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Molecular phylogenetic analysis of genera in the family Plakobranchidae (Mollusca: Opisthobranchia: Sacoglossa)

Anna L. Bass[1] and Stephen A. Karl[2]

Department of Biology, SCA 110, University of South Florida, 4202 E. Fowler Ave., Tampa, Florida 33620 USA

[1] To whom correspondence should be addressed: Email: abass@mail.usf.edu

[2] Current address: The Hawai'i Institute of Marine Biology, University of Hawai'i, Manoa, P.O. Box 1346, Kane'ohe, Hawai'i, 96744, Email: skarl@hawaii.edu

Abstract – Genera in the largest family of the suborder Sacoglossa (Mollusca; Opisthobranchia), the Plakobranchidae Gray, 1840, have been systematically revised numerous times since the 1800s. Several authors have questioned the validity and inter-relationships of the genera *Tridachia* Moerch, 1863, *Tridachiella* MacFarland, 1924, *Elysiella* Bergh, 1872, *Pattyclaya* Marcus, 1982, *Elysia* Risso, 1818, and *Thuridilla* Bergh, 1872. For many other groups, molecular data have proven fruitful in determining the systematic relationships of organisms for which few suitable morphological characters are available. Using DNA sequence data from one nuclear (Histone 3) and two mitochondrial genes (Cytochrome Oxidase subunit I and large ribosomal subunit), we infer the phylogenetic relationships among five of the seven recently recognized genera within the Plakobranchidae. These data question the monophyly of the genus *Elysia* and suggest that further divisions within the genus may be necessary. Additionally, it appears that the family Boselliidae Marcus, 1982 may be, at least, paraphyletic since one member of the genus *Bosellia* Trinchese, 1891, clusters within the family Plakobranchidae.

Key words: Opisthobranchia, Sacoglossa, Plakobranchidae, molecular phylogenetics

INTRODUCTION

Jensen (1996) and Mikkelsen (1998) using a cladistic analysis of morphological characters assessed the higher-level relationships within the suborder Sacoglossa. These analyses indicated that Limapontioidea Gray, 1847 is a sister group to the superfamily Plakobranchoidea Rang, 1829, which contains the family Plakobranchidae Gray, 1840. The family Limapontiidae Gray, 1847 is currently recognized as a member of the superfamily Limapontioidea (Jensen 1996; Mikkelsen 1998).

The family Plakobranchidae, with anywhere from 90–100 species, is the largest in the order Sacoglossa (Jensen 1997a, b). Since the 1800s members of this family have been variously divided into as few as nine and as many as 14 genera (Jensen 1992). Many of these revisions resulted in the designation of monotypic genera and families (e.g., *Plakobranchus*, is the sole member of the Plakobranchidae *sensu* Marcus 1982). Based on anatomical descriptions of a greater number of specimens and the identification of characters associated with internal organs, Jensen (1992) argued for the retention of five genera, but not the monotypic family, Plakobranchidae. Unfortunately, specimens of the monotypic genera *Tridachia* and *Tridachiella* were not included in Jensen's study and no conclusions as to their status could be drawn. Based on an assessment of 12 morphological characters measured in representatives of all seven genera, Gosliner (1995) demonstrated that *Thuridillla* and *Plakobranchus* cluster together. The remaining five genera, however, formed a polyphyletic clade (Figure 1). Based on these observations, Gosliner proposed retention of the genera *Thuridilla*, and *Plakobranchus* but synonymizing the remaining taxa as *Elysia*. Since these studies, no further attempts to determine the phylogenetic relationships of Plakobranchidae genera or species have been made.

