Phylogeny of members of the rockfish (*Sebastes*) subgenus *Pteropodus* and their relatives

Z. Li, A.K. Gray, M.S. Love, T. Asahida, and A.J. Gharrett

Abstract: The *Sebastes* Cuvier, 1829 subgenus *Pteropodus* Eigenmann and Beeson, 1893 includes six species from the northeastern Pacific Ocean (NEP) and four species from the northwestern Pacific Ocean (NWP). Several NEP species assigned to other subgenera are similar to NEP *Pteropodus* species. Restriction site variation in the mitochondrial NADH dehydrogenase subunit 3 and 4 genes and the 12S and 16S ribosomal RNA genes were used to evaluate their relationships. Phylogenetic reconstruction showed that six NEP species of *Pteropodus* formed a monophyletic group that also included three NEP species currently assigned to other subgenera: *Sebastes atrovirens* (Jordan and Gilbert, 1880) (subgenus *Mebarus* Matsubara, 1943) and *Sebastes auriculatus* Girard, 1854 and *Sebastes dalli* (Eigenmann and Beeson, 1894) (both subgenus *Auctospina* (Eigenmann and Beeson, 1894)). The small average nucleotide divergence (0.0124 per nucleotide) observed among members of this group of species was similar to that observed among species of the monophyletic subgenus *Sebastomus* Gill, 1864 (0.0089 per nucleotide). The NWP species of *Pteropodus* did not cluster with their NEP consubgeners but, generally, were similar to other NWP species. We recommend that *S. atrovirens*, *S. auriculatus*, and *S. dalli* be included in subgenus *Pteropodus* with the other NEP species and that the NWP species of *Pteropodus* be removed from the subgenus. Our results indicate that the morphological characteristics used to distinguish species often may not be useful for phylogenetic analysis.

Résumé : Le sous-genre *Pteropodus* Eigenmann et Beeson, 1893 de *Sebastes* Cuvier, 1829 contient six espèces du nord-est du Pacifique (NEP) et quartre espèces du nord-ouest du Pacifique (NWP). Plusieurs espèces du NEP placées dans d'autres sous-genres sont très semblables aux espèces du *Pteropodus* du NEP. Nous avons utilisé la variation des sites de restriction des sous-unités 3 et 4 des gènes mitochondriaux de la NADH déshydrogénase et des gènes 12S et 16S de l'ADN ribosomique pour évaluer leurs relations. Une reconstruction phylogénétique montre que les six espèces de *Pteropodus* du NEP forment un groupe monophylétique qui contient aussi trois espèces du NEP qui sont couramment assignées à d'autres sous-genres : *Sebastes atrovirens* (Jordan et Gilbert, 1880) du sous-genre *Mebarus* Matsubara, 1943, ainsi que *Sebastes auriculatus* Girard, 1854 et *Sebastes dalli* (Eigenmann et Beeson, 1894), tous deux du sous-genre *Auctospina* (Eigenmann et Beeson, 1894). La faible divergence moyenne des nucléotides (0,0124 par nucléotide) observée parmi les membres de ce groupe d'espèces est semblable à celle trouvée chez les espèces du sous-genre monophylétique *Sebastomus* Gill, 1864 (0,0089 par nucléotide). Les espèces de *Pteropodus* du NWP ne se regroupent pas avec celles du NEP, mais sont généralement semblables aux autres espèces due NWP. Nous recommandons que *S. atrovirens*, *S. auriculatus* et *S. dalli* soient inclus dans le sous-genre *Pteropodus* avec les autres espèces du NEP et que les espèces de *Pteropodus* du NWP soient retirées du sous-genre. Nos résultats indiquent que les caractères morphologiques servant à distinguer les espèces peuvent ne pas être utiles pour l'analyse phylogénétique.

[Traduit par la Rédaction]

Introduction

More than 100 species of rockfish in the genus *Sebastes* Cuvier, 1829 are distributed worldwide. They are most abundant in the northwestern and northeastern Pacific Ocean, but also occur in the North Atlantic Ocean and the Southern Hemisphere (Love et al. 2002). The large number of species and their morphological similarities create challenges for identification, especially for larval and juvenile forms. The difficulty in identifying species impedes efforts to study their life histories and also complicates efforts to determine the systematic relationships within the genus. Since the mid-1800s, when taxonomists began their efforts at delineating species within this genus, numerous revisions have been made in their taxonomic status, primarily because the taxonomists did not agree on which characters were important indicators of phylogenetic relationships. In the past century, many *Sebastes* species have been assigned to multiple genera or subgenera, the numbers of which have risen and fallen over the years. The 22 subgenera currently recognized (Kendall 2000) include 11 that occur in the northeastern Pacific, 5 in the northwestern Pacific, 5 spanning both sides of the north Pacific, and 1 in the north Atlantic. The largest group, *Sebastomus* Gill, 1864, includes about 15 species. Six subgenera are monotypic.

Received 15 August 2005. Accepted 13 February 2006. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 15 April 2006.

Z. Li, A.K. Gray,[1] and A.J. Gharrett.[2] Juneau Center, School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 11120 Glacier Highway, Juneau, AK 99801, USA.
M.S. Love. Marine Science Institute, University of California, Santa Barbara, CA 93106-6150, USA.
T. Asahida. School of Fisheries Sciences, Kitasato University, Sanriku, Ofunato, Iwate 022-0101 Japan.

[1]Present address: National Marine Fisheries Service, Auke Bay Laboratory, 11305 Glacier Highway, Juneau, AK 99801, USA.
[2]Corresponding author (e-mail: ffajg@uaf.edu).

 doi:10.1139/Z06-022

A variety of morphological characters, such as coloration, cranial spine pattern, cranial structure, and various meristic counts and morphometric ratios have been used as the basis for species identification, as well as for phylogenetic assignment to subgenus. Many of these characters provide good markers for species identification because there is abundant variation for each of the characters. Such variation usually permits species that closely resemble each other to be distinguished. However, these characters are often not appropriate for cladistic analysis. Kendall (2000) failed in his attempt to reconstruct a phylogeny for *Sebastes* subgenera based on morphological characters. He attributed the failure to the nature of the characters that were available.

