**Abstract.**—Five rockfish juveniles (15.0-30.4 mm SL) collected in midwater trawls from offshore banks off the coast of California were identified by using a combination of morphological and molecular characters. All had pigment patterns consistent with members of the subgenus Sebastomus, but each required the use of molecular markers for species identification. Using DNA sequence data from the mitochondrial cytochrome b, we identified the juveniles as a transforming larva of Sebastes constellatus and a transforming larva and three pelagic juveniles of S. ensifer. We provide detailed descriptions of the specimens and compare our results with the developmental stages of other species of Sebastes of the subgenus Sebastomus. We found some differences in structure and pigmentation that might allow identification of these young stages by traditional means, but more descriptive work is necessary. The use of molecular tools can thus be successfully used to complement traditional identification efforts to solve problems unassailable by morphological and pigment characters alone.

# Molecular identification and description of pelagic young of the rockfishes Sebastes constellatus and Sebastes ensifer

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Live-bearing rockfishes of the genus Sebastes constitute the largest genus of scorpaeniform fishes with about 110 species worldwide, 72 of which reside in the Northeast Pacific (Eschmeyer and Herald, 1983; Kendall, 1991; Nelson, 1994). In this geographic region, rockfishes are very important in the bottom trawl fishery as well as in recreational fisheries (Lenarz, 1986; Leet et al., 1992; Low, 1993). Newborn rockfishes are extruded as first-feeding larvae from viviparous females (Yoklavich and Boehlert, 1991), and they rank among the most frequent and abundant of all fish larvae in plankton collections off the coasts of California and Oregon (Moser et al., 1993; Moser, 1996; Doyle<sup>1</sup>). The juvenile stages of Sebastes are also important ecologically as prey of larger fishes and birds (Love et al., 1991; Moser and Boehlert, 1991; Ainley et al., 1993). In addition to their biological significance, rockfish larvae and early juveniles have received much attention for their potential use in estimating spawning biomass and recruitment (Moser and Butler, 1987; Hunter and Lo, 1993; Ralston and Howard, 1995).

Species identification has been the most challenging aspect in the study of the early life history stages of Sebastes. For instance, of the 59 species distributed in the California Cooperative Oceanic Fisheries Investigations (CalCOFI) region, complete developmental series are available for only thirteen and partial series are available for an additional eight (Moser, 1996). Several factors have influenced this lack of a complete developmental series, including a very large number of sympatric species, the preponderance of small larvae, and the limited number of taxonomic characters identifiable in the early stages (Sakuma and Laidig, 1995; Moser, 1996; Moser et al., 1977). Alternative methods involving the analysis of electrophoretic patterns or DNA

<sup>&</sup>lt;sup>1</sup> Doyle, M. 1992. Patterns in distribution and abundance of ichthyoplankton off Washington, Oregon, and northern California (1980-1987). U.S. Dep. Commer., NOAANMFS Alaska Fish. Sci. Center Process Report 92-14, 344 p.

have been proposed to overcome this obstacle (e.g. Seeb and Kendall, 1991; Rocha-Olivares, 1998b). In our paper we describe new developmental stages of two species of *Sebastes* identified using mitochondrial DNA (mtDNA) sequence data.

# Materials and methods

### Sample collection

Specimens were collected in the vicinity of Tanner (32°69'N119°12'W) and Cortes (32°61'N119°33'W) Banks in August 1995 during a rockfish sampling cruise aboard the Scripps Institution of Oceanography RV Robert Gordon Sproul. Four specimens were sampled in oblique tows with a 5-m<sup>2</sup> Isaacs-Kidd midwater trawl. The fifth specimen was retrieved, intact, from the digestive tract of an adult greenspotted rockfish, Sebastes chlorostictus, caught with hook and line. Fish were preserved in 95% ethanol. Except for one specimen that completely dried out upon evaporation of the preserving fluid, the shrinkage effect of ethanol preservation on the length of the specimens has been assumed to be negligible owing to their relatively large size (15.0-30.4 mm SL, Radtke, 1989). The dehydrated specimen was rehydrated in water before description.