The historical volatility of the taxonomy of the Plakobranchidae is not particularly surprising. Overall, members of the family appear to be extremely conservative in terms of variation in body form. As for others in the Sacoglossa, and mollusks in general, members of the family possess few morphological characters capable of reliably inferring evolutionary relationships. Many of the named species are based on the inspection of a single individual and often rely on malleable characters such as coloration and radular tooth morphology. Coloration or patterns of color are given in species descriptions, but only from live specimens as preservatives destroy all color except for black pigments. In addition, diet may also affect

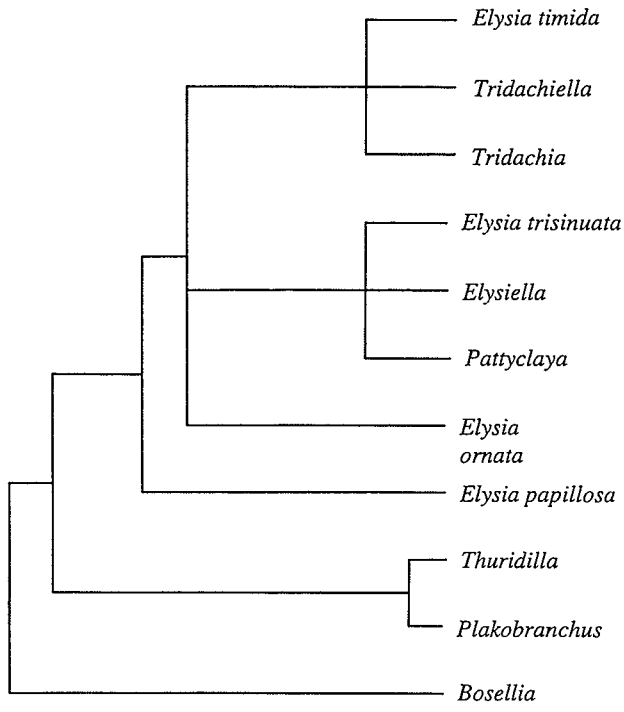

Figure 1 Systematic relationships among genera of Plakobranchidae as proposed by Gosliner (1995).

color (Marcus 1980) thus reducing its reliability for systematic purposes. Other common characters are the presence of papillae on head, rhinophores and parapodia. Radular teeth characteristics are included in a majority of descriptions, but also may be unreliable for species identification since tooth shape can be directly affected by diet (Jensen 1993). Populations of many of the species experience large temporal fluctuations in density (e.g., *E. chlorotica*; Bleakney, 1996), however, there has been little to no assessment of intra-specific or inter-seasonal variation in taxonomic characters. Some of the most useful characters are subtle differences of internal organs found through detailed anatomic examination (Jensen 1992). The shape of the pharynx, shape of the reproductive systems, and the presence or absence of a pharyngeal pouch might be the best way to differentiate between species. Accurate quantification of internal soft-body characteristics, however, is exceptionally time consuming and requires a significant investment in training. It may be advantageous, therefore, also to develop complementary approaches to aid in the reconstruction of sacoglossan relationships.

Here, we present a systematic analysis of the Plakobranchidae based on nuclear and mitochondrial DNA sequence data of five of the seven genera presented in Gosliner (1995). Specifically, we test the previously proposed systematic relationship of the genera, the monophyly of the genus *Elysia*, and the relationship of the Plakobranchidae to the family Boselliidae Marcus 1982. Two members of the family Limapontiidae are included as outgroups. Two genes from mitochondrial DNA genome, cytochrome oxidase subunit I (COI) and large ribosomal subunit (16S) were targeted due to their reported use in the resolution of family to genus level divergences (Folmer *et al.* 1994; Medina and Walsh 2000; Simon *et al.* 1994). We included data from the nuclear gene Histone 3 to examine its utility in resolving deeper level divergences observed after the collection of the mitochondrial DNA data. H3 has been used successfully to determine deep level taxonomic relationships in gastropods (Colgan *et al.* 2000) and insects (Danforth *et al.* 2005).

