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EVOLUTIONARY HISTORY OF THE PARROTFISHES: BIOGEOGRAPHY, ECOMORPHOLOGY, AND COMPARATIVE DIVERSITY

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Abstract.—The family Scaridae comprises about 90 species of herbivorous coral reef, rock reef, and seagrass fishes. Parrotfishes are important agents of marine bioerosion who rework the substrate with their beaklike oral jaws. Many scarid populations are characterized by complex social systems including highly differentiated sexual stages, territoriality, and the defense of harems. Here, we test a hypothesis of relationships among parrotfish genera derived from nearly 2 kb of nuclear and mitochondrial DNA sequence. The DNA tree is different than a phylogeny based on comparative morphology and leads to important reinterpretations of scarid evolution. The molecular data suggest a split among seagrass and coral reef associated genera with nearly 80% of all species in the coral reef clade. Our phylogenetic results imply an East Tethyan origin of the family and the recurrent evolution of excavating and scraping feeding modes. It is likely that ecomorphological differences played a significant role in the initial divergence of major scarid lineages, but that variation in color and breeding behavior has triggered subsequent diversification. We present a two-phase model of parrotfish evolution to explain patterns of comparative diversity. Finally, we discuss the application of this model to other adaptively radiating clades.

Key words.—Adaptive radiation, biogeography, ecomorphology, evolution, parrotfishes, sex reversal.

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Parrotfishes (family Scaridae) are among the most colorful and numerically dominant inhabitants of coral reefs and seagrass beds (Sale 1991). Scarids are circumtropically distributed, although the majority of taxa are confined to the Indo-Pacific region. There are roughly 90 parrotfish species partitioned into 10 genera (Parenti and Randall 2000), with more than half of all species in a single genus (*Scarus*). Individuals have beaklike oral jaws of fused teeth used to remove algae and detritus from the substratum. As such, scarids have been recognized as significant agents of marine bioerosion (Bellwood and Choat 1990; Bellwood 1995a,b) and determinants of benthic community structure (Lewis and Wainwright 1985).

Early taxonomic efforts were confounded by ontogenetic changes in size, shape, and coloration that correspond to discrete sexual stages. Parrotfishes display a bewildering repertoire of social and mating behaviors, most of which include protogynous (female first) sex reversal (Choat and Robertson 1975; Robertson and Warner 1978). Some populations live as groups of females within the territory of a single large and brightly colored male. If this male is removed, the largest female of the harem can reverse sex, adopting male coloration and behavior. The intrinsic and extrinsic controls of sex reversal have been studied extensively in reef fishes (Ghiselin 1969; Fishelson 1970; Shapiro 1980) and have led to various models of the process (Robertson 1972; Ross et al. 1983; Warner 1988; Shapiro 1989; Lutnesky 1994). This phenomenon and its ecological antecedents are particularly interesting given the diversity of vertebrate sex determining mechanisms that may be elicited by variation in a common set of genes (Morais da Silva et al. 1996; Raymond et al. 1998; Arango et al. 1999; Smith et al. 1999).

Bellwood (1994) revised the taxonomy of parrotfishes, proposing a phylogeny of genera based on comparative morphology (Fig. 1). Area cladograms suggest the family has experienced a complex biogeographic history. Scarids likely originated in the tropical Tethys Sea, and may have differentiated before as well as after its closure (~14 million years ago) isolated eastern (proto-Indo Pacific, i.e., *Cetoscarus*, *Bolbometopon*, *Chlorurus*, *Hipposcarus*, *Scarus*) from western (proto-Atlantic, Caribbean and eastern Pacific, i.e., *Cryptotomus*, *Nicholsina*, *Sparisoma*) populations. The earliest known fossil (a *Calotomus* species) from the mid-Miocene of Europe (Bellwood and Schultz 1991) confirms the presence of the family in the Tethys at the appropriate time. A scarid origin prior to the mid-Miocene is also supported by the estimated age of the genus *Sparisoma* derived from molecular data (14–35 million years ago, Bernardi et al. 2000). Subsequent to the closure of the Tethys, *Scarus* is thought to have colonized the world's oceans from the Indo-Pacific, perhaps before (Bellwood 1994) and/or after the rise of the Panamanian isthmus (~3 million years ago).

The evolution of habitat association and feeding mode appears less complicated. The phylogeny suggests a gradual shift from browsers living in seagrasses to excavators inhabiting rock and/or coral reefs to scrapers found exclusively in association with coral. In this context, *Sparisoma* is hypothesized to be a transitional genus because species are found in all habitat types and exhibit the full range of feeding modes (cf. Bernardi et al. 2000). A specific implication of this account is that reef-dwelling parrotfish genera (*Cetoscarus*, *Bolbometopon*, *Chlorurus*, *Hipposcarus*, and *Scarus*) are of relatively recent origin (Bellwood 1994).

