

Genetic structure and different color morphotypes suggest the occurrence and bathymetric segregation of two incipient species of *Sebastes* off Argentina

Leonardo A. Venerus · Javier E. Ciancio ·
Carla Riva-Rossi · Elizabeth A. Gilbert-Horvath ·
Atila E. Gosztonyi · John Carlos Garza

Received: 5 April 2013 / Revised: 11 May 2013 / Accepted: 16 May 2013
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Abstract Rockfishes of the genus *Sebastes* are extensively distributed in the Pacific and Atlantic oceans. Although the occurrence of two morphologically similar species in the Southern Hemisphere, *Sebastes oculatus* and *Sebastes capensis*, is now clearly established, the taxonomic status and phylogeographic patterns for the genus in the region have not yet been completely resolved. In this study, we provide new insights into the taxonomy and evolutionary relationships of rockfishes inhabiting the Southwestern Atlantic Ocean, off the coast of mainland Argentina, by combining mitochondrial DNA (mtDNA) control region sequences, microsatellite data, and color pattern analyses. Differences in coloration (“dark” and “light” fish) together with bathymetric segregation between color morphotypes were evident from fish collection and literature review. In addition, the mtDNA phylogenetic analysis and Bayesian clustering analysis using microsatellite data separated the fish into two distinct groups ($F_{ST}=0.041$), most likely representing incipient species. Our results suggest that speciation-by-depth

in the absence of physical barriers could be a widespread mechanism of speciation in *Sebastes* from both the Northern and Southern Hemispheres. Nevertheless, the degree of genetic differentiation found, added to the large number of individuals displaying high levels of admixture, points to the occurrence of incomplete reproductive barriers between color morphotypes. Beyond the taxonomic and phylogeographic implications of our findings, the occurrence of distinct groups of *Sebastes* off the coast of Argentina being targeted by different fisheries (angling and trawling) has consequences for the design and implementation of appropriate fishery regulations to avoid overharvest of either group.

Keywords Sebastidae · Phylogeography · Rocky reef fish · Incomplete reproductive isolation · Incipient speciation · Morphotypes

Introduction

The genus *Sebastes* Cuvier (1829) (Osteichthyes: Sebastidae) is among the 10 most speciose marine fish genera, with at least 110 species inhabiting a large variety of habitats, mainly in the North Pacific Ocean (Love et al. 2002; Eschmeyer et al. 2010). Rocha-Olivares (2004) suggested that the evolutionary success and adaptive radiation in this genus is facilitated by its type of reproduction, which includes internal fertilization and viviparity, and rely on the porous boundaries between sympatric species, as evidenced by the occurrence of hybridization and incomplete reproductive isolation (e.g., Roques et al. 2001). The occurrence of taxonomically problematic sibling species (Knowlton 1993) is fairly common within *Sebastes* and recent work has reported genetic divergence between co-

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-013-1061-2) contains supplementary material, which is available to authorized users.

L. A. Venerus (✉) · J. E. Ciancio · C. Riva-Rossi ·
A. E. Gosztonyi
Centro Nacional Patagónico—Consejo Nacional de
Investigaciones Científicas y Técnicas (CENPAT—CONICET),
Boulevard Brown 2915 (U9120ACD),
Puerto Madryn, Chubut, Argentina
e-mail: leo@cenpat.edu.ar

E. A. Gilbert-Horvath · J. C. Garza
National Marine Fisheries Service, Southwest Fisheries
Science Center, 110 Shaffer Road,
Santa Cruz, CA 95060, USA

occurring color morphotypes (Kai et al. 2002; Narum et al. 2004; Orr and Blackburn 2004; Gharrett et al. 2006) and between taxa exhibiting subtle differences in meristics and/or morphology or even habitat preferences (Hyde et al. 2008; Ingram 2011; Muto et al. 2011).

The North Pacific has been hypothesized to be the origin and center of speciation of *Sebastodes* (Eschmeyer 1969). Current theories propose that representatives of the ancestral taxon colonized the North Atlantic through a transarctic dispersal route, then expanded to the Southern Hemisphere by crossing the Eastern Tropical Pacific and spread around the Pacific and Atlantic coasts of South America, probably through a unique invasion event or at least during a short time interval by the same evolutionary lineage (Rocha-Olivares et al. 1999a), and ultimately reached Tristan da Cunha and Gough islands, and the coast of South Africa, through the West Wind Drift (Eschmeyer 1969; Eschmeyer and Hureau 1971; Andrew et al. 1995). Shape differences in sagittal otoliths among the four species of *Sebastodes* from the North Atlantic and six from the North Pacific, as well as the affinity of the Southern Hemisphere *Sebastodes capensis* otoliths to North Pacific species, support the zoogeographic hypothesis described above (Stransky and MacLellan 2005).

In the Southern Hemisphere, the lack of obvious morphological differences among rockfishes from different localities led some authors to consider all forms studied as belonging to only one species: *S. capensis* (Gmelin 1789), which has precedence over *Sebastodes oculatus* Valenciennes 1833 (in Cuvier and Valenciennes 1833; Chen 1971; Kong Uriba 1985). While *S. oculatus* is most commonly used by Argentinean ichthyologists to refer to rockfishes occurring off the Atlantic coast of South America, others continue using *S. capensis* for all *Sebastodes* fishes inhabiting the Southern Hemisphere. This situation gives rise to nomenclatural confusion in the taxonomic and fisheries literature, leading, in the most extreme cases, to the misclassification of *Sebastodes* species in studies aimed at analyzing ecological traits within a biogeographic framework (e.g., differences in ecto- and endoparasitic fauna: González and Moreno 2005; González et al. 2006).

Studies of mitochondrial DNA (mtDNA) sequence variation (Rocha-Olivares et al. 1999a, b; Hyde and Vetter 2007; Nuñez et al. 2010) have contributed significantly to disentangling the austral *Sebastodes* systematic puzzle, while also shedding some light on the zoogeographical pattern of the genus within the Southern Pacific and Atlantic oceans. Rocha-Olivares et al. (1999a, b) provided the first quantitative analysis of genetic data that supported the existence of two southern species: *S. capensis*, occurring around the Malvinas/Falkland, Tristan da Cunha and Gough islands, and off South Africa, and *S. oculatus*, occurring around the Malvinas/Falkland Islands and off Chile. They also suggested the occurrence of two *S. oculatus* lineages in South America: one occurring mostly in the Atlantic and

the other in the Pacific Ocean, although considerable overlap existed between them, particularly around the Malvinas/Falkland Islands. Hyde and Vetter (2007) analyzed seven mitochondrial and two nuclear genes and also considered both *S. capensis* and *S. oculatus* to be valid species, with an estimated divergence time of ~344,000 years before present (ybp) (scaled from their Fig. 5). More recently, Nuñez et al. (2010) used mtDNA sequences to test the boundaries between the Atlantic and Pacific lineages of *S. oculatus*, extending the analysis of Rocha-Olivares et al. (1999b) by including samples from Peru and the northern Patagonian coast of Argentina. Although they confirmed the occurrence of the two southern *Sebastodes* species, they did not find support for two different lineages within *S. oculatus*, concluding that the lack of inter-oceanic differentiation could stem from the occurrence of significant gene flow, mediated by the extensive larval dispersal along the South American coast driven by the Humboldt Current and the West Wind Drift.

However, none of these genetic studies took into account differences in coloration or other morphological features. Moreover, comprehensive studies examining the spatial distribution of color morphotypes or an integrative analysis combining information about color, morphology, genetics, and their correlations for southern rockfishes are lacking. Definitive answers to the questions of how many austral species of *Sebastodes* exist today and how they are distributed are therefore still pending (Chen 1971; Eschmeyer and Hureau 1971; Kong Uriba 1985; Kendall 2001; Stransky and MacLellan 2005).

Rockfishes constitute an important component of coastal recreational (Venerus 2006) and off-shore commercial trawl fisheries along the Patagonian Shelf (Bovcon and Cochia 2007; Góngora 2010) and are one of the most common and conspicuous fishes of the northern Patagonia rocky-reef fish assemblage (Galván et al. 2009b; Irigoyen et al. 2013). Yet the proper management of *Sebastodes* populations in the Southwestern Atlantic is hampered by the lack of clarity about their systematics and genetic relationships. The aim of the present work is therefore to investigate the genetic population structure of rockfishes in the Southern Atlantic Ocean off Argentina, as inferred from nuclear microsatellite loci and mtDNA sequence data. We also evaluate and discuss the genetic results in relation to the occurrence of different color morphotypes and their geographic distribution on the Argentinean continental shelf.

Materials and methods

Sample collection

Samples from a total of 164 adult *Sebastodes* (Table 1) were collected from San Matías Gulf (~41°32'S 64°20'W), near

the northern distribution limit for *Sebastodes* along the Argentinean coast (Sánchez and Acha 1988; Galván et al. 2009b), San José Gulf (~42°20'S 64°20'W), Nuevo Gulf (~42°43'S 64°34'W), the Argentinean shelf off Península Valdés (~43°33'S 60°11'W), Camarones Bay (~44°47'S 65°39'W), and waters off Santa Cruz Province (~46°03'S 67°36'W). Most fish were sampled during beach monitoring of recreational angling and spear-fishing, and caudal fin tissue samples were taken in the field and preserved in 96 % ethanol or air-dried for ease of transport and storage. Some fish were sampled by pole-hooking (Irigoyen and Venerus 2008), in which a small piece of the caudal fin (~1 cm²) was sectioned underwater by scuba divers, before releasing the fish. Off-shore samples were obtained during the oceanographic survey CONCACEN 2009, on-board the R/V “Puerto Deseado.” In addition, 16 adult fish belonging to a closely related species, *Sebastodes constellatus* (Jordan and Gilbert 1880), were collected from the North Pacific Ocean near Monterey Bay, CA, USA (~36°56'N 122°16'W) and were included in the microsatellite analysis as an outgroup in a population differentiation (F_{ST}) analysis.

We also reviewed the coloration patterns for the *Sebastodes* from the Southern Pacific and Atlantic oceans, described in

the scientific literature and in naturalists’ reports, and mapped the occurrence of different color morphotypes of *Sebastodes* off the coast of Argentina from two main sources: (1) analysis of 74 fish (28 lots) preserved in ethanol in the ichthyological collection of the Centro Nacional Patagónico (CNPICT) (Online Resource 1) and (2) compilation of published color descriptions and photographs of fresh fish in which the exact capture location was available.

