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Molecular Systematics, Zoogeography, and Evolutionary Ecology of the Atlantic Parrotfish Genus *Sparisoma*

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Parrotfishes of the genus *Sparisoma* (Scaridae) are ecologically important tropical reef fishes restricted to the Atlantic Ocean. We investigated phylogenetic relationships among the eight extant species within this genus using mitochondrially encoded 12S and 16S ribosomal genes. Our molecular data support the view that (i) *Sparisoma* originated ~14–35 million years ago (mya), probably in the tropical western Atlantic, off Brazil; (ii) there have been at least four discrete bouts of cladogenesis within the genus, with the most recent one (~2.8–5.6 mya) involving four events in both the east and the west Atlantic and across the Atlantic; and (iii) the genus invaded the eastern Atlantic on two different occasions, probably by at least two different routes. The data also offer support for Bellwood's ideas concerning the evolutionary changes in adult feeding patterns and habitat use within Scarids. Specifically, they support the evolutionary position of the ecological traits of *Sparisoma* as intermediate within the family.

Key Words: **parrotfish; Scaridae; *Sparisoma;* molecular phylogeography; fish ecology**

INTRODUCTION

Although relatively recently derived (15–20 million years ago (mya); Bellwood, 1994, 1996a; Bellwood and Schultz, 1991), parrotfishes (Scaridae) have rapidly evolved into a diverse and influential group of herbivores on modern coral reefs (Choat, 1991; Choat and Bellwood, 1991). As the dominant consumers of benthic primary production on reefs (e.g., Hatcher and Larkum, 1983), their rasping of the benthos shapes algal communities (e.g., Hay, 1991), erodes reefs (e.g., Hutchings, 1986; Bellwood, 1995), and contributes significantly to sedimentary processes (e.g., Bellwood, 1996b). Despite the obvious relevance of scarids to coral reef evolution and ecology, phylogenetic relationships within this speciose group remain unresolved (Choat and Bellwood, 1991; Bellwood, 1994).

Recently, Bellwood (1994) published a revision of the parrotfishes and proposed specific hypotheses to explain the evolutionary history of the family (see also Bellwood, 1996a). He suggested that (i) scarids arose in seagrass habitats and subsequently moved onto coral reefs and (ii) an ancestral browsing feeding mode (biting pieces of plants without disturbing the substratum) subsequently led to feeding modes in which the surface is disturbed—initially with an excavating mode (major surface disturbance) and subsequently with a scraping mode (a paedomorphic trait involving minor surface disturbance) among the most recently derived coral reef dwellers.

The parrotfish genus *Sparisoma* is one of three genera (*Cryptotomus, Nicholsina,* and *Sparisoma*) restricted to or primarily found in the Atlantic and immediately adjacent areas. These genera became separated from the Indo-Pacific scarids probably after the closing of the eastern Tethys, about 13–15 mya (Bellwood, 1994). *Sparisoma* apparently split off from other scarids somewhere between 5 and 14 mya (Bellwood, 1994). Analysis of evolutionary histories can be used to address questions about the spatio-temporal pattern of speciation and to determine evolutionary shifts in habitat use and feeding patterns. These aspects are of particular interest in *Sparisoma,* given its widespread geographic distribution throughout the tropical and subtropical Atlantic, its species richness, being the largest Atlantic Scarid genus, and its ecological diversity, presenting the full range of feeding modes and patterns of habitat use in the genus.

Currently, there are eight recognized species of *Sparisoma: S. atomarium, S. aurofrenatum, S. chrysopterum, S. cretense, S. radians, S. rubripinne, S. strigatum,* and *S. viride. S. cretense* and *S. strigatum* are

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moderately large (320–400 mm, Standard Length) species that are restricted to the eastern Atlantic. *S. cretense* occurs in the Macaronesian Archipelagos (Canaries, Azores, and Cape Verde Islands), the northwest coast of Africa, and the Mediterranean Sea (Gonzalez *et al.*, 1993), whereas *S. strigatum* is endemic to Saint Helena and Ascension Islands, in the middle of the south Atlantic (Schultz, 1958; Bellwood, 1994). Tropical west Atlantic members of the genus include two relatively small (140–180 mm) species, *S. atomarium* and *S. radians;* one mid-sized (240 mm) fish, *S. aurofrenatum;* and three larger (340–390 mm) species, *S. chrysopterum, S. rubripinne,* and *S. viride* (Robertson and Warner, 1978; Randall, 1996; Debelius, 1997). Whereas *S. radians* and *S. rubripinne* have been reported to occur in the east and west Atlantic (Bohlke and Chaplin, 1993 for both species; Randall, 1996 and Robins *et al.*, 1986 for *S. rubripinne*), *S. rubripinne* is abundant and evidently resident at Sao Tome off the west African coast (D.R.R., pers. obs.), but east Atlantic *S. radians* seem to represent stray individuals rather than an established population. The six west Atlantic species occur widely throughout that region, including both the Caribbean and Brazil (Böhlke and Chaplin, 1993; Randall, 1996; Robins *et al.*, 1986).

