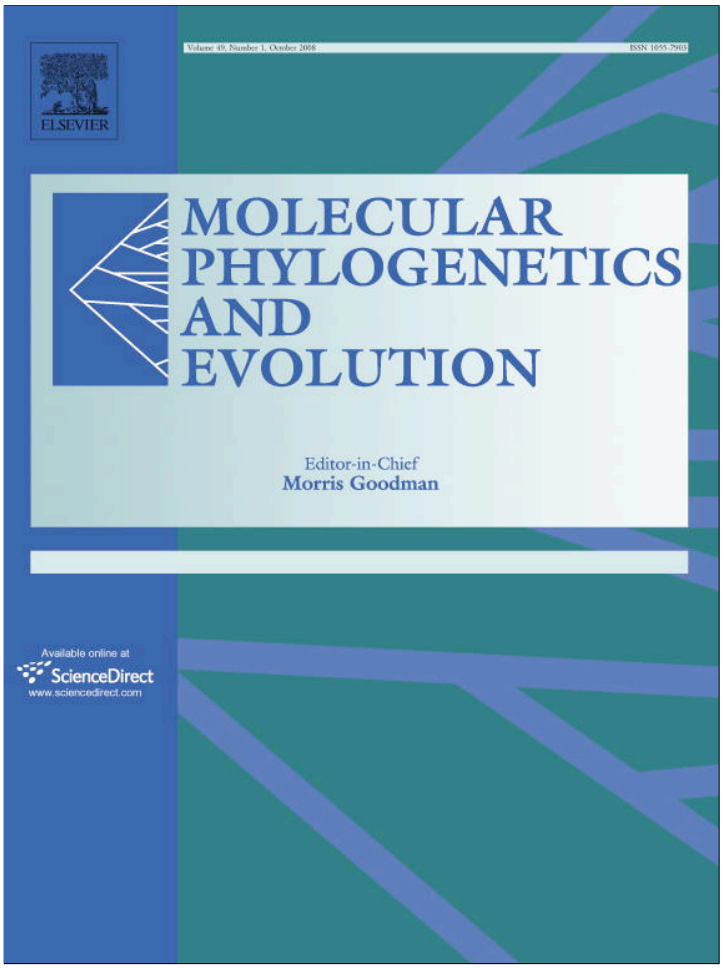

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Short Communication

Multiple origins of pelagicism within Flabelligeridae (Annelida)

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1. Introduction

Most pelagic clades are believed to have originated from benthic ancestors and open water habitat is thought to have remained open to invasion throughout evolutionary history (Rigby and Milsom, 1996). Many metazoan lineages contain at least one pelagic example, possibly due to the relative openness of the pelagic habitat. In most cases, the transition from benthic to pelagic habit is assumed to be accompanied by dramatic changes in the morphology and ecology of the animal as seen in the highly modified forms of pelagic species (e.g. *Phylliroe*, a nudibranch, *Tomopteris*, a polychaete, *Carolinites*, a trilobite) and in the varied pelagic larval forms of benthic animals. Broad scope analyses of pelagic invasion and radiation have been made for groups such as Foraminifera (e.g. Darling et al., 1997) and cladoceran (e.g. Sacherová and Hebert, 2003) and copepod (Bradford-Grieve, 2002) crustaceans, but there are few single-lineage examples using extant species. By examining individual invasion events and comparing the changes that occur in those lineages, we can learn about the "toolbox" they are working with, or the variability possible, and about the evolutionary constraints within the lineage. More importantly, these findings allow us to understand the selective pressures and evolutionary processes at work in the pelagic realm.

The majority of polychaetes are thought of as benthic, however, there are many polychaetes that swim periodically, larval and reproductive stages that utilize the water column for a portion of their life history, and several lineages that are holopelagic, or spend their entire lives in the water column (Rouse and Pleijel, 2001). Most holopelagic polychaete taxa belong within Phyllodocida and by the fact of the multiplicity of divergent clades, can be assumed to represent multiple transitions from benthic to pelagic habit (Rouse and Pleijel, 2003; Halanych et al., 2007). Possibly, the highly mobile nature of the Phyllodocida transfers well to active life in the water column.

There are a few non-phyllodocid pelagic polychaetes, notably, *Poeobius meseres* Heath, 1930, and *Chaetopterus pugaporcinus* Osborn et al., 2007. Recently, Burnette et al. (2005) placed *P. meseres* firmly within Flabelligeridae. As many pelagic organisms nested inside primarily benthic groups tend to show, *Poeobius* differs dramatically from its benthic relatives, so much so that for over 60 years its affinities were debated.

