

Congruence between mitochondrial genes and color morphs in a coral reef fish: population variability in the Indo-Pacific damselfish *Chrysiptera rex* (Snyder, 1909)

J. A. Drew · G. R. Allen · M. V. Erdmann

Received: 23 September 2009 / Accepted: 5 January 2010 / Published online: 28 January 2010
© Springer-Verlag 2010

Abstract The Pomacentrid fish *Chrysiptera rex* (Snyder 1909) is a small conspicuous member of Indo-Pacific coral reefs. Despite having planktonic larvae, which would seem to facilitate genetic and morphological homogeneity, it possesses three distinct color variations, which are geographically restricted. To investigate the presence of possible incipient speciation, samples were taken from three geographically distinct areas including the South China Sea, the Philippines and Indonesia. Phylogenetic analysis of these morphotypes resulted in congruence between color and genetic data sets, with separation by color type. Each of the color variants possessed a unique genetic signal at two mitochondrial loci, but the color variants were invariant across a nuclear gene. This study highlights the importance of range wide sampling when characterizing a species and argues that multiple lines of evidence should be used when evaluating the taxonomic and conservation status of coral reef organisms.

Keywords Color variation · Phylogeography · Collections based research · Taxonomy

Communicated by Biology Editor Dr. Ruth Gates

J. A. Drew (✉)
Biodiversity Synthesis Center, The Field Museum,
1400 South Lake Shore Ave, Chicago, IL 60605, USA
e-mail: jdrew@fieldmuseum.org

G. R. Allen
Department of Aquatic Zoology, Western Australian Museum,
Locked Bag 49, Welshpool DC, Perth, WA 6986, Australia