### Molecular analyses

Total genomic DNA was extracted and purified from liver or muscle tissue with a GlasPac/GS (U.S. National Scientific Supply CO., San Rafael, CA) DNA purification kit as described in Rocha-Olivares (1998b). Universal primers, and versions customized for Sebastes, were used for the polymerase chain reaction (PCR) and automated cycle sequencing (see Rocha-Olivares, 1998a for a complete list of primers). A region spanning 781 base pairs (bp) of the mitochondrial cytochrome b (cyt-b) was amplified by PCR as described in Rocha-Olivares et al. (1999a). Briefly, 50-mL reactions were performed following Kocher et al. (1989), with 100 ng of genomic DNA and 2 units of Taq DNA polymerase (Perkin Elmer Cetus, Foster City, CA, or Gibco BRL, Rockville, MD). Thermal cycling was performed as follows: hot start at 90°C for 2 min., followed by 36 cycles of 50 s at 94°C; 2 min at 51°C; 1.5 min at 72°C, and a final extension of 3 min at 72°C to ensure complete amplification of products. PCR products were purified with microconcentrators (Microcon® 100, Millipore, Bedford, MA) or purification columns (QIAquick® 250, Qiagen, Valencia, CA) following manufacturers' protocols. Automated DNA sequencing was performed with ABI PRISM (Perkin Elmer Cetus) DyeDeoxy® dRhodamine chemistry on an ABI 377 DNA sequencer in 12 µL reactions (30–100 ng double stranded PCR product, 3 pmol primer, 1.6–2.0 µL terminator ready reaction mix); cycle sequencing annealing was 10 seconds at 55°C; we followed the manufacturer's protocol in respect to all other experimental conditions. Sequence data were obtained by sequencing both DNA strands of the PCR products.

# Molecular identification

The mtDNA data from the unknown pelagic young was aligned with a database of orthologous sequences obtained from adult specimens of congeneric species generated at the genetics and physiology laboratory of the Southwest Fisheries Science Center in La Jolla (Rocha-Olivares, 1998a).<sup>2</sup> Because the morphological and pigmentation patterns of the specimens revealed that they belonged to the subgenus Sebastomus (Chen, 1971; 1975), the DNA sequence data were compared to 89 sequences obtained from all 15 species of this subgenus (Rocha-Olivares et al., 1999a; 1999c). Species identification was determined on the basis of the most similar haplotype among the species compared. Sequence comparisons were carried out by using pair-wise measures of sequence divergence calculated as the total number of different nucleotides.

# Results

## Molecular identification

The mtDNA sequence data confirmed morphological observations that the five pelagic young were members of the subgenus *Sebastomus*. The number of conspecific DNA sequences used in the reference database ranged from 1 to 13 (S. notius, S. lentiginosus, n=1; S. spinorbis, n=3; S. capensis, n=4; S. oculatus, S. exsul, S. helvomaculatus, n=5; S. rosaceus, n=6; S. chlorostictus, S. ensifer n=7; S. umbrosus, S. constellatus, S. simulator, S. eos, n=8; S. rosenblatti, n=13). Two juveniles (CA3 and CA4) had identical mtDNA cyt-*b* sequences. On the basis of the number of nucleotide differences, one specimen (CA1, Fig. 1) was identified as starry rockfish, S. constellatus, and the other four as swordspine rockfish, S. ensifer (CA2-CA5, Fig. 1). The mitochondrial haplotypes of three specimens (CA1, CA3, and CA4) were identical to adult reference sequences, providing unequivocal

<sup>&</sup>lt;sup>2</sup> Rocha-Olivares, A., and R. D. Vetter. 1998. Unpubl. data.



evidence of species identity. The others differed from reference data by 1 to 3 nucleotides (Fig. 1).