Flotidae Buzhinskaya, 1996 is a family with three described species (Salazar-Vallejo and Zhadan, 2007), *Buskiella abyssorum* McIntosh, 1885, *Flota flabelligera* Hartman, 1967, and *F. vitjasi* Buzhinskaya, 1977, all of which are pelagic. They have been closely allied to Flabelligeridae (McIntosh, 1885; Hartman, 1967; Rouse and Pleijel, 2003; Salazar-Vallejo et al., in press) because they show flabelligerid features such as extensive epidermal papillae (also shared with acrocirrids), annulated chaetae, and a retractable head. Flotids are sometimes linked to *Poeobius* (Rouse and Pleijel, 2003; Halanych et al., 2007) because of their substantial gelatinous sheath, reduced number of segments (*Poeobius* with 11 pairs of ganglia and two septa, adult flotids with nine chaetigers), and similar ecology. Because of these similarities, it has been assumed that the flotids and *Poeobius* represent a single transition from benthic to pelagic habit within the otherwise decidedly benthic Flabelligeridae (Rouse and Pleijel, 2003).

Here, we examine the phylogenetic position of *Flota*, find it to be part of Flabelligeridae, and assess pelagic transitions within Flabelligeridae.

2. Materials and Methods

2.1. Taxa

The flotid available for this project was most similar to *Flota vitjasi*, which is described from the Kuril–Kamchatka Trench in the northwest Pacific. The species used here differs from the description of *F. vitjasi* by having two pairs of gonopores located

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at the anterior margins of the fourth and fifth chaetigers instead of the single pair reported by Buzhinskaya (1977). The species is currently under review and the genus under revision by Erik Theusen (pers. comm.) and thus is not described here. This species is common off the coast of central California below 2000 m.

Flota sp. was collected by the ROV *Tiburon* outside Monterey Bay, California (specimen 1, 20 Jun. 2006 from 2816 m in 2879 m deep water at 36° 33.06′N, 122° 30.61′W; specimen 2, 28 Nov. 2007 from 2899 m in 3190 m deep water at 35° 50.42′N, 122° 40.13′W; voucher, 23 Jun. 2006 from 3452 m in 3508 m deep water at 36° 20.08′N, 122° 55.00′W). A separate voucher specimen was selected because, even if relaxed prior to removing tissue, the body distorts dramatically making it difficult to observe the features required for identification.

All flabelligerid sequences currently available were included in this project (Table 1) in order to resolve the relationships within Flabelligeridae and the relationship to the proposed sister group, Flotidae (Salazar-Vallejo et al., in press). Acrocirrids (*Flabelligena* and *Macrochaeta*) were used as the outgroup based on the findings of Struck et al. (2007), Rousset et al. (2007), and morphology (frontal palps, prostomium on top of peristomium, most with compound neurochaetae, epidermal papillae, and large (gonadal) papillae on one to three chaetigers of the anterior eight; Banse, 1969; Rouse and Pleijel, 2001; Salazar-Vallejo et al., 2007). Only vouchered and non-redundant (specimens collected from different localities) sequences available from GenBank were included in the analysis. Various sequences were concatenated for the combined analyses only if the specimens were collected from the similar localities (Table 1).

The acrocirrid, *Flabelligena* sp., included here is an undescribed species. This species differs from all described *Flabelligena* in the possession of two pairs of branchiae, a pair of gonopores in the seventh chaetiger (described as large ventral papillae by Aguirrezabalaga and Ceberio, 2006), two spinulose notochaetae per notopodium, two to three compound hooded hooks per neuropodium, and lacking eyes. Likewise, *Macrochaeta* sp. included here is an undescribed species most similar to *M. clavicornis* (M. Sars, 1835). They differ in the number of notochaetae per chaetiger and their genetics (*COI* uncorrected distance, 16.7%).

2.2. Genetic data collection

Tissue was placed in chilled 95% ethanol or RNAlater (Ambion, Austin, TX) following collection. Genomic DNA was extracted from specimens using DNAzol® Genomic DNA Isolation Reagent (Molecular Research Center, Inc., Cincinnati, OH) with modifications to the manufacturer's instructions (Osborn et al., 2007).

An approximately 1800 base pair fragment of small subunit ribosomal (*18S*) DNA was amplified with universal primers mitchA and mitchB or TimA and TimB (Table 2). An approximately 1100 base pair fragment of large subunit ribosomal (*28S*) DNA was amplified with universal primers LSUD1F and D3ar or C1 and R4. An approximately 650 base pair fragment of the mitochondrial Cytochrome Oxidase I (*COI*) gene was amplified using universal primers HCO2198 and LCO1490. An approximately 360 base pair fragment of Cytochrome *B* (*CytB*) was amplified from universal primers 424F and 876R (Table 2). An approximately 350–500 base pair fragment of mitochondrial small subunit ribosomal (*16S*) DNA was amplified either from universal primers arL and brH or annelid specific primers AnnF and AnnR (Table 2). Twenty-five microliter reactions were carried out using either Illustra PuReTaq Ready-To-Go PCR Beads (GE Healthcare, Uppsala, Sweden) or Promega GoTaq Green (Madison, WI). Amplification profiles were optimized for each extraction (Table 2).

