ELSEVIER

Molecular ecology, speciation, and evolution of the reef fish genus *Anisotremus*

Giacomo Bernardi [a,*], Yvette R. Alva-Campbell [a], João L. Gasparini [b], Sergio R. Floeter [c]

[a] Department of Ecology and Evolutionary Biology, University of California Santa Cruz, 100 Shaffer Road, Santa Cruz, CA 95060, USA
[b] Departamento de Ecologia e Recursos Naturais, Universidade Federal do Espírito Santo, Vitória, ES 29060-900, Brazil
[c] Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina, Florianopolis, SC 88010-970, Brazil

ABSTRACT

Grunts (family Haemulidae) in the genus *Anisotremus* comprise 10 described species which occur predominantly on coral reefs and subtropical rocky reefs in the Neotropics of the Tropical Eastern Pacific the Caribbean and adjacent waters. In this study, the phylogenetic relationships for all described species were examined based on one mitochondrial gene (cytochrome *b*) and one nuclear marker (the first intron of the ribosomal protein S7). Closely related species of the genus *Haemulon* and the single representative of the genus *Genyatremus*, *G. luteus* were also included in the analysis, following the suggestion, based on morphological data, that they may be closely related to, or possibly placed within, *Anisotremus*. Two sweetlips, genus *Plectorhinchus*, which belong to the recognized sister subfamily Plectorhynchinae, were used as outgroups. Two species pairs in the ingroup genus *Anisotremus* are geminate species, which presumably diverged after the rise of the Isthmus of Panama, thus providing an internal molecular clock calibration. This allowed for a comprehensive evolutionary scenario for the genus. Our data were consistent with two independent allopatric speciation events (geminate species), and one likely sympatric speciation event. Our data were also consistent with the placement of *Genyatremus* and *Haemulon* within the genus *Anisotremus*, thus breaking its monophyletic status. As proposed for other coral reef species, *Anisotremus* seems to have colonized coral reefs from non-coral reef habitats. In addition, ancestral species seem to have been widespread, since species that are currently found in Brazilian and Pacific waters are basal on the phylogenetic tree. Finally, this study adds to the hypothesis of a secondary evolutionary center in the New World.

© 2008 Elsevier Inc. All rights reserved.

1. Introduction

The family Haemulidae (grunts) comprises approximately 150 mostly marine species of fishes. Similarly to the closely related snappers (Lutjanidae), grunts generally school during the day and then disperse to forage individually at night. Grunts are broadcast spawners, with a pelagic larval phase that typically lasts in the range of 25–35 days. Several species then recruit to brackish or mangrove habitats (Nagelkerken et al., 2000; de la Moriniere et al., 2003; Mumby et al., 2004).

Grunts are divided into two subfamilies, the Plectorhynchinae (sweetlips), and the Haemulinae (grunts) (Johnson, 1980; Nelson, 2006). Haemuline grunts are primarily found in the New World and are represented by eight genera including *Haemulon* and *Anisotremus*. The genus *Anisotremus* comprises 10 described species, which are found in the Neotropics on both sides of the Central American divide. Recently, a morphological study proposed that the genus *Genyatremus*, which contains a single species, the Toroto grunt, *Genyatremus luteus*, should either be included in the

genus *Anisotremus*, bringing the number of species to 11, or alternatively not to consider *Anisotremus* as a monophyletic group (Tavera Vargas, 2006).

Anisotremus provides an unusual opportunity to study unique aspects of sympatric and allopatric speciation, while allowing for an internal molecular clock calibration. Indeed, the genus *Anisotremus* includes two geminate pairs, species that are presumed to have diverged after the rise of the Isthmus of Panama, approximately 3.5 Mya (million years ago) (Jordan, 1908). The two trans-Isthmian pairs include porkfish *Anisotremus virginicus* and black margate *A. surinamensis* which are morphologically similar to their presumed geminates in the Tropical Eastern Pacific (TEP), the panamic porkfish, *A. taeniatus* and the burrito grunt *A. interruptus*, respectively. In addition, two species live sympatrically in the TEP, the blackbarred grunt, *A. dovii* and the Carruco sargo, *A. pacifici*. These species are morphologically extremely similar, with a characteristic barred pattern, share the same sandy/muddy bottom habitat and a large range overlap (the Pacific Coast of Mexico to Peru), thus raising the possibility of sympatric speciation. Finally, one species, the sargo *A. davidsonii*, is a Baja California disjunct (Bernardi et al., 2003; Bernardi and Lape, 2005), which comprises populations that are geographically separated by the Baja

* Corresponding author. Fax: +1 831 459 4882.
E-mail address: bernardi@biology.ucsc.edu (G. Bernardi).

California Peninsula by being confined to the northern Sea of Cortez and the Pacific Coast of the Peninsula.