Parrotfish live in a variety of habitats with a diversity of diets, feeding modes, and social organizations (e.g., Robertson and Warner, 1978; Bellwood and Choat, 1990; Bellwood, 1994; McAfee and Morgan, 1996). Species of *Sparisoma* are found in a particularly broad range of tropical marine habitats (Bellwood, 1994), including seagrass beds, rocky reefs, and coral reef-fringe and back-reef areas of coral rubble and sand, as well as zones dominated by live coral (e.g., Robertson and Warner, 1978; Randall, 1996; Petrakis and Papaconstantinou, 1990; Gonzalez *et al.*, 1993; Bellwood, 1994; McAfee and Morgan, 1996).

Of the 14 scarids found in the Caribbean (Randall, 1996), 6 are species of *Sparisoma* that are ecologically important components of coral reef communities in that area. Species of *Sparisoma* have been the subject of a wide variety of ecological, evolutionary, and biochemical studies during the past 30 years (e.g., Robertson and Warner, 1978; Lobel and Ogden, 1981; Cardwell and Liley, 1991; Koltes, 1993; Bruggemann *et al.*, 1994). Relatively less is known about the two eastern Atlantic species (e.g., Petrakis and Papaconstantinou, 1990; Gonzalez *et al.*, 1993). Recent observations of *S. cretense* (E. Azzurro) and *S. strigatum* (D. R. Robertson) reveal that both species associate primarily with rocky reef habitat, in which they feed by browsing and scraping (sensu Bellwood and Choat, 1990) turf algae from rocky substrata. *S. cretense* also forages in deeper seagrass habitats, in which it browses on epiphitized blades of the seagrass *Posidonia oceanica*.

Molecular approaches have proven useful in elucidating phylogenetic relationships and patterns of evolutionary change in the ecology of organisms in cases in which classical morphology-based methodologies fall short (e.g., Ritchie *et al.*, 1996). Our goal here is to compare aspects of *Sparisoma* ecology with data on the biogeographic distribution and molecular phylogeny of the genus based on the mitochondrially encoded 12S and 16S ribosomal genes to assess (i) the dynamics of speciation in space and time within the genus and (ii) the validity of Bellwood's (1994) ideas about the pattern of evolutionary change in feeding modes and habitat use among the Scarids.

MATERIALS AND METHODS

Sampling and DNA Extraction

We obtained individuals from each species within *Sparisoma* by spear while free-diving. *S. cretense* was collected from the Island of Lampedusa, Italy. *S. strigatum* was collected from both Ascension and Saint Helena Islands. All the other *Sparisoma* species and *Cryptotomus roseus* were collected from near the Smithsonian field station in San Blas, Panama. Additionally, samples of *S. rubripinne* were also collected at Sao Tome Island, on the equator off the west coast of Africa. Samples of *Nicholsina denticulata* and *N. usta colletti,* were collected off Isla San Pedro Martir, Gulf of California (Mexico) and Sao Tome, respectively (Fig. 1). Liver or gill tissue was immediately dissected from collected specimens and preserved in either 95% ethanol or a buffer solution at ambient temperature in the field and then stored at 4°C in the laboratory. Tissues were digested overnight at 55°C in 500 ml of extraction buffer (NaCl 400 mM, Tris 10 mM, EDTA 2 mM, SDS 1%). We then purified the DNA by standard chloroform extraction and isopropanol precipitation (Sambrook *et al.*, 1989).

Polymerase Chain Reaction (PCR) Amplification

Amplification of the mitochondrially encoded 12S and 16S ribosomal gene regions was accomplished with the following primers: 12SAL and 12SBH, and 16SAR and 16SBR (Kocher *et al.*, 1989). Each 100-ml reaction contained 10 to 100 ng of DNA, 10 mM Tris–HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 units of *Taq* DNA Polymerase (Perkin–Elmer, Norwalk, CT), 150 mM each dNTP, and 0.3 mM each primer and was amplified with a cycling profile of 45 s at 94°C, 45 s at 48°C, and 1 min at 72°C for 35 cycles. After purification (following the manufacturer's protocol; ABI, Perkin–Elmer), sequencing was performed in both directions with the primers used in the PCR amplification on an ABI 373 automated sequencer (Applied Biosystems, Foster City, CA).