M. V. Erdmann
Conservation International, Indonesia Program, Densipar Bali,
Indonesia

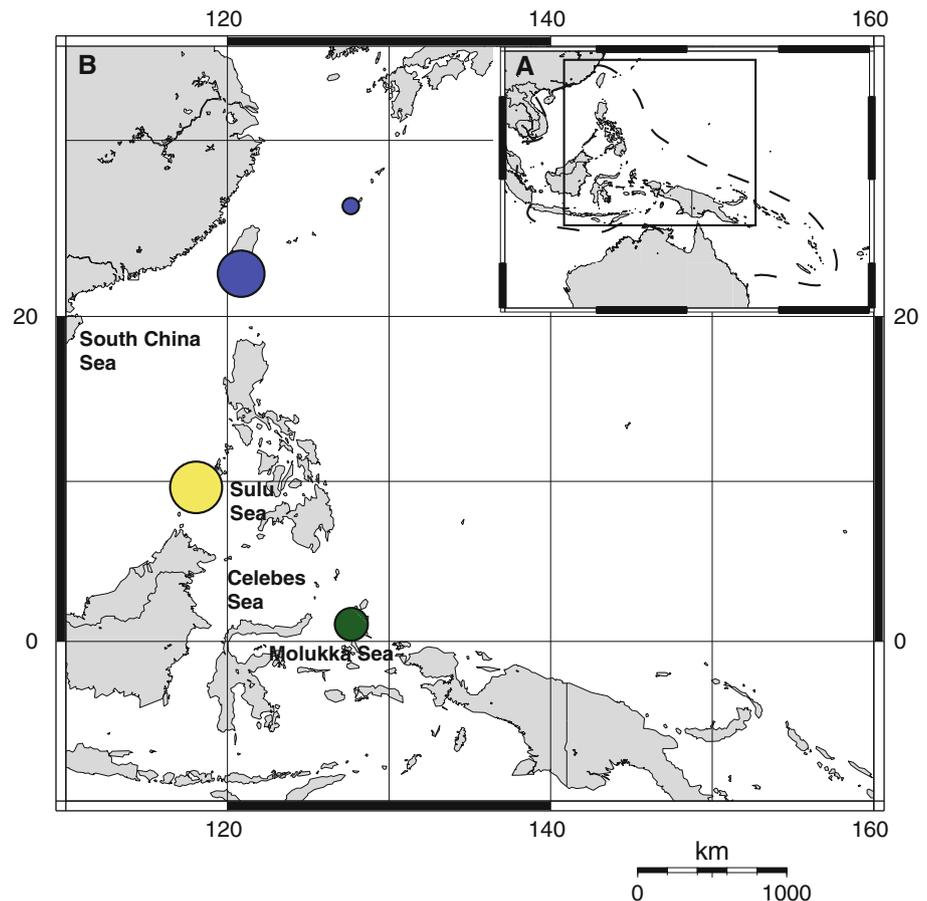
Introduction

The delineation of species is of fundamental importance to the identification and conservation of biodiversity (Vecchione and Collette 1996), and species are often used as a common denominator in comparing and prioritizing conservation areas (Roberts et al. 2002; Allen 2007). However, the question of what species are, and what characters should be used to define species boundaries has vexed biologists for generations (Avice and Wollenberg 1997; Ghiselin 2002; Wiley 2002; Coyne and Orr 2004).

Many coral reef fish possess vibrant colors; it is not surprising that coloration has often been used as a first pass at species identification. This has proven problematic for many coral reef groups that possess highly variable color phases (e.g., Scaridae, Labridae), and there are numerous examples where males and females of the same species have each been assigned different specific names (Herre 1935; Parenti and Randall 2000). However, for species not known to have sex-associated color changes, coloration has frequently been used to delineate species boundaries (Ready et al. 2006; Puebla et al. 2007; Drew et al. 2008).

Allopatrically distributed populations that possess unique color morphs have been useful models for research into the rate and processes underlying speciation. Small isolated populations may experience rapid mutation eventually becoming nascent species via the combined effects of founder speciation (Mayr 1947) reinforced by assortative mating (Domeier 1994) and selective pressures (Noonan and Comeault 2009). Furthermore in some species, peripheral populations can experience evolutionary trajectories independent from populations found within the core of a species range (Barraclough and Volger 2000; Drew and Barber 2009).

Fig. 1 **a** Range of *Chrysiptera rex* as outlined by dashed line. **b** Sampling locations. Size of circle is proportional to sample size. Colors of circles refer to colors of fish as indicated in Fig. 2



To determine whether color morphs correspond to distinct clades, we examined geographically isolated populations of the damselfish *Chrysiptera rex* (Pomacentridae, Snyder 1909). This species is common throughout its range, usually found in mild surge on semi-exposed reefs at depths of about 2–5 m. It occurs solitarily or in small groups of loosely scattered individuals (Fig. 1a). Their diet consists primarily of current-borne zooplankton. Typical of other Pomacentrids, it guards a nest of eggs in rocky crevices and after a pelagic larval duration of 15–20 days (Bay et al. 2006) often recruits in large masses (Sale et al. 1984). There are no known sex biases in dispersal and the fish is a benthic spawner. *Chrysiptera rex* is notable for the extreme color variations expressed by several geographically distinct populations (Fig. 2).

Materials and methods

Samples were collected from Taiwan, Halmahera Indonesia, El Nido and Anilao, Philippines and the Ryukyu Islands Japan—the type locality for this species (Fig. 1b). Genomic DNA was extracted using either a PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN) or

with Chelex (Walsh et al. 1991). We amplified the rapidly evolving mitochondrial control region using the primers CrA & CrE (Lee et al. 1995) and cytochrome b using the primers CytB-5 and CytB-H (Quenouille et al. 2004). Additionally, we amplified a segment of the nuclear RAG2 gene using the primers RAG2F1 and RAG2R2 (Westneat and Alfaro 2005) to assess genetic diversity at an independent locus. Thermocycling parameters were as follows: control region, denaturing at 94°C for 5 min followed by 40 cycles of denaturing at 94°C (30 s), annealing at 50°C (30 s), extension 72°C (40 s) and a final extension at 72°C for 3 min. For CytB, we used identical parameters to the control region but used a touchdown reaction for annealing where the annealing temperature was dropped 0.4°C per cycle over 20 cycles, from an initial temp of 58°C to 45°C, followed by 15 additional cycles at 45°C. For RAG2, we followed the control region protocol except we used an annealing temperature of 55°C.

