

Morphometric convergence and molecular divergence: the taxonomic status and evolutionary history of *Gymnura crebripunctata* and *Gymnura marmorata* in the eastern Pacific Ocean

W. D. SMITH*†, J. J. BIZZARRO*, V. P. RICHARDS‡, J. NIELSEN‡§,
F. MÁRQUEZ-FARÍAS||** AND M. S. SHIVJI‡

*Pacific Shark Research Center, Moss Landing Marine Laboratories, Moss Landing, CA 95039, U.S.A., †The Guy Harvey Research Institute, Oceanographic Center, Nova Southeastern University, Dania Beach, Florida 33004, U.S.A., and ‡Instituto Nacional de la Pesca, Centro Regional de Investigación Pesquera de Mazatlán, Calzada Sábalo-Cerritos S/N, CP. 82010, Mazatlán, Sinaloa, México

(Received 17 September 2007, Accepted 1 April 2009)

To clarify the taxonomic status of *Gymnura crebripunctata* and *Gymnura marmorata*, the extent of morphological and nucleotide variation between these nominal species was examined using multivariate morphological and mitochondrial DNA comparisons of the same characters with congeneric species. Discriminant analysis of 21 morphometric variables from four species (*G. crebripunctata*, *G. marmorata*, *Gymnura micrura* and *Gymnura poecilura*) successfully distinguished species groupings. Classification success of eastern Pacific species improved further when specimens were grouped by species and sex. Discriminant analysis of size-corrected data generated species assignments that were consistently accurate in separating the two species (100% jackknifed assignment success). Nasal curtain length was identified as the character which contributed the most to discrimination of the two species. Sexual dimorphism was evident in several characters that have previously been relied upon to distinguish *G. crebripunctata* from *G. marmorata*. A previously unreported feature, the absence of a tail spine in *G. crebripunctata*, provides an improved method of field identification between these species. Phylogenetic and genetic distance analyses based on 698 base pairs of the mitochondrial cytochrome *b* gene indicate that *G. crebripunctata* and *G. marmorata* form highly divergent lineages, supporting their validity as distinct species. The closely related batoid *Aetoplatea zonura* clustered within the *Gymnura* clade, indicating that it may not represent a valid genus. Strong population structuring (overall $\Phi_{ST} = 0.81$, $P < 0.01$) was evident between *G. marmorata* from the Pacific coast of the Baja California peninsula and the Gulf of California, supporting the designation of distinct management units in these regions.

© 2009 The Authors

Journal compilation © 2009 The Fisheries Society of the British Isles

Key words: Gymnuridae; mitochondrial DNA; phylogeography; sexual dimorphism.

†Author to whom correspondence should be addressed at present address: Oregon State University, Department of Fisheries & Wildlife, Corvallis, OR 97331, U.S.A. Tel.: +1 541 737 4531; fax: +1 541 737 3590; email: wade.smith@oregonstate.edu

§Present address Biology Department, Cox Science Center, University of Miami, Coral Gables, Florida 33124, U.S.A., **Universidad Autonoma di Sinaloa, Facultad de Ciencias del Mar, Mazatlán, Sinaloa, México

INTRODUCTION

Intraspecific variation and sexual dimorphism may complicate taxonomic designations, particularly among species with similar morphology. Although stingrays of the family Gymnuridae are readily distinguished from other rays because of disc widths (W_D) that are nearly twice that of body length and strong dorso-ventral compression, the conservative morphology displayed within the family has confounded taxonomy at both generic and specific levels. The butterfly rays (family Gymnuridae) currently comprise two genera, *Aetoplatea* (Valenciennes in Müller & Henle) and *Gymnura* (Kuhl in van Hasselt). The validity of the genus *Aetoplatea*, as well as that of several species within *Gymnura*, however, has been questioned because of the limited interspecific differentiation and uncertainty regarding the extent of intraspecific variation in their external morphology (Isouchi, 1977; Compagno, 1999a; Vossoughi & Vosoughi, 1999).

Three species of *Gymnura* have been reported from the eastern Pacific Ocean: *Gymnura afuerae* (Hildebrand, 1946), *Gymnura crebripunctata* (Peters) and *Gymnura marmorata* (Cooper). The ranges of these species are poorly known because their occurrences have been infrequently reported. Records of *G. afuerae* are known only from Ecuador and Peru (Hildebrand, 1946; Bearez, 1996). *Gymnura crebripunctata* has been identified from Mexico, Panama, Ecuador and Peru (Evermann & Radcliffe, 1917; Bearez, 1996; Cota-Gomez *et al.*, 1998), whereas *G. marmorata* is assumed to be continuously distributed from southern California, U.S.A. to Peru (McEachran & Notarbartolo di Sciara, 1995).

The taxonomic status of *G. crebripunctata* and *G. marmorata* is uncertain. The validity of *G. crebripunctata* was first questioned by Breder (1928) who suggested that the species does not appear to 'differ in significant characters' from *G. marmorata*. His discussion, however, focused on an expanded description of *G. marmorata* rather than a direct, comparative assessment of these species. Currently, the most comprehensive guides to batoids of the eastern central Pacific Ocean (McEachran & Notarbartolo di Sciara, 1995) and Mexico (Castro-Aguirre & Espinosa-Pérez, 1996; Castro-Aguirre *et al.*, 1999) maintain *G. crebripunctata* as a valid species. Castro-Aguirre & Espinosa-Pérez (1996) addressed Breder's (1928) concern but concluded that *G. crebripunctata* is a valid species that could be differentiated from *G. marmorata* by the absence of a ventral dermal tail fold. Additional diagnostic characters reported for distinguishing *G. crebripunctata* from *G. marmorata* are pre-orbital lengths that exceed inter-orbital widths in the former species (McEachran & Notarbartolo di Sciara, 1995). These features, however, may vary considerably between sexes. For example, female *G. marmorata* have been observed to possess well-developed dermal tail folds in contrast to males (Breder, 1928), and mature male *Gymnura micrura* (Bloch & Schneider) are known to develop longer, more acutely pointed snouts than their female counterparts (Bigelow & Schroeder, 1953). Therefore, these characters may be unreliable for taxonomic identification. Despite conflicting conclusions about the validity of *G. crebripunctata*, a critical evaluation of its taxonomic status has not been conducted.

Until recently, the importance of batoids in small-scale, coastal artisanal fisheries of the Mexican Pacific Ocean was largely unrecognized (Márquez-Farías, 2002; Bizzarro *et al.*, 2007). Rays numerically dominate landings at many sites throughout the Gulf of California and comprise significant components of bycatch in trawl

fisheries (Flores *et al.*, 1995; García-Caudillo *et al.*, 2000). *Gymnura* spp. are among the most commonly landed rays in the artisanal elasmobranch fishery of this region (Villavicencio-Garayzar, 1995; Bizzarro *et al.*, 2007) and may represent a large proportion of incidental batoid landings. The lack of taxonomic clarity for *Gymnura* in the Mexican Pacific Ocean constrains the advancement of even basic research on the distribution, biology and population structure of these rays. Such details are essential for developing effective management strategies.

To clarify the taxonomic uncertainty surrounding eastern Pacific butterfly rays, morphological and mitochondrial cytochrome *b* (*cyt b*) gene nucleotide character variation was assessed within and between the nominal species *G. crebripunctata* and *G. marmorata* in comparison with other congeneric species. Analyses revealed these two taxa to be evolutionarily highly divergent lineages, supporting their validity as separate species. Molecular analyses also identified a strong phylogeographic break between *G. marmorata* populations from the Pacific coast of the Baja California peninsula and the Gulf of California.

MATERIALS AND METHODS

MORPHOMETRIC ANALYSIS

Morphometric information was obtained from *G. crebripunctata* and *G. marmorata* specimens accessioned into the collections of the American Museum of Natural History (AMNH), California Academy of Sciences (CAS), Museum für Naturkunde der Humboldt-Universität zu Berlin (ZMB), Natural History Museum of Los Angeles County (LACM), Scripps Institution of Oceanography (SIO) and National Museum of Natural History (USNM). The holotype of *G. crebripunctata* (ZMB 7068) was among the specimens examined but that of *G. marmorata* was lost or destroyed from the CAS collections in the 1906 San Francisco earthquake. The congeneric species *G. micrura* and *Gymnura poecilura* (Shaw) were frequently available and measured to assess the extent of phenotypic variation within and among valid species. The holotype of *G. afuerae* (USNM 77709) was also examined and represented the only specimen available for this nominal species in these museum collections.

A minimum of 21 external characters were measured and the sex was determined for each specimen (Fig. 1). Specimens within the known size range of near-term embryos were excluded from analysis. Measurements were made point to point to the nearest 0.05 mm or to the nearest 1.0 mm when characters exceeded 150 mm. A total of one *G. afuerae*, 17 *G. crebripunctata*, 26 *G. marmorata*, 24 *G. micrura* and 22 *G. poecilura* were examined from museum collections (Table I).

