Marine Biology (2004) 144: 369–375
DOI 10.1007/s00227-003-1199-0

RESEARCH ARTICLE

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Evolution of coral reef fish *Thalassoma* spp. (Labridae). 1. Molecular phylogeny and biogeography

Received: 12 November 2002 / Accepted: 6 August 2003 / Published online: 1 October 2003

Abstract Wrasses in the genus *Thalassoma* comprise 27 recognized species that occur predominantly on coral reefs and subtropical rocky reefs worldwide. The phylogenetic relationships for 26 species were examined based on two mitochondrial genes (cytochrome *b* and 16S rRNA) and one nuclear intron (the first intron of the ribosomal protein S7). Two closely related species, the bird-wrasses (*Gomphosus varius* Lacepède, 1801 and *G. caerulaeus* Lacepède, 1801), were also included in the analysis. These species grouped within the genus *Thalassoma*. *Thalassoma newtoni* (Osório, 1891) from Sao Tome, which is generally synonymized with the Atlantic/Mediterranean *Thalassoma pavo* (Linnaeus, 1758) appears to be a valid species. Using a molecular clock, the genus was estimated to have originally diverged 8–13 million years ago, with *Thalassoma ballieui* (Vaillant and Sauvage, 1875) from Hawaii and *Thalassoma septemfasciata* Scott, 1959 from Western Australia as the ancestral species. Approximately 5–10 million years ago, a sudden burst of speciation resulted in seven clades, which were resolved with the sequence data. The terminal Tethyan event and the closing of the Isthmus of Panama were probably the major historical factors shaping the evolution of species in the genus *Thalassoma*. These data on the spatio-temporal pattern of speciation in the Indo-Pacific indicate that peripheral species have been generated at various times throughout the history of the genus, and that none of the widespread species are relatively young. Thus, there is no clear support for centrifugal (youngest at the periphery) versus centripetal (oldest at the periphery) modes of generation of species, two theories which have been used to account for geographic gradients in species diversity.

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00227-003-1199-0.

Communicated by J.P. Grassle, New Brunswick

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Introduction

Wrasses in the genus *Thalassoma* form a distinctive group (family Labridae, subfamily Julidini; Gomon 1997). They are abundant worldwide in circumtropical and subtropical coral and rocky substrates at shallow depths, generally to 25 m. Several species have been intensively studied from a variety of perspectives (e.g. Warner 1982; Kramer and Imbriano 1997; Barry and Hawryshyn 1999; Swearer et al. 1999), making comparative analyses especially interesting.

Of the 24 species discussed by Allen (1995), all have a generalized body shape typical of julidin wrasses. Although adult sizes range from 135 mm standard length (SL) for the smallest species (*Thalassoma noronhanum*) to >460 mm SL in the largest (*T. purpureum*), generating some allometric differences, the morphology of all the species is exceptionally homogeneous (Heiser 1981; Rocha et al. 2001). However, like other julidins, *Thalassoma* spp. vary widely in color pattern and hue, both inter- and intra-specifically. The ontogenetic and adult color patterns and hues (as well as locality data) are most useful in distinguishing species and

understanding their evolutionary relationships (Heiser 1981). Indeed, those few phylogenetic relationships among *Thalassoma* spp. that can be inferred from studies aimed chiefly at identifying sister species groups are based primarily on color pattern similarities (Randall and Edwards 1984; Allen and Robertson 1994; Randall 1994, 1995; Allen 1995; Randall et al. 1996).

A reliable phylogeny is a prerequisite for a critical analysis of such relationships. In other taxa, where morphological data (including color pattern and hue) shed little light on relationships, molecular approaches have proven useful (Avise 1994; Seehausen et al. 1999; Crochet et al. 2000; Knowlton 2000). Here, we generate a molecular phylogeny based on two mitochondrial genes (cytochrome *b* and 16S rRNA) and one nuclear intron (ribosomal protein S7) for 26 (out of 27) recognized taxa. In addition to the 26 *Thalassoma* species in this study, we included both species of bird-wrasse, genus *Gomphosus*. The juveniles of the bird-wrasse *Gomphosus varius* have been described as a distinct species of *Thalassoma* (*T. stuckiae*), due to their morphological similarities to many species of *Thalassoma* (Whitley 1959). Furthermore, courtship behavior is virtually identical in *Gomphosus* spp. and *Thalassoma* spp. (Heiser 1981).

