mode. Both *O. kennerlyi* and *O. bakeri* have a planktotrophic ophiopluteus. The feeding larva of *O. kennerlyi* develops from an egg of 105 µm (Lambert & Austin, 2007; Strathmann, 1987). 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; Strathmann et al., 2020).

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 to 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 (Figures 5B',I'; Key 24).

Reproductive timing. Larvae of *Ophiopholis* spp. occurred in the plankton from October to April, although the timing of their appearance varied from year to year (Figure 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 | Amphiuroidae LJUNGMAN 1867

We collected nine species of amphiuroid larvae in the plankton and were able to match eight of these to adult specimens via DNA barcoding: *Amphiodia* aff. *digitata*, *Amphiodia urtica*, *Amphiodia* aff. *periercta*, *Amphiodia* sp. opaque, *Amphiodia* sp. orange belly, *Amphiodia* sp. tan, *Amphipholis pugetana* and *Amphiura arcystata*. The remaining species is an 'orphan larva' without molecular matches to adult specimens, but it groups with species of *Amphiplus* (sp. vitellaria). See Supplementary Table S1; Figure S5.

Amphiuroids represent just over half of the larvae described here, which is in accordance with the diversity of Amphiuroidae, the largest of the brittle star families. We identified the larvae from four genera (out of 26 genera in the family): 6 species from *Amphiodia*, and one species each from *Amphipholis*, *Amphiplus*, and *Amphiura*.

There are far more *Amphiodia* spp. in the northeastern Pacific north of California than the three previously known (*A. occidentalis*, *A. periercta*, and *A. urtica*; see Lambert & Austin, 2007). The molecular and developmental data from our Oregon samples suggest that *A. urtica* is most closely related to four other species: *A. sp. opaque*, *A. sp. orange belly*, *A. sp. tan*, and *A. aff. digitata* (Supplementary Figure S3). *Amphiodia occidentalis* has two distinct taxa that are only 90% similar at COI and are sisters to *A. aff. periercta* (Supplementary Figure S3). Other species of *Amphiodia* known south of our sampling area include three species (*akosmos*, *digitata*, and *psara*) in southern and central California, and at least five more species along the Pacific coast of Mexico (*assimilis*, *platyspina*, *sculptilis*, *tabogae*, and *violacea*) (Granja-Fernández

et al., 2015, 2017; Hendler & Bundrick, 2001; Hendler, 2007; Lambert & Austin, 2007).

The most frequently encountered amphiuroids in our region were the direct developing *A. aff. periercta*, the reduced pluteus of *A. sp. opaque*, and the feeding ophioplutei of *A. urtica* and *A. pugetana* (Figure 3). The larvae of *A. aff. digitata*, *A. sp. vitellaria*, *A. arcystata*, *A. sp. orange belly*, and *A. sp. tan* were observed rarely, with none to a few per year.

We observed several developmental patterns amongst the amphiuroids of the NE Pacific, including planktotrophy, facultative planktotrophy, lecithotrophy via a vitellaria, and pelagic direct development. The most common brittle star known to brood its young in the NE Pacific is the cosmopolitan species *Amphipholis squamata* (Hugall et al., 2024; Strathmann, 1987), which was not described here because it lacks a planktonic form. Another brooding species, *Amphiodia akosmos*, is known only from the intertidal of the Monterey Peninsula, Santa Cruz County, and Farallon Island (Hendler, 2007; Hendler & Bundrick, 2001).

Three of the nine planktonic stages of amphiuroids had planktotrophic development via an ophiopluteus, *Amphiodia aff. digitata*, *A. urtica*, and *Amphipholis pugetana*. These plutei had a transparent epidermis with red pigmentation on the distal ends of the posterolateral arms and curved posterolateral arms. The ophioplutei of the genus *Amphiodia* generally have anterolateral arms that exceed the height of the posterolateral arms (Figures 2D–H; Key 10b, 14). The skeletal rods may have an orange-red tint and often bear conspicuous thorns, especially on the posterolateral and anterolateral arm rods. Amphiuroid ophioplutei have short postoral and posterodorsal arms that do not exceed the height of the mouth.

Remarkably, we observed six amphiuroids with abbreviated development, including three species that develop via a reduced pluteus (*Amphiodia* spp. *opaque*, *orange belly*, and *tan*), one that develops via a nonfeeding vitellaria (*Amphioplus sp. vitellaria*), and the two others that develop directly into juveniles in the plankton (*Amphiodia aff. 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, but we were not able to confirm this feeding pattern. Other abbreviated forms of development known from the family Amphiuroidae include *Amphioplus abditus* (Hendler, 1973) and *Amphiura chiajei* (Hendler, 1975), with several more species inferred to have abbreviated development based on egg size (Hendler, 1991, 1995).

The amphiuroid ophioplutei and reduced plutei from Oregon metamorphosed in a similar manner: posterodorsal arms (in feeding ophioplutei) and postoral arms are resorbed in the formation of a pentaradial juvenile. The posterolateral and anterolateral arms are retained in the rudiment-stage larva, but the left anterolateral is highly reduced and assumes a near-horizontal angle just anterior to the juvenile rudiment. This metamorphic phenotype appears similar to that of *Amphiura filiformis* (Mortensen, 1931) but differs from *Amphipholis kochii*, which appears to resorb both anterolateral arms (Yamashita, 1985). The retention of an intact right anterolateral arm during

metamorphosis was also observed in *Ophiura sarsii* (see Section 3.4.5.4) and *Ophionereis fasciata*; in the latter it has been suggested to represent the transition between pluteus and vitellaria (Selvakumaraswamy & Byrne, 2023).

We grouped descriptions of amphiuroid species by larval form to facilitate comparison. We describe the planktonic stages of nine species in the following order: planktotrophic ophioplutei of *A. aff. digitata*, *A. urtica*, and *A. pugetana*; reduced plutei of *A. spp. opaque*, *orange belly*, and *tan*; and pelagic direct development in *A. aff. periercta*, *Amphioplus sp. vitellaria*, and *Amphiura arcystata*.

3.4.3.1 | *Amphiodia aff. digitata* NIELSON, 1932

Species identity. We collected two larvae from the Charleston docks and obtained sequences from one that matched those of two small adult specimens collected off Cape Arago, Oregon. The small adults resembled *Amphiodia aff. digitata* in having a peripheral row (of uppermost ventral) scales bordered by hyaline spines (Hendler, 1996). These two specimens were 99% and 97% similar respectively, for COI from the larva we barcoded from the Charleston plankton (Supplementary Table S1; Figure S3). The 16S for these adults were also 100% and 99% similar, respectively, to the 16S sequence of the larva.

Distribution and local sites. *Amphiodia digitata* occurs from Point Conception, California to Mexico at depth of 18–280 m and with the type locality off La Jolla, California (Hendler, 1996; Nielsen, 1932). The two small individuals that we collected from the Cape Arago Shelf at approximately 100 m using a dredge resemble *A. digitata*. As the species identification is uncertain and we encountered larvae of this species only twice, there is insufficient data to label this as a range expansion for *A. digitata*.

Larval description. The ophiopluteus of *A. aff. digitata* has a transparent epidermis and grows to have eight arms. The skeletal rods may be tinted orange to red, and the anterolateral and posterolateral rods are curved and bear thorns (Figures 6A,B). *Amphiodia aff. digitata* can be recognized by its curved posterolateral arms and the presence of recurrent rods in the posterior girdle, which are not found on any other amphiuroid ophiopluteus in the northeastern Pacific Ocean. The body rods are just longer than the end rods. The distal ends of postoral and posterodorsal arms do not grow past the mouth in the anterior direction, a feature of amphiuroid ophioplutei that distinguishes them from other feeding ophioplutei of the region.

Similar species. The ophiopluteus of *A. aff. digitata* is very similar to that of *A. urtica* in shape and color but can be distinguished by the presence of recurrent rods in the posterior girdle (Figures 6A',B'), which are absent in *A. urtica* (Figure 6E"). In Oregon and Washington, we encountered larvae of *A. urtica* much more frequently than larvae of *A. aff. digitata*.

Development mode. This larva appears to be planktotrophic, based on the large size of the larva and its transparent epidermis through which we observed a complete digestive system. Hendler 1996 reports an egg size of 160 µm and suggested abbreviated development, but we saw no evidence of that type of development. *Amphiodia aff. digitata* forms a clade with another planktotroph, *A. urtica*, and two putative facultative planktotrophs, *A. sp. tan* and *A. sp.*

orange belly (Supplementary Figure S3).

Reproductive timing. We observed one, maybe two larvae of this species. The positively barcoded individual was collected on Jan 24, 2024 (Figure 6A), and an unconfirmed, photographed, larva on December 4, 2020 (Figure 6B).

3.4.3.2 | *Amphiodia urtica* (LYMAN 1860)

Species identity. We collected larvae from Charleston, Oregon and Friday Harbor, Washington whose COI sequences were 2% different from those of adults from southern California, British Columbia, Canada, and the Cape Arago, Oregon (Supplementary Table S1; Figure S3). In our tree of amphiuroids from the northeast Pacific, *Amphiodia urtica* is sister to the clade composed of *A. aff. digitata*, *A. sp. orange belly* and *A. sp. tan*.

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.

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 (Figure 6C). The postoral arms emerge next, supporting a portion of the ciliated band ventral to the mouth (Figure 6D). Lastly the posterodorsal arms grow out from the bases of the anterolateral arms to make an eight-armed ophiopluteus (Figure 6E). As the larva grows the posterolateral arms become curved and bear thorns (Figure 6E'); 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 are straight and positioned at 45° angles relative to the larval anterior-posterior axis. The body rods are connected by thorny transverse rods and the end rods are pointed but not touching (Figure 6E"). The body rods are nearly twice the length of the end rods.

The juvenile rudiment develops to the left of the digestive system. The lobes of the left coelom are visible (Figure 6F). 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 (Figure 6F).

Similar species. The ophiopluteus of *A. urtica* is strikingly similar to that of *A. aff. digitata* in the shape of its skeleton, especially the posterolateral and anterolateral arms (Figures 6B,E). *Amphiodia urtica* can be recognized by the absence of recurrent rods in the posterior girdle (Figure 6E"), which are present in *A. aff. digitata* (Figure 6B'). Both *Amphiodia urtica* and *A. sp. orange belly* can have thorns protrud-

ing from the postoral arms at the same location as the origin of the recurrent rods in *A. aff. digitata*.

Although *A. urtica* is very similar to *A. aff. digitata*, we observed the latter only twice. The early pluteus of *A. urtica* is more likely to be confused with other common planktotrophic species, such as *Ophiopholis* spp. or *Amphipholis pugetana*. The anterolateral arms of *A. urtica* (and presumably *A. aff. digitata*) and *A. pugetana* have a greater height than the posterolateral arms in the early pluteus, which distinguishes the early plutei of this family from those of *Ophiopholis* (see Key, 5, 14 and *Ophiopholis* spp. above). Later in development, the posterolateral arms of *Ophiopholis* spp. are straight while those of amphiuroid plutei are curved (Figures 2H,I)

The pluteus of *A. urtica* may also be confused with *A. pugetana*. As the plutei mature they develop several characteristics that can be used to tell them apart but are very similar as four-armed plutei. Both larvae have red-orange skeletons covered in transparent epidermis, with an accumulation of red pigmentation at the distal ends of the posterolateral arms (Figures 6C,I). The posterolateral arm rods of *A. pugetana* are curved across their length, in contrast to the proximally curved rods of *A. urtica* (Key 10). The posterolateral and anterolateral arm rods of *A. urtica* bear many thorns, especially later in development (Figure 6E), while thorns are absent or nearly imperceivable in *A. pugetana* (Figure 2). The body rods of the early plutei of *A. urtica* and *A. pugetana* form an angle of 90°. As the pluteus of *A. pugetana* develops, the body rods take on a wider angle relative to the midline until they are nearly horizontal (Figure 6K'). The end rods of *A. urtica* are over half the length of the body rods, whereas the end rods of *A. pugetana* are much shorter than the body rods. The transverse rods are thorny in *A. urtica* and unadorned in *A. pugetana*.

The epidermis of *A. urtica* adheres closely to the larval skeleton and follows the curvature of the arms. *Amphipholis pugetana* has thicker arms than *A. urtica* due to its inflated epidermis, which becomes more apparent as the larva develops, especially at the distal tips of the anterolateral arms, where the ciliated band bends medially (Figure 6K). *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. *Amphiodia urtica* develops as a planktotrophic ophiopluteus. Prior reports for the life history of this species are limited to egg size, approximately 120 µm 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 (Figures 6G,H).

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).