Sequence Analysis

Sequences from all eight known species of *Sparisoma* were analyzed. *C. roseus*, *N. denticulata*, and *N. usta*

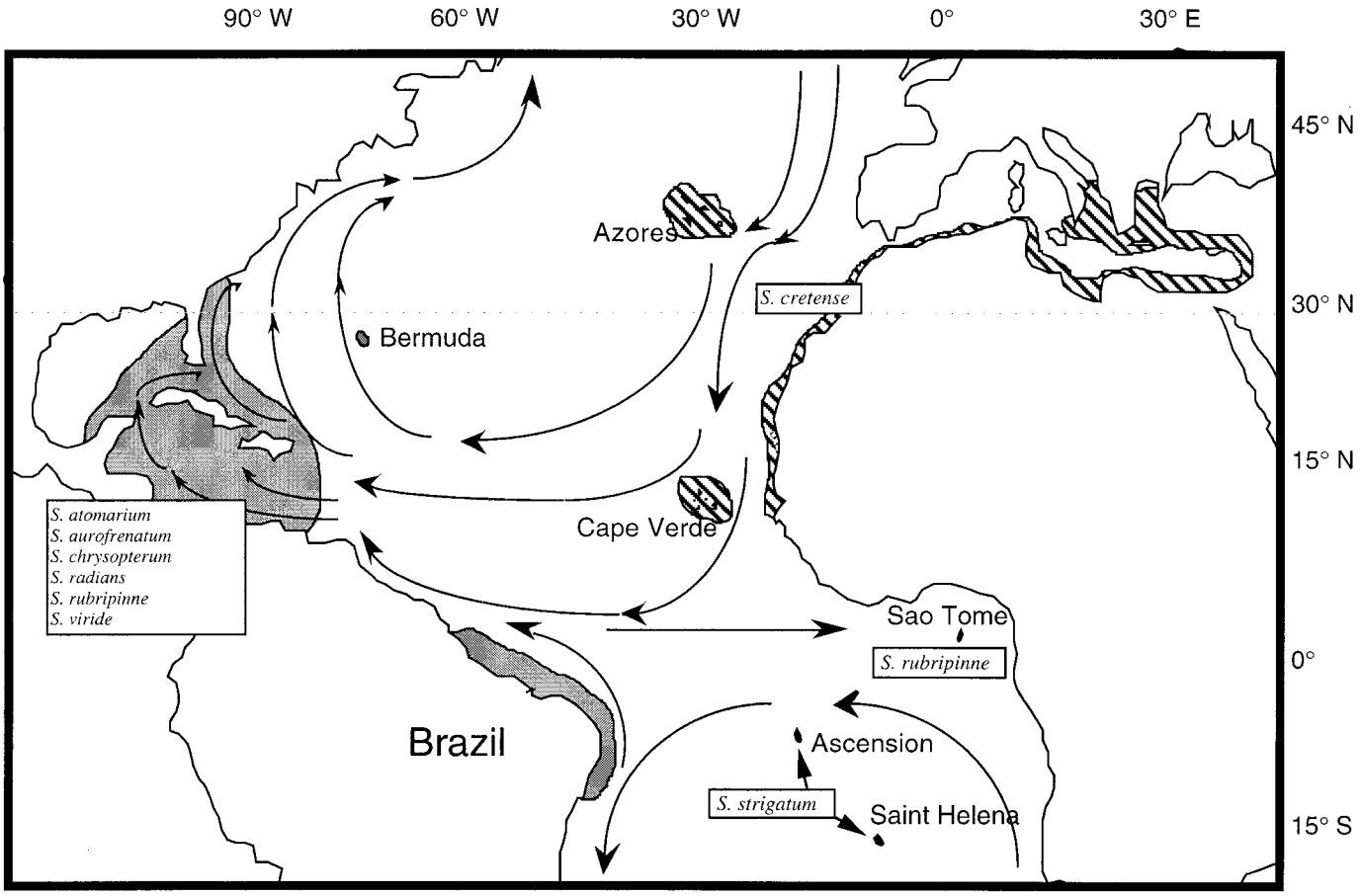

FIG. 1. Biogeographic distribution of the parrotfish genus *Sparisoma* in the Atlantic Ocean. *S. cretense* is found throughout the tropical eastern Atlantic and Mediterranean Sea. *S. strigatum* is endemic to Saint Helena and Ascension islands. Five western Atlantic species (*S. atomarium*, *S. aurofrenatum*, *S. chrysopterum*, *S. radians*, and *S. viride*) occur throughout the Caribbean and to the "hump" of Brazil. *S. rubripinne* occurs in Brazil, the Caribbean, and the eastern Atlantic island of Sao Tome. Arrows indicate major current flow patterns.

colletti were used as outgroups, following Bellwood (1994). We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the sequences. Phylogenetic relationships were assessed by maximum-parsimony (MP), neighbor-joining (NJ), and maximum-likelihood methods implemented by the Software package PAUP (Phylogenetic Analyses Using Parsimony, version 4.0; Swofford, 1998). Most-parsimonious trees were obtained using a branch and bound search. Statistical confidence in nodes was evaluated using 2000 nonparametric bootstrap replicates (Felsenstein, 1985; Hedges, 1992; Hillis and Bull, 1993). Homogeneity tests using 100 replicates were performed using PAUP. Alternative phylogenetic topologies were compared using topology-dependent tail permutation tests (T-PTP) (Faith, 1991; Trueman, 1996; Faith and Trueman, 1996; but see Swofford *et al.*, 1996) and Kishino and Hasegawa tests (Kishino and Hasegawa, 1989) implemented by PAUP.