Materials and methods

Tissue samples and DNA extraction

The sampling localities and geographic range of the sequenced specimens are provided in Table 1 and Fig. 1. The species *Halichoeres semicinctus* and *Coris julis*, which belong to the same wrasse subfamily (Julidinae) as the *Thalassoma* spp., were used as outgroups (Gomon 1997). Two individuals of each species were analyzed, except for *T. duperreyi* for which a single specimen was available. *Thalassoma heiseri* (endemic to the Pitcairn Island group) was the only *Thalassoma* species that was not included in our study. Liver or muscle tissue of adult fish was extracted immediately upon capture of the fish and preserved in 95% ethanol, then stored at 4°C in the laboratory. Tissues were digested overnight at 55°C in 500 µl of extraction buffer (NaCl 400 mM, Tris 10 mM, EDTA 2 mM, SDS 1%). We purified the DNA by standard chloroform extraction and isopropanol precipitation (Sambrook et al. 1989).

Polymerase chain reaction (PCR) amplification

Amplification of the mitochondrial cytochrome *b* (cyt*b*) and 16S ribosomal gene (16S) regions was accomplished using the primers GLUDG-L, CB3H and 16SAR, 16SBR, respectively, following Kocher et al. (1989). In the case of cyt*b*, after amplifying several individuals, a specific primer was designed to replace GLUDG-L, namely CYTTHAL-5′ AAC GGA GCA TCN TTC TTC TTT 3′. These primer sets amplify a 560-bp region of the cyt*b* and a 503-bp region of the16S gene. The nuclear marker used in this study was the first intron of the ribosomal protein S7 (S7), a single locus gene (Chow and Hazama 1998). This region was amplified using the universal fish primers of Chow and Hazama (1998). All amplifications (25 µl) contained 10–100 ng of DNA, 10 mM Tris HCL (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U of *Taq* DNA polymerase (Perkin-Elmer, Norwalk, Conn.), 150 mM of each dNTP, and 0.3 mM of each primer, and used a cycling profile of 45 s at 94°C, 45 s at 50°C, and 1 min at 72°C, for 35 cycles. Automated sequencing was performed in both directions with the primers used in the amplification using an ABI 3100 automated sequencer (Applied Biosystems, Foster City, Calif.).

Sequence analysis

We used the computer program Clustal V implemented by Sequence Navigator (Applied Biosystems) to align the mitochondrial and nuclear sequences. Phylogenetic relationships were assessed using the neighbor-joining (Kimura-2-distances) method (Nei 1987) and the maximum-parsimony method implemented by the software package PAUP (phylogenetic analyses using parsimony, version 4.0, Swofford 1998). Phylogenetic relationships were obtained for the three separate datasets (cyt*b*, 16S, S7), and each is presented in the Electronic Supplementary Material. Topological confidence was evaluated with 1000 bootstrap replicates (Felsenstein 1985). In both neighbor-joining and maximum-parsimony methods, bootstrapping analysis was performed with equal weighting of transitions and transversions. Alternative tree topologies were tested using the Kishino and Hasegawa (1989) method and the topology-dependent tail permutation test (Faith 1991). Consistency among the three markers was determined using permutation tests. All statistical tests were performed using the PAUP package. Testing for the actual presence of polytomies (as opposed to data artifacts) was done using the method of Walsh et al. (1999).

Results

Sequences and molecular phylogeny

Out of the 1839 aligned base pairs (560 for the cyt*b* region, 503 for the 16S region, 776 for the S7 region), 232 could not be aligned unambiguously and were therefore removed from the analysis (GenBank accession numbers: cyt*b*: AY328856–AY328885; 16S: AY328983–AY329012; S7: AY329640–AY329669). Of the remaining 1607 bp, 881 were variable, and 598 were phylogenetically informative. The two individuals sequenced for each species were identical, except for *Thalassoma purpureum* for which the Easter Island and Rangiroa individuals differed by a single substitution. One previous molecular study based on cyt*b* sequences focused on the relationships of three *Thalassoma* species (Mikami and Machida 1999), but because there were only 30 bp in common between our study and theirs, the sequences could not be directly compared. In addition, the *Thalassoma* spp. sequences presented by Mikami and Machida appeared to be contaminated (possibly with human DNA following a GenBank BLAST search).

