



“Little Red Jellies” in Monterey Bay, California (Cnidaria: Hydrozoa: Trachymedusae: Rhopalonematidae)

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Several species of small, red, deep-sea Trachymedusae have been described and then re-described over the past 20 years, leading to some confusion in the scientific literature. This paper provides an overview of three genera (*Benthocodon*, *Crossota*, and *Pectis*) in the family Rhopalonematidae (Cnidaria: Hydrozoa) that have been observed and examined both in the field and in the laboratory. Twenty years of *in situ* observations in Monterey Bay indicate that two of the genera, *Benthocodon* and *Pectis*, are often associated with the benthic boundary layer and can occur in dense patches. They have been observed resting on soft sediments with their subumbrellar surface down but are also found swimming up to several 100 m above the bottom. Individuals in the genus *Crossota* tend to be solitary and more pelagic in nature. Although *Crossota* may be found near the bottom as well, down to depths of 4,000 m, they have not been observed resting on the bottom. The three genera are morphologically similar and difficult to distinguish from each other. As a group, they are small (<5 cm) and sometimes darkly pigmented, making *in situ* identifications challenging. We show that these three genera can be differentiated morphologically and we provide a key to the genera and species common in Monterey Bay. Further, the genera differ in their depth distribution and behavior. Molecular genetics suggest that the genera and species are distinct from each other but that their taxonomy needs revision. This paper reviews the generic characteristics along with species identifications and provides images and video (Supplementary Material) that may be helpful in identification.

Keywords: Trachymedusae, *Crossota*, *Pectis*, *Benthocodon*, Monterey Submarine Canyon, ROV, phylogenetics

INTRODUCTION

The Trachymedusae are a diverse group of jellyfish, most of which live below the euphotic zone (Mayer, 1910). In Monterey Bay, California, they can be found in the water column all the way to the ocean floor at 4,000 m. This marks both the deepest extent of the Monterey Submarine Canyon as well as the depth limitation for the remotely operated vehicles (ROVs) used in this study. Three genera (*Pectis* Haeckel, 1879; *Benthocodon* Larson and Harbison, 1990; and *Crossota* Vanhöffen, 1902) in the family Rhopalonematidae are commonly reddish in hue, similar in size, and have been difficult to distinguish from one another. The most common species: *Benthocodon pedunculata* (Bigelow, 1913) and *Pectis profundicola* (Naumov, 1971) are benthopelagic and, although

patchy, have been recorded in numbers up to 80/m² (Larson et al., 1992). They often swim in short bursts, usually followed by bouts of sinking (Larson and Harbison, 1990; Larson et al., 1992; Matsumoto et al., 1997). *Crossota* species are typically found singly in the water column (Thuesen, 2003). Previous observations from human occupied vehicles and remotely operated vehicles consisted mostly of comments about "little red jellies" and the occasional specimen collection. Based on their abundance and mobility, these genera likely represent an ecologically important group (Alldredge, 1984). A similar conclusion was reached by Thuesen and Childress (1994) based on their physiological research which indicated that the metabolism of some deep-sea medusae is close to that of deep-sea fishes and crustaceans.

Representatives of *Benthocodon*, *Crossota*, and *Pectis* are abundant in the deep waters off Monterey and we present a morphological key and a molecular phylogenetic analysis that facilitates identification of each genus and species common to Monterey Bay. Lastly, we summarize field and laboratory observations that provide a better understanding of the natural history of these deep-sea jellies.

MATERIALS AND METHODS

Field Observations and Collection

Observations and collections were made using remotely operated vehicles from the Monterey Bay Aquarium Research Institute (MBARI) (Robison et al., 2017). The ROV *Ventana* is depth rated to 1,850 m. ROV *Tiburón* was, and ROV *Doc Ricketts* is, depth rated to 4,000 m (<http://www.mbari.org/at-sea/vehicles/>). High definition video cameras are mounted on these vehicles and the video signal is conveyed to the surface support vessel (ROV *Ventana*—R/V *Point Lobos* and R/V *Rachel Carson*; ROV *Tiburón* and ROV *Doc Ricketts*—R/V *Western Flier*) through optical fibers at the core of the ROV's tether. At the surface, the video signal is viewed on a high-resolution monitor and was recorded initially on digital BetaCam tape, then MBARI switched to a High Definition camera system and recorded on High Definition tape, and we are now recording the video digitally. The observer's comments and descriptions are recorded on the audio track of the recording. Environmental data (depth, location, temperature, dissolved oxygen, and salinity) for each dive are collected and, after each dive, this information is integrated into a database. These data and all frame grabs from video images collected by our ROVs, are annotated into a comprehensive relational database (<http://www.mbari.org/vars>) that is available to the public.

Specimens were collected in 6.5 l "detritus" samplers, designed for the gentle capture of delicate material in midwater (Youngbluth, 1984), or in a "suction" sampler consisting of a transparent funnel and 2 m of flexible tubing leading back to 12 separate 6-l collection cylinders within the ROV toolsled. For the detritus samplers, the ROV pilot positions the vehicle so that the open cylinder of the sampler encloses the medusa, then the doors at either end are gently closed by hydraulic rams. For the suction sampler, the funnel, attached to a clear plexiglass tube and flexible tubing, can be extended in front of the ROV (<https://www.mbari.org/high-frequency-suction-samplers/>). When a medusa

is near the wide opening of the funnel, the suction pump is turned on and the animal is gently collected and deposited into a sample container.

Molecular Genetic Data

Tissue samples for molecular analysis were collected with the ROVs and either frozen at -80°C or pressed onto Whatman FTA cards (Sigma-Aldrich, St. Louis, MO). Genomic DNA was extracted from frozen tissue samples using a modified CTAB-DTAB protocol (Gustinich et al., 1991). Approximately 300 μl of tissue was placed into a 2 ml Eppendorf tube and 600 μl of "blood lysis buffer" (8% DTAB, 1.5 M NaCl, 100 mM Tris-Cl, pH 8.6, 50 mM EDTA) was added. The tissue was incubated at 68°C for 5 min and then 900 μl of chloroform were added to deproteinize the tissue. The tube was inverted 2–3 times and then spun at 10,000 rpm for 2 min. The supernatant was removed (discarding the lower portion and being careful to avoid the tissue detritus at the interface of phases) and DNA was precipitated from the supernatant by adding 900 μl of ultrapure water and 100 μl of a 5% CTAB solution that contained 0.4 M NaCl. This was followed by gentle inversion of the tube and centrifugation at 10,000 rpm for 2 min. The resulting pellet was resuspended in 300 μl 1.2 M NaCl and 750 μl EtOH were added, the sample was gently mixed and spun at 10,000 rpm for 10 min to precipitate the DNA again, followed by a rinse with 300 μl 70% EtOH. Additional extractions were performed using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol for animal tissue extractions with some modifications: after the addition of Buffer AL, 200 μl of cold isopropanol was added to each sample. In addition, two elutions were done on each sample for a total of 70 μl genomic DNA. Genomic DNA was also obtained from FTA paper following the manufacturer's protocol. The resulting pellets were resuspended in 50 μl of ultrapure water; 1–2 μl of genomic DNA were used for either 30 or 10 μl PCR reactions.