We sought to further explore the evolutionary history of

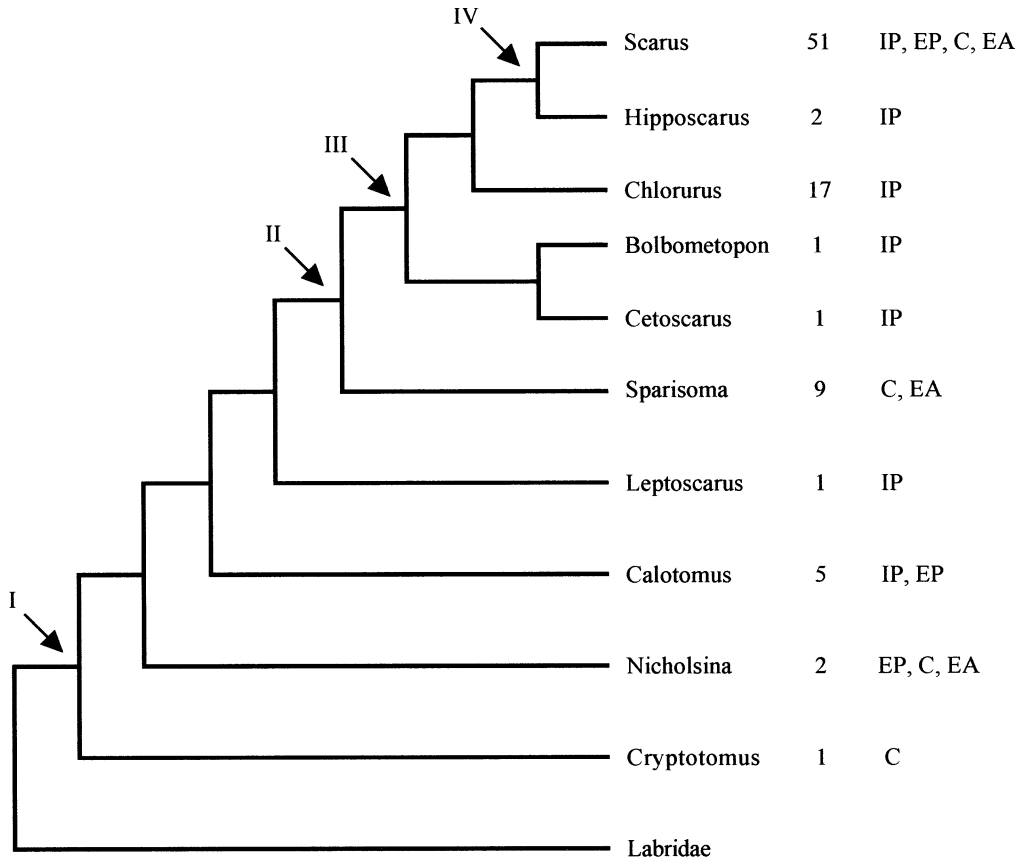


FIG. 1. Cladogram depicting parrotfish relationships derived from comparative morphology (Bellwood 1994). For each of the 10 parrotfish genera, the number of species in each genus, and present distribution is indicated. IP, Indo-Pacific; EP, eastern Pacific; C, Caribbean; EA, eastern Atlantic. Node numbers; I, the origin of parrotfishes, in seagrasses, browsing feeding mode; II, the transition onto rock and coral reefs; species of the genus *Sparisoma* are found in seagrasses and on reefs and use either browsing, excavating, or scraping feeding modes; III, evolution of taxa found only on coral reefs; IV, evolution of the scraping feeding mode from excavating ancestors.

parrotfishes by investigating characteristics of morphology, behavior, and ecology in the context of independent phylogenies reconstructed from DNA. We analyzed nearly 2 kb of sequence from four loci: the nuclear *Tmo-4C4* gene (Streelman and Karl 1997; Streelman et al. 1998), the mitochondrial cytochrome *b*, and ribosomal 12S and 16S genes (Kocher et al. 1989). Trees derived from all genes were largely concordant with one another. Topological differences between the DNA and morphology trees (Bellwood 1994), however, have major ramifications for the evolutionary history of parrotfishes. In particular, we find an ancient divergence of parrotfish lineages along an ecological axis (reef vs. seagrass). We synthesize these data with results from studies of other adaptively radiating clades and come to the conclusion that the phylogenetic signature of ecological speciation is common and strong.

MATERIALS AND METHODS

Samples and Sequences

Table 1 lists the species, collecting locations, polymerase chain reaction (PCR) primers, and annealing temperatures used in this study. Fishes were collected from the wild (unless noted) by pole spear, beach seine, gill net, or rotenone. Muscle samples were frozen in liquid nitrogen or placed directly in 95% ethanol prior to transport to the University of South Florida or the Field Museum of Natural History. We sampled representatives of each scarid genus and multiple members of species rich genera (e.g., *Scarus*). Labrid fishes were used as outgroups because previous work (Kaufman and Liem 1982; Bellwood 1994; Gomon 1997; Streelman and Karl 1997) suggested that wrasses are the closest parrotfish relative.

Total cell DNA was extracted from individual fish following standard phenol/chloroform procedures (Ausubel et al. 1993) or the PureGene extraction protocol (Gentra Systems, Minneapolis, MN). DNA was quantified by ultraviolet spectroscopy and diluted to 50 ng/μl. After PCR, 5 μl of the reaction mix was assayed for the amount and fidelity of amplification by agarose gel electrophoresis. Free nucleotides and unincorporated primers from successful amplifications were removed by centrifugal filtration with Ultrafree-MC (30,000 NMWL; Millipore, Bedford, MA) filter units or the GeneClean protocol (Bio 101, Carlsbad, CA). Both strands of the resulting purified DNAs were cycle sequenced using Prism Dye Terminator Cycle Sequencing Ready Reaction Kits, Prism dRhodamine Kits, or Prism BigDye Kits according to manufacturer protocols (P. E. Biosystems, Foster City, CA). Reactions were ethanol precipitated and then electrophoresed on ABI 310, 373, or 377 automated sequencers

TABLE 1. Species, collecting locations, polymerase chain reaction primers (5′–3′), and annealing temperatures (AT).