Phylogenies obtained with the mitochondrial cyt*b*, 16S, and the S7 gene were almost identical. Data from the three markers were found to be statistically indistinguishable (permutation tests), and the topologies obtained independently for each marker were found not to be statistically different either by a topology-dependent tail permutation test (T-PTP) (see Electronic Supplementary Material). We therefore combined the three datasets (1607 bp). Seven most-parsimonious trees were obtained with the maximum-parsimony method, one of them being identical to the neighbor-joining tree.

Table 1 Species names, common names (most widely used, descriptive), distribution, and collection localities of *Thalassoma* spp. and outgroup species used in the present study. Specimens were collected by: G.R. Allen (*GA*), E. Azzurro (*EA*), G. Bernardi (*GBe*), G. Bucciarelli (*GBu*), J.B. Heiser (*JH*), N.L. Crane (*NC*), S. Planes (*SP*), and D.R. Robertson (*DRR*)

Species (author)	Common name	Distribution	Sampling locality	Date of collection
Ingroup				
T. amblycephalum (Bleeker, 1856)	Twotone wrasse	Indo-Central Pacific	Moorea, Fr. Polynesia (GBe)	Sep 2000
T. ascensionis (Quoy & Gaimard, 1834)	Ascension wrasse	Ascension Island	Ascencion Island (DRR)	Jun 1997
T. ballieui (Vaillant & Sauvage, 1875)	Blacktail wrasse	Hawaii	Hawaii (DRR)	Sep 1998
T. bifasciatum (Bloch, 1791)	Bluehead wrasse	Caribbean	San Blas, Panama (DRR)	Oct 1996
T. cupido (Temminck & Schlegel, 1845)	Nishikibera	Japan to Taiwan	Akajima, Japan (DRR)	May 1997
T. duperreyi (Quoy & Gaimard, 1824)	Saddle wrasse	Hawaii	aquarium trade (GBe)	Apr 2003
T. genivittatum (Valenciennes, 1839)	Mascarene wrasse	South-East Africa	Reunion Island (DRR)	Mar 1996
T. grammaticum Gilbert, 1890	Green sunset wrasse	Tropical eastern Pacific	Clipperton Island (DRR)	Jun 1998
T. hardwicke (Bennett, 1830)	Sixbar wrasse	Indo-Central Pacific	Moorea, Fr. Polynesia (GBe)	Sep 2000
T. hebraicum (Lacepède, 1801)	Goldbar wrasse	East Africa	Zanzibar, Tanzania (NC)	Aug 1999
T. heiseri Randall & Edwards, 1984	Heiser's wrasse	Pitcairn Islands group	Not sampled	
T. jansenii (Bleeker, 1856)	Jansen's wrasse	Central Indian Ocean to W. Pacific	Lizard Island, Australia (DRR)	Sep 1997
T. loxum Randall & Mee, 1994	Slantband wrasse	Oman	Masirah Island, Oman (SP)	Dec 1998
T. lucasanum (Gill, 1862)	Cortez rainbow wrasse	Tropical eastern Pacific	Guaymas, Sea of Cortez (GBe)	Jul 1998
T. lunare (Linnaeus, 1758)	Moon wrasse	Indo-Central Pacific	Rangiroa, Fr. Polynesia (GBe)	Sep 2000
T. lutescens (Lay & Bennett, 1839)	Sunset wrasse	Indo-Central Pacific	Moorea, Fr. Polynesia (GBe); Easter I. (DRR)	Sep 2000, Jun 1997
T. newtoni (Osório, 1891)	Newton's wrasse	West Africa	Sao Tome (DRR)	Apr 1996
T. noronhanum (Boulenger, 1890)	Noronha wrasse	F. de Noronha, Brazil	Fernando de Noronha (DRR)	May 1994
T. pavo (Linnaeus, 1758)	Turkish wrasse	Mediterranean, E. Atlantic	Ustica, Italy (EA)	Feb 1999
T. purpureum (Forsskål, 1775)	Surge wrasse	Indo-Pacific	Rangiroa, Fr. Polynesia (NC); Easter I. (DRR)	Jun 1997
T. quinquevittatum (Lay & Bennett, 1839)	Five-stripe wrasse	Indo-Central Pacific	Moorea, Fr. Polynesia (GBe)	Sep 2000
T. robertsoni Allen, 1995	Clipperton wrasse	Clipperton Island	Clipperton Island (DRR)	Jun 1998
T. rueppellii (Klunzinger, 1871)	Rueppell's wrasse	Red Sea	Aquarium trade (JH)	Jan 2000
T. sanctaehelenae (Valenciennes, 1839)	Saint Helena wrasse	Saint Helena Island	Saint Helena Island (DRR)	Jun 1997
T. septemfasciata Scott, 1959	Seven banded wrasse	Western Australia	Perth, W. Australia (GA)	Jul 1999
T. trilobatum (Lacepède, 1801)	Christmas wrasse	Indo-Central Pacific	Moorea, Fr. Polynesia (NC)	Sep 2000
T. virens Gilbert 1890	Green wrasse	Revillagigedo, Clipperton	Clipperton Island (DRR)	Jun 1998
Gomphosus varius Lacepède, 1801	Pacific Bird wrasse	Pacific Ocean	Moorea, French Polynesia (GBe)	Sep 2000
Gomphosus caerulaeus Lacepède, 1801	Indian Bird wrasse	Indian Ocean	Zanzibar, Tanzania (GBe)	Aug 1999
Outgroup				
Coris julis (Linnaeus, 1758)	Rainbow wrasse	Mediterranean, E. Atlantic	Naples, Italy (GBu)	Feb 1998
Halichoeres semicinctus (Ayres, 1859)	Rock wrasse	California, Sea of Cortez	Baja California (GBe)	Jun 1997