Cluster analyses (unweighted pair-group method using averages [UPGMA]) performed on the pairwise distance matrices generated from the molecular data clustered CA1 with *S. constellatus* and CA2-CA5 with *S. ensifer* (not shown).

## **Description of specimens**

# Sebastes constellatus (Jordan and Gilbert 1880) Transforming larva: CA1 (15.0 mm SL), Figure 2A

**Literature** Transforming larvae and pelagic juveniles of *S. constellatus* have not been described in the published literature.<sup>3</sup> Larval stages up to notochord flexion (~7.1 mm) show features typical of preflexion larvae of other *Sebastomus* species: robust head and body with strong serrated parietal ridges and spines; strong postocular ridge and spine; heavy pigment on top of head, on jaws, and on paired fins, with heaviest pigment on distal edge of pectoral fin; postanal ventral midline series of 11–17 melanophores (Moser et al., 1977; Moser, 1996).

**General morphology** Body moderately deep and compressed; head, jaws, and eyes large (Table 1).

**Fins and other meristic features** Full complements of spinous and soft rays present; dorsal- and analfin rays moderate in length; anteriormost 5 scales of lateral line just beginning to form but visible only after staining with alizarin red-S (Table 2).

**Spination** Supraocular crest and associated spines (preocular, supraocular, postocular) prominent; parietal crests well developed, weakly serrate parietal spine slightly longer than nuchal spine; tympanic spine not yet formed on the upper margin of sensory canal pore but a slight prominence, indicating initial spine development, is noticeable; posterior preopercular series well developed with spine at angle longer than others and finely serrated along the longitudinal flange (dorsal and ventral margins of spine smooth); first and third spines weakly developed in anterior preopercular series; first and second lower infraorbitals present; first and fourth spines in upper series visible only after staining; presence of other spines as listed in Table 3.

<sup>&</sup>lt;sup>3</sup> A fully pigmented "pelagic juvenile" (length not given) ascribed to *S. constellatus* is illustrated and briefly described *in* Laroche, W. A. 1987. Guide to the larval and juvenile rockfishes (*Sebastes*) of North America. Unpublished manuscript. Stonefish Environm. & Taxon. Serv., Enosburg Falls, VT 05450.