MATERIALS AND METHODS

Individuals from five of the seven genera in Plakobranchidae, two species from Bosellidae and two species from a single genus in the Limapontiidae (Table 1) were collected, anesthetized with $MgCl_2$ and stored in 95% EtOH. Samples were collected between 2000 and 2003. Two population samples were collected for *Elysia papillosa* and *Tridachia crispata*. Total cell DNA was isolated from a small portion of the anterior end of the animal subjected to a non-boiling chelex method (Walsh *et al.* 1991) and used in subsequent polymerase chain reaction (PCR) amplifications of three genes. All treated samples were diluted with 1X TE (10 mM Tris, 1 mM EDTA; pH 7.5) and stored at -20°C.

Segments of two mitochondrial genes (~700 bp of cytochrome *c* oxidase subunit I [COI] and ~450 bp of the large subunit RNA [16S]) and one nuclear gene (~380 bp of Histone 3 [H3]) were PCR amplified with primer pairs, respectively, LCO1490 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and HCO2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Folmer *et al.* 1994), 16Ssar 5′-CGC CTG TTT ATC AAA AAC AT-3′ and 16sa 5′-CTC CGG TTT GAA CTC AGA TC-3′ (Kessing *et al.* 1989), and H3F 5′-ATG GCT CGT ACC AAG CAG ACV GC-3′ and H3R 5′-ATA TCC TTR GGC ATR ATR GTG AC-3′ (Colgan *et al.* 2000). Amplification reactions (25 or 50 mL) consisted of 1X buffer (Enzypol LTD., Denver, CO), 1.5 units of Enzypol Plus 2000 polymerase (Enzypol LTD., Denver, CO), 0.2 mM each dNTPs, 3.0 mM $MgCl_2$ (COI and 16S) or 1.5 mM $MgCl_2$ (H3), 0.5 mM of each primer, 1.0 M Betaine, 0.12 mg/mL of bovine serum albumen, and 2.0–4.0 mL of template. The cycling conditions for the COI and 16S primers consisted of 1 min. at 95°C followed by 35–45 cycles of 30 sec. at 95°C, 45 sec. at 48°C, and 1 min. at 72°C with a final extension of 3 min. at 72°C. Cycling conditions for the H3 primers consisted of 1 cycle of 4 min. at 95°C, 1 min. at 50°C, and 1 min. at 72°C, followed

Table 1 Material examined in the analysis including family and species designation, brief locality description, number of sequences or individuals used and type of genetic data available for the analysis.

Taxa	Locality	#	Gene
Bosellidae			
Bosellia marcusi	Bahamas	1	COI, 16S, H3
Bosellia mimetica	Bahamas	3	COI, 16S, H3
Limapontiidae			
Costasiella kuroshimae	Guam	1	COI, 16S, H3
Costasiella ocellifera	Bahamas	1	COI, 16S, H3
Plakobranchidae			
Elysia papillosa	Bahamas, Florida Keys	4, 4	COI, 16S, H3
Elysia ornata	Philippines	1	COI, 16S, H3
Elysia timida	Florida Keys	2	COI, 16S, H3
Elysiella pusilla	Hawaii	2	COI, 16S
Plakobranchus ocellatus	Guam	1	COI, 16S, H3
Thuridilla carlsoni	Hawaii	2	COI, 16S, H3
Thuridilla undula	Philippines	1	COI, 16S, H3
Tridachia crispata	Belize, Florida Keys	6, 10	COI, 16S, H3[1]

[1] Majority rule consensus sequences for Belize and Florida Keys are based on one and three individuals for 16S and six and ten individuals for CO1, respectively.

[2] Histone 3 sequence data from the Florida population only.

by 35 cycles of 45 sec. at 95°C, 30 sec. at 52°C, and 45 sec. at 72°C and a final extension of 3 min. at 72°C. A template free reaction was included for the detection of contamination.