Laboratory analysis

Microsatellite data

Genomic DNA was extracted from fin clips using DNeasy 96 Tissue Kits (Qiagen, Inc.) on a Qiagen BioRobot 3000 automated workstation, using a modified mouse-tail protocol. We employed a suite of 24 microsatellite loci that were found in previous experiments to amplify successfully and exhibit polymorphism across multiple *Sebastodes* species (Pearse et al. 2007; Gilbert-Horvath, unpublished data). These loci included di-, tetra-, and pentanucleotide microsatellite repeats that were originally described in seven different species of *Sebastodes* (Online Resource 2). Polymerase chain reaction (PCR)

Table 1 Number of individuals and origin of samples used for genetic studies involving *Sebastodes* spp. from the Southern Hemisphere. One fish caught within San Matías Gulf (~170 m depth) and all fish caught

off Península Valdés (this study) were of the light morphotype; all the remaining fish genotyped were dark (see the text for details)

Region	Sample site	Number	Reference
South Pacific	Huacho, Peru	6	Nuñez et al. (2010)
	Iquique, Chile	5	Nuñez et al. (2010)
	Antofagasta, Chile	5	Nuñez et al. (2010)
	Coquimbo, Chile	9	Nuñez et al. (2010)
	Valparaíso, Chile	1	Rocha-Olivares et al. (1999a)
	Valparaíso, Chile	10	Rocha-Olivares et al. (1999b)
	Valparaíso, Chile	1	Hyde and Vetter (2007), Hyde (personal communication)
	Aysén, Chile	5	Nuñez et al. (2010)
	Punta Arenas, Chile	1	Nuñez et al. (2010)
Southwest Atlantic	Malvinas/Falkland Islands	17	Rocha-Olivares et al. (1999b)
	San Matías Gulf, Argentina	5	This study
	San José Gulf, Argentina	53	This study
	Nuevo Gulf, Argentina	37	This study
	Nuevo Gulf, Argentina	7	Nuñez et al. (2010)
	Argentinean shelf, off Península Valdés	25	This study
	Camarones Bay, Argentina	38	This study
	Argentinean shelf, off Santa Cruz Province	6	This study
Mid- and Southeast Atlantic	South Africa	1	Rocha-Olivares et al. (1999a)
	South Africa	5	Rocha-Olivares et al. (1999b)
	South Africa	1	Hyde and Vetter (2007)
	Tristan da Cunha Island	5	Rocha-Olivares et al. (1999b)
	Cape Town, South Africa	9	Nuñez et al. (2010)

primers were labeled with a fluorescent dye (6-FAM, TET, or HEX) for detection on an ABI 377–96 automated DNA sequencer (Applied Biosystems, Inc.). Three panels, composed of eight loci each, were designed such that each panel could be electrophoresed as a set, based on empirically determined PCR product size ranges and dye combinations. Nineteen of the 24 loci were optimized for multiplex PCR (duplex or triplex reactions), and five were amplified in single-locus reactions (Online Resource 2). PCR was performed in 15 µl reaction volumes using 4 µl of genomic template DNA diluted 1:20 in 5 µM Tris buffer. Each PCR contained 1X PCR Buffer II, 1.6 mM MgCl₂, 0.25 U AmpliTaq DNA Polymerase (Applied Biosystems), 0.2 or 0.1 µM of each primer (single marker or multiplex reaction, respectively), and 0.1 mM of each dNTP. Specifics of the thermal cycling conditions are available from the authors upon request. Allele size calls were derived using GeneScan v3.0 and Genotyper v2.1 software (Applied Biosystems). To control for genotyping error, allele calls were independently made by two people, and any discrepancies then resolved through review by a third person and/or removal of discrepant genotypes from the dataset. Finally, as missing data can indicate compromised sample quality that may negatively affect the ability to accurately infer biological patterns, only individuals that were successfully genotyped at 12 or more of the 24 polymorphic loci were retained for further analysis; this resulted in the removal of 22 data-poor samples from the microsatellite dataset.

Mitochondrial DNA

We generated sequences of the mtDNA control region for 10 individuals collected on the Argentinean shelf, off Península Valdés ($n=5$) and within Camarones Bay ($n=5$). DNA was extracted with a standard proteinase K/phenol–chloroform procedure (Sambrook and Russell 2001) and a 557-base pair (bp) fragment amplified using the primers 5' TCAAAGCT TACACCAGTCTGTAAACC 3' (L15926; Kocher et al. 1989) and 5' CCTGAAGTAGGAACCAGATG 3' (H16498; Meyer et al. 1990). Amplified DNA templates were purified with the GENE CLEAN Purification Kit (Qbiogene, Inc.), and 20 ng of purified PCR product was used in cycle sequencing reactions following ABI PRISM BigDye Terminator protocols (Applied Biosystems). Sequencing was performed on an ABI PRISM 3130 automated capillary sequencer and both DNA strands were sequenced. Sequences were edited and aligned with the MEGA v5 software (Tamura et al. 2011) and then imported into DnaSP v5 (Librado and Rozas 2009) to identify unique haplotypes. Both Muscle and Clustal alignments were performed (both yielded almost identical results), and then the alignments were further adjusted by hand. Sequences of unique haplotypes were deposited in GenBank (accession numbers KC797495–KC797503).

We obtained partial D-loop sequences from GenBank for *S. capensis* (AF031503), *S. oculatus* (AF031502), *S. constellatus* (AF031505), *Sebastes notius* (AF031510), *Sebastes lentiginosus* (AF031509), *Sebastes umbrosus* (AF031516), *Sebastes exsul* (AF031514), *Sebastes spinorhynchus* (AF031515), *Sebastes chlorostictus* (AF031504), *Sebastes eos* (AF031506), *Sebastes rosenblatti* (AF031511), *Sebastes helvomaculatus* (AF031508), *Sebastes simulator* (AF031513), *Sebastes ensifer* (AF031507), *Sebastes rosaceus* (AF031512), *Sebastes serranoides* (AF031498), *Sebastes inermis* (ab071260), *Sebastes maliger* (AF031500), *Sebastes schlegelii* (NC005450), *Sebastes paucispinis* (AF031499), and *Sebastes ruberrimus* (AF031501) (Rocha-Olivares et al. 1999a; Kai et al. 2002; Kim and Lee 2004). These sequences were included as outgroups to make our analysis comparable to previous phylogenetic studies (i.e., Nuñez et al. 2010). Additionally, published sequences were obtained from 58 individuals from six locations off the Pacific coast of South America (*S. oculatus*), 24 individuals from two locations off the Atlantic coast of South America (*S. oculatus*), 19 individuals from two locations off the coast of South Africa (*S. capensis*), and from five individuals collected from Tristan da Cunha Island (*S. capensis*) (Rocha-Olivares et al. 1999b; Nuñez et al. 2010; summarized in Table 1).

Statistical analysis

Microsatellite analysis

Descriptive statistics were quantified as observed number of alleles at each locus, allelic richness (estimated with rarefaction), the local inbreeding coefficient (F_{IS}), observed (H_o), and expected heterozygosity (H_e) in FSTAT v2.9.3.2 (Goudet 2005). Probabilities for Hardy–Weinberg and linkage equilibria were calculated using an approximation of an exact test based upon the Markov chain Monte Carlo (MCMC) algorithm of Guo and Thompson (1992) and implemented in GENEPOL v3.4 (Raymond and Rousset 1995). Significance probabilities at individual loci were corrected for type I error using the sequential Bonferroni correction for multiple comparisons (Rice 1989).

Multilocus genotypes were used to infer population structure and to cluster *Sebastes* individuals of different color morphotypes (see “Results”) and collected at different sites. This analysis was conducted using the Bayesian clustering method in STRUCTURE v2.3.4 (Pritchard et al. 2000) and without reference to a priori defined groups. The analysis evaluated hypotheses of $K=1$ –10 genetic clusters in the dataset and was run under an admixture model with correlated allele frequencies using a 50,000 burn-in period and one million MCMC iterations. Maximum K of 10 was used so as to evaluate potential substructure within locations. Individual, fractional ancestry proportions (q -values) or likelihoods that an individual belongs to a given genetic

cluster (K) are also estimated: the higher a q -value, the more likely the individual belongs to a given genetic cluster. For each value of K , 10 iterations were performed and the most likely true value of K estimated based on the mean log-likelihoods $\text{LnP}(D)$ and the ΔK method proposed by Evanno et al. (2005), which predicts the most likely value of K in a given dataset based on second order rates of change between successive values of K , both implemented in Structure Harvester v0.6.1 (Earl and vonHoldt 2012). The software CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007) was used to deal with “label switching” by performing alignments of the q -values for the chosen number of genetic clusters, and individual plots of mean q -values (fractional membership or admixture proportions) were visualized with the program DISTRUCT v1.1 (Rosenberg 2004). Genetic differentiation between the clusters inferred by STRUCTURE was estimated using Wright’s F -statistics (F_{ST}), as implemented in GENETIX v4.0 (Belkhir et al. 1996–2004). The statistical significance of F_{ST} values was tested using 10,000 permutations. Finally, factorial correspondence analysis (FCA) in GENETIX v4.0 was used to visualize the genetic relationships among the fish from clusters inferred by STRUCTURE.

Phylogenetic analysis

In order to compare the population structure and differentiation patterns observed at microsatellite loci with the previous genetic studies of austral *Sebastes* species, a phylogenetic analysis was performed combining our novel mtDNA sequence data with published *Sebastes* sequences. The combined genetic data were used to test fit of 24 different evolutionary models using MrModeltest v2.3 (Nylander 2004) with the Akaike information criterion (Akaike 1974). The best fitting model was a general time reversible one (Rodríguez et al. 1990), with an empirically derived proportion of invariant sites (I) and gamma shape distribution (Γ). To assess the genetic relationships among southern and some northern Pacific rockfishes, a Bayesian inferred phylogenetic tree was generated using Metropolis-coupled MCMC analysis, implemented in MRBAYES v3.2 (Ronquist and Huelsenbeck 2003). Two independent runs of four Markov chains were allowed to proceed for one million cycles using the default heating values. The first 25 % was discarded to eliminate data from the “burn-in” phase, where the posterior probabilities may have not yet reached stationarity. Runs were checked for convergence using an average standard deviation of split frequencies below 0.01 as the diagnostic criterion. Run convergence was also checked with Tracer v1.4 (Rambaut and Drummond 2007). A control region sequence of the closely related species *Helicolenus lengerichi* Norman 1937 (a member of the same subfamily, Sebastinae, as *Sebastes*) was used as an outgroup.

Results

Color morphotypes and bathymetric distribution

Two color morphotypes, found in both live and preserved fishes, were distinguished in rockfish caught off Argentina (Fig. 1). Both color morphotypes have a few irregular white to pinkish spots below the dorsal fin and above the lateral line and bear the general pigmentation pattern that characterizes the austral *Sebastes*: individuals are darker in the upper part of the back and lighter towards the belly (see a review of color descriptions for austral *Sebastes* in Online Resource 3). Each morphotype is, however, clearly distinguishable by the overall color of the fish. For the “dark” morphotype, which match most color descriptions for *Sebastes* off Chile (e.g., Cuvier and Valenciennes 1833; De Buen 1960; Chen 1971; Kong Uribe 1985), the body is generally chestnut- to dark-brown, although this pigmentation pattern is slightly variable among sites; for example, a general pinkish background tone is more evident in some fish (Fig. 1, DF and DP). In contrast, the “light” morphotype has the same general pigmentation pattern described for live fish from Tristan da Cunha Island and South Africa (Smith 1845; Pappe 1853; Barnard 1927; Smith 1949; Eschmeyer 1969; Andrew et al. 1995): the body is bright orange to reddish above the insertion of the pectoral fin and fades to white shaded with pink in the ventral area of body and head (Fig. 1, LF and LP). Although differences between color morphotypes are too marked to be confounded, one preserved fish from the Ría Deseado (47°45'S 65°53'W) could not be unambiguously assigned to either color morphotype, presumably due to preservation (Online Resource 1).