As expected, differences among the seven trees were all located in regions that were weakly supported (see details below). The seven most-parsimonious trees were 2252 steps long (consistency index = 0.586). One of these most-parsimonious trees and the bootstrap supports for both maximum-parsimony and neighbor-joining methods are shown (Fig. 2).

Phylogenetic relationships

DNA sequences resolved our samples into eight well-supported clades. *Thalassoma ballieui* and *T. septemfasciata* formed a strongly supported clade (clade 1, Fig. 2), which was the sister clade to all other studied taxa. The monophyly of the remaining *Thalassoma*/*Gomphosus* species was also strongly supported (Fig. 2). Within this group, samples could be divided into seven major clades that were strongly supported, although their interrelationships were not. Those seven clades grouped the following species: clade 2 included the two bird-wrasse species *Gomphosus varius* and *G. caerulaeus*. Clade 3 was fully resolved and well supported (Fig. 2). It included several smaller subclades: *T. amblycephalum*, *T. robertsoni*, and *T. lucasanum* formed one subclade; *T. cupido*, *T. loxum*, and *T. trilobatum* formed another subclade, which grouped with the *T. purpureum*/*T. virens* subclade. *T. lunare* was also included in clade 3 and was ancestral to all the species named above. Clade 4 included *T. duperreyi*, *T. genivittatum*, *T. lutescens*, and *T. rueppellii*, which formed a subclade. *T. hebraicum* was included in clade 4 in an ancestral position. Clade 5 included the two widespread species, *T. hardwicki* and *T. jansenii*. Clade 6 included only the five-stripe wrasse, *T. quinquevittatum*. Clade 7 included the western Atlantic species *T. bifasciatum* and *T. noronhanum*.