Amplicons were purified using sterile nanopure water and 30,000 MW Millipore filters (Millipore Inc., Bedford, MA). The mass of the amplicons was determined by comparing ethidium bromide staining intensity of 2.0–5.0 mL of each purified reaction relative to a standard mass DNA ladder (Invitrogen Life Technologies, Carlsbad, CA). Cycle sequencing reactions (Amersham ET-Terminator Kit, Amersham Biosciences Corp., Biscataway, NJ) were conducted with approximately 100 ng of purified PCR product according to manufacturers specifications and fluorescently labeled products were size sorted and visualized using an ABI 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). The sequences from both strands of the amplicon were compared and edited (when needed) using Sequencher (v4.1; Gene Codes Corp., Ann Arbor, MI). The 16S gene region fragments were aligned using Clustal W (Higgins *et al.* 1996) and the resulting alignment was verified by eye and to identify hyper-variable regions where no one alignment appeared superior to another. The open reading frame for the COI and H3 genes were used to aid in proper alignment.

Maximum likelihood analysis as implemented in PAUP* (v4.0b10; Swofford 1998) was conducted on a best-fit model (TVM+I+G) of all the data as selected by Modeltest with Akaike information criteria (v3.06; Posada and Crandall 1998). Heuristic tree searches were conducted on ten trees generated from a stepwise addition (with random taxon addition) and tree-bisection-reconnection (TBR) branch swapping. In all analyses, gaps were treated as missing data. To evaluate the relative divergence levels among taxa, the best-fit model of evolution as selected in Modeltest using Akaike information criteria (v3.06; Posada and Crandall 1998) was determined for all genes combined excluding putative 3rd positions of the COI fragment. Putative third positions of the COI fragment were excluded after the indication of saturation of both transitions and transversions for all species as evidenced by a nonlinear relationship in a plot of the number of substitutions versus uncorrected pairwise percent sequence difference. The two intra-specific level samples were treated in the same manner to facilitate overall comparisons. Estimates of divergence were generated using a maximum likelihood estimator with PAUP*. Average divergence estimates were compared using a Student's t-test (Sokal and Rohlf 1995) to assess sequence divergence at various taxonomic ranks.

A Bayesian framework was used to determine clade support among the species of interest (Mr. Bayes v3.0b4; Huelsenbeck and Ronquist 2001). The data was divided into seven partitions: putative 1st, 2nd and 3rd codon positions of COI, putative 1st, 2nd, and 3rd codon positions of H3, and all positions of 16S. Under this partition framework, 4,000,000 generations were run and sampled every 1,000 generations. Model parameters, proportion of invariant sites, gamma distribution and nucleotide substitution rates, were estimated independently for each partition using Mr. Bayes default values of four chains and 0.2 for the chain temperature parameter. Four chains starting from random trees were run to determine if independent chains had converged and on average a "burnin" of 2,000 was used to determine posterior probability values.

Alternative tree topology hypotheses were tested

using a one-tailed Kishino-Hasegawa (K-H) maximum likelihood test as implemented in PAUP*. To test the hypothesis that Bosellidae is a monophyletic family, constrained (*Bosellia mimetica* + *Bosellia marcusi*) and unconstrained maximum likelihood trees were generated and compared.

RESULTS

At total of 1,417 nucleotides of sequence data collected from a total of 25 individuals from 12 species was used in the analysis (Table 1). For two species, *Elysia papillosa* and *Tridachia crispata*, majority rule consensus of the sequence data from multiple (2–10) individuals from two locations were included. Both 16S and CO1 sequences were collated from four individuals from the Bahamas and Florida Keys populations of *Elysia papillosa* and the majority rule consensus sequences were used in the analyses. For *Tridachia crispata* the 16S majority rule consensus sequences are based on one and three individuals from the Florida Keys and Belize, respectively. The CO1 majority rule consensus sequences are based on ten and six individuals from the Florida Keys and Belize, respectively. All three genes were assayed from at least one individual from 13 species but sequence data was obtained for only two genes (COI and 16S) from *Elysiella pusilla* due to technical difficulties in amplifying H3 from this species (Table 1). A small region of the 16S gene (i.e., 50 nucleotides) appeared to be highly variable and concerns about site homology resulted in it being excluded from the analysis.