RESULTS

Mitochondrial DNA Sequences

Sequences were obtained for all individuals investigated and deposited at GenBank under the following Accession Nos.: U95761, U95762, and U95765–U95778. No differences were found between the two to three individuals of the same species that we sampled (including the two *S. strigatum* individuals from Saint Helena Island and Ascension Island). Tree topologies were found to be identical when 12S and 16S data were used independently. Furthermore, homogeneity tests indicated that 12S and 16S datasets were congruent. Data for 12S and 16S sequences were therefore combined for the remainder of the analysis. A total of 913 bp were compared among the 11 species under investigation (393 bp for the 12S region and 520 bp for the 16S region). Of these 913 bases, 174 were variable and 67 were phylogenetically informative. Few insertions or

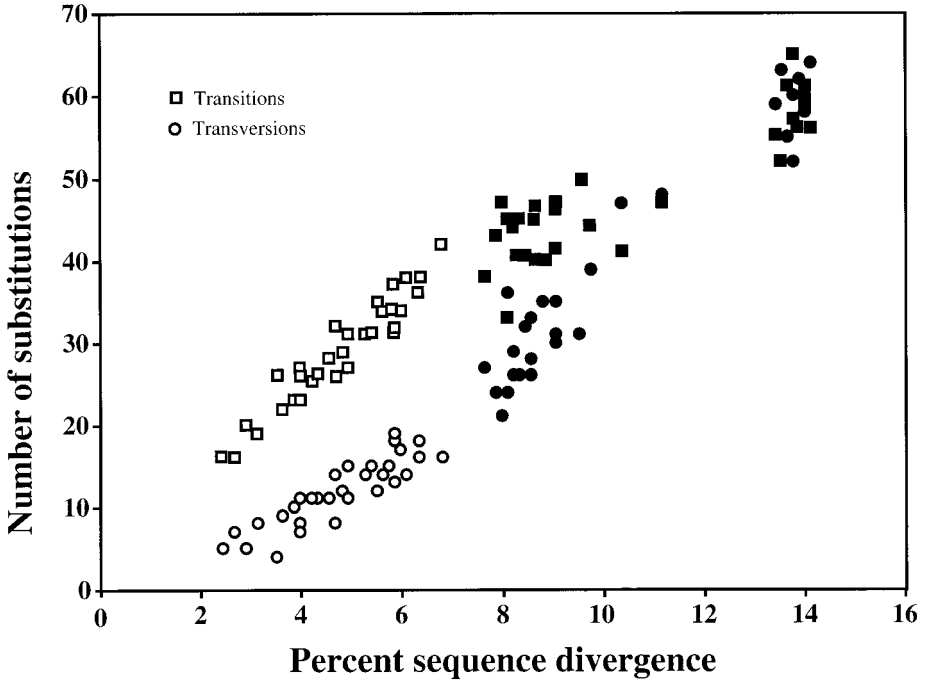

FIG. 2. Absolute numbers of transitions and transversions versus percentage divergences for all pairwise comparisons. Open symbols represent ingroup comparisons; solid symbols represent outgroup/ingroup comparisons.

deletions (indels) were observed. These indels were included in our analysis and were counted as one single step each, regardless of size. Their removal or inclusion in the analysis did not change the topology of the resulting phylogenetic tree. A plot of transitions and transversions versus genetic distance indicated that saturation was not reached within the ingroup and was starting to be present when comparing ingroup and outgroup (Fig. 2). Thus, divergence times were estimated using transversions only. Transitions exceeded transversions (ratio of transitions over transversions = 3, Table 1). When various weighting schemes were attempted (1:2, 1:3, 3:1), topologies remained unchanged; thus, the following analysis was performed without weighting characters.