Morphometric characters of pelagic yo	ung of three Sebastes (s	T subgenus Sebastom	<b>able 1</b> <i>us</i> ) species. Average values (j	n percent) are given for each p	roportion, followed by the
standard deviation and the range. Prof. length.	oortions for S. <i>helvomacu</i>	<i>ulatus</i> derived from	data in Kichardson and Laroc	.he (1979). Abbreviations: HL= F	1ead length; SL= standard
		Transforming speci	mens	Pelagic juv	/eniles
	S. constellatus n = 1	S. ensifer $n = 1$	S. helvomaculatus n = 5	S. ensifer $n = 3$	S. helvomaculatus n = 8
Proportions	15.0  mm SL	19.8 mm SL	17.8–18.6 mm SL	27.3–30.4 mm SL	19.8-41.6  mm SL
Body depth at pectoral fin base/SL	33.3	33.3	$31.6 \pm 0.50 (31.0 - 32.1)$	$32.9 \pm 1.23 (31.5 - 33.9)$	$31.2 \pm 1.57 (28.4 - 32.9)$
Body depth at anus/SL	26.0	26.8	$23.8 \pm 0.86 \ (23.1 - 25.3)$	$27.1 \pm 1.15 (26.0 - 28.3)$	$23.2 \pm 1.24 (21.2 - 25.0)$
Snout to anus length/SL	64.0	63.1	$61.4 \pm 2.12 (59.8 - 64.5)$	$63.6 \pm 2.12 (61.2 - 65.1)$	$62.8 \pm 2.44 \ (59.8 - 66.0)$
Snout to pelvic fin origin/SL	41.3	39.9	$41.7 \pm 4.15 (38.0 - 47.3)$	$40.3 \pm 1.85 (38.5 - 42.2)$	$42.8 \pm 3.17 (39.3 - 48.2)$
Head length/SL	37.3	39.9	$40.3 \pm 2.62 (36.4 - 43.0)$	$32.5 \pm 0.52 (31.9 - 32.9)$	$40.1 \pm 1.48  (37.5 - 41.9)$
Eye diameter/HL	39.3	38.0	$34.4 \pm 2.73 (32.0 - 37.5)$	$36.8 \pm 0.79 (36.0 - 37.5)$	$33.6 \pm 1.76 (30.9 - 36.9)$
Upper jaw length/HL	50.0	46.8	$43.4 \pm 2.80 \ (39.7 - 47.2)$	$51.8 \pm 2.29 (49.4 - 54.0)$	$45.2 \pm 2.63 (40.2 - 47.6)$
Snout length/HL	26.8	26.6	$32.3 \pm 4.50 \ (25.0 - 36.0)$	$25.6 \pm 1.29 (24.7 - 27.1)$	$31.7 \pm 2.52 (26.7 - 34.2)$
Interorbital distance/HL	30.4	24.1	$24.9 \pm 2.04 (23.3 - 28.4)$	$26.4 \pm 1.57 (25.0 - 28.1)$	$21.6 \pm 3.83  (13.3 - 25.6)$
Angle gill raker length/HL	17.9	16.5	$15.0 \pm 0.97 (13.8 - 16.4)$	$17.9 \pm 0.88 (17.0 - 18.8)$	$14.2 \pm 1.10 (12.7 - 15.7)$
Longest dorsal spine length/HL	28.6	36.7	$33.2 \pm 3.77 \ (30.7 - 37.5)$	$40.3 \pm 1.21 (39.3 - 41.7)$	$30.9 \pm 2.95 (28.0 - 35.7)$
Longest dorsal ray length/HL	ray broken	39.2	$36.8 \pm 4.05 (32.5 - 41.7)$	$44.7 \pm 3.26 (42.0 - 48.3)$	$35.8 \pm 2.23 (31.7 - 38.1)$
Longest anal spine length/HL	tip broken	31.7	$31.3 \pm 3.34 (28.4 - 36.1)$	$45.5 \pm 2.64 (42.7 - 47.9)$	$32.4 \pm 5.69 (23.8 - 38.8)$
Pectoral fin length/SL	22.7	broken	$26.2 \pm 1.28 (25.1 - 28.3)$	$26.5 \pm 1.53 (24.9 - 28.0)$	$25.3 \pm 3.44 \ (16.8 - 26.9)$
Pectoral fin base depth/SL	9.3	8.6	$9.6 \pm 0.33 \ (9.0 - 9.8)$	$8.5 \pm 0.42 (8.1 - 8.9)$	$9.0 \pm 0.34  (8.2 - 9.3)$
Pelvic fin length/SL	17.3	20.7	$19.6 \pm 1.44 (17.9 - 21.4)$	$19.5 \pm 1.06 (18.3 - 20.2)$	$19.2 \pm 1.37 (17.3 - 21.4)$
Pelvic spine length/SL	16.0	16.2	$18.1 \pm 0.76 (17.3 - 19.1)$	$16.0 \pm 0.57 (15.4 - 16.5)$	$16.4 \pm 2.01 \ (13.5 - 18.9)$
Parietal spine length/HL	8.6	5.6	$11.5 \pm 2.30 (9.0 - 14.3)$	not present	$5.6 \pm 3.32 (1.1 - 9.5)$
Nuchal spine length/HL	6.1	5.6	$4.2 \pm 0.90 (3.0 - 5.4)$	$6.0 \pm 0.32 (5.6 - 6.3)$	$3.6\pm1.15(1.7-5.2)$
Preopercular spine length/HL	23.2	10.6	$18.3 \pm 1.94 \ (16.3 - 20.9)$	$13.3 \pm 0.38 \ (13.0 - 13.5)$	$11.7 \pm 6.25 (2.6 - 15.9)$

# Table 2

Meristics from transforming larval specimens and pelagic juveniles of *Sebastes* (subgenus *Sebastomus*) from southern California. CA 1, CA 2, etc. are identification labels for individual fish.