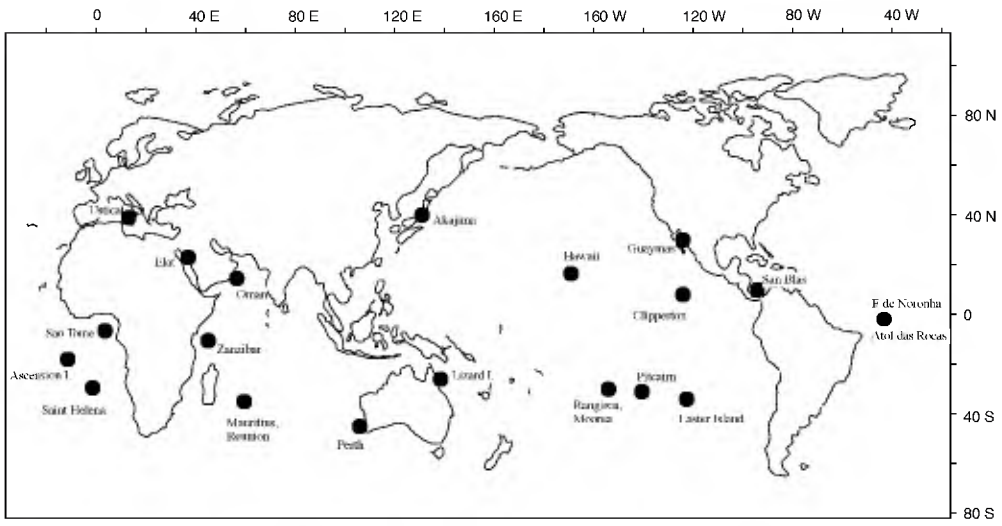

Fig. 1 Map of sampling locations mentioned in Table 1

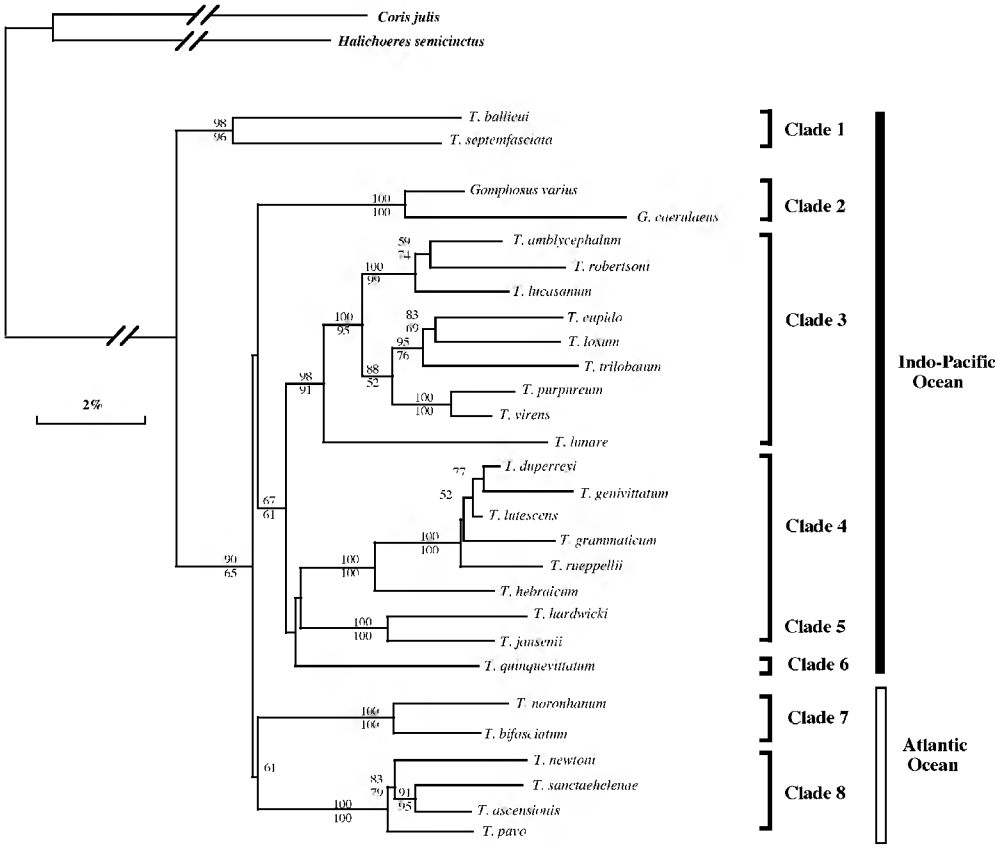

Fig. 2 Molecular phylogeny of *Thalassoma*/*Gomphosus* species based on mitochondrial (cyt*b* and 16S) and nuclear (S7) markers. One most-parsimonious tree is shown with bootstrap support (> 50%) indicated *on nodes*, maximum-parsimony *below the nodes*, neighbor-joining *above the nodes*. *Coris julis* and *Halichoeres semicinctus* were used as outgroups