Desirable cladistic characters are those with large, clear-cut changes rather than those with small, gradual ones (Kitching et al. 1998). However, many of the characters used for subgeneric grouping of *Sebastes* are either qualitative with gradual changes or quantitative. For example, the degree of development of cranial spines, which can differ among species, has been described as strong, well-developed, weak, small, or minute. However, because differences between conditions such as small and minute are not clearcut and depend on the judgment of the investigator, the degree of development cannot be translated into clear character states. For meristic and morphometric characters, the problem of continuous and intraspecific variation is apparent. Many species have overlapping ranges of morphological characters, which make character states difficult to define.

Kendall (2000) also noted that some of the characters used to delineate *Sebastes* species are correlated with each other and are adaptations for the particular type of habitat that a species occupies. Many species of *Sebastes* can be roughly separated into two categories, demersal and pelagic. Demersal species typically have a protruding lower jaw, concave interorbital area, heavy armature, thickened pectoral fin rays, and other characters that are adapted for a sedentary existence, whereas pelagic species have opposite states for these characters. For the bottom dwelling species, as well as the pelagic species, convergence toward their shared character states is expected regardless of ancestry.

Because the variable and adaptable nature of many of the characters that have been used to distinguish species make them unsuitable for phylogenetic determination, the subgenera based on them may be invalid. Consequently, subgeneric assignments need to be reviewed, and alternative approaches, such as molecular methods, are needed to help delineate phylogenetic relationships within the genus. *Sebastomus* is the only subgenus that has been investigated rigorously using both morphological and molecular methods. In that subgenus, morphological measurements (Chen 1971) and sequences of a mitochondrial cytochrome *b* gene (Rocha-Olivares et al. 1999) have shown that the subgenus is monophyletic.

Another subgenus, *Pteropodus* Eigenmann and Beeson, 1893, also appears to include a group of closely related species, based on both morphological and genetic data (Jordan and Evermann 1898; Seeb 1986; Rocha-Olivares et al. 1999). Developmental patterns of larvae and juveniles of *Pteropodus* also are similar within the group (Kendall 1991). Historically, the subgenus *Pteropodus* was erected as a genus for a group of species occurring in the northeastern Pacific. Eigenmann and Beeson (1893) placed 13 species in the genus and *Sebastes maliger* (Jordan and Gilbert, 1880) was the type species. Jordan and Evermann (1898) lowered the status of the genus to the subgeneric level and reduced the number of species to eight, including *Sebastes carnatus* (Jordan and Gilbert, 1880), *Sebastes caurinus* Richardson, 1844, *Sebastes chrysomelas* (Jordan and Gilbert, 1881), *Sebastes gilberti* (Cramer in Jordan, 1896), *S. maliger*, *Sebastes nebulosus* Ayres, 1854, *Sebastes rastrelliger* (Jordan and Gilbert, 1880), and *Sebastes vexillaris* (Jordan and Gilbert, 1880). Hubbs and Schultz (1933) synonymized *S. gilberti* (in subgenus *Pteropodus*) with *S. dalli* (in subgenus *Auctospina* Eigenmann and Beeson, 1893), and added a new species, *Sebastes litoris* (Hubbs and Schultz, 1933). They also remarked that, except for *S. rastrelliger*, the relationship among the species which they placed in the subgenus is "closer among themselves than with species placed in other subgenera". Both *S. vexillaris* and *S. litoris* were later synonymized with *S. caurinus* (Chen 1986). Matsubara (1943) added four species from the NWP: *Sebastes hubbsi* (Matsubara, 1937), *Sebastes longispinis* (Matsubara, 1934), *Sebastes nivosus* Hilgendorf, 1880, and *Sebastes trivittatus* Hilgendorf, 1880 to *Pteropodus*. The current species composition of the subgenus mainly follows that described in Jordan and Evermann (1898) and Matsubara (1943), and it now includes six species from the NEP (*S. carnatus*, *S. caurinus*, *S. chrysomelas*, *S. maliger*, *S. nebulosus*, and *S. rastrelliger*) and four species from the NWP (*S. hubbsi*, *S. longispinis*, *S. nivosus*, and *S. trivittatus*).

An allozyme study of numerous *Sebastes* species (Seeb 1986) suggested that members of the NEP *Pteropodus* are closely related. The study also suggested the addition of *S. auriculatus* (subgenus *Auctospina*), which clustered with members of *Pteropodus* in all phenograms, to the subgenus. Sequences of the cytochrome *b* gene of the mitochondrial DNA (mtDNA) corroborated the close relationship within the subgenus, and suggested the addition of three species from other subgenera to this group (Rocha-Olivares et al. 1999). Four species (*S. carnatus*, *S. caurinus*, *S. maliger*, and *S. rastrelliger*) of the subgenus clustered with *S. atrovirens* (subgenus *Mebarus*; Chen 1971) and with *S. auriculatus* and *S. dalli* (subgenus *Acutospina*, assigned by Eigenmann and Beeson 1894 and Jordan and Starks 1895, respectively).

We surveyed the mtDNA regions that included the NADH dehydrogenase subunit 3 and 4 genes and the 12S and 16S rRNA genes for species-specific markers, and analyzed the restriction site variation using maximum-parsimony, neighbor-joining, and maximum-likelihood methods to examine the relationship among species within the subgenus *Pteropodus* and its possible allies. The subgeneric assignments summarized in Kendall (2000) were considered to be those currently recognized, except for *Sebastes gilli* (Eigenmann, 1891), which is unassigned (A.W. Kendall, Jr., personal communication).

The questions we addressed were as follows: (*i*) are the presently recognized NEP *Pteropodus* species monophyletic; (*ii*) are there other NEP species that are monophyletic with the *Pteropodus* species; (*iii*) are the NEP and NWP species of *Pteropodus* monophyletic; (*iv*) if not, do the NEP and NWP species of *Pteropodus* each form separate monophyletic groups.