PCR products were visualized following electrophoresis on 1% agarose gel and enzymatically cleaned for sequencing by digestion in 5 U of exonuclease and 0.5 U of shrimp alkaline phosphatase for 30 min at 37°C followed by 15 min at 80°C. Direct sequencing of double-stranded PCR products was performed using Big Dye 3.1

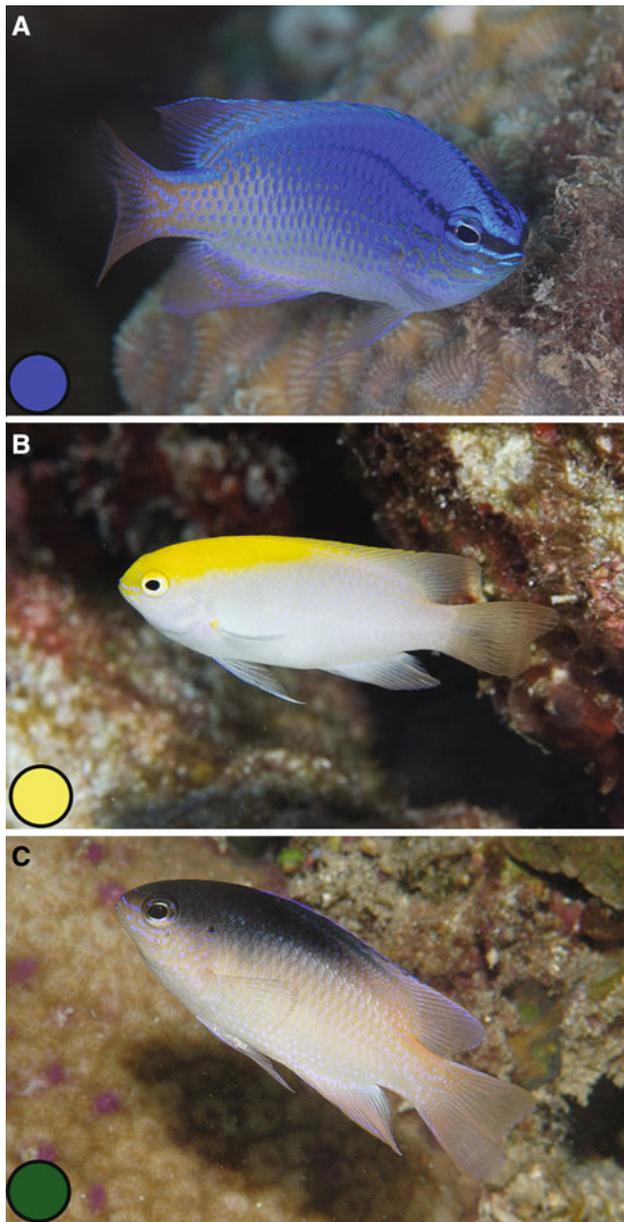


Fig. 2 **a** Underwater photograph of *Chrysiptera rex*, ~45 mm SL, Ishigaki Island, Ryukyu Islands (T. Uchida photo). **b** Underwater photograph of *Chrysiptera rex*, ~50 mm SL, Layang Layang Atoll (Sabah, Malaysia), South China Sea (G. Allen photo). **c** Underwater photograph of *Chrysiptera rex*, ~45 mm SL, Halmahera, Indonesia (G. Allen photo)

terminator chemistry (Applied Biosystems). Sequencing products were cleaned via 75% isopropanol precipitation and visualized on an ABI 3730 DNA Analyzer (Applied Biosystems). Forward and reverse sequences for each region were reconciled and compiled in Geneious Pro 4.4 with subsequent alignment by eye. All sequences have been deposited in Genbank (Ascension numbers GU562349–GU562387).

After determining that the HKY+G (control region) and SYM+I+G (CytB) were the most appropriate models of evolution using MrModeltest (Nylander 2004), phylogenetic analyses were conducted using MrBayes3.1.2 (Ronquist and Hulsenbeck 2003) as implemented in Geneious Pro 4.4. The phylogeny was determined after running the analysis for 2,000,000 generations. Stability of the analysis was assessed using the program AWTY. Posterior probabilities were generated after an initial burn in period of 500,000 generations.

Results

We recovered a 433-bp fragment of the control region for 21 samples, including 1 from Japan, 7 from Taiwan, 3 from Halmahera and 10 from the Philippines. Similarly, we obtained an 859-bp fragment of the cytochrome b locus for 17 samples (1 Japan, 6 Taiwan, 4 Halmahera and 6 from the Philippines). The phylogeny of the control region resulted in three well-supported clades (.65 and 1.0 Bayesian Posterior Probability, Fig. 3a) which was similar to the gene tree we recovered from the cytochrome b segment (1.0 Bayesian Posterior Probability for all clades, Fig. 3b) The nuclear RAG2 fragment, contained no phylogenetically informative characters.