Features counted	CA 1 S. constellatus transforming specimen	CA 2 S. ensifer transforming specime	CA 3 <i>S. ensifer</i> n pelagic juvenile	CA 4 <i>S. ensifer</i> pelagic juvenile	CA 5 <i>S. ensifer</i> pelagic juvenile
Standard length (mm)	15.0	19.8	30.4	30.1	27.3
Dorsal-fin spines and ray	s XIII, 13	XIII, 12	XIII, 13	XIII, 12	XIII, 13
Anal-fin spines and rays	III, 6	III, 6	III, 6	III, 6	III, 6
Pectoral-fin rays	17/17	17/17	17/17	17/17	17/17
Pelvic-fin spines and rays	I, 5	I, 5	I, 5	I, 5	I, 5
Gill rakers	7 + 19 = 26	9 + 25 = 34	11 + 25 = 36	11 + 27 = 38	11 + 25 = 36
Lateral line pores	5 visible	~10 visible	scales forming; some missing in serie	es ~40	39
Procurrent caudal-fin ray	s 9+9	10 + 11	10 + 11	11 + 11	11 + 11

**Pigmentation** Solid sheath over gut; solid patch over brain; shallow, internal, dorsal, and dorsolateral melanophores on snout and some at tip of lower jaw; large postorbital patch continues posteriad to cover opercle; patch of melanophores continuous with dorsal brain sheath, extending back on dorsum to sixth dorsal-fin spine and ventrad to lateral line region to form a saddle; separate patch below the saddle forming at horizontal septum region; melanophore on each side of dorsum at ninth dorsal-fin spine; bar at caudal peduncle complete except at ventral midline; melanistic pigment scattered over pectoral and pelvic fins.

### Sebastes ensifer Chen 1971

# Transforming larva: CA2 (19.8 mm SL), Figure 2B Pelagic juveniles CA5 (27.3 mm), Figure 2C; CA3 (30.4 mm SL) and CA4 (30.1 mm SL) not illustrated

**Literature** Transforming larvae and pelagic juveniles of *S. ensifer* have not been described in the published literature.<sup>4</sup> A 4.2-mm first-feeding larva has been illustrated (Moser et al., 1977; Moser, 1996), showing features typical of first-feeding *Sebastomus* larvae as summarized above for *S. constellatus*.

**General morphology** Body moderately deep and compressed; head, jaws, and eyes large; relative head

<sup>4</sup> A 35.5-mm pelagic juvenile ascribed to Sebastes ensifer is illustrated and briefly described in Laroche, W. A. 1987. Guide to larval and juvenile rockfishes (Sebastes) of North America. Unpublished manuscript. Stonefish Environm. & Taxon. Serv., Enosburg Falls, VT 05450. length, and eye diameter decrease after transformation to pelagic juveniles; relative interorbital distance dorsal spine and ray length, and anal spine length increase after transformation (Table 1).

**Fins and other meristic features** Full complements of spinous and soft rays present; dorsal and anal-fin rays moderate in length; anterior scales in lateral line series forming, ~10 visible after staining in transforming specimen, full complements of lateral line scales forming in pelagic juveniles (Table 2).

**Spination** Supraocular crest and associated spines well developed; parietal crests well developed, weakly serrate, and parietal and nuchal spines subequal in transforming specimen; in pelagic juveniles the nuchal spine forms the terminus of the parietal ridge and only a remnant of the parietal spine can be seen; tympanic spine present; posterior preopercular series well developed with spine at angle longer than others but not serrated; anterior preopercular spines absent; first and second lower infraorbitals present; upper infraorbitals faintly visible after staining in transforming specimen but not in pelagic juveniles; cleithral spine present in pelagic juveniles but not in transforming specimen; presence of other spines as listed in Table 3.