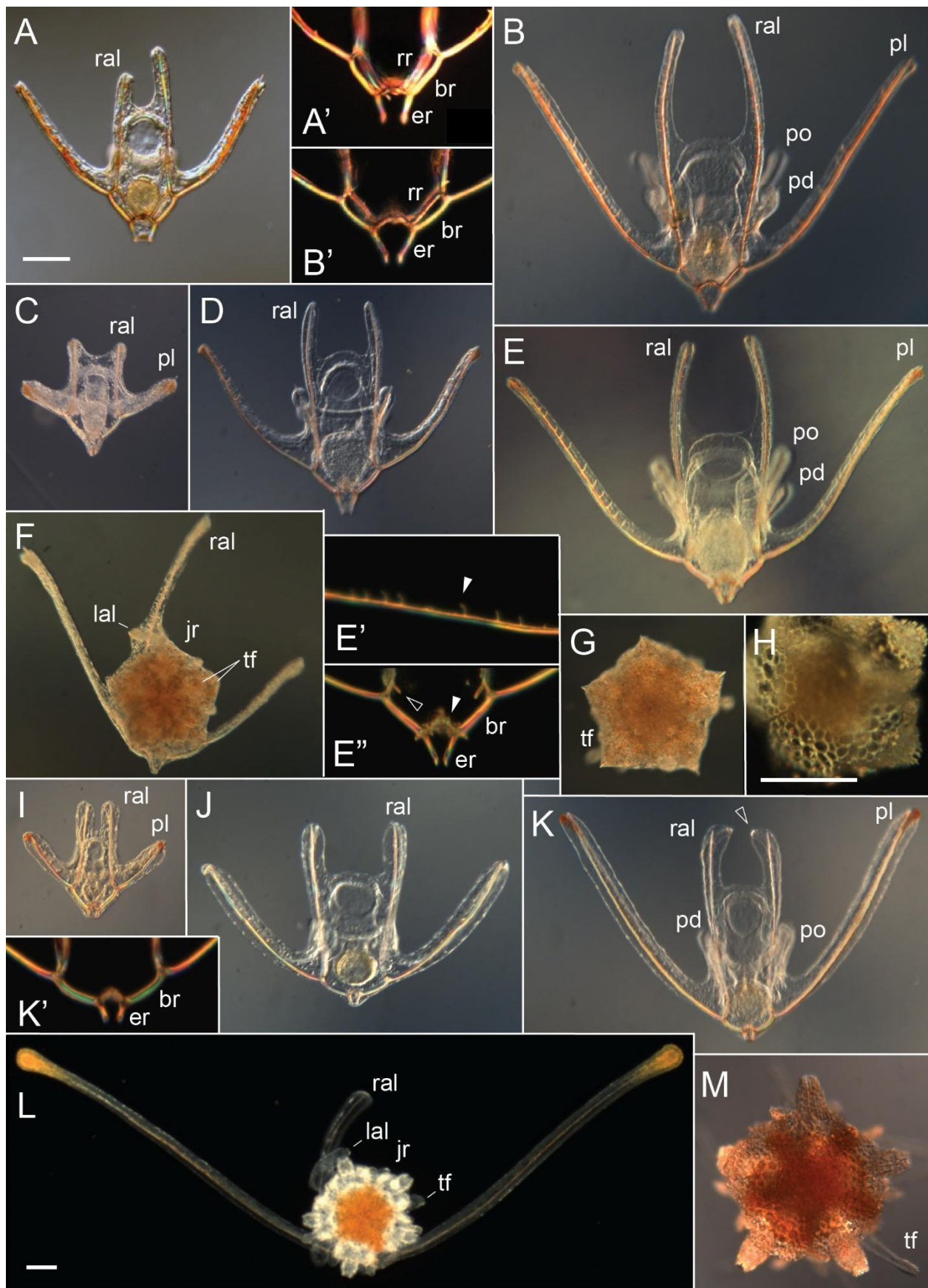


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FIGURE 6. Planktotrophic plutei of Amphiridae: *Amphiodia* aff. *digitata* (A,B), *Amphiodia urtica* (C–H) and *Amphipholis pugetana* (I–M). Secondary and tertiary views of the same individual are marked with an apostrophe ('), or a quotation mark ("), respectively. **A.** Oral view of the early pluteus of *A. aff. digitata*, with (A') the posterior girdle made of body (br), recurrent (rr), and end rods (er) in cross-polarized light at the same scale. **B.** Aboral view of another individual, not confirmed to be *A. aff. digitata* by barcoding but with similar morphology and recurrent rods (B'). **C.** 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. **D.** Early eight-armed (oral view), and **E.** advanced eight-arm pluteus (oral view) with anterolateral, posterolateral, postoral (po), and posterodorsal (pd) arm pairs. (E') Closeups of thorns on posterolateral arms, and (E") Posterior girdle, respectively, in cross-polarized light. The posterior girdle is composed of the transverse rods (filled arrowhead), body rods, and end rods. Also present are thorns (open arrowhead) that grow from the postoral rods. **F.** Oral view of a rudiment-stage larva with left (lal) and right anterolateral arm (ral) and juvenile rudiment (jr) bearing tube foot buds (tf). **G.** The juvenile of the same individual, one day later. **H.** A closeup of the stereom of another individual in cross-polarized light. **I.** Early pluteus of *Amphipholis pugetana* with red pigmentation at the distal tip of the posterolateral arms. **J, K.** 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). **L.** Rudiment-stage larva with right anterolateral arm (ral), juvenile rudiment (jr) with tube feet (tf), scale 100 μ m. **M.** Juvenile in partially cross-polarized light highlights the stereom of the disk plates and spine tips. Scale bar in **A** is 100 μ m and the same for **A–G, I–K, M**. Scale bars in **H** and **L** are 100 μ m.

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

3.4.3.3 | *Amphipholis pugetana* (LYMAN 1860)

Species identity. Larvae we identified as *Amphipholis pugetana* were $\geq 97\%$ similar for COI to adults collected locally or from California or British Columbia (BOLD bin: AAD7203). Two other samples from southern California, also called *A. pugetana* (OR347653, OR347652), were more distant (94% similar) to our specimens. Two other samples from California identified as *A. squamata* (DISA998-19, DISA840-19) also grouped with the *A. pugetana* clade and probably represent misidentifications (Supplementary Table S1; Figure S4).

Two species of *Amphipholis* occur in the northeast Pacific (26 *Amphipholis* spp. total): *A. pugetana* and the cosmopolitan brooder *A. squamata*. Our adult specimens of *A. pugetana* and *A. squamata* do not group together (Supplementary Table S1; Figure S4), and the genus *Amphipholis* is probably polyphyletic (Hugall et al., 2024; O'Hara et al., 2017). The placement of *Amphipholis* relative to *Amphiodia* and *Amphiura* is uncertain and the family Amphiridae requires revision (O'Hara et al., 2017).

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.

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, even in the early pluteus (Figure 6).

The posterolateral arms are curved, and any thorns on the skeletal rods 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 (Figure 2); the anterolateral rods taper to a sharp point and never bear thorns, unlike those of *Amphiodia* spp. (e.g., *A. aff. digitata*, *A. urtica*, *A. sp. opaque*, *A. sp. orange belly*, and *A. sp. tan*). The epidermis and ciliated band on the tips of anterolateral arms may extend medially, giving this larva a bunnyeared appearance (Figure 6K). The body rods are relatively short, curved, and nearly perpendicular to the larval anterior-posterior axis.

The transverse rods are short and unadorned. The end rods are much shorter than the body rods and run parallel to one another, not touching at their distal ends. The postoral and posterodorsal arms remain short, never terminating above the larval mouth.

Similar species. *Amphipholis pugetana* is most likely to be confused with *Ophiopholis* spp. or *Amphiodia urtica*, especially as an early pluteus (see sections above).

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 (Figure 6L).

Juvenile description. The juvenile has a roughly pentagonal disc with five arm tips that end bluntly (Figure 6M). 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 amphirid 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.

3.4.3.4 | *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* (Supplementary Table S1; Figure S3; see also Nakata & Emlet, 2023). COI sequences of our larval samples were $< 2\%$ different from adult specimens collected in Washington and British Columbia and $> 12\%$ different from the next closest clade, *Amphiodia* sp. orange belly (section 3.4.3.5 below).

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 and the San Juan Islands (Nakata & Emlet, 2023). At least 10 larval, juvenile, and adult stages have been collected in British Columbia, barcoded, and assigned to BOLD bin AEB6823 along with our larvae.

Embryonic description. Eggs are ca. 135 μ m in diameter. Eggs and embryos of this species are orange to salmon in color and have a thick hyaline layer (Figure 7A). This hyaline layer can be seen between

blastomeres at the 2–8 cell stages, like *A. aff. periercta* (Emlet, 2006). Embryos usually hatch after 24 hours as blastulae. Gastrulae are ovoid, wider toward the posterior and more pointed in the anterior, and uniformly ciliated. The first spicules develop as a pair in the posterior of the gastrula (Figure 7A').

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 triradial spicules (Figure 7B'). 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 (Figure 7D'). The anterolateral arms can be straight or curved (Figures 7C',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.

Similar species. The reduced pluteus of *A. sp. opaque* is not likely to be confused with its planktotrophic congeners (see above) due to the opaque coloration of the epidermis that somewhat obscures the internal anatomy of the larva. Furthermore, the larva is of small size and has short arms.

The four reduced plutei (*A. sp. opaque*, *A. sp. orange belly*, *A. sp. tan*, and *Ophiura luetkenii*) described in this study have opaque coloration of orange, salmon, pink, and tan. *Amphiodia* sp. opaque and *A. sp. tan* are uniform in color across their larval bodies and arms. salmon-colored in the former (Figure 7C) and tan in the latter (Figure 7N). In *A. sp. orange belly* and *O. luetkenii* the larval body is orange, and the posterolateral arms are white (Figures 7H and 11F).

The best way to distinguish *A. sp. opaque* from *A. sp. tan* is to compare the heights of the anterolateral and posterolateral arms. In *A. sp. opaque* the anterolateral arms are shorter in length than the posterolateral arms and therefore do not exceed the height of the posterolateral arms; in *A. sp. tan* their relative lengths are reversed, with long anterolateral arms that are of greater height than the posterolateral arms (Key 14). The posterior girdles also make a useful comparison: the pluteus of *A. sp. opaque* has straighter body rods that curve medially at the posterior, and longer, more prominent end rods (Figure 7D') than in the single individual of *A. sp. tan* we collected (Figure 7O'). One other aspect that differs between the two species is rate of development: *A. sp. tan* develops more quickly and has large coeloms even in the early pluteus (Figure 7M). These are not developed in *A. sp. opaque* until the six-armed pluteus.

Development mode. *Amphiodia* 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 suc-

cess, and larger juveniles when given food (Nakata & Emlet, 2023).

Juvenile description. The juvenile is pentagonal (Figure 7E), very similar to congeners with reduced plutei: *A. sp. orange belly* and *A. sp. tan* (Figures 7L,R).

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. A few larvae have also been observed in fall (October, November). In Friday Harbor, WA, larvae were collected in late summer (July, August, and September), but this was the only season we were able to 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).

3.4.3.5 | *Amphiodia* sp. orange belly

Species identity. We collected four larvae of *Amphiodia* sp. orange belly (Figure 3). Adult populations of *A. sp. orange belly* have not been identified near Charleston, Oregon, but two adults collected from the Straits of Juan de Fuca (off Vancouver Island) are < 1% different for *COI* from our larva (MVCRD114-20, MVCRD115-20). DNA barcoding and larval morphology places the larva within the genus *Amphiodia*, sister to *A. sp. tan* (Figure S3, Table S1).

Distribution and local sites. Adult populations in Oregon are not known, but two adults have been collected in the Straits of Juan de Fuca.

Larval description. Orange belly develops via a six-armed pluteus. The early pluteus is similar to that of *A. sp. opaque* (Figure 7B), but with a well-developed left coelom (Figure 7F). The reduced pluteus is small (Figures 7G,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 (Figure 7G-K). The pluteus has an open mouth and hollow stomach and intestine.

