

# Phylogenetics of Acrocirridae and Flabelligeridae (Cirratuliformia, Annelida)

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When seven deep-sea, swimming cirratuliforms were recently discovered, the need for a thorough phylogenetic hypothesis for Cirratuliformia was clear. Here, we provide a robust phylogenetic hypothesis for the relationships within Acrocirridae and increase the taxon sampling and resolution within Flabelligeridae based on both molecular (*18S*, *28S*, *16S*, *COI* and *CytB*) and morphological data. Data partitions were analyzed separately and in combination. Acrocirridae and Flabelligeridae were reciprocally monophyletic sister groups when rooted by cirratulids. The seven recently discovered species form a clade within Acrocirridae and will be designated as four genera based on phylogenetic relationships and apomorphies. A revised diagnosis is provided for *Swima*, restricting the genus to three species distinguished by a thick gelatinous sheath, transparent body, simple nuchal organs, a single medial subulate branchia, and four pair of small segmental branchiae modified as elliptical, bioluminescent sacs. *Helmetophorus* and *Chauvinelia* are maintained as separate genera based on morphological differences. Evidence for flabelligerid branchiae being segmental is provided, the papillae on segment two of most acrocirrids is confirmed to be the nephridiopores, and scanning electron microscopy is used to examine acrocirrid spinous chaetae in comparison with flabelligerid segmented chaetae.

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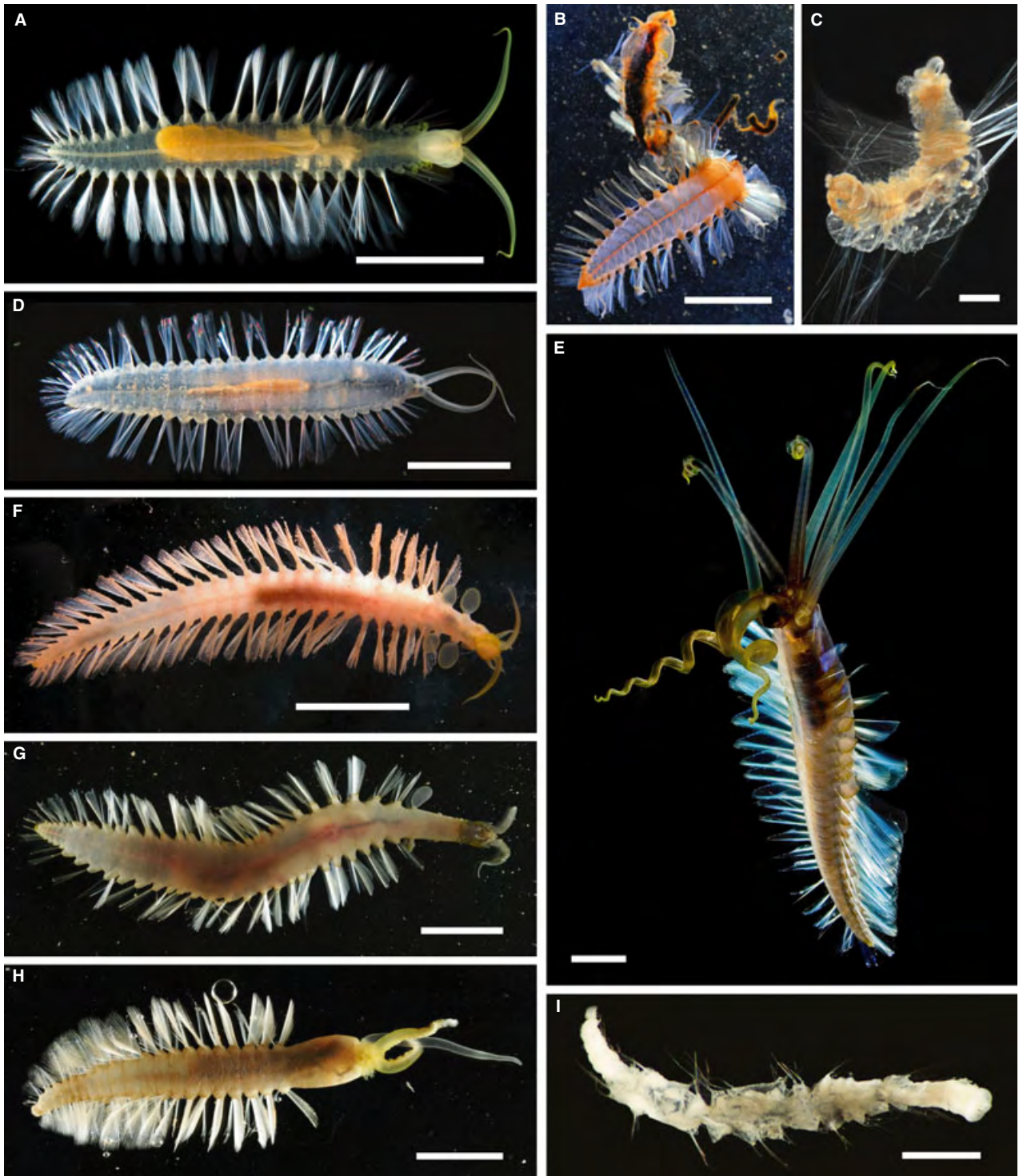
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## Introduction

The recent discovery of seven new members of Acrocirridae (Fig. 1; Table 1; Osborn *et al.* 2009) emphasized the need for a thorough phylogenetic treatment of Cirratuliformia. Addition of these new species to Acrocirridae, one of which, *Swima bombiviridis*, was formally described in Osborn *et al.* (2009), markedly increased the morphological and size diversity within the group. The striking differences observed between the recently discovered swimming species and other known acrocirrids were not surprising considering the pelagic (living in open water, not associated with the seafloor) and benthopelagic (living both in the water column and associated with the seafloor) lifestyles of the new species. Interestingly, some acrocirrids *Helmetophorus rankini* Hartman 1978; *Chauvinelia* spp. and possibly even some *Acrocirrus* were previously suspected of being able to swim (Banse 1969; Averincev 1980; Kirkegaard 1982). Also, several flabelligerids are pelagic, *Buskiella*

*abyssorum* McIntosh 1885 (Mesnil 1899; Fauvel 1916), *Flota* spp. and *Poebius meseres* Heath 1930, and these likewise differ dramatically from their benthic relatives. These swimming flabelligerids and acrocirrids possess features, e.g. thick gelatinous sheathes and long, clavate papillae, numerous elongate chaetae (*P. meseres* excepted), broad, flat chaetae (two of the recently discovered species), transparent bodies, and bioluminescence that may be homologous or convergent. Yet to understand the origin of the unusual structures found in the various swimming species and to understand the evolutionary transformations that took place in the transition from benthic to pelagic, a well-resolved evolutionary history of Cirratuliformia is needed.

Rouse & Fauchald (1997) found Acrocirridae, Cirratulidae, Ctenodrilidae, Fauveliopsidae, Flabelligeridae, Flotidae, Poebiiidae and Sternaspidae formed a clade later named Cirratuliformia (Rouse & Pleijel 2001). Poebiiidae



**Fig. 1** Swimming acrocirrids. —A. *Swima bombiviridis*, photo C. W. Dunn. —B. ‘Tawi-tawi Bomber’, photo L. P. Madin. —C. *Chauvinelia arctica* ZMUC POL-0159. —D. ‘Shining Bomber’ missing all elliptical branchiae. —E. ‘Squidworm’ missing a branchia, photo M. Aw. —F. ‘Horned Bomber’ missing three elliptical branchiae. —G. ‘Tiburon Bomber’ missing six elliptical branchiae, photo F. Pleijel. —H. ‘Juanita worm’ missing seven branchiae, photo F. Pleijel. —I. *Helmetophorus rankini* type USNM-46749. Scale bars: A–B and D–H = 10 mm, C and I = 1 mm.