The nuclear large subunit ribosomal RNA (LSU or 28S) gene was amplified (initial denaturation at 94°C for 2 min; 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 70 s; final elongation at 72°C for 7 min) using slightly modified (LSUD1F ACCCGCTGAATTTAAGCATA; D3Ca ACGAA CGATTTGACACGTCAG or LSUD4Ra AACCAGCTACTA GRYGGTTCGAT) universal primers (Scholin and Anderson, 1994). The PCR product was either sequenced directly from a gel cut or cloned into TOPO TA vector. In both cases, the results from multiple PCR reactions or plasmid DNAs from 2 to 5 individual clones were sequenced individually and as a pool with the SequiTherm EXCEL II Long-Read DNA sequencing kit (Epicenter Technologies) and analyzed on a LI-COR 4200 IR2 instrument. Some of the clones were also sequenced with the BigDye Terminator v.2 sequencing kit and analyzed on an ABI 3100 instrument.

The nuclear small subunit ribosomal RNA (SSU or 18S) gene was amplified using the modified universal primers *mitchA* and *mitchB* from (4 cycles of 94°C for 60 s, 58°C for 60 s, 72°C for 2 min, followed by 29 cycles of 94°C for 60 s, 64°C for 60 s, 72°C for 90 s; Medlin et al., 1988). All products were bi-directionally sequenced using BigDye[®] Terminator v3.1 Cycle Sequencing Kit

on an ABI 3100 or ABI 3500 sequencer (Applied Biosystems, Foster City, CA, USA).

Two mitochondrial genes were amplified and sequenced, the mitochondrial cytochrome oxidase one (COI) gene and the gene encoding the large RNA subunit of the mitochondrial ribosome (16S). COI were amplified using the forward primer of Leray et al. (2013) and the Folmer et al. (1994) reverse primer (16 cycles at 94°C for 10 s, starting annealing temperature of 62°C for 30 s that was lowered by 1°C during each subsequent cycle, and 68°C for 60 s, followed by 25 cycles of 94°C for 10 s, 46°C for 30 s, and 68°C for 60 s with a final elongation of 72°C for 10 min). 16S sequences were generated following the protocols described in Bentlage et al. (2018).

Phylogenetic Analyses

All sequences were assembled in Geneious (various versions; Biomatters Limited, NZ, RRID:SCR_010519) and a dataset was compiled that comprised newly generated and previously published sequences of Trachymedusae and Narcomedusae (Table 1 and Figure 1). Individual loci were aligned using MAFFT (v7.205; Katoh and Standley, 2013). Unreliably aligned positions were identified and excluded using Gblocks (Talavera and Castresana, 2007, RRID:SCR_015945), as implemented in the alignment viewer Seaview (version 4; Gouy et al., 2010, RRID:SCR_015059), allowing for smaller blocks, gap positions, and less strict flanking positions. The four resulting alignments were concatenated using the catfasta2phym1 script available from <https://github.com/nylander/catfasta2phym1>.

Partitionfinder (version 1.1.0; Lanfear et al., 2012) was used to identify the appropriate substitution models and best partitioning scheme for inferring phylogenetic relationships from the concatenated alignment; partitioning schemes were evaluated and ranked using the Bayesian information criterion (Bic). Partitioning schemes tested were the following: (1) all loci evolve under the same model (no partitioning), (2) all loci evolve under a different model of evolution (separate 16S, COI, 18S, and 28S partitions), (3) mitochondrial (16S and COI) and nuclear (18S and 28S) loci evolve under a different model, (4) nuclear loci (18S and 28S) evolve under one model while the mitochondrial loci (16S and COI) can be best modeled by separate models, and (5) mitochondrial loci (16S and COI) evolve under one model while the nuclear loci (18S and 28S) can be best modeled separately. The best partitioning scheme was then encoded in RAxML (version 8.2.7; Stamatakis, 2014, RRID:SCR_006086) to infer the maximum likelihood phylogeny. Robustness of the phylogeny was evaluated using 1,000 non-parametric bootstrap replicates using RAxML's rapid bootstrapping algorithm (-fa-m GTRGAMMAI-p \$RANDOM-x \$RANDOM-# 1000). Following the phylogenetic framework presented in Bentlage et al. (2018), the resulting phylogeny was rooted on the trachymedusan family Halicreatidae.

RESULTS

Phylogeny and Taxonomy

We have observed, collected, and sequenced several specimens of *Pectis profundicola* previously identified incorrectly as

Benthocodon pedunculata (Larson et al., 1992; Matsumoto et al., 1997) and as *Voragonema pedunculata* (Bouillon et al., 2001; Lindsay and Pagès, 2010), several species of *Crossota*, and *Benthocodon pedunculatus* (see discussion for the change in the species name from *B. pedunculata* to *B. pedunculatus*).

We increased taxon sampling compared to the recent phylogeny of Bentlage et al. (2018) with respect to *Crossota*, *Benthocodon*, and *Pectis*, and provide information to help discriminate between the three genera while expanding on the taxa and sampling in recent work (Bentlage et al., 2018). We support the earlier finding (Bentlage et al., 2018) that *Crossota* is not a monophyletic clade (Figure 1) with *Crossota millsae* (Thuesen, 2003) being most closely related to *Tetrorchis erythrogaster* (Bigelow, 1909). Note that *C. millsae* is paraphyletic with respect to *Tetrorchis*, albeit at low bootstrap support. Overall, the remainder of the genus *Crossota*, other than *C. millsae*, were paraphyletic with respect to *Benthocodon*, *Pectis*, and *Vampyrocrossota*. *Crossota* is separated according to nominal species, supporting their distinction based on morphology (Figures 2, 3). A putatively undescribed species (*Crossota* n. sp.—Figure 3) photographed and collected in the Gulf of California appears to be the closest relative of *Vampyrocrossota* and a specimen from Bentlage et al. (2018) that appears to have been misidentified (*Crossota alba* auct). *Benthocodon* and *Pectis*, the other two genera of interest in this contribution are nested firmly within a clade of *Crossota* species but some nodes remain poorly supported. Monophyly of *Benthocodon* and *Pectis* cannot be confirmed or rejected until additional species in these genera have been sampled.

Vertical Distribution and Behavior

Over 20 years of *in situ* observations in Monterey Bay have revealed that *Crossota*, *Benthocodon*, and *Pectis* exhibit distinct depth distributions.

All species of *Crossota* that we observed found in the water column at depths from 500 to 4,000 m and were never observed on the bottom, although they have been seen swimming in the benthic boundary layer. *Crossota* swims with a series of strong pulsing contractions, usually followed by a period of quiescence.

Benthocodon species are generally found in deeper waters (>2,500 m) although the type specimen (*Crossota pedunculata*) was collected in <100 m of water. While *Benthocodon hyalinus* is found in the water column or near the bottom, *Benthocodon pedunculatus* is typically found drifting above the sediment (up to 100 m from the bottom) or either on or just above the bottom (see Supplemental Video). Those *Benthocodon pedunculatus* specimens that are not on the sediment, generally pulse consistently but weakly every few seconds (see Supplemental Video). They are negatively buoyant and sink between pulses, thus maintaining a somewhat constant position on or just above the bottom. They are capable of stronger bouts of swimming, but these tend to be followed by longer sinking/rest periods. Abundance of these medusae was as high as 11 per square meter (based on video transects—Figure 4).

In Monterey Bay, *Pectis* specimens can be found throughout the water column with *P. tatsunoko* in the water column and *P. profundicola* associated with the benthic boundary layer

TABLE 1 | Genbank accession numbers for sequence data used in phylogenetic reconstruction.