A genetic study, based on six *Anisotremus* species confirmed the close relationship between the presumed geminate species (Bernardi and Lape, 2005). However, in an exhaustive study on groupers (Serranidae) in the genus *Alphestes*, Craig et al. (2004) discovered that presumed geminate species were not actual sister species, thus raising the issue of correct taxon sampling. A complete phylogeny of the genus *Anisotremus* is therefore necessary to settle that question. Such a phylogeny would allow us to assess the monophyletic status of *Anisotremus*, evaluate the sister relationships between presumed geminate species, understand patterns of speciation, both in allopatry and in sympatry, as well as proposing a biogeographic scenario and an evolutionary history for the genus. Coral reef fishes have been suggested to often be derived from non-coral reef habitats (Bellwood and Wainwright, 2002), a situation that could potentially have occurred in *Anisotremus*.

To achieve this goal, we sampled 92 individuals that included all 10 described species in the genus *Anisotremus*, and one *Genyatremus luteus*, and established phylogenetic relationships based on nuclear and mitochondrial molecular markers. In order to address the monophyletic question of the genus, we used individuals from the very closely related grunt genus *Haemulon*, where we chose a presumed geminate pair for an additional opportunity for molecular clock calibration (Rocha et al., 2008). Finally, we used two harlequin sweetlip individuals (*Plectorhinchus chaetodonoides*) as outgroups, since they belong to the Plectorhynchinae, which is the sister subfamily of the Haemulinae.

2. Materials and methods

2.1. Collections and DNA samples

Sampling sizes and locations are listed in Table 1 and Fig. 1. Harlequin sweetlip, *Plectorhinchus chaetodonoides* from the subfamily Plectorhynchidae were used as outgroups and were obtained from a commercial importer. Several grunts from the closely related genus *Haemulon* were sampled. Individuals from the Caribbean included blue striped grunt, *Haemulon sciurus* and sailor's choice, *H. parra*. Species from the TEP included the presumed geminate of *H. parra*, the mojarra grunt, *H. scudderii* and an undescribed species, *Haemulon* sp.n. *Genyatremus luteus* was collected by Franck Besnard in French Guiana (Table 1). After collection, samples were immediately placed in 95% ethanol and stored at ambient temperature in the field, and then at 4 °C in the lab. Muscle or liver tissue was later dissected from these samples. Total genomic DNA was prepared from 75 to 150 mg of muscle or liver tissue by proteinase K digestion in lysis buffer (10 mM Tris, 400 mM NaCl, 2 mM EDTA, 1% SDS) overnight at 55 °C. This was followed by purification using chloroform extractions and alcohol precipitation (Sambrook et al., 1989).

2.2. PCR amplification and sequencing

Twenty six (out of 104) mitochondrial cytochrome *b* sequences were from Bernardi and Lape (2005). Amplification of the additional cytochrome *b* segments was performed using the universal primers GLUDGL (5′-TGA CTT GAA RAA CCA YCG TTG-3′) and CB3H (5′-GGC AAA TAG GAA RTA TCA TTC-3′) (Palumbi et al., 1991) with an annealing temperature of 52 °C. Amplification of the 1st intron of the nuclear S7 ribosomal protein used the primers S7RPEX1F (5′-TGG CCT CTT CCT TGG CCG TC-3′) and S7RPEX2R (5′-AAC TCG TCT GGC TTT TCG CC-3′) (Chow and Hazama, 1998) with an annealing temperature of 56 °C. After purification following the

Table 1
Collection localities for *Anisotremus* spp., *Genyatremus luteus*, and *Haemulon* spp.

Species	Sampling site	n	Label
Anisotremus caesius	Mazatlan, Mexico	3	PNM
	Playa Troncoles, Costa Rica		PTC
	Fish Market, Panama City, Panama	1	MMP
Anisotremus davidsonii	Pacific Coast		
	California, USA		
	Catalina Island	1	CAT
	San Diego	1	SDI
	Baja California, Mexico		
	Punta Eugenia	1	PEU
	Punta San Roque	1	PSR
	Isla San Roque	1	ISR
	Bahia Asuncion	1	ASU
	Gulf of California, Mexico		
	Bahia de Los Angeles	1	BLA
	Punta Cholla	1	PCH
	Bahia Kino	2	BKI
Anisotremus dovii	Mazatlan, Mexico	3	PSM, EIM
	Zijuantenejo, Mexico	5	ZFI
	Fish Market, Panama City, Panama	5	MMP
Anisotremus interruptus	Bahia de Los Angeles, Mexico	2	BLA
	Zijuantenejo, Mexico	2	ZFI
	Mazatlan, Mexico	2	PNM
	Playa Troncoles, Costa Rica	4	PTC
	Puntarenas, Costa Rica	1	PAR
	Isla Conadora, Panama	1	RCP
	Fish Market, Panama City, Panama	6	MMP
Anisotremus moricandi	Guarapari Islands, Brazil	5	BRA
Anisotremus pacifici	Puntarenas, Costa Rica	2	PAR
	Panama City, Panama	1	MMP
Anisotremus scapularis	Lima, Peru	2	PER
Anisotremus surinamensis	Florida, USA	3	FLO
	Panama	1	PAN
	Los Roques, Venezuela	1	VEN
	Fernando de Noronha, Brazil	1	FDN
Anisotremus taeniatus	Cabo Pulmo, Mexico	2	CPU
	Puntarenas, Costa Rica	4	PAR
	Panama City, Panama	8	PAN, MMP
Anisotremus virginicus	USA, Florida (commercial)	2	CA
	Turneffe Atoll, Belize	5	BEL
	San Blas Islands, Panama	3	PAN
	Guarapari Islands, Brazil	5	BRA
Genyatremus luteus	Ilet Mamelles, French Guiana	1	CAY
Haemulon parra	Belize, Turneffe Atoll	3	BEL
Haemulon sciurus	Belize, Turneffe Atoll	1	BEL
Haemulon scudderi	Puntarenas, Costa Rica	4	PAR
Haemulon n.sp.	Zijuantenejo, Mexico	4	ZFI
Outgroups			
Plectorhinchus chaetodonoides	Commercial origin	2	COM

