Biochemical systematics of fishes of the genus *Stegastes* (Pomacentridae) from the Southern Marianas

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Abstract. The biochemical systematics of the subset of species of *Stegastes* (Pisces: Pomacentridae) available from coral reefs surrounding Guam, USA, was studied using three species of *Pomacentrus* as the outgroup. Analyses of allozymic variation at 14 presumptive loci (10 of which were polymorphic) using various evolutionary tree-construction techniques identified *S. fasciolatus* as the sister species of an assemblage comprised of *S. albifasciatus, S. nigricans, S. lividus*, and *S. aureus* possess 12 dorsal spines [DS XII; (DS = number of dorsal spines)], whereas *S. fasciolatus, S. emeryi*, and *S. apicalis* possess 13 (DS XIII), and *S. gascoynei* possesses 14 (DS XIV). Implicit to the proposed classification scheme was the suggestion that differences in a single character-state merit the subdivision of a genus of reef fishes.

In coral reefs surrounding Guam, the southernmost of the Mariana Islands, two putative subgeneric species complexes identified by Allen are represented by *Stegastes fasciolatus* (DS XIII) and *S. albifasciatus, S. nigricans, S. lividus*. It was therefore of interest to test the validity of the proposed partitioning of the genus *Stegastes* by examining, using alternative criteria, the systematics of the readily available subset of species. Based on previous pilot studies demonstrating fixed allelic differences at protein-coding loci among Atlantic damselfishes, characterization of allozymic variability among the four species and suitable outgroup taxa was deemed a feasible and appropriate approach for reexamining the proposed classification scheme.

Three species of the genus *Pomacentrus* – *P. pavo, P. amboinensis*, and *P. vaiuli* – were chosen for outgroup comparison on the basis of presumably robust morphologic criteria separating *Stegastes* and *Pomacentrus* and because of the availability of these species from waters surrounding Guam. Fishes of the genus *Stegastes* Jenyns exhibit uniserial dentition and scalation of the suborbital area and lack a notch between the preorbital and suborbital areas, whereas fishes of the genus *Pomacentrus* Lacede have biserial dentition, a notch between preorbital and suborbital areas, and a naked (nonscaled) suborbital (Allen 1975, Emery and Allen 1980). Inclusion of more than one species as an outgroup also afforded the possibility of assessing preliminarily the genetic relationships between *P. pavo* (subgenus *Pomacentrus*) and *P. amboinensis* and *P. vaiuli* (subgenus *Pseudopomacentrus*).
Materials and methods

Sample sizes and collection sites for each of the seven study species were as follows: Stegastes fasciatus (n=25; 144°52'48"N, 13°35'54"E); S. albifasciatus (n=25; 144°46'24"N, 13°16'00"E); S. nigricans (n=25; 144°46'24"N, 13°16'00"E); S. lividus (n=25; 144°51'6"N, 13°30'6"E); Pomacentrus pufo (n=24; 144°45'55"N, 13°27'54"E); P. amboinensis (n=22; 144°39'12"N, 13°27'00"E); P. vaiuli (n=21; 144°45'55"N, 13°27'54"E). The collection of samples of each species from single geographic locations was justifiable based on results of previous studies demonstrating micro- and macrogeographic homogeneity of gene frequencies among damselfish samples (Lacson 1990, 1992). Fish were collected using hand nets and an anesthetic [10% quinaldine (Sigma, St. Louis), 85% ethanol, and 5% water], transported live to the laboratory, and then frozen at ~70°C. Methods of tissue excision and homogenization, starch-gel electrophoresis, and histochemical staining were largely those described by Morizot and Schmidt (1990).

Preliminary starch-gel electrophoretic screens using two buffer systems [TC (0.135 M tris; 0.043 M citrate) pH 7.0; TEB (0.5 M, tris; 0.0016 M EDTA; 0.65 M borate), pH 8.0] revealed interspecific protein polymorphisms at ten presumptive loci. Multiple loci were assigned numerical designations with the most anodally migrating isozyme designated "1" and less anodal locus products receiving progressively greater numerical designations. Alleles at most presumptive loci were assigned numerical designations expressing the mobility of their respective products relative to the mobility of the most common allomorph (designated as "00") among samples of the OTUs (operational taxonomic units) (Fig. 1). Modification of this system of allele designation was necessary for the locus adenosine deaminase (ADA*) at which the most anodal electromorph was designated "000": the rationale for the different allele coding system for ADA* will be discussed further in the "Results" section. Banding patterns consistent with the known subunit structure of each enzyme and models of Mendelian inheritance were recorded as genotypes. Diagnostic polymorphic proteins, corresponding locus abbreviations and enzyme commission numbers and buffer systems utilized for optimal resolution of banding patterns were as follows: adenine deaminase (ADA*; E.C. 3.5.4.4; TEB pH 8.0), glucoisomerase (GPI-I*, GPI-2*; E.C. 5.3.1.9; TC pH 7.0), lactate dehydrogenase (LDH-I*; E.C. 1.1.1.27; TC pH 7.0), malate dehydrogenase (MDH-I*, MDH-2*; E.C. 1.1.1.37; TC pH 7.0), mannosphosphate isomerase (MPI*; E.C. 5.3.1.18; TC pH 7.0), peptidase [PEP-A*] (substrate was leucine-tyrosine; E.C. 3.4.11; TEB pH 8.0), phosphoglucone dehydrogenase (PGD*, E.C. 1.1.1.44; TEB pH 8.0), and phosphoglucomutase (PGM-2*; E.C. 2.7.5.1; TC pH 7.0). Products of other presumptive loci were monomorphic. These were: (1) a more cathodal LDH (presumably LDH-2*); (2) an anodal SOD [presumably SOD-1* (superoxide dismutase; E.C. 1.15.1.19)]; (3) a more anodal PEP-A (presumably PEP-A-1*; detected on TC pH 7.0); and (4) a more anodal PGM (presumably PGM-3*). A second cathodal SOD, a third more cathodal LDH (presumably LDH-3*), and a third more cathodal MDH (presumably MDH-3*), produced unresolved banding patterns.

The FREQPARS program of Swofford and Berlocher (1987) was used to construct an evolutionary tree using allele frequencies at polymorphic loci under the principle of maximum parsimony: the FREQPARS program selects a tree topology with a minimum number of branch and clade transformations. The BIOSYS-1 program of Swofford and Selander (1981) was used to: (1) calculate estimates of genetic distances (D; Rogers 1972, as modified by Wright 1978) among all pairwise combinations of OTUs (with data from monomorphic loci included); (2) construct evolutionary trees following the distance Wagner (DISWAG) methodology (Farris 1972) with outgroup rooting; and (3) generate an UPGMA phenogram from a matrix of estimates of genetic distances.

The most parsimonious cladogram was constructed by methods described by Wiley (1981); presumptive alleles detected in both the outgroup and ingroup were considered primitive. Ordering of alleles into transformation series was performed using outgroup criteria described by Farris (1982).

Results

Among the products of the ten diagnostic loci, 64 electromorphs were identified within the seven species studied (Table 1). The mean number of alleles per locus per species ranged from 1.1 in Pomacentrus amboinensis to 1.5 in P. vaiuli. Within each species, percentage of polymorphic loci (0.95 criterion) ranged from 30% in Stegastes albifasciatus to 0.0% in S. nigricans. The maximum number of alleles detected at a locus (all species considered) was 9 (GPI-2*), whereas the minimum was 5 (MDH-I*, MDH-2*, and PEP-A*). The greatest number of fixed allelic differences among species was detected at ADA*; one