	Species	18S	28S	16S	COI	Lab ID	Collection code	Collection date	Latitude	Longitude	Locality	Voucher
Narcomedusae	<i>Aeginona brunnea</i>	KY007602				DLSI443	20100627-1-ORI-4-Ab	27-Jun-2010	34° 29.39' N	140° 1.63' E	South-east of Sagami Bay, Japan	
	<i>Aeginopsis laurentii</i>	MG979347	MG979317	MG979377		GR10.122		***	***	***	Russ Hopcroft	
	<i>Aeginura grimaldii</i>	KY007605	MG979307	KY007590		DLSI451	KT10-24_St3	24-Oct-2010	27° 14.54' N	127° 5.40' E	Izena Hole, Japan	
	<i>Bathylkorus bouilloni</i>	MG979341	MG979308	MG979371		PS11406.4		***	***	***	Russ Hopcroft	
	<i>Cunina frugifera</i>	AF358059	KY077276	MG979372		AGC1026		Nov-1998	33° 10' N	118° 30' W	Catalina Channel, CA, USA	
	<i>Cunina octonaria</i>	KY007606	MG979310	KY007592		DLSI188	KT07-18_ORI-2-E	31-Jul-2007	35° 0' N	139° 20' E	Sagami Bay, Japan	
	<i>Pegantha martagon</i>	MG979344	MG979314	MG979375	MN068291	DLSI040	stUM15, RMT-D-8-2	28-Jan-2004			Dhugal Lindsay	
	<i>Pegantha cf. martagon</i>	MG979343	MG979313	MG979374		ss4ed.1		***	***	***	Karen Osborn	
	<i>Pegantha rubiginosa</i>	MG979345	MG979315	MG979376	MN068292	DLSI250	MOC1-12-7	***	***	***		
	<i>Pseudaegina rhodina</i>	EU247813			MN068275	RBMOC1001N7		13-Apr-2006	33° 31.47' N	69° 53.46' W	WNW of Bermuda	
	<i>Solmaris sp.</i>	MG979346	MG979316		MN068298	DLSI359	St. P-MTD-6 Cruise-KT09-4	24-Apr-2009	35° 0' N	139° 20' E	Sagami Bay, Japan	
	<i>Sigiweddellia sp.</i>	KY007607	MG979309	KY007593	MN068296	DLSI022	IO60320C, net 1	***	***	***	Dhugal Lindsay	
	<i>Solmundaegina nematophora</i>	AF358058	AY920789	EU293997		AGC1045		Sep-1997	33° 37' N	118° 21' W	off San Pedro CA, USA	
	<i>Solmundella bitentaculata</i>	EU247812	EU247795	EU293998	MN068300	Sch085	2001.05.03.1	3-May-01	43° 41.16' N	7° 19.02' E	Villefranche-sur-Mer, Ligurian Sea, Mediterranean	
	<i>Solmissus albescens</i>	MG979342	MG979311	MG979373		AGC506		27-Oct-2009	43° 36' N	7° 10.56' E	Bay of Angels, Cote d'Azur, France	
	<i>Solmissus incisa</i>	KY007609	MG979312	KY007596		DLSI361	HD519SS4	01-Mar-2006	35° 0.26' N	139° 21.40' E	Sagami Bay, Japan	
	<i>Solmissus marshalli</i>	AF358060	AY920790	EU294001	MN068299	AGC1025		Nov-1998	33° 10' N	118° 30' W	Catalina Channel, CA, USA	
	<i>Tetraplatia chuni</i>	MG979348	MG979318	KY007599	MN068301	DLSI261	I060322b-3	22-Mar-2006	34° 57.40' N	140° 13.50' E	Off Kamogawa, Japan	
	<i>Tetraplatia volitans</i>	DQ002501	DQ002502	EU293999		AGC139	V2508	2-Apr-2004	36° 31.89' N	122° 30.46' W	Monterey Bay Canyon, CA, USA	KUMIP 314322
Trachymedusae	<i>Arctapodema sp.</i>	MG979356	MG979327	MG979383	MN068279	DLSI358	St. P-MTD-3 Cruise-KT09-4	23-Apr-2009	35° 0' N	139° 20' E	Sagami Bay, Japan	

(Continued)

TABLE 1 | Continued

Species	18S	28S	16S	COI	Lab ID	Collection code	Collection date	Latitude	Longitude	Locality	Voucher
<i>Aglantha digitale</i>	MG979354	MG979324	EU293985		AGC208		Jun-2004	48° 32.82' N	123° 0.78' W	Friday Harbor, WA, USNM USA	1073329
<i>Aglaura hemistoma</i>	EU247820	MG979325	EU293984	MN068277	06Jap2301		7-Aug-2006	33° 41.63' N	135° 20.275' E	Seto Lab beach, Shirahama, Wayakama, Japan	
<i>Amphogona apicata</i>	MG979355	MG979326	MG979382	MN068278	DLSI282	I060326a-0	***	***	***	Off Oshima, Japan	
<i>Benthocodon pedunculatus</i> (1)			MN065485		T894SS1	3,500 m	20-sept-2005	36° 19.48' N	122° 54.1' W	Monterey Canyon, CA, USA	
<i>Benthocodon pedunculatus</i> (2)	MG979357	MG979328	MG979384		BB015	T1106SS12, 3,582 m	28-Jul-2007	36° 7' N	123° 33' W	Monterey Canyon, CA, USA	USNM 1452215
<i>Benthocodon pedunculatus</i> (3)	MK547161		MN065486		T894SS4	3,500 m	20-sept-2005	36° 19.47' N	122° 54.1' W	Monterey Canyon, CA, USA	
<i>Botrynema brucei</i>	EU247822	EU247798	EU293982			I060319bN4	19-Mar-2006	35° 0.6' N	139° 19.8' E	Sagami Bay, Japan	
<i>Botrynema ellinorae</i>	MG979349	MG979319	MG979378		PS11109.4		***	***	***	Russ Hopcroft	
<i>Colobonema sericeum</i>	MG979358	MG979329	MG979385		BB012	Tucker Trawl	28-Jul-2007	36° 09.40' N	123° 33.7' W	Monterey Canyon, CA, USA	
<i>Crossota alba</i> (1)	MK547162		MN065487	MN094795	DR716SS1	2,955 m	28-Feb-2015	25° 26.27' N	109° 50.43' W	Carmen Basin, Gulf of California, Mexico	
<i>Crossota alba</i> (2)				MN068303	DR90SS3	1,535 m	23-Oct-2009	36° 38.31' N	122° 07.27' W	Monterey Canyon, CA, USA	
<i>Crossota alba</i> auct.	MG979359	MG979330	MG979386		BB014	T1105SS4, 1,363 m	27-Jul-2007	36° 41.48' N	122° 34.99' W	Monterey Canyon, CA, USA	
<i>Crossota brunnea</i> (1)	MG979360	MG979331	MG979387	MN068280	DLSI093	stUM9, 1YGPT1000	***	***	***	Dhugal Lindsay	
<i>Crossota millsae</i> (1)	MG979361	MG979332	MG979388		BB009	T1106SS8, 2,281 m	28-Jul-2007	36° 07' N	123° 33' W	Monterey Canyon, CA, USA	
<i>Crossota millsae</i> (2)	MK547164		MN065488	MN068307	DR216SS5	2,837 m	18-Nov-2010	36° 33.2' N	122° 30.8' W	Monterey Canyon, CA, USA	
<i>Crossota millsae</i> (3)			MN065489	MN068306	DR88D2	2,115 m	21-Oct-2009	36° 20.3' N	122° 55.0' W	Monterey Canyon, CA, USA	

(Continued)