**Table 1** Characteristics of swimming acrocirrids

Taxa	Authority	Type locality	Depth (m)	Size <sup>a</sup> (mm)	Subulate branchiae	Main branchiae	Nuchal organs	Neurochaetae	Apomorphy
<i>Swima bombiviridis</i>	Osborn et al. 2009	Monterey Canyon, NE Pacific	2732–3744	28	Single medial	Elliptical, small <sup>b</sup>	Straight ridge	Capillary	Transparent anterior gut
Shining Bomber	This report	Monterey Canyon, NE Pacific	3267–3625	30	Single medial	Elliptical, small <sup>b</sup>	Straight ridge	Capillary	Dark pigmented anterior gut
Tawi-tawi Bomber	This report	Celebes Sea, Philippines	2836	45	3 in 1 row	Elliptical, small <sup>b</sup>	Straight ridge with single 180° bend	Compound	3 subulate branchiae, broad, flat compound neurochaetae
Horned Bomber	This report	Astoria Canyon, NE Pacific	1863	42	6 pairs in 3 rows	Elliptical, large <sup>b</sup>	Convoluted ridge <sup>c</sup>	Compound	Number and arrangement of subulate branchiae
Tiburon Bomber	This report	Juan de Fuca Ridge, NE Pacific	2367	59	8 pairs in 4 rows	Elliptical, large <sup>b</sup>	Convoluted ridge <sup>c</sup>	Compound	Notochaetae broad and flat with sharp point and 90 degree elbow at tip
<i>Helmetophorus rankini</i>	Hartman 1978	Weddell Sea, Antarctica	3111	6	3 pairs in 2 rows	Elliptical, small <sup>b</sup>	Convoluted ridge <sup>c</sup>	Capillary	Helmet-like cephalic hood
<i>Chauvinella biscayensis</i>	Laubier 1974	Bay of Biscay, NE Atlantic	4455	16	3 pairs in 2 rows	Unknown	Convoluted ridge <sup>c</sup>	Compound	Unknown
<i>Chauvinella arcica</i>	Averincev 1980	Polar Sea, Canada	3290–3380	20	0–3 pairs in 3 rows	Unknown	Convoluted ridge <sup>c</sup>	Compound	Foliose palps
Squidworm	This report	Celebes Sea, Philippines	2028–1912	90	None	Elongate	Convoluted ridge extending onto oppositely branched, free-standing structures	Capillary	Broad, flat notochaetae abruptly tapered to minute tip
Juanita worm	This report	Juan de Fuca Ridge, NE Pacific	2257–2291	41	None	Elongate	Convoluted ridge extending onto oppositely branched and spiral, free-standing structures	Pseudocompound	3 elongate featureless anterior segments, nuchal organs extending onto spiral, free-standing structures, pseudocompound neurochaetae

<sup>a</sup>Maximum length of types; <sup>b</sup>Small is less than half width body at widest, large is more than half width body at widest; <sup>c</sup>With multiple 180° bends.

and Flotidae were recently synonymized with Flabelligeridae (*Poebius* by Burnette *et al.* 2005; *Flota* by Osborn & Rouse 2008) and Ctenodrilidae placed within Cirratulidae (Bleidorn *et al.* 2003a; Rouse & Pleijel 2003). The question of the affinities of Sternaspidae and Fauveliopsidae remain unresolved, but Struck *et al.* (2007) and Rousset *et al.* (2007) both found these taxa to be sistergroups and not closely related to other Cirratuliformia. Thus, Cirratuliformia could be considered to consist of three families, Cirratulidae, Acrocirridae and Flabelligeridae. These three families share several distinctive features including: a pair of large anterior nephridia, chlorocruorin as a blood pigment, and a heart body (Banse 1969), as well as paired palps (Rouse & Fauchald 1997), though whether any of these are apomorphic is not yet resolved as all of these features are found in other polychaete groups (Rouse & Fauchald 1997).

Cirratulidae and, to an extent, Flabelligeridae were for many years 'dumping grounds' for taxa of uncertain affinities and both suffer from poorly understood phylogenies. Acrocirridae was erected from two genera previously included in Cirratulidae, *Acrocirrus* and *Macrochaeta*, by Banse (1969) who noted their similarity to flabelligerids on one hand and to the cirratulid genus *Dodecaceria* on the other. Acrocirridae grew with Orensanz (1974) transferring *Flabelligella* from Fauveliopsidae and Gillet (2001) then splitting *Flabelligella* when erecting *Flabelligena*. Laubier (1974) introduced *Chauvinelia biscayensis* as a flabelligerid, but Averincev (1980) moved the genus to Acrocirridae when describing a second species (*Chauvinelia arctica*). George & Hartmann-Schröder (1985) aligned *H. rankini* with Phyllodocida, but Glasby & Fauchald (1991) transferred it to Flabelligeridae. *Helmetophorus rankini* was then transferred, along with *Flabelliseta* (another flabelligerid), to Acrocirridae by Salazar-Vallejo *et al.* (2007). Thus, Acrocirridae currently consists of eight genera: *Acrocirrus*, *Macrochaeta*, *Flabelligella*, *Flabelligena*, *Chauvinelia*, *Helmetophorus*, *Flabelliseta* and *Swima*. Phylogenetic hypotheses based on morphological characters alone (Kristensen 1997; Rouse & Pleijel 2003; Salazar-Vallejo *et al.* 2008) have returned various hypotheses for relationships within Flabelligeridae and Acrocirridae and there has been no attempt to date on Cirratulidae. Fauchald & Rouse (1997) found no apomorphies to support monophyly of Acrocirridae, yet Rouse & Pleijel's (2003) Cirratuliformia analysis recovered the clade in all shortest trees, with the homoplastic presence of 'pseudocompound' chaetae being an apomorphy for the clade. Salazar-Vallejo *et al.* (2007) offered an amended diagnosis, and Salazar-Vallejo *et al.* (2008) found the spinous nature of the chaetal surface was an apomorphy for Acrocirridae.

Analyses of DNA sequence data have also been used to assess relationships of Flabelligeridae and Acrocirridae

(Burnette *et al.* 2005; Osborn & Rouse 2008; Osborn *et al.* 2009), but many questions remain, and a combined molecular and morphological approach has not yet been attempted. Here, we use morphological (29 characters) and molecular [*18S*, *28S*, *16S*, *COI* and Cytochrome B gene (*CytB*) sequences] evidence to reconstruct the evolutionary history of 17 flabelligerids and 11 acrocirrids, with seven cirratulids as nominal outgroups. Our objectives were to: (i) place the seven recently discovered acrocirrids in a stable phylogenetic framework in preparation for the description of the six remaining undescribed species (Osborn, Haddock & Rouse *in review*; Osborn, Madin & Rouse *in review*; Osborn & Pleijel *in prep.*), (ii) use comparative methods to determine structure, function and homology of morphological features of acrocirrids and flabelligerids, (iii) elucidate historical relationships within Flabelligeridae and Acrocirridae and (iv) generate an evolutionary framework on which to assess questions about adaptations for life in the water column.

## Materials and methods

### Specimens

Specimens of the seven recently discovered acrocirrids were recovered in varying conditions and preserved using several methods (70 or 95% ethanol, 2% sodium cacodylate buffered glutaraldehyde, 4–20% formalin, or frozen in liquid nitrogen). The delicate nature of these species makes them particularly prone to preservation artifacts that vary by individual and fixation method. Relaxation in magnesium chloride in freshwater isotonic with seawater prior to fixation yielded the least distortion. Fixation in chilled 95% ethanol yielded specimens that were brittle, with the gelatinous sheath dehydrated to a point such that all external features could be clearly viewed. Fixation in formalin and glutaraldehyde yield specimens that looked more like live specimens. Contraction of the body occurred to some degree in all specimens, however, even unanesthetized specimens did not retract their heads to the point that they appear to have a cephalic hood. The portion of the body surrounding the anterior loop of the gut was consistently the first to deteriorate when the animal died or was damaged. Palps and short, elliptical or long, cylindrical branchiae were easily lost during handling but free-standing nuchal organs, digitate and subulate branchiae were not.