Material examined for comparative morphometric analysis included the following specimens. *G. afuerae*: USNM 77709; *G. crebripunctata*: CAS 11394, CAS 11587, CAS 53963 (accession number associated with three specimens), CAS C6 W-53-310, LACM W50-20 (accession number associated with three specimens), LACM 51-36, LACM 52-40 (accession number associated with two specimens), LACM 52-45, LACM 58-47 (accession number associated with two specimens), USNM 028298, ZMB 7068; *G. marmorata*: CAS 17755 (accession number associated with two specimens), CAS 30827, CAS C6 W54-365, LACM W49-422 (accession number associated with two specimens), LACM W52-45, SIO H47-53-6E, SIO H-48 29, SIO H-50 291, SIO 01-180, SIO 55-69-6A, SIO 64-79 (accession number associated with two unidentified *Gymnura* spp. specimens), SIO 63-801, SIO 60-357-6A, SIO 64-236-6A, SIO 63-801, USNM 26770 (accession number associated with four specimens, two females and one male examined), USNM 62382, USNM 62384, USNM 62394; *G. micrura*: LACM 2462, USNM 51940 (accession number associated with four specimens), USNM 51897, USNM 116452 (accession number associated with six specimens), USNM 127073, USNM 127298, USNM 127299, USNM 127300, USNM 127334, USNM 131275, USNM 143221, USNM 155734, USNM 156435, USNM 158588, USNM 160832, USNM

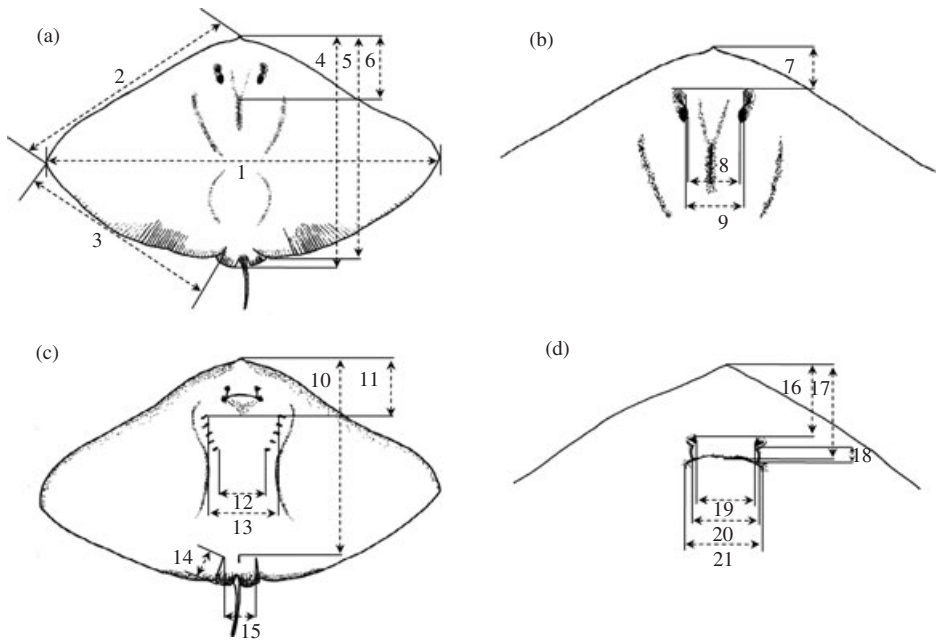


FIG. 1. Morphometric characters and associated abbreviations used in this study. (a) 1, disc width (W_D); 2, anterior pectoral length (L_{AP}); 3, posterior pectoral length (L_{PP}); 4, body length (L_B); 5, disc length (L_D); 6, head length (L_H). (b) 7, pre-orbital snout length (L_{POBS}); 8, inter-orbital width (W_{IO}); 9, interspiracular width (W_{IS}). (c) 10, snout to vent length (L_{SV}); 11, snout to first gill length (L_{SG1}); 12, fifth gill transverse distance (D_{G5}); 13, first gill transverse distance (D_{G1}); 14, anterior pelvic length (L_{APV}); 15, pelvic span (S_P). (d) 16, pre-narial length (L_{PN}); 17, pre-oral snout length (L_{POLS}); 18, nasal curtain length (L_{NC}); 19, inter-narial width (W_{IN}); 20, nasal curtain width (W_{NC}); 21, mouth width (W_M).

0452502 and *G. poecilura*: CAS 42168 (accession number associated with 11 specimens, two examined), CAS G-20, LACM 38314-23 (accession number associated with two specimens), LACM 38317-6, LACM 38311-32, LACM 38312-26 (accession number associated with two specimens), LACM 38318-10, LACM 38130-50, LACM 38133-47 (accession number associated with three specimens), LACM 38130-50 (accession number associated with two specimens), LACM 38317-6, USNM 206134, USNM 222602, USNM 222605, USNM 222610 and USNM 222612. Accession numbers could not be located for one *G. marmorata* specimen from the CAS collection (collected 1949, Baja California, Mexico) and one housed at SIO (collected from Sebastian Vizcaíno Bay, Baja California Sur, Mexico) that were used in this study.

An additional 44 *G. crebripunctata* and five *G. marmorata* were collected and examined from fishery landings in Mazatlán and Bahía Almejas, respectively, to increase the sample sizes for morphometric analyses (Table I). Tissue samples from a subset of the unpreserved, fishery-derived *G. crebripunctata* ($n = 24$) and all *G. marmorata* ($n = 5$) were included in the molecular analysis to verify species identifications made in the field. Female, male, immature and mature specimens were represented within each species grouping.

Morphological affinities among species and individual specimens were evaluated using forward stepwise canonical discriminant analysis (DA). This procedure maximizes between-group variation, minimizes within-group differences and identifies which variables contribute most to group separation (McGarigal *et al.*, 2000). Analysis was conducted on two groupings of morphometric data. To compare gradients of character variation and overall species classification success based on the selected morphological variables, all specimens were initially grouped based on museum or field-based species designations. The potential for

TABLE I. Sources and size composition of *Gymnura* spp. specimens included in morphometric analyses. Size range presented as disc width (W_D)

Species	Fishery-derived	Museum	Total <i>n</i>	Female size range (mm) (<i>n</i>)	Male size range (mm) (<i>n</i>)
<i>G. afuerae</i>	0	1	1		504
<i>G. crebripunctata</i>	44	11	55	332–812 (31)	290–463 (24)
<i>G. marmorata</i>	5	26	31	227–947 (16)	266–600 (15)
<i>G. micrura</i>	0	24	24	172–397 (9)	217–431 (15)
<i>G. poecilura</i>	0	22	22	265–633 (10)	251–649 (12)

sexual differences to influence the identification and classification of *G. crebripunctata* and *G. marmorata* was examined in a second DA in which female and male specimens of these nominal species were assigned to separate groups. To expand the size range and increase the sample size for this analysis, data from the unpreserved, fishery-derived specimens were combined with those obtained from museum specimens (Table I). The only available specimen of *G. afuerae* was included as an unweighted group to allow character comparisons with other eastern Pacific gymnurids. Morphometric data were $\log_{10}x + 1$ transformed to reduce heterogeneity in variance and analysed using SYSTAT statistical software (Ver. 10, SPSS Inc.; www.spss.com). Morphometric relationships among groups were illustrated by plots of resulting canonical scores for each DA. Proximity and orientation of groups or individuals in canonical space reflect morphological similarities or differences identified through DA.

Discrimination among species based on morphometric variables may be confounded by intraspecific variation of characters that are strongly influenced by size (Rohlf & Bookstein, 1987; McCoy *et al.*, 2006). Distinguishing morphological patterns can be obscured by allometric changes in morphometric relationships that are associated with developmental events such as sexual maturation. To evaluate differences in shape between *G. crebripunctata* and *G. marmorata* and reduce intraspecific growth effects, Burnaby's (1966) method of size-correction was applied to the $\log_{10}x + 1$ transformed multivariate data sets of both species. This procedure preserves intraspecific geometric relationships of morphometric characters while extracting overall variation related to body size (W_D , in this case) (Klingenberg, 1996; McCoy *et al.*, 2006). A third forward stepwise DA was computed using the resulting size-corrected data for *G. crebripunctata* and *G. marmorata*.

Overall performance of DA was measured by the squared canonical correlation (r_c^2), which reflects the proportion of total variation in a canonical function that is explained by differences among group means (McGarigal *et al.*, 2000). The utility of resulting canonical functions to discriminate among groups was evaluated using the results of leave-one-out jackknifed classification summaries. Accuracy of group assignments were further assessed using a chance-corrected criterion, Kappa, applied to the original classification matrix because of unequal group sizes (McGarigal *et al.*, 2000). Kappa values of zero indicate that classification accuracy represents no improvement over that of random chance, and values of one signify perfect discrimination.

Relationships of each character with respect to W_D and sex were initially plotted for *G. crebripunctata* and *G. marmorata* to assess the influence of sex and size on these variables using untransformed morphometric data. Results are presented only for characters that have been previously reported as diagnostic features (IOW, POBSL; Fig. 1) or those which contributed most significantly to group separation based on DA. Analysis of covariance (ANCOVA) was applied to determine if differences between the resulting linear regressions were statistically significant.

MOLECULAR ANALYSIS

Tissue samples were obtained from *G. crebripunctata* and *G. marmorata* landed in artisanal elasmobranch fisheries, industrial shrimp fisheries and fishery-independent surveys conducted

TABLE II. Collection locations and sample sizes (*n*) of *Gymnura* spp. and *Aetoplatea* spp. included in molecular analysis

Species	Location	<i>n</i>
<i>A. zonura</i>	Taiwan	1
<i>G. australis</i>	Eastern Australia	1
<i>G. altavela</i>	U.S.A., Atlantic	1
<i>G. crebripunctata</i>	Mexico, Sinaloa	24
<i>G. japonica</i>	Japan (GenBank)	1
<i>G. marmorata</i>	Mexico, Bahía Almejas	9
	Mexico, Ensenada	11
	Mexico, Sonora	10
<i>G. micrura</i>	U.S.A., Gulf of Mexico	1
<i>G. poecilura</i>	Oman	1

between 1999 and 2005 in western Mexico, with additional specimens ($n = 4$) contributed by fisheries researchers in Sonora, México (Table II and Fig. 2). These samples included a subset of the *G. crebripunctata* ($n = 24$) and *G. marmorata* ($n = 5$) specimens that were also included in morphometric analyses as well as additional specimens that were collected exclusively for molecular analysis. Sex and W_D were typically recorded, but sex information was not available for all specimens. Tissue samples were also obtained from *Aetoplatea zonura* (Bleeker), *Gymnura altavela* (L.), *Gymnura australis* (Ramsay & Ogilby), *G. micrura* and *G. poecilura* (Table II). All samples were stored in 95% ethanol.

Genomic DNA was extracted from 25 mg of tissue using the DNeasy Tissue Kit (Qiagen Inc.; www.qiagen.com). The following primers were designed for polymerase chain reaction (PCR) amplification and sequencing 698 base pairs (bp) of the *cyt b* gene: 163F (5'-CACTACACCGCAGACATCTC-3') and 998R (5'-GCCGCCGATTCATGTTAGGAT-3').