There was spatial segregation between color morphotypes (Fig. 2), with the dark form found in all coastal fish caught in shallow waters (<~40–50 m depth) between Puerto Lobos, San Matías Gulf, and Caleta Olivia, San Jorge Gulf (Fig. 2). In Camarones Bay, however, color variations among fish were more evident: while all fish were undoubtedly dark, some individuals showed a lighter pale brown to dark orange tone. Conversely, the light form occurred mostly offshore, at depths greater than ~80–100 m (the maximum recorded depth for the species off Argentina was 271 m). The only exception to this general pattern was three light fish between 136 and 235 mm total length (TL), caught in shallow waters within the Ría Deseado, and another two light fish (273–312 mm TL) angled off Caleta Sara at <40 m (Online Resource 1). Although we have insufficient data from fish caught at intermediate depths, between about 50 and 80 m, each collection lot (from shallower than 50 m or deeper than 80 m) containing more than one preserved individual (between 2 and 16 fish) consisted of either all dark or all light individuals (Online Resource 1).

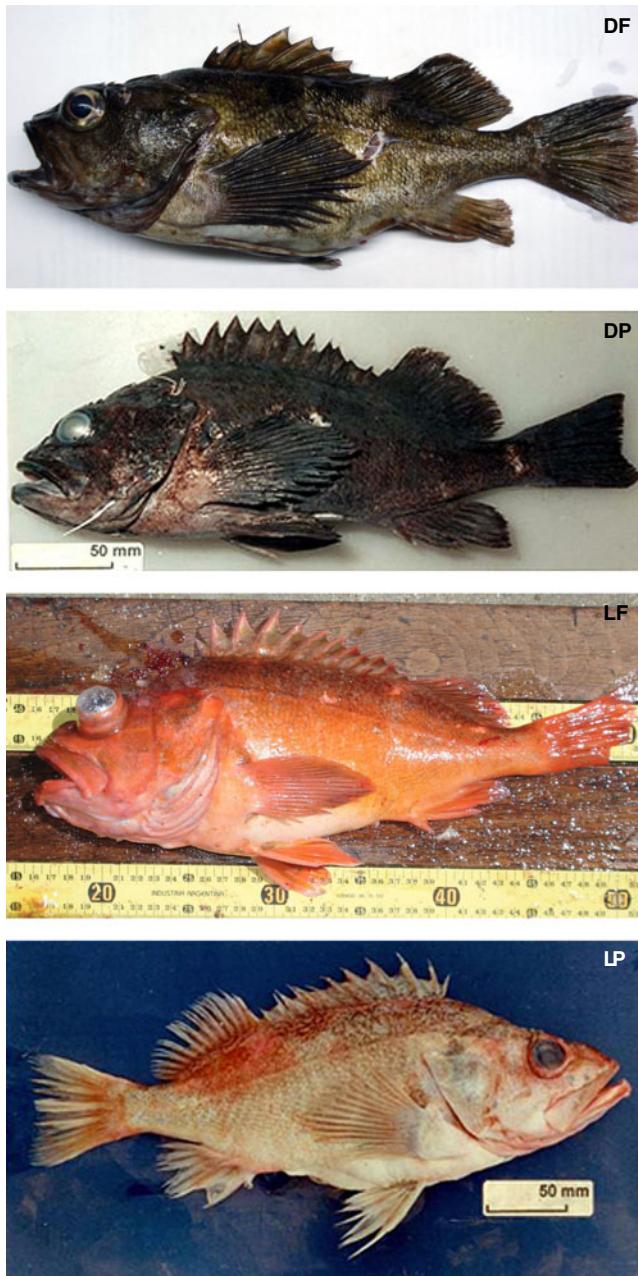


Fig. 1 Dark (D) and light (L) morphotypes of *Sebastes* caught off Argentina. Fresh (F) and preserved (P) individuals are indicated. DF: 26 cm total length (TL); <10 m depth, Puerto Lobos, San Matías Gulf, 8 Oct 2012. DP: 31 cm TL; Cracker Bay, Nuevo Gulf; depth and date of capture unknown. LF: 34 cm TL; 102 m depth, ~300 km SE Península Valdés (43°33'S 60°11'W), 23 Nov 2009; LP: 37 cm TL; Argentine Sea; exact location, depth and date of capture unknown. Photos: DF (LAV); DP and LP (AEG); LF (M. Delpiani)

Microsatellite data

Genetic diversity and summary statistics

All samples from austral *Sebastes* were fixed for a single allele at loci Sal2, Sme13, and Sth45, and these loci were

thus removed from further analyses. The remaining 21 loci had moderate to high levels of polymorphism (Table 2) compared with values in other *Sebastes* species genotyped for the same loci. The maximum number of alleles observed per locus was 21 (mean=8.57) and the maximum allelic richness was 13.08 (mean=5.35). Overall H_o for each locus ranged from 0.038 to 0.877 (mean=0.500) and was always lower than H_e , which ranged from 0.061 to 0.900 (mean=0.538). Over all loci, the inbreeding coefficient (F_{IS}) was 0.063, ranging from -0.089 to 0.879 for individual loci (Table 2). Locus Spi4 had a significant heterozygote deficit after correction for multiple tests, most likely caused by null alleles, but the remaining 20 loci conformed to Hardy–Weinberg expectations when all sites were pooled together. Only one pair of loci (Sal4 and Ssc51) showed significant linkage disequilibrium after correction for multiple comparisons.

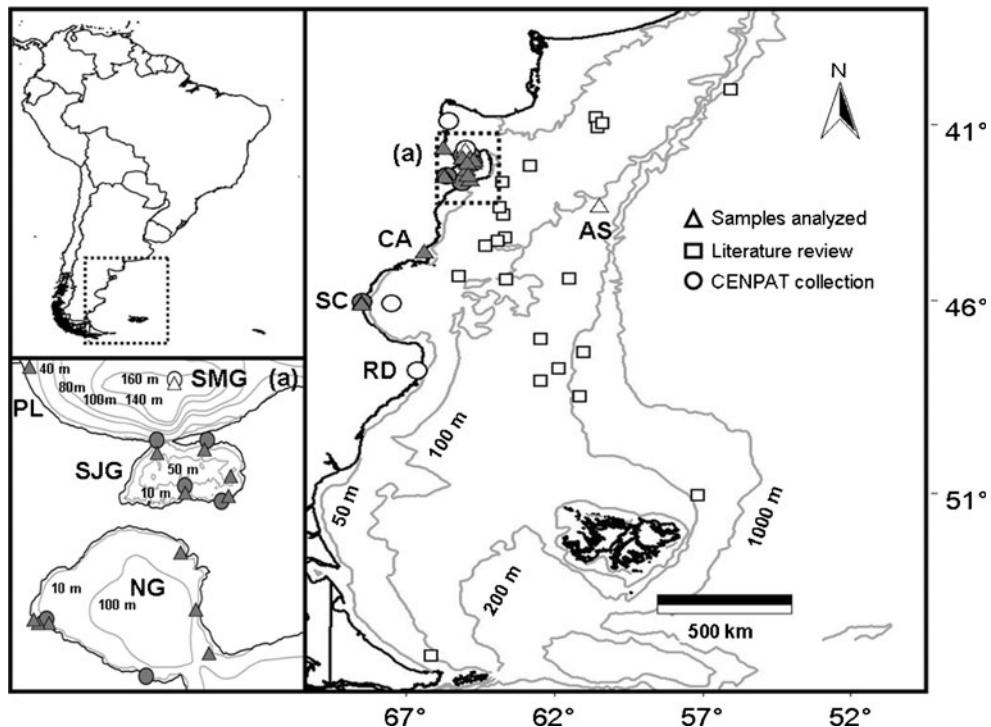
Population structure

STRUCTURE results indicated that $K=2$ was the most likely number of genetic groups represented by the data (Fig. 3a, b). These clusters largely corresponded to fish with differences in color and sampled in areas with different bottom depth and were not constrained by sampling site. All dark individuals caught at depths <40–50 m, irrespective of the sampling site, were assigned to cluster 1 with partial inferred ancestry (mean q -value)=0.69 (range=0.45–0.89). Conversely, all light fish caught off Península Valdés (102 m depth) were assigned to cluster 2, also with partial inferred ancestry, with mean q -value=0.79 (range=0.62–0.89) (Fig. 3c). One individual caught at 170 m near the mouth of San Matías Gulf had higher fractional ancestry in cluster 2 and, thus, was assigned to this cluster, while the remaining fish from this location, sampled at depths <15 m, were assigned to cluster 1. When each collection site was considered separately, STRUCTURE found only a single cluster at each site (only sites with sample size ≥ 25 were analyzed in this manner, data not shown).

Comparison of allelic richness and heterozygosity for the two primary inferred clusters revealed similar levels of variation for both measures. Only locus Spi4 showed significant deviations from Hardy–Weinberg equilibrium in both inferred clusters. However, six pairs of loci in the dark morphotype and two pairs in the light morphotype had significant linkage disequilibrium after correction for multiple tests. Most of these deviations were resolved when we analyzed the dark type separately for each location, with the exceptions of pairs Sme11–Sra16-5 and Sra15-8–Sra7-2, which remained significant in Camarones Bay and San José Gulf, respectively. The deviations remained significant for the light morphotype (pairs Sra15-8–Sal3 and Sal3–Ssc51).

The separation into two population clusters inferred by STRUCTURE was supported by a significant F_{ST} estimate

Fig. 2 Distribution of dark (gray) and light (open) *Sebastes* morphotypes off Argentina, based on different data sources. Panel *a* shows finer scale results around Península Valdés. Each symbol may represent more than one fish. Literature review included light fish cited in Cotrina et al. (1976), Nakamura et al. (1986), and recorded in the Barcode of Life Database (identified as *S. oculatus*): FARG191-06, FARG192-06, FARG193-06, FARG194-06, and FARG195-06. Acronyms for locations are as follows: SMG San Matías Gulf, PL Puerto Lobos, SJG San José Gulf, NG Nuevo Gulf, CA Camarones Bay, SC Caleta Olivia (Santa Cruz), AS Argentine shelf off Península Valdés, RD Ría Deseado



(0.041; p value <0.001), and the divergence between these groups and the outgroup species *S. constellatus* was much higher, with an F_{ST} of 0.222 with the dark morphotype and 0.208 with the light morphotype. Population structure in the *Sebastes* off the coast of Argentina was also clearly reflected in the FCA (77 % of the total variance explained, 60 % by the first two axes), in which the dark individuals clustered separately from the light ones (Fig. 4). Within the dark cluster, individuals collected at different coastal sampling locations (i.e., San Matías, Nuevo and San José gulfs, Camarones Bay, and off Santa Cruz) formed three closely related and overlapping subclusters, with some individuals from each location placed closer to individuals from another group than to their own.

Phylogenetic lineages

Nine distinct mtDNA haplotypes were recovered from the 10 partial control region sequences obtained. Three of them corresponded to previously published sequences (Atlan01, Atlan02, and Camar13 are similar to haplotypes Tristan05, Safrica01, and Gfnvo13, respectively), while the remaining six haplotypes were novel: Camar03, Camar09, Camar15, Camar20, Atlan06, and Atlan05/Atlan12 (the latter two are identical) (Online Resource 4).