Clade 8 included the mid-Atlantic to eastern Atlantic group *T. ascensionis*, *T. sanctaehelenae*, *T. newtoni*, and *T. pavo*. Clades 7 and 8 from the Atlantic were grouped together, but this grouping was weakly supported (61% of neighbor-joining bootstraps, < 50% for maximum parsimony).

Relationships among the seven clades were weakly supported as they corresponded to very short internal branches (thus generating more than one most-parsimonious tree). These polytomies did not result from saturation effects (plots transitions/transversions vs. divergence, not shown), and statistical tests showed that these polytomies were not due to data artifacts (Walsh et al. 1999). Polytomies were more likely to correspond to a group of divergence events occurring within a brief period of time, whose ordering was not resolved.

As shown in Fig. 2, the bird-wrasse genus *Gomphosus* nests within the genus *Thalassoma*. To test for the monophyletic status of the genus *Thalassoma*, we constrained our tree to place the genus *Gomphosus* outside the genus *Thalassoma* and re-ran the analysis to produce the shortest tree consistent with that topology. This new

topology was 98 steps longer than the most-parsimonious topology. Both Kishino–Hasegawa and T-PTP tests showed that these two topologies were significantly different, and that our data rejected ($P < 0.0001$) placing the genus *Gomphosus* outside *Thalassoma*.

Molecular clocks and divergence times

In order to estimate *Thalassoma* spp. divergence times, we restricted our analysis to the cyt*b* marker, as it is the most widely used for fish divergence time estimates (Meyer 1993; McCune and Lovejoy 1998). Considering the generally used molecular clock of 1.5–2.5% sequence divergence per million years, we found that the first splitting event in the genus, i.e. the divergence between the *T. ballieui*/*T. septemfasciata* clade and the remaining *Thalassoma* spp. occurred ~8 to 13 million years ago (Mya). The burst of divergence that produced polytomies between the seven clades described above is estimated to have occurred ~5 to 10 Mya.

Discussion

As mentioned in the "Introduction", several coral reef fish groups, including parrotfishes and wrasses, are very difficult to identify using morphological characters alone. Colors are heavily relied upon for proper identification (Bernardi et al. 2002). The genus *Thalassoma* is probably the most striking example, with most species sharing overlapping meristic and morphometric characters (Heiser 1981). Occasionally, authors have suggested possible relationships between a subset of species, again primarily based on coloration patterns. Here, we review those suggestions in light of our findings.

Randall and Edwards (1984) considered a closely related group of six Indo-Pacific species exhibiting similar color patterns: *Thalassoma cupido*, *T. rueppellii* (then called *T. klunzingeri*), *T. purpureum*, *T. trilobatum*, *T. quinquevittatum*, and *T. heiseri*. They considered *T. purpureum* and *T. trilobatum* to be closely related. Similarly they considered *T. heiseri* and *T. cupido* to be closely related. Our phylogeny is mostly concordant with these findings, with *T. cupido*, *T. trilobatum*, and *T. purpureum* being in the same clade. Interestingly, initial phases of *T. trilobatum* and *T. purpureum* are almost indistinguishable, yet they are not closest relatives. In contrast with that study, we did not find *T. rueppelli* in the same clade as the previous taxa, and our data could not group *T. quinquevittatum* with any other species with great confidence. Allen and Robertson (1994) placed *T. virens* close to *T. purpureum* (confirmed by Randall1995), and *T. grammaticum* close to *T. lutescens*. Both placements are in agreement with our findings. Allen (1995) considered *T. robertsoni*, *T. lucasanum*, and *T. amblycephalum* to be closely related, which is also what we found. Randall (1994) suggested that *T. loxum* was close to *T. cupido*, and this is also well supported by the molecular phylogeny. Overall there is a remarkable match between the predictions based on coloration patterns and the molecular phylogeny presented here.