Table 1. Names and subgeneric assignments of species used in the analyses.

Common name	Species	Subgenus	Authority	Range
Rougheye rockfish	*aleutianus*	*Zalopyr*	(Jordan and Evermann, 1898)	NEP/NWP
Kelp rockfish	*atrovirens*	*Mebarus*	(Jordan and Gilbert, 1880)	NEP
Brown rockfish	*auriculatus*	*Auctospina*	Girard, 1854	NEP
Gopher rockfish	*carnatus*	*Pteropodus*	(Jordan and Gilbert, 1880)	NEP
Copper rockfish	*caurinus*	*Pteropodus*	Richardson, 1844	NEP
Black-and-yellow rockfish	*chrysomelas*	*Pteropodus*	(Jordan and Gilbert, 1881)	NEP
Light dusky rockfish	*ciliatus*	*Sebastosomus*	(Tilesius, 1813)	NEP
Starry rockfish	*constellatus*	*Sebastomus*	(Jordan and Gilbert, 1880)	NEP
Calico rockfish	*dalli*	*Auctospina*	(Eigenmann and Beeson, 1894)	NEP
Splitnose rockfish	*diploproa*	*Allosebastes*	(Gilbert, 1890)	NEP
Greenstriped rockfish	*elongatus*	*Hispaniscus*	Ayres, 1859	NEP
Yellowtail rockfish	*flavidus*	*Sebastosomus*	(Ayres, 1862)	NEP
Bronzespotted rockfish	*gilli*	?	(Eigenmann, 1891)	NEP
Rosethorn rockfish	*helvomaculatus*	*Sebastomus*	Ayres, 1859	NEP
Yoroi-mebaru	*hubbsi*	*Pteropodus*	(Jordan and Hubbs 1925)	NWP
Mebaru	*inermis*	*Mebarus*	Cuvier in Cuvier and Valenciennes, 1829	NWP
Togotto-mebaru	*joyneri*	*Mebarus*	Günther, 1878	NWP
Quillback rockfish	*maliger*	*Pteropodus*	(Jordan and Gilbert, 1880)	NEP
Blue rockfish	*mystinus*	*Sebastosomus*	(Jordan and Gilbert, 1881)	NEP
China rockfish	*nebulosus*	*Pteropodus*	Ayres, 1854	NEP
Tiger rockfish	*nigrocinctus*	*Sebastichthys*	Ayres, 1859	NEP
Goma-soi	*nivosus*	*Pteropodus*	Hilgendorf, 1880	NWP
Bocaccio	*paucispinis*	*Sebastodes*	Ayres, 1854	NEP
Canary rockfish	*pinniger*	*Rosicola*	(Gill, 1864)	NEP
Grass rockfish	*rastrelliger*	*Pteropodus*	(Jordan and Gilbert, 1880)	NEP
Yelloweye rockfish	*ruberrimus*	*Sebastopyr*	(Cramer, 1895)	NEP
Bank rockfish	*rufus*	*Acutomentum*	(Eigenmann and Eigenmann, 1890)	NEP
Stripetail rockfish	*saxicola*	*Allosebastes*	(Gilbert, 1890)	NEP
Halfbanded rockfish	*semicinctus*	*Allosebastes*	(Gilbert, 1897)	NEP
Ezo-mebaru	*taczanowski*	*Mebarus*	Steindachner, 1880	NWP
Usu-mebaru	*thompsoni*	*Mebarus*	(Jordan and Hubbs, 1925)	NWP
Shima-zoi	*trivittatus*	*Pteropodus*	Hilgendorf, 1880	NWP
Harlequin rockfish	*variegatus*	*Allosebastes*	Quast, 1971	NEP
Kitsune-mabaru	*vulpes*	*Neohispaniscus*	Döderlein in Steindachner and Döderlein, 1884	NWP
Hilgendorf's rosefish	*Helicolenus hilgendorfi*		(Döderlein in Steindachner and Döderlein, 1884)	NWP

Note: NEP, northeastern Pacific Ocean; NWP, northwestern Pacific Ocean.

Methods and materials

Species examined

Thirty-four *Sebastes* species and *Helicolenus hilgendorfi* (Döderlein in Steindachner and Döderlein, 1884) were included in the analysis (Table 1). The foci of this study are species of the subgenera *Pteropodus*, *Mebarus*, *Auctospina*, and *Neohispaniscus* Matsubara, 1943. *Pteropodus* species included in this study were *S. carnatus*, *S. caurinus*, *S. chrysomelas*, *S. maliger*, *S. nebulosus*, and *S. rastrelliger* from the NEP, and *S. hubbsi*, *S. nivosus*, and *S. trivittatus* from the NWP. The *Mebarus* species were *S. atrovirens* from the NEP, and *Sebastes inermis* Cuvier in Cuvier and Valenciennes, 1829, *Sebastes joyneri* Günther, 1878, *Sebastes taczanowski* Steindachner, 1880, and *Sebastes thompsoni* (Jordan and Hubbs 1925) from the NWP. The *Auctospina* species were *S. auriculatus* and *S. dalli*, both from the NEP. The single species of *Neohispaniscus* included was *S. vulpes*, which occurs in the NWP. The species of *Auctospina*, *Mebarus*, and *Neohispaniscus* were included to test the monophyly of *Pteropodus*. Another 17 species from 10 other subgenera were chosen to provide a genus-wide perspective; *H. hilgendorfi* was used as the outgroup. In general, five individuals were analyzed to represent each species. A single haplotype (if intraspecific variation occurred) was used for each of the 10 species chosen to represent other subgenera.

DNA isolation and amplification

A sample of heart tissue from each specimen was preserved in either 95% ethanol or a DNA preservation solution composed of 20% (*v*/*v*) dimethyl sulfoxide (DMSO), 0.25 mol/L ethylenediaminetetraacetic acid (EDTA) at pH 8.0, and saturated with NaCl (Seutin et al. 1991).

