Are the Subgenera of *Sebastes* Monophyletic?

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Abstract

We examined genetic relationships among *Sebastes* rockfishes to evaluate the subgeneric relationships within *Sebastes*. We analyzed restriction site variation (12S and 16S rRNA and NADH dehydrogenase-3 and -4 genes) by using parsimony and distance analyses. Seventy-one *Sebastes* species representing 16 subgenera were included. Thirteen subgenera were represented by more than one species, and three subgenera were monotypic. We also evaluated three currently unassigned species. The only monophyletic subgenus was *Sebastomus*, although some consistent groups were formed by species from different subgenera. The northeastern Pacific species of *Pteropodus* clustered with one northeastern Pacific species of the subgenus *Mebarus* (*S. atrovirens*) and two northeastern Pacific species of the subgenus *Auctospina* (*S. auriculatus* and *S. dalli*) forming a monophyletic group distinct from northwestern Pacific

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Pteropodus species. The subgenera *Acutomentum* and *Allosebastes* were polyphyletic, although subsets of each were monophyletic. *Sebastes polyspinis* and *S. reedi*, which have not yet been assigned to subgenera, are closely related to two other northern species, *S. crameri* (subgenus *Eosebastes*) and the *S. ciliatus/variabilis* complex (subgenus *Sebastosomus*), which differed from other species assigned to their subgenera. These and other molecular studies show promise in determining the phylogenetic relationships among *Sebastes* species.

Introduction

The genus *Sebastes* is a species-rich and ecologically diverse group. Currently about 100 species are recognized; members of this genus are currently assigned to 21 subgenera, including six that are monotypic (Kendall 2000). Historically, morphological, meristic, and morphometric characters have been used for species identification and subgeneric determination. Abundant variation in many characters has provided means to separate most morphologically similar species. However, some characters that are subjective and possibly adaptive, are inappropriate for cladistic analysis (Li et al. 2006a). Consequently, the current subgeneric groupings need to be reevaluated using alternative methods that consider characters more suitable for cladistic analysis. Molecular methods have been used to test the validity of some subgenera of *Sebastes*. Comparison of sequences of a mitochondrial cytochrome *b* gene led to the conclusion that the subgenus *Sebastomus* as defined by Chen (1971) was monophyletic (Rocha-Olivares et al. 1999). All members of a monophyletic group descend from a common ancestor, i.e., they are part of the same genetic lineage. Relationships among species of another subgenus, *Pteropodus*, have also been evaluated (Kai et al. 2003, Li et al. 2006a). Our analysis of restriction site variation in the NADH-dehydrogenase subunit -3 and -4 genes (ND3/ND4 region) and the 12S and 16S rRNA genes (12S/16S region) of the mitochondrial DNA (mtDNA) showed that the northeastern Pacific members of *Pteropodus*, along with three northeastern Pacific species from two other subgenera, form a monophyletic group (Li et al. 2006a). However, the northwestern Pacific members of *Pteropodus* were distinct from the northeastern Pacific *Pteropodus* species and need to be evaluated further and reassigned (Li et al. 2006a).

In this paper, we extended our evaluation of *Sebastes* subgenera validity to 71 species and 16 subgenera. We used the subgeneric assignments summarized in Kendall (2000), except for *S. gilli*, which is unassigned (A.W. Kendall, pers. comm.). In addition, we examined the extent to which intraspecific variation interferes with phylogenetic determination by surveying variation in large samples of individuals from five of

the 71 species. Restriction site variation was used to generate maximum parsimony and neighbor-joining trees, and to estimate the extent of nucleotide divergences within and among subgenera.

Materials and methods

Species studied

Seventy-one species from 16 *Sebastes* subgenera were examined (Table 1). *Sebastolobus alascanus* and *Helicolenus hilgendorfi* were included as outgroup species. The northeastern Pacific *Sebastes* species and *Sebastolobus alascanus* were captured in the Gulf of Alaska and along the coast of California. The northwestern Pacific *Sebastes* species and *H. hilgendorfi* were collected along the coasts of Japan. Generally, five individuals were used to represent each species. In several instances, fewer individuals were used because of limited availability. Many additional individuals of *S. alutus*, *S. aleutianus*, *S. borealis*, *S. caurinus*, and the *S. carnatus/S. chrysomelas* complex were included to evaluate the influence of intraspecific variation on the analysis.

DNA amplification

A sample of heart tissue of each specimen was preserved in either 95% ethanol or a DNA preservation solution (Seutin et al. 1991). Total genomic DNA was extracted using Puregene™ DNA isolation kits (Gentra Systems Inc., Minneapolis). The mitochondrial ND3/ND4 and 12S/16S regions were amplified by polymerase chain reaction (Gharrett et al. 2001, Li et al. 2006b).

Restriction site analysis

A restriction site map was developed for both the ND3/ND4 and 12S/16S regions for endonucleases: *Bst*N I, *Bst*U I, *Cfo* I, *Dde* I, *Hin*d II, *Hin*f I, *Mbo* I, *Msp* I, *Rsa* I, and *Sty* I. All restriction sites were mapped from fragment sizes observed in double digests (Li et al. 2006b).

We attempted to find species-specific variation to separate *S. emphaeus*, *S. variegatus*, *S. wilsoni*, and *S. zacentrus* by identifying restriction site differences in published cytochrome *b* sequences (Rocha-Olivares et al. 1999). Although we expected to observe species-specific restriction digest patterns, the digests failed to delineate the species, probably because the nucleotide differences to which restriction enzymes were applied are the result of intraspecific variation shared by the four species. We also examined variation in the NADH-dehydrogenase subunit-5 and -6 genes for these four species. Although additional intraspecific variation was observed, there was little useful interspecific variation.

Table 1. Names, abbreviation, subgenus assignments, and range of species included.