Columns represent the number of individuals included in the study, and the abbreviations used in Figs. 1 and 2.

manufacturer's protocol (ABI, Perkin-Elmer), sequencing was performed in both directions with the primers used in the PCR amplification on an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). Heterozygous individuals were found to be very rare, and when present, only one allele was scored per individual (we did not find individuals with more than one heterozygotic site, thus making the calling of the two alleles possible).

2.3. Phylogenetic analyses

We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the DNA

sequences. Phylogenetic relationships were assessed by Maximum Likelihood (ML, GARLI software, Zwickl, 2006), Maximum Parsimony (MP, PAUP* software, Swofford, 2003), and Neighbor-Joining (NJ, PAUP software), methods. For Maximum Likelihood topologies, we conducted 10 independent runs in GARLI, using default settings and the automated stopping criterion, terminating the search when the ln score remained constant for 20,000 consecutive generations. The best likelihood of those runs was retained and is presented here. Maximum Parsimony searches included 100 random addition replicates and TBR branch swapping with the multrees option. Neighbor-Joining reconstructions used distances based on substitution models obtained with Modeltest (HKY+G) (Posada and Crandall, 1998; Huelsenbeck and Ronquist, 2001). Statistical confidence in nodes was evaluated using 2000 non-parametric bootstrap replicates (Felsenstein, 1985) (100 replicates for Maximum Likelihood in GARLI, using the automated stopping criterion set at 10,000 generations). Topological differences were tested using a Shimodaira and Hasegawa test (Shimodaira and Hasegawa, 1999) implemented by PAUP, based on resampling of estimated log-likelihoods tests (RELL, 1000 replicates).

2.4. Genetic divergence and gene flow

Molecular clock enforcements were tested using a Shimodaira and Hasegawa test (Shimodaira and Hasegawa, 1999) implemented by PAUP. Genetic divergence was estimated using distances based on substitution models obtained with Modeltest (HKY+G). In order to account for polymorphism in each species (or population), divergence was estimated as the average pairwise distance between species (or population) minus the average pairwise distance within a species (or population).

3. Results

3.1. Sequences

The 5′-end portion of the mitochondrial cytochrome *b* was sequenced for 104 individuals (Table 1; Fig. 1). Among *Anisotremus* species, out of 692 aligned base pairs, 236 bp were variable and 230 bp were informative. As expected the first intron of the nuclear S7 ribosomal protein was less variable. Out of 423 bp, 78 bp were variable and 69 bp were informative. All sequences were deposited in GenBank (Accession Nos.: S7 EU694180–EU694280, Cytb EU694281–EU694384).

3.2. Phylogenetic relationships

Overall phylogenetic relationships using mitochondrial and nuclear molecular markers were not found to be significantly different (Shimodaira–Hasegawa test, $p = 0.25$), thus the two datasets were combined and analyzed together. In addition, all three search methods (ML, MP, NJ) resulted in topologies that were statistically equivalent (S–H test, $p = 0.5$). The phylogenetic relationships of individuals based on a Maximum Likelihood search are presented in Fig. 2.