The posterolateral arms are somewhat straight but curved proximally. The posterolateral and anterolateral arm rods bear thorns (Figure 7K'). 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. orange belly* is most similar in size and shape to its congeners, *A. spp. opaque* and *tan*, but differs in color by having an orange body with white anterolateral and posterolateral arms. *Ophiura luetkenii* also has white posterolateral arms, but the anterolateral arms are orange. Furthermore, the larva of *O. luetkenii* can be distinguished from the reduced plutei of *Amphiodia* by its body rods, which are of equal or greater length than the short posterolateral rods (Key 13). The anterolateral rods of *A. spp. orange belly* and *tan* are longer than the posterolateral rods in the six-armed

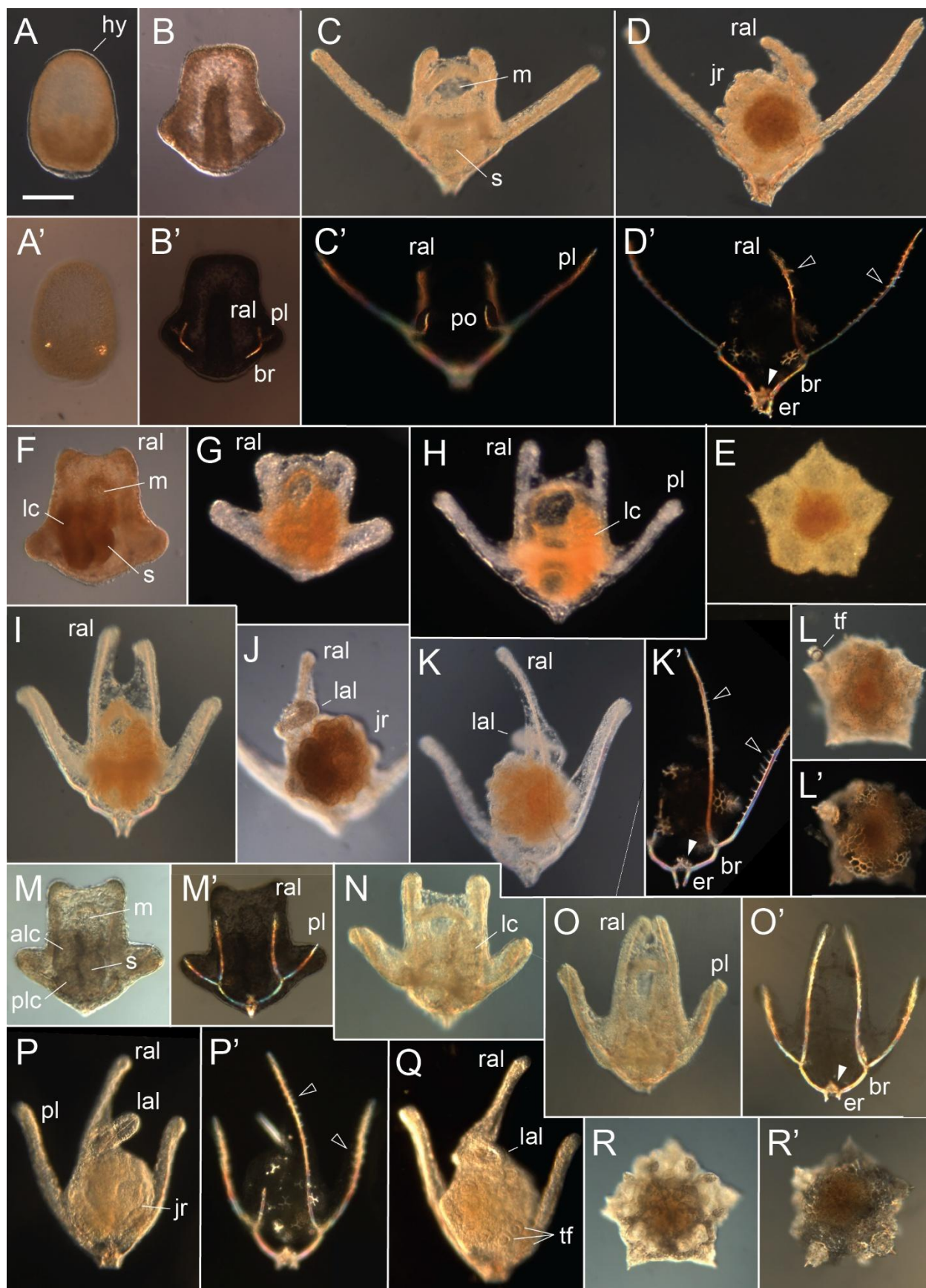


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FIGURE 7 Reduced plutei of three species of *Amphiodia*: (A–E) *Amphiodia* sp. opaque, (F–L) *Amphiodia* sp. orange belly, and (M–R) *Amphiodia* sp. tan. Secondary views of the same individual are marked with an apostrophe ('). Scale bar in A is 100 μ m 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 body rods (br) and rods that will support the larval right anterolateral (ral) and posterolateral (pl) arms. **C.** Oral view of the six-armed pluteus with functional mouth (m) and stomach (s). **C'.** In cross-polarized light, the anterolateral, postoral (po), and posterolateral 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 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, right, and left anterolateral arm (lal), 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 postero-lateral 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'**). The scale bar is 100 μ m, same scale for **A–R**.

pluteus, a feature that can distinguish those species from *A. sp. opaque* (Figure 2D–F; Key 14). The curvature of the arms and occurrence of thorns on the arm rods call to mind that of *A. urtica* (Figure H).

Development mode. We suspect that this larva is a facultative planktotroph, like *A. sp. opaque*, but have lacked 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. (Figure 7L).

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 (Figure 3).

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

3.4.3.6 | *Amphiodia* sp. tan

Species identity. We know this species from a single larva that groups with *Amphiodia* sp. orange belly and *A. aff. digitata* but is genetically and morphologically distinct (Figure S3, Table S1). A single adult specimen identified as *Amphiodia urtica* from California (CAS172824) was > 97% similar to our larva for *COI* and only 90% similar to other adults of *A. urtica* from Oregon, California, and British Columbia (Figure S3, Table S1).

Distribution and local sites. Adult populations from Oregon remain unknown. The adult specimen that was a close molecular match was from San Francisco Bay (California Academy of Sciences CAS172824; T. O'Hara, pers. comm.).

Embryonic description. The single specimen was collected in September 2020, as a tan blastula and recognized as an ophiroid embryo from its thick hyaline coat. When first collected, 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 (Figure 7M).

The final ophiopluteus has curved posterolateral and anterolateral arms, that bear small thorns (Figure 7O'). 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 (Figure 2D).

The rudiment stage larva is distinct from the other amphirid reduced plutei. The rudiment-stage larva retains part of the left anterolateral rod as well as the right anterolateral rod (Figure 7P), although the left rod eventually disappears as development proceeds (Figure 7Q).

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, in combination with the proportions of the larval skeleton, make *A. sp. tan* recognizable. The body rods and posterolateral rods are C-shaped, unlike the straighter versions in *A. sp. opaque*. The anterolateral arm rods are longer than the posterolateral rods, and thus the anterolateral arms exceed the posterolateral arms in height (see Key 14). In comparison to *A. sp. opaque*, which is usually salmon in color but ranges from pink to tan, *A. sp. tan* has large left coeloms even as an early pluteus. Furthermore, in the rudiment stage larva of *A. sp. tan* the left anterolateral arm is retained in addition to the right.

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. (Figure 7R).

Reproductive timing. We collected one blastula on September 19, 2022 (Figure 4).

Planktonic duration. The larva we observed developed into a juvenile in approximately 11 days.

3.4.3.7 | *Amphiodia* aff. *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*, aff. *periercta*, and a population from Haida Gwaii, BC (Supplementary Table S1; Figure S3).

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 (1987); and material collected from Olga later in the 1990's was confirmed as *A. occidentalis* by G. Hendler (Los Angeles County Museum of Natural History). The same larval 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 *COI* sequences were $\geq 12\%$ different, not a species-level match with those adult specimens of *A. occidentalis* from Oregon or the *A. occidentalis* collected subsequently from the original site at Olga, WA. (Supplementary Table S1; Figure S3; BOLD bin AAD7270). 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 ($< 2\%$) to this planktonic form (Supplementary Table S1; Figure S3; BOLD bin AEA1696). Based on comparison of this adult material with species descriptions and with material of *A. periercta* from the USNM (E13853), G. Hendler (NHMLAC, pers. comm., November 2023) has provisionally identified the adult and its larval phenotype as *A. aff. periercta*. Our adults lack a border of raised scales at the periphery of the disk that is described for *A. periercta* (Clark, 1911). Until *COI* sequences are obtained from specimens that unambiguously match the description of *A. periercta* H.L. Clark 1911, our species remains only provisionally assigned to this species.

Distribution and local sites. *Amphiodia periercta* H.L. CLARK 1911 occurs from the intertidal to 92 m depth from Unalaska (Aleutian) Island, Alaska to central California, (Lambert & Austin, 2007). We 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 190 μm in diameter, suggestive of their abbreviated development. See Emlet (2006) for a full description.

Larval description. Embryos and unhatched juveniles of *A. aff. periercta* are easily recognized by the thick hyaline layer surrounding their cells as eggs and embryos (Figures 8A,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 (Figure 8D').

Similar species. The unusual developmental pattern of *A. aff. periercta* means that it is not mistaken for any other ophiuroids whose

development has been described. There is one other directly developing amphiuroid in the region, *Amphiura arcystata*. *Amphiodia* aff. *periercta* is distinct from *A. arcystata* in the presence of a peripheral ring of skeletal spicules, and the absence of the interradian bulges and skeletal spicules that cross over the developing disk in *A. arcystata*.

Development mode. *Amphiodia* aff. *periercta* is a pelagic direct developer. It may represent the only of its kind and was described in detail as *A. occidentalis* by Emlet (2006).

Juvenile description. After hatching, the scallop-shaped early juvenile has a ring of skeletal elements supporting its periphery, and a stomodeal invagination (Figure 8E).

Development into a juvenile with moving podia and jaws is achieved in 7 to 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. aff. periercta* are initially pentagonal but their terminal arms plates soon develop into pointed rays (Figure 8F).

Reproductive timing. All stages, from eggs, to embryos and hatched juveniles of *A. aff. 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. aff. periercta* spawns frequently throughout the winter (Figure 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. aff. periercta* (R. Emlet, personal observation). 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).

3.4.3.8 | *Amphioplus* sp. vitellaria

Species identity. The species identity of this larva is uncertain, but our larvae group with a clade including adult specimens of *Amphioplus* spp. from the Northeastern Pacific in three BOLD bins: AFH3793, AFH0914, AGA5835 (Supplementary Table S1; Figure S5). The *COI* sequences from our larvae are $< 1\%$ different from a subadult (BOLD process ID: MVCRD185-20) collected off Vancouver, Canada, and all

share BOLD bin AFH0914. Our larval sequences are $\geq 6\%$ different from those of adults in BOLD bin AFH3793, and sequences in this last bin are ca. 99% similar to those from specimens identified as *Amphioplus strongyloplax* and *A. macraspis*, (SIO E6874 and RCBM 019-00543-011, respectively, T. O'Hara, pers. comm.). Our larval COI sequences are 6–8% different from two adult specimens collected from 450 m depth on the Oregon Shelf (OOPH049-23, OOPH067-24; Supplementary Figure S5). The specimen OOPH049-23 (BOLD bin AGA5835) consisted of the body disk without oral frame or arms and could be identified only to genus (G. Hendler, pers. comm.). We did not assign this larva to a known species given its separation from samples identified as *A. strongyloplax* and *A. macraspis* (Supplementary Figure S5). Instead, we gave this larva the provisional name of *Amphioplus* sp. vitellaria until additional adult material can be collected and examined. The genus *Amphioplus* is polyphyletic and requires revision (O'Hara et al., 2017).

There are six specimens in BOLD listed under the name *A. macraspis* (DSPEC471-08, DSPEC471-08, DSPEC544-08, DSPEC545-08, DSPEC552-08, and DSPEC553-08) but their COI sequences are < 1% different our specimens identified as *Amphiodia occidentalis* (see section 3.4.3.7 for more detail) and the same specimens are listed as *A. cf. occidentalis* in GenBank (see also Supplementary Table S1; Figure S5).

Distribution and local sites. Two species of *Amphioplus* are known from the Oregon region, *A. strongyloplax* and *A. macraspis*. *Amphioplus macraspis* is widespread in the north Pacific, in Asia and from Haida Gwaii to Washington in the eastern Pacific, 1–876 m depth. *Amphioplus strongyloplax* has a greater range in the eastern Pacific, ranging from the Gulf of Alaska to the Mexican border, 40–623 m (Lambert & Austin, 2007). Adults of both species have been reported from benthic surveys off of Coos Bay, Oregon (Henkel & Gilbane, 2020), but molecular data is not available for those Oregon specimens. Two specimens identified as *A. macraspis* and *A. strongyloplax* are from British Columbia and California, respectively (T. O'Hara, pers. comm.; Supplementary Table S1; Figure S5).

Embryonic description. The blastulae and larvae of this species can be recognized by the presence of lipid droplets and dark green speckled pigmentation, visible through the tan epidermis (Figures 8G,H).

Larval description The embryos develop directly into a nonfeeding vitellaria with anterior and posterior ciliated bands and disjunct ciliated bands that wrap around the middle of this elongate spheroidal larva (Figure 8I).

Similar species. The vitellaria of *Amphioplus* sp. can be distinguished from the other nonfeeding amphiuroids of southern Oregon by the presence of disjunct ciliary bands around its middle and continuous bands at the anterior and posterior. The pebbled appearance of the tan epidermis, flecked with dark green, is distinctive and present across development.

The only other species to have a vitellaria stage in this region was *Ophiopteris papillosa*, whose vitellaria is preceded by a feeding 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 10), whereas *A. sp. vitellaria* is light pink to tan-green, 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 Amphiuroidae (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*, *Ophioplepis*, and *Ophiacantha* (Brogger et al., 2013; Hendler, 1979, 1982, 1995; Komatsu & Shosaku, 1993; Mortensen, 1938; Selvakumaraswamy & Byrne, 2000). At least two species, *Ophiopozia 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 (Figure 8J).

Reproductive timing. *Amphioplus* sp. vitellaria occurs rarely in December and January in the Charleston plankton (Figure 3). We usually find them as single or a few individuals, not in high abundance.

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

3.4.3.9 | *Amphiura arcystata* H. L. CLARK 1911

Species identity. We have barcoded eight of these larvae since 2012, and sequences generated for COI are > 99.5% similar to a single young adult, identified as *Amphiura arcystata* (Supplementary Table S1; Figure S5; BOLD bin AFN8895). We found this adult in one of our aquaria, and this individual may have grown from a larva that we had put into the aquarium. The next most closely related specimens were adults of *Amphichondrius granulatus* (OOPH005-18) and *Amphipholis pugetana* (OOPH052-23), each < 81% similar at COI.