In order to resolve relationships of the seven recently discovered species to known Cirratuliformia, a range of benthic representatives of cirratuliforms were newly sequenced. All non-redundant and reliable flabelligerid and acrocirrid sequences from GenBank were included in this project, as well as several cirratulids to represent the span of cirratulid diversity (Table 2). Only taxa for which

**Table 2** GenBank and voucher accession numbers. Shaded pairs were concatenated. GenBank numbers in bold indicate new sequences

Taxon	18S	28S	COI	CytB	16S	Voucher	Locality
<b>Acrocirridae</b>							
<i>Acrocirrus validus</i> Marenzeller 1879	FJ944491	–	FJ944525	FJ944538	–	SIO BIC A1290	Hayama, Sagami Bay, Japan <sup>a</sup>
<i>Flabelligena</i> sp.	EU694120	EU694121	EU694126	EU694135	EU694113	SIO BIC A1126	Pacific Antarctic Ridge <sup>b</sup>
<i>Macrochaeta clavicornis</i> (Sars 1835)	EU791461	–	EU791463	–	<b>HQ326957</b>	SIO BIC A1087	Vattenholmen, Sweden <sup>b</sup>
<i>Macrochaeta clavicornis</i> (Sars 1835)	–	DQ779696	–	–	–	SMNH 75829	Bohuslän, Sweden <sup>b</sup>
<i>Macrochaeta</i> sp.	EU700414	–	EU694125	EU694136	EU694114	SIO BIC A1127	Belize <sup>b</sup>
<i>Swima bombiviridis</i> (PB51)	<b>GQ422143</b>	<b>GQ422144</b>	FJ944527	FJ944540	FJ944506	SIO BIC A1282	Monterey, California <sup>a</sup>
Shining bomber (sp. 1, PB32)	FJ944497	–	FJ944531	FJ944544	–	SIO BIC A1285	Monterey, California <sup>a</sup>
Shining bomber (sp. 1, P1)	–	FJ944519	–	–	–	SIO BIC A1286	Monterey, California <sup>a</sup>
Tawi-tawi Bomber (sp. 2)	FJ944499	FJ944520	FJ944533	FJ944546	FJ944511	NMA 04327	Celebes Sea, Philippines <sup>a</sup>
Horned Bomber (sp. 3)	FJ944500	FJ944521	FJ944534	FJ944547	FJ944512	SIO BIC A1287	Astoria Canyon, Oregon <sup>a</sup>
Tiburon Bomber (sp. 4)	FJ944501	FJ944522	FJ944535	FJ944548	FJ944513	SIO BIC A1288	Juan de Fuca Ridge, Oregon <sup>a</sup>
Squidworm (sp. 5)	FJ944503	FJ944524	FJ944537	FJ944550	FJ944515	NMA 04342	Celebes Sea, Philippines <sup>a</sup>
Juanita worm (sp. 6)	FJ944502	FJ944523	FJ944536	FJ944549	FJ944514	SIO BIC A1289	Juan de Fuca Ridge, Oregon <sup>a</sup>
<b>Flabelligeridae</b>							
<i>Brada villosa</i> (Rathke 1843)	EU791460	EU791462	–	–	<b>HQ326962</b>	SIO BIC A1161	Fiskebäckskil, Sweden <sup>b</sup>
<i>Brada villosa</i> (Rathke 1843)	–	–	–	AY727747	–	USNM 1073357	Trondheimsfjord, Norway <sup>c</sup>
<i>Brada</i> sp.	<b>HQ326967</b>	<b>HQ326968</b>	<b>HQ326970</b>	<b>HQ326974</b>	<b>HQ326963</b>	SIO BIC A1845	Central California, USA
<i>Coppingeria</i> cf. <i>longisetosus</i> Haswell 1892	<b>HQ326966</b>	–	<b>HQ326971</b>	<b>HQ326975</b>	<b>HQ326960</b>	SIO BIC A1846	Spencer Gulf, South Australia
<i>Diplocirrus glaucus</i> (Malmgren 1867)	AY708534	DQ790031	–	AY727751	–	USNM 1073353	Gullmarsfjorden, Sweden <sup>c,d</sup>
<i>Diplocirrus glaucus</i> (Malmgren 1867)	–	–	–	–	FJ944504	SIO BIC A1139	Kristineberg, Sweden <sup>a</sup>
<i>Flabelligerina ockeri</i> Salazar-Vallejo 2007	EU694119	–	EU694127	EU694137	EU694111	SIO BIC A1129	La Jolla, California, USA <sup>b</sup>
<i>Flabelligera affinis</i> Sars 1829	AY708532	–	–	–	–	USNM 1073354	Gullmarsfjorden, Sweden <sup>c</sup>
<i>Flabelligera affinis</i> Sars 1829	–	DQ779688	–	–	DQ779614	SAM E3562	Iceland <sup>e</sup>
<i>Flabelligera affinis</i> Sars 1829	AY708531	–	–	AY727755	–	USNM 1073355	Central California, USA <sup>c</sup>
<i>Flabelligera commensalis</i> Moore 1909	<b>HQ326965</b>	–	–	<b>HQ326972</b>	<b>HQ326959</b>	SIO BIC A1843	Malibu, California, USA
<i>Flabelligera infundibularis</i> (Johnson 1901)	EU694118	–	EU694131	EU694133	EU694112	SIO BIC A1128	Astoria, Oregon, USA <sup>b</sup>
<i>Flabelligera mundata</i> Gravier 1906	<b>HQ326964</b>	–	<b>HQ326969</b>	<b>HQ326973</b>	<b>HQ326958</b>	SIO BIC A1844	South Orkney Islands, Antarctica
<i>Flota</i> sp.	EU694116	EU694110	EU694128	EU694134	EU694110	SIO BIC A1131	Monterey, California, USA <sup>b</sup>
<i>Ilyphagus octobranchus</i> Hartman 1965	–	–	–	AY727749	–	USNM 1073351	Woods Hole, Massachusetts, USA <sup>c</sup>
<i>Pherusa plumosa</i> (Müller 1776)	AY708529	–	–	AY727756	–	USNM 1073348	Woods Hole, Massachusetts, USA <sup>c</sup>
<i>Pherusa plumosa</i> (Müller 1776)	AY708528	DQ790056	–	AY727752	–	USNM 1073356	Central California, USA <sup>c,d</sup>
<i>Piromis</i> sp.	–	–	–	–	<b>HQ326961</b>	LACM-AHF POLY 2264	Santa Monica, California, USA
<i>Poeobius meseres</i> Heath 1930	EU694115	EU694123	EU694130	EU700415	–	SIO BIC A1130	Monterey, California, USA <sup>b</sup>
<i>Poeobius meseres</i> Heath 1930	–	–	–	–	DQ779631	SAM E3563	Monterey, California, USA <sup>b</sup>
<i>Therochaeta collarifera</i> Ehlers 1887	AY708527	–	–	AY727753	–	USNM 1073350	Woods Hole, Massachusetts, USA <sup>c</sup>
<b>Cirratulidae</b>							
<i>Aphelochaeta marioni</i> (Saint Joseph 1894)	DQ779639	–	–	–	DQ779602	SAM E3559	Iceland <sup>e</sup>
<i>Dodecaceria concharum</i> Örsted 1843	AY577891	DQ209242	DQ209262	–	–	SAM E3355	Iceland <sup>f,g</sup>
<i>Dodecaceria concharum</i> Örsted 1843	–	–	–	–	FJ965555	SIO BIC A1141	Bohuslän, Sweden <sup>a</sup>
<i>Caulleriella</i> sp.	–	DQ779679	–	–	DQ779606	SAM E3560	Iceland <sup>e</sup>
<i>Caulleriella parva</i> Gilland 1979	AF448151	–	–	–	–	–	Concarneau, France <sup>h</sup>
<i>Cirratulus cirratus</i> (Müller 1776)	DQ779645	DQ779683	–	–	DQ779609	SAM E3561	Iceland <sup>e</sup>
<i>Cirratulus spectabilis</i> (Kinberg 1866)	AY708536	DQ790029	–	AY727746	–	USNM 1073359	Snug Harbour, Washington, USA <sup>c,d</sup>
<i>Cirriformia tentaculata</i> (Montagu 1808)	AY611456	AY611443	–	–	–	–	Banyuls, France <sup>i</sup>
<i>Ctenodrilus serratus serratus</i> (Schmidt 1857)	AY340426	AY340388	–	–	AY340452	–	Massachusetts, USA <sup>e</sup>

<sup>a</sup>Osborn et al. 2009 supplement; <sup>b</sup>Osborn & Rouse 2008; <sup>c</sup>Burnette et al. 2005; <sup>d</sup>Struck et al. 2007; <sup>e</sup>Rousset et al. 2007; <sup>f</sup>Rouse et al. 2004; <sup>g</sup>Osborn et al. 2007; <sup>h</sup>Bleidorn et al. 2003b; <sup>i</sup>Rousset et al. 2004.