PCR products were sequenced directly after spin column purification (Ultrafree-DA columns, Millipore, Billerica, MA) or after clean up with ExoSAP-IT (GE Healthcare, Uppsala, Sweden) following the manufacturers' protocols. All direct sequencing was carried out using the same primers that were used in the amplification, with the addition of four internal primers for *18S* (600F, a2.0, 4FBK, bi; Table 2). All sequencing was carried out by Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) at the University of Hawaii at Manoa using Applied Biosystems BigDye terminator chemistry and an ABI 3730XL sequencer. *CytB* sequences are forward reads only because sequencing reactions were never successful with reverse primers (Table 2; also tried 825R referenced in Burnette et al., 2005). Sequences were deposited in GenBank (Table 1).

Table 1
Specimen information and accession numbers

Taxon	*18S*	*28S*	*COI*	*CytB*	*16S*	Voucher	Locality
Ingroup							
Brada villosa (Rathke, 1843)	EU791460	EU791462	–	–	–	SIO BIC A1161	Fiskebäckskil, Sweden
Brada villosa (Rathke, 1843)	–	–	–	AY727747	–	USNM 1073357[a]	Trondheimsfjord, Norway[a]
Brada villosa (Rathke, 1843)	AY708535	–	–	–	–	USNM 1073349[a]	Central California, USA[a]
Diplocirrus glaucus (Malmgren, 1867)	AY708534	DQ790031	–	AY727751	–	USNM 1073353[a,b]	Gullmarsfjorden, Sweden[a,b]
Flabelliderma ockeri Salazar-Vallejo, 2007	EU694119	–	EU694127	EU694137	EU694111	SIO BIC A1129	La Jolla, California, USA
Flabelligera affinis Sars, 1829	AY708531	–	–	AY727755	–	USNM 1073355[a]	Central California, USA[a]
Flabelligera affinis Sars, 1829	AY708532	–	–	–	–	USNM 1073354[a]	Gullmarsfjorden, Sweden[a]
Flabelligera affinis Sars, 1829	–	DQ779688	–	–	DQ779614	SAM E3562	Iceland
Flabelligera infundibularis (Johnson, 1901)	EU694118	EU694124	EU6694131	EU694133	EU694112	SIO BIC A1128	Astoria, Oregon, USA
Flota sp., specimen 2	EU694117	–	EU694129	EU694132	–	SIO BIC A1131	Monterey, California, USA
Flota sp., specimen 1	EU694116	EU694110	EU694128	EU694134	EU694110	SIO BIC A1131	Monterey, California, USA
Ilyphagus octobranchus Hartman, 1965	AY708530	–	–	AY727749	–	USNM 1073351[a]	Woods Hole, Massachusetts, USA[a]
Pherusa plumosa (Mueller, 1776)	AY708529	–	–	AY727756	–	USNM 1073348[a]	Woods Hole, Massachusetts, USA[a]
Pherusa plumosa (Mueller, 1776)	AY708528	DQ790056	–	AY727752	–	USNM 1073356[a,b]	Central California, USA[a,b]
Poeobius meseres Heath, 1930	EU694115	EU694123	EU694130	EU700415	–	SIO BIC A1130	Monterey, California, USA
Poeobius meseres Heath, 1930	–	–	–	–	DQ779631	SAM E3563	Monterey, California, USA
Therochaeta collarifera Ehlers 1887	AY708527	–	–	AY727753	–	USNM 1073350[a]	Woods Hole, Massachusetts, USA[a]
Ougroup							
Macrochaeta clavicornis (Sars, 1835)	EU791461	–	EU791463	–	–	SIO BIC A1087	Vattenholmen, Sweden
Macrochaeta clavicornis (Sars, 1835)	–	DQ779696	–	–	–	SMNH 75829	Bohuslån, Sweden
Macrochaeta sp.	EU700414		EU694125	EU694136	EU694114	SIO BIC A1127	Belize
Flabelligena sp.	EU694120	EU694121	EU694126	EU694135	EU694113	SIO BIC A1126	Pacific Antarctic Ridge

Shaded pairs were concatenated in the combined analyses.
[a] Burnette et al. (2005).
[b] Struck et al. (2007), Struck, pers. comm.