Bayesian phylogenetic analysis yielded a tree highly consistent with that reported by Rocha-Olivares et al. (1999b). The tree recovered two major monophyletic clades supported by high posterior probability values that corresponded well with the austral *Sebastes* species: *S. capensis* and *S. oculatus*.

Within the latter, Atlantic and Pacific subclades (sensu Rocha-Olivares et al. 1999b) were also evident, but they were supported by only moderate posterior probabilities. In agreement with Rocha-Olivares et al. (1999b), haplotypes from Tristan da Cunha Island and South Africa were grouped into the *S. capensis* clade, ~60 % of the haplotypes from the Malvinas/Falklands Islands grouped into the Atlantic *S. oculatus* subclade and almost all of the haplotypes from the coasts of mainland Chile and Argentina grouped into the Pacific *S. oculatus* subclade (Fig. 4).

Three of the nine haplotypes recovered in this study—Atlan01, 02, and 06—fell within the *S. capensis* clade; four—Atlan05/12 and Camar09, 15, and 20—grouped with the Atlantic *S. oculatus* haplotypes; and the remaining two—Camar03 and 13—fell within the Pacific *S. oculatus* subclade (Fig. 5). The mtDNA haplotypes of the *S. oculatus* and *S. capensis* clades were not reciprocally monophyletic, as some light-colored fish, with a pigmentation pattern reminiscent of *S. capensis* and assigned to the light cluster on the basis of microsatellite data, fell within the *S. oculatus* clade. Specifically, within the genetic cluster (based on microsatellite loci) composed of dark fish, only *S. oculatus* mtDNA haplotypes (e.g., Camar03, 09, 13, 15, and 20) were found. However, in the light cluster, composed almost exclusively of individuals caught offshore, haplotypes similar to those of both *S. capensis* (e.g., Atlan01, Atlan02, and Atlan06) and *S. oculatus* (Atlan05/12) were found, the latter assigned to the *S. oculatus* Atlantic subclade (Fig. 4). Most fish carrying *S. oculatus* Atlantic subclade haplotypes were collected within Camarones Bay. Despite the observed color variation in dark rockfishes

Table 2 Microsatellite descriptive statistics for the samples of *Sebastes* off Argentina. Columns indicate the total number of alleles observed (Na), allelic richness (Ar), observed heterozygosity (H_o), expected heterozygosity (H_e), F_{IS} estimates following Weir and Cockerham (1984), and the significance of Hardy–Weinberg deficits. F_{IS} for loci Spi4 remained significant after sequential Bonferroni correction (indicated in bold)

Locus	Na	Ar	H_o	H_e	F_{IS}
Seb9	2.00	1.73	0.038	0.061	0.383
Sme13 ^a	1.00	1.00	0.000	0.000	NA
Sme4	20.00	12.05	0.845	0.861	0.020
Spi6	21.00	13.09	0.877	0.900	0.026
Spi14	11.00	7.34	0.713	0.797	0.105
Sra15-23	4.00	3.13	0.616	0.639	0.036
Sra15-8	7.00	6.38	0.667	0.725	0.081
Sth37	4.00	3.52	0.570	0.522	−0.093
Sal2 ^a	1.00	1.00	0.000	0.000	NA
Sal3	7.00	3.53	0.282	0.287	0.018
Seb25	6.00	4.08	0.474	0.506	0.063
Sme11	5.00	3.78	0.281	0.292	0.036
Sme3	18.00	7.84	0.548	0.554	0.010
Sme5	8.00	5.56	0.629	0.638	0.014
Spi4	6.00	2.75	0.063	0.518	0.879
Sra16-5	10.00	6.23	0.769	0.785	0.021
Sal4	5.00	4.08	0.321	0.322	0.004
Seb37	4.00	2.30	0.318	0.349	0.089
Spi12	2.00	2.00	0.300	0.313	0.043
Sra6-52	4.00	3.38	0.414	0.438	0.056
Sra7-2	17.00	9.34	0.811	0.819	0.010
Ssc1	3.00	2.12	0.214	0.231	0.074
Ssc51	16.00	8.20	0.740	0.736	−0.006
Sth45 ^a	1.00	1.00	0.000	0.000	NA
ALL ^b	8.57	5.35	0.500	0.538	0.089

^a Monomorphic loci

^b Average values across loci obtained from nonmonomorphic loci only

among and within sites, and although branch support in our mtDNA tree was moderate, the microsatellite analysis did not support the occurrence of a distinct Atlantic lineage within *S. oculatus*.

Discussion

Color morphotypes and bathymetric distribution

In this study, we report for the first time the occurrence of distinct color morphotypes in the nominal species *S. oculatus* off the coast of Argentina and describe differences in their distribution and genetic composition. These morphotypes were spatially segregated, with distribution patterns related to bottom depth. The dark morphotype was caught exclusively

in shallower waters <40–50 m depth (mostly <30 m), while the light one was generally found in deeper water, commonly >80–100 m. The occurrence of different color morphotypes in austral *Sebastes* has previously gone unnoticed, likely because surveys of these fishes in the Southwestern Atlantic were based exclusively on trawling over the inner shelf (>20–30 m depth; e.g., Menni and López 1984), or from shallow coastal areas within the northern Patagonian San Matías, San José, and Nuevo gulfs (e.g., Galván 2008; Galván et al. 2009a, b, 2012). The only mention of differences in pigmentation patterns of *Sebastes* off the coast of Argentina in the scientific or technical literature was in an identification guide that describes *S. oculatus* as being reddish, but darker, almost black, in shallow waters (Bovcon and Cochía 2007).

Additional evidence points to the observed pattern of two bathymetrically segregated morphotypes: dark-shallow versus light-deep fish. For example, rockfishes caught by trawling in offshore waters off Argentina are commonly confused with another Sebastid, “rubio”, *Helicolenus lahillei* Norman 1937 (Cousseau and Perrotta 2000), which is red with brown spots on the back and spines of the dorsal fin (Norman 1937; Cousseau and Perrotta 2000). Moreover, all rockfish caught offshore (between 42°29'S and 50°42'S; depth range >60–271 m) during an oceanographic survey conducted from November 1973 to January 1974 were identified as *H. lahillei* (Cotrina et al. 1976), although it is now well known that the southern distributional limit of *Helicolenus* in the Southwestern Atlantic is ~41°S (Otero et al. 1982; Cousseau and Perrotta 2000). Those fish were most likely misidentified light-colored *Sebastes* (see also Sánchez and Acha 1988). Nakamura et al. (1986) included in their description of *S. oculatus* a photograph of one individual corresponding to the light morphotype described here. The specimens examined by those authors were caught at depths between 95 and 148 m off Argentina and between 130 and 665 m off Chile. However, the collection number, and therefore the exact collection site, of the photographed specimen is not indicated in either the publication or in Nakamura’s original notes (Y. Kai, personal communication¹), but it was undoubtedly caught at a depth >90 m.

Genetic structure and phylogeography

Microsatellite data indicated that two genetic groups of *S. oculatus* are present in the Southwestern Atlantic Ocean off the coast of mainland Argentina, and these groups matched the observed differences in coloration and depth distribution. Bathymetric segregation between sister species with overlapping latitudinal distributions is indeed fairly common within the genus *Sebastes* (Hyde et al. 2008; Ingram 2011). Nevertheless, a large number of individuals displayed high

¹ Dr. Yoshiaki Kai, Maizuru Fisheries Research Station, Field Science Education and Research Center, Kyoto University

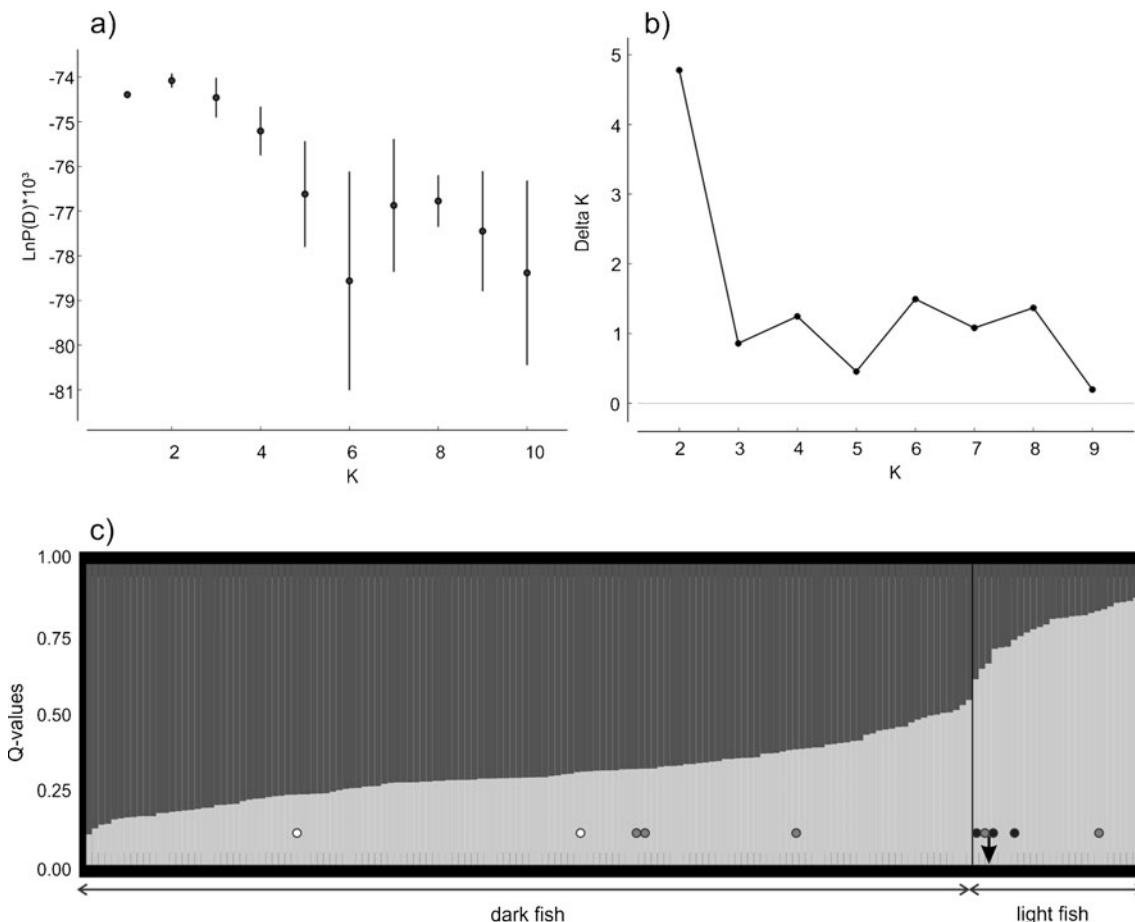


Fig. 3 Bayesian clustering analysis performed in STRUCTURE. **a** $\text{Ln } P(D)$, mean (\pm standard deviation) of log-likelihood values for each value of $K=1-10$ (10 independent runs per K), the number of putative populations. **b** Mean absolute difference of the second order rate of change with respect to K ($\Delta(K)$, following Evanno et al. 2005). **c** Proportional membership (sorted q -values) of Southern Atlantic Ocean rockfish in the two genetic clusters inferred by STRUCTURE.