Phylogenetic Analysis

A single most-parsimonious tree, identical to the neighbor-joining tree and to the maximum-likelihood tree, was obtained (tree length = 230 steps, consistency index = 0.75). Since no previous phylogenetic study of this group is available, this tree cannot be compared with a morphology-based phylogeny. Figure 3 shows the single most-parsimonious tree (obtained by

TABLE 1

Transitions and Transversion Substitutions in *Sparisoma*

		1	2	3	4	5	6	7	8	9	10	11	12
1	*C. rosaceus*	—	47	41	56	65	55	57	59	56	52	61	61
2	*N. denticulata*	48	—	28	47	45	38	47	43	45	41	45	47
3	*N. usta. colletti*	47	11	—	44	46	40	40	40	42	33	50	44
4	*S. aurofrenatum*	64	30	39	—	36	25	29	26	35	23	32	31
5	*S. atomarium*	52	24	31	18	—	34	34	38	42	34	38	37
6	*S. chrysopterum*	59	27	32	11	15	—	16	20	27	19	27	23
7	*S. radians*	60	26	35	12	14	7	—	26	32	22	31	26
8	*S. rubripinne*	60	24	33	8	14	5	4	—	16	22	37	30
9	*S. rubsaotome*	62	28	35	12	16	7	8	4	—	25	38	31
10	*S. viride*	63	29	36	11	17	8	9	7	11	—	31	26
11	*S. cretense*	58	26	31	18	16	15	14	12	14	19	—	16
12	*S. strigatumA*	55	21	26	15	13	10	11	9	11	14	5	—

Note. The absolute number of 12S rRNA and 16S rRNA transitions (above the diagonal) and transversions (below the diagonal) of *Sparisoma* (ingroup) and *Nicholsina* and *Cryptotomus* (outgroup) species are shown.

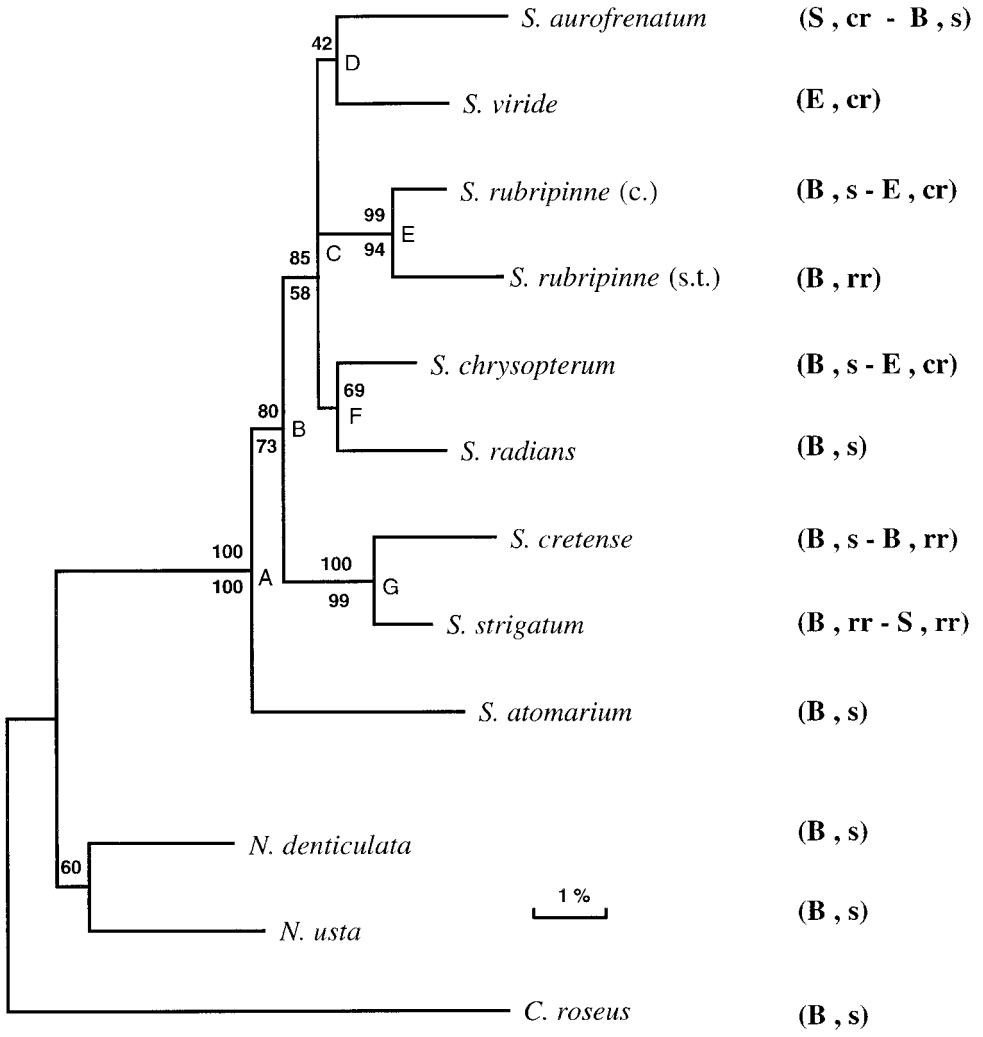

FIG. 3. Phylogram for the genus *Sparisoma*. Most-parsimonious phylogenetic tree based on 12S and 16S ribosomal genes. *S. rubripinne* was collected in the Caribbean (c.) and in Sao Tome, West Africa (s.t.). Numbers on nodes indicate results of bootstrapping (2000 replicates) with results for MP (above) and NJ (below) the nodes. Only values above 40% are shown. Foraging mode (sensu Belwood and Choat, 1990) is indicated on the right: B, browsing; E, excavating; S, scraping. Habitat: rr, rocky reef; s, seagrass; cr, coral reef.