The genus *Anisotremus* was found not to be monophyletic (a monophyletic *Anisotremus* resulted in a statistically significantly worse topology than the one presented here, SH test $p < 0.01$). This was due to the fact that *Haemulon* species and the Torroto grunt, *Genyatremus luteus* (also called Croupia), grouped within the genus *Anisotremus* (Fig. 2). A larger sampling effort in the Haemulid family is necessary to determine if additional taxa are going to intervene within the topology presented here.
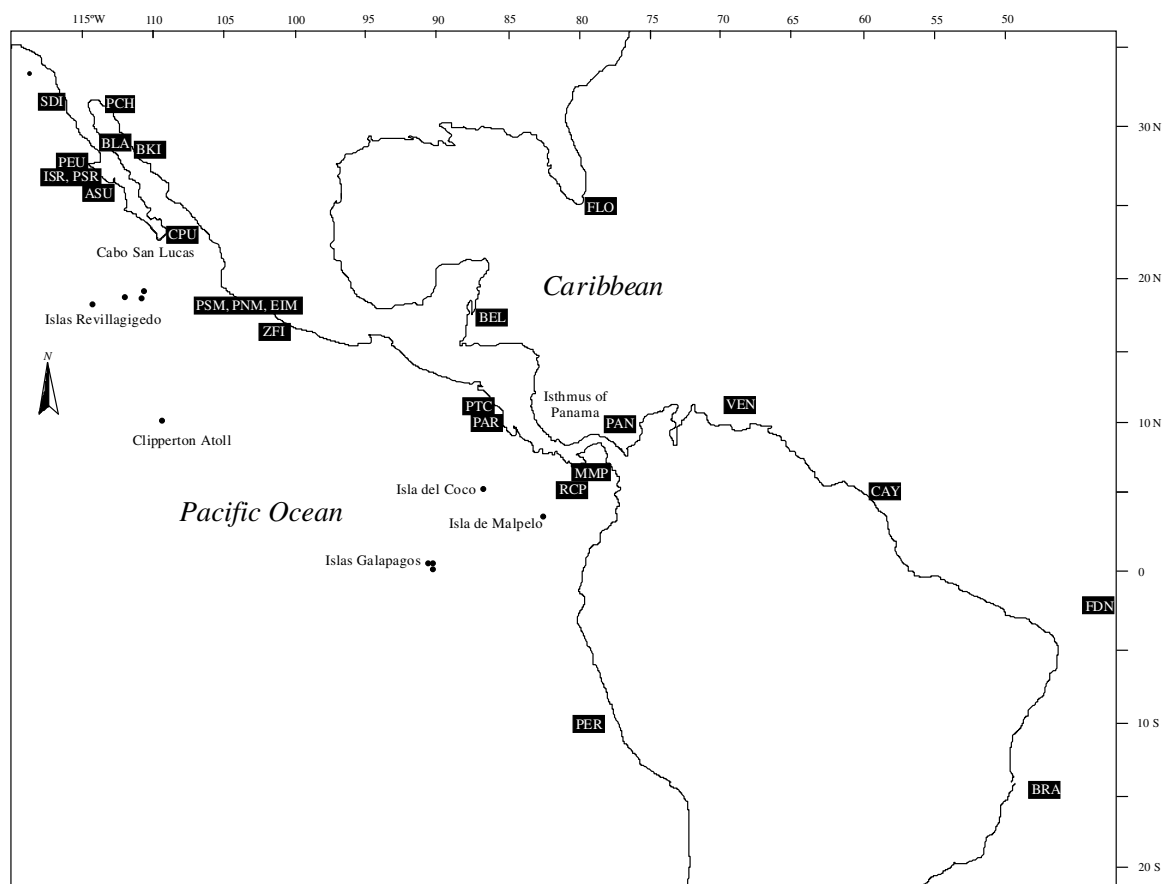

