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Deep-Sea Research I

Morphological, molecular, and in situ behavioral observations of the rare deep-sea anglerfish *Chaunacops coloratus* (Garman, 1899), order Lophiiformes, in the eastern North Pacific

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ABSTRACT

In situ observations and collections of *Chaunacops coloratus* (Garman, 1899) from seamounts in the eastern North Pacific Ocean lend new behavioral, morphological and molecular data to an undersampled, deep-sea group of fishes in the order Lophilformes. Seven observations were made at Davidson Seamount, 130 km southwest of Monterey, CA, and from the Taney Seamount chain, 290 km west of Moss Landing, CA, from depths ranging from 2313 to 3297 m. Specimens were collected at both locations. Morphometric and meristic analyses were performed to identify individuals to the species level. These observations of *C. coloratus* provide greater latitude and depth distributions in the eastern North Pacific Ocean than previously known. Detailed habitat information indicated the fish occurred near manganese-encrusted volcanic talus slopes, a highly rugose habitat. Video observations revealed possible ontogenetic color changes in which small fish were blue and large fish were red. Video recorded rapid, vertical swimming as an escape response and maneuvering, or walking, with pectoral and pelvic fins and esca deployment. Phylogenetic analyses used here verify what has been known since Garman first described *C. coloratus* in 1899, that *Chaunacops* are closely related; molecular tools complement previous knowledge and genetic information created has been submitted to GenBank for further use by the scientific community.

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1. Introduction

The lophiiform family Chaunacidae has two genera, *Chaunacops* and *Chaunax* (Caruso, 1989a,b, 1999; Caruso et al., 2006; Ho and Shao, 2010); with 3 species belonging to the former and 13 to the latter. Chaunacids are characterized as having globose heads, open sensory and lateral line canals, and loose skin covered by small spine-like scales. Color, which has been noted as an important distinguishing characteristic, has generally been described as pink, reddish orange, or rose (Garman, 1899; Caruso, 1989b). Members of the family are considered benthic, living at reported depths from 90 to over 2200 m with nearly global distribution, albeit absent from both poles.

Chaunacops (Chaunax) coloratus (Garman, 1899) was first described from a single specimen collected at the Cocos Ridge at a depth of 1789 m during the *Albatross* expedition of 1891. Initially

assigned to *Chaunax*, Garman (1899) commented that *C. coloratus* was different enough from other members of *Chaunax* to warrant the establishment of the subgenus *Chaunacops*; however, this subgenus was never used in the description of the type species. Caruso (1989a) subsequently erected the genus *Bathychaunax*, using a significant number of additional characters to separate *Bathychaunax* (*Chaunax*) *coloratus* and *Bathychaunax* (*Chaunax*) *roseus* from all other species of *Chaunax*, using *B. coloratus* as the type species for the new genus. Caruso (1989b) then described a third member of *Bathychaunax*, *B. melanostomus*, from the Indian Ocean and re-described *B. coloratus* using 11 additional specimens from the eastern Indian Ocean that were collected at depths between 1250–1733 m.

Upon discovery of Garman's original comment regarding *Chaunacops*, Caruso et al. (2006) formally elevated the genus *Chaunacops* making *Bathychaunax* its junior objective synonym. Thus, three species are currently recognized within the genus including *C. coloratus* (Garman, 1899), *C. roseus* (Barbour, 1941), and *C. melanostomus* (Caruso, 1989b).

Several phylogenetic studies using morphological and/or molecular techniques have been conducted in an effort to understand the

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relationship of fishes within the Lophiiformes and to assess the relationship of the Lophiiformes to other Teleostei (Shedlock et al., 2004; Pietsch and Orr, 2007; Miya et al., 2010; Matschiner et al., 2011). Using mitochondrial genomes, Miya et al. (2010) demonstrated monophyly for the Lophiiformes, with the suborder Lophioidei being basal. Mitochondrial data (Miya et al., 2010) also nullifed any previously hypothesized relations of the Lophiiformes with the Batrachoidiformes, suggesting instead a percomorph affinity and possible sister relationship with the Tetraodontiformes. A close relationship between Lophiiformes and Tetraodontiformes has also been supported by nuclear markers (Li, 2007; Matschiner et al., 2011). To date, no phylogenetic studies have included specimens of *Chaunacops*.

In this paper, we use video observations and two collected specimens to: (1) describe the habitat, in situ behavior, and color of *C. coloratus;* (2) increase the known geographic and depth distribution of this rarely encountered species (e.g., this species is not listed in Miller and Lea (1972), Eschmeyer et al. (1983), Nelson et al. (2004), Love et al. (2005), or Love (2011)); and (3) use mitochondrial and nuclear gene sequences to further assess phylogenetic relationships within the Chaunacoidei.