Table 2
Amplification and sequencing primers with associated amplification protocols

Name	Sequence	Source
18S[a]		
mitchA	5′-CAA CCT GGT TGA TCC TGC CAG T-3′	Medlin et al., 1988 Gene
mitchB	5′-TGA TCC TTC CGC AGG TTC ACC TAC-3′	Medlin et al., 1988 Gene
TimA	5′-AMC TGG TTG ATC CTG CCA G-3′	Norén & Jondelius, 1999 Cladistics
TimB	5′-TGA TCC ATC TGC AGG TTC ACC T-3′	Norén & Jondelius, 1999 Cladistics
600F	5′-GGT GCC AGC AGC CGC GGT-3′	Norén & Jondelius, 1999 Cladistics
a2.0	5′-ATG GTT GCA AAG CTG AAA C-3′	Giribet et al., 1999 Phil. Trans. R. Soc.
4FBK	5′-CTG GAA TTA CCG CGG CTG CTG G-3′	Norén & Jondelius, 1999 Cladistics
Bi	5′-GAG TCT CGT TCG TTA TCG GA-3′	Giribet et al., 1999 Phil. Trans. R. Soc.
28S[b]		
C1	5′-ACC CGC TGA ATT TAA GCA T-3′	Lê et al., 1993 Mol. Phy. Evol.
R4	5′-GTT CAC CAT CTT TCG GGT CCC AAC-3′	Struck et al., 2006 Syst. Biol.
LsudiF	5′-ACC CGC TGA ATT TAA GCA TA-3′	Lenaers et al., 1989 J. Mol. Evol.
D3aR	5′-ACG AAC GAT TTG CAC GTC AG-3′	Lenaers et al., 1989 J. Mol. Evol.
16S[c]		
arL	5′-CGC CG TTT ATC AAA AAC AT-3′	Palumbi et al., 1991 Fool's Guide
brH	5′-CCG GTC TGA ACT CAG ATC ACG T-3′	Palumbi et al., 1991 Fool's Guide
AnnF	5′-GCG GTA TCC TGA CCG TRC WAA GGT A-3′	Sjölin et al., 2005 Mol. Phy. Evol.
AnnR	5′-TCC TAA GCC AAC ATC GAG GTG CCA A-3′	Sjölin et al., 2005 Mol. Phy. Evol.
COI[d]		
LCO-1490	5′-TCA ACA AAT CAT AAA GAT ATT GG-3′	Folmer et al., 1994 Mol. Mar. Biol. Biotech.
HCO-2198	5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′	Folmer et al., 1994 Mol. Mar. Biol. Biotech.
CytB[e]		
424F	5′-GGW TAW GTW YTW CCW TGR GGW CAR AT-3′	Boore & Brown, 2000 Gen. & Dev.
876R	5′-GCR TAW GCR AAW ARR AAR TAY CAY TCW GG-3′	Boore & Brown, 2000 Gen. & Dev.

[a] 35 ramping cycles of: 94 °C for 60 s, 56–64 °C for 60 s, 72 °C for 90 s, initial denaturation at 94 °C for 3 min, final extension at 72 °C for 5 min.
[b] 30–35 cycles of 94 °C for 60 s, 57–60 °C for 60 s, 72 °C for 80 s, initial denaturation at 94 °C for 3 min, final extension at 72 °C for 5 min, optionally with 5 cycles of 94 °C for 60 s, 52 °C for 60 s, 72 °C for 80 s preceding the 30 cycles above.
[c] 30–35 cycles of 94 °C for 40 s, 45–49 °C for 40 s, 72 °C for 45 s, initial denaturation at 94 °C for 3 min, final extension at 72 °C for 5 min, optionally with 5 cycles of 94 °C for 40 s, 45 °C for 40 s, 72 °C for 45 s preceding the 30 cycles above.
[d] 5 cycles of: 94 °C for 30 s, 45 °C for 90 s, 72 °C for 60 s, 30 cycles of 94 °C for 30 s, 51 °C for 90 s, 72 °C for 60 s, initial denaturation at 94 °C for 60 s, final extension at 72 °C for 5 min.
[e] 35 ramping cycles of 94 °C for 45 s, 42 °C for 45 s, 72 °C for 45 s, initial denaturation at 94 °C for 3 min, final extension at 72 °C for 5 min, 4 μl of 25 mM $Mg(OAc)_2$ was used to each reaction.

2.3. Analysis

Table 1 shows what genes were included in all analyses. Sequences were aligned with MUSCLE (Edgar, 2004) and proofread by eye in MacClade v.4.04. No data was excluded from analyses. The alignments were deposited in GenBank (Table 1) and are also available from Treebase. Individual genes were analyzed both singly and concatenated into a combined analysis. A large number of target sequences are missing from the analysis due to the inclusion of all available flabelligerid sequences from GenBank and the unavailability of the tissue or extracts from which those sequences were derived.

Parsimony analyses were conducted with PAUP* 4.0b10. Parsimony trees were reconstructed from an equally weighted character matrix and the heuristic search option, using the tree-bisection-reconnection branch-swapping algorithm, and 1000 random addition replicates. Gaps were treated as missing data because of the missing sequences. Bootstrap values were obtained with the same settings as the parsimony searches (100 replicates).