In addition to our adult found in an aquarium, there was a single specimen listed as *A. arcystata* from Japan in GenBank (LC749655; Okanishi et al., 2023) with data for 16S; this specimen was > 12% different from 16S in our larvae, juvenile, and adults. Clark (1911) suggested that specimens of *A. arcystata* from Japan and Monterey

belonged to different species, which would concur with the molecular data we present here.

There are two other species of *Amphiura* known to occur in the northeastern Pacific Ocean: *A. carchara* and *A. diomedae* (Lambert & Austin, 2007), and we have not encountered their larvae. COI for *A. diomedae* (OOPH015-18) is only < 78% similar to that of *A. arcystata*, and a single 16S sequence *A. carchara* (Okanishi et al., 2023) is < 72% similar to that of *A. arcystata*.

Distribution and local sites. *Amphiura arcystata* occurs on both sides of the northern Pacific Ocean, from Monterey Bay, California to Mexico, 15–844 m and in Japan, 108–848 m (D'yakonov, 1967; Hendler, 1996). The presence of the short-lived larva suggests that

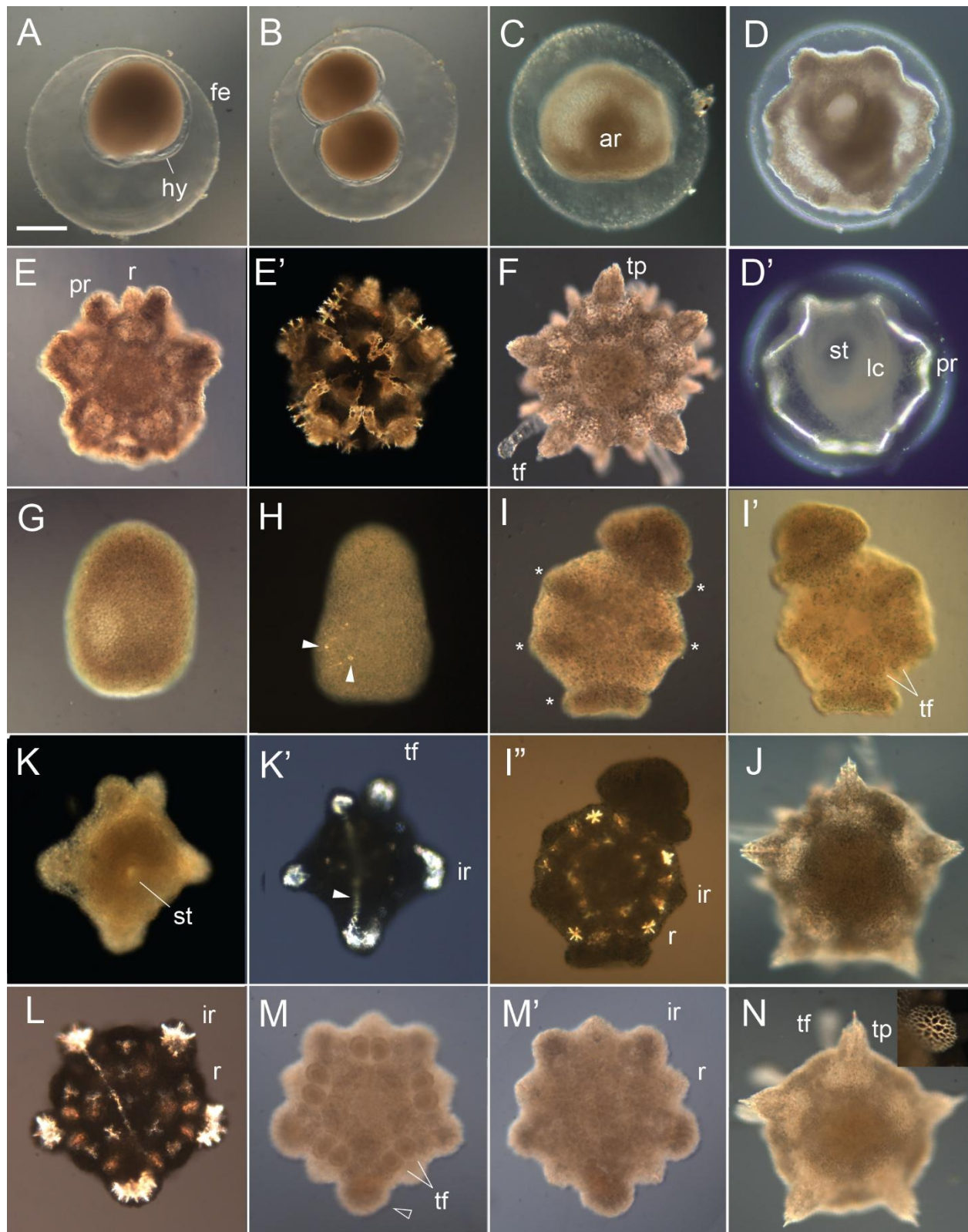


FIGURE 8 Three nonfeeding planktonic forms of Amphiuroidae: (A–F) *Amphiodia* aff. *periercta*, (G–J) *Amphiplus* sp. *vitellaria*, and (K–N) *Amphiuira arcystata*. Secondary and tertiary views of the same individual are marked with an apostrophe (') or quotation marks ("), respectively. **A.** The fertilized egg of *Amphiodia* aff. *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. aff. periercta*. and **D'** in cross-polarized light. The embryo has a stomadeal invagination (st), left coelom (lc) and peripheral rods (pr) of skeleton. **E, 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 *Amphiplus* sp. *vitellaria*, taken of the same individual one day apart. Note the pebbled exterior appearance due to many oil droplets

FIGURE 8 Legend continued on next page.

FIGURE 8 Legend continued

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. The scale bar is 100 μ m, same scale for **A–N.**

adults are close to Oregon and may be a possible range expansion. We have not yet discovered adult populations locally.

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 (Figure 8K' and 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 interradial bulges and the juvenile arms soon develop between them (Figure 8M). 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 surfaces.

Similar species. The directly developing juvenile of *A. arcystata* could be mistaken for that of *Amphiodia* aff. *periercta*, which also develops directly in the plankton (see discussion above). However, the larva of *A. aff. periercta* has a peripheral ring of many spicules (> 20) while the larva of *A. arcystata* has a line of skeletal elements (< 10) that cross the larval body (Key 18). *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 ($n = 3$), and at least one planktotroph (Fenaux, 1970; Falkner & Byrne, 2006; Hendler, 1991, 1995; Mortensen, 1920a, 1924, 1933b, 1936; O'Hara et al., 2014).

Juvenile description. Similar to other amphiurids, the juvenile is pentagonal with 5 short, pointed terminal arm plates (Figure 8N).

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

Planktonic duration. 6 days ($n = 3$).

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

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 (Supplementary Table S1; Figures S6 and S7), the ophioplutei of these species greatly resemble one another but may be distinguished by features of the skeleton and style of metamorphosis.

Ophiacantha diplasia and *Ophiopteris papillosa* both develop as an eight-armed, feeding ophiopluteus 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 feeding larvae to be described for their families. *O. diplasia* is the first ophiopluteus from the family Ophiacanthidae LJUNGMAN 1867; at least twelve other ophiacanthids with known development are brooders, including five other *Ophiacantha* spp. (Martín-Ledo et al., 2013; Mortensen, 1920b, 1933a; 1936) *O. papillosa* is the first pluteus that transforms into a vitellaria from Ophiopteridae O'HARA, STÖHR, HUGALL, THUY & MARTYNOV, 2018. Ophiopteridae contains a single other species, *O. antipodum*, which has a small egg associated with planktotrophic development (Naughton et al., 2014).

3.4.4.1 | Ophiacantha diplasia H. L. CLARK 1911

Species identity. We encountered several larvae of various developmental stages in El Niño years 2015, 2019, and 2024 (Figure 3). Barcoding revealed COI sequence for five larvae to be > 99% similar to sequences from three adult specimens that we identified as *Ophiacantha diplasia* and had collected off Cape Arago and off the Stonewall Bank (Supplementary Table S1; Figure S6). Larvae and adults share one bin in BOLD (AFN8124). The next closest sequences (*Ophioplocus japonicus* and *Ophiopristis procera*) to those of our larvae were only < 82% similar (Supplementary Table S1; Figure S6). The genus *Ophiacantha* is large, polyphyletic, and requires revision (O'Hara et al., 2018).

Distribution and local sites. *Ophiacantha diplasia* occurs from Haida Gwaii to Southern California at depths of 71–1178 m on silty sand or hard rock (Astrahantseff & Alton, 1965; Lambert & Austin, 2007).

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 (Figure 9B), and straight distally in the advanced ophiopluteus (Figures 9C,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 girdle is composed of body rods, ventral and dorsal recurrent rods, transverse rods, and end rods. In the early pluteus the rods of the posterior girdle are similar in length; in the advanced pluteus the transverse and end rods are longer, more robust, with the end rods overlapping at their distal ends as they curve medially (Figure 9D'). The right dorsal transverse rod may bear a median process, but we have also observed individuals that lack such a process. The arm 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 ophiocomid ophioplutei (Byrne, Cisternas, O'Hara, & Selvakumaraswamy, 2024; 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 eight larval arms are retained until metamorphosis (Figure 9E).

Similar species. In the northeastern Pacific, the pluteus of *O. diplasia* is most likely to be confused with that of *Ophiopteris papillosa*. The advanced larvae of both species have posterolateral arms that are of similar length to the other arms and are held at a narrow angle, with vibratile lobes at the base of the arms, first giving the impression of an echinopluteus; however, examination of the placement of the pluteus arms shows them to be ophioplutei. The larval epidermis of both species is transparent and accented with touches of yellow green at the distal tips of the arms. Viewed in 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 skeletal elements (Key 8). In the advanced ophiopluteus of *O. diplasia* the transverse and end rods lengthen to accommodate the posterior of the larva, whereas in *O. papillosa* the transverse rods become separated as the larva grows. 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 (Figures 9B–D). During rudiment formation *O. diplasia* retains all eight larval arms (Figure 9E), a highly unusual metamorphic phenotype for ophiuroids (Selvakumaraswamy & Byrne, 2023). Developmental pattern is known for at least twelve other ophiacanthids, all of which are brooders (Martín-Ledo et al., 2013; Mortensen, 1920b, 1933a; 1936). According to our molecular data, *O. diplasia* is not closely related to other *Ophiacantha* spp. or brooding ophiacanthids with molecular data. Phylogenetic resolution and additional developmental data are needed to understand the evolution of developmental patterns in this large and diverse family.

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 in 2015, 2019, and 2024 which were all years with El Niño conditions on the Oregon coast.

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 until metamorphosis.

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 of *COI* and *16S* mitochondrial genes and comparison with sequences for material borrowed from NHMLAC or adult specimens from GenBank and BOLD (Supplementary Table S1; Figure S7). From six larvae or newly metamorphosed juveniles we obtained five *16S* sequences and four *COI* sequences. The *16S* sequences were all $\geq 99\%$ similar to each other and to a *16S* sequence from an adult obtained from the NHMLAC. Our *COI* sequences from larvae were $\geq 98\%$ to sequences from 14 public records in BOLD and GenBank (Supplementary Table S1; Figure S6; BOLD bin ACI1163). The family Ophiopteridae contains just two species, *Ophiopteris papillosa* found along the west coast of North America, and *O. antipodum* found around New Zealand.

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 at low intertidal to 140 m depth (Austin & Hadfield, 1980; Lambert & Austin, 2007).

Larval description. A planktotrophic larva was predicted by the small egg size, diameter 100 μm , of the species (Pearse, 1994). The larvae of *O. papillosa* have a transparent epidermis, sometimes with yellow to green pigmentation on the distal ends of the larval arms and on the ciliary bands (Figures 10A,B). The posterolateral arms are curved in the six-armed pluteus (Figure 10A'). In later pluteus stages the posterolateral arms are not markedly longer than the others, and postoral and posterodorsal arms are shorter in height than the anterolateral arms. The larval arms may bend outward at their distal end; except those of the anterolateral arms may point medially. The advanced pluteus has large vibratile lobes at the posterior (Figures 10C,C'). The posterior girdle is without recurrent rods; the transverse and end rods are roughly equal in length and are shorter than the body rods. The transverse and end rods are touching or nearly touching in the early pluteus but separate as the larva grows to accommodate the larval gut of the advanced pluteus (Figure 10D).

After approximately a month, the ophiopluteus develops into a vitellaria (Figure 10E). 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 (Figures 10E,E'). The anterior most ciliated band wraps around the distal ends of remnants the anterolateral arms. The juvenile arms are multisegmented and develop folded toward the center of the oral side.