2. Materials and methods

Seven in situ observations of C. coloratus were made during expeditions to Davidson (2002, one specimen; Fig. 1, DeVogelaere et al., 2005) and Taney Seamounts (2010, six specimens) using the remotely operated vehicles (ROVs) Tiburon (2002) and Doc Ricketts (2010). These ROVs are equipped with studio-quality digital video cameras, digital still cameras, and a variety of sensors (e.g., CTD, O_2) and sampling gear (e.g., manipulator arm, suction sampler). Observations were recorded to digital video tape and later analyzed using the Video Annotation and Reference System (VARS, Schlining and Jacobsen Stout, 2006), a software and database system containing over 4000,000 observations of organisms, geologic features, and equipment deployed during MBARI's 25 years of deep-sea research. Organism color, behavior, and habitat were recorded and merged with physical data collected by the ROV so that position, depth, and water chemistry are known for each observation. Parallel red lasers (640 nm), spaced 29 cm apart, were used to estimate organism size for observations where no physical collection was made.

Two specimens were collected using the ROV suction sampler; one of these was preserved in 10% seawater formalin (2002, MBARI sample T426-A8, CAS 216055) and the other was preserved in 95% ethanol (2010, MBARI sample D177-A32, CAS 232088). A tissue sample was removed and frozen at -80 °C from the formalin-preserved specimen before being fixed. Both specimens were subsequently accessioned into the ichthyology collection at the California Academy of Sciences and their catalog numbers (CAS) are provided above. Morphometrics and meristics were performed on the two preserved specimens using the characters of C. (Bathychaunax) coloratus in Caruso (1989a), where standard length (SL) is traditionally measured and tail length is described as percentages of the following measures with regard to SL: TL1 is the distance between the urogenital papilla and the end of the hypural plate; TL2 is the distance between the posterior end of the soft-dorsal fin base and the hypural plate; TL3 is the distance between the posterior end of the anal fin base and the hypural plate.

2.1. Molecular methods

DNA extraction, PCR, and sequencing followed the methods of Johnson et al. (2008). PCR primers and reaction conditions are described in Table 3. Sequences were then edited with Geneious Pro (v.5.4.2) (Drummond et al., 2010).

2.2. Phylogenetic analyses

Datasets from Pietsch et al. (2009), Roa-Varón and Ortí (2009), Steinke et al. (2009), Miya et al. (2010, which included whole mitochondrial genomes of fishes in the Order Lophiiformes), Matschiner et al. (2011), and Arnold and Pietsch (2012) were downloaded from GenBank into the program Geneious Pro. Additional gene fragments of *Chaunax* spp. were included when available for the various loci. Individual GenBank numbers of exact taxa used are available within supplemental gene tree figures. When existing alignments were not submitted, sequences were aligned using MUSCLE within GENEIOUS PRO and edited by eye. Individual gene fragments were then analyzed separately. Appropriate evolutionary



Fig. 1. Map showing region where video observations and samples were collected: Taney Seamounts (six video observations, one specimen collected) and Davidson Seamount (one video observation, one specimen collected).

models were selected using JMODELTEST (v1.0.1, Guindon and Gascuel, 2003; Posada, 2008). Phylogenies of each gene fragment were first analyzed separately for each locus, then concatenated and partitioned for mitochondrial gene fragments and run in MRBAYES (v3.1.1, Ronquist and Huelsenbeck, 2003). Mitochondrial and nuclear loci were not combined because there were not enough overlapping data between the datasets. Bayesian analyses involved six chains and were run for at least 50 million generations with a printing, sampling frequency, and burn-in period of 1000. Analyses were run five times each and data were visualized using TRACER v1.3 (Rambaut and Drummond, 2003) to determine the appropriate burn-in period and ensure data had reached convergence. Trees were visualized using FIGTREE v.1.0 (Morariu et al., 2008; http://tree.bio.ed.ac.uk/software/figtree/). All nodes with less than 0.95 posterior probability support were represented as basal polytomies.

3. Results

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3.1. Morphometrics and meristics

Morphometric and meristic analyses of collected specimens in this study agreed well with past work but also highlighted differences (Table 1; Garman, 1899; Caruso, 1989b). Pectoral fin rays of our specimens differed in number from those reported by Caruso (1989b) of 11–12, yet agreed with Garman's (1899) original description of the holotype, which had 14 rays. Compared to Caruso (1989b), morphometric analyses revealed that CAS 216055 (T426-A8) TL1 is within the ranges listed for *C. melanostomus*, TL2 is within the ranges listed for *C. roseus*, and TL3 was in the ranges listed for *C. coloratus*; for CAS 232088, (D177-A32) TL1, TL2, and TL3 are all in the ranges listed for *C. melanostomus*.

Table 1	
Morphometrics and merestics for	r two collected specimens of C. coloratus.

Specimen #	T426-A8	D177-A32
Morphometrics		
Total length	172 mm	78 mm
Standard length	140 mm	61 mm
Buccal cavity	Dusky	Pale
Tail Lengths		
TL1	49.2% (69 mm)	45.1% (27.5 mm)
TL2	16.4% (23 mm)	29.5% (18 mm)
TL3	17.8% (25 mm)	27.8% (17mm)
Meristics		
Dorsal rays	11	11
Anal rays	6	5
Pectoral rays	14	14
Caudal rays	8	8
Branched	6	6
Unbranched	2	2

Tabl	e	2

Details of in situ observations of C. coloratus.