Common name	Species[a]	Abbr.	Subgenus	Range
Rougheye rockfish	*aleutianus*	*ale*	*Zalopyr*	NE-NW Pacific
Pacific ocean perch	*alutus*	*alu*	*Acutomentum*	NE-NW Pacific
Kelp rockfish	*atrovirens*	*atr*	*Mebarus*	NE Pacific
Brown rockfish	*auriculatus*	*ari*	*Auctospina*	NE Pacific
Aurora rockfish	*aurora*	*aro*	*Eosebastes*	NE Pacific
Redbanded rockfish	*babcocki*	*bab*	*Rosicola*	NE Pacific
Shortraker rockfish	*borealis*	*bor*	*Zalopyr*	NE-NW Pacific
Silvergray rockfish	*brevispinis*	*bre*	*Acutomentum*	NE Pacific
False jacopever	*capensis*	*cap*	*Sebastomus*	NE Pacific/ S. Hemisphere
Gopher rockfish	*carnatus*	*car*	*Pteropodus*	NE Pacific
Copper rockfish	*caurinus*	*cau*	*Pteropodus*	NE Pacific
Greenspotted rockfish	*chlorosticus*	*dhl*	*Sebastomus*	NE Pacific
Black-and-yellow rockfish	*chrysomelas*	*chr*	*Pteropodus*	NE Pacific
Dusky rockfish	*ciliatus/ variabilis*	*cil*	*Sebastosomus*	NE Pacific
Starry rockfish	*constellatus*	*con*	*Sebastomus*	NE Pacific
Darkblotched rockfish	*crameri*	*cra*	*Eosebastes*	NE Pacific
Calico rockfish	*dalli*	*dal*	*Auctospina*	NE Pacific
Splitnose rockfish	*diploproa*	*dip*	*Allosebastes*	NE Pacific
Greenstriped rockfish	*elongatus*	*elo*	*Hispaniscus*	NE Pacific
Puget Sound rockfish	*emphaeus*	*emp*	*Allosebastes*	NE Pacific
Swordspine rockfish	*ensifer*	*ens*	*Sebastomus*	NE Pacific
Widow rockfish	*entomelas*	*ent*	*Acutomentum*	NE Pacific
Pink rockfish	*eos*	*eos*	*Sebastomus*	NE Pacific
Gulf rockfish	*exsul*	*exs*	*Sebastomus*	NE Pacific
Yellowtail rockfish	*flavidus*	*fla*	*Sebastosomus*	NE Pacific
Bronzespotted rockfish	*gilli*	*gil*	?	NE Pacific
Chilipepper	*goodei*	*goo*	*Sebastodes*	NE Pacific
Rosethorn rockfish	*helvomaculatus*	*hel*	*Sebastomus*	NE Pacific
Squarespot rockfish	*hopkinsi*	*hop*	*Acutomentum*	NE Pacific
Yoroi-mebaru	*hubbsi*	*hub*	*Pteropodus*	NW Pacific
Mebaru	*inermis*	*ine*	*Mebarus*	NW Pacific
Shortbelly rockfish	*jordani*	*jor*	*Sebastodes*	NE Pacific
Togotto-mebaru	*joyneri*	*joy*	*Mebarus*	NW Pacific
Freckled rockfish	*lentiginosus*	*Len*	*Sebastomus*	NE Pacific
Cowcod	*levis*	*Lev*	*Hispaniscus*	NE Pacific
Mexican rockfish	*macdonaldi*	*mac*	*Acutomentum*	NE Pacific

[a]Genus *Sebastes* unless otherwise noted.

Table 1. (Continued.)

Common name	Species[a]	Abbr.	Subgenus	Range
Quillback rockfish	*maliger*	*mal*	*Pteropodus*	NE Pacific
Black rockfish	*melanops*	*mep*	*Sebastosomus*	NE Pacific
Blackgill rockfish	*melanostomus*	*mes*	*Eosebastes*	NE Pacific
Vermillion rockfish	*miniatus*	*min*	*Rosicola*	NE Pacific
Blue rockfish	*mystinus*	*mys*	*Sebastosomus*	NE Pacific
China rockfish	*nebulosus*	*neb*	*Pteropodus*	NE Pacific
Tiger rockfish	*nigrocinctus*	*nig*	*Sebastichthys*	NE Pacific
Goma-soi	*nivosus*	*niv*	*Pteropodus*	NW Pacific
Speckled rockfish	*ovalis*	*ova*	*Acutomentum*	NE Pacific
Bocaccio	*paucispinis*	*pau*	*Sebastodes*	NE Pacific
Chameleon rockfish	*phillipsi*	*phi*	*?*	NE Pacific
Canary rockfish	*pinniger*	*pin*	*Rosicola*	NE Pacific
Northern rockfish	*polyspinis*	*pol*	*?*	NE Pacific
Redstripe rockfish	*proriger*	*pro*	*Allosebastes*	NE Pacific
Grass rockfish	*rastrelliger*	*ras*	*Pteropodus*	NE Pacific
Yellowmouth rockfish	*reedi*	*ree*	*?*	NE Pacific
Rosy rockfish	*rosaceus*	*rsa*	*Sebastomus*	NE Pacific
Greenblotched rockfish	*rosenblatti*	*rsb*	*Sebastomus*	NE Pacific
Yelloweye rockfish	*ruberrimus*	*rbr*	*Sebastopyr*	NE Pacific
Flag rockfish	*rubrivinctus*	*rbv*	*Hispaniscus*	NE Pacific
Bank rockfish	*rufus*	*ruf*	*Acutomentum*	NE Pacific
Stripetail rockfish	*saxicola*	*sax*	*Allosebastes*	NE Pacific
Halfbanded rockfish	*semicinctus*	*sem*	*Allosebastes*	NE Pacific
Olive rockfish	*serranoides*	*srd*	*Sebastomus*	NE Pacific
Treefish	*serriceps*	*srp*	*Sebastocarus*	NE Pacific
Pinkrose rockfish	*simulator*	*sim*	*Sebastomus*	NE Pacific
Spiny-eye rockfish	*spinorbis*	*spi*	*Sebastomus*	NE Pacific
Ezo-mebaru	*taczanowski*	*tac*	*Mebarus*	NW Pacific
Usu-mebaru	*thompsoni*	*tho*	*Mebarus*	NW Pacific
Shima-zoi	*trivitattus*	*tri*	*Pteropodus*	NW Pacific
Honeycomb rockfish	*umbrosus*	*umv*	*Sebastomus*	NE Pacific
Harlequin rockfish	*variegatus*	*var*	*Allosebastes*	NE Pacific
Kitsune-mebaru	*vulpes*	*vul*	*Neohispaniscus*	NW Pacific
Pygmy rockfish	*wilsoni*	*wil*	*Allosebastes*	NE Pacific
Sharpchin rockfish	*zacentrus*	*zac*	*Allosebastes*	NE Pacific
Helicolenus	*Helicolenus hilgendorfi*	*Hh*		NW Pacific
Shortspine thornyhead	*Sebastolobus alascanus*	*Sa*		NE Pacific

Phylogenetic analysis

The rate of nucleotide substitution per nucleotide (d_r) was calculated for all pairs of haplotypes following Nei and Tajima (1981) and Nei and Miller (1990, eq. 4) using REAP (McElroy et al. 1990). Nucleotide substitutions per nucleotide were calculated for all pairs of *Sebastes* spp., between *Sebastes* spp. and *Helicolenus hilgendorfi*, between *Sebastes* spp. and *Sebastolobus alascanus*, and between *H. hilgendorfi* and *Sebastolobus alascanus*. Pairwise restriction site differences were calculated using Arlequin 2.0 (Schneider et al. 2000). One hundred neighbor-joining trees (Saitou and Nei 1987) using PHYLIP 3.57c (Felsenstein 1993) were estimated by using randomized orders of the taxa. Maximum parsimony analyses were performed using heuristic searches with PAUP 4.0b10 (Swofford 1998). Because the likelihood of the loss of a site is higher than the gain of a site, three character-weighting schemes were used. The weight of gaining a site was analyzed as (1) equal to that of losing a site, (2) twice that of losing a site, and (3) four times that of losing a site. The following search parameters were used: exclude uninformative characters, retain minimal tree from each replicate, collapse zero-length branches, tree-bisection-reconnection branch swapping in effect, steepest descent not enforced, and save all optimal trees. One hundred replicates were performed for each of the three weighting schemes, and the multiple maximum parsimony trees generated for each scheme were combined to produce a 50% majority consensus tree.