Fig. 1. Distribution map and sampling locations of *Anisotremus*, *Genyatremus* and *Haemulon*. Labels refer to sampling locations described in Table 1.
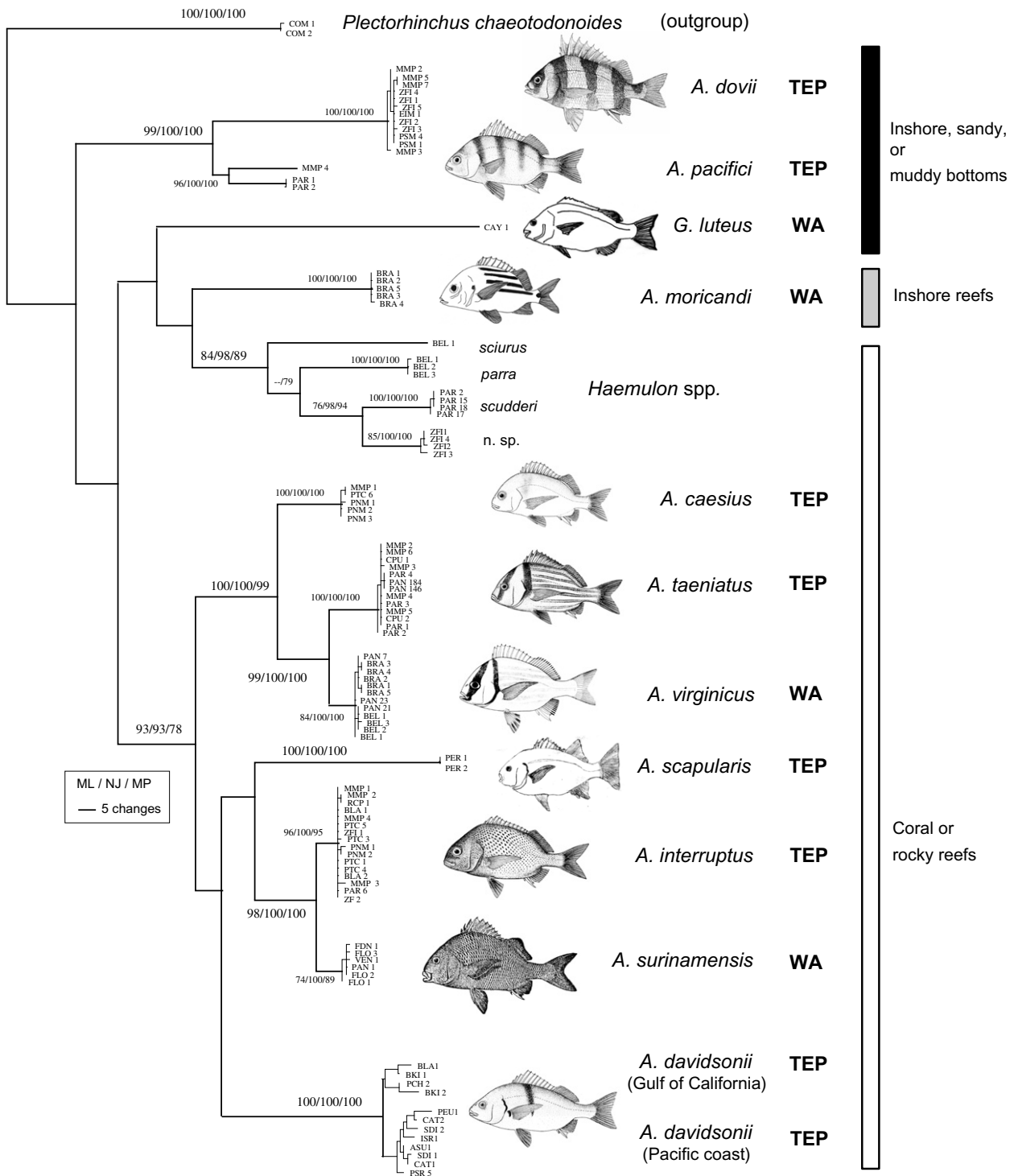
Fig. 2. Molecular phylogeny of *Anisotremus* based on mitochondrial and nuclear molecular markers (cytochrome *b* and the 1st intron of the ribosomal protein S7) using the Maximum Likelihood method (Maximum Parsimony and Neighbor-Joining reconstructions resulted in the same topology). Labels are described in Table 1. Bootstrap support is shown when above 50%, for the three methods used, Maximum Likelihood, Neighbor-Joining, and Maximum Parsimony, in that order. Harlequin sweetlip, *Plectorhinchus chaetodonoides*, individuals were used as outgroups. Drawings of fish species are from FAO (or our own for *A. moricandi*, *A. scapularis*, and *G. luteus*) and are drawn to approximate relative scale of adult size.

While *Anisotremus* was found not to be monophyletic, within our dataset, very well supported patterns were found: (1) The two sympatric sandy/bottom species *A. dovii* and *A. pacifici* grouped together in a very well supported clade. (2) High bootstrap (both markers and with all methods) supported the grouping of seven *Anisotremus* species. Within this group. (3) The presumed geminate species grouped together: *Anisotremus virginicus* with *A. taeniatus*

and *A. surinamensis* with *A. interruptus*. (4) *Anisotremus caesius* was sister to the *A. taeniatus*–*A. virginicus* clade (strong support), and *A. scapularis* was sister to the *A. surinamensis*–*A. interruptus* clade (weak support). (5) As expected, the two Baja California disjunct populations of *A. davidsonii* grouped together, confirming the results of Bernardi and Lape (2005). There was also some weak support for two more patterns. *Genyatremus luteus*, which occurs

from Colombia to Brazil in French Guiana and adjacent areas, and lives at the sand, mud/rock interface was the sister species to the Brazilian nearshore species *A. moricandi*. In addition, the four sandy/muddy bottom, nearshore fishes, *G. luteus*, *A. moricandi*, *A. dovii*, and *A. pacifici*, as well as individuals from the genus *Haemulon*, were found to be ancestral to the rocky/coral reef species. Finally, the undescribed species, *Haemulon* sp.n. was found to be closer to *H. scudderii* than its presumed geminate *H. parra*, thus indicating that the presumed geminates *H. parra* and *H. scudderii* are not sister taxa and cannot be used for molecular clock calibrations (see Rocha et al., 2008).

3.3. Genetic divergence and temporal divergence

Our data did not show any departure from a molecular clock ($p = 0.209$ for mtDNA and $p = 0.189$ for nuclear DNA) indicating that a molecular clock could not be rejected. In addition to a molecular clock, prior knowledge of mutation rates is necessary to estimate divergence times. In this study, rate calibration was obtained using two congeneric trans-Isthmian geminate species pairs. Based on mitochondrial cytochrome *b* sequences, and using the evolution model obtained using Modeltest (HKY+G), the divergence between *A. virginicus* and its geminate species *A. taeniatus* was found to be 1.7 times higher than the divergence between the other pair of geminate species, *A. surinamensis* and *A. interruptus*. Divergence of *A. taeniatus* and *A. virginicus* was also found to be greater than for *A. surinamensis* and *A. interruptus* for the nuclear marker (ratio 24:1, Model selected, F81+G). However, the very low level of nuclear divergence in the latter species pair prevents an accurate estimate of divergence and was therefore not used for later analyses.