3.2. Habitat utilization patterns

In nearly all instances (6 out of 7), *C. coloratus* was observed in areas of relatively high-relief topography composed of manganeseencrusted talus (Clague et al., 2000; Clague et al., 2009) that created many rocky crevices and shallow depressions, which were filled with sediment. Within this habitat, we often observed *C. coloratus* with one pectoral fin in sediment and one upon rock, giving the appearance of being wedged between the two substrates. One specimen was observed in very low-relief habitat that consisted of small manganese nodules lying on top of soft sediment with no discernible slope. Depths of observations (Table 2) averaged 2570 m (range 2313–3297 m, n=7). Oxygen concentration averaged 1.59 ml/L (range 1.477–1.85 ml/L, n=6) and temperature averaged 1.68 °C (ranged 1.61–1.72 °C, n=6). Salinity varied little and averaged 34.64 psu (n=6).

3.3. Color, morphology, and behavioral observations

The observed color of *C. coloratus* varied (Fig. 2), but in general was dominantly blue (n=3) or red (n=4). Blue specimens had an average length of 110 mm (range 78–133 mm, n=3) while red specimens had an average length of 184 mm (range 152–213 mm, n=4). In most specimens the pigmentation of the esca appeared similar or identical to the surrounding tissue of the illicium and illicial cavity. However, in the specimen noted in observation 5 (Fig. 2g), the esca was white, in distinct contrast to the red illicium and illicial cavity.

During 5 of the 7 observations, C. coloratus swam vertically (oriented head up), apparently to avoid the ROV. During observation 2. a specimen was successfully tracked for 232 s by the ROV such that altitude/depth changes per time could be analyzed (Video 1). This specimen ascended from a resting position on the seafloor approximately 3.2 m in a rapid ascent using dorsal, caudal, and anal fins for propulsion and tucking all other fins in, close to the body. This change in altitude was accomplished in 90 s giving an estimated velocity of 0.036 m/s (2.16 m/min) for this initial ascent. After the initial ascent, the fish oriented horizontally at \sim 3.2 m altitude for 86 s, appearing to rest, then proceeded to ascend another \sim 1.2 m. In total, this specimen ascended 4.4 m off the seafloor in 232 s, with an overall estimated velocity, including the 86 s of rest, of 0.021 m/s (1.26 m/min). After 232 s it appeared exhausted, visibly gasping with its mouth, and fully extending its opercula. Observations were suspended when the fish appeared to cease motion and orient horizontally.

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For two other observations, *C. coloratus* moved upon the seafloor by maneuvering or "walking" with its pectoral and pelvic fins. During observation 1, the fish moved forward \sim 8 cm in the following sequence: (1) dorsal fin side-to-side thrusting; (2) caudal fin thrusting; (3) pectoral fin maneuvering; and (4) pelvic fin

Observation#	MBARI Sample	Total length (mm)	Color	Latitude	Longitude	Depth	Light	Oxygen	Salinity	Temperature
1	D0175-02HD	152	Red	36.842170	- 125.600105	2585.59	92.94	1.58	34.64	1.69
2	D0175-03HD	213	Red	36.841730	-125.601524	2572.66	92.96	1.59	34.64	1.69
3	D0175-06HD	120	Blue	36.838940	-125.608250	2358.38	92.94	1.52	34.64	1.71
4	D0177-07HD-A32	78	Blue	36.854200	-125.616220	2313.01	92.87	1.48	34.63	1.72
5	D0178-02HD	199	Red	36.715010	- 125.247310	3297.26	92.79	1.85	34.66	1.61
6	D0177-05HD	133	Blue	36.851948	-125.614105	2407.77	92.83	1.56	34.64	1.69
7	T0426-07-A8	172	Red	35.704327	-122.779980	2458.78	-	-	-	-



Fig. 2. In situ images from observations of *C. coloratus* showing color morphs, behavior, and type of habitat. Note: Observation 1 of a red color morph (Fig. 2(a)); Close-up image from observation 1 showing illicium and esca (highlighted with red circle) retracted (Fig. 2(b)) and in "fishing" position (Fig. 2(c)); Observation 2, in resting, descending position after ascent and that all fins and rays of dorsal, anal, and caudal fins are clearly visible (Fig. 2(d)). Observation 3 shows a blue color morph (Fig. 2(e)). Observation 4 shows the suction sampler in the fore ground (Fig. 2(f)). Observation 5 (Fig. 2(g)) shows clearly the red morph. Observation 6 shows the largest blue color morph (Fig. 2(h)). Finally, observation 7 depicts the red color morph first collected at the Davidson Seamount in 2002 (Fig. 2(i)).

maneuvering (Video 2). After moving forward a bit, it maintained contact with the seafloor and stabilized itself using its pectoral and pelvic fins. Once stable, and while not in motion, this individual perched above the sediment on its small, thoracic pelvic fins and the illicium moved forward and exposed a shaggy, white esca. The esca was deployed in the 'fishing' position for approximately 25 s, then retracted into the illicial cavity (Fig. 2b, c and Video 3). During observation 5, the fish was observed maneuvering or walking forward, albeit slowly, using a combination of pectoral and pelvic fins, while occasionally thrusting forward with the dorsal and caudal fin (Video 4). This specimen also walked backwards, briefly, using pectoral and pelvic fins.