Total genomic DNA was extracted using Puregene™ DNA isolation kits (Gentra Systems Inc., Minneapolis, Minnesota). Two target regions of mtDNA were amplified using the polymerase chain reaction (PCR). The ND3/ND4 region begins in the glycyl tRNA gene and spans the NADH dehydrogenase subunit 3, arginyl tRNA, NADH dehydrogenase subunit 4L, and NADH dehydrogenase subunit 4 genes ending in the histidyl tRNA gene. The 12S/16S region starts

near the phenylalanyl tRNA end of the 12SrRNA gene, and runs through the valyl tRNA gene to near the leucyl tRNA end of the 16S rRNA gene. Primers for target regions have been used to detect species differences in northern Pacific rockfish (Gharrett et al. 2001; Li et al. 2006). The lengths for the ND3/ND4 and 12S/16S regions are 2385 and 2430 base pairs (bp), respectively, based on the aggregate restriction map.

Amplification was accomplished using the following PCR thermal cycling profile: 1 cycle for 5 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 52 °C, and 3 min at 72 °C using *Taq* polymerase. For the ND3/ND4 region, a 50 μL reaction required 2 mmol/L of $MgCl_2$, 0.2 mmol/L of each dNTP, 0.2 μmol/L of each primer, and 2 units of *Taq* polymerase. The 50 μL reaction mix for the 12S/16S region differed from that of the ND3/ND4 region in that the concentration of $MgCl_2$ was increased to 2.5 mmol/L.

Restriction site analysis

Subsamples of the PCR products from both regions were first digested singly with 10 different restriction endonucleases: *Bst*U I, *Cfo* I, *Dde* I, *Hin*f I, *Mbo* I, *Msp* I, and *Rsa* I have 4-nucleotide recognition sites; *Bst*N I has an ambiguous 5-nucleotide recognition site; and *Hin*d II and *Sty* I have ambiguous 6-nucleotide recognition sites. The subsamples were then double-digested to pinpoint the restriction sites. Fragments from all digests were separated on 1.5% agarose gels (one part agarose (Sigma, St. Louis, Missouri) and two parts Synergel™ (Diversified Biotech Inc., Boston, Massachusetts)) in 0.5× TBE buffer (TBE is 90 mmol/L of Tris – boric acid and 2 mol/L of EDTA at pH 7.5). A 100-bp ladder was used as the molecular weight standard. The agarose gels were stained with ethidium bromide and photographed on an ultraviolet light transilluminator. Digests that produced fragments, which were too small to be measured accurately using an agarose gel, were separated on 8% polyacrylamide gels (29:1 of acrylamide:bisacrylamide) in 2× TAE buffer (TAE is 40 mmol/L of Tris – acetic acid and 1 mmol/L of EDTA at pH 8). The polyacrylamide gels were stained with SYBR Green I Nucleic Acid Stain™ (Molecular Probes, Eugene, Oregon). A 25-bp ladder was used as the molecular weight standard.

All restriction sites were mapped with fragments observed in double digests. A restriction site map included all observed restriction fragment patterns. Each variant digest pattern was designated by a letter. A composite haplotype (a 20-letter code) was determined for each individual. The composite haplotype data were previously used to construct a key and the fragment size data are available there (Li et al. 2006). The composite haplotypes, represented as presence or absence of each restriction site as binary codes for the species considered in this study, can be obtained at the following Web site: http://ak.aoos.org/data/archive/2006/0000003 (Appendixweb.xls file in the data directory).

Phylogenetic analysis

Variation in DNA sequences was detected indirectly by the presence or absence of restriction sites. Nucleotide substitution per site (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. 1992). Ninety-nine neighbor-joining trees (Saitou and Nei 1987) using PHYLIP version 3.57c (Felsenstein 1993) were estimated by using randomized orders of the taxa. A maximum-likelihood tree was constructed using RESTML in PHYLIP version 3.57c (Felsenstein 1993) with the assumption that recognition sites of all enzymes were 4 bp long.

Maximum-parsimony analyses were performed using heuristic searches with PAUP* version 4.0b10 (Swofford 1998). All character states were regarded as unordered. Because of the higher likelihood of losing than gaining a restriction site, three gain/loss weighting schemes were applied. The weight of gaining a site was as follows: (*i*) equal to that of losing a site; (*ii*) twice that of losing a site; and (*iii*) four times that of losing a site. The following PAUP* version 4.0b10 search parameters were included: 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. Ten thousand replicates were performed for the 1:1 weighting scheme and 1000 replicates were made for each of the 1:2 and 1:4 schemes. The multiple maximum-parsimony trees generated for each scheme were combined to produce a 50% majority consensus tree, but each node was labeled with its percentage of consensus.

Results

Restriction site analysis

We mapped 172 restriction sites (Li et al. 2006). Of these, 96 were unique to the genus *Sebastes*, 9 were unique to *H. hilgendorfi*, and 67 were shared between the 2 genera. Fifty-six composite haplotypes were observed. Eleven species had intraspecific variation. The most variable species were *S. dalli*, *S. hubbsi*, and *S. trivittatus*, each of which had four variants. *Helicolenus hilgendorfi* was represented by a single haplotype.