Total PCR volumes were 50 μ l and contained 1 μ l of the extracted genomic DNA, 5 μ l 10 \times PCR Buffer, 50 μ M of each dNTP, 0.25 μ M of each primer and 0.75 units of HotStar Taq™ DNA Polymerase (Qiagen Inc.). PCR was performed in a Mastercycler Gradient (Eppendorf Inc.; www.eppendorf.com) thermal cycler as follows: 95° C initial heating for 15 mins to activate the hot start DNA polymerase, followed by 35 cycles of 94° C for 1 min, 50° C for 1 min, 72° C for 1 min and a 5 min final extension step at 72° C. For a few individuals that did not amplify well initially, the number of cycles was increased to 41, the 50° C annealing step increased to 2 mins and the 72° C extension step increased to 3 mins. A negative control (no genomic DNA) was included in each PCR set to check for reagent contamination. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc.) and sequenced in both directions using the Applied Biosystems (AB) BigDye Terminator v3.1 Cycle Sequencing Kit and an AB 3130 Genetic Analyser (www.appliedbiosystems.com). Bases were called using AB Sequencing Analysis Software version 5.2. Cytochrome *b* sequences for the gymnurid species and haplotypes of the batoids included in this study are available from GenBank, where associated accession numbers are as follows: *Aetobatus narinari* (Euphrasen) (FJ010561), *A. zonura* (FJ010562), *G. altavela* (FJ010565), *G. australis* (FJ010563), *G. crebripunctata* (FJ010567–FJ010574), *G. marmorata* (FJ010575–FJ010585), *G. micrura* (FJ010566) and *G. poecilura* (FJ010564). The *Gymnura japonica* (Temminck & Schlegel) *cyt b* sequence included in the analyses was obtained from GenBank (accession number AB021503).

PHYLOGENETIC AND STATISTICAL ANALYSES

MacCLADE version 4.03 (Maddison & Maddison, 2001) was used to align and edit individual sequences. The alignment was translated using GENEDOC version 2.6.02 (Nicholas *et al.*, 1997). Sequences were checked for correct vertebrate mtDNA amino acid coding and aberrant start and stop codons.

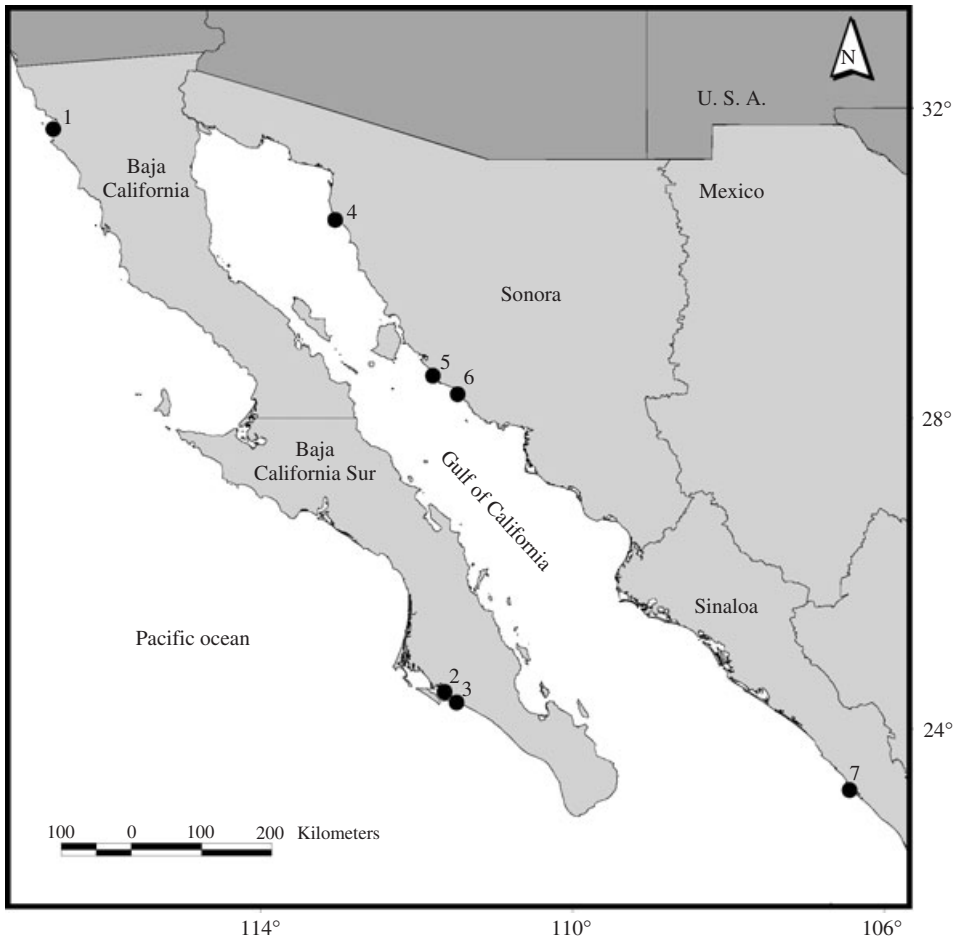


FIG. 2. Collection sites for *Gymnura crebripunctata* and *Gymnura marmorata*: 1, Punta Banda (Ensenada); 2, Puerto Viejo (Bahía Almejas); 3, Loma Amarillo (Bahía Almejas); 4, Desemboque (Sonora); 5, El Sahuimaro (Sonora); 6, El Choyudo (Sonora); 7, Mazatlán (Sinaloa).

Maximum likelihood (ML) and neighbour joining (NJ) phylogenetic reconstructions were performed using PAUP version 4.0b10 (Swofford, 2002). ML heuristic searches obtained starting trees *via* stepwise addition using 10 random addition sequence replicates and branch swapping was performed using tree bisection-reconnection (TBR). Using the Akaike information criterion (AIC), Modeltest version 3.06 (Posada & Crandall, 1998) selected the TrN+I+G model of nucleotide substitution as the best fit for the data. This model was used in the ML and NJ analyses.

Bayesian phylogenetic reconstructions were performed using MrBayes version 3.0b4 (Huelsenbeck & Ronquist, 2001). Markov-chain Monte-Carlo (MCMC) sampling was initiated with a random tree and run for one million generations. Metropolis coupling with one cold and three heated chains was used to improve MCMC sampling. The data were partitioned according to codon position and trees sampled every 100 generations. The GTR+I substitution model (selected using the AIC in MR Modeltest version 2.2; Nylander, 2004) was implemented in the tree search, with the first 30 000 trees discarded as burn in.

Statistical support for branch nodes was assessed using non-parametric bootstrap analyses (100 replicates for ML; 2000 replicates for NJ) (Felsenstein, 1985) and posterior probabilities

for the Bayesian analysis. The ML heuristic bootstrap searches obtained starting trees *via* stepwise addition using the as-is option. Branch swapping was performed using nearest neighbour interchange (NNI).

Mean pairwise uncorrected (p) and Kimura two-parameter (K2P) corrected genetic distances were calculated among *G. crebripunctata* and *G. marmorata* phylogenetic groupings and between *Gymnura* congeners using MEGA3 (Kumar *et al.*, 2004). Patristic distances were calculated from the ML tree using the programme PATRISTIC version 1.0 (Fourment & Gibbs, 2006). Genetic population structure among *G. marmorata* sampled from Ensenada, Bahía Almejas and Sonora was examined by an analysis of molecular variance (AMOVA) as implemented in Arlequin version 2.000 (Schneider *et al.*, 2000).

RESULTS

MORPHOMETRIC ANALYSIS

Discriminant analysis using museum-based identifications distinguishes four species groupings (Fig. 3). The western Pacific and Indian Ocean congener *G. poecilura* is clearly differentiated from other groups; however, notable overlap of data points is evident among *G. crebripunctata*, *G. marmorata* and, to a lesser extent, *G. micrura*. Ten of the initial 21 characters were determined to be valuable diagnostic features among the groups in stepwise analysis (Wilks' lambda = 0.02); W_{IS} , W_{IO} , W_{IN} , S_P , W_M , L_{AP} , L_B , L_{PN} , L_{NC} and D_{G1} (Fig. 1) (ranked highest to lowest by discriminating power; F -to-remove). *Gymnura afuerae* falls outside of the confidence ellipses of each species grouping but is most closely associated with *G. crebripunctata*. Canonical discriminant functions one (49.2%) and two (33.4%) account for 82.6% of the total variance in group variation. Differences among group means explains 81% of the total canonical variation ($r_c^2 = 0.81$). Jackknifed classification accurately predicts 100% of *G. crebripunctata*, 92% of *G. marmorata*, 91% of *G. micrura* and 100% of *G. poecilura*. Group classification is 95% better than could be expected based on random assignment of individuals to each group (Kappa = 0.95).

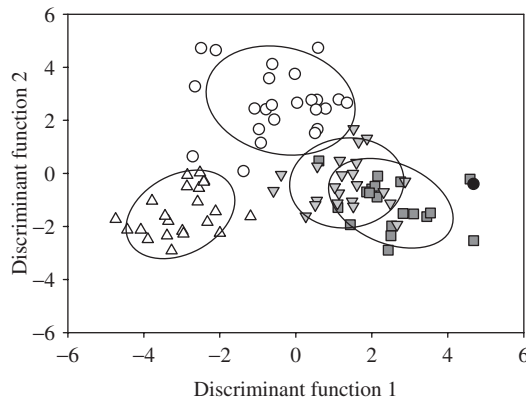


FIG. 3. Plot of case scores from forward step discriminant analysis (DA) of *Gymnura afuerae* (●), *Gymnura crebripunctata* (■), *Gymnura marmorata* (▽), *Gymnura micrura* (○) and *Gymnura poecilura* (△). Groupings based on museum identifications. Bivariate 95% confidence ellipses are depicted.