Coral reef organisms utilize color for multiple purposes including mimicry (Cheney and Marshall 2009), sexual selection (Gray and McKinnon 2007), intraspecific communication (DeMartini and Donaldson 1996) and camouflage (Marshall 2000). Therefore, coloration is subject to a variety of potentially conflicting selectional constraints (DeMartini and Donaldson 1996) and congruence between morphological and genetic data sets is not always to be expected. For example, recent studies have shown that broadly distributed marine species can possess a surprisingly high degree of genetic variation even when there is considerable conservation in morphology and meristics (Barber et al. 2006; Mathews 2006; Crandall et al. 2008; DeBoer et al. 2008; Hyde et al. 2008). Alternatively, there have been several instances where the morphological or color variations in fishes are not reflected in neutral genes (McMillan et al. 1999; Rocha et al. 2007). For example, in *Hypoplectrus* spp. groupers (Serranidae), 11 putative species, currently defined largely by color variations, have not been separated using mitochondrial markers (McCartney et al. 2003; Ramon et al. 2003; Garcia-Machado et al. 2004; Puebla et al. 2007; Barreto and McCartney 2008, but see Puebla et al. 2008). Similarly, in the pomacanthid *Centropyge loriculus*, there were no diagnosable genetic differences between three geographically restricted color morphs (Schultz et al. 2006).