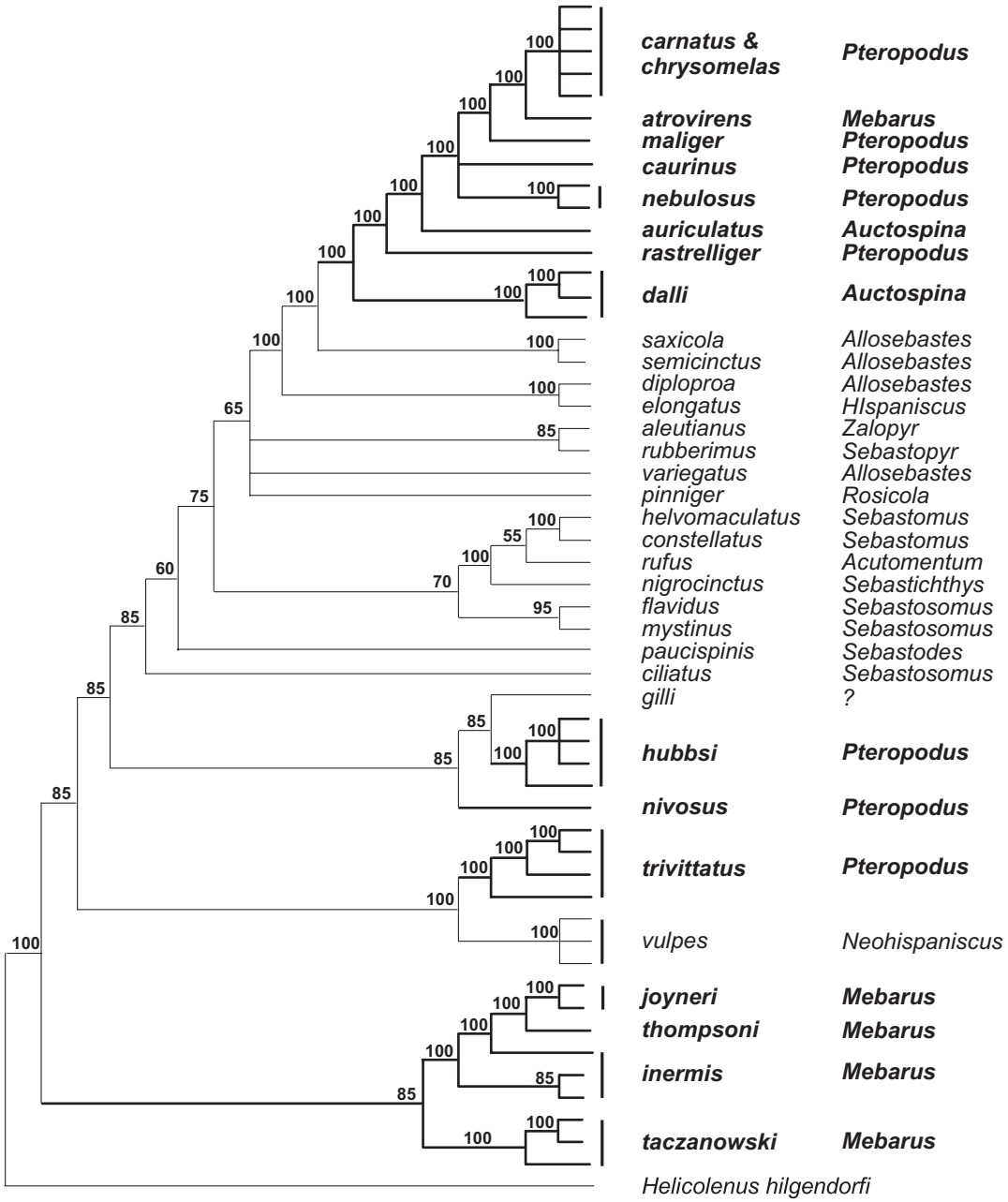
Differences between haplotypes ranged from 0 restriction site differences (between a *S. carnatus* variant and a *S. chrysomelas* variant) to 52 restriction site differences, which represented 0.0961 nucleotide substitutions per site (between a *S. hubbsi* variant and *H. hilgendorfi*). Between the *Sebastes* species and *H. hilgendorfi*, the average rate of nucleotide substitution was 0.0782 per site. Among the *Sebastes* species, the average rate of nucleotide substitution was 0.0339 per site. Within the group that included the *Pteropodus* species and *S. atrovirens*, *S. auriculatus*, and *S. dalli*, the average rate of nucleotide substitution was 0.0127 per site. Within-species variation ranged from 0.002 substitutions per site (shared by several species) to a maximum of 0.0125 per site in *S. inermis*.

Phylogenetic analysis

Of the 172 restriction sites, 91 were polymorphic, 36 were monomorphic, and 45 were autapomorphic. The ND3/ND4 region was more variable than the 12S/16S region and included 109 restriction sites, of which 8 were monomorphic and 33 were autapomorphic. The 12S/16S region had a total of 63 restriction sites, of which 28 were monomorphic and 12 were autapomorphic.

For the maximum-parsimony analysis, the three schemes that assigned different weights to loss and gain of a restriction