NMA, National Museum of the Philippines, Manila; SIO-BIC, Scripps Institution of Oceanography, Benthic Invertebrates Collection, La Jolla, California; USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC; LACM-AHF POLY, Los Angeles County Museum of Natural History, Allen Hancock Foundation Polychaete Collection, Los Angeles, California.

sequence data was available were included in analyses with the exception of *Flabelligella*, *Flabellisetia*, *Helmetophorus* and *Chauvinelia* for which it was critical to assess relationship to the new taxa and for which only morphological data could be generated. *Fauveliopsis*, *Laubieriopsis* and *Sternaspis* that were previously thought to be within Cirratuliformia (Rouse & Pleijel 2001; Salazar-Vallejo *et al.* 2008) were not included because they may only be distantly related to the taxa considered here (e.g. Rousset *et al.* 2007; Struck *et al.* 2007). Of Cirratuliformia, the new species were most morphologically similar to acrocirrids and flabelligerids and least similar to cirratulids. Cirratulids were chosen as the root based on morphological (Rouse & Fauchald 1997; Rouse & Pleijel 2003; Salazar-Vallejo *et al.* 2008) and molecularly based (Burnette *et al.* 2005; Rousset *et al.* 2007; Struck *et al.* 2007) attempts to resolve relationships within Annelida.

The following specimens were examined for comparison with the seven recently discovered swimming acrocirrids:

*Helmetophorus rankini* Hartman, 1978

*Holotype*. USNM-46748, three paratypes USNM-46749, National Museum of Natural History, Smithsonian Institution, Washington, DC.

*Chauvinelia biscayensis* Laubier, 1974

*Holotype*. MNHN-1263, Muséum National d'Histoire Naturelle, Paris, three specimens were collected but only one was deposited.

*Chauvinelia arctica* Averincev, 1980

*Non-type*. ZMUC POL-0159. This single specimen was described by Kirkegaard (1982) from the FRAM I Drift Ice Expedition off of NE Greenland from Station 18 at 2300–3370 m and is deposited in the Zoological Museum, University of Copenhagen. The species was described from 17 specimens collected from the Canadian Trench in the Arctic at 3296–3380 m depth. These type specimens (Zoological Institute, Russian Academy of Sciences, Saint Petersburg) were unavailable for comparison.

The six recently discovered and as yet unnamed swimming acrocirrids are here given colloquial names so that their relationships and morphology can be individually discussed (Fig. 1; Table 1).

### Sequence data

Genomic DNA was extracted from specimens using a Qia-gen DNeasy tissue kit (Valencia, CA, USA). An approximately 1800 base pair fragment of the small subunit ribosomal DNA (*18S*) was amplified with the universal primers mitchA and mitchB (Medlin *et al.* 1988) or TimA and TimB (Norén & Jondelius 1999) with 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. Approximately 1100 base pairs of

the large subunit ribosomal DNA (*28S*) were amplified with the universal primers LSUD1F and D3ar (Lenaers *et al.* 1989) or C1 and R4 (Lê *et al.* 1993 and Struck *et al.* 2006; respectively) with 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. A 350–500 base pair fragment of the mitochondrial small subunit ribosomal DNA (*16S*) was amplified either using the universal primers 16SarL and 16SbrH (Palumbi *et al.* 1991) or the annelid specific primers AnnF and AnnR (Sjölin *et al.* 2005) with 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. Approximately 650 base pairs of the mitochondrial *COI* gene were amplified using universal primers HCO2198 and LCO1490 (Folmer *et al.* 1994) with 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. Approximately 360 base pairs of the *CytB* were amplified via the universal primers 424F and 876R (Boore & Brown 2000) with 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)<sub>2</sub> was added to each reaction. 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, USA). Amplification profiles were optimized for each extraction.

The PCR products were sequenced directly after spin column purification (Ultrafree-DA columns; Millipore, Billerica, MA, USA) following the manufacturers' protocols. All sequencing was carried out using the same primers that were used in the amplification, with the addition of four internal primers for *18S* (600F, 4FBK Norén & Jondelius 1999; a2.0, bi; Giribet *et al.* 1999). All sequencing was carried out by Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) at the University of Hawaii at Manoa using Applied Biosystems (Calsbad, CA, USA), Big-Dye terminator chemistry and an ABI 3730XL sequencer. *CytB* sequences are forward reads only because sequencing reactions were never successful with reverse primers (also 825R referenced in Burnette *et al.* 2005 was tried unsuccessfully). Sequences were deposited in GenBank (Table 2).

### Morphology

A 29 character morphological matrix was compiled (Appendix S1) after consideration of previous morphologi-

cal analyses (Rouse & Fauchald 1997; Rouse & Pleijel 2003; Salazar-Vallejo *et al.* 2008) in order to examine relationships of the new taxa to each other, to Flabelligeridae, and to Acrocirridae. The matrix included all species for which we had sequence data, as well as four species for which sequence data could not be obtained, but whose relationships to the new species were of interest (*C. arctica*, *H. rankini*, *Flabellisetia incrusta* Hartman 1978; and *Flabelligella* sp.). Characters were coded from type material whenever possible or from non-type material, original descriptions or recent revisions. See Appendix S1 for characters and the supplemental online material for discussion of characters. The matrix is also available at Treebase (<http://www.treebase.org>).

### Analyses

Sequences were concatenated only when from the same extraction, except in eight cases for which extracts were not available (Table 2 shaded pairs). Sequences were aligned with MUSCLE 3.6 (Edgar 2004) using default settings and proofread by eye in MacClade v.4.04 (Maddison & Maddison 2000). Possibly ambiguously aligned bases were not excluded from ribosomal genes alignments in favour of total evidence. Individual ribosomal alignments consisted of the following: *18S* was 2177 base pairs, 1409 constant or uninformative, 768 informative; *28S* was 1139 base pairs, 503 constant or uninformative, 636 informative; *16S* was 567 base pairs, 275 constant or uninformative, 292 informative. Third positions were not saturated for *COI* with respect to the ingroup or outgroup (DAMBE; Xia *et al.* 2003), thus all positions were included in the analysis (684 base pairs, 413 constant or uninformative, 271 informative). *CytB* third positions were saturated with respect to both ingroup and outgroup taxa and thus were excluded from all final analysis runs (390 base pairs, 130 excluded, 181 constant or uninformative, 79 informative; inclusion of third positions had no impact on support or topology in the combined analyses).

Bayesian analyses of the data sets were conducted using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) through the Cornell University Computation Biology Service Unit. Standard procedures based on Modeltest 3.5 (Posada & Crandall 1998) were implemented in PAUP\* 4.0b10 to select the most appropriate models for individual genes. The relative fit of models was assessed by the Akaike information criterion. Smaller values of Akaike information criterion are preferred (Akaike 1974; Posada & Crandall 2001) and the General Time Reversible + Proportion Invariant + Gamma (GTR + I +  $\Gamma$ ) represented the optimal model with respect to all genes. Partitions were unlinked in the concatenated analyses. Each Markov chain, three heated and one cold, was started from a random tree

and all four chains were run simultaneously for 5 to 60 million generations, with trees being sampled such that the resulting data set from each run contained at least 10 000 data points after burn-in. AWTY (Wilgenbusch *et al.* 2004) was used to determine if a sufficient number of generations had been completed for posterior probabilities to stabilize, as well as to determine amount of required burn-in before inference from the Markov chain Monte Carlo data sets were made. Numerous repeated analyses converged on similar parameter estimates.

Maximum likelihood analyses were conducted with RAxML 7.2.6 (Stamatakis 2006) on the CIPRES Portal (Miller *et al.* 2009) as a partitioned dataset (each gene and each codon position as in the Bayesian analyses) and the General Time Reversible + Gamma (GTR +  $\Gamma$ ) model was used. ‘Multiparametric’ bootstrapping (command-b = non-parametric bootstrapping) with random seed value: 12345 was carried out in each case with a minimum of 100 replicates, also using GTR + G.