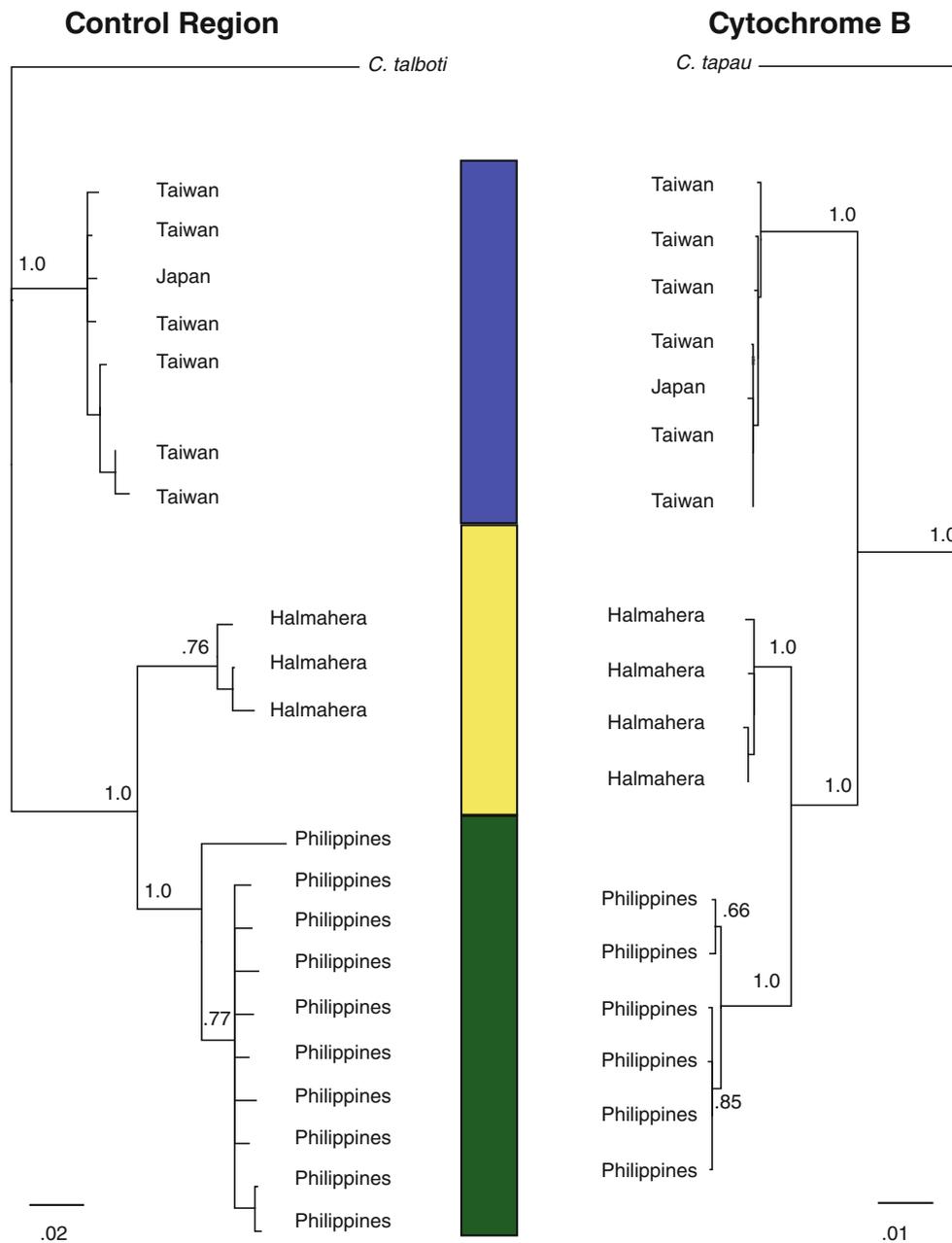


Fig. 3 Phylogenetic reconstructions with Bayesian analysis of mtDNA control region (*left*) and cytochrome b data (*right*). Nodal support is presented from posterior probability from 2,000,000 generations. *Bar color* represents color of individual samples as indicated in Fig. 2

In this study, we have found that color and geography are congruent with genetic structuring in the coral reef fish *C. rex*. Thus, while we acknowledge small sample sizes, our data show that for this species, color is a consistent surrogate for genetic similarity. The mtDNA loci predictably both present the same topology, where the clade containing all of the samples from the East China Sea is sister to the clade containing all the samples from Halmahera and the Philippines. We were not able to make

any hypotheses based on the nDNA locus, which had no phylogenetically informative characters.

Interestingly, each of these color variations is associated within specific enclosed seas in the western Pacific. The geologic history of the region is highly volatile with both large scale plate migrations (Hall 2002) and episodic changes in eustatic sea-level change (Voris 2000), which could have provided ample opportunities for both vicariance and dispersal. Specifically, during Pleistocene low sea-level

periods, the Sulu, Celebes and Molukka seas were isolated from other water bodies (Carpenter and Springer 2005), providing a possible mechanism for the recent divergence between the Halmahera and Philippine populations.

The taxonomic status of these populations clearly warrants further investigation. Each of the populations has a diagnosable color variation as well as autapomorphic substitutions at two mitochondrial gene regions. Because taxonomic revisions carry with them a host of legal, social and philosophical implications, we argue for a pluralistic approach toward taxonomy including genetic, morphological and meristic evidence (Ghiselin 2002; Paquin and Hedin 2004; Meirl and Mace 2007). Given the degree of differences, both in coloration and genes, the populations from Halmahera and the Philippines represent independent evolutionary units and possibly new species.

Previous studies have demonstrated that seemingly broadly distributed populations of fish are in actuality complexes of more geographically restricted, and genetically distinct populations (Drew et al. 2008; Drew and Barber 2009). Regardless of the ultimate taxonomic rank ascribed to the Halmahera and Philippines populations, the data indicate limited connectivity among all three populations and suggest that they are evolutionarily and ecologically independent lineages. Additionally, our study highlights the importance of making range wide collections to fully document the diversity encompassed by a species (Wallace 1863). Given the variability, we have seen in our limited sampling, a more thorough investigation throughout the range (Fig. 1a) may identify additional color morphs.

Chrysiptera rex live in some of the most threatened coral reefs in the world and opportunities to observe them in unperturbed systems are rapidly disappearing (Allen 2007). Effective scaling of conservation measures relies on both accurate delineation of population connectivity and identification of concordant patterns of endemism (Rocha et al. 2007; Almany et al. 2009). To successfully quantify connectivity and endemism, we must first accurately describe the evolutionary history of the species in question. The identification of three genetically differentiated populations indicates a lack of broad scale connectivity across the range of this species, and that conservation measures should be more finely focused on regional or even more localized scales.