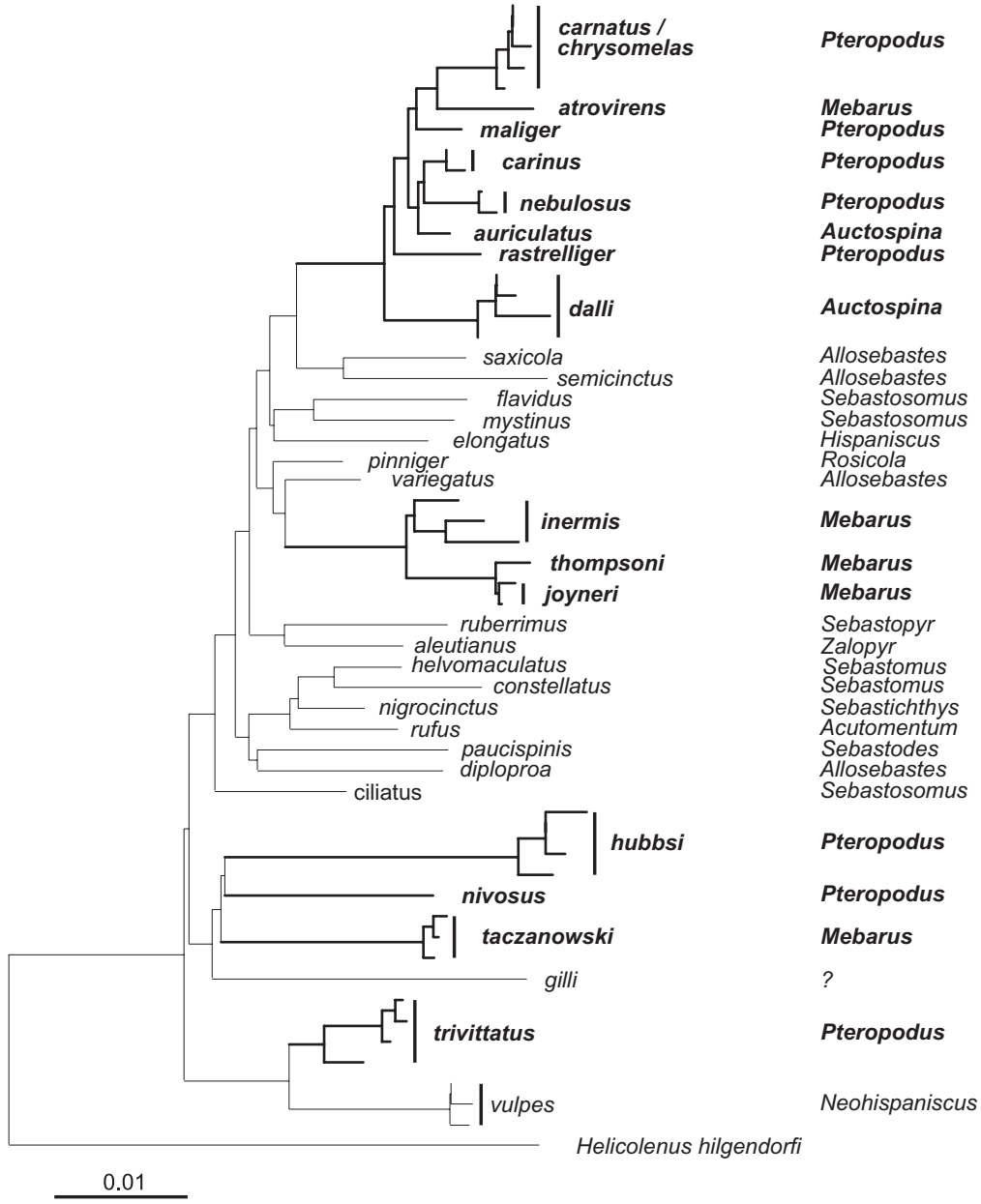
Fig. 1. The 50% majority consensus tree generated from 20 maximum-parsimony trees under the 1:1 scheme. Vertical lines reflect multiple haplotypes for a species. Numbers at the nodes indicate the proportion of trees with that node.



site resulted in: (*i*) 20 most parsimonious trees, each with a total length of 274 steps for the 1:1 scheme; (*ii*) 720 trees, each with a total length of 376 steps for the 1:2 scheme; and (*iii*) 1230 trees, each with a total length of 484 steps for the 1:4 scheme. For the 1:1 scheme, a 50% majority consensus tree was produced from the multiple maximum-parsimony trees generated in the heuristic searches (Fig. 1). Trees for the other maximum-parsimony analyses are not presented.

The NEP species of *Pteropodus* formed a strict (100% consensus) monophyletic clade that included *S. atrovirens* (subgenus *Mebarus*) and *S. auriculatus* and *S. dalli* (both in subgenus *Auctospina*) in all three maximum-parsimony cladograms (see Fig. 1 for the 1:1 weighting scheme, the others are not presented). The NEP *Pteropodus* species *S. atrovirens*, *S. auriculatus*, and *S. dalli* occurred on the same branch in the neighbor-joining (Fig. 2) and maximum-likelihood trees (Fig. 3). The closest relationship among these species was between *S. carnatus* and *S. chrysomelas*, which shared many haplotypes that made them virtually undifferentiated. The two species consistently formed a subclade with *S. atrovirens* and *S. maliger*. No other clear relationship was evident within the group. *Sebastes saxicola* (Gilbert, 1890) and *Sebastes semicinctus* (Gilbert, 1897) were adjacent to the NEP *Pteropodus* group in some trees, suggesting that they may be closely related to the NEP *Pteropodus* species and their relatives.

Fig. 2. Neighbor-joining tree (Saitou and Nei 1987). The scale bar indicates the proportion of nucleotide substitutions. Vertical lines reflect multiple haplotypes for species.