TABLE 1 | Continued

Species	18S	28S	16S	COI	Lab ID	Collection code	Collection date	Latitude	Longitude	Locality	Voucher
<i>Crossota millsae</i> (4)	MK547165			MN068308	DR437ss12	2,555 m	25-Oct-2012	36° 32.10' N	122° 30.30' W	Monterey Canyon, CA, USA	
<i>Crossota norvegica</i>	MG979362	MG979333	MG979389	MN068281	PS11-217		***	***	***	Russ Hopcroft	
<i>Crossota n. sp.</i>	MK547166		MN065490		DR726D4	2,992 m	12-Mar-2015	22° 54.59' N	108° 6.55' W	Mazatlan Basin, Gulf of California, Mexico	
<i>Crossota rufobrunnea</i> (1)	EU247823	EU247799	EU293987		AGC1061		***	***	***	Allen Collins	
<i>Crossota rufobrunnea</i> (2)	EU247824	EU247800	EU293986	MN068282		I060319bN4	19-Mar-2006	35° 0.6' N	139° 19.8' E	Sagami Bay, Japan	
<i>Crossota rufobrunnea</i> (3)			MN065492		DR90SS3	1,516 m	23-Oct-2009	36° 38.31' N	122° 7.27' W	Monterey Canyon, CA, USA	
<i>Crossota rufobrunnea</i> (4)	MK547167		MN065493	MN068305	DR716SS3	2,955 m	28-Feb-2015	25° 26.27' N	109° 50.43' W	Carmen Basin, Gulf of California, Mexico	
<i>Crossota rufobrunnea</i> (5)			MN065494		D794T	Tucker Trawl	12-Aug-2015	36° 44.99' N	122° 6.2' W	Monterey Canyon, CA, USA	USNM 1284404
<i>Halicreas minimum</i>	MG979350	MG979320	EU293983		I060320A-N1		19-Mar-2006	35° 0.6' N	139° 19.8' E	Sagami Bay, Japan	
<i>Haliscera conica</i>	EU247825	EU247797	EU293981		AGC1057		Sep-1997	33° 37' N	118° 21' W	off San Pedro, CA, USA	
<i>Haliscera bigelowi</i>	MG979351	MG979321	MG979379		BB013	T1105SS8, 1,106 m	27-Jul-2007	36° 41.48' N	122° 34.99' W	Monterey Canyon, CA, USA	
<i>Halitrepes maasi</i>	MG979352	MG979322	MG979380		BB004	T1105DS4, 9,878 m	27-Jul-2007	36° 41.48' W	122° 34.99' W	Monterey Canyon, CA, USA	
<i>Pantachogon haeckeli</i> (1)			MN065495		DR787SS5	950 m	8-Aug-2015	36° 41.23' W	122° 2.26' W	Monterey Canyon, CA, USA	
<i>Pantachogon haeckeli</i> (2)	AF358062	AY920792	KY077291		AGC1049		***	***	***	Allen Collins	
<i>Pantachogon haeckeli</i> (3)	MK547168	MK537356			BB010	Tucker Trawl	28-Jul-2007	36° 9.4' N	123° 33.7' W	Monterey Canyon, CA, USA	
<i>Pectis profundicola</i> (1)	MG979363	MG979334	MG979390		DR793SS7	607 m	12-Aug-2015	36° 44.39' N	122° 6.12' W	Monterey Canyon, CA, USA	

(Continued)

TABLE 1 | Continued

Species	18S	28S	16S	COI	Lab ID	Collection code	Collection date	Latitude	Longitude	Locality	Voucher
<i>Pectis profundicola</i> (2)	MK547171				DR719SS12	945 m	12-Aug-2015	36° 44.99' N	122° 6.19' W	Monterey Canyon, USNM CA, USA	1284394
<i>Ptychogastria polaris</i>	MG979353	MG979323	MG979381	MN068293	DLS1257	2K1285SS3e	***	***	***	Dhugal Lindsay	
<i>Rhopalonema velatum</i>	EU247819	EU247804	EU293992	MN068294	Sch355	Vfr05.6	4-Apr-2005	43° 41.16' N	7° 19.02' E	Villefranche-sur-Mer, Ligurian Sea, Mediterranean	
<i>Sminthea arctica</i>	MG979364	MG979335	MG979391	MN068297	PS11334.4		***	***	***	Russ Hopcroft	
<i>Sminthea eurygaster</i>	KY077284		KY077293		DLS1090	stUM6, WP2	***	***	***	Dhugal Lindsay	
<i>Tetrorchis enythrogaster</i>	KY077285	KY077274	EU293995		T981SS5	492 m	12-May-2006	36° 36.9' N	122° 22.35' W	Monterey Canyon, CA, USA	
<i>Vampyrocrossota</i> sp.	MK547172			MN068302	T842SS2	1,192 m	7-Apr-2005	35° 29.60' N	123° 52.0' W	Monterey Canyon, CA, USA	

***Location not provided.

Accession numbers in bold were generated as part of this study. Collection IDs provide information regarding source of specimen (some information is still pending). DR refers to the ROV Doc Ricketts; T refers to the ROV Tiburon, and V refers to the ROV Ventana with the numbers following representing the dive number and then the collection method (DS, Detritus Sampler; SS Suction Sampler; T, Tucker Trawl) followed by the number of the collection container.

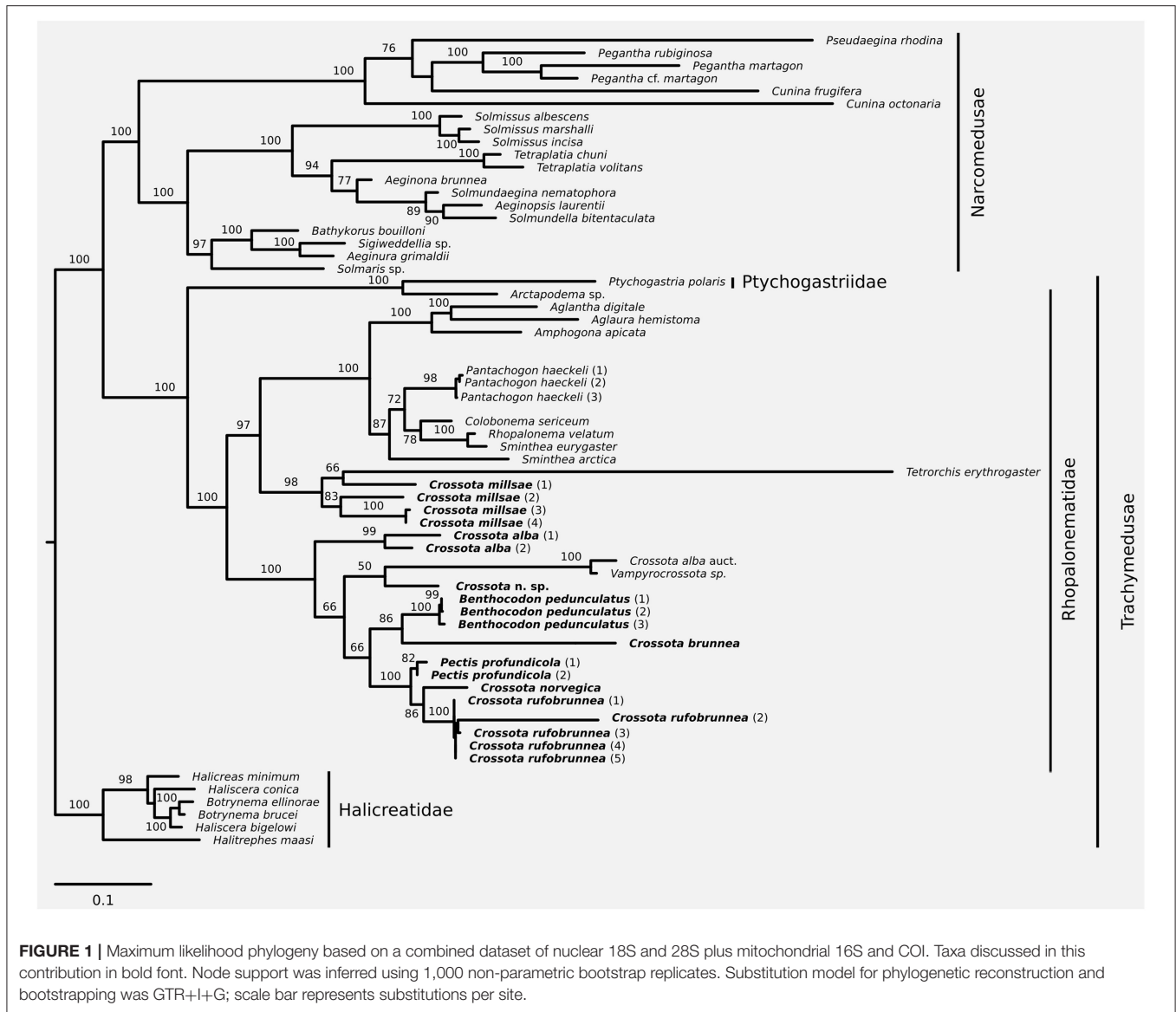
(Matsumoto et al., 1997) similar to *Benthocodon pedunculatus* but are usually observed shallower than the latter near the bottom in depths <3,000 m. *Pectis profundicola* specimens have longer escape bouts of pulsing and shorter rest periods between pulsing bouts than *Benthocodon pedunculatus* (see **Supplemental Video**).