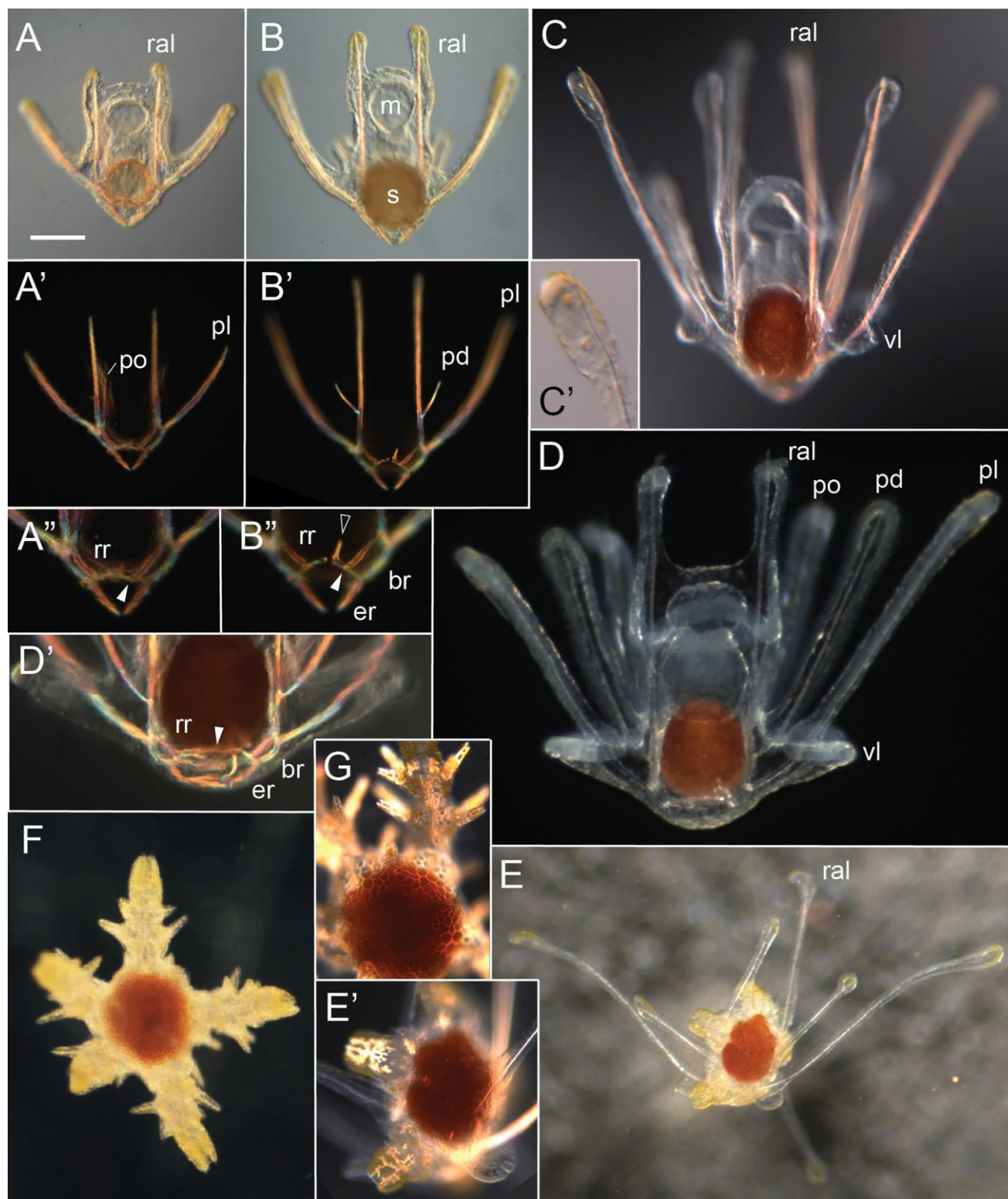


FIGURE 9 Ophioplutei and juveniles of *Ophiacantha diplasia*, all in aboral view. Secondary and tertiary views of the same individual are marked with an apostrophe (') and quotation mark (''), respectively. **A**, **B**. Aboral views of six- and eight-armed ophioplutei, respectively (postoral arms cannot 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 μ m and applies for **B**, **D**, and **E**. No scale for **C**, **F**, **G**.

Similar species. As an ophiopluteus, *O. papillosa* resembles *O. diplasia* but can be identified by examination of the posterior girdles, especially the absence of recurrent rods, which are present in *O. diplasia* (see Similar Species of *O. diplasia* for more). The vitellaria stage is unlikely to be confused with that of another species as there is only one other vitellaria that occurs in this region, that of *Amphioplus* sp. vitellaria (Section 3.4.3.8), which differs from *O. papillosa* in several ways. First, the vitellaria of *Amphioplus* sp. is opaque tan with flecks of green while that 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 (Figure 10E'), a feature absent in *Amphioplus* sp. vitellaria (Key 19).

Development mode. *Ophiopteris papillosa* has a feeding ophiopluteus that transforms into a nonfeeding vitellaria before metamorphosis into a juvenile. *O. papillosa* has a small egg (102 µm) and planktotrophic development (Pearse, 1994; Rumrill & Pearse, 1985), but this is the first description of its transformation into a vitellaria prior to metamorphosis into a juvenile. The transformation of an ophiopluteus into a vitellaria is known previously from *Ophiocomella pumila* (Mladenov, 1985b) and *Breviturma dentata* (Byrne, Cisternas, O'Hara, & Selvakumaraswamy, 2024). The vitellaria of *O. papillosa* differs from the latter two in its partial retention of a pair of larval arms at the anterior of the larva. This is the first observation of this developmental pattern in the family Ophiopteridae. *Ophiopteris papillosa* has a single congener, *O. antipodum*, which 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*; *O. pumila* and *B. dentata* have small eggs like that of their planktotrophic congeners (Byrne, Cisternas, O'Hara, & Selvakumaraswamy, 2024).

Juvenile description. The juvenile of this species develops from the vitellaria larva. At metamorphosis its arms have two segments plus the terminal plate. The juvenile may be motile while still bearing remnants of the ciliated bands (Figure 10F).

Reproductive timing. We found a small number of 8-armed ophioplutei in the winter of 2015, from November to February, and rarely in April. We also found similar numbers in February 2024 (Figure 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 & TROSCHER 1840

We observed the ophioplutei of four species from the family Ophiuridae, all of which were identified using DNA barcoding (Supplementary Table S1; Figures S8–S10). 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 *Ophiecten hastatum*, a deep-water species that occurs at depths of

1000 m and greater. The planktotrophic plutei of this family all had relatively short body rods and recurrent rods; this feature was greatly altered in the reduced pluteus of *O. luetkenii*, which has very long body rods and no recurrent rods.

The ophiurid plutei observed here varied in their patterns of metamorphosis. The rudiment-stage larva of the planktotrophic *Ophiecten hastatum* consisted of a juvenile suspended between two intact posterolateral arms; in the planktotrophic *Ophiura sarsii*, the rudiment stage larva was slightly opaque, and the developing juvenile was held by both posterolateral arms as well as the right anterolateral arm; finally, in the reduced pluteus of *Ophiura luetkenii*, only the postoral arms were resorbed, leaving the juvenile rudiment supported by both posterolateral and anterolateral arms. The latter two examples from *Ophiura* represent new metamorphic pathways relative to existing paradigms (Selvakumaraswamy & Byrne, 2023).

3.4.5.1 | *Ophiecten hastatum* LYMAN 1878

Species identity. *Ophiecten hastatum* is reported from deep water in the North Atlantic, Eastern Pacific, and the Southern Ocean (off S. Africa and southern Australia) and is a species complex with four bins in BOLD with public data. O'Hara and Thuy (2022), citing genetic evidence in the supplement of Christodoulou et al. (2019), state that this taxon is a species complex and needs to be re-evaluated. We observed two plutei from this species, collected in January 2015, and mid-February 2024. The first ophiopluteus (Figure 2M) was a > 98.5% match at the COI locus with four adult sequences from British Columbia, Canada that were identified as *O. hastatum* (Supplementary Table S1; Figure S5), and was placed with them in BOLD bin AAC6367. The other bins of *O. hastatum* are < 96% match to our larva. We did not obtain COI for the second larva, but 16S sequences for the two larvae were similar (> 98%). COI sequence trees show the next most closely related species outside of the *O. hastatum* complex are Antarctic taxa *O. amitinum* and *O. megaloplax*, at < 94% COI sequence similarity.

Distribution and local sites. *Ophiecten hastatum* has a cosmopolitan distribution at bathyal to abyssal depths in both northern and southern hemispheres. It occurs at over 2000 m depth in the NE Atlantic, 1408–2877 m in tropical eastern Pacific, and 916–2877 m in British Columbia and Washington (Gage et al., 2004; Kyte, 1969; Lütken & Mortensen, 1899). We have not identified local populations of adult *O. hastatum*.

Larval description. The two larvae we collected were large, eight-armed ophioplutei and obligately planktotrophic. The arms of both larvae were long and bilaterally asymmetric in length, especially the posterolateral arms, and may reflect a bias likely created by the process of rudiment formation as in some echinoids (Hodin et al., 2016). 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 (Figure 2M). The posterior girdle is supported by end rods, relatively short body rods and recurrent rods, end rods, and transverse rods that lack thorns or medial processes. The ventral and dorsal recurrent rods lie largely over one another so it's difficult to see both pairs (Figure 11A').

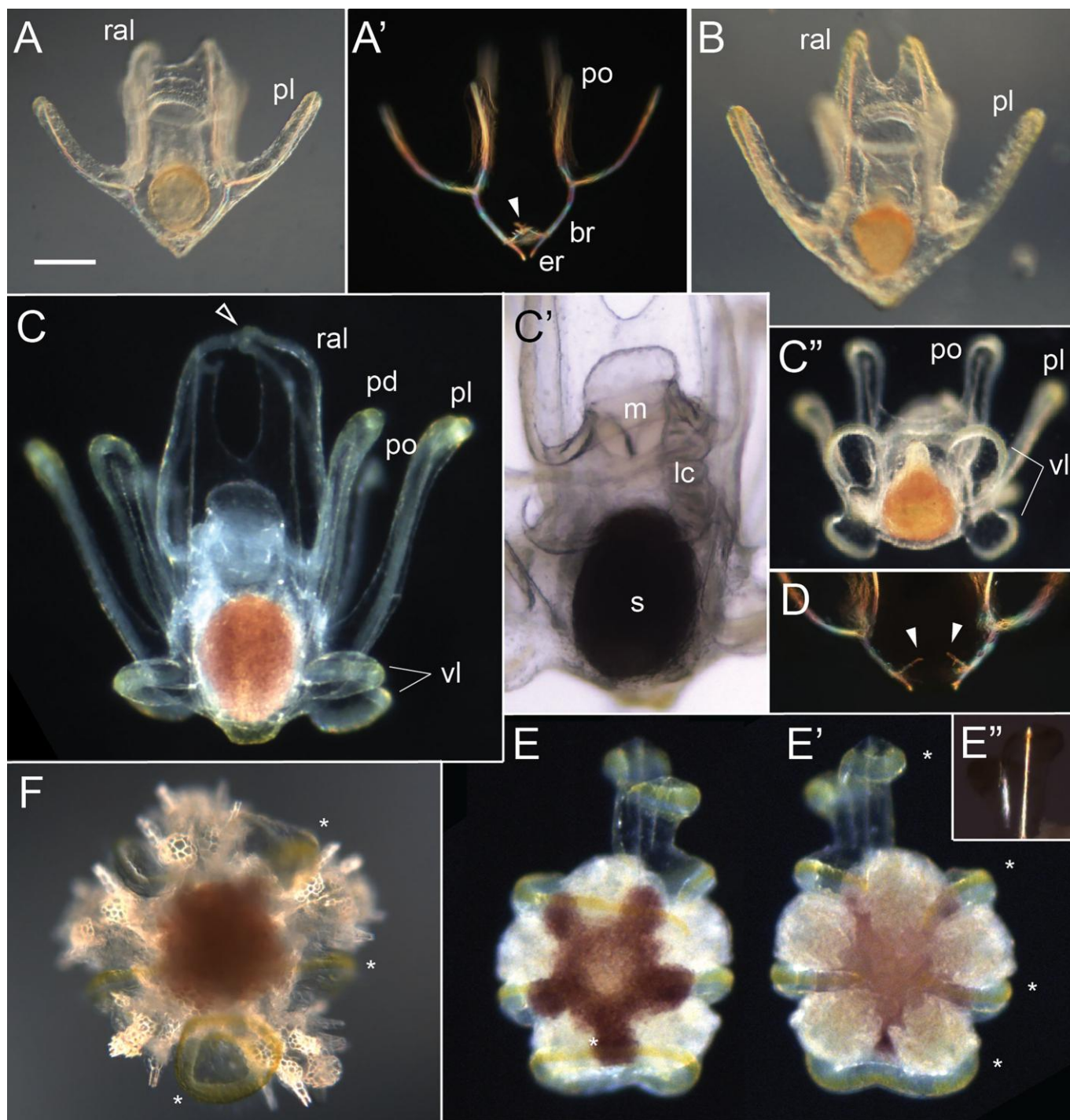


FIGURE 10 Ophiopluteus, vitellaria, and juvenile of *Ophiopteris papillosa*. Secondary and tertiary views of the same individual are marked with an apostrophe (') and quotation mark ("), respectively. **A**. An oral view in DIC optics and **A'**. partially polarized light which highlights the larval skeleton of a six-armed pluteus at time of collection and **B**. eight days later. Six-armed larva with right anterolateral (ral), posterolateral (pl), and postoral (po) arms. **C**. The advanced eight-arm pluteus shown in aboral view has anterolateral, 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 posterior girdle of the late ophiopluteus, another individual, in which the transverse rods do not articulate (arrowheads). **E**. The eight-armed ophiopluteus develops into a vitellaria, shown in aboral view and oral view (**E'**) with the ciliated bands marked with * **E''**. Anterior portion of larva in partial cross-polarized light, showing two arm rods from the pluteus. **F**. An early juvenile, still bearing remnants of the yellow ciliated bands (*). The scale bar is 100 μ m and applies to **A-F**.