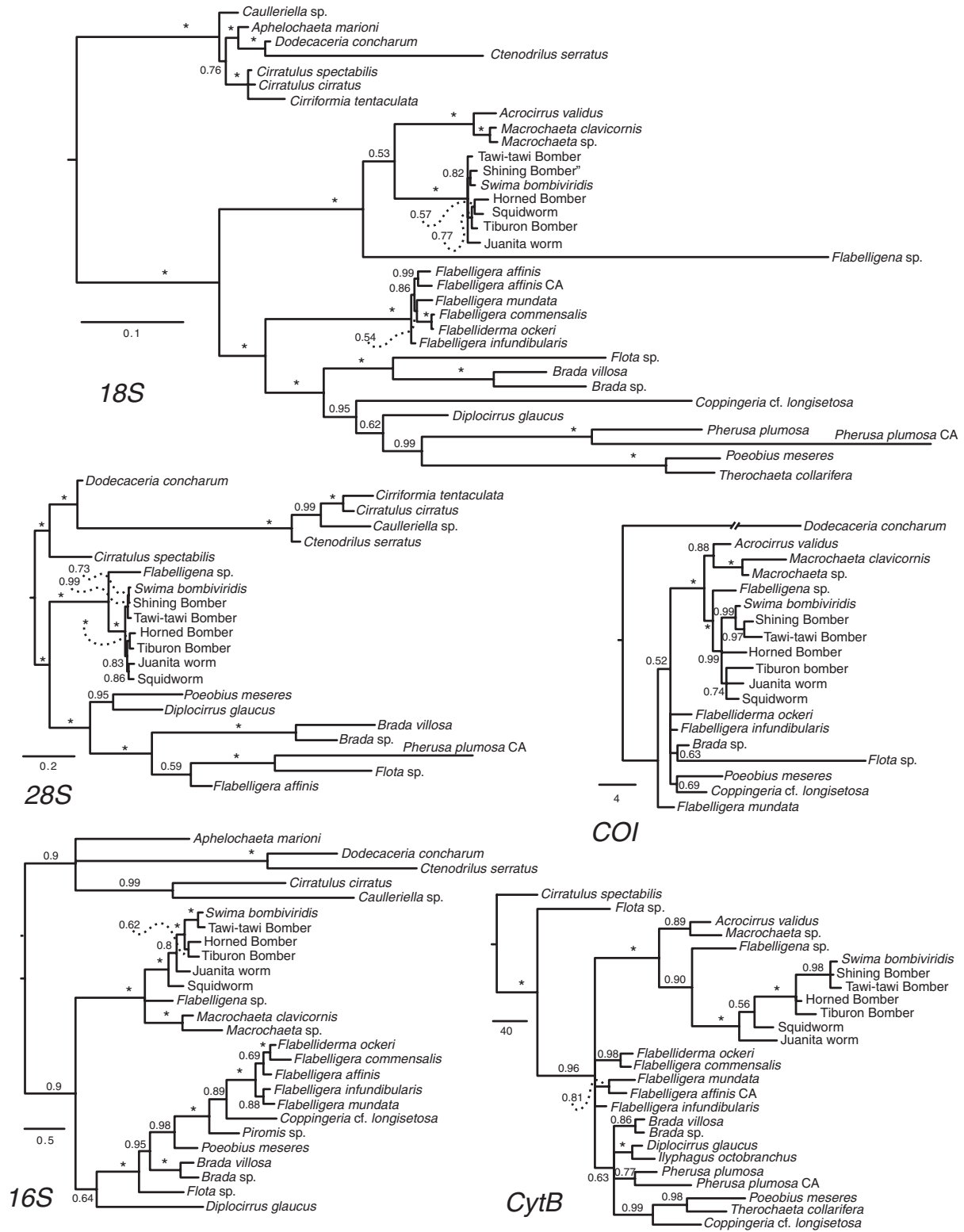
Parsimony analyses of molecular data were conducted with PAUP\* 4.0b10 (Swofford 2002) using an equally weighted character matrix, the heuristic search option, the tree-bisection-reconnection branch-swapping algorithm, and 1000 random addition replicates. Gaps were treated as missing data. Bootstrap values were obtained with the same settings as the parsimony analysis (1000 replicates).

Parsimony analyses were run on the morphological matrix alone, on the combined morphological and molecular matrix containing only species for which there were both type of data, and on the combined morphology and molecular matrix containing all species. Parsimony settings were as described above from molecular data. Bayesian analyses with settings as described above were also run on the combined molecular and morphological matrixes with the addition of a rate variable model used for the morphology partition.

## Results

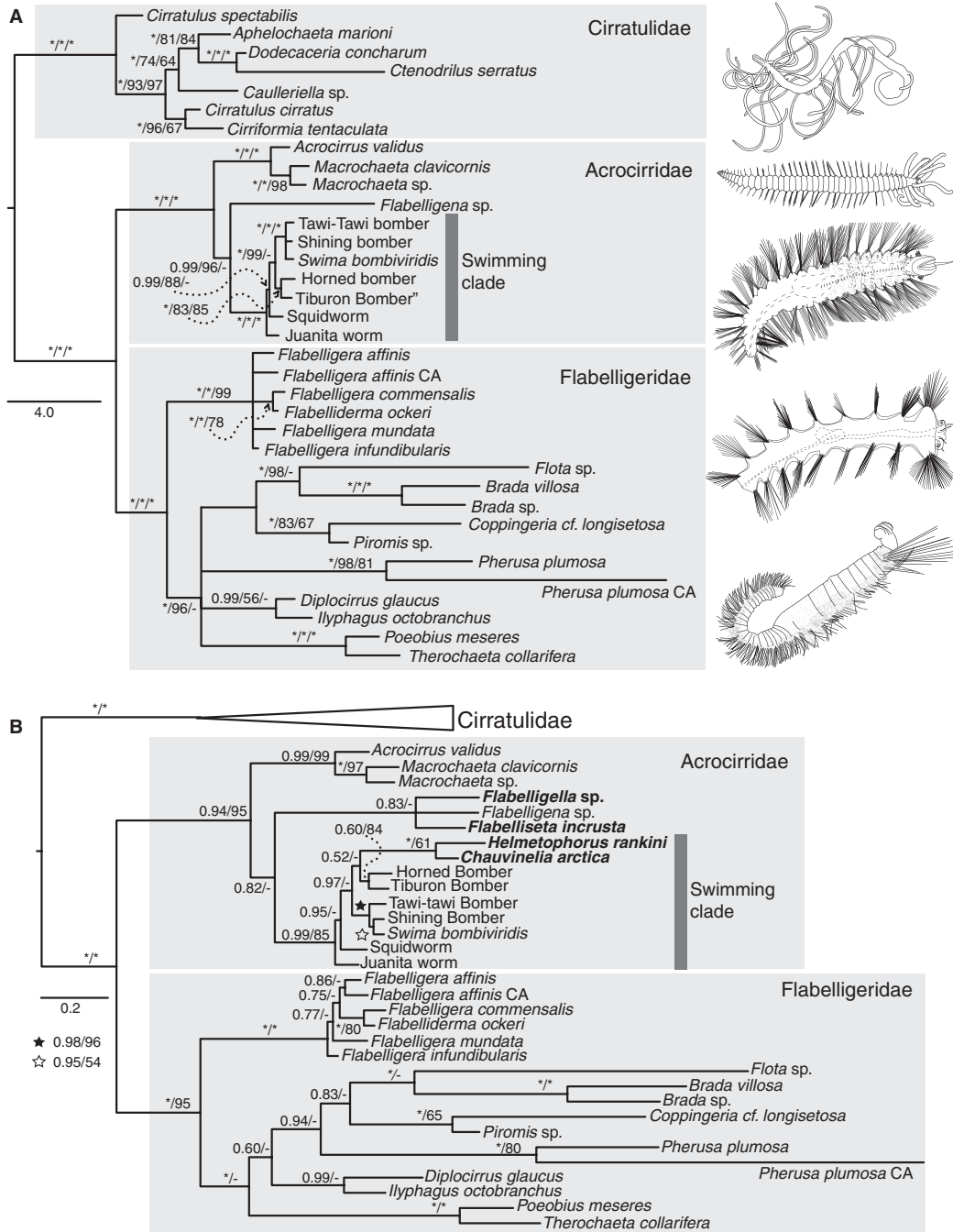
### Gene trees

Some general themes were seen in the individual gene trees (Fig. 2). Strong support was consistently found for the seven recently discovered species forming a clade (containing *S. bombiviridis*, ‘Shining’, ‘Tawi-tawi’, ‘Horned’, and ‘Tiburon Bombers’, ‘Juanita worm’ and ‘Squidworm’) and for that clade being within the monophyletic Acrocirridae. The monophyly of Flabelligeridae and their sister relationship to Acrocirridae was recovered in all but the *COI* and *CytB* analyses. The position of *Flabelligena* sp. was variable in the various genes, though it was always outside of both the *Acrocirrus/Macrochaeta* clade and the clade containing the seven recently discovered swimming species (hereafter referred to as the ‘Swimming clade’ Fig. 3). A



**Fig. 2** Majority rule consensus trees from Bayesian analyses of individual genes. Protein coding genes were partitioned by first, second and third positions and third positions were excluded in the *CytB* analyses. Support values are shown as posterior probabilities. Asterisks indicate 1.0 posterior probabilities.