DISCUSSION

As more research vehicles spend time in the deeper waters of the ocean collecting specimens and video footage, it is likely that more species within the three genera of *Crossota*, *Benthocodon*, and *Pectis* will be observed and described. This paper is not intended to be a full taxonomic species review of the three genera but is an attempt to provide guidelines for placing initial observations into the appropriate genus as well as provide some preliminary information for those who want to delve deeper into the species within each genus. Full species descriptions will require additional specimen collections and close examination of taxonomic and molecular characters. Nonetheless, phylogeny and imagery provided here demonstrate that the diversity of these taxa is larger than currently described, even in such a well-studied location as the Monterey Submarine Canyon. An issue that remains to be resolved is that the classification of rhopalonematids does not align with its phylogenetic history, as shown by the analyses presented here (**Figure 1**) and previously (Bentlage et al., 2018).

Naumov (1971) described a new genus and the type species (*Voragonema profundicola*), based upon a single specimen. He used the presence of prominent centripetal canals as a defining feature rather than the presence of a peduncle. Bouillon et al. (2001) noted that (Naumov, 1971) original description of *Voragonema* was incomplete, with some of the characters based solely on the holotype illustration that lacked detail. To address this issue, Bouillon et al. (2001) redescribed the genus *Voragonema* and described a new species, *V. laciniata*. Galea et al. (2016) proposed that *Pectis antarctica* (Haeckel, 1879) possesses all of the published characteristics of *Voragonema* and appears identical to *Voragonema laciniata* (Bouillon et al., 2001). The genus name *Pectis* has precedence over *Voragonema* and, following Galea et al. (2016), *Pectis* is used in this paper when referring to specimens previously identified as *Voragonema*. While *Pectis pedunculata* is in the literature, the type specimen is the same for both this species and *Benthocodon pedunculatus* and upon examination (by the primary author GIM), the type specimen is lacking centripetal canals and belongs in the genus *Benthocodon* and not *Pectis*, therefore *Pectis pedunculata* cannot be considered as valid.

The genus *Crossota* was erected in 1902 by Vanhöffen as a trachymedusa with a distinctive dark red coloration, several rows of tentacles, and 8 pendant gonads hanging adjacent to the stomach. Vanhöffen (1902) described two species (*C. norvegica* and *C. brunnea*) differentiating *C. brunnea* (the type species) from *C. norvegica* by the latter's brighter red coloration. Both Vanhöffen (1902) and Maas (1906) originally grouped *Crossota* with *Ptychogastria* (Allman, 1878) due to the arrangement of the tentacles (despite the fact that *Crossota* lacks



the clusters of tentacles and adhesive pads that are characteristic of *Ptychogastris*). Bigelow (1913) described two new species (*C. alba* and *C. pedunculata*) and noted that the presence of a peduncle in these two new species might be sufficient basis to erect a new genus. *Ptychogastris* has since been placed into the Ptychogastridae, separate from *Crossota* (Rhopalonematidae) and the two genera were shown not to be monophyletic (Grange et al., 2017; Bentlage et al., 2018). *Ptychogastris* can be found in Monterey Bay on both hard and soft sediment with a transparent exumbrella and different colored tentacles making them relatively easy to distinguish from *Benthocodon*, *Pectis*, or *Crossota*.

The third genus of “little red jellies” encountered in the Monterey Submarine Canyon is *Benthocodon*, a genus described by Larson and Harbison (1990) based upon: (1) the presence of a peduncle, and (2) gonads that are continuous along the

upper part of the radial canals with the proximal part of the gonad somewhat detached. Larson and Harbison (1990) erected *Benthocodon* to accommodate a new species, *B. hyalinus* described from a single specimen (the type species, Holotype USNM 87603) and a photograph published by Curtsinger (1986) and incorrectly identified as *Arctapodema* (taken near McMurdo Station Antarctica). Larson and Harbison (1990) also moved *Crossota pedunculata* (Bigelow, 1913) into the genus *Benthocodon* along with their newly described species *B. hyalinus*. Unfortunately, Larson and Harbison (1990) did not match the gender of *Benthocodon* with the species name upon moving *Crossota pedunculata*. To match gender (male), the correct spelling is *Benthocodon pedunculatus* which is how it is referred to in this paper.

At this time, we recognize two species of *Pectis* (*P. profundicola* and *P. tatsunoko*), five species of *Crossota* (*C.*