Results

Restriction site analysis

A total of 215 restriction sites were detected in the ND3/ND4 and 12S/16S regions (Li et al. 2006b). The faster evolving ND3/ND4 region had 141 sites, and 74 sites were in the more conserved 12S/16S region. Of the total 215 sites, 97 were unique to *Sebastes* species, 21 were unique to *Sebastolobus alascanus*, seven were unique to *Helicolenus hilgendorfi*, and one was shared only by *Sebastolobus alascanus* and *H. hilgendorfi*.

Site differences in the two mtDNA regions yielded 132 composite haplotypes (Li et al. 2006b). Individuals of the *Sebastes* species had 127 haplotypes, *Sebastolobus alascanus* had four haplotypes, and *H. hilgendorfi* had a single haplotype. Thirty-four of the 71 species displayed intraspecific variation and were represented by more than one composite haplotype.

In several instances, haplotypes were identical among species. These were (1) a variant of *S. carnatus* and a variant of *S. chrysomelas* were identical; (2) *S. chlorostictus*, *S. eos*, and *S. rosenblatti* shared a haplotype; (3) haplotypes of *S. ciliatus/variabilis*, *S. crameri*, and a vari-

ant of *S. polyspinis* were the same; and (4) *S. emphaeus*, a variant of *S. variegatus*, and a variant of *S. wilsoni* were the same. Also, haplotypes of several species differed by a single restriction site. Single site differences were observed in four instances: (1) between *S. entomelas* and *S. mystinus*; (2) between *S. hopkinsi* and *S. ovalis*; (3) between *S. zacentrus* and the *S. emphaeus/S. variegatus/S. wilsoni* complex; and (4) between *S. reedi* and the *S. ciliatus/variabilis/S. crameri/S. polyspinis* complex.

Differences in restriction sites between haplotypes ranged from 0 between some pairs of species (e.g., *S. carnatus* and *S. chrysomelas*) to 71 sites between *Sebastolobus alascanus* and *S. inermis*, and *Sebastolobus alascanus* and *S. joyneri*.

Nucleotide divergence within variable *Sebastes* species averaged 0.0027 substitutions per nucleotide. Nucleotide divergence between *Sebastes* species averaged 0.0285 substitutions per nucleotide, ranging from 0 (as mentioned above for several pairs of species) to 0.0664 per nucleotide between *S. hubbsi* and *S. jordani*. Nucleotide divergence between *Sebastes* species and *H. hilgendorfi* averaged 0.0767 substitutions per nucleotide. Nucleotide divergence between *Sebastes* species and *Sebastolobus alascanus* averaged 0.1047 substitutions per nucleotide, and the largest was observed between *S. hubbsi* and *Sebastolobus alascanus*, at 0.1226 substitutions per nucleotide. The average nucleotide divergence within subgenera ranged from 0.0089 substitutions per nucleotide for the subgenus *Sebastomus* to 0.0370 substitutions per nucleotide for the subgenus *Sebastodes* (Table 2).

Six new restriction sites in the ND3/ND4 region were detected for the individuals included for investigation of intraspecific variation, which resulted in 10 additional haplotypes for *S. aleutianus* ($n = 39$), four for *S. alutus* ($n = 60$), nine for *S. borealis* ($n = 78$), 10 for *S. carnatus* and *S. chrysomelas* ($n = 98$), and five for *S. caurinus* ($n = 78$).

Phylogenetic analysis

A neighbor-joining tree (NJ) (Fig. 1) was constructed from all haplotypes, including those observed for the additional specimens, to evaluate the influence of intraspecific variation on the tree. Multiple haplotypes for each species formed many short terminal branches, but did not obscure interspecific differences. Each additional haplotype of *S. aleutianus*, *S. alutus*, *S. borealis*, *S. carnatus*, and *S. chrysomelas* clustered with the original haplotypes observed for those species. Haplotypes of *S. aleutianus* separated into two clusters at the tip of a branch.

Subsequent analyses included only the original five specimens of each species so that all species would be represented by about the same number of individuals. In the ND3/ND4 region, 109 sites were polymorphic, six were monomorphic, and 26 were autapomorphic (variation was observed in only a single haplotype). In the 12S/16S region, 45 sites were

Table 2. Number of *Sebastes* species analyzed in each subgenus, total number (Kendall 2000), and average nucleotide divergence (substitutions per nucleotide) within each subgenus. The subgenera are abbreviated in Figs. 1-4.

Subgenus	Abbr.	No. species analyzed in subgenus	No. species in subgenus, Kendall 2000	Average divergence
Acutomentum	*Acuto*	7	7	0.0308
3 species[a]				0.0144
Allosebastes	*Allo*	8	13	0.0203
4 species[b]				0.0028
Auctospina	*Aucto*	2	2	0.0129
Eosebastes	*Eoseb*	3	3	0.0184
Hispaniscus	*Hispan*	3	3	0.0272
Mebarus	*Mebar*	5	7	0.0270
NWP clade[c]				0.0226
Neohispaniscus	*Nhispan*	1	2	N/A
Pteropodus	*Ptero*	9	10	0.0291
NEP clade[d]				0.0124
Rosicola	*Rosi*	3	3	0.0179
Sebastichthys	*Sich*	1	1	N/A
Sebastocarus	*Scar*	1	1	N/A
Sebastodes	*Sode*	3	5	0.0370
Sebastomus	*Stom*	14	15	0.0089
Sebastopyr	*Spyr*	1	1	N/A
Sebastosomus	*Stoso*	5	5	0.0224
3 species[e]				0.0101
Zalopyr	*Zpyr*	2	3	0.0171
Unassigned		3		
5 N. Pacific species[f]				0.0084

[a] *S. hopkinsi*, *S. ovalis*, *S. rufus*.
[b] *S. emphaeus*, *S. variegatus*, *S. wilsoni*, *S. zacentrus*.
[c] w/o *S. atrovirens*.
[d] NEP *Pteropodus* w/*S. atrovirens*, *S. auriculatus*, *S. dalli*.
[e] *S. flavidus*, *S. melanops*, *S. serranoides*.
[f] *S. alutus*, *S. ciliatus/variabilis*, *S. crameri*, *S. polyspinis*, *S. reedi*.
NEP = northeastern Pacific; NWP = Northwestern Pacific.

polymorphic, 25 were monomorphic, and four were autapomorphic. A total of 154 sites provided information for the parsimony analysis.

Three schemes assigned different weights to loss and gain of a restriction site in the parsimony analyses. The 1:1 loss/gain scheme produced 18,529 equally parsimonious trees, each with a total length of 536 steps. The 1:2 scheme produced 11,279 trees, each with a total length of 782 steps. The 1:4 scheme produced 1,324 trees, each with a total length of 1,082 steps. For each scheme, a 50% majority consensus tree was produced (Figs. 2-4).