Bayesian analyses of the data sets were conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). Standard procedures based on Modeltest 3.5 were implemented in PAUP to select the most appropriate models for the analyses. The relative fit of models was assessed by the Akaike Information Criterion (AIC). Smaller values of AIC were preferred (Posada and Crandall, 2001) and the GTR + I + Γ represented the optimal model with respect to the *18S*, *28S*, and *CytB* data, GTR + SS with respect to the *COI* data, and the GTR + Γ with respect to the *16S* data. Sequence data was partitioned into unlinked individual genes and in the cases of the protein coding genes, into first, second, and third positions. Each Markov chain, three heated and one cold, was started from a random tree and all four chains ran simultaneously for 3–5 million generations, with trees being sampled so that the resulting data set from each run contained at least 10,000 data points after at least 30% had been discarded as burnin. AWTY (Wilgenbusch et al., 2004) was used to check if analyses were run until stationarity, or convergence of each chain, was reached before inference from the MCMC data set was made. Several repetitions of each analysis converged on similar parameter estimates.

3. Results

Flotids are nested within Flabelligeridae in all analyses (Figs. 1 and 2). A parsimony analysis of the combined data set was run with *Flota* constrained to the sister group of the Flabelligeridae. This resulted in two most parsimonious trees that were 108 steps longer than the two most parsimonious trees without the constraint, increased homoplasy (e.g. rescaled consistency index was 0.027 higher in the unconstrained analysis), and a significantly less parsimonious explanation of the data (Templeton's test, $p < 0.0001$).

The addition of several taxa to the matrix did not change the sistergroup relationship of *Poeobius* and *Therochaeta* found by Burnette et al. (2005). *18S* and *28S* data strongly support a sister relationship between *Flota* and *Brada* while the *COI* analysis suggested a sister relationship to the morphologically similar *Flabelligera/Flabelliderma* clade (Fig. 2), though *COI Brada* sequences were not available.

The two pelagic species included are not most closely related according to any of the genetic markers (Fig. 2). A parsimony analysis of the combined data set was run with *Poeobius* and *Flota* constrained to sister taxa. This resulted in two most parsimonious

trees that were 180 steps longer than the two most parsimonious trees without the constraint, increased homoplasy (e.g. rescaled consistency index was 0.048 higher in the unconstrained analysis), and a significantly less parsimonious explanation of the data (Templeton's test, $p < 0.0001$).

Three pairs of specimens that were identified based on morphology as belonging to the same species in the original study (Burnette et al., 2005), but were collected from widely different locations, were shown to be decidedly different from each other. Specimens identified as *Pherusa plumosa,* but collected from different ocean basins varied 16% and 24% in their available *18S* and *CytB* sequences, respectively. Similarly, *Brada villosa* specimens varied 7% in their *18S* sequences and specimens identified as *Flabelligera affinis* differed 1% in their *18S* sequences. *Flabelligera affinis* and *Flabelligera infundibularis* were synonymized by Pettibone (1954) but differed by 1%, 15%, 11%, and 19% in their *18S, 28S, 16S*, and *CytB* sequences, respectively.

4. Discussion

4.1. Flotids included in Flabelligeridae

Our results clearly show that flotids are nested within flabelligerids. This finding confirms McIntosh's suggested placement of *Buskiella* within Flabelligeridae (as Chloraemidae; 1885) and likewise Hartman's original placement of *Flota* (1967). In addition to the molecular sequence data, several morphological features unite Flabelligeridae and the flotids. Flabelligerids have the blood pigment chlorocruorin (Banse, 1969), which gives some a yellow–green color. They have an eversible head, consisting of the prostomium with a pair of grooved palps, peristomium, and numerous branchiae. The "trifid organ" found in flotids requires further work to determine its homology with the palps, branchiae (Salazar-Vallejo and Zhadan, 2007), or both. Flabelligerids typically possess some sort of body covering modified by the