3.4. Molecular analyses

All sequence data were submitted to GenBank (JN235966-81). All phylogenetic analyses showed *C. coloratus* is most closely related to *Chaunax* spp. with full Bayesian support (Fig.3, S1–S8). Mitochondrial analyses on mtDNA fragments (*COI, CytB*, and ND4), and two rRNA

fragments (16S and 12S) included only members of the Lophiiformes because more data were available than for nuclear loci (Figs. 3 and S1-S5). In concatenated and individual fragment analyses a high degree of saturation caused unresolved polytomies among distantly related taxa. Analyses on individual loci were largely incongruent, especially among more distantly related species. Relationships among closely related taxa were better resolved, including those within the Chaunaucidae. Concatenated gene trees revealed the Chaunacoidei (=Chaunacidae) were most closely related to the Ceratioidei. These findings reflected what Miya et al. (2010) showed based on mitochondrial genomes and what Shedlock et al. (2004) showed based only on rRNA 16S sequences. Our analysis on 16S was uninformative because deeper relationships resulted in basal polytomies. Novel results indicated that within the Chaunacidae, Chaunax spp. were most closely related to C. coloratus, which agrees with earlier hypotheses that used morphological characteristics to establish this relationship.

Nuclear analyses included three single copy protein-coding genes (*Tbr-1, Ptr,* and *ENC-1*, Figs. S5–S8) that included representative taxa



Fig. 3. Bayesian phylogenetic analysis of Lophiformes fishes on concatenated mitochondrial gene-fragments including:*COI* (652 bp, GTR+1+G), *ND4* (968 bp, SYM+1), *12S* (420 bp, GTR+1+G), *16S* (432 bp, GTR+1+G), *CytB* (353 bp, TIM2+1+G). Small numbers at the nodes represent Bayesian posterior probability values. Support values below 0.95 represented as basal polytomies. Line drawings reproduced with permission of M. Miya from Miya et al. (2010). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from the Tetraodontiformes, Perciformes, Scorpaeniformes, Ophidiiformes, Polymixiiformes, and the Lophiiformes. Within these analyses, the Lophiiformes were most closely related to the Tetraodontiformes and *Chaunax suttkusi* was most closely related to *C. coloratus*. Unfortunately sequence data for only a few members of the Lophiiformes were available for these loci, therefore severely limiting resolution among members of the group.

4. Discussion

The usefulness of video collected by ROV's in describing new species (Reiswig and Lee, 2007; Williams and Lundsten, 2009;

Mah et al., 2010), species behavior (Luck and Pietsch, 2008; Robison and Reisenbichler, 2008), and whole faunas (Lundsten et al., 2009a,b, 2010) is well documented. The ability of ROV's to record in situ imagery as well as collect voucher specimens for further analysis adds value to biological characterizations of deep-sea organisms and habitats which was simply not possible in the past (e.g., Garman, 1899). However, and particularly when related to fishes, ROV surveys have been shown to alter behavior because of avoidance or attraction (Cailliet et al., 1999; Trenkel et al., 2004; Stein et al., 2005, 2006). The strength of either often varies depending on species or physical parameters; moreover, avoidance by some fast-moving fish species appears to be common among both trawl and submersible surveys. We observed avoidance during five of the encounters reported here. During the other two encounters the individuals showed no obvious response to the ROV.

Morphological and meristic analyses of these two specimens agreed well with the type description of Garman (1899), however, we noted differences from those reported by Caruso (1989b), who suggested that a good deal of morphological similarity exists between the 11 eastern Indian Ocean *C. coloratus*, the single eastern Pacific specimen of *C. coloratus*, and the 8 western Atlantic specimens of *C. roseus*. However, he could neither verify nor nullify the conspecifity of all three populations until more eastern Pacific species were acquired. The differences identified here and in Caruso (1989b) indicate a need to further evaluate whether the eastern Indian and eastern Pacific populations of *C. coloratus* are different populations or species. The relationship among these three populations is ideally suited to further analyses using molecular tools.

While color has been noted as an important character used in identifying chaunacids, ontogenetic color changes may occur. The two smallest specimens were steely-blue (obs. 3 and 4) and the four longest specimens (obs. 1, 2, 5, 7) were red. One specimen (obs. 6), categorized as blue, had a bluish body background with rose fins. That specimen number 6 was longer than the two blue specimens but shorter than all four red specimens is consistent with an ontogenetic color change, most likely taking place between 125-140 mm in length (Table 2). Ontogenetic color changes are described for many organisms, including fishes, for numerous reasons (Booth, 1990). Transparency has been identified as advantageous to midwater organisms, and, most likely, for the planktonic larval chaunacids. A recent study by Zylinski and Johnson (2011) shows that the midwater octopus Japatella heathi actively changes from transparent to red, using chromatophores. when exposed to different light regimes. We hypothesize that the blue and red color morphs observed here are indicative of a transition from an assumed transparent larval form, to juvenile blue, and, finally, to the adult red form. Red would be an advantageous color for an ambush predator that employs a likely bioluminescent lure to attract its prey. Red coloration would conceal the predator, rendering it virtually invisible even if using a glowing lure.