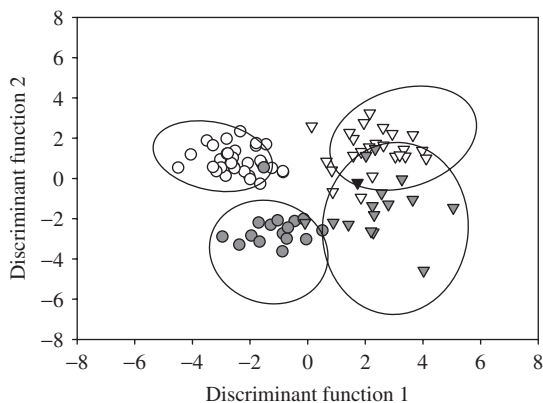


FIG. 4. Plots of individual case scores from forward step DA of eastern Pacific *Gymnura* grouped by species and sex: male *Gymnura afuerae* (∇), female *Gymnura crebripunctata* (\circ), male *Gymnura crebripunctata* (∇), female *Gymnura marmorata* (\bullet) and male *Gymnura marmorata* (\blacktriangledown). Bivariate 95% confidence ellipses are depicted.

Distinct groupings of *G. crebripunctata* and *G. marmorata* were identified by DA using sex-based classifications within species (Fig. 4). Optimal group separation was achieved using seven of the 21 initial characters (Wilks' lambda = 0.04); L_{PP} , L_D , L_{POLs} , L_{APV} , D_{G1} , W_{IN} and W_M (Fig. 1) (ranked highest to lowest by discriminating power; F -to-remove values). A cumulative dispersion of 98.3% is explained by the first (66.4%) and second (31.9%) canonical discriminant functions. Group means (centroids) of females are more strongly oriented with L_{PP} , W_M and W_{IN} (Fig. 1) than males. Male groupings of both species reflect analogous morphological patterns, indicating strong similarity in the characters of L_{POLs} and L_{APV} (Fig. 1). Correlation among the seven discriminating variables indicates that 84% of the total canonical variation can be attributed to differences among group means ($r_c^2 = 0.84$). Jackknifed assignment success is 100% for female *G. crebripunctata*, 88% for male *G. crebripunctata*, 94% for female *G. marmorata* and 67% for male *G. marmorata*. Chance-corrected predictability of overall group membership is 87% (Kappa = 0.87). Plots of individual canonical scores reveal considerable overlap between morphometric relationships of male *G. crebripunctata* and *G. marmorata*. Canonical scores of male and female *G. marmorata* are relatively more broadly dispersed than in *G. crebripunctata*. The male holotype of *G. afuerae* is oriented within the confidence ellipses of male *G. crebripunctata* and *G. marmorata* based on plots of the resulting canonical scores.

Gymnura crebripunctata and *G. marmorata* were successfully distinguished by DA of size-corrected morphometric variables (Fig. 5). Although 16 of the initial 21 characters used in the forward stepwise DA contribute to group discrimination based on F -to-remove values (Wilks' lambda = 0.004; L_{NC} , L_{APV} , D_{G5} , S_P , W_M , W_{IN} , L_{PN} , L_{POBS} , D_{G1} , W_{IO} , L_D , L_{POLs} , W_{IS} , L_H , W_{NC} and L_{SV} ; Fig. 1), L_{NC} provides a much greater contribution to overall discriminatory power than that generated by the other variables. A cumulative dispersion of 100% is explained by the first canonical discriminant function. The r_c^2 further indicates that 99% of the total canonical variation can be attributed to differences among group means ($r_c^2 = 0.99$).

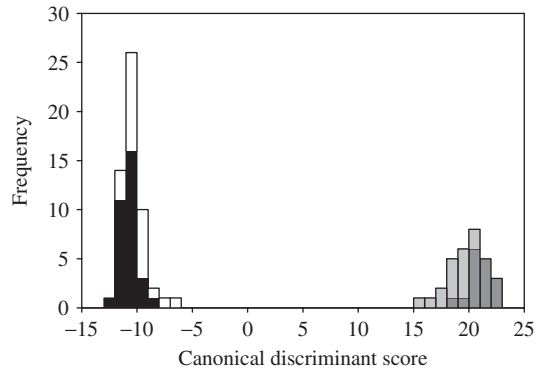


FIG. 5. Canonical scores obtained from size-corrected DA of morphometric variables from female *Gymnura crebripunctata* (■), male *G. crebripunctata* (□), female *Gymnura marmorata* (▒) and male *G. marmorata* (▨).

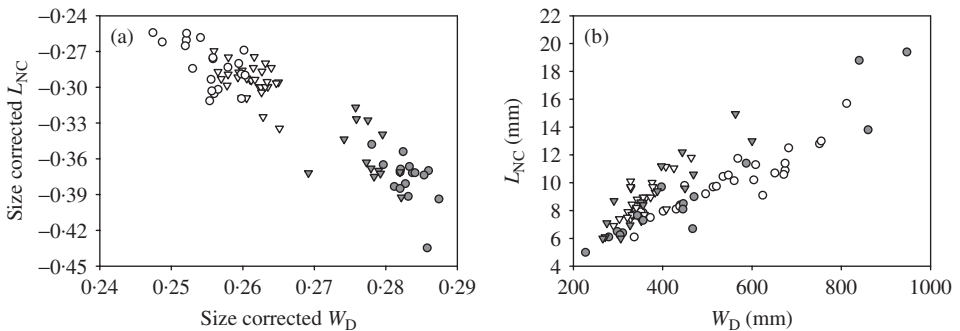


FIG. 6. Species-specific relationships between (a) size-corrected values of nasal curtain length (NCL) and disc width (W_D) and (b) the original values of these same characters: female *Gymnura crebripunctata* (○), male *G. crebripunctata* (▽), female *Gymnura marmorata* (●) and male *G. marmorata* (▼).

Jackknifed species assignment correctly places all specimens to their respective species. Likewise, chance-corrected predictability of overall group membership is 100% ($Kappa = 1.0$). Plots of size-corrected W_D and L_{NC} reveal separation of *G. crebripunctata* from *G. marmorata*, however, patterns of differentiation are not evident from similar plots using the raw variables (Fig. 6).

Each of the 21 morphometric characters (Fig. 1) examined displays positive allometry in relation to W_D based on univariate regression analysis of female and male *G. crebripunctata* and *G. marmorata*. Sexual dimorphism is evident for each species in several characters and is especially pronounced among larger specimens. The most strongly dimorphic characters (Fig. 1) are L_{APV} [Fig. 7(a), (b)] and L_{POBS} [Fig. 7(c), (d)]. Differences between sex and L_{APV} (ANCOVA, $n = 55$, $P > 0.05$) and L_{POBS} (ANCOVA, $n = 55$, $P > 0.05$) are not determined to be significant in *G. crebripunctata*. A significant interaction is detected between sex, L_{APV} (ANCOVA, $n = 31$, $P < 0.01$) and L_{POBS} (ANCOVA, $n = 31$, $P < 0.001$) in *G. marmorata*. Sexual dimorphism is not evident in L_{PP} , the most influential character identified in the sex-based DA, for either *G. crebripunctata* (ANCOVA, $n = 55$, $P > 0.05$)

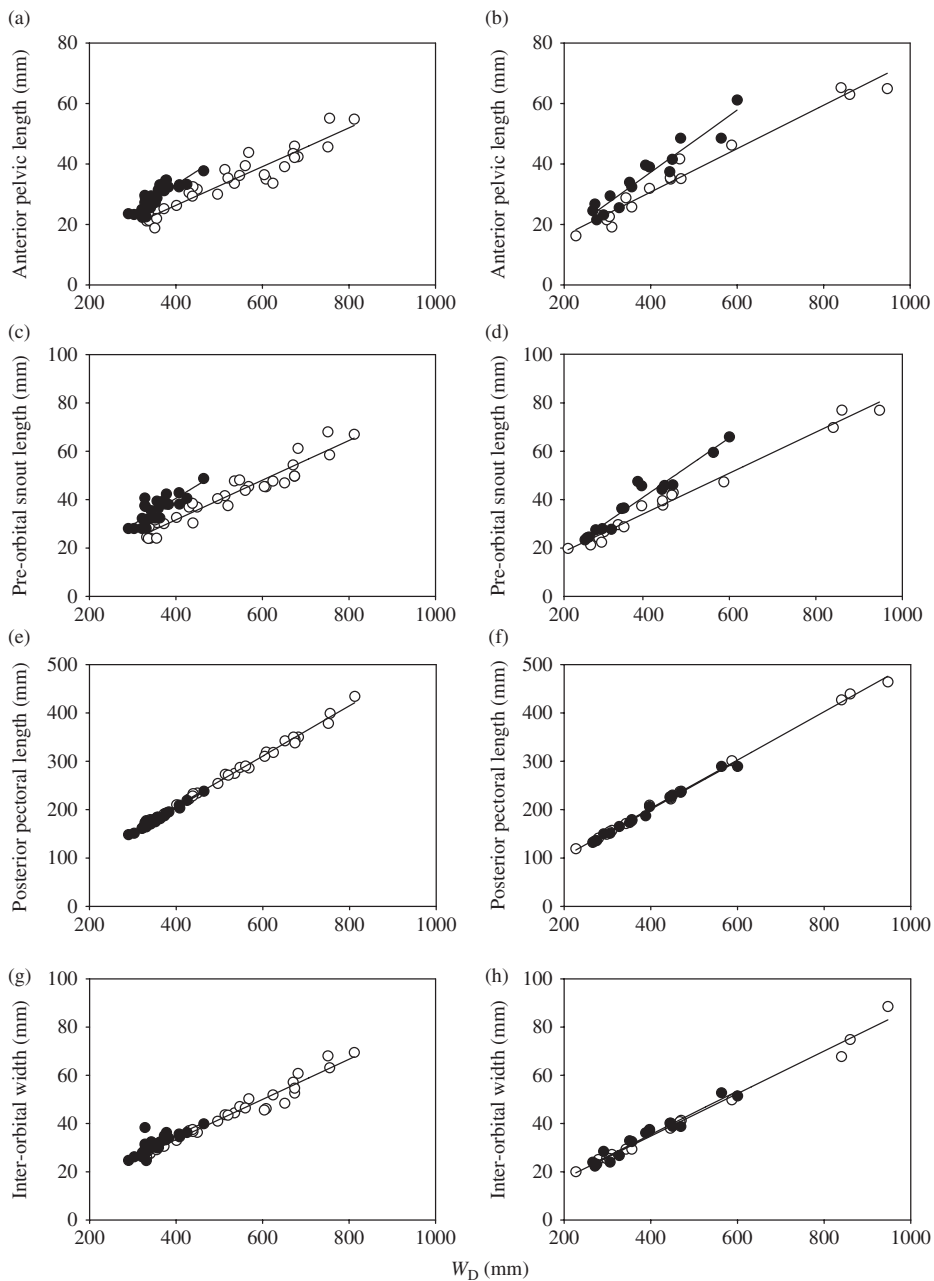


FIG. 7. Relationships between (a) and (b) anterior pelvic length (L_{AP}); (c) and (d) pre-orbital snout length (L_{POBS}); (e) and (f) posterior pectoral length L_{PP} , (g) and (h) inter-orbital width (W_{IO}) (g, h) and disc width W_D for female (○) and male (●) (a), (c), (e), (g) *Gymnura crebripunctata* and (b), (d), (f), (h) *Gymnura marmorata*. Female *G. crebripunctata*, $n = 31$; male *G. crebripunctata*, $n = 24$; female *G. marmorata*, $n = 16$; male *G. marmorata*, $n = 15$. The trend lines were inserted in the figures as visual aids.