Species	Collecting Location
Family Labridae	
Choerodon fasciatus	Lizard Island, Australia
Pseudodax moluccanus	Solomon Islands
Family Scaridae	
Cryptomus roseus	Glovers Reef, Belize
Nicholsina usta	Virginia Key Beach, Miami
Calotomus carolinus	Phillipines
Leptoscarus vaigiensis	Philippines
Sparisoma aurofrenatum	St. Thomas, Virgin Islands
Sparisoma chrysopterum	Haiti
Sparisoma viride	Little Africa Reef, Dry Tortugas
Cetoscarus bicolor	Phillipines
Bolbometopon muricatum	Phillipines
Chlorurus oedema	Phillpines
Chlorurus sordidus	Lizard Island, Australia
Hipposcarus longiceps	Phillipines
Scarus coelestinus	Little Sambo Reef, Florida Keys
Scarus flavipectoralis	Lizard Island, Australia
Scarus frenatus	Lizard Island, Australia
Scarus guacamaia	Little Sambo Reef, Florida Keys
Primers and Temperatures	
Tmo-4C4	
Tmo-4C4F: CCTCCGGCCTTCCTAAAACCTCTC	
Tmo-4C4R: CATCGTGCTCCTGGGTGACAAAGT	
AT = 50–55°C	
Cytochrome *b*	
L14724: CGAAGCTTGATATGAAAAACCATCGTTG	
H15149: AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA	
for *Calotomus carolinus*	
GLUDG: TGATCTGAAAAACCACCGTTGTA	
B3: CCCTCAGAATGATATTGTTCCTCA	
AT = 48–52°C	
12S	
12Sa: AAACTGGGATTAGATACCCCACTAT	
12Sb: GAGGGTGACGGGCGGTGTGT	
AT = 47–51°C	
16S	
16Sar: CGCCTGTTTATCAAAAACAT	
16Sbr: CCGGTCTGAACTCAGATCACGT	
AT = 47–51°C	

(Applied Biosystems, Perkin-Elmer, Foster City, CA). All sequences have been deposited in GenBank (accession nos. AY081063–AY081133 and AY081211).

Sequence Alignment and Phylogenetic Analysis

Sequences were aligned by eye using the programs SeqEd (Applied Biosystems) or Sequencher (GeneCodes, Ann Arbor, MI). For the 12S and 16S ribosomal genes, a teleost model of secondary structure (Orti et al. 1996) was used to identify putative stems and loops. Gaps and ambiguously aligned regions were excluded from phylogenetic analysis. Alignments in the form of NEXUS files are available from JTS. We used PAUP 4.0b4a (Swofford 1999) and PUZZLE 2.5 (Strimmer and von Haeseler 1996) to analyze the sequence data in various ways. First, to evaluate the suitability of each gene for the task of reconstructing scarid evolution, we examined the types and frequencies of mutations that accumulated with divergence. For each gene, we graphed the number of different mutation types (e.g., third position transitions for coding loci, stem transitions for ribosomal genes) and the transition:transversion ratio for all pairwise comparisons of taxa versus genetic distance. We also calculated the observed nucleotide frequencies for each locus and tested whether individual sequences deviated from this pattern (i.e., stationarity).

The incongruence length difference (ILD) test (Farris et al. 1995) was used to determine whether significant conflict existed among the four data partitions (12S, 16S, cytochrome *b*, and *Tmo-4C4*). To explore the degree of conflict between our DNA and previously collected morphological data, we also performed pairwise ILD tests using Bellwood's (1994) data matrix and individual as well as combined gene partitions. The heuristic search option, with two random addition sequence replicates and 500 total replicates, was used to generate the null distribution for these tests. Following Cunningham (1997), invariant sites were excluded and a significance level of 0.01 was employed. None of the gene partitions were found to conflict with one another ($P > 0.19$ for all pairwise comparisons), so we combined data from all genes for phylogenetic analysis.

We used Modeltest (Posada and Crandall 1998) to justify an appropriate likelihood model for the concatenated gene set. This program computationally implements the strategy outlined in Huelsenbeck and Crandall (1997), applying a series of likelihood-ratio tests to determine the most parameter-rich model supported by the data. In turn, we performed a heuristic search with 25 random addition sequence replicates and TBR branch swapping under a Tamura-Nei (1983) model with rate heterogeneity and invariant sites.

We used two methods to estimate support for our phylogeny: character bootstrapping and Bayesian analysis. Under the maximum-likelihood criterion, we generated 1000 bootstrap replicates of the dataset. Using MrBayes (Huelsenbeck 2000) we ran a Markov chain for 60,000 generations, sampling every 100 generations. The first 10,000 generations were discarded to insure that the chain reached stationarity. To guarantee that the Markov chain was appropriately sampling from the posterior distribution, we repeated this procedure 10 times and monitored for chain convergence. Because all chains converged on the same distribution, we combined the results of all 10 chains (excluding burn-in chains) to calculate final posterior probabilities. Posterior probabilities of clades were estimated using the majority-rules consensus of the remaining 500,000 sampled trees (Larget and Simon 1999).

RESULTS AND DISCUSSION

Molecular Characterization

Sequence alignment resulted in a total of 1815 characters (16S = 550, 12S = 355, *Tmo-4C4* = 511, cytochrome *b* = 399). Approximately one-third of these (608) were variable across the dataset (16S = 166, 12S = 133, *Tmo-4C4* = 143, cytochrome *b* = 166) and about one-quarter (432) were informative (16S = 114, 12S = 88, *Tmo-4C4* = 91, cytochrome *b* = 139). Ribosomal sequences were similar in nucleotide frequency (A = 0.2858, 0.2868; C = 0.2549, 0.2518; G = 0.2358, 0.2329; T = 0.2236, 0.2285 for 16S and 12S, re-

spectively). *Tmo-4C4* sequences were biased against cytosine (A = 0.2868, C = 0.1876, G = 0.2682, T = 0.2623) as reported for cichlids (Streelman et al. 1998) and cytochrome *b* sequences were biased against guanine (A = 0.2505, C = 0.2917, G = 0.1664, T = 0.2915) as summarized for other fishes (Meyer 1993; Lydeard and Roe 1997). Putative loops were rich in adenine for both 16S and 12S (0.3687, 0.3532) and biased against guanine (0.1757, 0.1959), reflecting the findings of Orti et al. (1996). Putative first positions of the *Tmo-4C4* gene were rich in guanine (0.4000), second positions were rich in adenine and thymine (0.3572, 0.3148) and biased against guanine (0.1206), and third positions were rich in thymine (0.3109). Third positions of the cytochrome *b* gene were rich in cytosine (0.4029) and biased against guanine (0.0662), again as noted previously (Lydeard and Roe 1997). Stationarity was violated only for cytochrome *b* third positions, where *Nicholsina*, *Cryptotomus*, and the outgroup *Pseudodax* differed in base composition from other taxa (P = 0.0451, 0.0001, 0.0145, respectively). Nearly all substitution types accumulated linearly with sequence divergence; the exception was third position transitions in the cytochrome *b* gene.