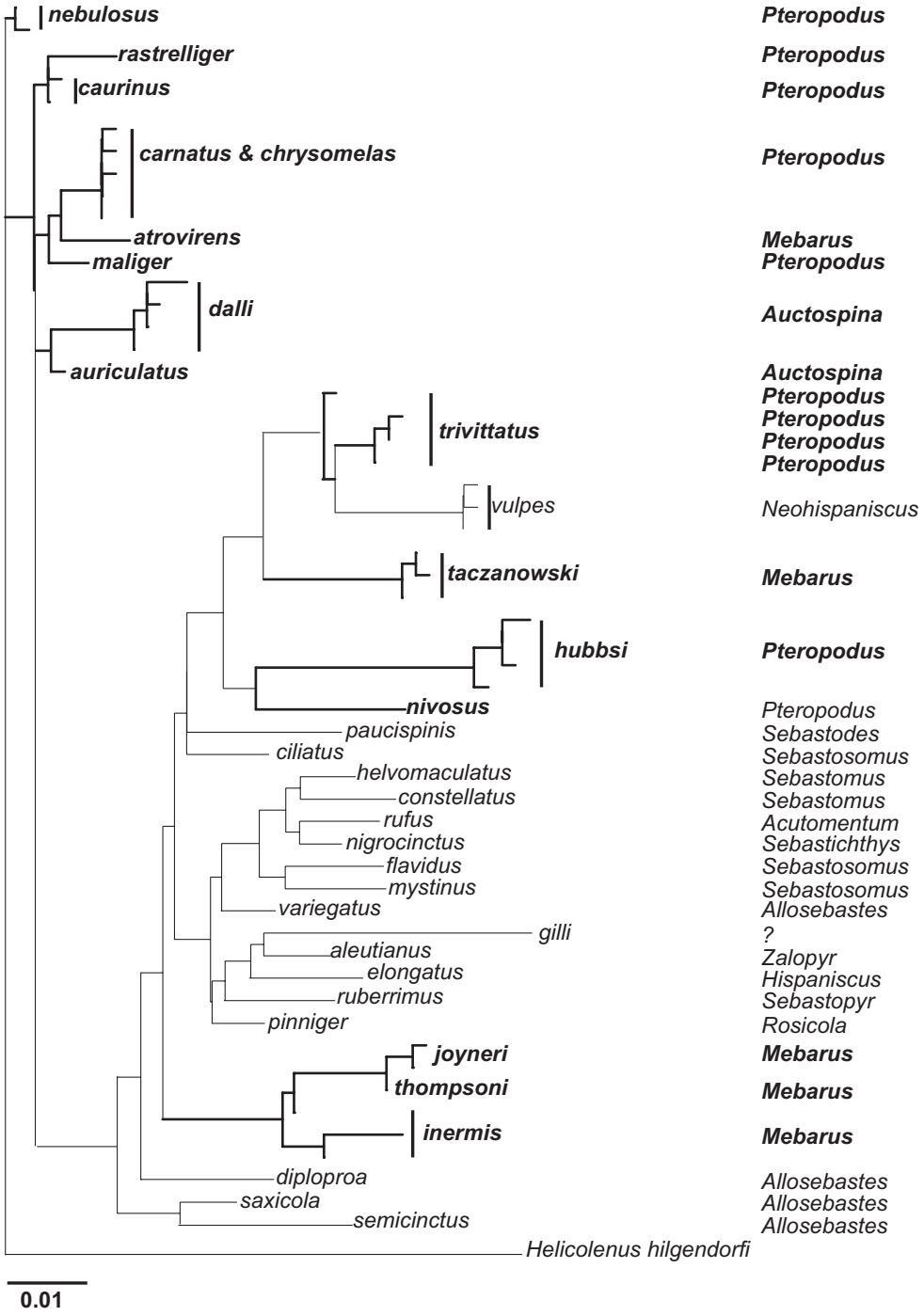
The NWP species were not monophyletic and formed three or more branches in the different analysis. Two monophyletic branches (100% consensus in the maximum-parsimony trees) were evident in the NWP species: the three *Mebarus* species (*S. inermis*, *S. joyneri*, and *S. thompsoni*) and *S. trivittatus* and *S. vulpes*. Both of these branches were distinct from *S. hubbsi* and *S. nivosus*.

There were no other obvious clusters in this data set, mainly because the other species were chosen as representatives of their subgenera to provide context for the species in focus. An unexpected observation was the position of *S. gilli*, which was near the NWP species in all but the maximum-likelihood tree, in which it clustered with *Sebastes aleutianus* (Jordan and Evermann, 1898). The position of *S. gilli* is probably spurious and resulted from long-branch attraction because it had five autapomorphic sites.

Discussion

The NEP members of *Pteropodus* were not monophyletic with the NWP members in any of the phylogenetic trees and should not be included in the same subgenus. Biogeography of the NEP and NWP groups of *Pteropodus* supports their separation; these species do not overlap in range. The NEP *Pteropodus* species with the widest distribution, *S. auriculatus* and *S. caurinus*, range from the Gulf of Alaska to Baja California, whereas the NWP species are limited to waters around Japan and Korea.

Fig. 3. Maximum-likelihood tree (Felsenstein 1993). The scale bar indicates the proportion of nucleotide substitutions. Vertical lines reflect multiple haplotypes for a species.



NEP *Pteropodus* species and relatives

Sebastes atrovirens (subgenus *Mebarus*), *S. auriculatus* and *S. dalli* (both in subgenus *Auctospina*), and the NEP *Pteropodus* species formed a monophyletic clade in all three types of phylogenetic analyses. In addition, we observed little nucleotide substitution among the species in this group. The average nucleotide substitution rate was 0.0124 per site, which is similar to that observed among the species of the subgenus *Sebastomus*, which was 0.0089 per site (Li 2004). The similarity in the mtDNA ND3/ND4 and 12S/16S regions among members of this group of species strongly suggests that it is monophyletic.

Two other studies of *Sebastes* species observed the same relationships. Seeb (1986) used a transformed Nei's unbiased distance to measure genetic similarity of nuclear allozyme loci within the subgenera. The mean distance within the subgenus *Pteropodus*, which was represented by *S. carnatus*, *S. caurinus*, *S. chrysomelas*, *S. maliger*, and *S. nebulosus*, was the lowest of any of the subgenera she considered. *Sebastes auriculatus* clustered consistently with these species,

which prompted the author to suggest its addition to *Pteropodus*. Rocha-Olivares et al. (1999) observed that *S. auriculatus*, as well as *S. atrovirens* and *S. dalli*, were monophyletic with *S. carnatus*, *S. caurinus*, *S. maliger*, and *S. rastrelliger*. In a related study that incorporated the data in Rocha-Olivares et al. (1999), Kai et al. (2003) considered these species an NEP clade and suggested that the species currently assigned to *Pteropodus* and *Mebarus* are not monophyletic.

Earlier taxonomists also have noted the close relationship between the NEP *Pteropodus* species and *S. atrovirens*, *S. auriculatus*, and *S. dalli*. *Sebastes atrovirens* was first described by Jordan and Gilbert (1880), placed in *Pteropodus* by Eigenmann and Beeson (1894), and amended to *Mebarus* by Chen (1985). However, the rationale for Chen's amendment was not clearly spelled out. Barsukov (1991) disagreed with Chen's assignment of *S. atrovirens* to the subgenus *Mebarus*, because he believed it did not share some characteristics relating to depth, morphology, and dispersal with three NWP *Mebarus* species (*S. inermis*, *S. joyneri*, and *S. thompsoni*).