The location and depth of these observations of *C. coloratus* significantly increase the previous known geographic and bathymetric ranges for the species. Our observations ranged from 2313–3297 m whereas previous collections ranged from 1250–1789 m. Additionally, observation 5 represents a depth record for the family Chaunacidae at 3297 m. These observations also

represent a northward geographic range extension for *C. coloratus* in the eastern North Pacific of over 5500 km. That such great species ranges exist in the deep sea is not uncommon (McClain et al., 2009) and cosmopolitan distribution for seamount faunas has been hypothesized (Wilson and Kaufman, 1987) and implied for deep-sea fishes elsewhere (Lundsten, 2009b).

C. coloratus was usually observed motionless on the seafloor and associated with highly rugose, rocky substrate. The unevenness in terrain most likely provides some level of protection with regard to predator avoidance while, at the same time, offers some concealment when lying in wait for prev. The presence of the ROV elicited a vertical, rapid burst of swimming (an escape response: Pietsch and Grobecker, 1987) 5 out of 7 times. Thus, it is likely that it is more common for this species to lie motionless on the seafloor, "fishing" for prey. It is also possible that burst swimming from the seafloor could be used to ambush prey higher up in the water column; this was not observed but cannot be ruled out as a means of prey capture. It appears that C. coloratus can also walk upon the seafloor as has been described for other fishes including antennariids (Pietsch and Grobecker, 1987; Edwards, 1989) and skates and rays (Lucifora and Vessallo, 2002; Macesic and Kajiura, 2010). Lucifera and Vessallo (2002) describe walking in skates as similar to terrestrial vertebrate locomotion and presume that walking may be energetically less expensive than swimming and that water displacement may be minimized, precluding detection by predators or prey. These explanations may be applicable here.

The illicium and esca were deployed, apparently in fishing mode, for 25 s during observation 1 (Fig. 2b, c and Video 3). Once stabilized after briefly maneuvering (see description above), the illicium and esca were tentatively moved forward into what appeared to be a fishing position. The esca appeared shaggy, mop-like, and lighter in color than the darker red skin and illicial cavity of this specimen. After 25 s, the illicium and esca were retracted into the illicial cavity. A rapid current was visible and moved from the tail toward the head.

This family of anglerfishes does not exhibit male parasitism as part of the reproductive modality (Pietsch, 1976, 2005). Thus, use of habitat might be related to locations where these fishes undergo mating. Subsequent recruitment to the adult, deep-sea habitat from the epipelagic and pelagic anglerfish larval distribution is unknown. This is another area in which research needs to be focused on the chaunacids.

Within the family Chaunacidae there are two recognized genera (*Chaunax* and *Chaunacops*) and approximately 15 species. Our results parallel what Miya et al. (2010) and Shedlock et al. (2004) found, where mitochondrial sequence data showed the

Table 3

PCR primers and methods for gene loci in Chaunacops

Locus	Product	Primers	Ref. ^a	PCR methods	Length (bp)			
COI	Cytochrome-c-oxidase subunit-I	COIF/R	Nelson and Fisher (2000)	b	1200			
16S	Mitochondrial 16S	16SAR/BR	Palumbi (1996)	Fast PCR ^c	~ 650			
ND4	NADH dehydrogenase subunit 4	ARGBL/Nap2H	Arevalo (1994)	Fast PCR ^c	~ 800			
12S	Mitochondrial 12 S	12sF/R	Kocher et al. (1989)	Fast PCR ^c	~ 450			
CytB	Cytochrome Oxidase subunit B	424 F/876 R	Boore and Brown (2000)	Fast PCR ^c	\sim 380			
Ptr	Hypothetical protein LOC564097 locus	PtrF458/R1248 PtrF463/R1242	Li et al. (2007)	Fast PCR ^c	\sim 770			
Tbr-1	Nuclear coding T-box brain I	Tbr1F1/R820 Tbr1F86/R811	Li et al. (2007)	Fast PCR ^d	~ 900			
ENC-1	Nuclear coding (similar to) ectodermal-neural cortex I	ENC1F85/R982 ENC1F88/R975	Li et al. (2007)	Fast PCR ^d	~885			

^a References in literature cited.

^b PCR program: 95 °C/10 min; 35 × [94 °C/1 min, 55 °C/1 min; 72 °C/2 min], extension at 72 °C/7 min.

^c Fast PCR: Amplitaq Gold Fast PCR Master Mix, UP (Life Technologies Corp., Carlsbad, CA) and the protocol for the *taq* supplied by manufacturer of Veriti thermal cycler with an annealing temperature at 50 °C (Life Technologies Corp., Carlsbad, CA).