maximum-parsimony), with superimposed data from a 50% consensus tree of 2000 bootstrap replicates performed with both maximum-parsimony and neighbor-joining methods. When a maximum-likelihood search with a molecular clock assumption was performed, a similar tree was obtained. The only difference between this tree and the most-parsimonious tree was that *S. aurofrenatum* and *S. viride* were not found to form a monophyletic clade but were paraphyletic sister taxa. This tree was not found to be significantly different from the tree presented in Fig. 3 (Kishino–Hasegawa test, $P = 0.2$), indicating that it is legitimate to consider that substitution rates were constant among lineages and that a molecular clock can be used (Huelsenbeck and Rannala, 1997).

The sequences from this study were aligned with the complete sequences of the 12S rRNA (Springer and Douzery, 1996) and the 16S rRNA (Gutell *et al.*, 1985) of the cow, for which the secondary structure of these genes is known. These reference sequences were used as models for the folding structure of the rRNAs, and putative loops and stems were identified for *Sparisoma*. More variation, in terms of both substitutions and indels, was observed in the loop than in the stem regions of *Sparisoma*. Since double-stranded stems are not independently evolving regions, we constructed phylogenies based solely on loop regions. These new datasets produced identical trees as the complete datasets; thus, complete datasets were used for the remainder of the analysis.

Our data indicate that *S. atomarium* is the sister clade of the remaining *Sparisoma* species. The two eastern Atlantic species, *S. cretense* and *S. strigatum*, formerly grouped together in the genus *Euscarus* (Jor-

dan and Evermann, 1896), were sister taxa in 100% (100% NJ, 99% MP) of the bootstrap replicates. The grouping of mostly western Atlantic species (*S. aurofrenatum, S. viride, S. rubripinne, S. chrysopterum,* and *S. radians*) within a single clade was marginally supported by bootstrap analysis (85% NJ, 58% MP) but highly supported by T-PTP ($P < 0.01$) and Kishino–Hasegawa ($P < 0.01$) tests. Although these five species separated in three clades (Fig. 2), our data could not reliably evaluate the phylogenetic relationships among those clades. One of those three clades contained the *S. rubripinne* representatives from the eastern and western Atlantic. The grouping of those two forms was well supported (99% NJ, 94% MP). Levels of divergence between the sequences of sister taxa in the most recently derived clades ranged from 2.3% between the east and the west Atlantic *S. rubripinne* to 2.7% between *S. chrysopterum* and *S. radians* and 2.6% between *S. strigatum* and *S. cretense.*

Feeding Ecology

We followed Bellwood and Choat (1990) in categorizing feeding modes as browsing, scraping, and excavating. Feeding modes on rocky reefs, seagrasses, and coral reefs were correlated with our data, as shown in Fig. 3. The significance of the correlation between evolutionary history of *Sparisoma* and feeding modes is detailed in the Discussion below.

DISCUSSION

Phylogenetic Relationships

Based on biogeographical and geological data, Bellwood (1994) proposed that the genus *Sparisoma* diverged from the other scarids between ~5 and 14 mya. Although the use of molecular clocks remains controversial and, as such, should be used cautiously, our data, which are consistent with the presence of a molecular clock, are useful for addressing biogeographical questions pertaining to the evolutionary history of the genus *Sparisoma*. Applying the generally accepted rate of mitochondrial sequence divergence among bony fish 12S and 16S regions (0.14% transversion divergence per million years; Ritchie *et al.,* 1996) to the average transversion divergence of *Sparisoma* species (5.0%) from the other two genera and to the largest transversion divergence within *Sparisoma* (2.0%), we estimate that the divergence between the two outgroup genera *Nicholsina* and *Cryptotomus* and the genus *Sparisoma* occurred ~14.3 to 35 mya. This range of values places the origin of *Sparisoma* at a slightly earlier date than that proposed by Bellwood (1994). Assuming that our figures for the timing of the origin of the genus *Sparisoma* are valid, our data suggest that subsequent cladogenesis within that genus proceeded in the following chronological order. (i) First, a split between the ancestor of *S. atomarium* and the remaining species occurred 12 mya (transversion divergence = 1.8%; split A, Fig. 3). (ii) This was followed by a split 10 mya (transversion divergence = 1.4%) between the lineage leading to the two eastern Atlantic species (*S. cretense* and *S. strigatum*) and that leading to the five remaining west Atlantic species (split B, Fig. 3). (iii) Then, about 6.4 mya (average transversion divergence = 0.9%), that west Atlantic clade began to split into three lineages, although the exact pattern of those events remains unclear (split C, Fig. 3). (iv) Finally, ~2.8–5.6 mya, three cladogenic events occurred in different parts of the Atlantic, one within the west Atlantic clade, a split between *S. chrysopterum* and *S. radians* (transversion divergence = 0.8%), another that involved the divergence of *S. rubripinne* into isolated east and west Atlantic populations (transversion divergence = 0.4%), and another that involved the split of the two eastern Atlantic species, *S. cretense* and *S. strigatum* (transversion divergence = 0.6%) (splits F, E, G, Fig. 3). Interestingly, the most recent bout of speciation in *Sparisoma* coincides with a period of both accelerated extinction and accelerated speciation of corals in the Caribbean, about 1–4 mya (Johnson *et al.,* 1995; Budd and Johnson, 1996; Budd *et al.,* 1996). This indicates that events leading to speciation in Caribbean corals may have stimulated speciation in other taxa, such as *Sparisoma,* and had effects on cladogenesis that extended throughout the tropical west Atlantic and across to the eastern Atlantic.