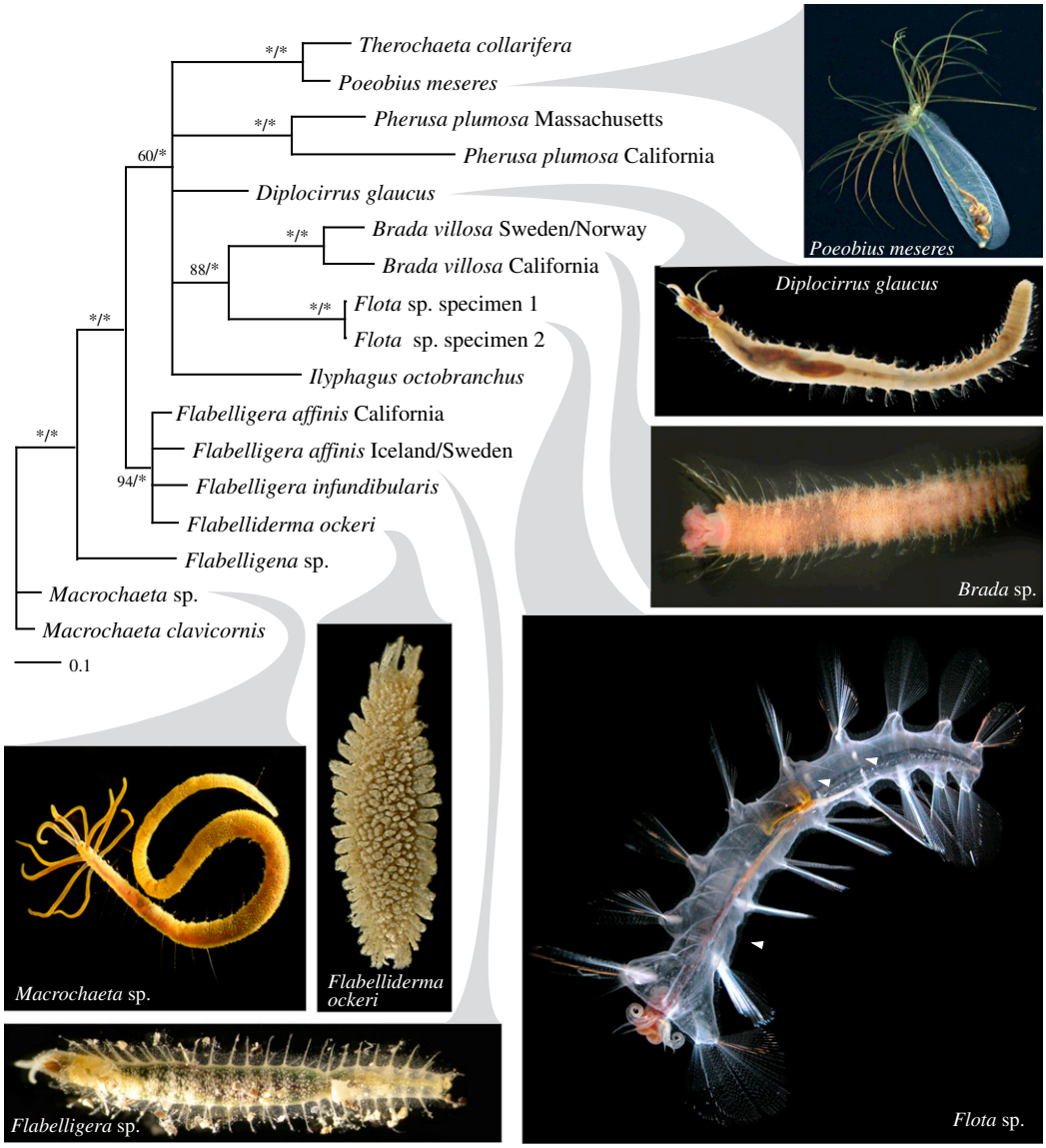

Fig. 1. Results of the combined Bayesian analyses of five genetic markers assessing the relationship of *Flota* sp. to Flabelligeridae. Eighty percent majority rule consensus tree of the Bayesian analyses with support indicated as bootstraps from the parsimony analysis, then posterior probabilities from Bayesian analyses. Asterisks indicate 100% bootstrap or 1.0 posterior probabilities. *Flota* sp., live dorsal view of voucher specimen, anterior in lower left. The head is everted such that the branchiae and lateral lips are visible. When alive the body was 91 mm long excluding the everted head. The segmentation of the chaetae is evident as reflective crossbars along the entire length of each chaeta. The dorsolateral and oblique muscle bands are visible through the transparent body wall. White arrows indicate the narrow gelatinous sheath (lowest) and developing male gonads in chaetigers 5 and 6 (upper pair). Exemplars of *Macrochaeta*, *Flabelligera*, *Brada*, *Flabelliderma ockeri*, *Diplocirrus glaucus*, and *Poeobius meseres* (*in situ*) are also shown. Photo credits: G.W.R., *Macrochaeta* sp., *Flabelligera* sp., *Brada* sp., *F. ockeri, D. glaucus*; K.J.O., *Flota* sp.; MBARI's ROV *Tiburon*, *P. meseres*.