Based on mitochondrial cytochrome *b* sequences, if we assume that the separation of *A. surinamensis* and *A. interruptus* occurred at the closure of the Isthmus of Panama, 3.1–3 Mya, the other geminates (*A. virginicus* and *A. taeniatus*) would have diverged 5.3–6.0 Mya. In contrast, if we consider *A. virginicus–A. taeniatus* to have diverged 3.1–3.5 Mya, then the divergence of *A. surinamensis* and *A. interruptus* would have occurred approximately 2 Mya. These alternative hypotheses give us a bracket of time that can then be applied to other branches. The divergence of the "genus" *Anisotremus* was found to have occurred between 11 and 12 Mya. The divergence between the sister species *A. dovii* and *A. pacifici* would have occurred approximately 6–7 Mya. The divergence of the internal clade (all species except *A. dovii*, *A. pacifici*, *G. luteus*, and *A. moricandi*) to have occurred between 7 and 8 Mya.

4. Discussion

Haemulidae are species of fish that clearly present some considerable taxonomic challenges. The genus *Anisotremus* comprises only 10 species, yet it offers unique possibilities in testing biogeographic, ecological and evolutionary hypotheses. Data presented here reject the monophyletic status of the genus. Thus a more comprehensive assessment of the family is necessary to settle this question. Recently, a morphological study of the genus was presented (Tavera Vargas, 2006). The overall relationships within the genus were consistent with our findings, and the poorly known coastal/brackish water monospecific genus *Genyatremus* (the Torroto grunt, *Genyatremus luteus*) was placed within *Anisotremus* in a basal position close to the *A. dovii–A. pacifici* clade. Our results were consistent with the morphological study, also placing *G. luteus* in a basal position, close to *A. dovii* and *A. pacifici*. While there is additional potential for paraphyly in the genus *Anisotremus*, very robust relationships clearly emerge from our study.

4.1. Allopatric and sympatric modes of speciation

The sister relationship of the two presumed geminate pairs, *Anisotremus virginicus–A. taeniatus*, and *A. surinamensis–A. interruptus*, was confirmed by our study and was very well supported (Fig. 2). In contrast, the sister relationship of the species *Haemulon parra–H. scudderi* was questioned by the presence of an intervening new species, and is consistent with Rocha et al. (2008). The genetic divergence between *A. virginicus* and *A. taeniatus* was found to be higher than between *A. surinamensis* and *A. interruptus*. This has been reported and discussed previously (Bermingham et al., 1997; Bernardi and Lape, 2005). Briefly, two potential reasons for this finding are 1. The divergence of *A. virginicus–A. taeniatus* occurred at the rise of the Isthmus of Panama (3.1–3.5 Mya), while the divergence of *A. surinamensis–A. interruptus* would have occurred more recently, during a breaching event dated approximately at 2 Mya. Alternatively, *A. surinamensis–A. interruptus* would have diverged at the rise of the Isthmus, and the divergence of *A. virginicus–A. taeniatus* would have preceded the rise of the Isthmus (about 5 Mya). In both situations, divergence of these species pairs is likely to have occurred during allopatric situations.

In contrast, the divergence of the barred species *Anisotremus dovii* and *A. pacifici* seems more puzzling. These species live in sandy/muddy bottoms, and have a large overlapping range. These species seem to also extensively overlap ecologically, although studying the ecology and behavior of sandy/muddy bottom fishes is generally difficult (Rocha et al., 2005). It seems likely that in this situation, sympatric speciation may have occurred. It is predicted that sympatric speciation should result in a relatively faster genetic divergence (McCune and Lovejoy, 1998), which may explain the large genetic divergence between these species that are morphologically virtually indistinguishable.

4.2. Ecological shifts

Coral reef fishes have been suggested to be generally derived from non-coral reef habitats, slowly colonizing new emerging habitats, an event followed by quick speciation events in a new set of ecological niches. While this hypothesis is appealing, few empirical cases have been evaluated. The "genus" *Anisotremus* seems to follow this scenario with non-coral reef species found in an ancestral position (Fig 2). Indeed, the Tropical Eastern Pacific *A. dovii*, and *A. pacifici*, live in muddy/sandy habitats, the Guianan *Genyatremus luteus* lives in sandy/muddy and in brackish habitats, and the Brazilian *A. moricandi* lives in "generally murky" coastal rocky reefs (see Acero and Garzón, 1982). In contrast, derived *Anisotremus* species are tropical coral reef species (but for *Anisotremus davidsonii* which seems to have secondarily colonized colder northern rocky reefs, Bernardi and Lape, 2005). Our phylogenetic results are therefore consistent with the scenario proposed by Bellwood and Wainwright (2002).