Acknowledgments This work was supported by the National Science Foundation under a Postdoctoral Fellowship in Bioinformatics (2008) to JAD with additional support by John D. and Catherine T. MacArthur Foundation support of the Encyclopedia of Life. This research was carried out in the Field Museum's Pritzker Laboratory for Molecular Systematics and Evolution operated with support from the Pritzker Foundation. Samples were kindly provided by Vanson Liu and Gento Shimohara. Hiroyuki Tanaka provided the photograph of *C. rex* from the Ryukyu Islands, with permission from the photographer Takeshi Uchida. We would like to thank A. Cabral,

K. Feldheim E. Jones Sbrocco and B. Sazenbacher, for their useful comments and suggestions.

References

- Allen GR (2007) Conservation hotspots of biodiversity and endemism for Indo-Pacific coral reef fishes. *Aquat Conserv* 18:541–556
- Almany GR, Connoll SR, Heath DD, Hogan JD, Jones GP, McCook L, Mills M, Pressey RL, Williamson DH (2009) Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs* 28:339–351
- Avise JC, Wollenberg K (1997) Phylogenetics and the origin of species. *Proc Natl Acad Sci USA* 94:7748–7755
- Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. *Evolution* 60:1825–1839
- Barraclough TG, Volger AP (2000) Detecting the geographical pattern of speciation from species-level phylogenies. *Am Nat* 155:419–434
- Barreto FS, McCartney MA (2008) Extraordinary AFLP fingerprint similarity despite strong assortative mating between reef fish color morphospecies. *Evolution* 62:226–233
- Bay LK, Buechler K, Gagliano M, Caley MJ (2006) Intraspecific variation in the pelagic larval duration of tropical reef fishes. *J Fish Biol* 68:1206–1214
- Carpenter KE, Springer VG (2005) The center of the center of marine shore fish biodiversity: the Philippine Islands. *Environ Biol Fish* 72:467–480
- Cheney KL, Marshall NJ (2009) Mimicry in coral reef fish: how accurate is this deception in terms of color and luminance? *Behav Ecol* 20:459–468
- Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Boston, MA
- Crandall ED, Frey MA, Grosberg RK, Barber PH (2008) Contrasting demographic history and phylogeographical patterns in two Indo-Pacific gastropods. *Mol Ecol* 17:611–626
- DeBoer TS, Subia MD, Ambariyanto, Erdmann MV, Kovitvongsa K, Barber PH (2008) Phylogeography and limited genetic connectivity in the endangered boring giant clam across the coral triangle. *Conserv Biol* 22:1255–1266
- DeMartini EE, Donaldson TJ (1996) Color morph-habitat relations in the arc-eye hawkfish, *Paracirrhites arcatus* (Pisces: Cirrhitidae). *Copeia* 1996:362–371
- Domeier ML (1994) Speciation in the serranid fish *Hypoplectrus*. *Bull Mar Sci* 54:103–141
- Drew JA, Barber PH (2009) Sequential cladogenesis of *Pomacentrus moluccensis* (Bleeker, 1853) supports the peripheral origin of marine biodiversity in the Indo-Australian Archipelago. *Mol Phylogenet Evol* 53:335–339
- Drew JA, Allen GR, Kaufman L, Barber P (2008) Regional color and genetic differences demonstrate endemism in five putatively cosmopolitan reef fishes. *Conserv Biol* 22:965–975
- Garcia-Machado E, Chevalier Monteagudo PP, Solignac M (2004) Lack of mtDNA differentiation among hamlets (*Hypoplectrus*, Serranidae). *Mar Biol* 144:147–152
- Ghiselin MT (2002) Species concepts: the basis for controversy and reconciliation. *Fish Fish* 3:151–160
- Gray SM, McKinnon JS (2007) Linking color polymorphism maintenance and speciation. *Trends Ecol Evol* 22:71–79
- Hall R (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *J Asian Earth Sci* 20:353–431
- Herre AWCT (1935) New fishes obtained by the Crane Pacific expedition. *Fieldiana Zool* 18:383–438