The only current members of the subgenus *Auctospina* are *S. auriculatus* and *S. dalli*. *Auctospina* was erected as a genus by Eigenmann and Beeson (1893), and initially included only *S. auriculatus* and *S. aurora*. In a diagram depicting the relationship among the eight rockfish genera recognized at the time, the authors indicated that *Auctospina* was more closely related to *Pteropodus* than to any other groups. Eigenmann and Beeson (1893) described *S. dalli* and placed it in *Pteropodus*, but it was subsequently moved to *Auctospina* by Jordan and Starks (1895) who considered *S. dalli* a subspecies of *S. auriculatus* based on the observation by Cramer (1895) that it "is probably a young *Sebastodes auriculatus* with coronal spines obsolete". However, Hubbs and Schultz (1933) disputed this, because the original descriptions of *S. dalli* could not be applied to *S. auriculatus*. Instead, they observed that many of *S. dalli*'s characters do occur in *S. gilberti*, which was described by Cramer (in Jordan 1896) who considered it an ally of *S. carnatus* and *S. chrysomelas*, and was placed in *Pteropodus* by Jordan and Evermann (1898). Hubbs and Schultz (1933) proposed that *S. gilberti* be synonymized with *S. dalli*, and that the name *dalli* be removed from the *auriculatus* group. The current status of *S. dalli* indicates that the first part of this proposal was accepted, whereas the second part was not. The retention of *dalli* in *Auctospina* is probably erroneous, since Hubbs and Schultz (1933) showed that the move of *S. dalli* to *Auctospina*, based on the presumed similarity of *S. dalli* to *S. auriculatus*, did not have any support. Therefore, it appears that *S. dalli* should be reinstated in *Pteropodus*.

Morphological and ecological similarities appear to support the grouping of the NEP *Pteropodus* species and *S. atrovirens*, *S. auriculatus*, and *S. dalli*. These species can generally be characterized by having a mottled color pattern and strong head spines. They generally occupy nearshore and shallow shelf areas, and are sympatric along most parts of the coast of California. Although these species appear to share physical and ecological similarities with the NWP species of *Pteropodus*, our results suggest that they do not share a recent common ancestor. The NEP and NWP species may have acquired the similarities as a result of convergent evolution. The results also underscore the assertion that many morphological characters are inappropriate for phylogenetic determination.

Our inability to delineate some species may reflect recent divergence. However, the nature of the data in this study does not permit a more detailed examination of the relationships within the subgenus. One approach to improving the ability to determine the fine-scale relationships among the species may be to examine additional regions of the mtDNA, particularly those fast evolving ones such as genes for the mitochondrial NADH dehydrogenase subunits 1, 2, 5, and 6. Information from nuclear genes would provide alternative perspectives of the relationships and probably increase resolution of the phylogenetic relationships.

In light of the genetic evidence, we recommend that *S. atrovirens* of *Mebarus* and *S. auriculatus* and *S. dalli* of *Auctospina* be placed in *Pteropodus*. Consequently, the subgenus *Auctospina* would be eliminated and the subgenus *Mebarus* would comprise only NWP species.

NWP *Pteropodus* species and others from NWP

Sebastes trivittatus, a *Pteropodus* species, was monophyletic (100% consensus) with *S. vulpes* of *Neohispaniscus* in the maximum-parsimony cladograms and occurred on the same branch in the neighbor-joining and maximum-likelihood trees. This pairing was corroborated by morphological similarities. Chen and Barsukov (1976) suggested that *S. trivittatus* and *S. nivosus* were erroneously placed in *Pteropodus* by Matsubara (1943) and that the two species belonged in *Neohispaniscus* with the *S. vulpes* complex, which included *S. ijimae* and *S. zonatus* and which have since been synonymized with *S. vulpes* (Ishida 1984). This is consistent with our observations for *S. trivittatus* but not for *S. nivosus*. *Sebastes trivittatus* was distinct from the other NWP *Pteropodus* species, *S. hubbsi* and *S. nivosus*, which showed no consistent relationship to each other or to other NWP species included.

The three *Mebarus* species (*S. inermis*, *S. joyneri*, and *S. thompsoni*) were monophyletic (100% consensus) in all three maximum-parsimony analyses and were members of the same branch in the neighbor-joining and maximum-likelihood trees. In a study based on sequences of the mitochondrial cytochrome *b* gene, a neighbor-joining tree also showed that these three species appeared to be closely related (Kai et al. 2003). Morphological similarities described in Matsubara (1943) and Ishida (1984) appear to support the genetic similarity reflected by this grouping. Incidentally, *S. inermis* may be a group of species because Chen (1985) and Barsukov (1991) showed that three different meristic types existed within *S. inermis*. Recent studies (Kai and Nakabo 2002; Kai et al. 2002) identified three different types of *S. inermis* that could be separated by both morphological and genetic differences. These morphotypes roughly corresponded with those of Chen (1985). The taxonomic status of the three types of *S. inermis* has not been determined; however, in our study, intraspecific variation within *S. inermis* was the largest observed and of the order of interspecific differences in NEP *Pteropodus*.

Sebastes taczanowski was distinct from other NWP species included in our study. Chen (1985) suggested that *S. taczanowski* and *Sebastes wakiyai* (Matsubara, 1934) (not included in this study) should be removed from *Mebarus*.

Chen stated that these two species were "very different from the *S. inermis* complex".

In all, our results indicate that neither the NWP species of *Pteropodus* nor the NWP species of *Mebarus* are monophyletic. We recommend that the NWP *Pteropodus* species included in this study (*S. hubbsi*, *S. nivosus*, and *S. trivittatus*) be removed from the subgenus, since they did not form a monophyletic group with the NEP *Pteropodus* species.

Because a number of NWP species were not included in this study, the ultimate subgeneric placements of those NWP species that were examined cannot be made. A genetic study that includes all of the NWP species is needed to resolve the phylogenetic relationships of every NWP species.

Acknowledgments

This work is part of the master's thesis of Z. Li. Dr. A.W. Kendall, Jr. contributed advice and insight that helped us develop this paper. This work was supported by the US Geological Survey (Biological Resources Division) Western Regional Office in Seattle, Washington, (R.W.O. #32) through the Alaska Cooperative Fishery and Wildlife Research Unit. A.J. Gharrett was supported by the Japan Society for the Promotion of Science while he was on sabbatical at Hokkaido and Kitasato universities in Japan.

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