4.3. Biogeographic patterns

The evolutionary history of the genus *Anisotremus* may originate as far back as the Eocene (approximately 35 Mya), where the first *Anisotremus* fossil may have been traced, based on fossilized otoliths (earbones) found in Louisiana (Nolf and Stringer, 2003). It is likely that ancestral *Anisotremus* species were widespread, since species that are currently found in Brazilian and Pacific waters are basal on the phylogenetic tree.

The genus *Anisotremus* as well as *Haemulon*, *Elacatinus*, *Rypticus*, and the families Chaenopsidae and Labrisomidae all have several species in the NW (New World, i.e. both sides of tropical America) and are thought to have originated and diversified in the NW (Guimarães, 1999; Hastings, 2000; Taylor and Hellberg, 2005;

Robertson et al., 2006; Floeter et al., 2008). Some of these taxa further invaded the Eastern Atlantic (e.g. *Rypticus*, *Labrisomus*) thus adding to the hypothesis of a secondary evolutionary center in the New World, the other being the East Indies Triangle (Briggs, 2003).

Despite the lack of a consistent temporal signal for speciation events among different reef fish genera, an important peak of diversification occurred during the late Miocene to the Pliocene (peaking from 2 to 8 Mya—Floeter et al., 2008). This is also where all speciation events occurred in the *Anisotremus* tree. A second burst of speciation events occurred after the Isthmus closure in many reef fish taxa (Floeter et al., 2008), but not in the genus.

4.4. Anisotremus

It is interesting to note that in both cases of Isthmus geminate pairs, the closest species to the geminate pair is from the TEP indicating a potential evolutionary origin of the group. Other than the Isthmus, the Amazon barrier (Floeter and Gasparini, 2000; Rocha, 2003) could also be invoked to explain the deep separation (7–8 Mya) of *Anisotremus moricandi* from the coral or rocky reef clade. *A. moricandi* could have evolved in the Brazilian coast (the larger part of its distribution) and later invading the southern Caribbean (Joyeux et al., 2001; Rocha, 2003; Rocha et al., 2007). However, ecological shifts from muddy waters to clear reefs may be a more plausible explanation, as suggested above.

4.5. Taxonomical issues

Our data raise several taxonomical issues. Individuals from the genus *Haemulon*, (which appears to be monophyletic, Rocha et al., 2008), and *Genyatremus luteus*, are embedded within the genus *Anisotremus*, thus breaking its monophyletic status. The Tropical Eastern Pacific species *A. dovii* and *A. pacifici* are closely related to each other, and basal to the *Haemulon, Genyatremus, Anisotremus* clade. It is likely that further examination of these relationships will place these two species into a different genus. Indeed, before current placement in the genus *Anisotremus*, these two species have been grouped within the genera *Conodon*, *Pomadasys*, and *Pristipoma*, thus underscoring their complex taxonomic history. Our data show very strong support for the grouping of 7 (out of 10) *Anisotremus* species, as shown in Fig. 2. It is likely that these species will represent the genus *Anisotremus*, "*sensu stricto*", although further work on the family is necessary at this stage.

5. Conclusion

The genus *Anisotremus* offers a unique opportunity to explore evolutionary patterns in tropical fishes. *Anisotremus* includes two pairs of trans-Isthmian geminate species, one pair of TEP sympatric species, and one Baja California disjunct species. In addition, the study of *Anisotremus* sheds light on the ecological shift between sandy/muddy rocky reefs to the colonization of coral reefs. The potential for molecular clock calibration, based on geminate species, also affords the testing of specific biogeographic hypotheses. Thus overall, the genus *Anisotremus*, and more generally the Haemulid family, provide a unique model for the study of marine reef fish evolution.

References

Acero, P.A., Garzón, F.J., 1982. Rediscovery of *Anisotremus moricandi* (Perciformes: Haemulidae), including a redescription of the species and comments on its ecology and distribution. Copeia 1982, 613–618.

Bellwood, D.R., Wainwright, P.C., 2002. The history and biogeography of fishes on coral reefs. In: Sale, P.F. (Ed.), Coral Reef Fishes. Dynamics and Diversity in a Complex Ecosystem. Academic Press, San Diego, pp. 5–32 (Chapter 1).

Bermingham, E., McCafferty, S.S., Martin, A.P., 1997. Fish biogeography and molecular clocks: perspectives from the Panamian Isthmus. In: Kocher, T.D., Stepien, C.A. (Eds.), Molecular Systematics of Fishes. Academic Press, pp. 113–128.

Bernardi, G., Lape, J., 2005. Tempo and mode of speciation in the Baja California disjunct fish species *Anisotremus davidsonii*. Mol. Ecol. 14, 4085–4096.

Bernardi, G., Findley, L., Rocha-Olivares, A., 2003. Vicariance and dispersal across Baja California in disjunct marine fish populations. Evolution 57, 1599–1609.

Briggs, J.C., 2003. Marine centres of origin as evolutionary engines. J. Biogeog. 30, 1–18.

Chow, S., Hazama, K., 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. Mol. Ecol. 7, 1255–1256.