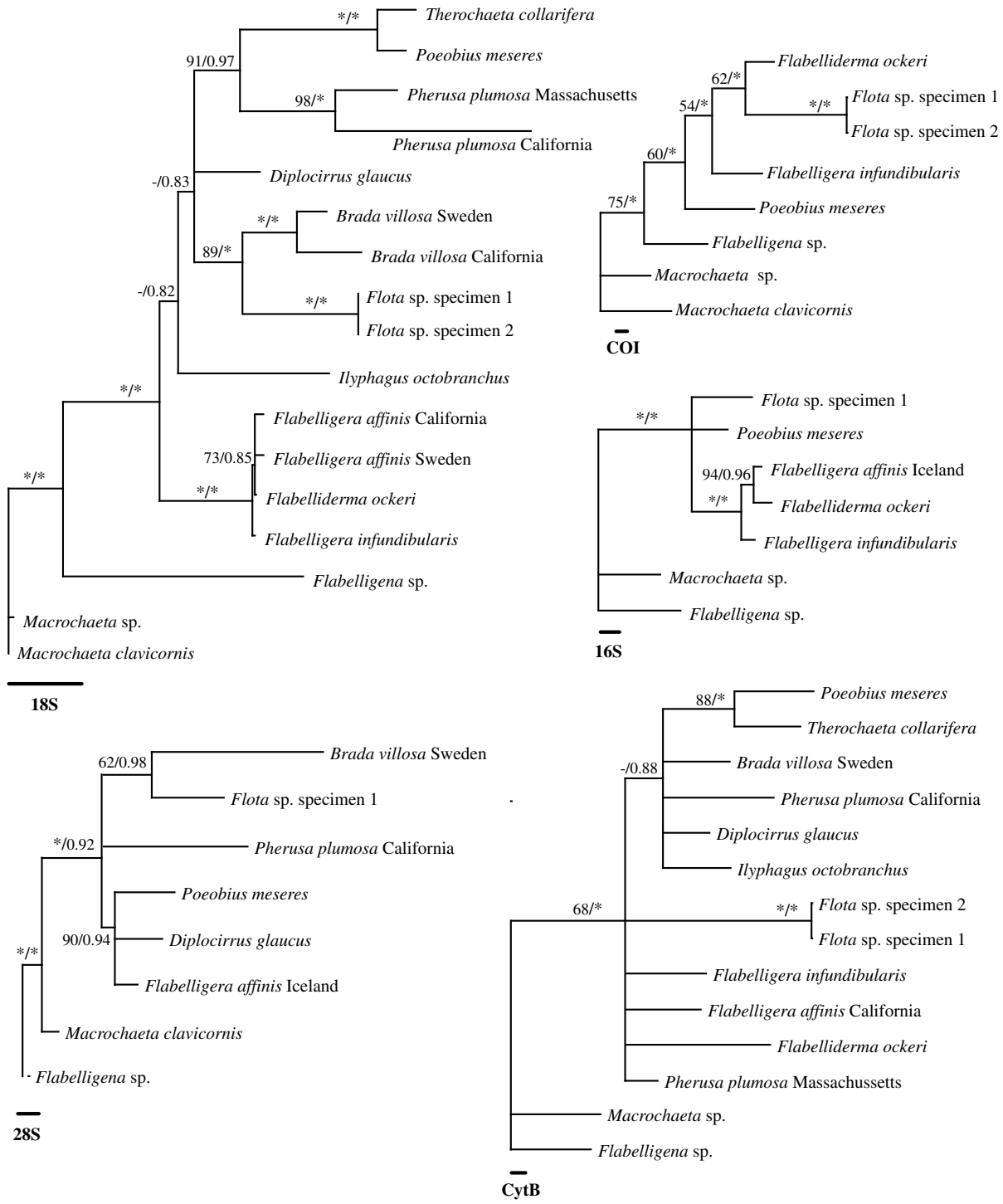

Fig. 2. Individual gene trees from Bayesian analyses. Eighty percent majority rule consensus tree of the Bayesian analyses with support indicated as bootstraps from the parsimony analysis, then posterior probabilities from Bayesian analyses. Asterisks indicate 100% bootstrap or 1.0 posterior probabilities, dashes indicate branches not supported by the parsimony analyses. Scale bars for *18S*, *28S*, and *16S* = 0.1 and *COI* and *CytB* = 10.

papillae; this ranges in form from foreign particles adhered to mucus on individual papillae (e.g. *Flabelliderma, Brada*, and *Pherusa*) to a solid mucus sheath covering the body and penetrated by numerous long papillae (*Poeobius*, *Flota*, and *Flabelligera*). Acrocirrids have body papillae, sometimes with fine adhered sediment as well, but as yet have not been found to form a gelatinous sheath. Salazar-Vallejo et al. (2007) additionally defined Flabelligeridae by the segmented, or articulated chaetae, a feature shared with *Flota* and *Buskiella*.

Flotidae was established by Buzhinskaya (1996) and later supported by Salazar-Vallejo and Zhadan (2007) based on the reduced and constant number of segments, branched palps (called trifid organs by SV & Z), and the nature of the ventral nerve cords. Buzhinskaya (1996) suggested that the "reduced head blade, oral tentacles, sacculate ventral pharyngeal organ, bibranchial parapodia with simple chaetae of two types, one or two pairs of nephridia between segments II and V, and funnel-shaped pygidium" characterized flotids and separated them

from Flabelligeridae. However, each of these characters is either shared with other flabelligerids, or is of questionable validity. For example, oral tentacles were not reported in the original description of the specimens (Buzhinskaya, 1977) and were not found by other researchers examining specimens from the same location (Salazar-Vallejo and Zhadan, 2007). The "nephridia" between the segments are actually gonopores and are known from other flabelligerids and acrocirrids, typically referred to as segmental organs, and most easily seen as small papillae projecting into the gelatinous sheath (Heath, 1930; Robbins, 1965; Hobson and Banse, 1981) or between segments in the case of acrocirrids (Banse, 1969). Salazar-Vallejo et al. (2007) additionally used the relative position of the first chaetiger, the relative size of the second chaetiger, and their pelagic habit to distinguish flotids from flabelligerids. Unfortunately, both chaetiger characters are preservation artifacts since there is no trace of them in live animals and significant distortion occurs during fixation and preservation. Thus, these chaetiger characters should not be used to distinguish this group from the Flabelligeridae.