Each bar represents an individual fish. Shading indicates the fraction of the individual's genotype attributed to cluster 1 (dark gray) and cluster 2 (light gray). The arrow indicates one individual caught within San Matías Gulf, at ~ 170 m depth (see the text for details). Individuals with mtDNA information are color-coded based upon mitochondrial clade membership (*S. capensis* lineage: black circles; *S. oculatus* Atlantic lineage: gray circles; *S. oculatus* Pacific lineage: white circles)

levels of admixture, suggesting recent and potentially ongoing hybridization. This, in turn, raises the question of what level of reproductive isolation exists across the distributional range of these two forms. It has been suggested that color polymorphism, in conjunction with internal fertilization, promotes assortative mating and may play a role in developing and maintaining reproductive isolation (Narum et al. 2004; Elmer et al. 2009).

It is possible that the pattern we describe arose not through incipient speciation in partial sympatry, but through secondary contact and hybridization between previously allopatric or otherwise divergent taxa. That explanation seems much less likely, though, given the relatively recent invasion of the Southern Hemisphere by the genus *Sebastes*, the lack of much phylogenetic diversity in Southern Hemisphere rockfishes, the very close relationship of the two taxa described here, the lack of strong disequilibrium or other characteristics of recent hybridization, and the presence of many similar incipient species pairs in other parts of this highly diverse genus.

Our finding of nominal *S. oculatus* populations in the Southern Hemisphere that are in the early stages of speciation, with distributions apparently stratified by depth, alongside another closely related species (*S. capensis*), suggests that the speciation processes in southern *Sebastes* involve similar mechanisms as those observed in the North Pacific and Atlantic oceans, where multiple closely related rockfish taxa live in close proximity, but are separated by depth and/or color patterns (e.g., Roques et al. 2001; Hyde et al. 2008).

The level of genetic divergence between color morphotypes of *S. oculatus* estimated from the microsatellite data in this study ($F_{ST}=0.041$) was higher than what has been observed for between-population comparisons of rockfish and nearly as high as that reported for some reproductively isolated sister species within the genus *Sebastes* ($F_{ST}=0.046$ between *Sebastes carnatus* and *Sebastes chrysomelas*, Narum et al. 2004; $F_{ST}=0.051$ between *Sebastes mystinus* types, Burford 2009). However, the degree of genetic differentiation found in

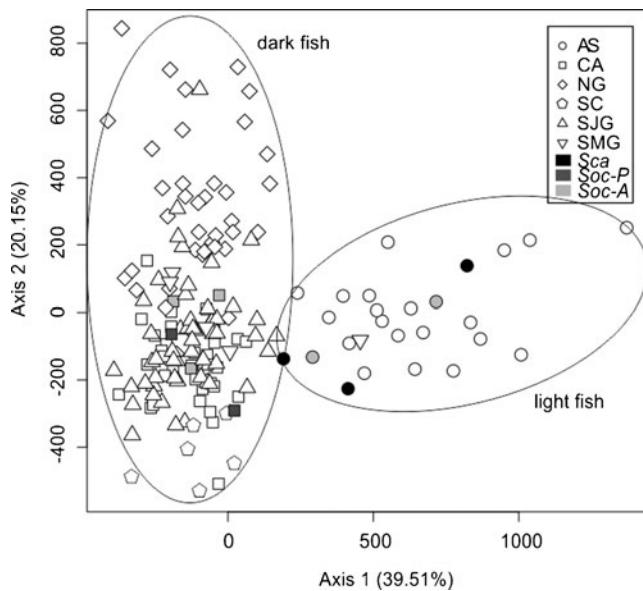


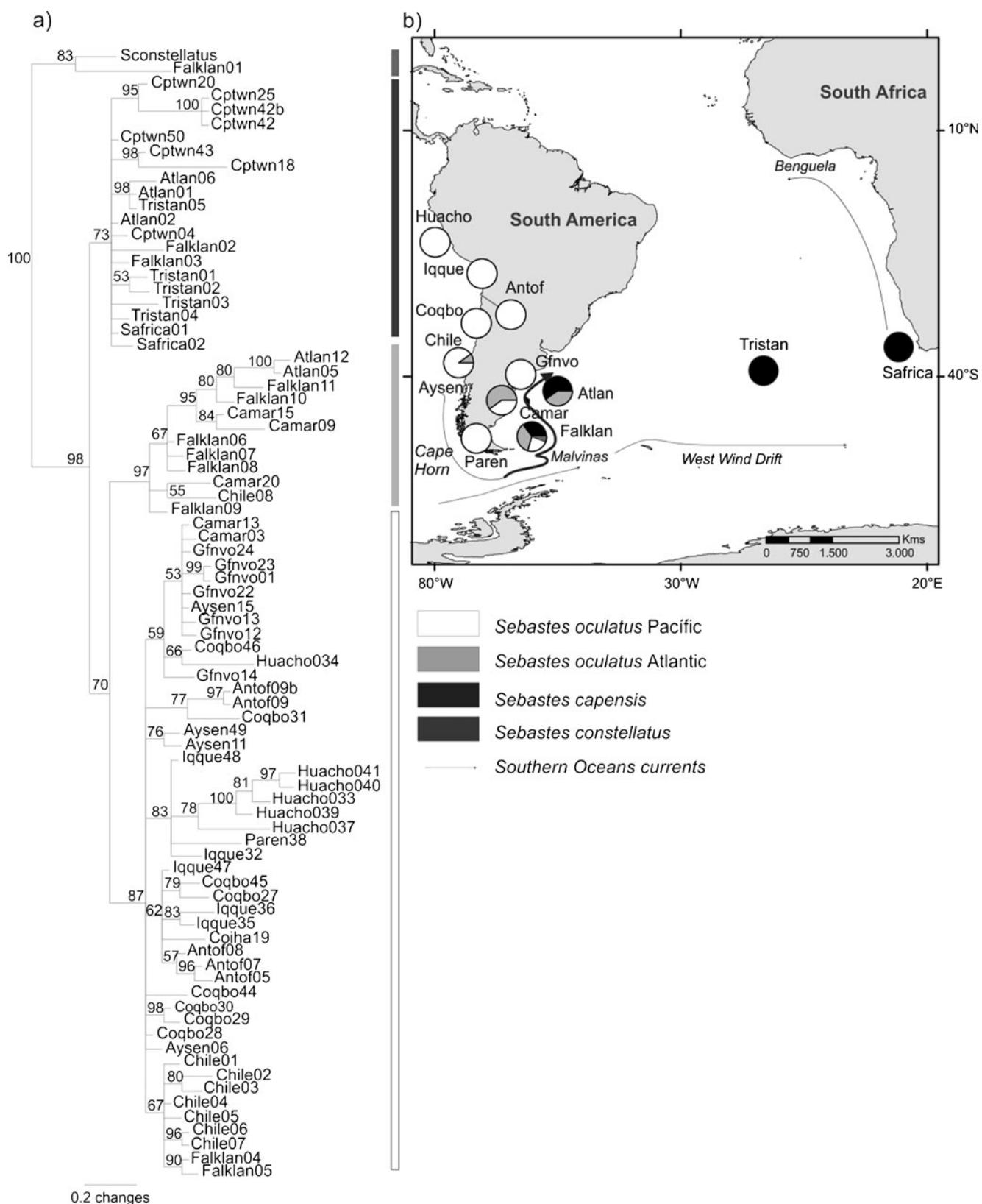
Fig. 4 Biplot representation of the 21 microsatellite-based FCA conducted on *Sebastes* specimens from the Southwestern Atlantic Ocean. Acronyms for locations as in Fig. 2. Individuals with mtDNA information are color-coded based upon mitochondrial clade membership (*SCA* *S. capensis* lineage, *SOC-P* *S. oculatus* Pacific lineage, and *SOC-A* *S. oculatus* Atlantic lineage). The ellipses indicate the two main clusters identified by STRUCTURE

this study does not support the hypothesis of complete reproductive isolation between the color morphotypes, as the value reported here is less than that found in pairwise comparisons of over 30 other pairs of species in the genus *Sebastes* (median $F_{ST}=0.347$; quartiles 0.281 and 0.422, data from Pearse et al. 2007). An alternate explanation for the low degree of genetic differentiation between the forms would be recent divergence between sister lineages. For example, divergence time estimates between *S. carnatus* and *S. chrysomelas*, and between the two forms of *S. mystinus*, ranged from ~500,000 to ~600,000 ybp, whereas the estimated divergence time between *S. constellatus* and the southern *Sebastes* species was approximately 650,000 ybp (Hyde and Vetter 2007; Burford et al. 2011). The estimated divergence time between the *S. capensis* and *S. oculatus* pair was even more recent, at ~344,000 ybp (Fig. 5 in Hyde and Vetter 2007). The divergence between the two forms of *S. oculatus* described here is likely even more recent and they are presumably undergoing incipient speciation. Incomplete lineage sorting between diverging lineages, such as those characterized by color morphotypes, due to recent descent from a large, genetically diverse, ancestral population could maintain genetic similarities between two distinct groups, resulting in genetic divergence lower than that between reproductively isolated species, but higher than expected between genetically differentiated populations (Arbogast et al. 2002).

Fig. 5 Subtree of Bayesian-derived phylogenetic tree based upon D-loop sequence data. Species other than *S. constellatus*, *S. capensis*, and *S. oculatus* are not shown. Branch lengths represent genetic distance and only measures of posterior support >50 are presented. Codes for published haplotypes, which identify sample locations, follow the designations given by Rocha Olivares et al. (1999b) and Nuñez et al. (2010). South Africa: Safrica; Tristan da Cunha: Tristan; Malvinas/Falkland Islands: Falklan; Valparaíso: Chile; Huacho: Huacho; Iquique: Ique; Antofagasta: Antof; Coquimbo, Coqbo; Aysen channels: Aysen; Punta Arenas: Paren; and Cape Town: Cptwn. Haplotypes generated in this study came from the Atlantic shelf off Península Valdés: Atlan and Camarones Bay: Camar. **b** Zoogeographic pattern of *Sebastes* lineages in the Southern Hemisphere. The pie charts depict the haplotype composition of the sampling sites. The occurrence of one fish from Malvinas/Falkland Islands having an *S. constellatus* haplotype was thoroughly discussed in Rocha Olivares et al. (1999b)

The mtDNA analysis provided strong support for the hypothesis of recent divergence between the dark and light color morphotypes described in this study. Phylogenetic analysis identified two distinct clades with high support largely partitioned by both coloration and depth. Three haplotypes, belonging to light-colored fish, clustered with sequences previously described for *S. capensis* collected from South Africa and Tristan da Cunha Island, while five haplotypes belonging to dark-colored individuals clustered with sequences described for *S. oculatus* collected off Argentina and Chile. Interestingly, the color morphotypes were not reciprocally monophyletic: two light-colored fish collected off Península Valdés and assigned with the microsatellite data to the light-off-shore cluster carried *S. oculatus* haplotypes. The weak phylogeographic signal within this lineage suggests long distance gene flow and considerable population connectivity along the South American coast, probably facilitated by both the Humboldt Current and West Wind Drift (Nuñez et al. 2010). Either incomplete lineage sorting due to recent divergence or one-way mtDNA introgression (Chan and Levin 2005) from *S. oculatus* into *S. capensis* might explain the observed discrepancy between nuclear and mitochondrial data, and both processes have been reported within *Sebastes* (e.g., Buonaccorsi et al. 2005; Gharrett et al. 2006; Hyde et al. 2008; Muto et al. 2011; Bunke et al. 2013). Power to elucidate patterns of introgression or incomplete lineage sorting in our study is limited, however, because of relatively small sample sizes. As previous mtDNA studies and this study are based on a small number of fish per sampling site, we cannot reject the possibility that a certain number of haplotypes were unsampled. Larger sample sizes thus might reveal that *S. capensis* and *S. oculatus* share more haplotypes than exposed here, resulting in a much lower mtDNA differentiation between both species.