There were many areas of congruence among the three consensus trees and the NJ tree. The NJ tree and the consensus trees were similar in that members of the subgenus *Sebastomus* formed a tight cluster, as did the northeastern Pacific members of *Pteropodus*, whereas many of the other species did not cluster according to their subgeneric assignments. Rather, species from different subgenera often formed small, separate clusters, and the relationships among the clusters were not always clear.

The species of the subgenus *Sebastomus* generally clustered together except for *S. rosaceus*, which often clustered with the group of species that phenotypically have conspicuous vertical bands: *S. babcocki*, *S. nigrocinctus*, *S. rubrivinctus*, and *S. serriceps*. This group of species formed a cluster near the *Sebastomus* cluster.

The northeastern Pacific *Pteropodus* species, including *S. chrysomelas*, *S. carnatus*, *S. caurinus*, *S. maliger*, *S. nebulosus*, and *S. rastrelliger*, clustered consistently with *S. auriculatus* and *S. dalli* of the subgenus *Auctospina*, and *S. atrovirens* of *Mebarus*. Three species clustered near but not consistently with the *Pteropodus* group: *S. saxicola* and *S. semicinctus* (both subgenus *Allosebastes*), and *S. elongatus* (subgenus *Hispaniscus*). The northwestern Pacific members of *Pteropodus*, *S. hubbsi*, *S. nivosus*, and *S. trivittatus*, as well as the northwestern Pacific members of *Mebarus*, *S. inermis*, *S. joyneri*, *S. taczanowski*, and *S. thompsoni*, did not cluster with their northeastern Pacific counterparts. Instead, they generally clustered with other northwestern Pacific species in the analysis. These results are in agreement with earlier observations (Kai et al 2003, Li et al. 2006a).

As for the species in other subgenera, four small consistent clusters of species representing three subgenera occurred in all phylogenetic trees. They were (1) *S. hopkinsi*, *S. ovalis*, and *S. rufus* (subgenus *Acutomentum*); (2) *S. emphaeus*, *S. variegatus*, *S. wilsoni*, and *S. zacentrus* (subgenus *Allosebastes*); (3) *S. inermis*, *S. joyneri*, and *S. thompsoni* (subgenus *Mebarus*); and (4) *S. saxicola* and *S. semicinctus* (subgenus *Allosebastes*). Each of these clusters was distinct from other species belonging to their subgenera. Two other groups formed with a little less consistency. *Sebastes miniatus* and *S. pinniger* (subgenus *Rosicola*) clustered together in all but the 1:1 consensus tree. *Sebastes flavidus*,

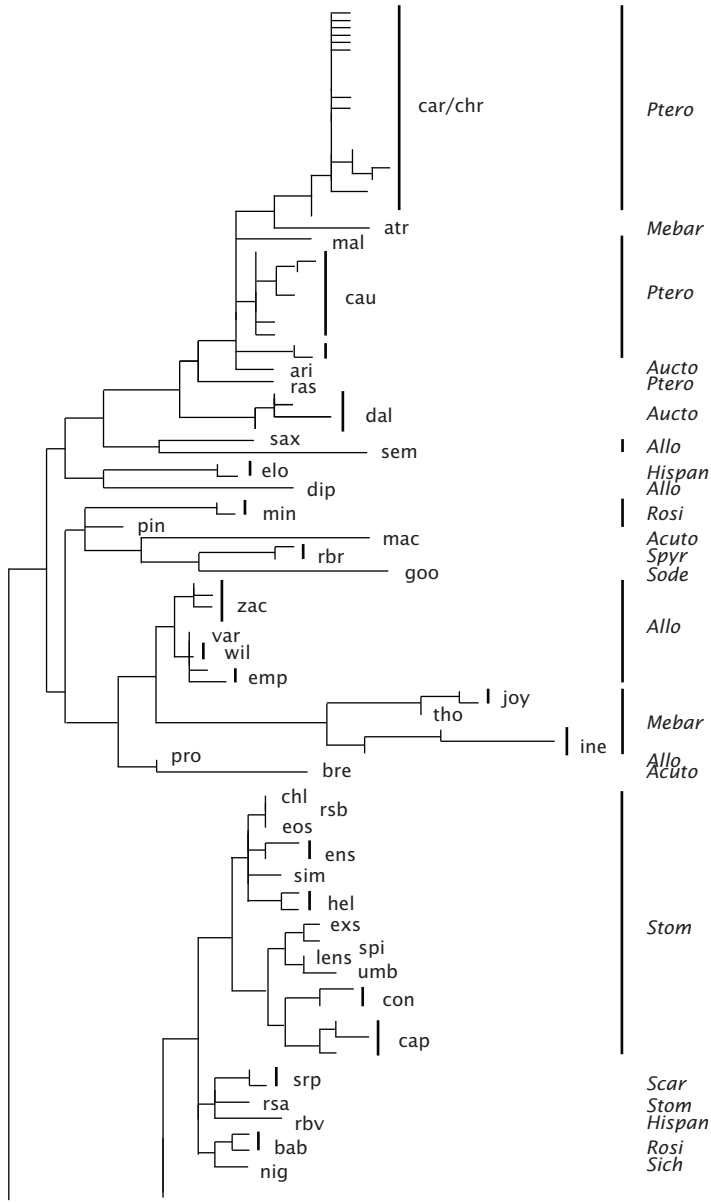

Figure 1. Neighbor-joining tree (Saitou and Nei 1987) based on restriction site variation in *Sebastes*. Haplotypes of additional samples were included for five species to examine the influence of intraspecific variation. Vertical lines reflect multiple haplotypes for a species. Abbreviations are in Tables 1 and 2.