**Pigmentation** *Transforming specimen*: solid sheath over gut; solid covering over brain; scattered melanophores on snout and some at tip of lower jaw; large postorbital and opercular patches; patch of melanophores continuous with dorsal brain sheath extending back on dorsum to 11th dorsal-fin spine

Spine	Specimen (standard length)							
	CA 1 (15.0 mm)	CA 2 (19.8 mm)	CA 3 (30.4 mm)	CA 4 (30.1 mm)	m) CA 5 (27.3 mm)			
Parietal	+	+	ns the parietal spine bine is becoming the	parietal spines are degenerating becoming the terminus of each				
Nuchal	+	+	+	+	+			
Anterior preopercular-1	☆	-	-	_	_			
Anterior preopercular-2	_	_	_	_	_			
Anterior preopercular-3	☆	_	_	-	_			
Posterior preopercular-1	+	+	+	+	+			
Posterior preopercular-2	+	+	+	+	+			
Posterior preopercular-3	+	+	+	+	+			
Posterior preopercular-4	+	+	+	+	+			
Posterior preopercular-5	+	+	+	+	+			
Opercular-upper	+	+	+	+	+			
Opercular-lower	+	+	+	+	+			
Interopercular	\$	Å	-	_	_			
Subopercular	_	-	-	_	_			
Preocular	+	+	+	+	+			
Supraocular	+	+	+	+	+			
Postocular	+	+	+	+	+			
Lower infraorbital-1	+	+	+	+	+			
Lower infraorbital-2	+	+	+	+	+			
Lower infraorbital-3	_	-	-	_	_			
Upper infraorbital-1	+	Å	-	_	_			
Upper infraorbital-2	_	\$	-	_	_			
Upper infraorbital-3	_	\$	-	_	_			
Upper infraorbital-4	\$	\$	-	_	_			
Nasal	+	+	+	+	+			
Coronal	_	-	-	_	_			
Fympanic	☆	+	+	+	+			
Pterotic	\$	-	-	_	_			
Upper posttemporal	+	+	+	+	+			
Lower posttemporal	+	+	坛	*	+			
Supracleithral	+	+	+	+	+			
Cleithral	_	-	+	+	+			

and ventrad to lateral line region to form a broad saddle; saddle continues ventrad faintly to horizontal septum; wide bar at caudal peduncle, complete except at ventral midline; scattered melanophores on pelvic fin and at base of pectoral fin. Embedded pigment, barely visible through the musculature of the body, lies on the upper surface of the vertebral column. *Pelagic juveniles*: general melanistic pigment filling in previously unpigmented regions except ventrally on head, chest, and region above anal fin; heavy along dorsum; saddle somewhat variegated, with pale regions that become white patches in adults; caudal peduncle bar enlarged anteriorly, blending with saddle, darkest on peduncle region with several chevrons forming; some pigment on paired fins.

# Table 4

Geographic ranges and meristic characters for the species of *Sebastes*, subgenus *Sebastomus*, in the eastern North Pacific. All species have three anal spines, one pelvic spine with five rays, and 8+7 principal caudal-fin rays. Data are derived primarily from Miller and Lea (1972), Chen (1971, 1975, 1986), Matarese et al. (1989), Moser (1996). Abbreviations: BCA = Baja California; C = central; CA = California; G of AK = Gulf of Alaska; G of CA = Gulf of California; N = northern; S = southern; WA = Washington. GR = gill rakers.