The topology of the Bayesian and ML trees were identical except that in the Bayesian analysis *Elysia ornata* was grouped with the *Elysia papillosa* clade (Figure 2) as opposed to at the base of the clade containing the remaining plakobranchids as was the case for the ML analysis. A K-H test indicated that the ML and Bayesian trees were not significantly different (K-H = 0.878) and clade credibility values for the Bayesian analysis generally were large. Similarly, the hypothesis of a monophyletic Boselliidae resulted in a less likely tree than a

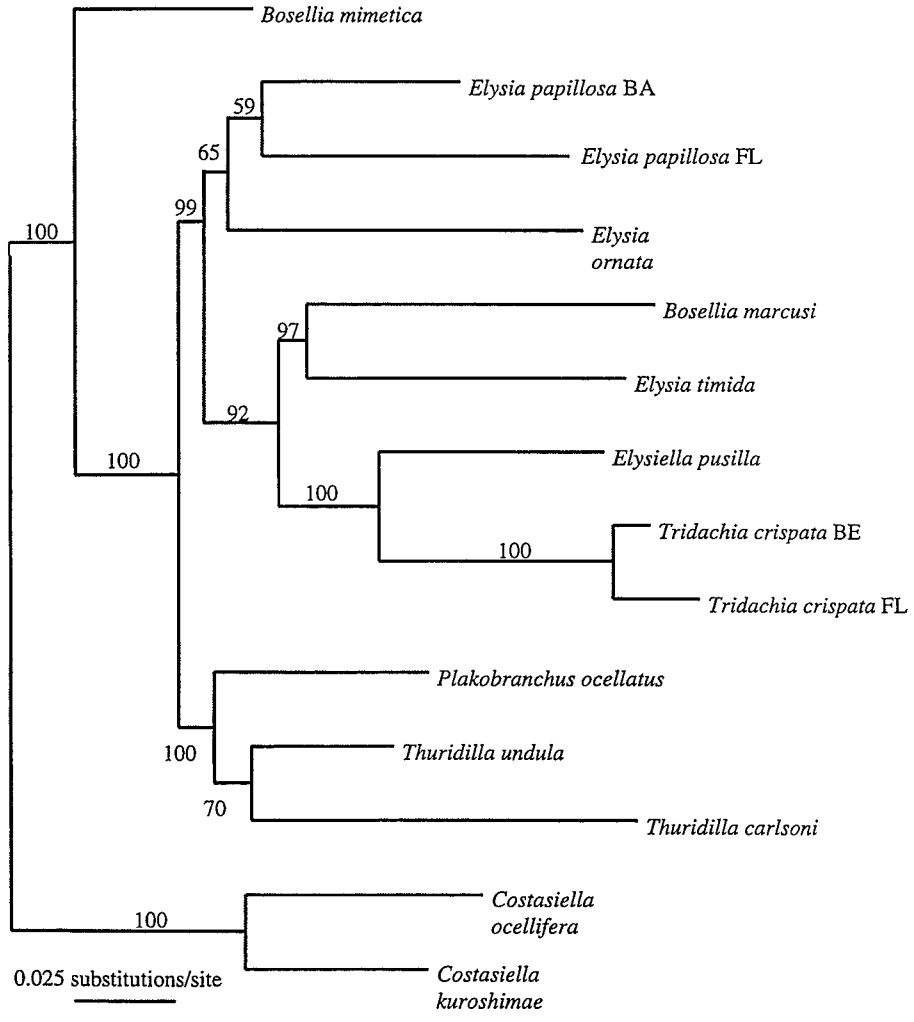

Figure 2 Maximum likelihood tree of the relationships among five of the seven genera used in Gosliner (1995), and members of the families Bosellidae and Limapontiidae. BE = Belize, BA = Bahamas and FL = Florida Keys. *Costasiella* species were used to root the tree. Bayesian posterior node probability values were estimated after an average burn-in of 2000.