^d Gene fragments were amplified in a nested PCR by using 1ul of product from the initial PCR in the second reaction with the internal primers.

Ceratiodei were most closely related to the Chaunacoidei. The order Lophiiformes radiated in the lower Cretaceous, however the genus *Chaunax* appears to have evolved recently, in the upper Cenozoic (Miya et al., 2010). It is plausible *Chaunacops* spp., the closest known relative of *Chaunax*, also radiated recently. More data using specimens from different localities would answer questions such as whether molecular diversity mirrors morphological diversity. Furthermore, molecular data could confirm if *C. coloratus* is a cosmopolitan species, found worldwide or if there is a species complex among eastern Pacific and Indian Ocean fishes.

Future studies should make use of the material examined here, which has been deposited at the California Academy of Sciences and the National Center for Biotechnology Information, and should attempt to retrieve genetic data from new and existing specimens in an effort to further understand the phylogenetic relationships between the three populations of *C. coloratus* and the three *Chaunacops* species (*C. coloratus, C. melanostomus,* and *C. roseus*).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dsr.2012.05.012.

References

- Arevalo, E., Davis, S.K., Sites, J.W.J., 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Syst. Biol. 43, 387–418.
- Arnold, R.J., Pietsch, T.W., 2012. Evolutionary history of frogfishes (Teleostei: Lophiiformes: Antennariidae): a molecular approach. Mol. Phylogenet. Evol. 62, 117–129, http://dx.doi.org/10.1016/j.ympev.2011.09.012.
- Barbour, T., 1941. Notes on pediculate fishes. Proc. New Eng. Zool. Club 19, 7-14.
- Boore, J.L., Brown, W.M., 2000. Mitochondrial genomes of *Galathealinum, Helob-della*, and *Platynereis*: sequence and gene arrangement comparisons indicate that Pogonophora is not a phylum and Annelida and Arthropoda are not sister taxa. Mol. Biol. Evol. 17, 87–106.
- Booth, C.L., 1990. Evolutionary significance of ontogenetic colour change in animals. Biol. J. Linn. Soc. 40 (2), 125–163.
- Cailliet, G.M., Andrews, A.H., Wakefield, W.W., Moreno, G., Rhodes, K.L., 1999. Fish faunal and habitat analyses using trawls, camera sleds and submersibles in benthic deep-sea habitats off central California. Oceanol. Acta 22, 579–592.