or *G. marmorata* (ANCOVA, $n = 31$, $P > 0.05$) [Fig. 7(e), (f)]. The diagnostic character typically associated with L_{POBS} for identification of these species, W_{IO} , does not differ significantly between the sexes (*G. crebripunctata*: ANCOVA, $n = 55$, $P > 0.05$; *G. marmorata*: ANCOVA, $n = 31$, $P > 0.05$) [Fig. 7(g), (h)].

PHYLOGENETIC ANALYSIS

All three phylogenetic approaches recovered four major clades with the same branching order among them (Fig. 8). The *G. crebripunctata* and *G. marmorata* individuals form two strongly supported, highly divergent lineages (clades C and D) that are largely concordant with geography. All specimens identified in the field as *G. crebripunctata* and obtained from Mazatlán form a single lineage (clade C). With one exception, all individuals from Ensenada, Bahía Almejas and Sonora identified in the field as *G. marmorata* also form a major monophyletic lineage (clade D). The single exception, an individual collected from Sonora, possesses a *cyt b* haplotype identical to *G. crebripunctata* from Mazatlán, placing it within clade C (Gm17SON; Fig. 8). The Pacific *Gymnura* congeners (*G. poecilura*, *G. australis* and *G. japonica*) and *A. zonura* form a third major lineage (clade A) with strong (Bayesian) to moderate (NJ) support. Relationships within this lineage, however, are unresolved. The Atlantic-Caribbean congeners (*G. altavela* and *G. micrura*) form a strongly supported, fourth major lineage (clade B). Interestingly, *G. marmorata* has a basal position in the phylogeny with *G. crebripunctata* individuals which appear more derived and group closely with the Atlantic *Gymnura* congeners.

STATISTICAL ANALYSIS

All three mean genetic distance measures between *G. crebripunctata* and *G. marmorata* individuals (ML patristic = 0.345; K2P = 0.187; $P = 0.163$) exceed all pairwise comparisons among Pacific and Atlantic congeners (Pacific: ML patristic = 0.306–0.222; K2P = 0.169–0.141; $P = 0.149$ –0.126) (Atlantic: ML patristic = 0.075; K2P = 0.065; $P = 0.062$) (Table III).

Clade D (*G. marmorata*) individuals exhibit strong genetic structuring between the Gulf of California and Pacific coast of the Baja California peninsula (Ensenada v. Sonora: $\Phi_{ST} = 0.82$; $P < 0.001$; Bahía Almejas v. Sonora: $\Phi_{ST} = 0.88$; $P < 0.001$). In contrast, there is no evidence of genetic structuring along the Pacific coast of the Baja California peninsula (Ensenada v. Bahía Almejas: $\Phi_{ST} = -0.01$; $P > 0.05$).

DISCUSSION

MORPHOMETRIC ANALYSIS

Classification success of nominal *Gymnura* species groupings based on jackknifed and chance-corrected procedures is high, indicating that these species may be distinguished using a combination of external morphological characters. Despite this morphological distinction among species, there is an overlap of bivariate confidence

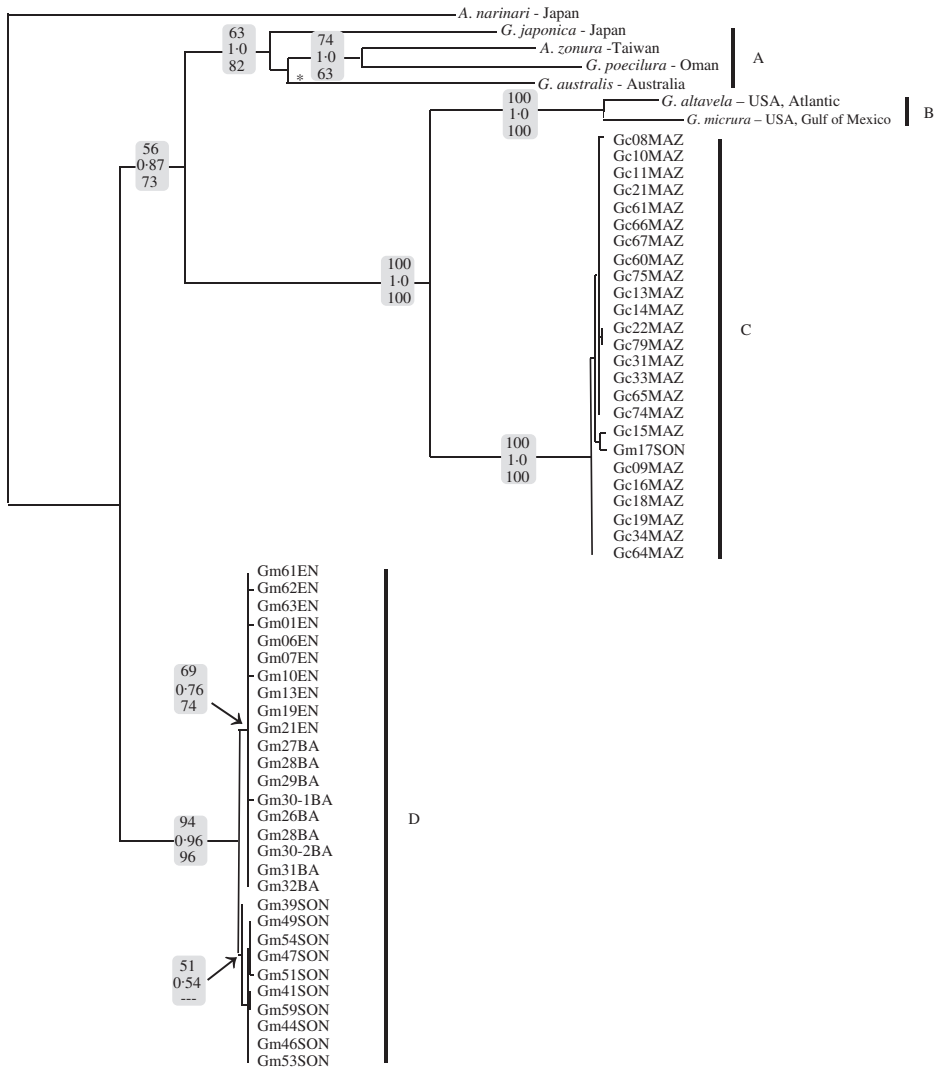


FIG. 8. Maximum likelihood (ML) phylogram (698 bp *cyt b*) depicting relationships among taxa. Geographic sources of taxa are indicated. Tree is rooted with the batoid *Aetobatus narinari* (family Myliobatidae). Clade support values are indicated in boxes over branches: ML bootstrap (top), Bayesian posterior probability (middle), neighbour joining (NJ) bootstrap (bottom), (---) indicates no support due to a collapsed node in the bootstrap consensus tree. * within clade A indicates a node that was collapsed in the bootstrap and Bayesian analyses. Specimens were designated as *Gymnura crebripunctata* (Gc) or *Gymnura marmorata* (Gm) based on the field identifications. Sample locations: Bahía Almejas (BA), Ensenada (EN), Mazatlán (MAZ) and Sonora (SON).

TABLE III. Pairwise genetic distances for *Aetoplatea zonura* and *Gymnura* spp. based on cytochrome *b* sequences. First line (bold) shows maximum likelihood patristic (branch length) distances generated using the TrN+I+G model of nucleotide substitution. Second and third lines show Kimura 2 parameter (K2P) and uncorrected (*p*) sequence distances respectively. Distances for *Gymnura crebripunctata* (clade C; Mazatlán) and *Gymnura marmorata* (clade D; Ensenada–Bahía Almejas/Sonora) are mean pairwise comparisons

	<i>G. japonica</i>	<i>A. zonura</i>	<i>G. australis</i>	<i>G. poecilura</i>	<i>G. altavela</i>	<i>G. micrura</i>	<i>G. crebripunctata</i>
<i>A. zonura</i>	0.278						
	0.150						
	0.133						
<i>G. australis</i>	0.278	0.280					
	0.157	0.166					
	0.139	0.146					
<i>G. poecilura</i>	0.304	0.222	0.306				
	0.162	0.141	0.169				
	0.143	0.126	0.149				
<i>G. altavela</i>	0.446	0.467	0.467	0.493			
	0.203	0.212	0.232	0.216			
	0.175	0.181	0.195	0.185			
<i>G. micrura</i>	0.460	0.481	0.481	0.507	0.075		
	0.215	0.202	0.236	0.229	0.065		
	0.183	0.173	0.198	0.193	0.062		
<i>G. crebripunctata</i>	0.412	0.433	0.433	0.459	0.225	0.239	
	0.212	0.202	0.206	0.210	0.147	0.155	
	0.181	0.174	0.178	0.181	0.131	0.138	
<i>G. marmorata</i>	0.288	0.309	0.309	0.335	0.379	0.393	0.345
	0.170	0.163	0.170	0.171	0.196	0.206	0.187
	0.149	0.144	0.149	0.151	0.169	0.176	0.163

ellipses, broad dispersion of individual data points and several outliers are evident for *G. crebripuncata*, *G. marmorata* and *G. micrura* (Fig. 3). Intraspecific variability of morphological characters is influenced by the combined effects of environment, genetics, geography, size (ontogenetic stage) and sexual dimorphism. Measurement error as well as preservation and storage effects (*i.e.* shrinkage and deformation) represent additional sources of variability that may contribute to the observed distributions of canonical discriminant scores. With the exception of two *G. micrura*, outliers consist of the largest and smallest individuals within each designated species group. Because of the potential for recommended diagnostic characters to be strongly influenced by size or sex, size-specific character variation was of particular interest and all morphological characters were initially examined without the removal of size effects.