Classification work involves a comprehensive approach based on morphological, behavioral, biogeographical, and genetic methods. Therefore, we are not proposing new interpretations here to the systematics of the genus *Thalassoma*. Our data, however, shed light on some relationships that warrant further work.

1. The species collected in Sao Tome is usually referred to as *Thalassoma pavo* (Gomon and Forsyth 1990; Seret 1990). This species, however, was originally described as *Thalassoma newtoni* (Osório 1891). Our data show *T. pavo* and *T. newtoni* to be genetically as distant as several other pairs of species, and, in addition, *T. newtoni* is closer to the mid-Atlantic island endemic species (*T. sanctaehelenae* and *T. ascensionis*) than it is to *T. pavo* (Fig. 2). Further work on this subclade (*T. pavo*, *T. newtoni*, *T. ascensionis*, and *T. sanctaehelenae*) is presented in an accompanying paper (Costagliola et al. 2003).
2. The two bird-wrasse species in the genus *Gomphosus* appear to be included in the genus *Thalassoma*. Bird-wrasses are fishes with very elongated snouts. Their unique adaptation sets them apart from the remaining *Thalassoma* spp., yet, as larvae and early juveniles, *Gomphosus* spp. do not have an elongated snout and can be very difficult to distinguish from *Thalassoma* spp. *Gomphosus* spp. should therefore be included in the genus *Thalassoma* as a specialized morphological variant.
3. One *Thalassoma* sp. individual was obtained from a pet store without geographic origin information (not included in this study). Its coloration did not match any described coloration for a *Thalassoma* species, and its sequence did not match any of the sequences described here. The individual looked most like *T. duperreyi* and *T. lutescens*. Its sequence was similar, but not identical to the sequence of *T. duperreyi*, ruling out the possibility of being a hybrid. Thus, it may correspond to an undescribed species.

Hybridization in fishes has been seen as a possible indicator of genetic similarity (Craig et al. 2000), and some *Thalassoma* spp. are known to hybridize. In the Red Sea, *T. rueppellii* was shown to hybridize with *T. lunare* (Randall and Miroz 2001). In the Hawaiian archipelago, *T. duperreyi* and *T. lutescens* are also known to hybridize frequently (Hoover 1993; Witte and Mahaney 2001; P. Lobel, personal communication). Finally, a hybrid initial phase *Thalassoma*/*Gomphosus* specimen was collected by G.R. Allen in September 1994 at Cassini Island, Western Australia. The *Thalassoma* sp. parent of the hybrid was later assigned to *T. lunare* by J.E. Randall (G.R. Allen, personal communication).

The molecular phylogeny presented here suggests that *T. duperreyi* and *T. lutescens* are genetically closely

related, and that *T. lunare*/*T. rueppellii*, and *T. lunare*/*Gomphosus* spp. are related but are not closest relatives. Thus, while hybridizations indicate that some parental species can interbreed, supporting the conclusion that the genus *Gomphosus* should be included in the genus *Thalassoma*, hybridization may not indicate that hybridizing species are always closest relatives.

Species in the genus *Thalassoma* provide an ideal system to study biogeographic patterns as they are distributed over a vast geographic range. The molecular phylogeny presented here raises some interesting questions that can be tested. Our data indicate that the genus *Thalassoma* originated approximately 8–13 Mya. It has been postulated that several groups of coral reef fishes evolved during the Terminal Tethyan Event (approximately 15 Mya) (Bellwood and Wainwright 2002). The genus *Thalassoma* may be an example of such an event. Furthermore, our data also indicate that 5–10 Mya, a speciation explosion occurred in the genus *Thalassoma*. This was accompanied by a large variety of coloration patterns, behavior differences, and vast geographic expansion. The separation of Indo-Pacific from Atlantic clades occurred after the first major divergence event (clade 1 divergence), but at about the same time as the major divergence of the seven more derived clades. Furthermore, within the Atlantic, western (e.g. *T. bifasciatum*) and eastern (e.g. *T. pavo*) clades seem to have separated at approximately the same time as the Atlantic and Indo-Pacific split. This may suggest that a common factor was involved in the two differentiating events. The rise of the Isthmus of Panama, which occurred approximately 3.5 Mya, has been proposed as the major historical event responsible for the separation of Atlantic and Indo-Pacific faunas. The eastern Pacific *T. lucasanum* was originally thought to have been separated by the rise of the Isthmus of Panama from its Caribbean geminate species *T. bifasciatum* (e.g. Bermingham et al. 1997), although Heiser (1981) pointed out that *T. amblycephalumi* and *T. lucasanum* were closer to each other than either was to *T. bifasciatum*. Our data show that eastern Pacific groups such as *T. lucasanum*, *T. robertsoni*, or *T. grammaticum* are not closely related to Atlantic taxa. In contrast, *T. lucasanum* and *T. robertsoni* were found to be more closely related to the Indo-Pacific *T. amblycephalum*.