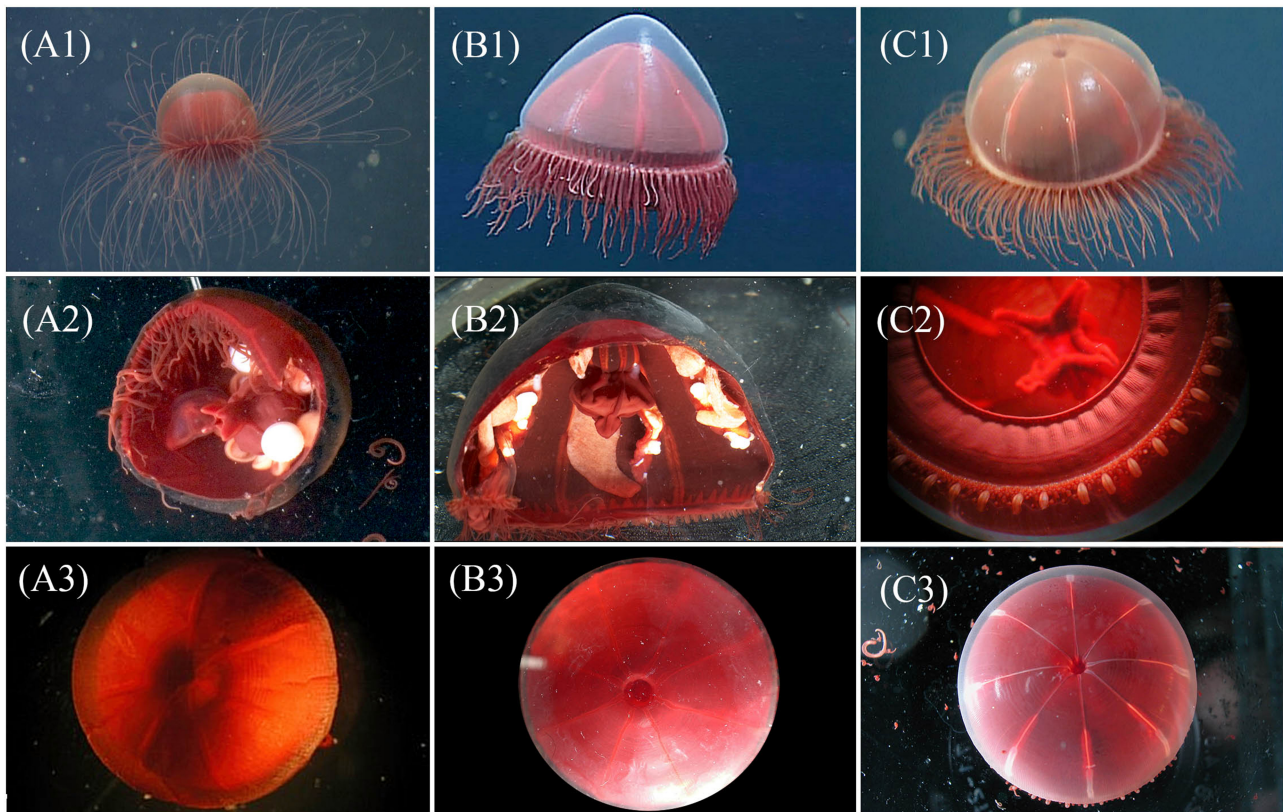


FIGURE 2 | Visual comparison for three genera of "little red jellies" provided for assistance with identification of genera, each image is a unique specimen: *Crossota* (A1-3), *Pectis* (B1-3), and *Benthocodon* (C1-3). Top row, left to right: *in situ* images for *C. rufobrunnea* (A1—DR457 @ 968 m in Monterey Bay), *P. profundicola* (B1—T622 @ 777 m in Monterey Bay), and *B. pedunculatus* (C1—T1113 @ 3,483 m in Monterey Bay, California) taken by MBARI ROVs. Of the three genera, only *Pectis* (B1) has centripetal canals and this individual has 8 in each quadrant, irregular in size and just above the canal ring. Row 2 (A2,B2,C2) provides closeup and partial dissections taken in lab for different individuals of *C. rufobrunnea* (A2—T1023SS1 @ 955 m in Astoria Canyon, Oregon), *P. profundicola* (B2—T1023SS2 @ 1,117 m in Astoria Canyon, Oregon), and *B. pedunculatus* (C2—T623b @ 3,607 m in Monterey Bay, California). Note the pendant gonads and absent peduncle of *Crossota* (A2), and the semi-pendant gonads and short peduncle for *Pectis* (B2). *Benthocodon* (C2) shows the folded, per-radial lips, tentacular arrangement, and well-developed velum. See **Figure 4** for a better view of the peduncle for which *Benthocodon pedunculatus* is named. Row 3 (A3,B3,C3) provides a dorsal view of the subumbrella region. The subumbrella for *C. rufobrunnea* (A3—T766 @ 1,375 m in Monterey Bay, California), unlike both *P. profundicola* (B3—T834SS9 @ 1,384 m in Monterey Bay, California), and *B. pedunculatus* (C3—T835SS5 @ 3,285 m in Monterey Bay, California), appears closed and does not allow a view through clear mesoglea into the peduncle.

alba, *C. norvegica*, *C. millsae*, *C. brunnea*, and *C. rufobrunnea*), and two *Benthocodon* species (*B. pedunculatus* and *B. hyalinus*) from the west coast of North America and in the Monterey Submarine Canyon in particular. We did not collect enough specimens of either *P. tatsunoko* or *B. hyalinus* to include them in the molecular analysis. Two specimens not found in Monterey—*Crossota norvegica* and a potential new *Crossota* species collected in the Gulf of California—are included in the phylogeny but *Pectis antarctica* is not included as we have not had access to specimens. All of the species in this paper can be identified using morphological characters taken from personal observations and existing descriptions. It is possible that further taxonomic work may result in either splitting up the different species of *Crossota* or combining genera (*Pectis*, *Crossota*, *Benthocodon*, and a fourth genus of a small black jelly-*Vampyrocrossota*) into a single genus. At this point, we do not have enough information to justify either approach.

Consistent identifications will be necessary to interpret the ecological data collected alongside deep-sea jellyfish specimens (cf. Lindsay et al., 2017).

At the generic level, the medusae discussed herein occupy different depth layers of the water column, providing insight into deep-living jelly community structure. Ultimately, accurate taxonomy will give a clearer picture of deep-sea community composition and assembly. For example, gelatinous zooplankton have been shown to be an important component of deep-water food webs as predators and prey (Choy et al., 2017), and clarifying species identities holds potential for further resolving the roles different genera and species play in these food webs (Grange et al., 2017).

The phylogeny presented herein provides further evidence that *Crossota* is a polyphyletic group. The taxonomic key presented reflects our understanding of the current classification status of "little red jellies" and some of their relatives. It