- Caruso, J.H., 1989a. Systematics and distribution of the Atlantic chaunacid anglerfishes (Pisces: Lophiiformes). Copeia 1989 (1), 153–165.
- Caruso, J.H., 1989b. A review of the Indo-Pacific members of the deep-water chaunacid anglerfish genus *Bathychaunax*, with the description of a new species from the eastern Indian Ocean (Pisces: Lophiiformes). Bull. Mar. Sci. 45 (3), 574–579.
- Caruso, J.H. 1999. Chaunacidae, pp. 2020–2022. In: Carpenter, K.E. and V.H. Niem. Species Identification Guide for Fisheries Purpose. The Living Marine Resources of the Western Central Pacific. Batoid Fishes, Chimeras and Bony Fishes, Part 1 (Elopidae to Linophrynidae). Food and Agricultural Organization, Rome.
- Caruso, J.H., Ho, H.C., Pietsch, T.W., 2006. *Chaunacops* Garman, 1899, a senior objective synonym of *Bathychaunax* Caruso, 1989 (Lophiiformes: Chaunacoidei: Chaunacidae). Copeia 2006 (1), 120–121.
- Clague, D.A., Reynolds., J.R., Davis, A.S., 2000. Near-ridge seamount chains in the northeastern Pacific Ocean. J. Geophys. Res. 105 (B7), 541–561 16.
- Clague, D.A., Paduan, J.B., Duncan, R.A., Huard, J.J., Davis, A.S., Castillo, P.R., Lonsdale, P., DeVogelaere, A., 2009. Five million years of compositionally diverse, episodic volcanism: construction of Davidson Seamount atop an abandoned spreading center. Geochem. Geophys. Geosyst. 10, Q12009, http:/ /dx.doi.org/10.1029/2009GC002665.
- DeVogelaere, A., R. Kochevar, M. Tamburi, G. Cailliet, E. Burton, S. Benson, and W. Douros. 2005. Exploring the Davidson Seamount: combining science, public outreach, and resource management. Pages 371–376, In: Magoon, O. T., H. Converse, B. Baird, B. Jines, and M. Miller-Henson (Editors). Proceedings, California and the World Ocean '02. American Society of Civil Engineers, (www.Pubs.Asce.org).
- Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T., and Wilson, A. 2010. Geneious v5.1, Available from http://www.geneious.com.
- Edwards, J.L., 1989. Two perspectives on the evolution of the tetrapod limb. Am. Zool. 29, 235–254.
- Eschmeyer, W.N., Herald, E.S., Hammann, H., 1983. A Field Guide to Pacific Coast Fishes of North America. Houghton Mifflin Co., Boston 336 p.
- Garman, S. 1899. Reports on an Exploration Off the West Coasts of Mexico, Central and South America, and off the Galapagos Islands, in charge of Alexander Agassiz, by the U.S. Fish Commission steamer "Albatross," during 1891, Lieut. Commander Z. L. Tanner, U.S.N., Commanding. XXVI. The Fishes. Memoirs of the Museum of Comparative Zoology 24.
- Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.
- Ho, H., Shao, K., 2010. A new species of *Chaunax* (Lophiiformes: Chaunacidae) from the western South Pacific, with comment on *C. latipunctatus*. Zootaxa 2445, 53–61.
- Johnson, S.B., Warén, A., Vrijenhoek, R.C., 2008. DNA barcoding of Lepetodrilus limpets reveals cryptic species. J. Shellfish Res. 27, 43–51.
- Kocher, T., Thomas, W., Meyer, A., Edwards, S., Paabo, S., Villablanca, F., Wilson, A., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Nat. Acad. Sci. U. S. A. 86, 6196–6200.
- Li, L.-C., 2007. Methods Mol. Biol. 402, 370–383, http://dx.doi.org/10.1007/978-1-59745-528-2_19, II, E.
- Love, M.S., 2011. Certainly More Than You Want to Know About the Fishes of the Pacific Coast—A Postmodern Experience. Really Big Press, Santa Barbara, California 649 pages.
- Love, M.S., C.W. Mecklenburg, T.A. Mecklenburg, and L.K. Thursteinson. 2005. Resource Inventory of Marine and Estuarine Fishes of the West Coast and Alaska: A Checklist of North Pacific and Arctic Ocean Species from Baja California to the Alaska-Yukon Border. U.S. Department of the Interior, U.S. Geological Survey, Biological Resources Division, Seattle, Washington, 98104, OCS Study MMS 2005-030 and USGS/NBII 3005-001 (<http://wfrc.usgs.gov>; <http://www.id.ucsb.edu/lovelab>).
- Lucifora, L., Vassallo, A., 2002. Walking in skates (Chondrichthyes, Rajidae): anatomy, behaviour and analogies to tetrapod locomotion. Biol. J. Linn. Soc. 77, 35–41.
- Luck, D.G., Pietsch, T.W., 2008. In-situ observations of a deep-sea ceratioid anglerfish of the genus Oneirodes Lütken (Lophiiformes: Oneirodidae). Copeia 2008 (2), 446–451.
- Lundsten, L., Barry, J.P., Cailliet, G.M., Clague, D.A., DeVogelaere, A.P., Geller, J.B., J.B., 2009a. Benthic invertebrate communities on three seamounts off Southern and Central California, USA. Mar. Ecol. Prog. Ser. 374, 23–32.
- Lundsten, L., McClain, C.R., Barry, J.P., Cailliet, G.M., Clague, D.A., De Vogelaere, A.P., 2009b. Ichthyofauna on three seamounts off southern and central California, USA. Mar. Ecol. Prog. Ser. 389, 223–232.
- Lundsten, L., Schlining, K.L., Frasier, K., Johnson, S.B., Kuhnz, L.A., Harvey, J.B.J., Clague, G., Vrijenhoek, R.C., 2010. Time-series analysis of six whale fall communities in Monterey Bay, California, USA. Deep Sea Res. Part I 57, 1573–1584.
- Nelson, K., Fisher, C., 2000. Absence of cospeciation in deep-sea vestimentiferan tube worms and their bacterial endosymbionts. Symbiosis 28, 1–15.
- Macesic, L.J., Kajiura, S.M., 2010. Comparative punting kinematics and pelvic fin musculature of benthic batoids. J. Morphol. 271, 1219–1228.
- Mah, C., Nizinski, M., Lundsten, L., 2010. Phylogenetic revision of the hippasterinae (Goniasteridae; Asteroidea): systematics of deep sea corallivores, including one new genus and three new species. Zoolog. J. Linn. Soc. 160, 266–301.