		Fin rays						
		Dorsa	ıl					
Species	Distribution	Spines	Rays	Anal	Pectoral	Vertebrae	GR (first arch)	Lateral line pores
S. chlorostictus	WA to C BCA	XIII	11-15	5-7	16–18	26-27	31–36	35-43
S. constellatus	C CA to S BCA	XIII–XIV	12 - 14	5 - 7	16 - 18	25-26	25-30	37 - 47
S. ensifer	C CA to C BCA	XIII	12 - 14	5 - 7	16 - 18	26	34-40	34 - 44
S. eos	C CA to C BCA	XIII	11 - 13	5 - 7	17 - 18	26	26-31	34-42
S. exsul	G of CA	XIII	12 - 13	5-6	16 - 18	26	32-37	35 - 43
S. helvomaculatus	G of AK to S CA	XII–XIV	12 - 14	6 - 7	15 - 18	26	28-33	34 - 45
S. lentiginosus	S CA to N BCA	XIII	12 - 13	6 - 7	16 - 18	26	34-39	33-41
S. notius	C BCA	XIII	11 - 13	5 - 7	15 - 18	26	33–38	34-44
S. rosaceus	N WA to C BCA	XIII–XIV	11 - 14	5 - 7	16 - 18	26-27	29-34	36-46
S. rosenblatti	C CA to C BCA	XIII–XIV	11 - 13	5-6	16 - 18	26	28-34	34-42
S. simulator	S CA to C BCA	XIII	12 - 14	5-6	16 - 18	26	28-33	33-40
S. spinorbis	G of CA	XIII	13 - 14	6	18	26	30–33	33–38
S. umbrosus	C CA to S BCA	XII–XIV	11–13	5–7	15–18	26	31–38	33-44

# Discussion

# **Molecular identification**

The genetic information encoded in the rockfish mitochondrial cytochrome b has been found useful in the study of phylogenetic relationships of the species in the genus Sebastes (Rocha-Olivares, 1998a). The degree of genetic variability is large enough to recognize diagnostic mutations characteristic of several species (Rocha-Olivares, 1998a; Rocha-Olivares and Vetter<sup>2</sup>). The use of this information in blind tests of species identification of adult Sebastes, independently determined by morphological and genetic techniques, has given satisfactory results (Rocha-Olivares and Lea<sup>5</sup>). Diagnostic mutations have been used to design a method of species identification based on multiplex PCR (Rocha-Olivares, 1998b). In our study we relied on raw DNA sequence data to determine the species identity of five wild-caught pelagic young of Sebastes. The very small sequence divergence of their mtDNA with that of known adults (ranging from zero to three bp) provided very strong evidence supporting the identity of the unidentified specimens. Moreover, the small intraspecific genetic variation, indicated by the reduced standard errors in Figure 1, suggests that the possibility of species misidentification with our molecular approach is also very small.

Faster-evolving regions of the mtDNA have been successfully used to study the genetic structure of rockfish populations in both hemispheres (Rocha-Olivares et al., 1999b; Rocha-Olivares and Vetter, 1999); therefore, it is conceivable that molecular tools can also be applied intraspecifically to study, for example, the relative contribution of juvenile recruits from genetically differentiated populations.

### **Species comparisons**

**Transforming specimens of S.** constellatus and S. ensifer The specimens of S. constellatus and S. ensifer are remarkably similar. The meristic similarity reflects the general overlap among species documented for the subgenus (Table 4; Chen, 1971, 1975). The distinctly lower gill raker count for the transforming specimen of S. constellatus, compared with that of S. ensifer specimens (Table 2), matches the gill raker counts of adults (Table 4; Chen, 1971). Slight differences in spination between the trans-

<sup>&</sup>lt;sup>5</sup> Rocha-Olivares, A., and Lea, R. N. 1998. Unpubl. data.

forming specimens of the two species (e.g. a longer serrate third preopercular spine in S. constellatus, more infraorbitals in S. ensifer only visible after staining) may be due to ontogenetic stage or individual variation (Table 3). However, the relatively longer fin rays in S. ensifer appear to be a species difference. The relative size and position of the parietal and nuchal spines differs in the two transforming specimens; however, this may be due to difference in stage of development. In the S. ensifer series the nuchal spine gradually replaces the parietal spine as the terminus of the parietal ridge and this may also occur in S. constellatus. Transforming specimens of the two species differ slightly in pigmentation (e.g. wider dorsal saddle and peduncle patch in S. ensi*fer*); however, this difference can not be confirmed without additional specimens.