The occurrence of light individuals off Argentina (above ~41°S), carrying *S. capensis* haplotypes, confirms that the *S. capensis* lineage is not restricted to the Tristan da



Cunha Island Group and South Africa (as stated by Eschmeyer and Hureau 1971), but instead is extensively distributed throughout the Southern Atlantic Ocean (Rocha-Olivares et al. 1999b). Based on their phylogenetic reconstruction, Rocha-Olivares et al. (1999b) concluded that the *S. capensis* clade is likely ancestral, a pattern consistent with our results, and hence predicted a dominance of *S. capensis* in the Southeastern Pacific, because that region would have been first colonized by the genus (Eschmeyer 1969; Eschmeyer and Hureau 1971). Intriguingly, no fish genetically assigned to the *S. capensis* lineage has been identified in Chilean or Peruvian waters (Rocha-Olivares et al. 1999a, b; Hyde and Vetter 2007; Nuñez et al. 2010), a pattern attributed to insufficient sampling (Rocha-Olivares et al. 1999b). However, in our review of color morphotypes (Online Resource 2), we found that several authors described *Sebastes* caught off Chile as having a reddish color pattern resembling the light morphotype (Jenyns 1840; Cunningham 1871; De Buen 1960; Kong Uribe 1985; Nakamura et al. 1986). The question of how many species of *Sebastes* there are in the Southeastern Pacific Ocean off Chile and Peru, and their identity, clearly merits further research.

More exhaustive sampling efforts are necessary to definitively resolve the phylogeographic history and taxonomy of *Sebastes* in the Southern Hemisphere, and such investigation should include collection of data on genetics, morphology, and habitat preferences. Nevertheless, our data revealed significant population structure and limitations to gene flow in rockfishes off Argentina, implying that the color morphotypes described in this study are undergoing the process of speciation. The distributional differences mean that the two groups are targeted by different fisheries using different gear: the dark-colored form is primarily angled and/or speared in recreational coastal fisheries along the northern and mid-Patagonian coast, whereas the light-colored rockfishes are mostly caught by trawlers offshore. The identification of two distinct groups of *Sebastes* off Argentina that are experiencing different fishery impacts, along with potentially significant differences in life history between the two forms, has implications for the design and implementation of appropriate fishery regulations to avoid overharvest of either group.

Acknowledgments We thank “Gringo” Durbas, M. San Emeterio, D. Galván, M. Delpiani, C. Fulvio Pérez, P. Useglio, G. Trobbiani, M. Trivellini, L. Rojas, L. Getino, M. López, L. Villanueva, J. Lancelotti, R. Kosaka, V. Apkenas, C. Columbus, R. Díaz, and the crew and scientific staff on board R/V “Puerto Deseado” for their help in the field or in the laboratory and/or for sample collection. Y. Huenchual, R. Huenchul, and E. Bahamonde helped us during the monitoring of Fiesta Nacional del Salmón de Mar (Camarones). N. Basso and M. Iglesias collaborated in mtDNA sequencing. We greatly appreciate the comments, photographs, and additional data generously provided by A. Rocha-Olivares, W. Eschmeyer, Y. Kai, J. Hyde, N. Muto, P. Heemstra, A. Irigoyen, “Lobo” Orensanz, D. Fernández, N. Bovcon, G. Piacentino, H. López, P. Toledo, E. Niklitschek, and M. Ehrlich. E. Crandall, the editor, and three anonymous reviewers made useful comments on an earlier version of the article. This work was supported

by grants from the ANPCyT (PICT 2006–1468) and CONICET (PIP 2010–2012) (both granted to LAV) and from Secretaría de Ciencia, Tecnología e Innovación de la Provincia de Chubut 2010–2011 (granted to P. Useglio, UNPSJB). Field work was partially conducted within a World Natural Heritage Site and authorized by Dirección General de Conservación de Áreas Protegidas del Chubut.

Conflict of interest The authors declare that they have no conflict of interest.

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Online Resource 1 List of material examined, deposited in the ichthyological collection of the Centro Nacional Patagónico (CNPICT). One dark reddish fish caught at Ría Deseado (Lot ID 1970/4) could not be assigned to either of the color morphotypes described

Species	Lot ID (CNPICT)	N	Length (mm TL)	Color morphotype	Capture location
<i>S. capensis</i>	1988 / 5	1	251	-	Tristan da Cunha Island, Mid Atlantic (RUSI Collection #34603)
	1994 / 1	1	217	-	Coast of Gough Island, Mid Atlantic (RUSI Collection #44807)
<i>S. oculatus</i>	1970 / 4	1	166	Undefined	Puerto Deseado Pier, Ría Deseado, Santa Cruz (47°45'S 65°53'W)
	1969 / 49	1	235	Light	Off Río Negro (41°18'S 64°55'W)
	1972 / 12	1	215	Light	Cañadón de las Brótolas, Ría Deseado, Santa Cruz (47°45'S 65°53'W)
	1972 / 13	1	136	Light	Puerto Deseado, Ría Deseado, Santa Cruz (47°45'S 65°53'W)
	1972 / 14	1	235	Light	Puerto Deseado, Ría Deseado, Santa Cruz (47°45'S 65°53'W)
	1993 / 49	2	277–384	Light	Off Chubut (Fish #10, 11)
	1994 / 19	2	349–375	Light	Argentine Sea
	1994 / 20	3	308–351	Light	Argentine Sea
	2001 / 26	1	243	Light	Argentine Sea, off-shore (45°50'S 61°08'W)
	2001 / 44	1	247	Light	Argentine Sea
	2002 / 08	2	273–312	Light	Off Caleta Sara, Chubut
	2003 / 18	1	320	Light	San Jorge Gulf, off Santa Cruz
	2003 / 57	2	139–313	Light	Argentine Sea
	2009 / 15	2	253–285	Light	off Chubut (43°33'S 60°11'W)
	2009 / 17	1	135	Light	San Matías Gulf, off Chubut (42°04'S 64°22'W)
1984 / 12	1	252	Dark	Cracker Bay, Golfo Nuevo, off Chubut	
1993 / 47	16	210–300	Dark	Golfo Nuevo, off Chubut (Fish #23–30, 32, 34, 36–39, 41, 42)	
1993 / 48	2	234–275	Dark	Golfo Nuevo, off Chubut	
1994 / 24	2	205–261	Dark	Argentine Sea	

Species	Lot ID (CNPICT)	N	Length (mm TL)	Color morphotype	Capture location
<i>S. oculatus</i> (Cont.)	1994 / 33	16	253–349	Dark	Caleta Olivia, off Santa Cruz (42°14'S 67°30'W) (Fish #w/n, 52–56, 58–64, 67, 72, 74)
	1994 / 38	2	118–305	Dark	Punta Quiroga, San José Gulf, Península Valdés (42°14'S 64°27'W)
	1995 / 27	2	305–323	Dark	Punta San Román, San José Gulf, Península Valdés (42°15'S 64°14'W)
	1996 / 21	1	207	Dark	San José Gulf, Península Valdés
	2004 / 11	1	137	Dark	Punta Este, Nuevo Gulf, off Chubut
	2007 / 2	4	210–267	Dark	Fracasso Beach, San José Gulf, Península Valdés (42°25'S 64°09'W)
	2007 / 3	3	219–265	Dark	Fracasso Beach, San José Gulf, Península Valdés (42°25'S 64°09'W)

Title Genetic structure and different color morphotypes suggest the occurrence and bathymetric segregation of two incipient species of *Sebastes* off Argentina, southwestern Atlantic

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Authors Leonardo A. Venerus, Javier E. Ciancio, Carla Riva-Rossi, Elizabeth A. Gilbert-Horvath, Atila E. Gosztonyi and John C. Garza

Affiliation Centro Nacional Patagónico – Consejo Nacional de Investigaciones Científicas y Técnicas (CENPAT – CONICET) (LAV)

E-mail leo@cenpat.edu.ar (LAV)

Online Resource 2 Summary statistics and references for 24 *Sebastes* microsatellite loci amplified in *Sebastes* from the Southwestern Atlantic (off Argentina). Each panel represents a set of markers electrophoresed together on an ABI377 sequencer. Details of PCR conditions and reaction volumes are available from the authors upon request

Panel	Locus	Fluorescent Dye Label	Repeat unit	Size range (bp)	Number of alleles	Multiplex PCR Combination	<i>Sebastes</i> species in which marker isolated	Marker Reference
1	Seb9	TET	dinucleotide	085-087	2	Sme13, Seb9	Atlantic redfish complex	Roques et al. 1999
	Sme13	6-FAM	tetranucleotide	115-131	4	Sme13, Seb9	<i>S. melanops</i>	L. Seeb, ADFG unpublished
	Sme4	TET	tetranucleotide	236-328	22	Sme4, Sra15-23	<i>S. melanops</i>	L. Seeb, ADFG unpublished
	Spi6	TET	non-stepwise	110-199	25	Spi6, Sth37	<i>S. pinniger</i>	Gómez-Uchida et al. 2003
	Spi14	HEX	tetranucleotide	270-412	20		<i>S. pinniger</i>	Gómez-Uchida et al. 2003
	Sra15-23	HEX	non-stepwise	114-127	5	Sme4, Sra15-23	<i>S. rastrelliger</i>	Westerman et al. 2005
	Sra15-8	6-FAM	tetranucleotide	290-330	8		<i>S. rastrelliger</i>	Westerman et al. 2005
	Sth37	6-FAM	dinucleotide	239-247	5	Spi6, Sth37	<i>S. thompsoni</i>	Sekino et al. 2000
2	Sal2	6-FAM	undetermined	095-123	2	Sal2, Spi4	<i>S. alutus</i>	Miller et al. 2000
	Sal3	HEX	pentanucleotide	098-153	9	Sal3, Sme3	<i>S. alutus</i>	Miller et al. 2000
	Seb25	HEX	dinucleotide	183-197	7	Seb25, Sme11	Atlantic redfish complex	Roques et al. 1999
	Sme11	TET	tetranucleotide	109-145	9	Seb25, Sme11	<i>S. melanops</i>	L. Seeb, ADFG unpublished
	Sme3	HEX	tetranucleotide	226-370	29	Sal3, Sme3	<i>S. melanops</i>	L. Seeb, ADFG unpublished
	Sme5	6-FAM	tetranucleotide	250-314	11		<i>S. melanops</i>	L. Seeb, ADFG unpublished
	Spi4	6-FAM	tetranucleotide	135-271	10	Sal2, Spi4	<i>S. pinniger</i>	Gómez-Uchida et al. 2003
	Sra16-5	TET	tetranucleotide	190-234	11		<i>S. rastrelliger</i>	Westerman et al. 2005