Phylogeny of the Parrotfishes

The ILD test revealed no conflict among genetic data partitions, thus justifying the combination of genes to create a concatenated molecular dataset. However, the test revealed significant conflict between previous morphological (Bellwood 1994) and our molecular data (M. Alfaro, J. T. Streelman, S. A. Karl, and M. W. Westneat, unpubl. ms.). Here, we focus on the phylogenetic results from DNA sequence. A Tamura-Nei model of sequence evolution with rate heterogeneity and invariant sites best described the concatenated gene set. Heuristic searches under this model recovered a single ML tree in all 25 replicate random addition sequences (Fig. 2). Other methods (e.g., parsimony, distance) converged on this topology.

The most striking pattern that emerges from phylogenetic analysis of DNA is a clear break between two clades of parrotfishes. These major groups correspond to genera that are seagrass (*Cryptotomus*, *Nicholsina*, *Leptoscarus*, *Calotomus*, *Sparisoma*) or coral reef (*Bolbometopon*, *Cetoscarus*, *Hipposcarus*, *Chlorurus*, *Scarus*) associated. This split contradicts Bellwood's (1994) hypothesis of parrotfish relationships but matches Schultz's (1958) division of the Scaridae into two subfamilies, the Scarinae (reef) and Sparisomatinae (seagrass). Nearly 80% of species are in the reef clade, with 76% in the group *Chlorurus* + *Scarus*. Using a transversion rate of 0.14% per million years for the 12S and 16S genes (Bernardi et al. 2000), we calculate that reef and seagrass clades diverged approximately 42 million years ago.

Origins and Ecology

Phylogenetic analysis of geographic distribution (Fig. 2) indicates that parrotfishes originated in the eastern Tethys Sea (the precursor of the Indo-Pacific), similar to other tropical marine organisms (Briggs 1999). Surprisingly, the phylogenetic signature of ecology is stronger than that of biogeography. The presence of basal, Indo-Pacific genera in each clade likely reflects the separation of reef and seagrass types before closure of the Tethys; this supposition is bolstered by the estimated age of divergence. An Eocene division of reef and seagrass clades predates the first scarid fossil by 30 million years. These results imply that both reef and seagrass habitats have been important throughout parrotfish evolution. The reef-seagrass bifurcation points to a primary role of ecology in driving the initial divergence of scarids.

Differences in species richness among major clades might be related to different evolutionary histories of coral reefs and seagrasses since the late Eocene. The Oligocene to early Miocene was a time of seagrass expansion (Brasier 1975) and coral reef fragmentation (Veron 1995). Since the mid-Miocene (~14 million years ago), the distribution of corals and seagrasses has been shaped differentially by fluctuations in sea level, sea surface temperatures, and ocean currents (Briggs 1974; Longhurst 1998). It is tempting to speculate that the gap in species richness among clades is due in part to a greater sensitivity of corals to environmental perturbations (Brasier 1975; Potts 1983). Increased fragmentation of reef habitats may have contributed to higher rates of diversification for reef-dwelling parrotfish species.

There is convincing evidence, however, that differences between coral reef and seagrass habitats cannot explain the variance in species richness among reef and seagrass clades. Seagrass beds are often located directly adjacent to coral reefs and the two environments have been linked in successional scenarios (McCoy and Heck 1976). It is likely that reef taxa have used seagrasses as refuges during times of coral extinction and individuals of many reef species spend at least part of their lives (usually as juveniles) in seagrass (Overholtzer and Motta 1999). In addition, the long pelagic larval stage and expansive range of contemporary populations (e.g., Shulman and Bermingham 1995) suggests that dispersal would have been sufficient to overcome local habitat fragmentation and extinction. In short, there seems to have been no recognizable vicariance between the two habitats. Rather, it is important to ask why seagrass and reef taxa have experienced unique evolutionary histories despite such spatial proximity.

Cranial Anatomy and Feeding Mode

The two major clades have followed distinct evolutionary paths in terms of cranial anatomy (Fig. 3). Species of the seagrass clade, with the exception of some *Sparisoma*, exhibit few modifications from the condition found in most labrids (Bellwood 1994). The oral jaws retain discrete teeth in oblique rows with no evidence of external cementation. The angular and dentary bones, which comprise the lower jaw, as well as the adductor mandibulae (cheek muscles responsible for closing the jaw) exhibit the typical labrid form (simple A1, 2 and 3, small Aω; see Bellwood 1994, figs. 4, 5, 21–25). However, some *Sparisoma* species, especially *S. viride*, share a suite of features characteristic of the reef clade.

In the reef clade (and some *Sparisoma*), the teeth of the oral jaws form a mosaic of vertical and oblique rows with the outer face of the jaws coated with cement. Cement covers the base of the teeth in *Bolbometopon* + *Cetoscarus* but is present almost to the cutting edge in the *Hipposcarus* + *Chlo-*