- Hyde JR, Kimbrell CA, Budrick JE, Lynn EA, Vetter RD (2008) Cryptic speciation in the vermilion rockfish (*Sebastes miniatus*) and the role of bathymetry in the speciation process. *Mol Ecol* 17:1122–1136
- Lee WJ, Howell WH, Kocher TD (1995) Structure and evolution of teleost mitochondrial control regions. *J Mol Evol* 41:54–66
- Marshall NJ (2000) Communication and camouflage with the same ‘bright’ colours in reef fishes. *Proc R Soc Biol Sci Ser B* 355:1243–1248
- Mathews LM (2006) Cryptic biodiversity and phylogeographical patterns in a snapping shrimp species complex. *Mol Ecol* 15:4049–4063
- Mayr E (1947) Ecological factors in speciation. *Evolution* 1:263–288
- McCartney MA, Acevedo J, Heredia C, Rico C, Quenouille B, Bermingham E, Mcmillan WO (2003) Genetic mosaic in a marine species flock. *Mol Ecol* 12:2963–2973
- McMillan WO, Weigt LA, Palumbi SR (1999) Color pattern evolution, assortative mating, and genetic differentiation in brightly colored butterflyfishes (Chaetodontidae). *Evolution* 53:247–260
- Meirl S, Mace GM (2007) New taxonomy and the origin of species. *PLoS Biol* 5:e194
- Noonan BP, Comeault AA (2009) The role of predator selection on polymorphic aposematic poison frogs. *Biol Lett* 5:51–54
- Nylander JAA (2004) mrModeltest v2. Evolutionary Biology Centre, Uppsala University
- Paquin P, Hedin M (2004) The power and perils of ‘molecular taxonomy’: a case study of eyeless and endangered *Cicurina* (Araneae: Dictynidae) from Texas caves. *Mol Ecol* 13:3239–3255
- Parenti P, Randall JE (2000) An annotated checklist of the species of the Labroid fish families Labridae and Scaridae. *Ichthyol Bull JLB Smith Inst Ichthyol* 68:1–97
- Puebla O, Bermingham E, Guichard F, Whiteman E (2007) Colour pattern as a single trait driving speciation in *Hypoplectrus* coral reef fishes? *Proc R Soc Biol Sci Ser B* 274:1265–1271
- Puebla O, Bermingham E, Guichard F (2008) Population genetic analyses of *Hypoplectrus* coral reef fishes provide evidence that local processes are operating during the early stages of marine adaptive radiations. *Mol Ecol* 17:1405–1415
- Quenouille B, Bermingham E, Planes S (2004) Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analysis of mitochondrial and nuclear DNA sequences. *Mol Phylogenet Evol* 31:66–88
- Ramon ML, Lobel PS, Sorenson MD (2003) Lack of mitochondrial genetic structure in hamlets (*Hypoplectrus* spp.): recent speciation or ongoing hybridization? *Mol Ecol* 12:2975–2980
- Ready JS, Sampaio I, Schneider H, Vinson C, Dos Santos T, Turner GF (2006) Colour forms of Amazonian cichlid fish represent reproductively isolated species. *J Evol Biol* 19:1139–1148
- Roberts CM, McClean CJ, Veron JEN, Hawkins JP, Allen GR, McAllister DE, Mittermeier CG, Schueler FW, Spalding M, Wells F, Vynne C, Werner TB (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science* 295:1280–1284
- Rocha LA, Craig MT, Bowen BW (2007) Phylogeography and the conservation of coral reef fishes. *Coral Reefs* 26:501–512
- Ronquist F, Hulsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574
- Sale PF, Doherty PJ, Eckert GJ, Douglas WA, Ferrell DJ (1984) Large scale spatial and temporal variation in recruitment to fish populations on coral reefs. *Oecologia* 64:191–198
- Schultz JK, Pyle RL, DeMartini EE, Bowen BW (2006) Genetic connectivity among color morphs and Pacific archipelagos for the flame angelfish, *Centropyge loriculus*. *Mar Biol* 151:167–175
- Snyder JO (1909) Descriptions of new genera and species of fishes from Japan and the Riu Kiu Islands. *Proc U S Natl Mus* 36:598–610
- Vecchione M, Collette BB (1996) The central role of systematics in marine biodiversity problems. *Oceanography* 9:44–45
- Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *J Biogeogr* 27:1153–1167
- Wallace AR (1863) On the physical geography of the Malay Archipelago. *J Royal Geo Soc London* 33:217–234
- Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR based typing from forensic material. *Biotechniques* 10:506–513
- Westneat MW, Alfaro MA (2005) Phylogenetic relationships and evolutionary history of the reef fish family Labridae. *Mol Phylogenet Evol* 36:370–390
- Wiley EO (2002) On species and speciation with reference to the fishes. *Fish Fish* 3:161–170