Based on the agreement of previous morphological work and the molecular evidence presented here, we suggest that *Buskiella abyssorum* be returned to Flabelligeridae, that *Flota flabelligera* and *F. vitjasi* be referred to Flabelligeridae, and that Flotidae is a junior synonym of Flabelligeridae Saint-Joseph, 1894.

4.2. Relationships within Flabelligeridae

The available *Brada* species form the sister group to *Flota*. Like *Flota*, *Brada* possess gonopores on one or two of the third through fifth chaetigers (Hobson and Banse, 1981; Blake, 2000). These structures have typically been referred to as nephridial papillae, but in the few cases that have been possible to examine, are associated with segments containing the gonads and are coelomoducts similar to those seen in *Poeobius* (Robbins, 1965). Additionally, *Brada* and *Flota* have only simple neurochaetae and neither genera have a cephalic cages (all chaetae are relatively the same length, Salazar-Vallejo et al., in press).

The Burnette et al. (2005) study included two specimens each of *Pherusa plumosa, Brada villosa*, and *Flabelligera affinis* from various ocean basins. Molecular evidence provided in their own paper showed that their *P. plumosa, B. villosa*, and *F. affinis* specimens each represented two species. Thus, what they referred to as *P. plumosa, B. villosa*, and *F. affinis* are probably cosmopolitan species complexes currently indistinguishable by morphology. This finding emphasizes the importance of placing vouchers in a public museum for all sequences placed in GenBank.

Although without strong support, our results indicate that the two specimens of *Flabelligera affinis* were more closely related to *Flabelliderma ockeri* than to *Flabelligera infundibularis (18S and 16S)*. *Flabelligera affinis* and *F. infundibularis* were synonymized by Pettibone (1954; supported by Blake, 2000; Blake and Ruff, 2007). *Flabelligera affinis* collected from Sweden (and possibly the specimen from Iceland) probably represents the species since it was collected near the type locality. Genetic differences between *F. affinis* from California and *F. infundibularis* from deep water (2815 m) off of Oregon indicate that there are two species off western North America, possibly separated by depth.

We assume that the *B. villosa* Sweden sequence represents the species because it was collected near the type locality. The *B. villosa* from California is probably *B. pilosa* Moore, 1906, a species synonymized with *B. villosa* by Pettibone (1954).

The extremely long branches separating the *Pherusa plumosa* specimens in the *18S* tree and lack of a relationship in the *CytB* data suggest, not only that the species with the "*plumosa*" morphology are different species, but that they may not be sister taxa. The *Pherusa plumosa* specimen collected from Massachusetts is assumed to represent the species because it was collected near the type locality. The *Pherusa plumosa* specimen collected from California is thought to represent *P. neopapillata* based on the relatively larger size of the dorsal papillae of this specimen (vouchers USNM 1073348 and 1073356 were examined). Further sampling across a range of other flabelligerids is required to determine the extent of the paraphyly and the relationships within Flabelligeridae.

4.3. Two pelagic origins

Poeobius and *Flota* are not sister groups as proposed by Rouse and Pleijel (2003), and thus it is unlikely that they are the result of a single invasion of the water column. Instead, it appears that they represent two separate origins of pelagicism. This conclusion is based on the assumption that the flabelligerid ancestor was benthic. This is justifiable because their sister group (Acrocirridae), as well as Cirratuliformia from which they are thought to stem, are all benthic (Rouse and Pleijel, 2001). Flabelligerid genera exhibit wide morphological diversity (Fig. 1) indicating that the clade is morphologically plastic. Changes in the form of the structures of the eversible head, the body papillae and associated gelatinous sheath, and the chaetae, are responsible for the majority of the variability found among genera. This plasticity may be why two groups were able to successfully invade the water column while most other polychaete groups have not.

The three genera possessing thick gelatinous sheaths (*Poeobius*, *Flota/Buskiella*, and *Flabelligera*) are not monophyletic and thus this character must be homoplastic. Additionally, the two holopelagic lineages show convergence in reduced segment number. This is also observed in a holopelagic chaetopterid (Osborn et al., 2007) and some tomopterid species (a holopelagic lineage of Phyllodocida). Further examination of morphological transitions associated with pelagic habit requires increased taxon sampling and better resolution of the flabelligerid tree.

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