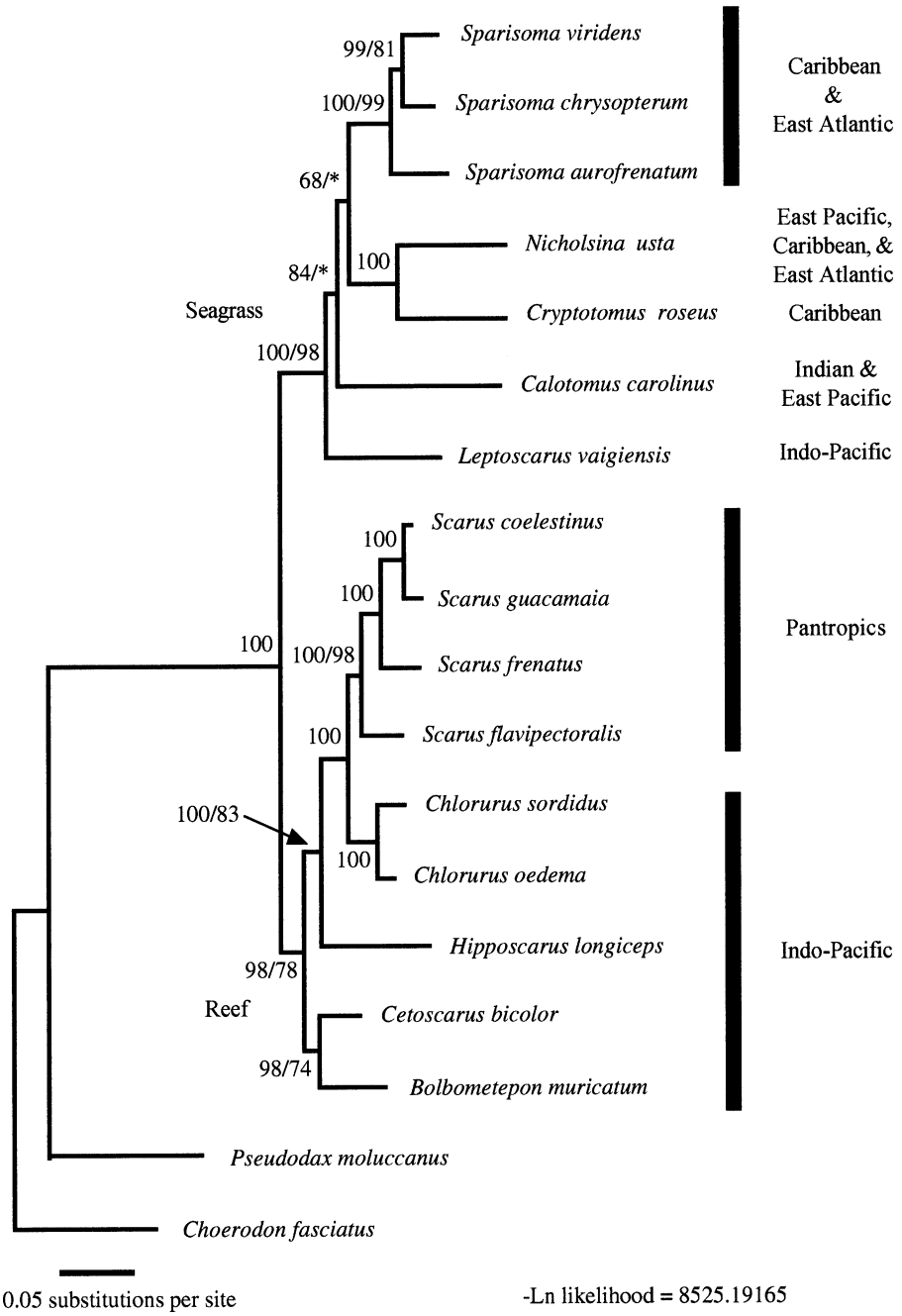


FIG. 2. Maximum-likelihood estimate of parrotfish phylogeny based on combined analysis of nuclear and mitochondrial genes. Numbers above nodes indicate support values from Bayesian analysis followed by bootstrap percentages from 1000 maximum-likelihood replicates. Bayesian support values indicate the posterior probability that the clade is correct (Larget and Simon 1999). An asterisk (*) indicates that the clade appeared in fewer than 50% of the bootstrap replicates, and a single value above a node indicates that bootstrap and Bayesian support values are identical.

rurus + *Scarus* clade (and *S. viride*). Members of this latter clade (but not *S. viride*) possess an unusual articulation between the dentary and angular. In reef species, the adductor mandibulae are likewise highly modified with the A3 inserting on the dentary in *Chlorurus* and *Bolbometopon* + *Cetoscarus*, whereas *Hipposcarus* and *Scarus* have a fused A1/A2. These modifications underscore the functional abilities of the taxa and their respective feeding strategies.

Browsers use the teeth of the oral jaws to remove epilithic algae, macroalgae, or seagrass from the substrate without scarring or scraping it (Bellwood 1994). Excavators remove pieces of the substratum while feeding; scrapers perform a nonexcavating bite restricted to the surface of the substratum. Excavators have robust jaws with simple and strong articulations among elements and relatively massive muscles able to generate a forceful bite with little kinesis. In contrast, scrapers have relatively mobile and complex jaw articulations with gracile musculature (Bellwood and Choat 1990; Bell-