**Fig. 3** —A. Ninety-five percent majority rule consensus tree from Bayesian analyses of five concatenated genes and the morphological matrix. Data were partitioned by each gene and the morphological matrix, and by first, second and third codon positions. *CytB* third positions were excluded. The topology of the RAxML tree (GTR + G model) did not differ from the Bayesian tree, however, the strict consensus tree from the parsimony analysis differed in that Squidworm and Juanita worm formed a clade with 91% bootstrap support and that clade formed a polytomy with *Swima sensu stricto* and the Horned/Tiburon Bomber clades. Support is indicated as posterior probabilities from the Bayesian analysis/bootstraps from the maximum likelihood analysis/bootstraps from the parsimony analysis. Asterisks indicate 1.0 or 100% support respectively. Illustrations of a representative cirratulid, acrocirrids *Acrocirrus validus* and *Swima bombiviridis*, and flabelligerids *Flota* sp. and *Pherusa plumosa* CA are given to the right. —B. Majority rule consensus tree from Bayesian analyses of five concatenated genes and 29 morphological characters including four species (bold) for which only morphological data was available (all other analysis parameters are the same as in A). Based on the limited information provided by the morphological data and the large amount of missing data for these four taxa, all support values decreased within Acrocirridae where the added taxa fell.

sister relationship between *Flabelliderma ockeri* Salazar-Vallejo 2007 and *Flabelligera commensalis* Moore 1909 was recovered for all genes (*18S*, *16S* and *CytB*) that included both species. The relatively long branch lengths within the non-*Flabelligera/Flabelliderma* flabelligerids *18S* and *28S* analyses were also notable.

The *18S* data provided poor resolution within the Swimming clade (Fig. 2). Within Flabelligeridae the *18S* data strongly supported the following: (i) distinction of *Flabelligera/Flabelliderma* from other flabelligerids, (ii) monophyly of each of the included *Pherusa*, *Brada*, and *Flabelligera/Flabelliderma* and (iii) sistergroup relationships between *Brada/Flota* sp., as well as between *P. meseres/Therochaeta collarifera* Ehlers 1887. Additionally *18S* data suggested a sister relationship between included *Pberusa* spp. and *P. meseres/T. collarifera*.

The *28S* data provided strong support for a clade containing Horned and Tiburon Bombers, as well as one containing *S. bombiviridis* and Shining and Tawi-tawi Bombers (Fig. 2). *Swima* is here restricted to those three species that consistently grouped together within the Swimming clade based on sequence data and that possess the following characters: transparent body, thick gelatinous sheath, nuchal organs as simple ridge with no more than one 180° bend, small ‘bombs’ (elliptical, segmental branchiae that bioluminesce when autotomized, measuring less than half widest body width), and single medial subulate branchia on head. Thus, *Swima sensu stricto* consists of *S. bombiviridis*, Shining and Tawi-tawi Bombers. Strong support was also found for a relationship between *Flota* sp. and the included *Pherusa*, a result not found with other genes.

The *16S* data provided a reasonable amount of variability across the acrocirrids and flabelligerids sampled, as seen by the relatively intermediate branch lengths compared to other genes sampled (Fig. 2). Strong support was provided for a sister relationship between *Swima sensu stricto* and the clade of Horned and Tiburon Bombers. Interestingly *16S* placed the *Flabelligera/Flabelliderma* clade as relatively derived among flabelligerids in contrast to the group’s more basal position in other gene trees.

While the *COI* data were useful in identifying species groups (Osborn *et al.* 2009; Osborn, Haddock and Rouse, *in review*), it provided little resolution of relationships for phylogenetic analyses (Fig. 2). *CytB* data provided good resolution within the portion of the tree that had short branches for other genes (i.e. within the Swimming clade), yet similar to *COI*, provided little information about more distant relationships. Analyses of *CytB* sequences, unlike those of *COI*, found similar relationships, although with low support, to that found in other genes.

### Morphology

Morphology alone provided little insight into the relationships within and between Flabelligeridae and Acrocirridae (Fig. S1). Body papillae united Flabelligeridae and Acrocirridae and distinguished them from Cirratulidae. The shape of the prostomium further distinguished cirratulids from acrocirrids and flabelligerids, as well as most flabelligerids from acrocirrids (*P. meseres* is similar to that of the acrocirrids). Monophyly of Flabelligeridae was recovered in the parsimony analysis of the morphological matrix (Fig. S1), supported by presence of the following: branchial membrane (with the exception of sharing this character with Squidworm and Juanita worm), retractable head, cephalic hood (present also in the acrocirrids *H. rankini* and *C. arctica*), and cephalic cage (though lost in *Brada* spp., *Flota* spp., *P. meseres* and *Diplocirrus* spp.). The texture of the chaetae (Fig. S2) distinguished the families considered here; cirratulids possess smooth chaetae, acrocirrids have fine denticles on the surface and flabelligerids have segmented or cross-barred chaetae (*Flabelligella* spp. may share this feature with flabelligerids). The parsimony analysis of the morphological matrix did not recover a monophyletic Acrocirridae, despite the chaetal apomorphy. The following characters were shared among various members of Acrocirridae and Flabelligeridae but were not found in Cirratulidae: sediment adhered to epidermis, gelatinous sheath, nephridiopore as a papillus, parapodia distinct projections from the body wall, and compound chaetae.

Elliptical main branchiae supported a clade containing *Swima sensu stricto*, Horned and Tiburon Bombers, *H. rankini*, and possibly *C. arctica*. *Helmetophorus rankini* and *Chauvinelia* spp. share several characteristics with *Swima sensu stricto* and Horned and Tiburon Bombers. All possess subulate branchiae occurring in one or more rows just behind or medial to the nuchal organs. These subulate branchiae are not found in any other Cirratuliformia examined and each species that possesses them also has at least one other branchial form. *Helmetophorus rankini*, *Swima sensu stricto* and Horned and Tiburon Bombers all share elliptical main branchiae (*Chauvinelia* spp. may as well, but it cannot be determined based on the material, branchial scars are found on at least two anterior, achaetous segments similar to that found in all species bearing elliptical branchiae). Additionally, *H. rankini*, *Chauvinelia* spp. *Swima sensu stricto* and Horned and Tiburon Bombers have fans of numerous long chaetae, a feature shared with the other two members of the Swimming clade as well (Juanita worm and Squidworm).

Squidworm and Juanita worm are distinct from other known acrocirrids based on their free-standing nuchal structures, a branchial membrane supporting four pairs of

elongate, tapered main branchiae and the presence of the nephridiopore on the branchial membrane. Additionally, these two species, along with *Swima sensu stricto* and Horned and Tiburon Bombers, share two notable (though convergent) features with Flabelligeridae, gonads in discrete anterior segments and looped guts (shared also with *Chauvinelia* spp. and *H. rankini*).