Thalassoma spp. may be widespread (e.g. *T. lunare*), have a restricted range (e.g. *T. duperreyi*), found at the periphery of the Indo-Pacific, or are more abundant in its central regions. We found that Indo-Pacific peripheral species (*T. ballieui*, *T. septemfasciata*, *T. robertsoni*, *T. lucasanum*, *T. cupido*, *T. loxum*, *T. virens*, *T. duperreyi*, *T. genivitattum*, *T. grammaticum*, and *T. rueppelli*) were derived from splits occurring across a range of times from ancient to quite recent. Thus, our data did not provide evidence that peripheral species are recent additions to ancestral widespread or central species (Jokiel and Martinelli 1992; Briggs 1999a, 1999b, 2003). The production of such peripheral species has occurred in at least four Indo-Pacific clades, with existing widespread species. Interestingly, the splits involving currently widespread species, *T. amblycephalum*, *T. trilobatum*, *T. purpureum*, *T. lunare*, on one hand, and *T. lutescens*, *T. hardwicki*, *T. jansenii*, *T. quinquevittatum*, on the other hand, are all fairly ancient. Widespread species are ancestral in some (e.g. *T. trilobatum* and *T. lunare*), but not all cases (e.g. *T. lutescens*). Taken together, these patterns suggest a mixture of centripetal (youngest in center) and centrifugal (youngest at edges) generation of species in the Indo-Pacific, with no recent generation of widespread species. A unique situation is found in Hawaii, where the two endemic species, *T. ballieui* and *T. duperreyi*, are found early and late in the evolution of the genus.

The use of more molecular markers may provide the appropriate tools to determine the precise succession of events that led to the unique radiation of *Thalassoma* spp.

Notes added in proof While this manuscript was in press, two studies came to our attention: 1. A phylogeny of the closely related genus *Halichoeres* was done by P. Barber and D. Bellwood. This study determined that the Caribbean species *Halichoeres maculipinna* was more closely related to *Thalassoma* spp. that to order *Halichoeres* species. When including *H. maculipinna* to our dataset, we found that it is close to *Thalassoma* but not within the genus. It is however, an ideal outgroup to be used. When *H. maculipinna* was used as an outgroup, our results were unchanged (we would like to acknowledge P. Barber and D. Bellwood for permission to use their data before publication). 2. A new species of *Thalassoma nigrofasciatum*, a new species of labrid fish from the south-west Pacific, Aqia 7(1), (1–8). This species is closely related to *T. jansenii* and is found in the Great Barrier Reef and adjacent areas. As such, our *T. jansenii* sample should be labeled *T. nigrofasciatum*. We sequenced the 12S rRNA, 16S rRNA and the cytochrome b regions for 2 individuals of bona fide *T. jansenii* (collected in June 1998 by DRR in Ishigaki, Japan). The two individuals had identical sequences. Their 16S sequence was identical to the Australian *T. nigrofasciatum* and differed by one nucleotide at the cytochrome b locus. We conclude that *T. nigrofasciatum* and *T. jansenii* are either very closely related species with no detectable genetic divergence at these two loci, or that *T. nigrofasciatum* is a color variant of *T. jansenii*.

Acknowledgements We would like to thank the people who provided samples and/or comments for this study: G. Allen, E. Azzurro, N. Crane, A. Edwards, and S. Planes. This research was partly supported by faculty research funds granted by the University of California Santa Cruz to G.B.

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