Table 2 Estimates of sequence divergence based on combined data. Putative third positions in the COI fragment were excluded due to saturation. Divergence was estimated under a maximum likelihood framework with model (TVM + I + G) determined using ModelTest and AIC (Posada and Crandall 1998) and PAUP* (Swofford 1998).

Taxonomic Level	(± SD)	Range	Expected Magnitude
Intra-specific[1]	0.058 ± 0.049	0.024 - 0.093	None – Small
Inter-specific Congeneric	0.160 ± 0.027	0.124 - 0.196	Small – Moderate
Inter-generic Confamilial	0.150 ± 0.036	0.094 - 0.209	Moderate – Large
Inter-familial	0.173 ± 0.044	0.115 - 0.245	Large – Very Large

[1] Estimates are based on more than two individuals from more than one population of *Elysia papillosa* and *Tridachia crispata*.

topology where *Bosellia marcusi* was clustered within the Plakobranchidae clade (K-H = 0.003, $p < 0.05$).

An analysis of the maximum likelihood distances for various hierarchical taxonomic classifications did not yield a strong correlation to taxonomic level (Table 2). Student's t-tests indicated that the there were significant differences in average divergence estimates in two of four comparisons. The average divergences between intra- and inter-specific congeneric categories were significantly different ($p = 0.013$) as were the averages between inter-generic confamilial and inter-familial categories ($p = 0.001$). The remaining categories, inter-specific congeneric versus inter-generic confamilial and inter-specific congeneric versus inter-familial, were not significantly different ($p = 0.615$ and $p = 0.180$, respectively).

DISCUSSION

One of the fundamental strengths of our approach is that DNA sequence data often can reveal significantly more variation than detailed internal morphological analyses and therefore our conclusions are based on a larger suite of variable characters taken from several members of each targeted taxa. The ability to include more than one individual of a taxa allows us to begin to address the degree of intra-group variation as well as sister group associations. Historically, descriptions of members of the family Plakobranchidae have been limited to single individuals with no comparisons among material from multiple locations (as noted in Jensen 1992). Both here, and as part of a larger study of variation within the family Plakobranchidae, we have sampled several individuals within putative single species and found for intraspecific comparisons both large (d = 9.2 %; *Elysia papillosa*) and small degrees of variation (d = 2.3%; *Tridachia crispata*) among populations of named species. Estimates of intra-specific variation are derived only for *E. papillosa* and *T. crispata* because these are the species for which we have more than two samples from more than one location. The significant amount of genetic differentiation found between *E. papillosa* in the Bahamas and the Florida Keys indicates that these clearly are not conspecifics. The assessment of morphological differences among taxa is without question essential. Nonetheless, the inclusion of molecular approaches can provide a wealth of data for systematic analysis as well as a divining rod for further investigations of interesting morphological characteristics. In this case, now that clear evolutionary divergence has been established through genetic analyses, investigation of the morphology of this species is likely to reveal previously overlooked physical differences.

Paraphyly and systematic relationships

This study supports the conclusions of Gosliner (1995) in reference to the question of paraphyly within the family Plakobranchidae since *Elysia* is clearly paraphyletic in our analysis. Furthermore, considering just the topology of the tree, our results are consistent with Gosliner's proposal to subsume *Elysiella* and *Tridachia* into *Elysia*. Even so, we do not support this proposal but instead believe that further generic designations within *Elysia* are appropriate. As indicated previously, there is a large degree of genetic divergence seen throughout the Plakobranchidae. The degree of divergence among the taxa in the *Elysia/Bosellia/Tridachia/Elysiella* clade (Figure 2) is considerable and equivalent to that seen among other genera (i.e., *Plakobranchus* and *Thuridilla*). Consider just the degree of genetic divergence seen among these taxa. If we were to support subsuming *Tridachia* and *Elysiella* into *Elysia* we should be equally compelled to subsume other legitimate generic (e.g., *Plakobranchus* and *Thuridilla*) as well as inter-familial (e.g., *Bosellia marcusi*) level designations due to similar topological arrangements and divergence levels. Alternatively, if we accept *Plakobranchus* and *Thuridilla* as valid genera then we also should accept generic level status for *Tridachia* and *Elysiella*. Further generic and species level designations within *Elysia* would also be necessary. This is consistent with Jensen's (1997a) proposal that the large number of described species in *Elysia* and the dearth of reliable taxonomic characters for these