### Combined analyses

There was little difference between the combined molecular only (Fig. S4) and combined morphology and molecular analyses (Fig. 3a). The seven new species formed a single, well-supported clade that was within Acrocirridae in all analyses (Fig. 3, Fig. S4), distinguished here as four groups. *Swima sensu stricto*, containing *S. bombiviridis*, Shining and Tawi-tawi Bombers, was well-supported in all analyses and was most derived relative to the other swimming forms. Tiburon and Horned Bombers were sister-group to *Swima sensu stricto* and were well-supported by all except the analysis including taxa with only morphological data (Fig. 3b), where support decreased for all clades within Acrocirridae. Two morphologically and molecularly distinct taxa were also found, Squidworm and Juanita worm. Parsimony analysis of the combined dataset suggested with low support that these groups may be sister to each other, but this result was not recovered in any other analyses. Instead, the relationship of these two groups remained unresolved within the Swimming clade (Fig. 3). Based on morphology, the swimming clade also includes *H. rankini* and *C. arctica* (Fig. 3b, Fig. S3), species that were most similar morphologically to Horned and Tiburon Bombers.

Within Acrocirridae, the included *Acrocirrus* and *Macrochaeta* species formed a well-supported clade in all analyses. The relationship of *Flabelligena* sp. to other acrocirrids was variable, generally falling out as sister to the swimming clade or in a basal polytomy with the *Acrocirrus/Macrochaeta* and Swimming clades (Figs. 3, Fig. S4). Similarly, the addition of *F. incrusta* and *Flabelligella* sp. to the analysis found them also in the acrocirrid basal polytomy (Fig. 3b).

The addition of molecular data for *Piromis* sp., *Coppingeria* cf. *longisetosus* Haswell 1892; an additional *Brada* sp., *Flabelligera mundata* Gravier 1906 and *F. commensalis* Moore 1909 provided further resolution within the Flabelligeridae and clarified the sister relationship between Flabelligeridae and Acrocirridae compared to that found in previous attempts (Osborn & Rouse 2008; Osborn *et al.* 2009). Notably Flabelligeridae was found to form a clade rather than a grade. As with previous results a *Flabelligera/Flabelliderma* clade was strongly supported, *Brada* spp. and *Flota* sp. remained sister to each other, as did *P. meseres* and

*T. collarifera* (Fig. 3). *Coppingeria* cf. *longisetosus* and *Piromis* sp. may be more closely related to *Brada/Flota* than to other flabelligerids, but support was not consistently strong across analyses (Fig. 3).

The addition of species for which only morphological data was available (*C. arctica*, *H. rankini*, *Flabelligella* sp. and *F. incrusta* Hartman 1978) to the combined molecular and morphological analyses did not change the topology of the tree with respect to the taxa for which both types of data were available (Fig. 3b). However, these additions decreased the support for all nodes within Acrocirridae where the additional taxa fell.

## Discussion

### Taxonomy

*Swima* was originally delineated as a clade of seven swimming species in the Osborn *et al.* (2009) tree, but is here restricted to only the three species (including the type species *S. bombiviridis*) with the following apomorphies: a medial subulate branchia, one row of subulate branchiae (sometimes consisting of only the medial branchia), nuchal organs as slightly raised ridges making no more than one 180° bend, and short segmental, ellipsoid branchiae. The description of the two undescribed *Swima* species, Tawi-tawi and Shining bombers is forthcoming (Osborn, Haddock and Rouse, *in review*). The remaining four species will be divided into three new genera (Horned and Tiburon Bombers forming one and Squidworm and Juanita worm each forming monotypic taxa) derived from morphology and a well-supported tree topology based on all currently available evidence. *Chauvinelia* and *Helmetophorus* are certainly related to these new groups but without genetic data and clear understanding of the nature of the achaetous anterior region of *H. rankini* and *Chauvinelia* spp., we retain these as separate genera.

*Helmetophorus rankini* is most similar to Horned and Tiburon Bombers, sharing the following characters: multiple rows of subulate branchiae, relatively large 'foliose' or elliptical segmental branchiae and nuchal organs as convoluted ridges winding among the bases of the subulate branchiae. *Helmetophorus rankini* differs from all other members of the Swimming clade in the following characters: its small size (6 mm long, 0.8 mm wide compared with the smallest species of the swimming clade, *S. bombiviridis*, which, even when preserved, reaches at least 11 mm length and 2.1 mm width), a uniramous first chaetiger (notochaetae only), a non-retractile oral tube and a cephalic hood projecting anteriorly on the dorsum. *Helmetophorus rankini* differs from Horned and Tiburon Bombers most notably in having only simple neurochaetae. The small size and poor condition of *H. rankini* specimens make it impossible to determine if more than two pairs of

elliptical branchiae were once present as is suspected. Also, the nature of the cephalic hood is questionable (see discussion of characters 1 and 2 in Supplemental Materials and Methods).

*Chauvinelia* spp. are also most similar to Horned and Tiburon Bombers, similarly possessing multiple rows of subulate branchiae and nuchal organs as convoluted ridges winding among the bases of the subulate branchiae. *Chauvinelia* spp. differ from Horned and Tiburon Bombers in possessing the following characters: a thick gelatinous sheath, semi-transparent body, and the inflated and elongate, anterior, featureless, achaetous segments. While Horned and Tiburon Bombers have achaetous, anterior segments, these segments are muscular, not inflated, have branchiae, and the head is never retracted inside of these segments irrespective of handling and fixation. The gelatinous sheath of Horned and Tiburon Bombers is thin, sometimes has a minute layer of fine sediment particles embedded in it, and is apparent only when it is damaged or around the bases of the chaetae. The branchial scars on the achaetous segments clearly drawn by Averincev (1980) and referred to as 'indistinct swellings' are difficult to distinguish on the infolded body wall of Kirkegaard's (1982) specimen (Fig. 1), but appear to be similar to the scars left by the ellipsoid branchiae of the Bombers. The nature of these lost branchiae is unknown for *Chauvinelia* species.

Retractability of the head and the nature of the cephalic hood of *Helmetophorus* and *Chauvinelia* are problematic when trying to understand these two taxa's relationships to members of the Swimming clade. Missing information due to the limited number of specimens known for each species the delicate nature of the worms that leads to poor condition upon collection and preservation, and the lack of information about the live animals are problematic.

In addition to their cephalic hoods, *Chauvinelia* spp. and *H. rankini* both have multiple rows of subulate branchiae surrounded by a convoluted ridge that forms the nuchal organs. These two taxa differ in the thickness of their gelatinous sheaths, with that of *Chauvinelia* spp. being relatively thick, while *H. rankini* has a thin sheath (see Supplemental Materials and Methods). Additionally, *Chauvinelia* spp. have compound neurochaetae while those of *H. rankini* are simple. These two species also differ in general size, *Chauvinelia* spp. being longer and wider (6–17.5 mm long and 1.2–2.8 mm wide) than *H. rankini* (less than 6 mm long and 1 mm wide). Salazar-Vallejo *et al.* (2007) were the first to compare these taxa and they did not consider synonymizing them despite their similarities. We also maintain them as separate taxa consistent with traditional morphological taxonomy. Until further specimens of *Chauvinelia* and *Helmetophorus* can be obtained from which genetic material can be extracted and the nature of the segmental branchiae and

cephalic hood can be determined, the question of their relationships to the remainder of the Swimming clade cannot be fully resolved.

### Morphology

The ontogeny of flabelligerid branchiae is unknown. Details of the circulatory system provided by Schlieper (1927) and Spies (1973) both suggest origin of the branchiae posterior to the peristomium. Additionally, Rouse & Pleijel (2001) argued that the branchiae are segmental because the single, large pair of nephridia opens onto the branchial membrane and if this were a peristomial structure, the position of the nephridiopores would be novel within Annelida. Our observations show that the nephridiopores of *Pherusa plumosa* CA are located at the bases of the second from lateral branchiae and are similarly located in Squidworm and Juanita worm. Likewise, the nephridiopore of *Dipplocirrus longisetosus* (Marenzeller 1890) is also at the bases of the second branchiae on the branchial membrane (Filippova *et al.* 2003). Acrocirrids have a single large pair of anterior nephridia and a pair of papillae on the second chaetiger that is the nephridiopore. Although these papillae have been clearly illustrated by most authors since Banse (1969) their connection to the nephridia has not been confirmed, likely due to the small size of many acrocirrids. Observations of the internal anatomy of members of the Swimming clade allowed recognition of the function of these structures. The location of the nephridiopore on the second segment in acrocirrids, the close relationship of acrocirrids to flabelligerids, the location of the nephridiopore at the base of the second branchiae in Squidworm and Juanita worm, and the location of the nephridiopore next to the second branchiae in *P. plumosa* CA and *D. longisetosus* all suggest that flabelligerid branchiae are likely segmental in origin.