The level of sequence divergence between the east and the west Atlantic forms of *S. rubripinne* is similar (see above) to that between sympatric west Atlantic sister species (*S. chrysopterum* and *S. radians*), as well as that between allopatric sister species (*S. cretense* and *S. strigatum*). This suggests that the east and west Atlantic populations of *S. rubripinne* probably are separate species. Morphological analysis of this situation is warranted. In general, the eastern Atlantic fauna has been much less studied and subject to less taxonomic work than the western counterpart.

Biogeography of Sparisoma—Its Origin and Trans-Atlantic Dispersal

The spatiotemporal patterns of speciation within *Sparisoma* are complex. The following set of scenarios can account for the present-day distributions of the known *Sparisoma* species: Both extant lineages derived from the earliest cladogenic event within the genus occur in the west Atlantic, but only one occurs in the east Atlantic. Although complex vicariant events may be evoked to explain this pattern, in the absence of any fossil evidence bearing on this question, the simplest explanation is that the genus originated in the western Atlantic. After diverging from *S. atomarium,* the main clade within *Sparisoma* gave rise to two groups of fishes, one that dispersed to and diversified in the eastern Atlantic (as *S. cretense* and *S. strigatum*)

and a second group that diversified in the western Atlantic (all remaining species), and from which one species (the immediate ancestor of *S. rubripinne*) subsequently dispersed eastward to the equatorial eastern Atlantic.

What are the most parsimonious scenarios that account for the present day distributions of the three *Sparisoma* species in the east Atlantic? Assuming that currents that produced those eastward transatlantic dispersals were essentially the same as those that exist now, there are three possible routes for such dispersal (see Fig. 1): (i) via the Gulf Stream from the Caribbean to the Macaronesian islands off northwest Africa, (ii) via the equatorial countercurrent from northern Brazil to equatorial Africa, and (iii) from southern Brazil to the central Atlantic islands. Of those three, the last route would seem to be the most difficult because the eastward movement of warm water from Brazil is stopped in the mid-Atlantic by convergence of that flow with cold currents flowing from the opposite direction, a convergence that occurs well to the south of the mid-Atlantic islands (Fig. 1).

Eastward dispersal on the equatorial countercurrent is the simplest mechanism that accounts for the occurrence of the sister of *S. rubripinne* in equatorial West Africa. The existing distributions of *S. cretense* and *S. strigatum* require a more complicated explanation because those species occur in the northern and southern hemispheres well away from the African equatorial region. The ranges of those two species are separated by 20 degrees of latitude (a distance equal to that between Brazil and West Africa), and current flows between those two areas are perpendicular to that north–south axis (Fig. 1). The existing *cretense/strigatum* pattern could have been accomplished in either of two ways. (i) First, eastward dispersal could have occurred by one transatlantic route by the common ancestor of the clade that split off from *S. atomarium*. This was followed by secondary dispersal within the eastern Atlantic and subsequent divergence of isolated *cretense* and *strigatum* populations. Such dispersal on either the northern or the southern route could have established a population in one hemisphere, with a secondary dispersal establishing a population in the other hemisphere. Alternatively, initial eastward dispersal by the equatorial route could have produced a population in equatorial Africa, from which there was secondary dispersal to both north and south. The first mechanism is the most parsimonious because it involves only two steps (two dispersal events), whereas the second involves four events—the initial dispersal to equatorial Africa, secondary dispersals both north and south, and the extinction of the common ancestor in equatorial Africa. (ii) Alternatively, the common ancestor of *cretense/strigatum* might have dispersed eastward by both the north and south Atlantic routes. However, this is the least parsimonious mechanism because it requires five events. First, a split produced the common ancestor of *cretense/strigatum* and the lineage that led to the bulk of the west Atlantic species in the west Atlantic before any eastward dispersal. Then, two dispersal events occurred followed by the extinctions in Brazil and in the Caribbean of the common ancestor of *cretense/strigatum*.