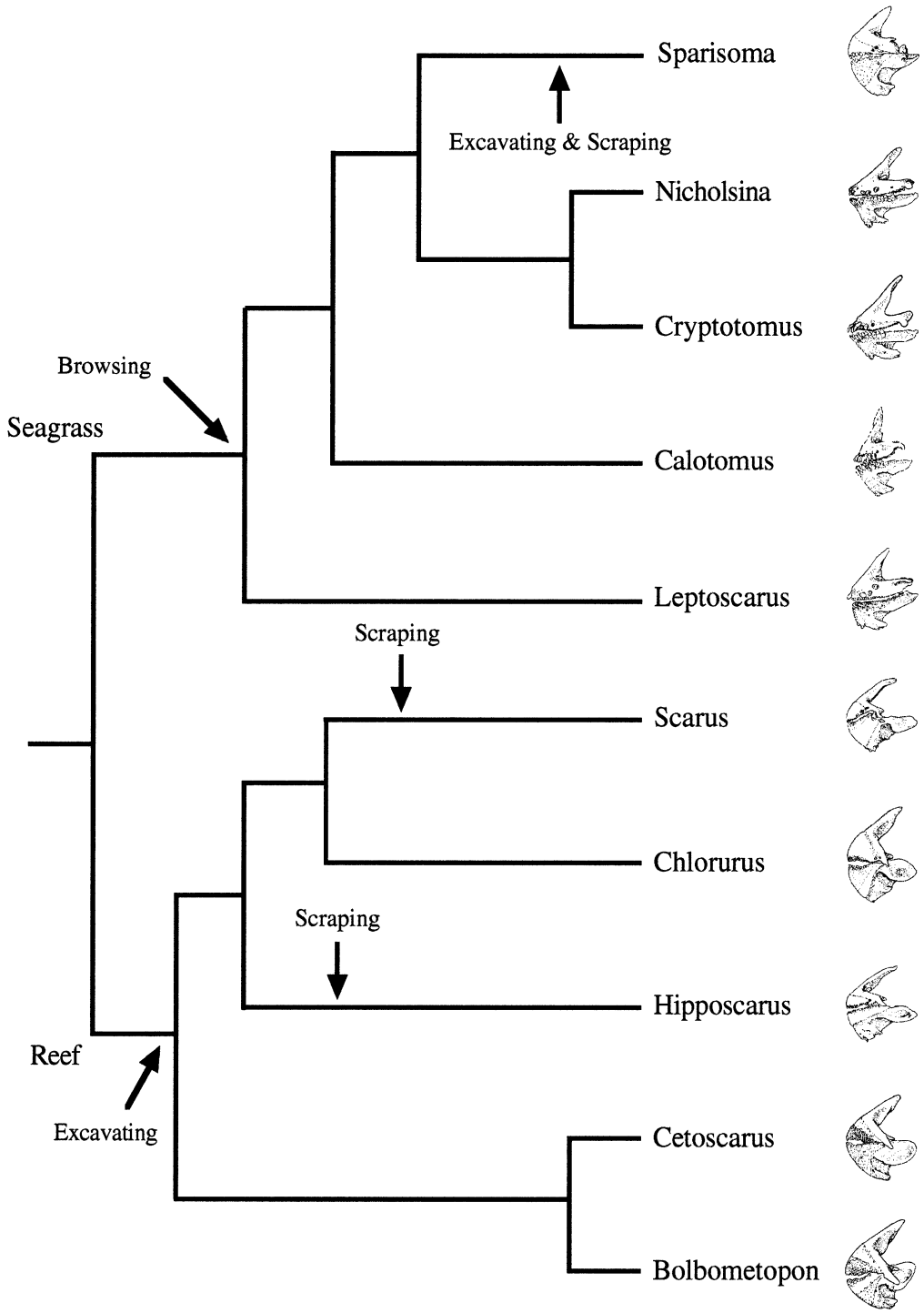


FIG. 3. Evolution of feeding mode among parrotfish genera. Shown at right are representative oral jaws (dentaries and premaxillae) for each genus (Bellwood 1994). Note that this is one of multiple equally parsimonious reconstructions of feeding mode evolution.

wood 1994). Excavators feed at low rates and prefer convex surfaces, whereas scrapers feed at high rates and show no obvious preference for habitat angle (Bellwood and Choat 1990; Bellwood 1995b; Alfaro and Westneat 1999).

Nearly all members of the seagrass clade are browsers (Table 2). The exception is the Atlantic/Caribbean genus *Sparisoma*, which consists of seagrass and reef associated species that exhibit browsing (e.g., *S. radians*), excavating (*S. viride*), and/or scraping (*S. aurofrenatum*) feeding modes (Bernardi et al. 2000). Genera in the reef clade are either excavators or scrapers (Bellwood and Choat 1990; Bellwood 1994). The ancestral scarid feeding mode is equivocal because we do not yet know the most closely related wrasse lineage. Although cranial anatomy has been characterized for

TABLE 2. General trends in the distribution of feeding behaviors and reproductive characteristics for parrotfish genera. See text for descriptions and references.

Genus	Feeding Mode	Sex reversal	Breeding territories	Harems	Males
Sparisoma	browsing, excavating, scraping	yes	yes	yes	functional diandry
Nicholsina	browsing	yes	no	no	monandry
Cryptotomus	browsing	yes	no	no	monandry
Calotomus	browsing	yes	no	no	diandry
Leptoscarus	browsing	no	no	no	monandry
Scarus	scraping	yes	yes	yes	diandry
Chlorurus	excavating	yes	yes	yes	diandry
Hipposcarus	scraping	yes	temporary	yes	diandry
Cetoscarus	excavating	yes	temporary	yes	diandry
Bolbometopon	excavating	yes	temporary	yes	diandry

many labrid taxa (Bellwood 1994; Gomon 1997), less is known about feeding mode, and this character is likely to be highly variable among groups. We infer that excavating and scraping have originated at least twice—in the reef clade and *Sparisoma*. Scraping may also have evolved independently within the reef clade (*Hipposcarus* and *Scarus*) or been lost in *Chlorurus*. The placement of *Sparisoma* within the seagrass clade challenges the hypothesis that it is an evolutionarily transitional taxon (Bernardi et al. 2000).

The observation that parrotfishes adopt various ways to feed in different habitats (Bellwood and Choat 1990) may explain the divergence of major clades. Differences in feeding performance among individuals may have forged strong habitat preferences that isolated reef from seagrass populations. Indeed, individuals from contemporary populations show preference for both habitat and food types (Bruggemann et al. 1994a; Overholtzer and Motta 1999) and compete for high-quality territories (Bruggemann et al. 1994b). This would suggest that parrotfishes are split into ecological clades in part because of associated differences in trophic morphology.