Although each approach to DA included in this study was generally successful in distinguishing *G. crebripuncata* from *G. marmorata*, analysis of size-corrected morphometric data provided the only unambiguous method of discriminating between the two species. Size variation has commonly been identified as having the greatest influence on variability within sets of morphological characters (Reist, 1985; Rohlf & Bookstein, 1987). As a result, underlying differences in shape and form are often masked without the removal of size effects (Rohlf & Bookstein, 1987; McCoy *et al.*, 2006). Of the 16 size-corrected characters (Fig. 1) found to contribute significantly to group separation, L_{NC} is the single-most useful determinant. Although DA of these size-corrected morphological variables provides a reliable method of distinguishing *G. crebripuncata* from *G. marmorata*, it does not serve as a practical tool for rapid identification of these species in the field. A plot of raw measurements of W_D and L_{NC} does not indicate distinctive separation of species [Fig. 6(b)] and ANCOVA similarly does not confirm that significant differences exist in these characters between species ($P > 0.05$). Examination of L_{NC} as a percentage of W_D indicates overlap between the species, with values for *G. crebripuncata* ranging between 1.46–2.16% and 1.43–2.21%. Given the subtle variation in morphology between these species, additional features should be identified that may facilitate improved field identification.

Ontogeny and sexual dimorphism strongly influence variation of several morphologic characters for both *G. crebripuncata* and *G. marmorata*. The relative dispersion of canonical scores for *G. crebripuncata* and *G. marmorata* groupings are similar in magnitude to that of *G. micrura*, a species with a documented pattern of ontogenetic changes associated with sexual dimorphism (Bigelow & Schroeder, 1953). Multivariate and univariate analyses of morphological data reveal sexually dimorphic variation among several characters within both *G. crebripuncata* and *G. marmorata*. Patterns of morphological differentiation are more similar in orientation and the extent of canonical dispersion when specimens are grouped by sex rather than by species (Fig. 4). Females and males of both species are generally well separated based on this DA, with overlap of the sexes occurring only among the smaller size classes. Snout length and associated characters (L_{POLs} , L_{POBS} , L_D ; Fig. 1) increase more rapidly with increasing male size in comparison to females of equivalent size within both species. Differences in L_{POBS} have been identified as a key character for distinguishing *G. crebripunctata* from *G. marmorata* (McEachran & Notarbartolo di Sciarra, 1995). Identifications based on this diagnostic technique, however, would separate mature males from females and immature males of both species rather

than distinguish either of the two species. Indeed, the illustrations associated with the key of McEachran & Notarbartolo di Sciara (1995) depict a female specimen for *G. marmorata* and a male as *G. crebripunctata*. The predominance of juvenile specimens and limited size range of male *G. crebripunctata* (Table I) in the current study restricted a complete analysis of sexually dimorphic patterns for this species. The distribution of the canonical scores of females and males within each species based on size-corrected DA, however, reveals an underlying variation in morphology between the sexes (Fig. 5), and the marginal *P*-value associated with L_{APV} [Fig. 7(a); $P > 0.05$] suggests that further patterns of sexual dimorphism are likely to be confirmed if the size range is expanded for *G. crebripunctata*.

Variables used in these analyses were restricted to 21 external characters, but internal morphometrics (e.g. chondrocranial measurements), meristic data (e.g. vertebral counts) or additional external measurements not considered in this study may prove equally valuable to or improve upon identifications of these species. Several characters that may have improved species identification were initially examined but were not incorporated into final morphometric analyses. Castro-Aguirre & Espinosa-Pérez (1996) relied on the presence or absence of the dermal folds on the dorsal and ventral surfaces as a primary diagnostic between *G. crebripunctata* and *G. marmorata*. Shrinkage and deformation of dermal tail folds as a result of preservation limited the ability to consistently and accurately measure these finer-scale features among museum specimens. Although it was not possible to incorporate this character into DA because dermal tail folds could not be reliably measured for all specimens, these characters were regularly examined. Ventral dermal tail folds were occasionally absent among *G. crebripunctata*, but dermal folds were frequently found to be present on the dorsal and ventral tail surfaces of both species. Among the unpreserved *G. crebripunctata* examined in this study, ventral dermal tail folds were evident in 52% of the females and 27% of the males. Females typically possess more prominent ventral tail folds than do males (female, $n = 14$, mean \pm s.d. ventral caudal fold length: 6.99 ± 2.15 mm, mean \pm s.d. ventral caudal fold height: 0.07 ± 0.11 mm; male, $n = 5$, mean \pm s.d. ventral caudal fold length: 5.34 ± 1.72 mm; mean \pm s.d. ventral caudal fold height: 0.04 ± 0.01 mm). Ventral caudal fold dimensions appear to be highly variable and are present as little more than a ridge on the holotype as well as several other male *G. crebripunctata*. Breder's (1928) observation that the extent of tail fold development varies between female and male *G. marmorata* appears to apply to *G. crebripunctata* as well. Because these folds can be present (although variable) in *G. crebripunctata*, the absence of ventral dermal folds should not be considered as a reliable character for distinguishing *G. crebripunctata* from *G. marmorata* as previously suggested.

Alternative characters were found to be unexpected, valuable aids for field identifications of *G. crebripunctata* and *G. marmorata* that are not reflected in the DA. Surprisingly, all *G. crebripunctata* examined (including the holotype) lacked tail spines. This easily visualized difference is not reported in the literature, as far as is known, and Garman (1913) references the presence of a spine in this species. Myliobatiform rays periodically shed and may possess multiple tail spines (Teaf & Lewis, 1987), but no evidence was found in the present study that spines had been shed or were ever present in *G. crebripunctata*. The congener *G. micrura* also lacks a tail spine (Bigelow & Schroeder, 1953), but was incorrectly described as possessing one later in life by Garman (1913). Given that spines are present on

G. marmorata unless they have been shed (or removed by fishermen to simplify handling) and absent in *G. crebripuncata*, their presence or absence should serve as a new diagnostic feature for distinguishing these species.

To aid in the identification of *G. crebripuncata* and *G. marmorata*, the following provisional key is provided for field diagnosis based on qualitative observations gained from this study. Additional and expanded diagnostic characters coupled with detailed re-descriptions are necessary to improve the recognition and distinction of these species.

(1) Many irregular, but closely spaced, small, white speckles evident along the dorsal anterior disc margin; tail spine absent; central ventral surface white with yellowish–copper colouration along disc Mazatlán butterfly ray *Gymnura crebripuncata*.

(2) Dorsal anterior disc margin dark, occasionally marked with a few discrete, white spots in some specimens; tail spine typically present; ventral surface predominately white with brown–grey shading along outer disc margins which is more prominent posteriorly California butterfly ray *Gymnura marmorata*.

The holotype of *G. afuerae* was not clearly differentiated from its eastern Pacific congeners by DA. Additional specimens were unavailable from the museum collections that were examined. Like *G. crebripuncata*, the holotype of *G. afuerae* lacks a tail spine and possesses similar dorsal and ventral markings and colour patterns. Hildebrand (1946) distinguished and described *G. afuerae* based on comparisons with two smaller, immature *G. crebripuncata* (including USNM 028298 examined in this study) which he assumed were synonymous with *G. marmorata*. The most notable differences reported by Hildebrand (1946) related to the shape of the disc, length of the snout, and proportions of the eyes, spiracles and inter-orbital width. In the present study, disc shape [L_{AP} ; Fig 7(a)] and snout length [L_{POBS} ; Fig 7(c)] were found to be sexually dimorphic characters among male *G. crebripuncata* and *G. marmorata* and are therefore unlikely to serve as primary diagnostic features among species. The type locality of *G. afuerae*, Lobos de Afuera Island in Peru, is a location in which *G. crebripuncata* has also been reported (Meek & Hildebrand, 1923). Although evidence is inconclusive, it appears that *G. afuerae* may be a synonym of *G. crebripuncata*. Further investigation is required to resolve the taxonomic distinctness of *G. afuerae*.

MOLECULAR DIVERGENCE, PHYLOGENY AND PHYLOGEOGRAPHY

The reciprocally monophyletic lineages formed by *G. crebripuncata* and *G. marmorata* and large genetic distances between them validate the taxonomic distinction between these species. The patristic genetic distance between *G. crebripuncata* and *G. marmorata* (0.345) substantially exceeds those between *G. crebripuncata* and the Atlantic Ocean and Gulf of Mexico congeners *G. altavela* (0.225) and *G. micrura* (0.239), and also those between several other taxonomically uncontroversial congener pairs (Table III). Based on these values and assuming comparable mutation rates for *cyt b*, it is inferred that the magnitude of divergence between *G. crebripuncata* and *G. marmorata* exceeds those that may be expected for intraspecific differences.

The Indo-West Pacific is a major center of origin and radiation of stingrays, including the Gymnuridae (Carvalho *et al.*, 2004; McEachran & Aschliman, 2004).

The phylogeny produced in this study reflects this relationship, as the basal (ancestral) lineages (e.g. clades A and D) are all Indo-Pacific in origin and the Atlantic lineages (*G. altevela* and *G. micrura*) are among the most derived. The basal position of *G. marmorata* implies a direct derivation from an Indo-Pacific lineage.