Panel	Locus	Fluorescent Dye Label	Repeat unit	Size range (bp)	Number of alleles	Multiplex PCR Combination	<i>Sebastes</i> species in which marker isolated	Marker Reference
3	Sal4	6-FAM	tetranucleotide	079-119	8		<i>S. alutus</i>	Miller et al. 2000
	Seb37	HEX	dinucleotide	215-223	5	Seb37, Sra6-52, Sra7-2	Atlantic redfish complex	Roques et al. 1999
	Spi12	TET	dinucleotide	096-104	3	Spi12, Sth45	<i>S. pinniger</i>	Gómez-Uchida et al. 2003
	Sra6-52	HEX	dinucleotide	123-137	6	Seb37, Sra6-52, Sra7-2	<i>S. rastrelliger</i>	Buonaccorsi unpublished
	Sra7-2	HEX	dinucleotide	123-181	18	Seb37, Sra6-52, Sra7-2	<i>S. rastrelliger</i>	Westerman et al. 2005
	Ssc1	6-FAM	non-stepwise	244-255	6	Ssc1, Ssc51	<i>S. schlegeli</i>	Yoshida et al. 2005
	Ssc51	6-FAM	dinucleotide	151-187	16	Ssc1, Ssc51	<i>S. schlegeli</i>	Yoshida et al. 2005
	Sth45	TET	dinucleotide	193-195	2	Spi12, Sth45	<i>S. thompsoni</i>	Sekino et al. 2000

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E-mail leo@cenpat.edu.ar (LAV)

Online Resource 3 Original descriptions of pigmentation patterns in *Sebastes* from the Southern Hemisphere

Sampling site	Capture depth	Color description	Preserved (P) or Fresh (F)	Reference
Cape of Good Hope, South Africa	W/d	<i>S. capensis</i> : “Dans leur état actuel, ces sébastes du Cap paraissent rougeâtres, avec des mouchetures blanchâtres, nombreuses sur la tête et sur le dos, et une teinte noire sur l'opercule; mais nous ne savons pas quelle était leur couleur dans l'état frais; cependant tout porte à croire qu'elle était rouge.”	P	Cuvier and Valenciennes (1829)
Cape of Good Hope, South Africa	W/d	<i>S. capensis</i> : “Cette Sébaste paraît rougeâtre dans son état vivant, avec des nuances jaunes et de nombreuses mouchetures blanches sur le dos. La queue est d'un rougeâtre plus intense à sa terminaison. L'œil est grand, jaune et bordé de rouge.”	F	Quoy and Gaimard (1835)
Seas around the Cape of Good Hope, South Africa,	W/d	<i>S. capensis</i> : “The upper surface of the head, its sides to the operculum, and the body above the lateral line, a tint intermediate between chestnut-brown and purplish red, which towards the caudal fin passes into light reddish orange. The edges of most of the scales are of a light pearly colour, and in addition to these slight variegations, there occur also a few irregular-shaped flesh-red spots distinctly indicated in the fresh fish. Lower parts of body below lateral line and hinder portion of operculum buff-orange shaded with aurora-red, and towards lateral line faintly clouded with crimson-red; the point of operculum carmine-red. Lower jaw, lower portion of operculum, space in front of pectoral and ventral fins, and the upper lips intermediate between brownish purple-red and lake-red. Dorsal fin between lake-red and chestnut-brown. Pectoral, ventral, and caudal fins aurora-red, the two first rayed with lake-red only very faintly towards lower part of pectorals, the caudal is rayed with light orange-coloured brown, and tinged with lake-red. Anal fin pale buff-orange rayed with brownish purple-red. Eyes bright brownish orange, with incomplete rings of bright gall-stone yellow and King's-yellow.”	F	Smith (1845)
Cape of Good Hope, South Africa	W/d	<i>S. capensis</i> : “Tile-red, with shades of orange, white, and yellowish-green; marked on the sides with a flesh-colored spots. Belly white, tinged with orange”.	F	Pappe (1853)
Cape Seas	W/d	<i>S. capensis</i> : “Reddish.”	P	Günther (1860)

Sampling site	Capture depth	Color description	Preserved (P) or Fresh (F)	Reference
Tristan da Cunha Island	<i>Discovery</i> St. 4, 40 m	<i>S. capensis</i> : “The coloration is described by Smith (1845), J.L.B. Smith (1949), and by Barnard (1927). Barnard says the colour is red, shading to orange below; several silvery-white or pinkish irregular spots on the sides above the lateral line; fins red; membrane of spinous dorsal fin mottled with brown; iris golden. The coloration of the preserved specimen (Fig. 13c) agrees with that described by Norman (1937, pp. 122-123) for a specimen of <i>S. oculatus</i> from Chile, with an indication of pale areas on the sides located in the same areas as shown schematically by Norman (1937, fig. 67). This also agrees with the pale areas shown by Smith (1845, fig. 1) and J.L.B. Smith (1949, pl. 85) for specimens of <i>S. capensis</i> from South Africa. The upper part of the back is dark between the pale areas.”	P / F	Eschmeyer (1969)
Tristan da Cunha Island; Cape Town, South Africa (1)	(1) 10 m (SIO 57-34)	<i>S. capensis</i> (this author referred all <i>Sebastes</i> from the Southern Hemisphere as <i>S. capensis</i>): “The Tristan da Cunha and Cape Town specimens are all light brown, but the light colour is likely due to preservation, as the African form has been illustrated by Smith (1953) as being dark dorsally and red ventrally.”	P	Chen (1971)
South Africa	W/d	<i>S. capensis</i> : “Reddish to brownish, with 5-6 pale spots on upper back.”	W/d	Smith and Heemstra (1991)
Tristan da Cunha and Gough Islands	W/d	<i>S. capensis</i> : “Variable, ranking from gold, through red, to orange with 5 or 6 pale spots on body at base of dorsal fin.”	W/d	Andrew et al. (1995)
Valparaíso, Chile	W/d	<i>S. oculatus</i> : “Le dessin que M. Gay nous a montré, la peint en rouge brun sur le dos, et en rose argenté sous le ventre. Quatre taches rosées brillent sur le brun du dos à la base de la dorsale. La première au pied du quatrième rayon épineux; la seconde sous le neuvième; la troisième au commencement de la portion molle, et la quatrième sous le dernier rayon de la nageoire: une cinquième tache est placée sur les flancs, à la hauteur de l'épaule et entre les deux premières taches. Les nageoires sont brunes, plus ou moins foncées et bordées de rose assez vif.”	W/d	Cuvier and Valenciennes (1833)
Valparaiso, Chile	W/d	<i>S. oculatus</i> : “Under surface, sides, branchial covering, and part of the fins, ‘tile and carmine red;’ dorsal scales pale yellowish dirty brown.” – D. In its present dried state, the colour is of a uniform brown. “It may be added further, that Mr. Darwin's notes make no mention of the four brilliant rose-coloured spots along the base of the dorsal fin, spoken of by Valenciennes.”	F/P	Jenyns (1840)

Sampling site	Capture depth	Color description	Preserved (P) or Fresh (F)	Reference
Fortune Bay, Chile (52°17'S 73°43'W)	W/d	<i>S. oculatus</i> : "In the evening one of the men caught a handsome species of <i>Sebastes</i> (<i>S. oculatus</i>), of a fin scarlet colour. This fish had been previously recorded from Valparaiso, so that it is distributed over more than twenty degrees of latitude."	F	Cunningham (1871)
Baverstock Island, Chile (2); ~300 km off the coast of Santa Cruz, at ~50 km S of Ría Deseado, Argentina (3); Islas Malvinas / Falkland Islands (4)	(2) 22 m; (3) 137 – 139 m; (4) 102 – 106 m.	<i>S. oculatus</i> : "Brownish; the back mottled with darker, the pigment tending to be concentrated into 4 or 5 dark blotches; 4 or 5 more or less definite rounded pale (pink in life) spots on each side, 3 or 4 immediately below the dorsal fin and another on the lateral line about level with the eighth and ninth dorsal spines; membrane of dorsal fin more or less dusky."	P	Norman (1937)
Montemar, Chile	W/d	<p><i>S. oculatus oculatus</i>: "En machos y hembras el color del cuerpo es amarillo oro con superposición de manchas negras y blancas; vientre y región ventral rojo minio, extendido a las aletas pares. En el lomo, por encima de la línea lateral, cinco zonas intensamente negras, que en ocasiones pueden desvanecerse, penetrando en ellas el tono amarillento de fondo; la más avanzada mancha la nuca y el comienzo de la dorsal, las siguientes se distribuyen detrás, mediada la zona espinosa de la dorsal y a su término sin llegar al final, hay otra en la parte blanda y la última, menos amplia, sobre el pedúnculo caudal.</p> <p>Manchas blanco puro, en otros ejemplares rosadas, se extienden por el lomo, una de ellas al pie de la reunión entre espinas y radios de la dorsal, otra, difusa, hacia mitad de la parte espinosa, menos aparente la tercera implantada al comenzar el pedúnculo caudal y la cuarta justo encima de la línea lateral, apenas visible.</p> <p>En la región ventral y en los flancos del cuerpo, bajo la línea lateral, destacan sobre el fondo amarillo, halos negruzcos y fina puntuación blanca ordenada en series longitudinales.</p> <p>Cabeza y labios negro rojizo, las membranas entre el premaxilar y el maxilar, y entre éste y el antorbitario, de color oliva. Cavidad bucal, en unos casos blanca con espacios amarillos o en otros con paladar ennegrecido y espacios blancos y verde oliva.</p> <p>Pectorales, por arriba oliváceo oscuro o rojo ennegrecido, con la mitad externa de los radios digitiformes blanco rosado pálido. Espinas y radios de las ventrales rojos y membrana entre ellos negro oliváceo.</p>	F	De Buen (1960)

Sampling site	Capture depth	Color description	Preserved (P) or Fresh (F)	Reference
		<p>Dorsal y caudal obscuras, prolongación del colorido del cuerpo, con la parte apical de las espinas dorsales suavemente rosada, pudiendo tener en ocasiones una zona central olivácea; las membranas interraciales de la caudal oliva oscuro.”</p> <p><i>S. oculatus darwini</i> (synonym of <i>S. oculatus</i>): “Uno de los ejemplares era de color rojo sangre, entremezclado en el lomo con negro, amarillo y rojo parduzco; membranas entre el maxilar y el premaxilar verde oliva; ventrales, parte avanzada de la anal y borde inferior de la caudal de rojo más intenso que el resto del pez.</p> <p>Otro de los ejemplares era rojo, ennegrecido en la cabeza, sobre el lomo, en los flancos y las aletas, con iris azul y plateado.”</p> <p><i>S. prognathus</i> (synonym of <i>S. oculatus</i>): “Rojo ladrillo negruzco, más oscuro el dorso de la cabeza y el lomo. Labios negruzcos, rojizos el maxilar y la parte baja de las piezas operculares.</p> <p>Membrana branquióstega blanco sucio con los labios ligeramente rosados. Aletas con el color negruzco del cuerpo.”</p> <p><i>S. hernandezi</i> (synonym of <i>S. oculatus</i>): “Negro oscuro uniforme, lo mismo el cuerpo que la cabeza y las aletas. También negros la mucosa bucal y el peritoneo.”</p> <p><i>S. oculatus oculatus</i>: “Tonalidad café con vientre más pálido; en el lomo, más oscuro, espacios blanquecinos al pie de la dorsal, los más avanzados de menor amplitud que el tercero colocado en los límites de la parte espinosa y blanda de la dorsal; bajo la mancha pálida media, inmediatamente sobre la línea lateral, hay un espacio blanquecino menos aparente. En los flancos red oscura de líneas inclinadas de atrás adelante, dejando reducidos espacios claros entre sus mallas. Aletas de color semejante al cuerpo, exentas de manchas. Cavidad bucal parduzca.”</p> <p><i>S. oculatus darwini</i> (synonym of <i>S. oculatus</i>): “Dorso, flancos del cuerpo y cabeza café claro. Manchas oscuras irregulares, invadidas por el color de fondo, se extienden por la parte dorsal, la más avanzada sobre la nuca, las dos siguientes, separadas o confundidas en una sola muy amplia bajo la parte espinosa de la dorsal, invadiendo los flancos hasta descender atravesando la línea lateral la mancha más adelantada de la pareja o la parte anterior de la mancha única; sigue otra bajo la dorsal blanda y la última en la parte alta del pedúnculo caudal. Estas manchas oscuras dejan espacios claros, cuatro en el borde del lomo y una más, que puede ser apenas aparente, a nivel del segundo e inmediatamente encima de la línea lateral. En la cabeza se oscurece la parte postorbitaria y superior del opérculo, el borde inferior de la mejilla en forma de banda y menos</p>	P (formalin)	De Buen (1960) (Cont.)