Similar species. Due to its large size the advanced eight-armed ophiopluteus of *O. hastatum* is not easily confused with any of the other plutei we observed. Only *O. spiculata* rivals it in size, but the length, angle, and coloration of the posterolateral arms can but used to distinguish between the two (Key 6).

Development mode. The larvae we found confirm that *Ophiecten 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, wherspawning 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, 1980, 1981; Thorson, 1934; Tyler & Gage, 1982).

Juvenile description. We kept the second larva, collected in February 2024, in the laboratory until metamorphosis. The juvenile was large and had arms with five segments (Figure 11B).

Reproductive timing. We collected this larva twice, on January 18, 2015, a warm water year, and February 16, 2024.

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

Species identity. We observed a single larva in May 2019, and two larvae in February 2024. The three larvae differed by < 1% at the COI locus from an adult specimen collected from the continental shelf off Oregon (BOLD bin AEK1023; Supplementary Table S1; Figures S9 and S10). Our COI sequences are also a > 99% match to COI sequences for 9 samples collected as plankton in the Straits of Georgia, British Columbia, Canada (QHAK-, BOLD bin AEK1023; Supplementary Table S1; Figure S9). Images were available for three of those specimens, showing them to be two ophioplutei and one juvenile (see more below). Named species with the next closest COI matches to our initial larva are <85% similar and include *Ophiuroglypha lymani*, *Ophiura ljunmani*, and *Ophiura robusta*.

Distribution and local sites. *Ophiura leptoctenia* was characterized by Clark (1911) as one of the most common ophiuroid species in the north Pacific, ranging from Korea to the Bering Sea and from Alaska to southern California, with a gap between Sakhalin and the western Aleutian Islands (Lambert & Austin, 2007). *Ophiura leptoctenia* occurs in large numbers off California (Astrahantseff & Alton, 1965). This species has a mostly bathyal depth range, 25–3239 m (Hendler, 1996; Lambert & Austin, 2007). We collected one adult from the Cape Arago shelf at 120 m depth.

Larval description. The advanced ophiopluteus of *O. leptoctenia* has eight arms, and a transparent epidermis that appears inflated due to its separation 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 (Figure 2N). The body rods are short and there are ventral and dorsal recurrent rods (Figure 11C'). The two ophioplutei from the same bin in BOLD as our larvae (QHAK3901-23, QHAK1571-22) appear to be more advanced than the ophioplutei we observed based on the length of the larval arms. The two ophioplutei

on BOLD share the recurrent rods that we consider characteristic of the planktotrophic plutei in this family. The larval arms have a slightly less inflated appearance in these later stages than in the early eight-armed plutei we observed in Oregon.

Similar species. The ophiopluteus of *O. leptoctenia* is very similar to that of *O. sarsii* (Figures 11C,I; Key 12). Both have eight arms, an inflated transparent epidermis, and a similarly structured posterior girdle with short body rods and recurrent rods and relatively long end rods (Figures 11C',I'). 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 (see Key 12). The pluteus of *Amphipholis pugetana* also has an inflated epidermis, but it is of a uniform width along the length of the arms and terminates with red pigmentation at the distal ends of the posterolateral arms. We noticed a small amount of pigmentation in the posterolateral arms of the BOLD match (QHAK1571-22) that is reminiscent of the arm pigmentation we observed in the advanced pluteus of *Ophiecten hastatum* (see section 3.4.5.1; Figure 2M).

Development mode. To our knowledge, this is the first report of the larva of *O. leptoctenia*. Hendler (1996) reported an egg diameter of ca. 200 µm 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. Two larvae collected in February 2024 had unpigmented, transparent arms and bodies with bright red guts probably due to the algae they had fed on in the plankton.

Juvenile description. We did not observe the juvenile of *O. leptoctenia*. There was, however, a single photo of a juvenile collected from the plankton of British Columbia, Canada (QHAK3794-23). This juvenile was transparent except for its orange gut, had two arm segments, and a honeycomb stereom. The stereom pattern is just like that of the juveniles of *O. luetkenii* and *O. sarsii* we observed.

Reproductive timing. We observed a single larva on May 8, 2019, two in February 2024, both were El Niño years.

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* (BOLD bin AAD8864), which is closely related to *O. sarsii* (Supplementary Table S1; Figures S9 and S10).

Distribution and local sites. *Ophiura luetkenii* occurs from the Bering Sea, Alaska, to Cedros Island, Baja California at depths of 9–1265 m (Hendler, 1996; Lambert & Austin, 2007). *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 repeatedly 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.

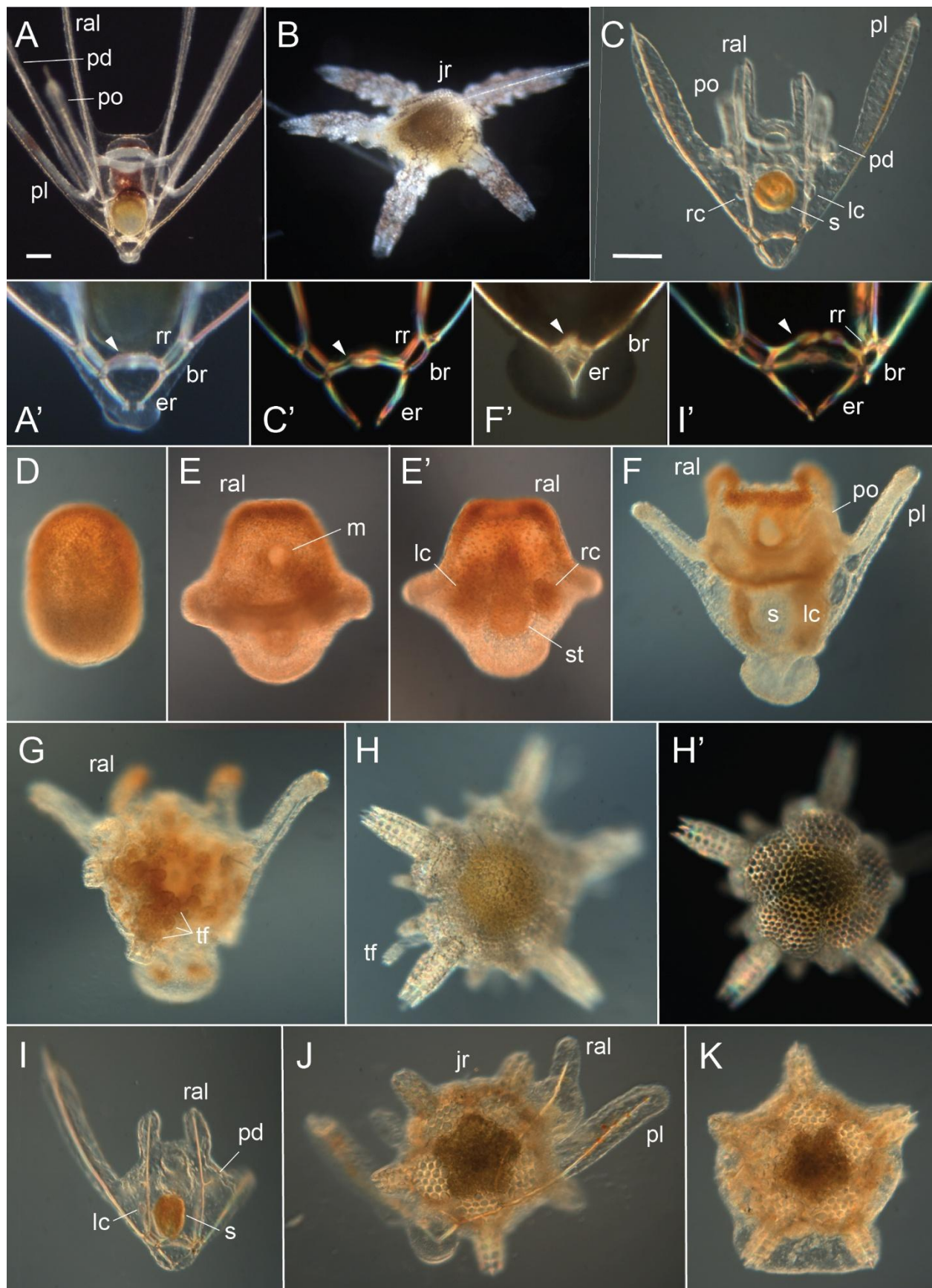


FIGURE 11 Legend is on the next page

FIGURE 11 Ophioplutei and juveniles of four species of Ophiuridae: (A,B) *Ophiocten hastatum*, (C) *Ophiura leptoctenia*, (D–H) *Ophiura luetkenii*, and (I–K) *Ophiura sarsii*. Secondary views of the same individual are marked with an apostrophe ('). **A.** Partial oral view of the eight-armed ophiopluteus of *O. hastatum*, same individual as Figure 2M, with right anterolateral (ral), posterodorsal (pd), postoral (po), and posterolateral (pl) arms. **A'.** Close-up of the posterior girdle with transverse rods (filled arrowhead), recurrent rods (rr), body rods (br), and end rods (er). **B.** A juvenile rudiment (jr) with larval rods still attached. **C.** Oral view of eight-armed ophiopluteus of *O. leptoctenia* in transmitted light with small right and left coeloms (rc, lc) and a stomach (s) full of microalgal food. Same individual as Figure 2N. **C'.** The posterior girdle in cross-polarized light. **D.** Oral view of *O. luetkenii* gastrula, **E.** oral and **E'.** aboral views of the early pluteus, and **F.** oral view of the six-armed reduced pluteus. The reduced pluteus has anterolateral (al), postoral (po), and posterolateral (pl) arms, a stomach (s), and left coelom (lc). **F'.** The posterior girdle in partial cross-polarized light shows the transverse rods (filled arrowhead), body rods (br), and end rods (er). **G.** 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. **H.** The juvenile of *O. luetkenii* in transmitted and (**H'**) cross-polarized light, aboral view. Note the honeycomb pattern of the primary plates and disc plate. Aboral views of *O. sarsii* (**I.**) pluteus, (**J.**) rudiment-stage pluteus, and (**K.**) it's juvenile a day later. Scale bar in **A** is 100 μ m; same scale for **B**. Scale bar in **C** is 100 μ m and approximately twice the scale in **A**; same scale for **C–K**.

The earliest stage we have observed from the plankton is the gastrula (Figure 11D), 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 (Figures 11E,E').

At the six-armed stage the pluteus the posterolateral arms are translucent white, and the body region, anterolateral and postoral arms are orange. The body rods are long, of equal or greater length than the posterolateral arms (Figure 2O). At the posterior end the epidermis is distinctively inflated around the body and end rods (Figure 11F). The juvenile rudiment bears tubefeet and both anterolateral arms are retained (Figure 11G).

Similar species. The reduced pluteus of *O. luetkenii* does not much resemble its planktotrophic congeners. Instead, it has an orange and white epidermis, long body rods, short posterolateral arms, and no recurrent rods.

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 (Key 13).

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 (Figure 11H). 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 sari* LÜTKEN 1855

Species identity. We identified the larva of *O. sarsii* from DNA barcoding with a *COI* sequence that was $\geq 99.8\%$ similar to those from three adults collected from the Oregon coast (Supplementary Table S1; Figures S9 and S10). BOLD identified 3 bins for *O. sarsii* in the N. Pacific, Arctic Ocean, and N. Atlantic (Genelt-Yanovskiy et al., 2021;

Laakmann et al., 2017; Layton et al., 2016). Our larval sequence was placed in bin AAD3481 that has samples from the eastern and western North Pacific and the western Arctic Ocean, and this bin is 94–5% similar to the next closest bin (ACO7183) from the eastern Arctic and north Atlantic. A fourth bin with subspecies *O. sarsii vadicola* (AEG4873) occurs in China and Japan (Li et al., 2020) and is only ca. 85% similar to our sample.

Distribution and local sites. *Ophiura sarsii* is a circumpolar species that occurs throughout the Arctic Ocean, the northern Atlantic, and in the Pacific from the Bering Sea to California, Japan and Korea, from 0–1460 m (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*.

Larval description. We collected two larvae on May 20, 2019, including a six-armed pluteus and a rudiment-stage larva of *O. sarsii* (Figures 11I,J). The ophiopluteus (Figure 11I) 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 are roughly equal in length with the transverse rods. The end rods are longer than the body rods and are nearly touching at their distal ends.

In the rudiment-stage larva the left anterolateral and postoral arms had been resorbed and there was a developing juvenile rudiment (Figure 11J). 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* (Figure 11I) is very similar to that of *O. leptoctenia* (see section above, Key 12).