Thus, unlike the situation with *S. rubripinne,* it is difficult to arrive at any fairly straightforward scenario that led to the present distributions of the sister species *S. cretense* and *S. strigatum*. While it is always possible that ocean current patterns were different when the ancestors of the two east Atlantic clades established themselves in that region, four additional pieces of information may also help resolve this issue: (i) determination of the phylogenetic relationships of putatively conspecific populations of *Sparisoma* in Brazil and the Caribbean, (ii) determination of the validity of reports (Böhlke and Chaplin, 1993) of *S. radians* in the east Atlantic and assessment of the phylogenetic status of any such population, (iii) analysis of phylogenetic relationships of other scarids that have resident populations on both sides of the Atlantic (e.g., *N. usta*), and (iv) assessment of the phylogenetic relationship of the eastern Atlantic scarid, *Scarus hoefleri* (the sole member of that genus in the Eastern Atlantic), to congeners in the west Atlantic.

Evolutionary Change in Feeding and Habitat Use in Sparisoma

How do our molecular data on patterns of speciation through time within *Sparisoma* bear on Bellwood's (1994) hypotheses regarding the evolutionary changes in habitat use and feeding mode within the family Scaridae and whether *Sparisoma* represents a transitional group? The following patterns of habitat use and feeding mode were obtained among the six species in the Caribbean. The ancestral *S. atomarium* is a browsing resident of seagrass and seagrass/reef interface habitats (Robertson and Warner, 1978). The five more recently derived Caribbean species include a broad range of types: (a) a small browsing species that is largely restricted to seagrass and macroalgal beds but makes occasional forays onto reefs to feed on macroalgae (*S. radians*—Robertson and Warner, 1978; Randall, 1967; Lobel and Ogden, 1981); (b) two relatively large species that feed both as browsers in seagrass and Sargassum beds and as excavators on coral reefs (*S. rubripinne* and *S. chrysopterum*—Robertson and Warner, 1978; Randall, 1967; McAfee and Morgan, 1996; K. E. Clifton, unpub. data); (c) one species that occurs mainly on coral reefs, on which it feeds as a scraper, but also occurs as a browser in seagrass/reef interface habitats (*S. aurofrenatum*—Randall, 1967; McAfee and Morgan, 1996; K. E. Clifton and D. R.

Robertson, unpub. obs.); and (d) one species that feeds only as an excavator on coral reefs (*S. viride*—Randall, 1967; McAfee and Morgan, 1996).

In the east Atlantic both coral reefs and seagrass beds are minor habitats. The two, recently derived, eastern Atlantic species, *S. cretense* and *S. strigatum*, feed mainly by browsing and/or scraping on rocky reefs, although *S. cretense* also makes occasional feeding forays into seagrass (in the Mediterranean). At Sao Tome, adults of the recently derived sister of *S. rubripinne* feed by browsing and scraping on rocky shores (and small coral patch reefs) and immediately adjacent sand areas (D.R.R., pers. obs.).

When viewed against the history of speciation within the genus, as indicated by our molecular data, patterns of variation in feeding mode and habitat use among and within the *Sparisoma* species offer partial support for Bellwood's (1994) ideas. First, the idea that the genus represents a transitional form is supported by the facts that the most derived species are found in all potential habitats and use a great variety of feeding modes and that most species, including the most ancestral one, *S. atomarium*, feed in a variety of habitats. Second, the idea that browsing preceded excavating on coral reefs is supported by the facts that *S. viride*, one of the most recently derived species, feeds exclusively by excavating in the Caribbean and that the most ancestral species, *S. atomarium*, is a browser. Third, the fact that *S. atomarium* is also a seagrass inhabitant in the Caribbean supports the idea that browsing in seagrasses is the ancestral condition. However, our conclusion about both the spatio-temporal pattern of speciation within *Sparisoma* in the Atlantic and the evolution of adult feeding pattern within this group must remain tentative because we lack data on the Brazilian members of the genus. Brazilian seagrasses belong to a different genus with a growth form different from that of the predominant species in the northwest Atlantic (*Thalassia testudineum*) (de Oliviera *et al.*, 1983; Larkum and den Hartog, 1989). Hence, *Sparisoma's* many uses of seagrasses as habitat and food could well differ in those two areas. Further, the phylogenetic relationships between putatively conspecific forms in the northwest and southwest Atlantic need to be assessed using molecular and morphological data.

Our results indicate that the combination of molecular approaches with ecological data allows useful explorations of a combination of spatio-temporal patterns of speciation and evolutionary ecology. It seems likely that similar approaches, with other members of the Scaridae for which ecological data are available, would help resolve the evolutionary development and radiation of this complex guild of important tropical herbivores.

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