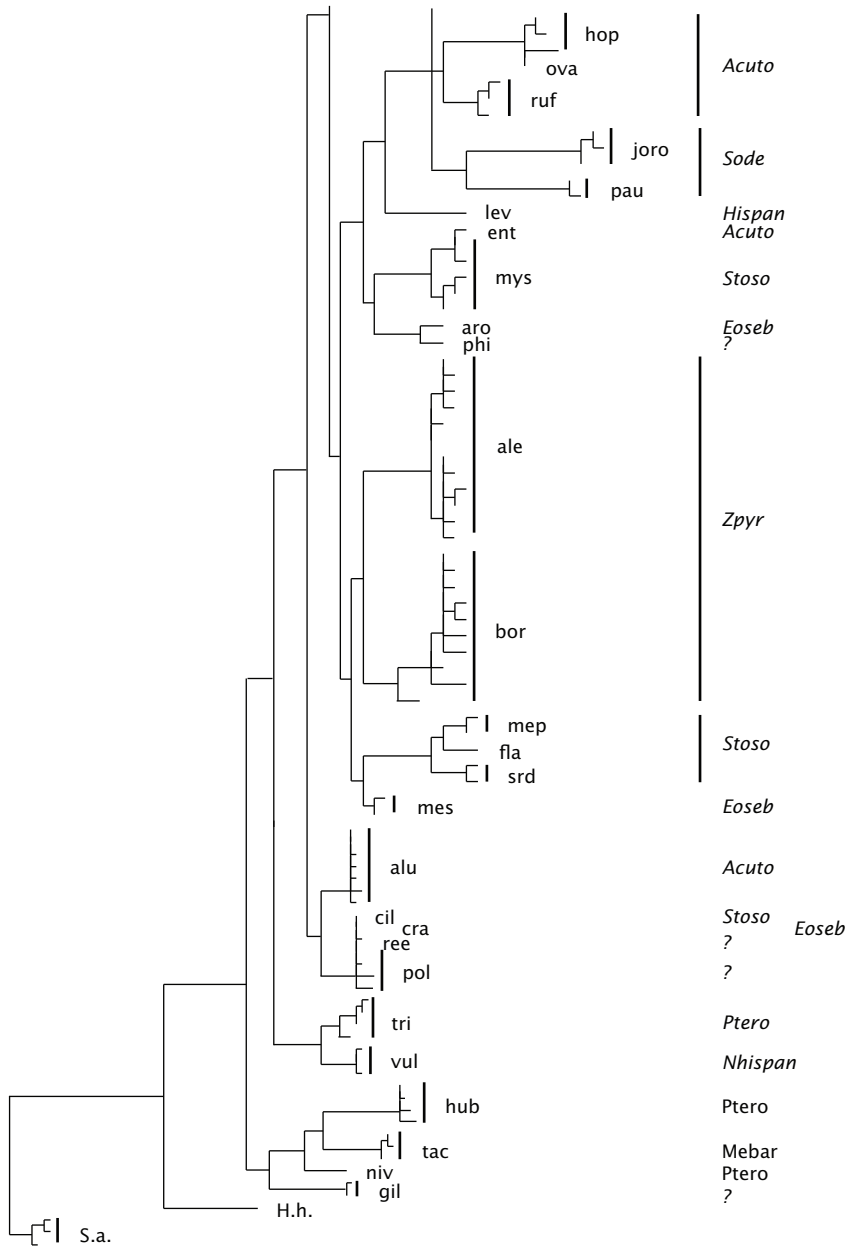

Figure 1. (Continued.)

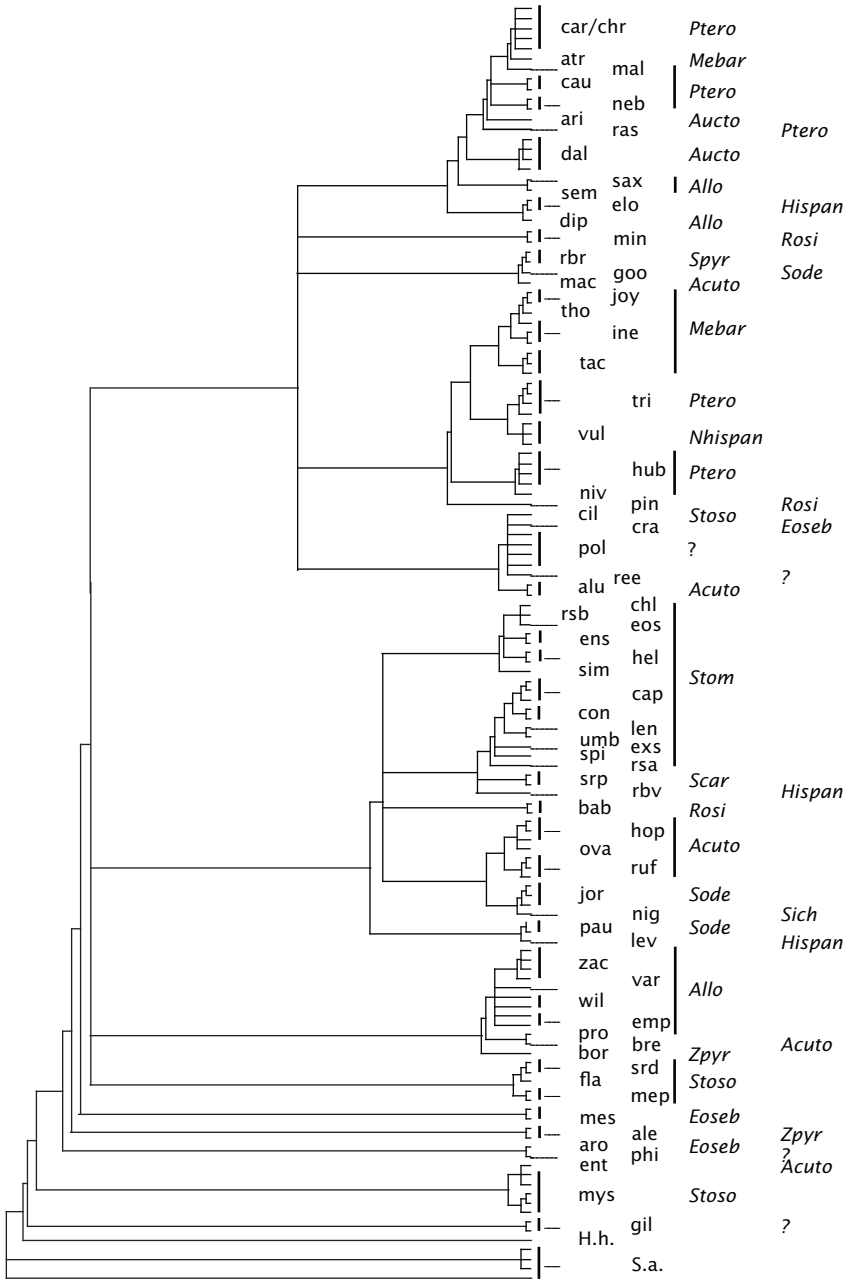

Figure 2. 50% majority consensus tree of 18,529 parsimony trees generated under the 1:1 weighting scheme. Vertical lines reflect multiple haplotypes for a species. Abbreviations are in Tables 1 and 2.