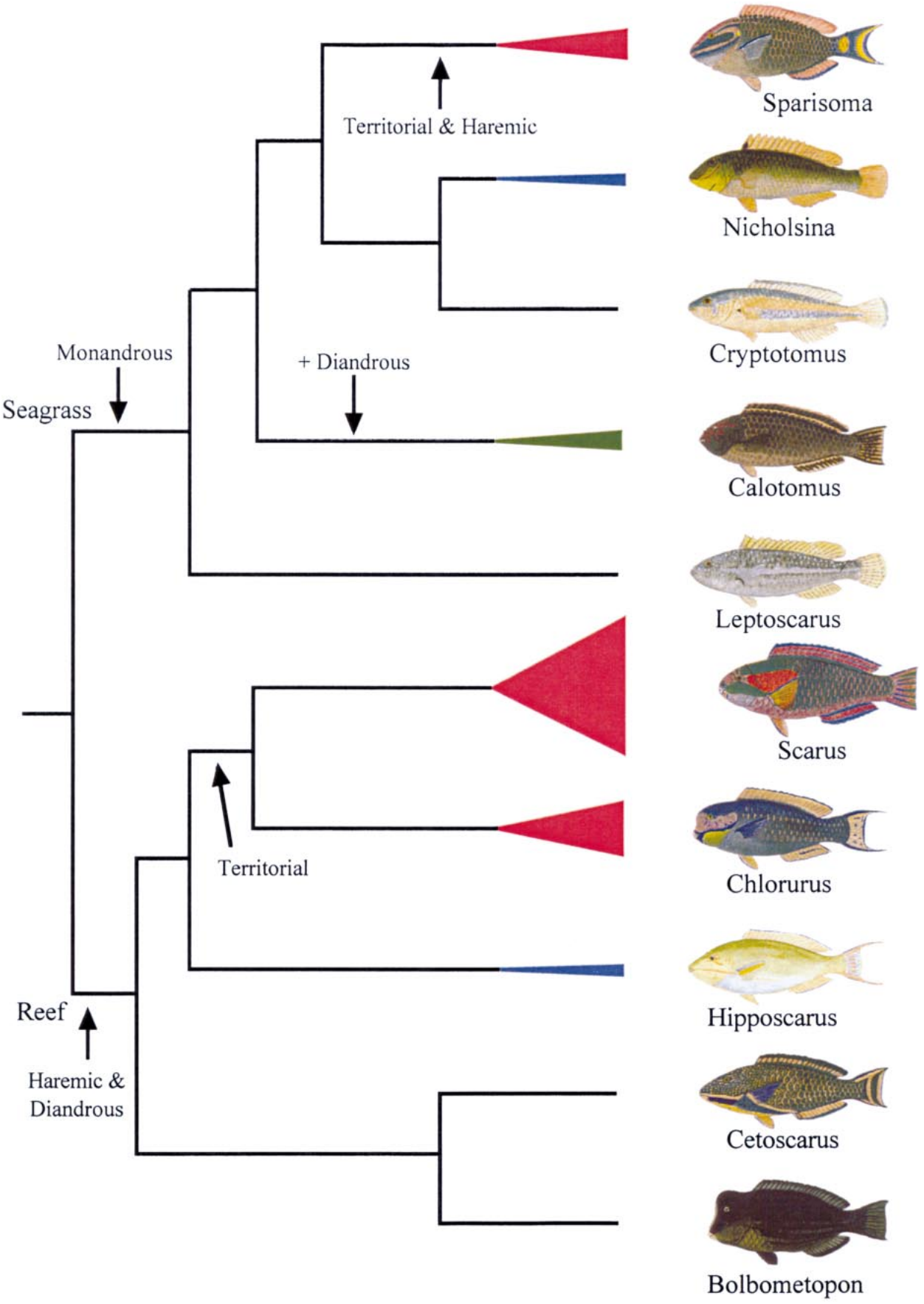
If seagrass and coral reef environments differed in the number of available habitats, we might expect collateral trends in comparative diversity among seagrass and reef clades. This has been a consistent explanation for trends in species diversity on tropical coral reefs versus other marine environments (Briggs 1974; Brasier 1975; Veron 1995). However, only two (*Scarus*, *Chlorurus*) of five genera in the reef clade have more than five species. This implies that any inherent properties of reef versus seagrass habitats or associated ecomorphologies will be unable, by themselves, to explain the observed diversity patterns. We suggest that ecomorphology has played a fundamental role in the initial divergence of parrotfishes but may be only tangentially involved in subsequent diversification.

Sexual Dimorphism, Sex Reversal, and Sexual Selection

Despite variability in mating and social behaviors within and between species (e.g., Choat and Robertson 1975; Robertson and Warner 1978; van Rooij et al. 996;a,b), there are interesting trends among genera and major clades (Fig. 4, Table 2). There are three ways to be a male parrotfish. First, individuals can be direct developing, primary males (true gonochores). Primary males can be of initial (usually drab, similar to females) or terminal phase (bright, colorful) coloration. Secondary males pass through a fully functional female stage; sex reversal can occur early or late in life, and such protogynous males can be of initial or (usually) terminal phase color pattern. Finally, some populations have prematurational males (secondary gonochores) of either phase whose testes develop from nonfunctional ovaries. Species with both primary and secondary males are called diandrous, those with only secondary males are monandrous.

All genera show sex reversal except *Leptoscarus* (Robertson et al. 1982). That *Leptoscarus* is the first genus to diverge from the seagrass clade raises the possibility that the ancestor of this group did not reverse sex; of course, it is also possible that sex reversal was lost along the branch to this taxon. Notably, all species examined in the seagrass clade are monandrous except *Calotomus spinidens*. Some species within the seagrass clade (e.g., *Sparisoma viride*, *S. chrysopterum*, *S. radians*) have prematurational males. In contrast, all taxa examined in the reef clade are diandrous, many with primary, secondary, and prematurational males.