- Matschiner, M., Hanel, R., Salzburger, W., 2011. On the origin and trigger of the notothenioid adaptive radiation. PLoS ONE 6 (4), e18911, http://dx.doi.org/ 10.1371/journal.pone.0018911.
- Morariu, V.I., Srinivasan, B.V., Raykar, V.C., Duraiswami, R., Davis, L.S., 2008. Automatic online tuning for fast Gaussian summation. Adv. Neural Inf. Proces. Syst. 21, 1113–1120.
- McClain, C.R., Lundsten, L., Ream, M., Barry, J.P., DeVogelaere, A.P., 2009. Endemicity, biogeograhy, composition, and community structure on a Northeast Pacific Seamount. PloS ONE 4 (1), e4141, http://dx.doi.org/10.1371/journal.pone.0004141.
- Miller, D.J., Lea, R.N., 1972. Guide to the coastal marine fishes of California. Calif. Dept. Fish Game Fish Bull. 157 249 p.
- Miya, M., Pietsch, T., Orr, J., Arnold, R., Satoh, T., Shedlock, A., Ho, H., Shimazaki, M., Yabe, M., Nishida, M., 2010. Evolutionary history of anglerfishes (Teleostei: Lophiiformes): a mitogenomic perspective. BMC Evolut. Biol. 10 (1). http://dx. doi.org/10.1186/1471-2148-10-58.
- Nelson, J.S., E.J. Crossman, H. Espinosa-Pérez, L.T. Findley, C.R. Gilbert, R.N. Lea, and J.D. Williams (Editors). 2004. Common and Scientific Names of Fishes from the United States Canada and Mexico, Sixth edition. American Fisheries Society, Special Publication 29, 386 pages.
- Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer and Associates Inc., Sunderland, Massachusetts, pp. 205–247.
- Pietsch, T.W., 1976. Dimorphism, parasitism, and sex: reproductive strategies among deep-sea ceratioid anglerfishes. Copeia 1976 (4), 781–793.
- Pietsch, T.W., 2005. Dimorphism, parasitism, and sec revisited: modes of reproduction among deep-sea ceratioid anglerfishes (Teleostei: Lophiiformes). Ichthyol. Res. 52, 207–236.
- Pietsch, T.W., Grobecker, D.B., 1987. Frogfishes of the World: Systematics, Zoogeography, and Behavioral Ecology. Stanford University Press, Stanford, xxii+420 pages.
- Pietsch, T., Orr, J., 2007. Phylogenetic relationships of deep-sea anglerfishes of the suborder Ceratioidei (Teleostei: Lophiiformes) based on morphology. Copeia 2007 (1), 1–34.
- Pietsch, T.W., Arnold, R.J., Hall, D.J., 2009. A bizarre new species of frogfish of the genus Histiophryne (Lophiiformes: Antennariidae) from Ambon and Bali, Indonesia. Copeia 2009 (1), 37–45.
- Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253-1256.
- Rambaut. A., and Drummond A.J. 2003. Tracer, version 1.3, University of Oxford, Rambaut Research Group. Available at <http://tree.bio.ed.ac.uk/software/tra cer/>.

- Reiswig, H.M., Lee, W.L., 2007. A new species of *Cladorhiza* (Porifera: Cladorhizidae) from S. California (USA). pp. 517–523. In: Custódio, M.R., Lôbo-Hajdu, G., Hajdu, E., Muricy, G. (Eds.), Porifera Research: Biodiversity, Innovation and Sustainability. Museu Nacional, Rio de Janeiro, Brazil, pp. 694 2007.
- Roa-Varón, A., Ortí, G., 2009. Phylogenetic relationships among families of Gadiformes (Teleostei, Paracanthopterygii) based on nuclear and mitochondrial data. Mol. Phylogenet. Evol. 52, 688–704, http://dx.doi.org/10.1016/ j.ympev.2009.03.020, ISSN 1055-7903.
- Robison, B.H., Reisenbichler, K.R., 2008. Macropinna microstoma and the paradox of its tubular eyes. Copeia 2008 (4), 780–784.
- Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
- Schlining, B., Jacobsen Stout, N. 2006. MBARI's video annotation and reference system. In: Proceedings of the Marine Technology Society/Institute of Electrical and Electronics Engineers Oceans Conference, Boston, MA, pp. 1–5.
- Shedlock, A., Pietsch, T., Haygood, M., Bentzen, P., Hasegawa, M., 2004. Molecular systematics and life history evolution of anglerfishes (Teleostei: Lophiiformes): evidence from mitochondrial DNA. Steenstrupia 28, 129–144.
- Stein, D.L., Felley, J.D., Vecchione, M., 2005. ROV observations of benthic fishes in the Northwind and Canada Basins. Arctic Ocean. Pol. Biol. 28, 232-237.
- Stein, D.L., Drazen, J.C., Schlining, K.L., Barry, J.P., Kuhnz, L., 2006. Snailfishes of the central California coast: video, photo-graphic and morphological observations. J. Fish Biol. 69, 970–986.
- Steinke, D., Zemlak, T.S., Hebert, P.D.N., 2009. Barcoding nemo: DNA-based identifications for the ornamental fish trade. PLoS ONE 4 (7), e6300 http://dx.doi.org/10.1371/journal.pone.0006300.
- Trenkel, V.M., Lorance, P., Mahévas, S., 2004. Do visual transects provide true population density estimates for deepwater fish? ICES J. Mar. Sci. 61, 1050–1056.
- Williams, G.C., Lundsten, L., 2009. The nephtheid soft coral genus *Gersemia* (Marenzeller, 1878) with the description of a new species from the northeast Pacific and a review of two additional species (Octocorallia: Alcyonacea). Zoologische Mededelingen. Leiden. 83 (34), 1067–1081.
- Wilson, R.R., Kaufmann, R.S., 1987. Seamount biota and biogeography. In: Keating, B.H., et al. (Eds.), Seamounts, Islands, and Atolls. American Geophysical Union, Washington, pp. 227–237.
- Zylinski, S., Johnsen, S., 2011. Mesopelagic Cephalopods switch between transparency and pigmentation to optimize camouflage in the deep. Curr. Biol. http://dxdoi.org/10.1016/j.cub.2011.10.014.