organisms warrant further divisions within *Elysia*. We propose to leave *Tridachia* and *Elysiella* as valid genera, and sister taxa, however, until a more thorough sampling and taxon analysis of *Elysia* can be completed. *Bosellia marcusi* should either be subsumed into genus *Elysia* or reassigned at the generic level thus resolving the paraphyly of both *Elysia* and *Bosellia*. The *Elysia ornata* + *E. papillosa* clade satisfies the condition of monophyly at similar divergence levels and can be left unchanged. We emphasize, however, that any decision at this time is preliminary and final revisions require a more comprehensive survey of members of the Plakobranchidae.

Our data confirm previous indications by Jensen (1996) and Mikkelson (1998) that the species *Bosellia mimetica* is closely related to Plakobranchidae. The family Bosellidae was erected because of the lack of parapodia and a different chromosome number (for *Bosellia mimetica*) relative to other plakobranchids (n=7 vs n=17; Marcus 1982; Mancini and Sordi 1965). Thompson and Jaklin (1988) also report that a chitinous penial stylet, "other features of the reproductive system (Sanders-Esser 1984)" and the absence of parapodia differentiate *Bosellia mimetica* from members of the genus *Elysia*. Even so, they preferred to keep *Bosellia* within a larger more "broadly defined Elysiidae" family. Our data clearly indicate that *B. mimetica* clusters outside but adjacent to the family Plakobranchidae. The divergence between *B. mimetica* and the Plakobranchidae (0.143 ± 0.025) is less than that for *B. mimetica* to the Limapontiidae (*Costasiella kuroshimae* and *C. ocellifera*; 0.164 ± 0.013) and from the Limapontiidae to the Plakobranchidae (0.219 ± 0.027).

The placement of *Bosellia marcusi* within the Plakobranchidae and not with its congener was an unexpected result (Figure 2). Marcus' original description of *B. marcusi* was based on several specimens collected in Florida (Marcus 1973). Subsequently, however, no further sightings of this species had been recorded until Thompson (1977) found specimens in Jamaica. While collecting in the Bahamas, we recovered a single specimen of *B. marcusi* and four specimens of *B. mimetica* from the same clump of *Halimeda* sp. Photographs taken of the Bahamian specimens (ALB) appear to match the original description by Marcus (1973) and both the description and drawing of Thompson (1977) (Colin Redfern, pers. comm.). The placement of *B. marcusi* within the plakobranchids and not with its congener in our analysis is robust as indicated by large posterior probability values at internal nodes leading to this clade. Constraining the tree to produce a monophyletic Boselliidae yields a significantly less likely tree than the unconstrained topology (i.e., Figure 2). Although *B. marcusi* shares characters with *B. mimetica* including eyes posterior to rhinophores and lack of parapodia, Marcus (1973) notes several differences. These include more slender radular teeth, an unpaired furrow on the right side, a true bursa copulatrix, and a short and wide penial stylet in *B. marcusi* (Marcus 1973). The strong affinity with *Elysia timida* indicates one of two possibilities; similar morphological and molecular characters due to convergence, or *B. marcusi* is incorrectly named. In reference to convergence of morphological characters, both *B. marcusi* and *E. timida* have slender radular teeth and both feed on genera of calcified green algae, either *Halimeda* or *Acetabularia*, respectively. Clearly, more specimens of *B. marcusi* are needed before a firm conclusion can be reached. Regardless, we do not believe that the final taxonomic status of *B. marcusi* is likely to change our conclusions concerning the plakobranchids.