Development mode. *Ophiura sarsii* develops via a small egg of 100–110 μ m 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 (Figures 11H,K), 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

Gorgonocephalus eucnemis (MÜLLER & TROSCHER 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 *diomedae* 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 *stimpsonii* (Clark, 1911) and the species name *eucnemis* occurs across 4 BOLD bins. Our own analysis shows several species within the genus to be closely related at the COI locus (within 6%; Figure S11, Table S1). The sequences from our larvae were identical to that of an adult we collected off Cape Arago and another from California (BOLD bin AAI5449) and a < 1% different from two other specimens collected off Cape Arago (Table S1). Sequences in another bin (AAE7308) of *G. eucnemis* found in the Salish Sea, in central British Columbia, and in the Bering Sea is only 93% similar to our samples, indicating that our Oregon samples are distinct from most of those further north (Supplementary Figure S11).

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 m (Lambert & Austin, 2007). No other basket stars occur in our region; though a congener, *G. diomedae*, occurs in Panama.

Embryonic description. Eggs are approximately 220 µm 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 (Figure 12A) and have a thick hyaline layer with a textured appearance like that of translucent spheres (Figure 12B'). 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.

Similar species. The embryo and juvenile of *G. eucnemis* is unlike any other species found in the plankton of the southern Oregon coast. The amphiuroids with abbreviated development – *Amphiodia* sp. opaque, *A. sp. tan*, *A. aff. periercta*, *Amphiplus* sp. vitellaria, and *Amphiura arcystata* – share the tan to salmon opaque coloration of *G. eucnemis* but differ from *G. eucnemis* in recognizable ways. As an embryo *G. eucnemis* has a thick hyaline layer with a bubble-like texture, a feature that is not present in any other species in the region. As a juvenile, *G. eucnemis* lacks any skeletal armature and does not have visible tube feet, other features unique to this species amongst those described in this study (Key, 20).

The early juvenile of *Amphiodia* aff. *periercta* is also pink in color,

unciliated, and is dorsal-ventrally flattened. They differ, however, in the structure of the five developing juvenile arms: those of *A. aff. periercta* are triangle-shaped, covered in scales and with obvious tube feet; those of *G. eucnemis* are lobate, without scales or visible tube feet, and with distal hooks (Figure 12E'). Furthermore, the nature of the hyaline layers differ, with that of *A. aff. periercta* smooth and vanishingly thin as the juvenile develops, and that of *G. eucnemis* with a bubble-like texture that is apparent even in the late juvenile (Figure 18E).

Development mode. Freely spawned, fertilized eggs of 220 µm give rise to nonfeeding embryos and juveniles (Emlet, unpublished data; Strathmann, 1987). Development is unknown for other members of *Gorgonocephalus*, but *G. caputmedusae* free-spawns eggs, although it is unknown if they develop into an ophiopluteus larva (Mortensen, 1927).

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 (Figure 12E). The arms of early juveniles of *Gorgonocephalus* that have not yet begun to branch. Juveniles are thought to associate with the soft coral *Alcyonium*, previously known as *Gersemia rubiformis* (Austin & Hadfield, 1980; Williams, 2013).

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 (Figure 3). Emlet (unpublished) has gotten adults to spawn in early February.

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

CONCLUSIONS

We observed planktonic developmental stages of 19 species from seven families of brittle stars from the southern coast of Oregon 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.

KEYS TO THE OPHIUROID PLANKTONIC FORMS OF THE NORTHEASTERN PACIFIC OCEAN

The characteristic feeding larva of ophiuroids is the ophiopluteus, a bilaterally symmetrical larva that usually has eight arms bearing a ciliated band. 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). In larvae with a transparent epidermis, many skeletal rods can be seen in transmitted light; in larvae with opaque coloration, cross-polarized light can be used to visualize skeletal elements. 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

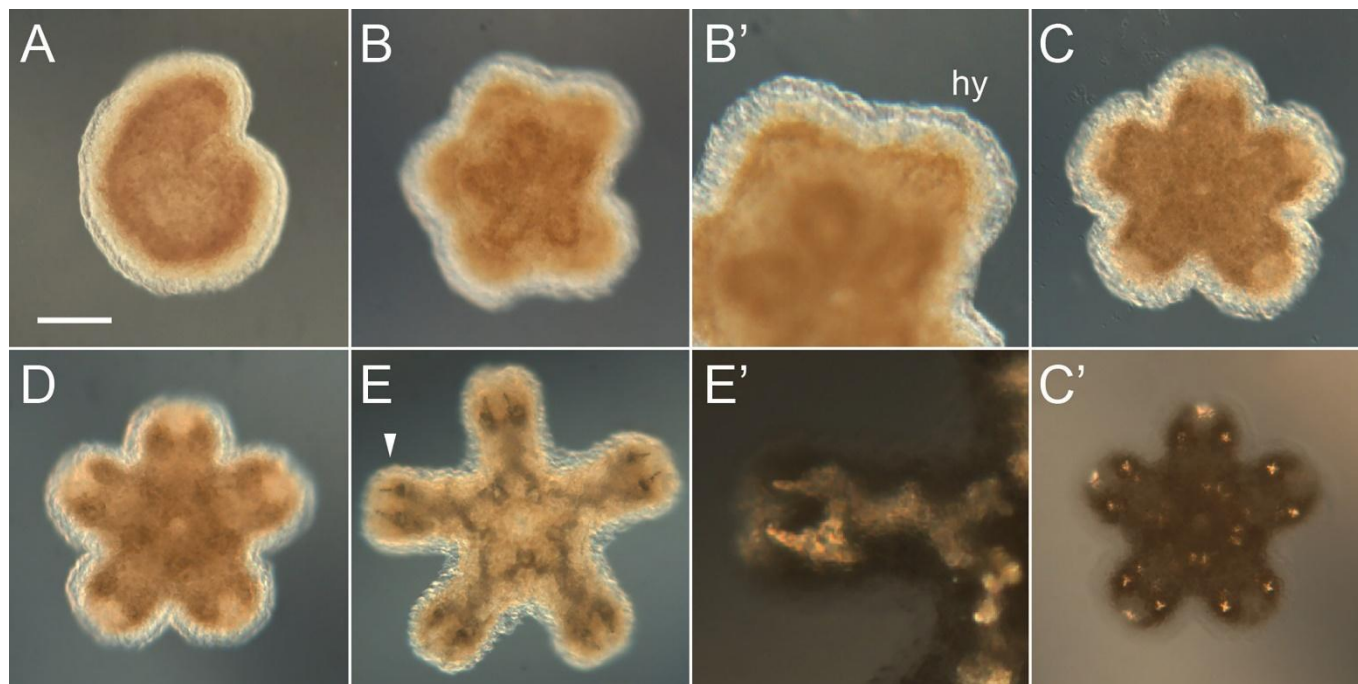


FIGURE 12 Planktonic stages of *Gorgonocephalus eucnemis*. Secondary views of the same individual are marked with an apostrophe ('). **A.** Embryo, lateral view; **B.** early juvenile, oral view; and **B'.** closeup view of hyaline layer (hy) with bubbled appearance. **C.** Same individual as **B** one day later; **C'.** is viewed in crossed-polarized light. **D.** Same individual as **B** two days later. **E.** Another individual with mobile arms tipped with hooks (filled arrowhead), also shown in (**E'**) cross-polarized light. The juvenile can be distinguished from those of other ophiuroids in the region by the absence of stereom in the central disc. Scale bar is 100 μ m, same scale for **A–E**.

ophiopluteus. These two spicules are first visible in cross-polarized light in the gastrula and early pluteus (Figure 1A) prior to the extension of the body rods and their connection via the transverse rods. A third larval spicule is reported in rare cases (see Mortensen, 1921). Each spicule has rods that support four arms on its side of the larva. The posterolateral rods – often the longest and most prominent pair, but not always – extend laterally and anteriorly, and distinguish an ophiopluteus from an echinopluteus (Figure 1B). The anterolateral rods extend anteriorly and support the mouth and adjacent ciliary band. The postoral rods extend ventrally to support a ciliary band in front of the mouth. Finally, the posterodorsal rods originate from the posterior portion of the anterolateral arm rods, project dorsally, and do not appear until later in development. In many species the postoral and posterodorsal arms are mirror imaged across the frontal plane, making it difficult to see both pairs at once (Figure 1B). 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 and contact along the larval midline with their counterparts from the opposite spicules. The transverse rods may bear one or more median processes. Some ophioplutei have pairs of recurrent rods that connect the bases of the postoral rods with the ventral transverse rods and the anterolateral rods with the dorsal transverse rods of the larva. Posterior to the transverse rods, the body

terminates in end rods. We refer to this informative region of the larval skeleton at the posterior of the larva as the ‘posterior girdle’ (Figure 1B).

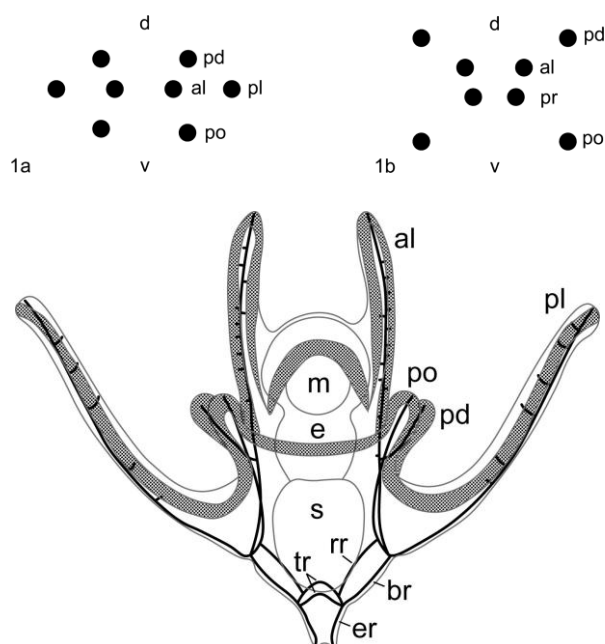
Like many echinoderms, ophiuroid plutei develop a juvenile starting as a series of coeloms on the left and right sides of the larval body, which merge and elaborate to form the juvenile rudiment (Selvakumaraswamy & Byrne, 2023; Sweet et al., 2022). In many species several larval arms may be reabsorbed in the process of rudiment formation, often leaving both posterolateral arms and the right anterolateral arm (Figure 1C), or just the posterolateral arms (this study; Selvakumaraswamy & Byrne, 2023). Juveniles metamorphosing from plutei are usually on the small end of the range found among all types of development.

Ophiuroids with abbreviated development usually develop to a juvenile more quickly than species with feeding plutei and can have small or relatively large post-larvae produced from moderate numbers of yolky eggs. Abbreviated developers are a developmentally heterogeneous group taking the form of reduced plutei, pelagic or brooded vitellaria, demersal larvae, or modified larvae that undergo metamorphosis in an attached fertilization envelope (Byrne et al., 2008; Byrne, Cisternas, O'Hara, Sewell, et al., 2024; Hendler, 1975). We have also observed several pelagic direct developers (e.g., Emlet, 2006). Reduced plutei have a modified or reduced morphology, and may have two, four or six larval 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 with a functional digestive tract (Allen & Podolsky, 2007; Nakata & Emlet, 2023).

The typical nonfeeding larva of ophiuroids is the vitellaria (Figure 1D), though some authors include doliolaria (McEdward & Miner, 2001). We reserve doliolaria as being barrel-shaped and having a series of transverse ciliary rings found among holothuroids, while ophiuroid vitellaria are dorsoventrally compressed and may have complete ciliary rings at the anterior and posterior, but the mediary ciliary bands are disjunct (Figure 1D). The vitellaria lacks a larval mouth, stomach, and anus as it does not feed but may bear the mouth of the incipient juvenile. Vitellaria larvae have been observed in 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 three species from the family Ophiocomidae (*Ophiocomella pumila*, *Ophiomastix wendtii*, and *Breviturma dentata*) a feeding ophiopluteus transforms into a vitellaria prior to metamorphosis (Byrne, Cisternas, O'Hara, & Selvakumaraswamy, 2024; Mladenov, 1985).

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 bursae of the adult disc until they crawl away as advanced juveniles (e.g., Byrne, Cisternas, O'Hara, Sewell, et al., 2024). Another, rarer type is pelagic direct development, in which embryos and early juvenile stages are planktonic (Emlet, 2006; Hendler, 1973; Patent, 1970).



Above is the anterior view of the generalized, relative position of larval arms in ophiuroids (1a) and echinoids (1b). Arms, from dorsal (d) to ventral (v) are posterodorsal (pd), anterolateral (al), posterolateral (pl) in 1a, preoral (pr) in 1b, and postoral (po).

The key is divided into ophioplutei, nonfeeding forms, and juveniles.