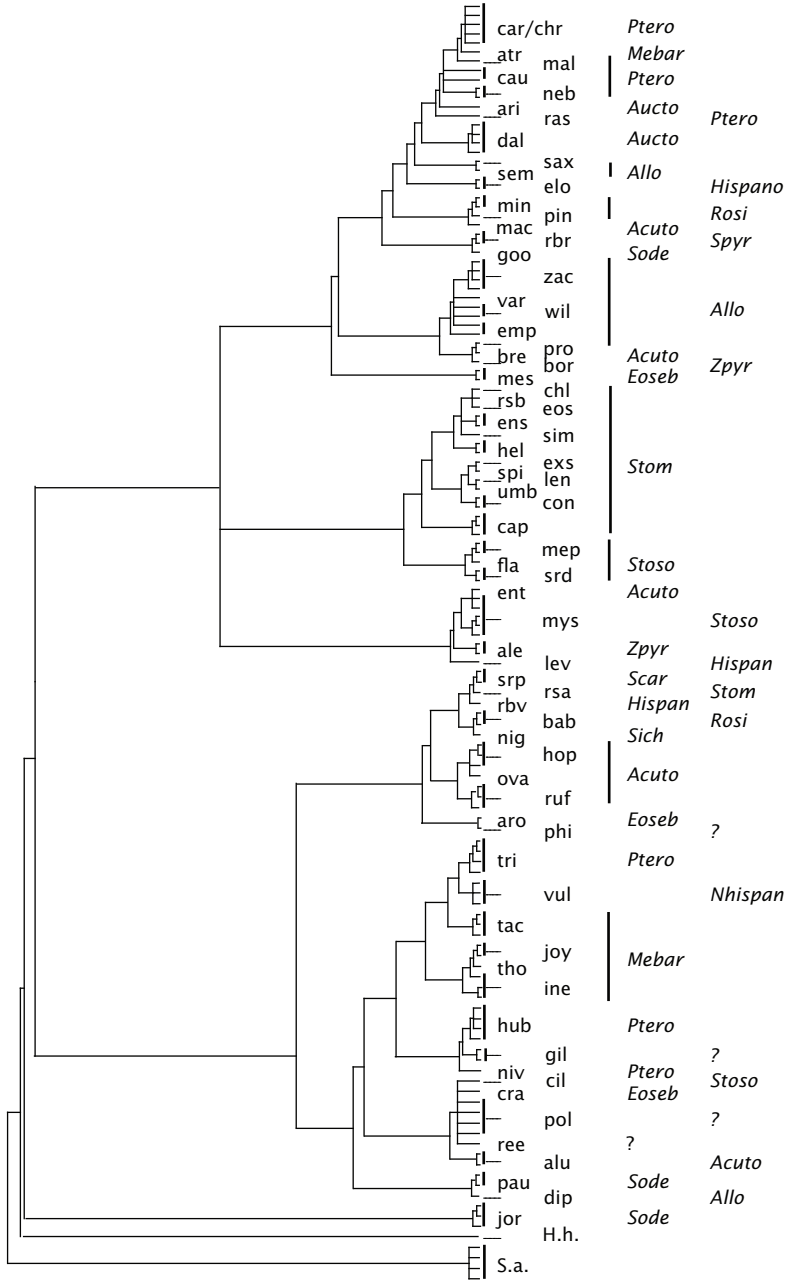

Figure 3. 50% majority consensus tree of 11,279 parsimony trees generated under the 1:2 weighting scheme. Vertical lines reflect multiple haplotypes for a species. Abbreviations are in Tables 1 and 2.

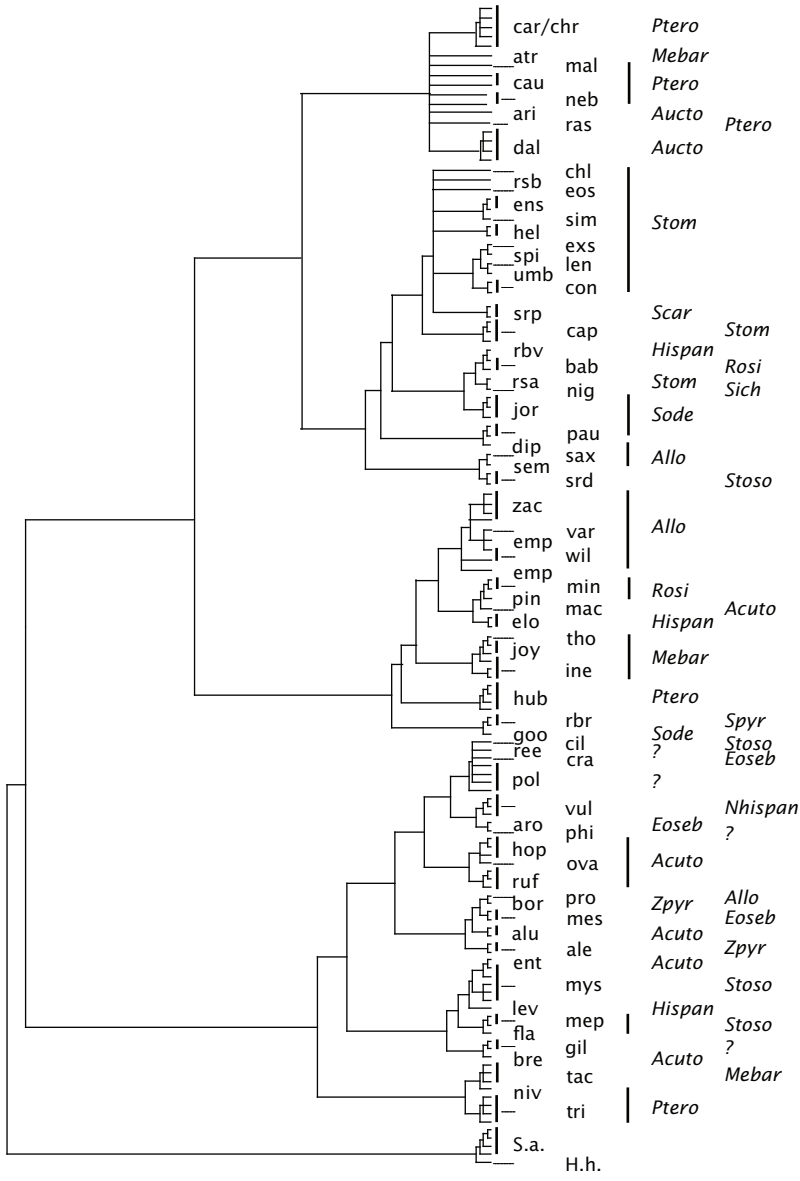

Figure 4. 50% majority consensus tree of 1,324 parsimony trees generated under the 1:4 weighting scheme. Vertical lines reflect multiple haplotypes for a species. Abbreviations are in Tables 1 and 2.

S. melanops, and *S. serranoides* (subgenus *Sebastosomus*) clustered together in all trees except for the 1:4 consensus tree. Other species previously assigned to these subgenera by other authors did not cluster with the species in their assigned subgenera.

Consistent groups were also formed by species from different subgenera. Four clusters appeared in all of the trees: (1) *S. mystinus* (subgenus *Sebastosomus*) and *S. entomelas* (subgenus *Acutomentum*); (2) *S. aurora* (subgenus *Eosebastes*) and *S. phillipsi* (unassigned); (3) *S. ruberrimus* (subgenus *Sebastopyr*) and *S. goodei* (subgenus *Sebastodes*); and (4) *S. ciliatus/variabilis* (subgenus *Sebastosomus*), *S. crameri* (subgenus *Eosebastes*), *S. polyspinis* (unassigned), and *S. reedi* (unassigned). *Sebastes alutus* (subgenus *Acutomentum*) clustered near the latter group in all but the 1:4 consensus tree. *Sebastes babcocki* (subgenus *Rosicola*), *Sebastes nigrocinctus* (subgenus *Sebastichthys*), *S. rubrivinctus* (subgenus *Hispaniscus*), and *S. serriceps* (subgenus *Sebastocarus*) also clustered closely and consistently, often with *S. rosaceus* (subgenus *Sebastomus*).