Craig, M.T., Hastings, P.A., Pondella, D.J., 2004. Speciation in the Central American Seaway: the importance of taxon sampling in the identification of trans-Isthmian geminate pairs. J. Biogeog. 31, 1085–1091.

de la Moriniere, E.C., Pollux, B.J.A., Nagelkerken, I., van der Velde, G., 2003. Diet shifts of Caribbean grunts (Haemulidae) and snappers (Lutjanidae) and the relation with nursery-to-coral reef migrations. Est. Coast. Shelf Sci. 57, 1079–1089.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Floeter, S.R., Gasparini, J.L., 2000. The southwestern Atlantic reef fish fauna: composition and zoogeographic patterns. J. Fish Biol. 56, 1099–1114.

Floeter, S.R., Rocha, L.A., Robertson, D.R., Joyeux, J.C., Smith-Vaniz, W.F., Wirtz, P., Edwards, A.J., Barreiros, J.P., Ferreira, C.E.L., Gasparini, J.L., Brito, A., Falcón, J.M., Bowen, B.W., Bernardi, G., 2008. Atlantic reef fish biogeography and evolution. J. Biogeog. 35, 22–47.

Guimarães, R.Z.P., 1999. Revision, phylogeny and comments on biogeography of soapfishes of the genus *Rypticus* (Teleostei: Serranidae). Bull. Mar. Sci. 65, 337–379.

Hastings, P.A., 2000. Biogeography of the tropical eastern pacific: distribution and phylogeny of chaenopsid fishes. Zool. J. Linnean Soc. 128, 319–335.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Johnson, G.D., 1980. The limits and relationships of the Lutjanidae and associated families. Bull. Scripps Inst. Oceanogr. 24, 1–114.

Jordan, D.S., 1908. The law of geminate species. Am. Nat. 42, 73–80.

Joyeux, J.C., Floeter, S.R., Ferreira, C.E.L., Gasparini, J.L., 2001. Biogeography of tropical reef fish: the South Atlantic puzzle. J. Biogeog. 28, 831–841.

McCune, A.R., Lovejoy, N.R., 1998. The relative rate of sympatric and allopatric speciation in fishes: tests using DNA sequence divergences between sister species and among clades. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms: Species and Speciation. Oxford University Press, New York.

Mumby, P.J., Edwards, A.J., Arias-Gonzalez, J.E., Lindeman, K.C., Blackwell, P.G., Gall, A., Gorczynska, M.I., Harborne, A.R., Pescod, C.L., Renken, H., Wabnitz, C.C.C., Llewellyn, G., 2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. Nature 427, 533–536.

Nagelkerken, I., van der Velde, G., Gorissen, M.W., Meijer, G.J., van't Hof, T., den Hartog, C., 2000. Importance of mangroves, seagrass beds and the shallow coral reef as a nursery for important coral reef fishes, using a visual census technique. Est. Coast. Shelf Sci. 51, 31–44.

Nelson, J.S., 2006. Fishes of the World, fourth ed. John Wiley and Sons Inc., New York. pp. 601.

Nolf, D., Stringer, G., 2003. Late Eocene (Priabonian) fish otoliths from the Yazoo Clay at Copenhagen, Louisiana. Louisiana Geol. Surv. Geol. Pamphlet 13, 1–23.

Palumbi, S., Martin, A., Romano, S., McMillian, W.O., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR. University of Hawaii, Honolulu. 46 pp.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Robertson, D.R., Karg, F., Moura, R.L., Victor, B.C., Bernardi, G., 2006. Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes.. Mol. Phyl. Evol. 40, 795–807.

Rocha, L.A., 2003. Patterns of distribution and processes of speciation in Brazilian reef fishes. J. Biogeog. 30, 1161–1171.

Rocha, L.A., Robertson, D.R., Roman, J., Bowen, B.W., 2005. Ecological speciation in tropical reef fishes. Proc. R. Soc. Lond. B 272, 573–579.

Rocha, L.A., Craig, M.T., Bowen, B.W., 2007. Phylogeography and the conservation of coral reef fishes. Coral Reefs 26, 501–512.

Rocha, L.A., Lindeman, K.C., Rocha, C.R., Lessios, H.A., 2008. Historical biogeography and speciation in the reef fish genus *Haemulon* (Teleostei: Haemulidae). Mol. Phyl. Evol 48, 918–928.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, second ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Swofford, D.L., 2003. PAUP: Phylogenetic analysis using parsimony (and other methods), version 4.0b 10. Sinauer Associates, Sunderland, Massachusetts.

Taylor, M.S., Hellberg, M.E., 2005. Marine radiations at small geographic scales: speciation in neotropical reef gobies (Elacatinus). Evolution 59, 374–385.

Tavera Vargas, J.J., 2006. Analisis filogenetico y biogeografico del genero Anisotremus Gill, 1861 (Perciformes: Haemulidae). M. Thesis. Instituto Politecnico Nacional La Paz, Mexico.

Zwickl, D.J., 2006. GARLI, Genetic Algorithm for Rapid Likelihood Inference, Version 0.951. Available from: <http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html>.