1a. Larva has bilateral symmetry and with four to eight arms supported by skeletal rods, the longest and most wideset is a single pair, positioned laterally ... 3, ophiopluteus

1b. Larva has bilateral symmetry and four to eight arms supported by skeletal rods, the longest and most wideset of which are two pairs and are positioned ventrally and dorsally... echinopluteus

1c. None of the above...2

2a. Larva with a disc- or ovoid-shaped body; may or may not have transverse ciliated bands ... 16, nonfeeding larva (including vitellaria)

2b. Motile disc or star with podia, arms or arm spines, and a functional mouth ... 20, juvenile

OPHIOPLUTEI

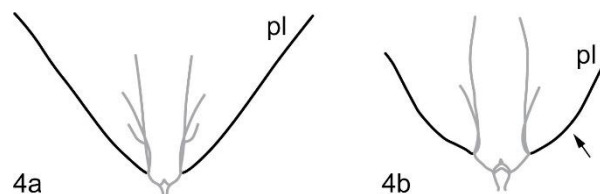
3a. Ophiopluteus with four to eight arms. Larval epidermis transparent, such that skeletal rods are visible through the epidermis under transmitted light (Figures 1 and 2); pigmentation, if present, is usually restricted to the arms, and often occurs at the distal ends or along the length of the posterolateral arms. Also, any pluteus of large size (> 1 mm) and eight arms ... planktotrophic ophioplutei, 4

3b. 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, 13

Planktotrophic plutei

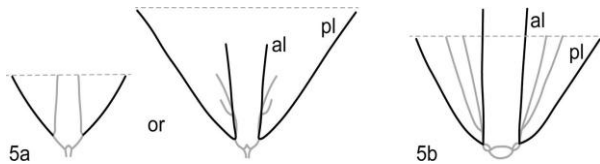
4a. The posterolateral (pl) rods – usually the longest, widest, and most conspicuous pair (but see 5b) – are more or less straight across their length ... 5

4b. The skeletal rods of the posterolateral arms are curved concave toward the larval body ... 9



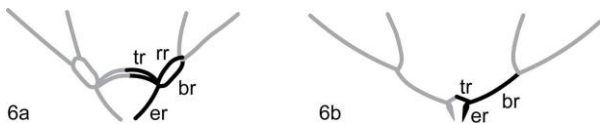
5a. The 'height' of the anterolateral rods (al) never exceeds that of the posterolateral rods; that is, a line connecting the tips of the posterolateral rods would just touch the distal tips or not intersect the anterolateral rods ... 6

5b. The anterolateral rods are 'taller' than the posterolateral rods; that is, a line connecting the tips of the posterolateral rods would bisect the anterolateral rods. The eight-armed pluteus is large, with vibratile lobes and a boxy profile, almost like that of an echinopluteus due to the length and height of the postoral and posterodorsal arms and their narrow angle ... late pluteus of Ophiacanthina (suborder), 8



6a. Recurrent rods (rr) present (Figure 11A). The transverse (tr) and end rods (er) are approximately the same length as the body rods (br). The 8-armed pluteus is several mm in size and may have posterolateral arms of asymmetrical length. Both posterolateral arms may each bear a band of reddish-brown coloration midway along its length (Figure 2M) ... *Ophiactis hastatum*

6b. Recurrent rods absent; the transverse and end rods are very short relative to the body rods ... 7



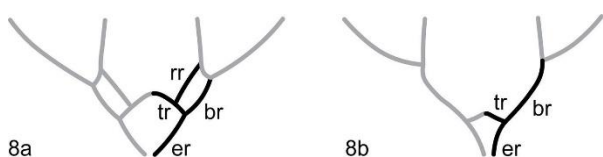
7a. Ophiopluteus 4-6 mm in size. The posterolateral rods up to five times the length of the anterolateral arms (Figure 2A). The posterolateral arms open wide, with an angle $\gg 90^\circ$ in the 8-armed pluteus, and nearly 180° in the rudiment-stage larva. The rudiment-stage larva resembles that of *Ophiopholis*, but generally much larger, spanning several millimeters in size. If present, there is a single median process (Figure 5A) ... *Ophiothrix spiculata*

7b. Ophiopluteus 1-2 mm in size. The posterolateral arms create an angle close to 90° : the angle of the posterolateral arms is $< 90^\circ$ in the 4- and 6-armed plutei (Figures 5C,D), $\geq 90^\circ$ in the 8-armed pluteus (Figures 2B,C and 5F,J), and $\gg 90^\circ$ in the rudiment-stage larva (Figure 5H). The posterolateral arms do not exceed three times the length of the anterolateral arms. If present, there are two median processes ... *Ophiopholis* spp.



8a. Recurrent rods present. In the six- and early eight-armed pluteus the transverse and end rods are approximately equal in length to body rods (Figures 9A' B', below). In the advanced ophiopluteus the transverse and end rods lengthen, and the end rods articulate to support the posterior of the larva (Figure 9D') ... *Ophiacantha diplasia*

8b. Recurrent rods absent; transverse and end rods shorter than body rods; transverse rods articulate in early pluteus (Figure 14A', below). In the advanced ophiopluteus the transverse rods do not articulate and are well separated to accommodate the larval gut (Figure 10D) ... *Ophiopteris papillosa*



9a. The epidermis is transparent with yellow-green pigmentation on the distal ends of the arms, especially the anterolateral and posterolateral pairs. Thorns absent from skeletal rods. The

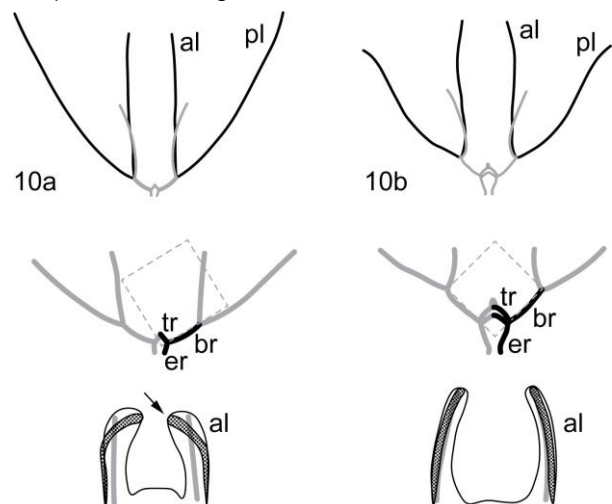
posterolateral arms are concave relative to the midline near their base, where they attach to the other skeletal rods. ... early pluteus of *Ophiacantha*, 8

9b. The epidermis is transparent with red pigmentation on the distal ends of the arms, especially the posterolateral pair. The skeletal rods may have an orange-red tint and often bear conspicuous thorns, especially on the posterolateral and anterolateral arm rods. The posterolateral rods are curved, concave toward the larval body. The height of the postoral arms never exceeds that of the mouth ... *Amphiridae*, 10

9c. The epidermis is completely transparent and lacks any pigmentation. The posterolateral rods are curved and concave toward the larval body. Thorns are absent. Recurrent rods present. The body rods are approximately equal in length with the transverse rods ... *Ophiuridae*, 12

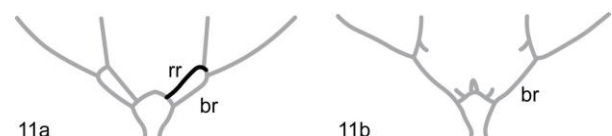
10a. The posterolateral rods are concave toward the larval body, and the curve is distributed along the length of the arm. The anterolateral arms are straight and parallel to one another. There is space between the skeletal rods and the epidermis. The epidermis and ciliated band on the tips of the anterolateral arms may extend medially, giving this larva a 'bunny-eared' appearance. The eight-armed pluteus has anterolateral and posterolateral arms of equal height (Figure 6K). The body rods of the six- and eight-armed pluteus are curved and form an angle $> 90^\circ$. End and transverse rods are short relative to the body rods. Thorns, if present, are small and triangle shaped ... *Amphipholis pugetana*

10b. The posterolateral rods are concave proximally near their base and the distal ends are straight or flaring. The anterolateral rods are curved. There is little space between the skeletal rods and the epidermis. Thorns are often present and may be prominent (Figure 6E). The body rods form an angle of $\sim 90^\circ$... 11



11a. Recurrent rods present ... *Amphiodia* aff. *digitata*

11b. Recurrent rods absent ... *Amphiodia urtica*



12a. The pluteus has a transparent epidermis that has an inflated appearance such that there is an obvious gap between the epidermis

and the skeletal rods, especially the posterolateral and anterolateral arms. The epidermis comes to a point at the distal ends of the posterolateral arms (Figure 11C) ... *Ophiura leptoctenia**

12b. The transparent epidermis is somewhat inflated, and is rounded at the distal ends of the posterolateral arms (Figure 11I)

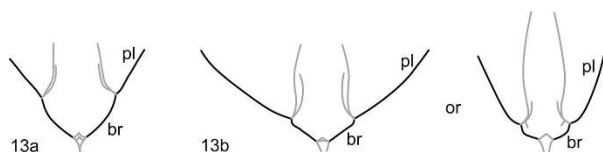
... *Ophiura sarsii**

*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*.

Reduced plutei

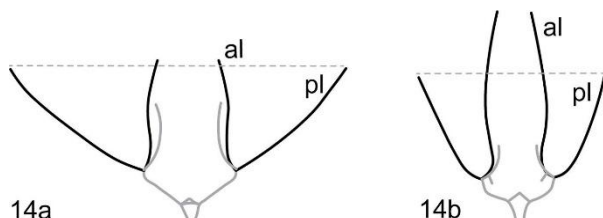
13a. Body rods long, of length equal to or greater than the short straight posterolateral arms; thorns are never present. The epidermis covering the body and anterolateral arms is orange and the epidermis of the posterolateral arms is white (Figure 11F) ... *Ophiura luetkenii*

13b. The body rods are shorter than the posterolateral rods. The posterolateral and anterolateral rods often bear thorns. The epidermis can vary from tan to pink to bright orange (Figure 7) ... Amphiridae, 14



14a. The anterolateral arm rods are shorter in length than the posterolateral rods; the anterolateral arms are equal in height to the posterolateral arms. The posterolateral arms are set at approximately a 90° angle and may be straight or curved (Figure 7C). The epidermis is uniformly tan to salmon in color. Planktonic development takes at least two weeks and the early pluteus lacks well-developed coeloms on the left side (Figure 7B) ... *Amphiodia* sp. opaque

14b. The anterolateral arm rods are longer than the posterolateral rods; the anterolateral arms exceed the height of the posterolateral arms. The posterolateral arms curve upwards such that they create an angle < 90°. Early pluteus with well-developed coeloms on the left side (Figures 7F,M); planktonic development proceeds in just over a week ... 15



15a. The epidermis is orange on the body and white on the anterolateral and posterolateral arms (Figures 7H,I) ... *Amphiodia* sp. orange belly

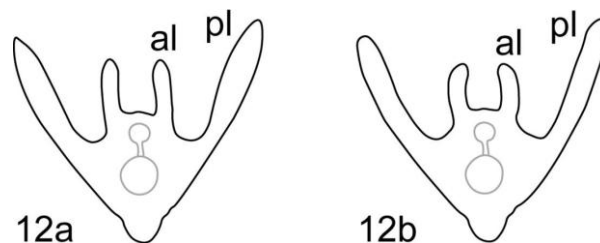
15b. The epidermis is uniformly tan in color across the whole larva (Figures 7N, O) ... *Amphiodia* sp. tan

NONFEEDING LARVAE

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

16a. Embryo with pentaradial symmetry ... 17

16b. Symmetry is semi-bilateral or unclear ... 18 17a. Embryo is flattened and round ... 18



17b. 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, 19

18a. 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 < 10 skeletal elements spanning an arc across the embryo; mouth centered (Figures 8K-M) ... *Amphiura arcystata*

18b. Embryo light pink in color, very flat with an uneven periphery; with a ring of ~ 20 skeletal elements around periphery of embryo; stomodeal pit not centered until pentaradial symmetry is well established (Figures 8D,E) ... *Amphiodia* aff. *periercta*

19a. Vitellaria light pink in color with darkened ciliated bands, approximately 300 µm. Ciliated bands five in number (Figure 8I) ... *Amphioplus* sp. vitellaria

19b. Vitellaria of approximately 400 µm, with a transparent epidermis, except for the ciliated bands which are yellow green. The white skeleton of the juvenile rudiment is visible, as is the red coloration of the gut. The anterior-most portion of the ciliated band is supported by remnants of the pluteus arms, as evidenced by the presence of two skeletal rods (Figure 10E") ... *Ophiopteris papillosa*

JUVENILES

20a. Juvenile arms without segments or scales, covered in pink epidermis, and lacking tube feet but with a pair of distal claws (Figure 12E) ... *Gorgonocephalus eucnemis*

20b. Juvenile with calcite plates and scales ... 21

21a. Juvenile arms with 2 or more segments ... 22

21b. Juvenile arms with 1 segment (terminal arm plate) ... 25 22a.

Arm segments without hooked spines ... 23

22b. A pair of hooked spines on the lateral sides of penultimate arm segment ... 24

23a. Yellow pigmentation present at the distal arm segments (Figure 9F) ... *Ophiacantha diplasia*

23b. Arms white throughout (Figure 10F) ... *Ophiopteris papillosa*

24a. Penultimate arm segments bearing spines with one hook (Figure 5B) ... *Ophiothrix spiculata*

24b. Penultimate arm segments bearing spines with two hooks (Figure 5I) ... *Ophiopholis* spp.



25a. Terminal arm plates are triangular to cone shaped (Figures 6G,M, 7E,L,R, and 8F,J,N) ... Amphiruridae

25b. 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 ... Ophiuridae, 26

26a. Larval body is orange (Figure 11H) ... *Ophiura luetkenii*

26b. Larval body is transparent to tan (Figure 11K) ... *Ophiura sarsii*

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SUPPORTING INFORMATION

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