Sampling site	Capture depth	Color description	Preserved (P) or Fresh (F)	Reference
		<p>intensamente los labios. Aletas, también oscurecidas, sin manchas especiales, más pálida la anal.”</p> <p><i>S. prognathus</i> (synonym of <i>S. oculatus</i>): “Lomo y cabeza café oscuro, labios ennegrecidos, también la región postorbitaria, el opérculo y la parte baja de las mejillas; vientre blanquecino y pálidos los radios digitiformes de las pectorales. En el ejemplar de más talla hay manchitas negruzcas bajo las pectorales, en la base de esas aletas y al pie de la anal.”</p> <p><i>S. unimaculatus</i> (synonym of <i>S. oculatus</i>): “Blanco lechoso. En el lomo nubosidades negruzcas con grandes espacios blancos interpuestos, la primera zona manchada está en la mitad anterior de la dorsal espinosa, la siguen más débiles bajo la parte blanda de la misma aleta y la última en el dorso del pedúnculo caudal. Son incoloras o blanco lechoso la caudal, la anal y las ventrales. En el limbo de las pectorales, en la parte ocupada por los radios ramosos, dos espacios suavemente ennegrecidos en el lado izquierdo del cuerpo, inexistentes en el lado opuesto. Borde superior de las espinas dorsales y sus membranas incoloros, el resto suavemente oscurecido con vermiculaciones blancas, existentes también en la parte baja de la dorsal blanda. Mancha intensamente negra en la mitad inferior del final de la dorsal, comprendida entre los radios décimo y último. Dorso de la cabeza suavemente ennegrecido, también las piezas operculares; labios incoloros; mentón manchado de negro.”</p>		De Buen (1960) (Cont.)
Callao, Perú (5); Montemar, Chile;	(5) 15-19 m (SIO 65-617)	<i>S. capensis</i> (this author referred all <i>Sebastes</i> from the Southern Hemisphere as <i>S. capensis</i>): “The South American species in isopropyl alcohol are all black, suggesting a rather dark color in life.”	P (isopropyl alcohol)	Chen (1971)
Chilean coast, 18°28'-50°50'S	W/d	<i>S. capensis</i> : (this author referred all <i>Sebastes</i> off Chile as <i>S. capensis</i>) “Pardo oscuro en la parte superior, haciéndose más claro hacia los flancos, casi abruptamente pasa a blanco en la parte ventral, partiendo de una línea trazada a nivel del origen del radio inferior de la pectoral, hasta llegar a la aleta anal. Por detrás de la aleta anal el color es rojo. Región gular y pecho, rojizos. Aleta dorsal y caudal, pardo amarillento. Mitad superior de la aleta pectoral, pardo oscuro. Tercio íñferodistal, negruzco, anteriormente rojizo. Pélvicas rojo-parduzca. Anal rojiza, especialmente hacia la porción distal, en ejemplares de 30 cm. En un especímen de 20 cm, los tonos, en general, son más oscuros y la pectoral es pardo uniforme. Área anal y postanal, anaranjada.”	W/d	Kong Uriba (1985)

Sampling site	Capture depth	Color description	Preserved (P) or Fresh (F)	Reference
Off Argentina, 48°00'-54°15'S and 62°00'-65°27'W (6), and Chile 41°19'- 52°45'S and 74°20'- 75°20'W (7)	(6) 95- 148 m; (7) 130- 665 m	<i>S. oculatus</i> : "Five dark blotches on dorsal part of body. Four to five spots on lateral part of body."	W/d	Nakamura et al. (1986)

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Title Genetic structure and different color morphotypes suggest the occurrence and bathymetric segregation of two incipient species of *Sebastes* off Argentina, southwestern Atlantic

Journal Naturwissenschaften

Authors Leonardo A. Venerus, Javier E. Ciancio, Carla Riva-Rossi, Elizabeth A. Gilbert-Horvath, Atila E. Gosztonyi and John C. Garza

Affiliation Centro Nacional Patagónico – Consejo Nacional de Investigaciones Científicas y Técnicas (CENPAT – CONICET) (LAV).

E-mail leo@cenpat.edu.ar (LAV)

Online Resource 4 Cont.

Accession number or reference	Haplotype	1	6	6	6	7	7	7	9	0	1	3	4	4	5	8	9	2	3	3	3	4	4	5	6	7	7	8	8	9	9	0	0	1	1	3	3	3	3	3	3	3	3	4	4	4
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KC797497	Atlan05	A	T	C	G	.	.	A	.	.	G	.	.	.	T									
KC797498	Atlan06	A								
KC797497	Atlan12	A	T	C	G	.	.	A	.	.	G	.	.	.	T										
KC797499	Camar03	A	.	.	G	.	.	G	.	.	.										
KC797500	Camar09	A	T	C	A	.	.	G	G	.	.	T													
KC797501	Camar13	A	.	.	G	G	.	.	.												
KC797502	Camar15	T	C	G	.	A	.	.	G	G	.	.	T															
KC797503	Camar20	C	G	.	A	T																

Online Resource 4 Cont.

Online Resource 4 Cont.

Accession number or reference		5	5	5	5	5	5	5	5	5
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		5	4	0	2	9	5	1	5	6
AF031503	<i>S. capensis</i>	T	T	C	G	T	-	-	-	-
AF031502	<i>S. oculatus</i>	-	-	-	-
GU136683	Antof05	C	T	G	T	T
GU136684	Antof07	T	T	G	T	T
GU136685	Antof08	T	T	G	T	T
GU136686	Antof09	T	T	G	T	T
GU136687	Antof9b	T	T	G	T	T
GU136680	Gfnvo01	-	-	-	-	-	-	-	-	-
GU136676	Gfnvo12	T	A	G	T	T
GU136678	Gfnvo13	T	T	G	G	T
GU136677	Gfnvo14	T	T	G	T	T
GU136679	Gfnvo22	-	-	-	-	-	-	-	-	-
GU136681	Gfnvo23	-	-	-	-	-	-	-	-	-
GU136675	Gfnvo24	-	-	-	-	-	-	-	-	-
GU136719	Aysen06	.	.	.	G	T	T	G	T	T
GU136720	Aysen11	T	T	G	T	T
GU136716	Aysen15	T	T	G	T	T
GU136718	Aysen19	.	-	-	-	-	-	-	-	-
GU136717	Aysen49	T	T	G	T	T
GU136706	Coqbo27	T	T	G	T	T
GU136703	Coqbo28	T	T	G	T	T
GU136704	Coqbo29	T	T	G	T	T
GU136702	Coqbo30	T	T	G	T	T
GU136705	Coqbo31	T	T	G	T	T
GU136708	Coqbo44	T	T	G	T	T
GU136707	Coqbo45	T	T	G	T	T
GU136710	Coqbo46	T	T	G	T	T
GU136712	Iorque32	C	.	.	T	G	T	T	G	T
GU136714	Iorque35	T	T	G	T	T
GU136715	Iorque36	T	T	T	T	C
GU136713	Iorque47	T	T	G	T	T
GU136711	Iorque48	T	T	G	T	T
GU136682	Paren38	C	A	.	.	T	T	G	T	T
GU136696	Huacho033	C	.	.	.	T	T	G	T	T
GU136698	Huacho034	T	T	G	T	T
GU136697	Huacho037	T	T	G	T	T
GU136699	Huacho039	C	A	.	.	T	T	G	T	T
GU136700	Huacho040	C	A	.	.	T	T	G	T	T
GU136701	Huacho041	T	T	G	T	T

Online Resource 4 Cont.

Accession number or reference	Haplotype	5	5	5	5	5	5	5	5	5
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GU136691	Cptwn20	-	-	-	-	-	-	-	-	-
GU136693	Cptwn25	T	T	G	T	T
GU136694	Cptwn42	T	T	G	T	T
GU136695	Cptwn42b	T	T	G	T	T
GU136689	Cptwn43	T	T	G	T	T
GU136690	Cptwn50	T	T	G	T	T
Rocha-Olivares et al. 1999	Safrica01	-	-	-	-	-
	Safrica02	-	-	-	-	-
	Tristan01	-	-	-	-	-
	Tristan02	-	-	-	-	-
	Tristan03	-	-	-	-	-
	Tristan04	-	-	-	-	-
	Tristan05	-	-	-	-	-
	Falklan01	-	-	-	-	-
	Falklan02	-	-	-	-	-
	Falklan03	-	-	-	-	-
	Falklan04	-	-	-	-	-
	Falklan05	-	-	-	-	-
	Falklan06	.	.	T	.	-	-	-	-	-
	Falklan07	.	.	T	.	-	-	-	-	-
	Falklan08	.	.	T	.	-	-	-	-	-
	Falklan09	-	-	-	-	-
	Falklan10	.	.	T	.	-	-	-	-	-
	Falklan11	.	.	T	.	-	-	-	-	-
	Chile01	-	-	-	-	-
	Chile02	-	-	-	-	-
	Chile03	-	-	-	-	-
	Chile04	-	-	-	-	-
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	Chile06	-	-	-	-	-
	Chile07	-	-	-	-	-
	Chile08	-	-	-	-	-
KC797495	Atlan01	T	T	G	T	T
KC797496	Atlan02	T	T	G	T	T

Online Resource 4 Cont.

Accession number or reference	Haplotype	5	5	5	5	5	5	5	5	5	5
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		5	4	0	2	9	5	1	5	6	7
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KC797498	Atlan06	.	.	T	.	.	T	T	G	T	T
KC797497	Atlan12	G	T	T	G	T	T
KC797499	Camar03	.	.	T	.	.	T	T	G	T	T
KC797500	Camar09	T	T	G	T	T
KC797501	Camar13	-	-	-	-	-	-	-	-	-	-
KC797502	Camar15	.	.	T	.	.	T	T	G	T	-
KC797503	Camar20	T	T	G	T	T

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