The phylogeny and genetic distances also show that despite their modern co-occurrence, *G. crebripuncata* and *G. marmorata* are distantly related, with *G. crebripuncata* being more closely related to the Atlantic congeners of *G. altevela* and *G. micrura* than any of the other Pacific gymnurids examined in this study. Furthermore, molecular clock calibration based on the *cyt b* genetic distance between disjunct populations of the batoid *A. narinari* from opposite sides of the Panamanian isthmus (i.e. Gulf of California v. Caribbean) indicates that *G. crebripuncata* and *G. marmorata* lineages split c. 40 MYA (V.P. Richards & M. Shivji, unpubli. data). These findings reveal that despite their morphological similarity and geographic proximity, *G. crebripuncata* and *G. marmorata* did not speciate from a common ancestor present on the west coast of the Americas. Rather, these findings suggest that the two species colonized the eastern Pacific Ocean independently. As a regional or global phylogenetic assessment of the Gymnuridae was not the objective of this study, further analyses incorporating larger sample sizes, multiple genetic markers, mitochondrial nucleotide diversity and a broader geographic basis are necessary to clarify the phylogenetic inferences and evolutionary history presented here.

The presence of a small caudal fin has served as a convenient external morphological basis for distinguishing *Aetoplatea* from *Gymnura* (Compagno, 1999b). Although the validity of these generic separations has been questioned (Compagno, 1999a), these concerns have not been evaluated through directed taxonomic investigations. The inclusion of *A. zonura* within a distinct Indo-Pacific clade (Fig 8: clade A) also containing *G. australis*, *G. japonica* and *G. poecilura*, and genetic distances between this species and other *Gymnura* taxa are consistent with observed patterns of interspecific differentiation within this family. These results, therefore, do not indicate a molecular basis for the placement of the species *zonura* in the genus *Aetoplatea*, and instead support its assignment to *Gymnura*. In contrast, phylogenetic analysis based on anatomical variation of four gymnurid rays placed *A. zonura* as the sister group of *G. marmorata*, *G. micrura* and *G. japonica* (González-Isáis & Domínguez, 2004). Unfortunately, the genus *Aetoplatea* and its two representative species have rarely been included in other phylogenetic analyses of myliobatiform rays. Additional analyses incorporating the holotypes of *A. zonura* and, in particular, *Aetoplatea tentaculata* [Müller & Henle (ex Valenciennes)] are needed to further evaluate the validity of the genus *Aetoplatea*.

The Gulf of California and Pacific coast of the Baja California peninsula are situated in a region of biological, geological and physical transition and complexity that encompasses the confluence of the San Diego Province of the temperate California Region and the Panamic Province of the tropical East Pacific Region (Brusca *et al.*, 2005). Recent, dynamic tectonic history and Pleistocene climatic events within this region of environmental transition have restricted gene flow, resulting in numerous documented endemic species and disjunct populations (Present, 1987; Bernardi *et al.*, 2003; Riginos, 2005). High pairwise Φ_{ST} values between *G. marmorata* from Ensenada and Sonora (0.82), and Bahía Almejas and Sonora (0.88), indicate strong population structuring between the Pacific coast of the Baja

California peninsula and the northern Gulf of California. High levels of population differentiation have also been reported for another batoid [*Rhinobatos productus* (Ayres); Sandoval-Castillo *et al.*, 2004] and among teleosts between these regions (Stepien *et al.*, 2001; Bernardi *et al.*, 2003), suggesting that a common vicariant event may have been responsible for the similar patterns of population structuring observed in these diverse species. Before the uplift and formation of the present-day Baja California peninsula *c.* 1 MYA (Fig. 2), the lower peninsula consisted of a chain of islands (Riginos, 2005; Lindell *et al.*, 2006). Closure of the mid-peninsular seaways following uplift of the peninsula largely eliminated connectivity and is hypothesized as a primary mechanism for disjunction and allopatric speciation between these regions (Huang & Bernardi, 2001; Bernardi *et al.*, 2003). Evidence for unidirectional gene flow in *R. productus* from the Pacific into the Gulf of California coupled with the observed strong population differentiation (*cyt b*; $\Phi_{ST} = 0.63$) between these regions led Sandoval-Castillo *et al.* (2004) to propose incipient allopatric speciation, qualifying Pacific and Gulf of California populations of *G. marmorata* as different evolutionarily significant units for conservation purposes. Levels of genetic differentiation between Pacific and Gulf of California *G. marmorata* populations exceeded those reported for *R. productus*, suggesting that *G. marmorata* from these regions should, at the very least, be considered as separate management units. These findings add to the case for further investigation into the potential for isolation and cryptic speciation in elasmobranchs of this region.

ECOLOGICAL AND CONSERVATION CONSIDERATIONS

The modern ranges of *G. crebripuncata* and *G. marmorata* are poorly known. Collection locations of material examined from museum collections confirm that these rays occur sympatrically, at least along the coast of Sonora. In this study, the observation of a 370 mm W_D male specimen putatively identified as *G. marmorata* from Sonora (Fig. 8), with a haplotype identical to *G. crebripuncata* from Mazatlán, was very likely due to misidentification of the specimen in the field. This sample was obtained from researchers conducting a survey off the Sonoran coast and identification was based largely on potentially misleading characters presented in McEachran & Notarbartolo di Sciara (1995) and Castro-Aguirre & Espinosa-Pérez (1996). Accepting this as a misidentified specimen of *G. crebripuncata*, these analyses provide supplementary evidence of the co-occurrence of *G. crebripuncata* and *G. marmorata* off Sonora ($30^{\circ} 37'36''$ N; $113^{\circ} 09'12''$ W).

Castro-Aguirre & Espinosa-Pérez (1996) hypothesized that *G. crebripuncata* replaces *G. marmorata* in more tropical, southern latitudes, implying a largely warm temperate distribution for *G. marmorata*. Similar patterns of replacement by congeners are exemplified in the eastern Pacific among other ray genera, including *Narcine* (Henle), *Myliobatis* (Cuvier), *Rhinobatos* (Linck) and *Zapteryx* (Jordan & Gilbert) (McEachran & Notarbartolo di Sciara). Although the present genetic analyses were restricted to specimens from Mexican waters, a single *G. crebripuncata* from Panama in a museum collection (CAS C6 W53-310) was encountered, verifying a range that includes equatorial regions for this species. A record of *G. crebripuncata* from the western Gulf of California indicates that the species may be more broadly distributed in this region (Cota-Gomez *et al.*, 1998).

Typically reported as being continuously distributed from southern California to Peru, the actual range of *G. marmorata* may be restricted to the extreme northern and southern limits of this assumed range if the assertion of Castro-Aguirre & Espinosa-Pérez (1996) is correct. Water temperature and sea level changes associated with Plio-Pleistocene glaciation events may have isolated populations and restricted gene flow and connectivity of populations across the region (Bowen *et al.*, 1998). It is also possible that records of *G. marmorata* along the Central and South American Pacific coast are the product of misidentification and the range of *G. marmorata* is much more restricted than previously thought. The occurrence of *G. marmorata* in these areas should be confirmed.

Exploitation of rays, including *Gymnura* spp., is increasing within Mexican waters (Bizzarro *et al.*, 2007), as well as globally (White & Dharmadi, 2007). This study serves to highlight basic, essential information on species identification, taxonomic status, population structure and distribution that is lacking for many species. The ability to accurately distinguish eastern Pacific gymnurids may provide a basis for improving fishery records and initiating directed life-history studies that are critical for the development of effective management strategies. Disjunct populations of *G. marmorata* may possess differing levels of resilience to fishing pressure. Further research on the connectivity and structure of *G. crebripuncata* and *G. marmorata* is necessary to evaluate the effect of exploitation on these species.

We thank D. Adams, G. Burgess, J. Carter, L. Castillo-Géniz, C. Conrath, S. du Pleiss, A. Henning, S. Kubis, D. Long, O. Sosa-Nishizaki, B. Whitaker and S. Wintner for assistance with sample collection and are grateful to fishermen throughout the Gulf of California and Bahía Magdalena lagoon complex for their co-operation in this project. D. Catania (California Academy of Sciences) and P. Bartsch (Museum fuer Naturkunde der Humboldt-Universität zu Berlin) were remarkably helpful with arrangements for shipping and examining specimens. S. Jewett (National Museum of Natural History), J. Seigel (Natural History Museum of Los Angeles County) and H.J. Walker (Scripps Institute of Oceanography Vertebrate Museum) were also generous with their assistance and access to museum specimens. H. Mollet provided a valuable translation of the original Peters (1869) description of *G. crebripuncata*. We thank M. Debiasse and R. Vargo for assisting with the genetics laboratory work. G. Cailliet, J. Cope, C. Rinewalt, D. Ebert and T. Trejo provided constructive input and assistance throughout this study. R. Sanders offered helpful support and created the illustration included as Fig. 1 of this publication. The manuscript was greatly improved as a result of the edits and suggestions of I. Harrison, D. Markle and three anonymous reviewers. Funding for this project was provided in part by the Guy Harvey Research Institute, Homeland (Marisla) Foundation, National Marine Fisheries Service *via* the National Shark Research Consortium and Pacific Shark Research Center, and PADI Project AWARE. Research was conducted in accordance with and under the approval of the San Jose State University IACUC Protocol #801.

References

- Bearez, P. (1996). Lista de los peces marinos del Ecuador continental. *Revista de Biología Tropical* **44**, 731–741.
- Bernardi, G., Findley, L. & Rocha-Olivares, A. (2003). Vicariance and dispersal across Baja California in disjunct marine fish populations. *Evolution* **57**, 1599–1609.