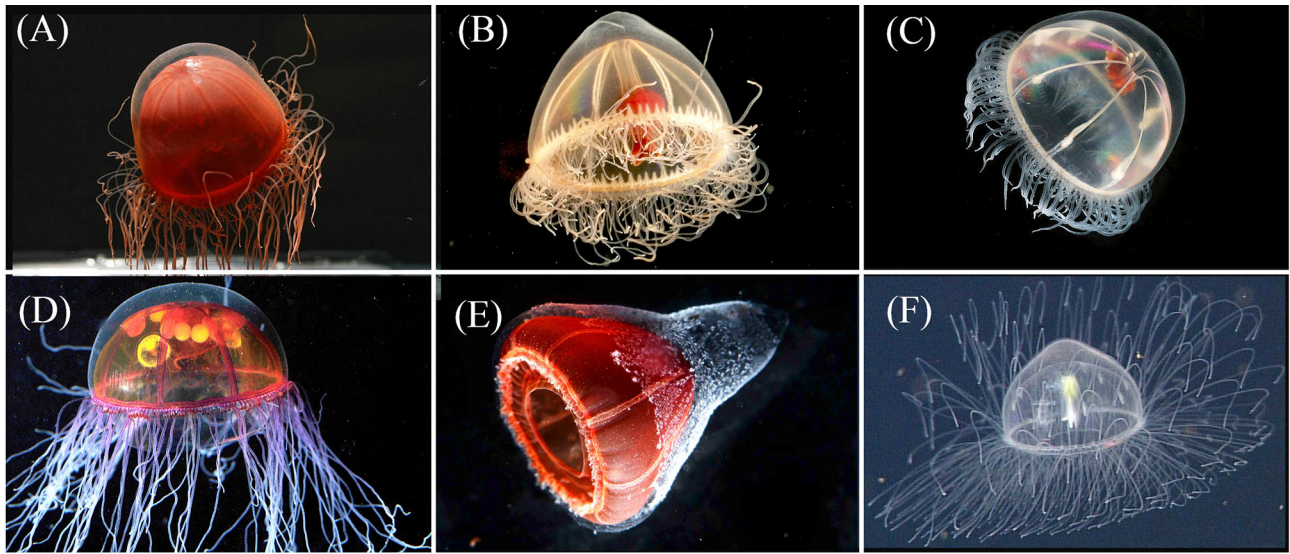


FIGURE 3 | Row 1: The exumbrella of *Crossota norvegica* [(A)—photo by K. Raskoff from 1,595 m in the Chukchi Basin, Arctic] appears more transparent than its congeners. *Pectis tatsunoko* [(B)—T1024SS11 from 1,028 m in Astoria Canyon, Oregon] has eight centripetal canals, often irregular in height along each quadrant and are more hemispherical in shape than *Benthocodon hyalinus* [(C)—photo by K. Raskoff, from 635 m along the Northwind Ridge, Arctic]. Note the presence of a mostly clear peduncle and red lips in both *P. tatsunoko* and *B. hyalinus*. Row 2 provides images of two additional species of *Crossota* that we have observed and were included in the molecular analysis: *Crossota millsae* [(D)—DR88 @ 2,115 m in Monterey Bay], *Crossota* n. sp. [(E)—DR726 @ 2,292 m in Mazatlan Basin, Gulf of California], and one that was not included in the molecular analysis *Crossota alba* [(F)—DR723 @ 2,600 m in Farallon Basin, Gulf of California]. Specimens (A) and (C) were collected in the Arctic *(Raskoff et al., 2010).

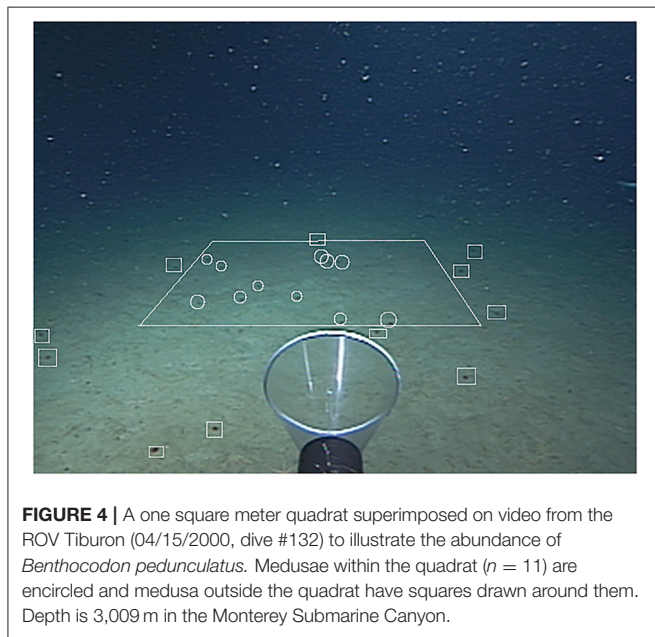


FIGURE 4 | A one square meter quadrat superimposed on video from the ROV Tiburon (04/15/2000, dive #132) to illustrate the abundance of *Benthocodon pedunculatus*. Medusae within the quadrat ($n = 11$) are encircled and medusa outside the quadrat have squares drawn around them. Depth is 3,009 m in the Monterey Submarine Canyon.

provides an operational framework for identifying these taxa until further taxonomic revisions may provide additional characters and/or a reinterpretation of known characters that align taxonomy with phylogeny in Trachymedusae. Coloration and exumbrellar furrows have previously been used as diagnostic characteristics for species identification of *Crossota*, *Benthocodon*, and *Pectis* but those features need to

be validated in the field and post-collection via photographs as both are frequently lost upon collection (Bigelow, 1913; Galea et al., 2016) and coloration is often lost or reduced upon collection (Figure 4) and/or preservation. *Crossota* specimens sometimes retain the deep red color of their subumbrella upon fixation while both *Pectis* and *Benthocodon* specimens often become more faintly colored, the latter to the point of transparency. Consequently, we omitted these coloration and exumbrellar furrows from our key to facilitate the identification of fixed specimens. Both *Pectis* and *Benthocodon* have abscission points at the base of the tentacles, *Crossota* does not.

Key* to the Three Genera (Figure 2)

- 1a. With distinct centripetal canals (Figures 2B1,B2)... *Pectis*
- 1b. Without distinct centripetal canals (Figures 2A1,C1)... 2
- 2a. With subumbrella appearing to have a distinct 'hole' that leads down to peduncle (Figure 2C3)... *Benthocodon*
- 2b. With subumbrella lacking a 'hole-like' invagination at the apex (Figures 2A3,B3)... *Crossota*

Generic Diagnosis

Genus *Pectis* (Haeckel, 1879)

Pectis (Haeckel, 1879): 266.

Voragonema (Naumov, 1971): 13

Rhopalonematidae with a thick, hemispherical bell. Eight pendant gonads, medusa buds may be present, centripetal canals, and a gastric peduncle. Tentacles in multiple rows

and while capable of autotomization, many tentacles remain after collection.

Valid Species

- *Pectis antarctica* (Haeckel, 1879) [Galea et al. (2016) considers *Voragonema laciniata* (Bouillon et al., 2001) as a junior synonym]—type species, 11–13 triangular centripetal canals but usually 12; 1,000–1,200 tentacles arranged in 5–6 rows; gonads wide, folded, thick-walled and slightly pendant distally (Haeckel, 1881, p. 5; **Figure 2**); does have a peduncle—Not included in this study as we have no specimens and have not observed this species in Monterey Bay.
- *Pectis profundicola* (Naumov, 1971). Original description based on trawled specimen from 6,800 to 8,700 m; Eight conical centripetal canals; ~500 tentacles; gonads unknown; does have a short peduncle; brown manubrium; and clear umbrella. Our specimens differ from the original described species by the latter having a clear subumbrella. We believe that the transparent exumbrella from the original description is likely a collection artifact as some of the preserved specimens that we have collected have lost their pigmentation and *in situ* images reveal that they did have pigmentation prior to collection (**Figures 3, 5**). Otherwise, our specimens match the incomplete description provided by Naumov (1971) although we have found specimens with more tentacles (1000–2000) and with irregular centripetal canals as well as specimens with seven, eight, and nine centripetal canals.
- *Pectis tatsunoko* (Lindsay and Pagès, 2010). Nine triangular centripetal canals originating from the ring canal; ~1,050 tentacles; 6–7 rows of tentacles, rose-orange-colored subumbrella. The coloration of the umbrella and tentacles is not maintained upon preservation. This species was not included in our molecular analysis as we did not have access to enough specimens.

Species Key for *Pectis*

- 1a. Eight centripetal canals present *P. profundicola*
- 1b. More than eight centripetal canals present..... 2
- 2a. More than ten rounded centripetal canals.....
..... *P. antarctica*
- 2b. Less than 10 pointed centripetal canals.....
..... *P. tatsunoko*

Genus Crossota (Vanhöffen, 1902)

Crossota (Vanhöffen, 1902): 72.

Rhopalonematidae with a hemispherical bell. Eight tubular or pendant gonads, medusa buds may be present. No centripetal canals. Tentacles in single or multiple rows and while capable of autonomization, many tentacles remain after collection.

Valid Species

- *Crossota brunnea* (Vanhöffen, 1902) Type species, multiple rows of tentacles (up to 84 tentacles/octant observed); gonads hanging from radial canals next to manubrium, still immature in specimens 17 mm in diameter.