**Transforming specimens and pelagic juveniles of** *S. ensifer* and *S. helvomaculatus* The species differ markedly in morphometry (Table 1). *S. ensifer* has a deeper-body than field-caught *S. helvomaculatus* described and illustrated in Richardson and Laroche (1979), as shown by the greater relative body depth measured at the anus (Table 1; Figs. 2 and 3). The

two species are different in other morphometric features. Eye diameter, jaw length, and fin ray and spine lengths in the dorsal and anal fins are relatively greater in transforming specimens and pelagic juveniles of *S. ensifer* compared with *S. helvomaculatus*, whereas relative snout length is greater in *S. helvomaculatus*. In pelagic juveniles, relative head length is greater in *S. helvomaculatus* than in *S. ensifer*, whereas relative interorbital length is less compared with that in *S. ensifer*. The two species differ in stage of development at length. The illustrated 18.4-mm transforming specimen of S. helvomaculatus lacks a melanistic saddle on the trunk, and the caudal peduncle bar is just beginning to form, whereas these features are well established in the 19.8-mm specimen of S. ensifer (Figs. 2 and 3). The pigment saddle is present in the 22.4-mm pelagic juvenile of S. helvomaculatus but does not extend ventrad more than about half-way to the lateral line. Moreover, the complex pattern of bars and clear areas present on late-stage pelagic juveniles of S. ensifer is not present on S. helvomaculatus (Fig. 3). Also, the



fornia: (A) S. constellatus, transforming larva, 15.0 mm SL; (B) S. ensi larva, 19.8 mm SL; (C) S. ensifer, pelagic juvenile, 27.3 mm SL.



ontogenetic changes in head spines (e.g. loss of infraorbitals and anterior preoperculars; decrease in the relative size of the parietals) occur in *S. helvomaculatus* larger than *S. ensifer* (Figs. 2 and 3).

**Pelagic juveniles of other** *Sebastomus* **species** A fieldcaught 21.0-mm pelagic juvenile of *S. chlorostictus* illustrated in Matarese et al. (1989) and Kendall (1991) is generally similar to specimens of *S. constellatus* and *S. ensifer* described in this study. In contrast to these species, the nape area in *S. chlorostictus*  appears to lack pigment. In *S. chlorostictus* the dark pigment saddle extends from the first to sixth dorsalfin spines, becomes wider mid-laterally on the trunk and appears to extend to the abdominal region. The caudal peduncle pigment is equally heavy and forms a complete band around the peduncle in the 21.0-mm specimen. Also, the dorsal and anal fin rays appear to be shorter than those in *S. ensifer*.

**Comments on the identification of pelagic young of Sebastomus** The transforming larvae and pelagic juveniles of S. constellatus and S. ensifer identified by molecular methods, although conforming to the general facies known for Sebastomus pelagic young, show some differences in morphological features and pigmentation that may permit their identification by traditional means. The possibility of identifying the pelagic young of this species-rich subgenus is further suggested by the striking differences between pelagic young of S. ensifer and S. helvomaculatus. Further advancement will require: 1) the collection of fresh specimens of pelagic young of all species of Sebastomus in the waters off California and Baja California; 2) the establishment of ontogenetic series positively identified by molecular methods; and 3) a detailed description and series of illustrations published for each species. Pelagic young of this subgenus are common constituents of the midwater fauna of the continental borderland of the Southern California Bight (Moser and Ahlstrom, 1978; Moser and Boehlert, 1991), and specimens are readily available from midwater trawls.

In conclusion, this paper reports a novel approach to the study of young rockfishes. We have used molecular data in conjunction with morphological descriptions to increase knowledge on the identification of elusive early life history stages of *Sebastes*.

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