Discussion

Intraspecific variation

Generally, all variants of a species clustered together, separate from other species in the neighbor-joining tree. In four instances, variants of one species clustered with another species. Variants of *S. carnatus* and *S. chrysomelas* clustered together; variants of *S. mystinus* clustered with *S. entomelas*; variants of *S. polyspinis* clustered with *S. ciliatus/variabilis*, *S. crameri*, and *S. reedi*; and variants of *S. emphaeus* and *S. wilsoni* clustered with *S. variegatus*. In addition, three morphotypes of *S. inermis* have concordant, but small mtDNA and AFLP differences that probably reflect species differences (Kai et al. 2002). These occurrences suggest that the species within the clusters diverged recently. Intraspecific variation did not obscure phylogenetic relationships. The 10 additional haplotypes of *S. aleutianus* formed two separate clusters on the neighbor-joining tree, which is consistent with the existence of two cryptic species (Gharrett et al. 2005).

Phylogenetic analysis

Thirteen subgenera that included more than one species were included in this study. They were *Acutomentum*, *Allosebastes*, *Auctospina*, *Eosebastes*, *Hispaniscus*, *Mebarus*, *Neohispaniscus*, *Pteropodus*, *Sebastocarus*, *Sebastomus*, *Sebastopyr*, *Sebastosomus*, and *Zalopyr*. Based on the subgeneric assignments currently recognized in Kendall (2000) (except for *S. gilli*), our results suggest that only the subgenus *Sebastomus* defined by Chen (1971) is monophyletic. The other subgen-

era are probably polyphyletic, because only a few species clustered with others in their subgenera. The three species in monotypic subgenera all clustered with species from other subgenera. In addition, three species currently not assigned to subgenera consistently clustered with species to which a subgenus had been assigned. Five subgenera will be discussed in detail: *Sebastomus*, *Pteropodus*, *Acutomentum*, *Allosebastes*, and *Sebastosomus*.

Sebastomus

The subgenus *Sebastomus* was erected by Gill (1864) for *S. rosaceus*, and the current species composition mainly follows Chen (1971). From morphology, Chen (1971) concluded that the 13 species *S. capensis*, *S. chlorostictus*, *S. constellatus*, *S. ensifer*, *S. eos*, *S. exsul*, *S. helvomaculatus*, *S. lentiginosus*, *S. notius*, *S. rosaceus*, *S. rosenblatti*, *S. simulator*, and *S. umbrosus*—but not *S. gilli*—form a monophyletic group. He also outlined the relationships among the species (Chen 1971). *Sebastes spinorbis* was described later and considered a close relative of *S. exsul* (Chen 1975). Allozyme patterns (Seeb 1986) of seven of the species included by Chen, and mitochondrial cytochrome *b* sequences (Rocha-Olivares et al. 1999) corroborate the monophyly of the subgenus.

We included all the members of *Sebastomus* except *S. notius* and observed that all the species clustered tightly. The average nucleotide divergence within *Sebastomus* is 0.0089 substitutions per nucleotide. The southern (*S. capensis*, *S. constellatus*, *S. exsul*, *S. spinorbis*, *S. lentiginosus*, and *S. umbrosus*) and northern (all the other species) lineages identified by Rocha-Olivares et al. (1999) were evident in our neighbor-joining tree and the 1:1 consensus tree. Within *Sebastomus*, three species—*S. chlorostictus*, *S. eos*, and *S. rosenblatti*—had identical composite haplotypes; and two pairs of species—*S. exsul* and *S. spinorbis*, and *S. lentiginosus* and *S. umbrosus*—were each separated by two restriction sites, suggesting that these species pairs have recently diverged. These species groupings were also observed by Rocha-Olivares et al. (1999). The type species, *S. rosaceus*, did not consistently group with other members of the subgenus, but instead grouped with the conspicuously banded species, *S. babcocki*, *S. nigrocinctus*, *S. rubrivinctus*, and *S. serriceps*. Rocha-Olivares et al. (1999) suggested that *S. babcocki*, *S. nigrocinctus*, and *S. rubrivinctus* may be a sister clade to *Sebastomus*.

Pteropodus

The northeastern Pacific species of *Pteropodus* (*S. carnatus*, *S. caurinus*, *S. chrysomelas*, *S. maliger*, *S. nebulosus*, and *S. rastrelliger*) clustered with *S. atrovirens*, *S. auriculatus*, and *S. dalli*, but were separate from the northwestern Pacific members of the subgenus (*S. hubbsi*, *S. nivosus*, and *S. trivittatus*) (Li et al. 2006b). The average nucleotide substitution

rate between species within the northeastern Pacific cluster is 0.0124 per nucleotide, the second lowest of all groups considered. The low level of species divergence, as well as the tight cluster these species formed, provides support for the addition of *S. atrovirens*, *S. auriculatus*, and *S. dalli* to the subgenus *Pteropodus*. A possible sister clade of these species is the group *S. saxicola*, *S. semicinctus*, and *S. elongatus*. The northwestern Pacific species of the subgenus did not form any consistent monophyletic groups in the phylogenetic trees (Li et al. 2006a). These results were in agreement with Kai et al. (2003).

Acutomentum

Eigenmann and Beeson (1893) established the subgenus *Acutomentum* for species with "much projecting" lower jaws, among other characteristics. The group originally included *S. ovalis*, the type species, as well as *S. melanostomus*, *S. rufus*, *S. alutus*, and *S. macdonaldi*. Jordan and Evermann (1898) added *S. entomelas*, *S. brevispinis*, *S. hopkinsi*, and *S. proriger*, and moved *S. melanostomus* to the subgenus *Eosebastes*. Chen (1975) moved *S. proriger* to the subgenus *Allosebastes*. Matsubara (1943) added four species from the northwestern Pacific: *S. flammeus*, *S. iracundus*, *S. scythropus*, and *S. baramenuke*. All seven northeastern Pacific species but none of the northwestern Pacific species currently in the subgenus were included in this analysis. The average nucleotide substitution rate within northeastern Pacific *Acutomentum* was 0.0308 per nucleotide (Table 2), among the highest of all subgenera considered.