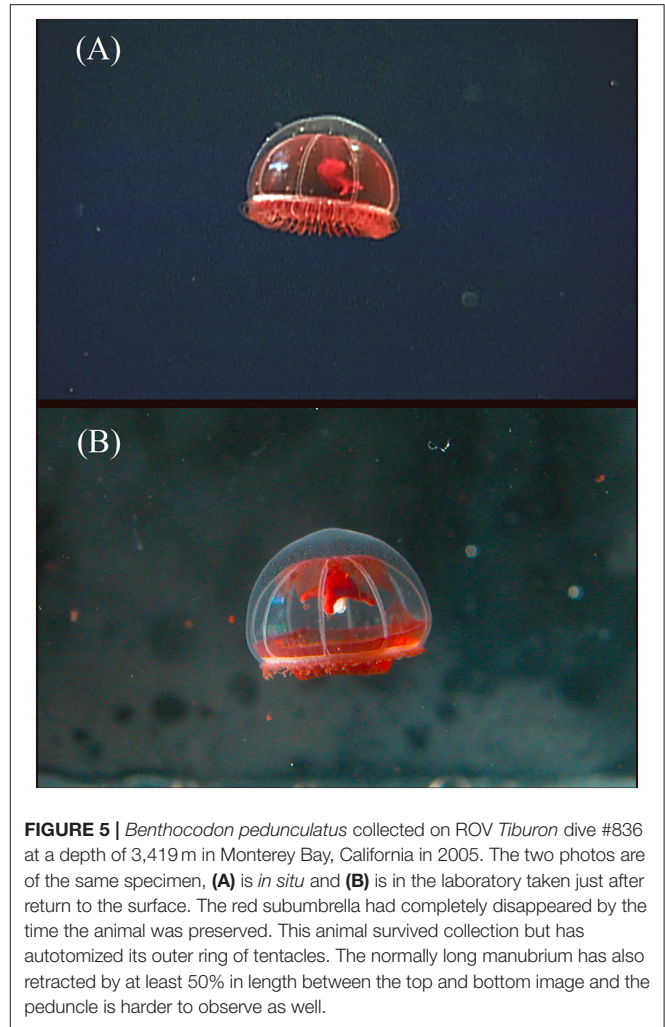


FIGURE 5 | *Benthocodon pedunculatus* collected on ROV *Tiburón* dive #836 at a depth of 3,419 m in Monterey Bay, California in 2005. The two photos are of the same specimen, **(A)** is *in situ* and **(B)** is in the laboratory taken just after return to the surface. The red subumbrella had completely disappeared by the time the animal was preserved. This animal survived collection but has autotomized its outer ring of tentacles. The normally long manubrium has also retracted by at least 50% in length between the top and bottom image and the peduncle is harder to observe as well.

- *Crossota norvegica* (Vanhöffen, 1902) synonymized with *C. brunnea* by Bigelow (1913)—*C. brunnea* var. *norvegica*. Fewer than 600 tentacles (up to 43/octant observed); eight pendant gonads from near the manubrium and as long as the manubrium in specimens 15–20 mm in diameter; umbrella deep reddish-brown.
- *Crossota rufobrunnea* (Kramp, 1913)—medusa buds (Thuesen, personal communication); 250+ tentacles in one row; eight pendant gonads hanging from radial canals next to short peduncle; umbrella reddish brown.
- *Crossota alba* (Bigelow, 1913)—~179 tentacles in three rows; eight sausage shaped hanging gonads midway between apex and ring canal; medusa buds not yet observed; long unpigmented peduncle; manubrium darkly pigmented (chocolate-brown to black); subumbrella unpigmented.
- *Crossota millsae* (Thuesen, 1993)—up to 220 tentacles in one row with clear abscission zones; eight sausage shaped pendant gonads hang from ~1/3 of the way down radial canal from apex; medusa buds sometimes present; no peduncle; bright pink to lavender color to manubrium and gonads; mesoglea clear; umbrella may have burnt-tangerine pigmentation.

Species Key for *Crossota*

- 1a. Gonads midway along radial canal; umbrella colorless.....*C. alba*
- 1b. Gonads located nearer manubrium than ring canal, umbrella pigmented.....2
- 2a. Gonads located approximately halfway between base of manubrium and mid-point of radial canal, umbrella with burnt-tangerine pigment.....*C. millsae*
- 2b. Gonads near base of manubrium, coloration not burnt-tangerine.....3
- 3a. With one row of 200–250 tentacles
.....*C. rufobrunnea* (umbrella deep reddish brown)
- 3b. With multiple rows of tentacles.....4
- 4a. With 600 or more tentacles in several rows; umbrella pale brown, immature at 17 mm diameter.....
.....*C. brunnea*
- 4b. Less than 600 tentacles, umbrella bright red/brown, mature at 18 mm in diameter.....*C. norvegica*

Genus *Benthocodon* (Larson and Harbison, 1990)

Benthocodon (Larson and Harbison, 1990): 22.

Rhopalonematidae with a thick, hemispherical bell. Eight linear to sinuous, flattened, partially pendant gonads occur on the eight radial canals; not known to have medusa buds; centripetal canals absent; manubrium with a well-developed gastric peduncle; tentacles numerous (about 800). Tentacles in multiple rows that tend to autotomize upon collection.

Valid Species

- *Benthocodon pedunculatus* Type species was first described as *Crossota pedunculata* (Bigelow, 1913) (Holotype USNM 31057). It was then reclassified as *B. pedunculata* (Larson and Harbison, 1990). *Benthocodon pedunculatus* lacks centripetal canals; short peduncle; 8 sausage shaped gonads only attached to radial canals for a short distance and mostly hanging distally; 3–4 rows of tentacles ~350 tentacles; reddish brown pigmentation on subumbrella. Has a deeper depth distribution than *Pectis profundicola* (down to at least 4,000 m in Monterey Bay). Dense aggregations often observed.
- *Benthocodon hyalinus* (Larson and Harbison, 1990). Peduncle $\frac{1}{4}$ or more the length of the manubrium; eight linear to sinuous gonads attached along most of the radial canals becoming pendant distally; lacks pigmentation on the subumbrella, and the gonads extend along most of the length of the radial canals, only at their distal ends are the 8 linear gonads pendant; more than 800 tentacles of different sizes but one type. Reaches up to 4 cm in diameter and has been observed in moderately dense aggregations just above the bottom. This species was not included in the

molecular analysis due to lack of sufficient specimens for DNA extraction.

DATA AVAILABILITY STATEMENT

Accession numbers (in bold) in **Table 1** represent sequences generated as part of this study. Specific sequence data can be found in GenBank using the provided accession number.

ETHICS STATEMENT

All specimens were collected under valid US Scientific Collecting Permit guidelines and restrictions.

AUTHOR CONTRIBUTIONS

GM, RS, and BR contributed to the conception and design of the study as well as the collection and identification of specimens. GM, KW, and BB performed the DNA extractions. BB performed the phylogenetic analysis. All authors contributed to the manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2019.00798/full#supplementary-material>

Supplemental Video | Four supplemental videos are provided. *Benthocodon pedunculatus* filmed in the water column on ROV *Tiburón* dive #837; *Benthocodon pedunculatus* filmed just above the seafloor during ROV *Tiburón* dive #894; *Pectis profundicola* filmed in the water column from ROV *Ventana* dive #4176; and *Crossota millsae* filmed in the water column during ROV *Doc Ricketts* dive #1049. Depths and identifications are on each video clip. All clips were filmed in Monterey Bay, California.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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