The phylogenetic distribution of monandry and diandry among scarids may be related to the population structure and social systems that have evolved in seagrasses versus reefs. Many reefal populations exhibit (permanently or temporarily) territorial, terminal phase males that control females for haremic or leklike breeding (Robertson and Warner 1978; Colin and Bell 1991). Territoriality is largely lacking in the seagrass clade except, once again, in the genus *Sparisoma*, in which both reef and seagrass species can be territorial and haremic (Robertson and Warner 1978). Applying the theory of sexual selection to reef fishes, Warner et al. (1975) suggested that the presence of relatively few territorial, breeding males can elicit an increasingly large advantage in male mating success from small differences in size. This discrepancy in mating success between males and females, as well as a sex ratio skewed heavily in favor of females, may have set the stage for the evolution of precociously developing (i.e., primary) and early sex-changing (secondary) males that attempt to gain reproductive access by interference. Diandry would have come to be associated with the presence of territorial terminal phase males, found almost exclusively in the reef clade. The exception to this rule is particularly insightful. In the genus *Sparisoma*, certain species are both territorial and haremic. Here, prematurational males are analogous in breeding be-


Territorial & Haremic
Monandrous
+ Diandrous
Seagrass
Territorial
Reef
Haremic &
Diandrous
Sparisoma
Nicholsina
Cryptotomus
Calotomus
Leptoscarus
Scarus
Chlorurus
Hipposcarus
Cetoscarus
Bolbometopon

havior (i.e., interference spawners) to the primary males of the reef clade, creating a pattern of functional diandry (Robertson and Warner 1978). We infer two important points. First, parrotfish social and breeding behavior is strongly associated with habitat. Second, if changes in social and breeding behaviors have been accompanied, as suggested, by sexual selection, we might expect differences in the elaboration of sensory cues among reef and seagrass taxa.

Indeed, the greatest degree of variation in male breeding color is found among reef taxa. In general, reef species exhibit more brightly colored terminal phase males, greater variance in color usage and patterning, and greater dimorphism in size and color, than seagrass species. Interestingly, *Leptoscarus vagiensis*, the only gonochoristic parrotfish, is grayish brown and shows almost no dichromatism between males and females. There seems to be a correlation among scarid genera in the degree of sexual dimorphism in color, behavioral and social complexity, and species richness. Species in genera *Scarus* and *Chlorurus* represent the apex of this trend; they are diandrous, often strongly territorial, often haremic, and divergently sexually dimorphic. In this respect, they are more like certain species of the genus *Sparisoma* (e.g., *S. aurofrenatum*, *S. viride*, *S. radians*) than other genera of the reef clade. These congruent patterns suggest that sexual selection may have been important in the diversification of scarids, and that this diversification is conspicuously tied to social and breeding behavior in the coral reef habitat.

Scarid Evolution: Decoupling Divergence and Diversification

We have shown that parrotfishes are split into two major groups along an ecological axis. A phylogenetic interpretation of organismal variation suggests that ecomorphology has been important in the initial divergence of parrotfish taxa, but that other traits, including breeding behavior and sexually dimorphic coloration, are likely to have been involved in subsequent diversification (i.e., the multiplication of species). We offer the following scenario of parrotfish evolution. Sometime prior to the mid-Miocene, scarids split into reef and seagrass clades. It is difficult to know what factors precipitated this divergence, but it was likely accompanied by changes in the oral jaw apparatus reinforced by differences in feeding preference and performance in the two habitat types. Recent authors have commented on the importance of ecology in speciation (Orr and Smith 1998) and have argued that divergence can occur when ties between morphology and habitat are strong (Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999).

Among parrotfishes in the reef habitat, territoriality and the haremic mating system increased skew in both mating success (favoring terminal phase males) and sex ratio (favoring female). Variance in male mating success led to the evolution of subversive male breeding strategies (diandry) and provided the raw material for sexual selection. Sexual selection, in turn, drove the elaboration of male breeding coloration and the diversification of reef species along the secondary axis of color/communication (Higashi et al. 1999). This scenario suggests that populations of species-rich genera (*Scarus*, *Chlorurus*, and *Sparisoma*) will exhibit the greatest variance in male breeding success. It also implies that divergence and subsequent diversification have been decoupled in scarid evolution—diversification is a contingent event that may depend on the presence of genetic variation in traits (e.g., color) other than those selected during divergence (e.g., jaw morphology).

It is noteworthy that recent studies have shown bifurcating evolutionary networks, evidence for basal splits along axes of ecomorphology, for Lake Malawi cichlids (Moran et al. 1994), Caribbean anoles (Losos et al. 1998), and Darwin's finches (Sato et al. 1999), similar to the phyletic pattern we detect for scarids. We suggest that the decoupled view of divergence and diversification, used here to explain trends in species richness between major groups of parrotfishes, is also explicative of perplexities noted among other radiating clades. Radiation in stages helps to explain why cichlid groups in lakes are species rich but those in rivers are not, why postglacial fishes (e.g., cisgoes, arctic charr, whitefish, sticklebacks), Caribbean anoles, and Darwin's finches have diverged but not diversified. In all cases, initial splitting proceeded along axes of ecology (e.g., sand/rock, benthic/limnetic, ground/trunk/canopy, ground/tree), and the degree of subsequent diversification is produced by unique combinations of place, time, and organismal variation. For instance, lacustrine cichlids are generally more site specific, territorial and colorful than riverine taxa, implying that diversification in lakes proceeded along the axis of color/communication after initial divergence in ecomorphology (Albertson et al. 1999). Darwin's finches, however, have diverged along the axis of ecomorphology but diversification has stalled. The paucity of variation in finch color and the observation that song is neither heritable (Grant and Grant 1997) nor independent of beak morphology (Podos 2001) imply that sexual selection has been less strong in finches than other taxa.

The decoupling of initial divergence and subsequent diversification, the importance of natural selection to the former and sexual selection to the latter, are hypotheses that can and should be tested with more than phylogenetic data. Our interpretation suggests that the explosive accumulation of species in adaptively radiating clades is the result of selection along axes of color and/or communication and not ecomorphology. We have inherited a long tradition of studying adaptive radiations as special cases of core evolutionary phenomena. The trick lies in identifying not only what makes the group of radiating clades exemplary, but also what distinguishes them.

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FIG. 4. Evolution of male coloration and social and breeding behavior. At right is a representative terminal phase male for each genus (reproduced with permission from Lieske and Meyers 1999). Wedges are proportional to the number of species in each genus. Data summarized from Choat and Robertson (1975); Robertson and Warner (1978); Robertson et al. (1982); Colin and Bell (1991); Bellwood (1994); Gladstone (1996); Parenti and Randall (2000).

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