- Bigelow, H. B. & Schroeder, W.C. (1953). *Fishes of the Western North Atlantic. Sawfishes, Guitarfishes, Skates, Rays, and Chimaeroids*, No. 1, Part 2. New Haven, CT: Yale University Press.
- Bizzarro, J. J., Smith, W. D., Márquez-Farías, J. F. & Hueter, R. E. (2007). Artisanal fisheries and reproductive biology of the golden cownose ray, *Rhinoptera steindachneri* Evermann and Jenkins, 1891, in the northern Mexican Pacific Ocean. *Fisheries Research* **84**, 137–146. doi: 10.1016/j.fishres.2006.10.016
- Bowen, B. W., Clark, A. M., Abreu-Grobois, F. A., Chaves, A., Reichart, H. A. & Ferl, R. J. (1998). Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) as inferred from mitochondrial DNA sequences. *Genetica* **101**, 179–189.
- Breder, C. M. Jr. (1928). Elasmobranch from Panama to lower California. *Bulletin of the Bingham Oceanographic Collection* **II**(1), 12.
- Brusca, R. C., Findley, L. T., Hastings, P. A., Hendrickx, M. E., Cosio, J. T. & van der Heiden, A. M. (2005). Macrofaunal diversity in the Gulf of California. In *Biodiversity, Ecosystems, and Conservation in Northern Mexico* (Cartron, J. L., Ceballos, G. & Felger, R. S., eds), pp. 179–202. New York, NY: Oxford University Press.
- Burnaby, T. G. (1966). Growth-invariant discriminant functions and generalized distances. *Biometrics* **22**, 96–110.
- Carvalho, M. R., Maisey, J. G. & Grande, L. (2004). Freshwater stingrays of the Green River formation of Wyoming (early Eocene), with the description of a new genus and species and an analysis of its phylogenetic relationships (Chondrichthyes: Myliobatiformes). *Bulletin of the American Museum of Natural History* **284**, 1–136.
- Castro-Aguirre, J. L. & Espinosa Pérez, H. (1996). *Listado faunístico de México. VII Catálogo sistemático de las rayas y especies afines de México (Chondrichthyes: Elasmobranchii: Rajiformes: Bateoideomorpha)*. México, DF: UNAM.
- Castro-Aguirre J. L., Espinosa Pérez H. & Schmitter-Soto J. J. (1999). *Ictiofauna estuarino-lagunar y vicaria de México*. México, DF: Limusa-Noriega Editores.
- Cooper, J. G. (1864). On new genera and species of Californian fishes—No. III. *Proceedings of the California Academy of Sciences* **1**, 108–114.
- Compagno, L. J. V. (1999a). Checklist of living elasmobranchs. In *Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes* (Hamlett, W. C., ed.), pp 471–498. Baltimore, MD: Johns Hopkins University Press.
- Compagno, L. J. V. (1999b). Gymnuridae. In *FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific. Volume 3. Batoid Fishes, Chimaeras and Bony Fishes Part 1 (Elopidae to Linophryniidae)* (Carpenter, K. E. & Niem, V. H., eds), pp. 1397–2068. Rome: FAO.
- Cota-Gómez, V. M., De La Cruz-Agüero, J. & Arellano-Martínez, M. (1998). Distributional notes on some marine fishes collected on southern Baja California, México. *Boletín de Investigaciones Marinas y Costeras* **27**, 103–105.
- Evermann, B. W. & Radcliffe, L. (1917). The fishes of the west coast of Peru and the Titicaca basin. *Bulletin of the United States National Museum* **95**, 1–166.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Flores, J. O., Rodríguez, M., Shimizu, M. & Machii, T. (1995). Evaluation of demersal fishery resources of the Gulf of California using Mexican shrimp trawlers. *Journal of the National Fisheries University* **44**, 9–19.
- Fourment, M. & Gibbs, M. J. (2006). PATRISTIC: a program for calculating patristic distances and graphically comparing the components of genetic change. *BMC Evolutionary Biology* **6**, 1. doi: 10.1186/1471-2148-6-1
- García-Caudillo, J. M., Cisneros-Mata, M. A. & Balmori-Ramírez, A. (2000). Performance of a bycatch reduction device in the shrimp fishery of the Gulf of California, México. *Biological Conservation* **92**, 199–205.
- Garman, S. (1913). The Plagiostoma (sharks, skates and rays). *Memoirs of the Museum of Comparative Zoology at Harvard College* **36**.
- González-Isáis, M. & Domínguez, H. M. M. (2004). Comparative anatomy of the superfamily Myliobatoidea (Chondrichthyes) with some comments on phylogeny. *Journal of Morphology* **262**, 517–535. doi: 10.1002/jmor.10260

- Hildebrand, S. F. (1946). A descriptive catalog of the shore fishes of Peru. *Bulletin of the United States National Museum* **189**, 71–74.
- Huang, D. & Bernardi, G. (2001). Disjunct Sea of Cortez-Pacific Ocean *Gillichthys mirabilis* populations and the evolutionary origin of their Sea of Cortez endemic relative, *Gillichthys seta*. *Marine Biology* **138**, 421–428.
- Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: A program for bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755.
- Isouchi, T. (1977). Butterfly ray *Gymnura bimaculata*, a junior synonym of *G. japonica*. *Japanese Journal of Ichthyology* **23**, 242–244.
- Klingenberg, C. P. (1996). Multivariate allometry. In *Advances in Morphometrics* (Marcus, L. F., Corti, M., Loy, A., Naylor, G. J. P. & Slice, D. E., eds), pp. 23–49. New York, NY: Plenum Press.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5**, 150–163.
- Lindell, J., Ngo, A. & Murphy, R. W. (2006). Deep genealogies and the mid-peninsular seaway of Baja California. *Journal of Biogeography* **33**, 1327–1331.
- Maddison, D. R. & Maddison, W. P. (2001). *MACCLADE 4: Analysis of Phylogeny and Character Evolution. Version 4.03*. Sunderland, MA: Sinauer Associates.
- Márquez-Farías, F. J. (2002). The artisanal ray fishery in the Gulf of California: development, fisheries research, and management issues. *Shark News* **14**, 1–5.
- McCoy, M. W., Bolker, B. M., Osenberg, C. W., Miner, B. G. & Vonesh, J. R. (2006). Size correction: comparing morphological traits among populations and environments. *Oecologia* **148**, 547–554. doi: 10.1007/s00442-006-0403-6
- McEachran, J. D. & Aschliman, N. (2004). Phylogeny of batoida. In *Biology of Sharks and their Relatives* (Carrier, J. C., Musick, J. A. & Heithaus, M. R., eds), pp. 79–113. New York, NY: CRC Press.
- McEachran, J. D. & Notarbartolo di Sciara, G. (1995). Peces Batoideos. In *Guia FAO para la identificacion de especies para los fines de la pesca centro oriental*. Vol. II (Fischer, W., Krupp, F., Schneider, W., Sommer, C., Carpenter, K. E. & Niem, V. H., eds), pp. 745–792. Rome: FAO.
- McGarigal, K., Cushman, S. & Stafford, S. (2000). *Multivariate Statistics for Wildlife and Ecology Research*. New York: Springer Science.
- Meek, S. E. & Hildebrand, S. F. (1923). The marine fishes of Panama. *Field Museum of Natural History Publication 215, Zoological Series Volume XV*.
- Nicholas, K. B., Nicholas, H. B. Jr & Deerfield, D.W. II (1997). GENEDOC: Analysis and visualization of genetic variation. *EMBNEW News* **4**, 14.
- Nylander, J. A. A. (2004). *MRMODELTEST version 2*. Uppsala, Sweden: Evolutionary Biology Centre, Uppsala University (Program distributed by the author).
- Peters, W. (1869). Über neue oder weniger bekannte Fische des Berliner Zoologischen Museums. *Monatsbericthe der Akademie Wissenschaften der Berlin* **1869**, 703–711.
- Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Present, T. M. C. (1987). Genetic differentiation of disjunct Gulf of California and Pacific outer coast populations of *Hypsoblennius jenkinsi*. *Copeia* **1987**, 1010–1024.
- Reist, J. D. (1985). An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Canadian Journal of Zoology* **63**, 1429–1439.
- Riginos, C. (2005). Cryptic vicariance in Gulf of California fishes parallels vicariant patterns found in Baja California mammals and reptiles. *Evolution* **59**, 2678–2690.
- Rohlf, F. J. & Bookstein, F. L. (1987). A comment on shearing as a method for “size correction”. *Systematic Zoology* **36**, 356–367.
- Sandoval-Castillo, J., Rocha-Olivares, A., Villavicencio-Garayzar, C. & Balart, E. (2004). Cryptic isolation of Gulf of California shovelnose guitarfish evidenced by mitochondrial DNA. *Marine Biology* **145**, 983–988. doi: 10.1007/s00227-004-1378-7
- Schneider, S., Roessli, D. & Excoffier, L. (2000). *ARLEQUIN, Version 2.000: a Software for Population Genetics Data Analysis*. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.

- Stepien, C. A., Rosenblatt, R. H. & Bargmeyer, B. A. (2001). Phylogeography of the spotted sand bass, *Paralabrax maculatofasciatus*: divergence of Gulf of California and Pacific coast populations. *Evolution* **55**, 1852–1862.
- Swofford, D. L. (2002). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sunderland, MA: Sinauer Associates.
- Teaf, C. M. & Lewis, T. C. (1987). Seasonal occurrence of multiple caudal spines in the Atlantic stingray, *Dasyatis sabina* (Pisces: Dasyatidae). *Copeia* **1987**, 224–227.
- Villavicencio-Garayzar, C. J. (1995). Distribución temporal y condición reproductiva de las rayas (Pisces: Batoidei), capturadas comercialmente en Bahía Almejas, BCS., México. *Revista de Investigaciones Científicas Serie Ciencias Marinas UABCS* **6**, 1–1.
- Vossoughi, G. H. & Vosoughi, A. R. (1999). Study of batoid fishes in northern part of Hormoz Strait, with emphasis on some species new to the Persian Gulf and Sea of Oman. *Indian Journal of Fisheries* **46**, 301–306.
- White, W. T. & Dharmadi (2007). Species and size compositions and reproductive biology of rays (Chondrichthyes, Batoidea) caught in target and non-target fisheries in eastern Indonesia. *Journal of Fish Biology* **70**, 1809–1837. doi: 10.1111/j.1095-8649.2007.01458.x