Species of the subgenus *Acutomentum* that we examined formed several distinct clusters, some with species in the subgenus, others with species from other subgenera. Three species, *S. hopkinsi*, *S. ovalis*, and *S. rufus*, consistently clustered together in all of the phylogenetic trees, indicating they are monophyletic. The average nucleotide substitution rate among these three species was 0.0144 per nucleotide; and the rate between *S. hopkinsi* and *S. ovalis* was extremely low, at 0.0031 substitutions per nucleotide. A pair of species, *S. entomelas* and *S. mystinus* (subgenus *Sebastosomus*), clustered consistently together, and the nucleotide divergence between them was also low, at 0.0055 substitutions per nucleotide. Rocha-Olivares et al. (1999) also observed the close relationship between *S. hopkinsi* and *S. ovalis*, and between *S. entomelas* and *S. mystinus*. *Sebastes brevispinis* and *S. proriger* (subgenus *Allosebastes*) clustered together in all but the 1:4 consensus tree. *Sebastes macdonaldi* occurred in various positions in the trees; it is unclear to which species it is most closely related. *Sebastes alutus* clustered near four northerly species, *S. ciliatus/variabilis* (*Sebastosomus*), *S. crameri* (*Eosebastes*), *S. polyspinis* (unassigned), and *S. reedi* (unassigned), in all but one of the trees but was distal from other *Acutomentum* species.

Allosebastes

The subgenus *Allosebastes* was erected for *S. sinensis* by Hubbs (1951), who regarded the "most notable character of this subgenus" to be "the reduction of the anal soft-rays to 5." Hubbs also described other distinct characters of *S. sinensis*, among them "the smooth, mostly cycloid scales; the swollen lower pectoral rays; the excessively long anal spines." *Sebastes sinensis* remained the only member of the subgenus until Chen (1975) added 11 northeastern Pacific species—*S. dalli*, *S. diploproa*, *S. emphaeus*, *S. proriger*, *S. rufinanus*, *S. saxicola*, *S. semicinctus*, *S. variegatus*, *S. wilsoni*, and *S. zacentrus*—and one northwestern Pacific species—*S. scythropus*. Chen (1971) considered *S. cortezi*, *S. peduncularis*, and *S. varispinis* relatives of the subgenus, and added them to the subgenus in 1985. Currently *S. dalli* is assigned to *Auctospina* (also see under *Pteropodus*) (Jordan and Starks 1895, Kendall 2000), and *S. scythropus* is assigned to *Acutomentum* following Matsubara (1943).

Chen (1975) gave a brief description of the characters shared among the species, including the cranial spine patterns, a banded color pattern, and the morphology of the gas-bladder muscles. However, these characters were not those considered by Hubbs (1951), and did not seem specific enough for subgeneric assignments (e.g., Kendall 2000). In addition, some species were described from few specimens (only two *S. peduncularis* and two *S. rufinanus* have ever been collected) or from samples of juveniles (*S. peduncularis* and *S. varispinis*). Chen admitted that the phylogenetic relationships he proposed were speculative. Our results do not support the monophyly of *Allosebastes*. We observed close relationships within two groups of species: *S. emphaeus*, *S. variegatus*, *S. wilsoni*, and *S. zacentrus* consistently clustered together and *S. saxicola* and *S. semicinctus* clustered with each other near the *Pteropodus* clade. *Sebastes proriger* clustered near the *S. emphaeus* group, relationships also suggested by Seeb (1986). *Sebastes diploproa* clustered with *S. elongatus* in the 1:1 consensus tree and the neighbor-joining tree, and clustered with *S. paucispinis* in the 1:2 and 1:4 consensus trees. From our data, it is unclear to which species *S. diploproa* is most related. Because a sample of the type species, *S. sinensis*, was unavailable for this study, it is difficult to determine which species added by Chen should be retained in the subgenus.

Sebastosomus

Three of the five members of *Sebastosomus*, *S. flavidus*, *S. melanops* (the type species), and *S. serranoides*, clustered together in three of the phylogenetic trees, suggesting that some species assigned to the subgenus are monophyletic. This group was also observed by Seeb (1986) and Rocha-Olivares et al. (1999). The other two *Sebastosomus* members, *S. crameri* and *S. mystinus*, were associated with complexes formed by species from other subgenera. *Sebastes crameri* consistently grouped with *S.*

ciliatus/variabilis, *S. polyspinis*, and *S. reedi*, which indicates that these species are closely related, and may warrant assignment to a single subgenus. *Sebastes alutus* appeared to be closely related to this group. The ranges of these species overlap off British Columbia and Southeast Alaska, and are all categorized as continental slope species. Love et al. (2002) reported that *S. reedi* are commonly taken with *S. alutus* and *S. crameri*. *Sebastes mystinus* clustered with *S. entomelas* in all trees, suggesting that they are closely related. Seeb (1986) and Rocha-Olivares et al. (1999) observed this grouping as well.

Other groups

Other notable groupings included *S. aurora* and *S. phillipsi*, and *S. ruberrimus* and *S. goodei*, both of which appeared in all of the phylogenetic trees. *Sebastes aurora* and *S. phillipsi* resemble each other morphologically, overlap in ranges, and are both deepwater species (Love et al. 2002). They appear to be closely related. Although *S. ruberrimus* and *S. goodei* cluster consistently, they differ from each other in coloration and body shape and differ at 11 restriction sites (0.021 substitutions per nucleotide). *Sebastes miniatus* and *S. pinniger* (both *Rosicola*) clustered together in all trees except for the 1:4 consensus tree. These species resemble each other in having a mottled pattern and prominent white markings along the lateral line, and they differ mostly in coloration. They are most likely closely related. This group was also observed by Rocha-Olivares et al. (1999).

Two subgenera in this study, *Pteropodus* and *Mebarus*, contain species from both the northeastern Pacific and the northwestern Pacific. The results provide strong evidence that the northeastern Pacific and northwestern Pacific members of these subgenera are not monophyletic. This may prove true for other trans-Pacific subgenera as well. Analysis of more northwestern Pacific species needs to be done to resolve the relationship between northeastern Pacific and northwestern Pacific species.

Although many consistent species groups were revealed in this study, relationships among other species remained unresolved, as are the relationships among the existing subgenera. Northeastern Pacific species that did not cluster consistently with any other species included *S. aleutianus*, *S. borealis*, *S. gilli*, *S. jordani*, *S. levis*, *S. macdonaldi*, *S. melanostomus*, and *S. paucispinis*. In addition, species within several groups have identical composite profiles. These observations point to the need to explore other mtDNA regions and that comparison of nuclear genes is needed to corroborate the results from analysis of mtDNA variation.

Our results were generally similar to those of Seeb (1986) from allozyme analysis and to those of Rocha-Olivares et al. (1999) and Kai et al. (2003) from sequence analysis of the mtDNA cytochrome *b* gene.

In many instances the results were identical. The concordance in results of the three studies gives strong support to some of the phylogenetic relationships we suggested, because they were observed using three different methods that examined different parts of the genome. The similarities also indicate that both sequencing and restriction site analysis can provide data for phylogenetic comparisons. Each method has its advantages and disadvantages. DNA sequencing can provide data that can be analyzed using a wider array of analytical tools and provide more detail; however, restriction site studies can efficiently survey longer spans of DNA at a relatively low cost. In this study, an estimated 901 nucleotides were recognized by the restriction enzymes, equivalent to about 19% of the 4,815 nucleotides examined in the two mtDNA regions, and 5.4 % of the mtDNA genome. In Rocha-Olivares et al. (1999), the stretch of DNA sequenced was about 750 bp.

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