Taxonomic rank and evolutionary history

If taxonomic rank reflects evolutionary history, then we expect a general increase in the magnitude of divergence from the intra-specific through inter-generic to the inter-familial levels (see Johns and Avise, 1998). In addition, although there likely is a range of divergence estimates within a taxonomic level, the ranges should not, in general, substantially overlap adjacent levels and this should be even less so for non-adjacent ones. For example, the upper end of the divergence range for intra-specific comparisons would not include the lower end of the inter-familial range. An absence of this pattern likely indicates problems with the divergence estimates or the assigned taxonomy. The divergence data presented here do not conform to this predicted pattern (Table 2). As mentioned previously, the magnitude of divergence seen between the Bahamas and the Florida Keys for *E. papillosa* is quite large and indicates that further taxonomic divisions are necessary for this species. This again is indicated in that the smallest inter-generic confamilial divergence estimate is nearly identical to the largest intra-specific comparison. Clearly, this level of divergence cannot simultaneously be indicative of divergence within species and between genera. Furthermore, the inter-specific congeneric and inter-generic confamilial ranges completely overlap. In fact, the former is completely subsumed by the latter. More importantly, some divergence estimates between species within a genus ($0.124 = d = 0.196$) are larger than those seen between families ($0.115 = d = 0.245$; Table 2). It could be that the taxonomic ranks are correct as currently formulated, but molecular evolutionary rates have varied across taxa or that evolutionary rates have stayed approximately constant, but taxonomic rankings are wrong or some combination of the two. We do not believe that variable evolutionary rate alone is sufficient

explanation because highly uneven branch lengths are not seen in the tree topology (Figure 2) and both presumably too large (e.g., inter-specific congeneric comparisons) and too small (e.g., inter-generic confamilial) divergence values are seen requiring both acceleration and deceleration. Even so, given the limited taxon sampling and preliminary nature of the analysis we are unable at this time to provide a definitive answer for the discrepancy observed between the current taxonomy and the observed genetic divergence estimates.

The large molecular divergence estimates within the Plakobranchidae are consistent with Jensen's (1997a) view that the genus *Elysia* needs further division at both the generic and specific levels. The apparent decoupling of external morphology (i.e., current taxonomy) with genetic divergence indicates that we may be greatly underestimating the number of taxa within this family. If these findings hold, they are likely to affect multiple aspects of sacoglossan biology. Investigations relying on an understanding of the evolution of this family would presumably assume that the current taxonomy accurately reflects evolutionary history. Where the two diverge, the result would be either an over- or under-estimate of taxonomic diversity and misleading evolutionary inferences. For example, if kleptoplasty were a key innovation leading to radiation of the Sacoglossa as suggested by Wägele (2004) there should be a concomitant increase in the numbers of species with the evolution of kleptoplasty. If estimates of the taxonomic diversity in the clade of interest or in reference clades are either over- or under-estimates then potential radiation events are likely to be overlooked. Regardless, a stable taxonomy is in itself a laudable goal in this taxonomically protean group. An excellent example is the 150-year taxonomic history of *ornata*-like species that have variously experienced, at least three generic splits, 13 species descriptions, and seven species synonymizations (Kelaart 1858; Pease 1860; Pease 1871; Bergh 1872; Eliot 1904; Eliot 1906; Baba 1936; Baba 1957; Thompson 1973; Thompson 1977; Carlson and Hoff 1978; Marcus 1980; Heller and Thompson 1983; Jensen 1992).

Overall, the family Plakobranchidae clearly presents interesting biological and systematic issues for further investigations. The results of this study generally conform to the currently accepted relationships of the various groups. Several previously proposed revisions, however, also appear to be supported. Primarily, the genus *Elysia* contains a considerably amount of evolutionary diversity not currently reflected in its morphology or its taxonomy and *Bosellia marcusi* is likely a member of the Plakobranchidae to the exclusion of at least some of its congeners. Specific and robust inferences based on the molecular data, however, will have to wait for the results from a more complete taxon sampling effort currently underway.

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