### Phylogenetics

Our analyses confirmed Acrocirridae as a clade including all seven recently discovered swimming species (Osborn *et al.* 2009), as well as *Helmetophorus* and *Chauvinelia* as hypothesized by Salazar-Vallejo *et al.* (2007). We found that the seven new species form a clade within Acrocirridae along with *Helmetophorus* and *Chauvinelia*. A revised diagnosis of Acrocirridae is provided to reflect this increased morphological diversity.

Our analyses also confirmed the validity of Flabelligeridae as a clade and their sister relationship to Acrocirridae. Addition of sequence data for seven key taxa since Osborn *et al.* (2009) resulted in recovery of Flabelligeridae as a clade and not a grade as was found previously. Numerous apomorphies also support Flabelligeridae as separate from Acrocirridae (Fig. S3).

Retention of *Flabelliderma* may render *Flabelligera* paraphyletic. This synonymy was indirectly suggested by earlier workers' confusion between the genera, recent transfer of species between them (e.g. Salazar-Vallejo 2007), and the instability of *F. commensalis* (Hartman 1969; Fauchald 1997; Light 1978; Salazar-Vallejo 2007). Salazar-Vallejo (2007) noted three main apomorphies for a *Flabelligera/Flabelliderma* clade, (i) chaetae of the cephalic cage arranged in a single row, forming a nearly complete circle around the head, (ii) head only moderately, if at all, eversible and (iii) pseudocompound neurohooks. Our finding of *Flabelliderma ockeri* Salazar-Vallejo 2007 as most closely related to *F. commensalis* suggests that either *F. commensalis* belongs in *Flabelliderma* or that *Flabelliderma* is nested within *Flabelligera* (Fig. 3). A detailed phylogenetic analysis containing multiple examples of *Flabelliderma* and *Flabelligera* is needed to resolve this question.

The position of *Flabelligella* and *Flabelligena* within Acrocirridae was unresolved. These two genera may be sister to either the *Acrocirrus/Macrochaeta* clade or the Swimming clade. Additionally, sequence data is needed for *F. incrusta*, *H. rankini* and *Chauvinelia* spp. to better understand their relationships within Acrocirridae. The long branches and unresolved relationships between the included flabelligerids beg further study with increased taxon sampling for DNA sequencing.

Finally, the ecological and behavioural information we have for the seven recently discovered members of the Swimming clade combined with these phylogenetic results suggest an evolutionary transition from the nearly benthic Juanita worm, through the benthopelagic Squidworm, Horned and Tiburon Bombers to the holopelagic members of *Swima sensu stricto*. This transition, together with the three separate origins of pelagic lifestyle within Cirratuliformia (Osborn *et al.* 2009) presents an ideal system to examine pelagic adaptations within Annelida.

### Systematics

Family ACROCIRRIDAE Banse, 1969

Type genus *Acrocirrus* Grube, 1873 by original designation.

*Diagnosis (emended)*. Free-living polychaetes with spinous chaetae that are most easily observed at distal tips. Notochaetae capillary, rarely lacking. Neurochaetae pseudocompound hooks or compound spinigers or hooks, rarely with posterior neurochaetae simple. Frontal grooved palps, easily lost but leaving obvious scars. Reduced prostomium dorsal on peristomium. Mouth opening subterminal, proboscis unarmed, with a buccal sac. With or without eyes. Nuchal organs straight, variously convoluted ciliated bands, or extending onto free-standing, oppositely

branched and/or spiral structures. Sometimes with one or more rows of subulate branchiae surrounded by nuchal ridges. Usually with achaetous anterior segments, sometimes with anterior segments fused. Sometimes with transparent, gelatinous sheath. Usually with four pairs anterior segmental branchiae, easily lost, dorsal to notopodia, long, digitiform to short, elliptical. Pair of large anterior nephridia, nephridiopores ventral to second pair segmental branchiae, often as elongate papillae. Gonoducts in two or three anterior segments or in numerous abdominal segments. Epidermis with papillae, interramal papillae prominent. Gut straight or looped.

*Swima* Osborn *et al.* 2009

Type species, *Swima bombiviridis*, by original designation

*Diagnosis (emended)*. Swimming acrocirrids with thick gelatinous sheath penetrated throughout by long, clavate papillae. Body transparent. One or more lollipop-shaped, interramal papillae projecting well beyond gelatinous sheath. With more than 30 long (more than body width) chaetae per parapodium. Eyes absent. Head not retractable. Nuchal organs just posterior to palps as simple, slightly raised ciliated ridges making no more than single 180° bend. Possessing single, medial subulate branchia either individually or as part of a single row of subulate branchiae immediately posterior to palps and anterior to segmental branchiae, not easily lost. Sometimes with single row of more than 30 digitiform branchiae just posterior to subulate branchiae. Nephridiopores as papillae on second achaetous segment. Four pairs of segmental branchiae modified as ellipsoid, bioluminescent structures, the second of which is attached to basal portion of nephridiopore papillae. Segmental branchiae small (largest less than half width widest body), easily lost, leaving obvious circular scars.

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analyses were run on the CIPRES Portal 2.2 at UCSD. Thanks goes to the crew of the R/V *Western Flyer*, the pilots of the ROVs *Tiburón* and *Doc Ricketts*, and the MBARI video lab staff for their dedication to deep-sea exploration. Evelyn York and Sara Tanner's expertise on the SEM was much appreciated.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Matrix of multistate coding scores of morphology. Unknowns are coded with ‘?’ and inapplicable with ‘-’.

**Fig. S1** Photos of various characters. —A. *Acrocirrus validus*, left lateral view showing four branchiae scars (numbered), the nephridiopore (white arrow), palp (p) and clusters of short interramal papillae (black arrow). —B. *Chauvinelia arctica* (ZMUC POL-0159) clavate interramal papillae. —C. *Chauvinelia biscayensis* holotype possible lollipop-shaped interramal papillae. —D. Shining Bomber lollipop interramal papillae. —E. *Flabelligera affinis* from Edithburgh, Australia with gelatinous sheath removed showing long clavate body papillae. —F. *Flabelligera affinis* from same collecting event as E, with gelatinous sheath intact. —G. *Poebobius meseres* showing thick gelatinous sheath (gs) and protruding body papillae (arrow). —H. Body of *Flabelligerina ockeri* (right) removed from its sediment encrusted gelatinous sheath (left). —I. *Swima* sp., dorsal view of head showing palps, medial subulate branchia (sb) and nuchal organ (arrow). —J. Juanita worm, dorsal view of head showing palp scar (p) and branchiae scars (numbered), spiral (spn) and oppositely branched (obn) free-standing nuchal organs. —K. Head of *Pherusa plumosa* CA showing a palp, branchia, eyes and nephridio-

pore papillus (arrow). —L. *Acrocirrus validus* head in dorsal view showing palps, branchiae, eyes, and convoluted nuchal ridges, photo F. Pleijel. —M. *Swima bombiviridis* ventral view of anterior showing nephridiopores (arrow indicates left nephridiopore), lollipop interramal papillae and subulate branchia. —N. Antero-dorsal view of head of Squidworm showing palps, branchiae, oppositely branched free-standing nuchal organs and nephridiopores (arrow indicates left nephridiopore), photo L. P. Madin.

**Fig. S2** Acrocirrid (A–H) and flabelligerid (I–L) chaetae. —A. Shining Bomber distal tip neurochaeta. —B. Juanita worm notochaeta. —C. Tiburon Bomber neurochaeta. —D. *Acrocirrus validus* notochaetae. —E. Tawi-tawi Bomber notochaeta distal tip. —F. Tawi-tawi Bomber distal element neurochaeta. —G. Squidworm distal portion neurochaeta. —H. Squidworm shaft of neurochaeta. —I. *Flota* sp. segment joint of notochaetae. —J. *Pherusa plumosa* CA medial portion notochaeta. —K. *Pherusa plumosa* CA basal portion notochaetae. —L. *Flabelligera mundata* neurochaeta.

Scale bars: A = 2  $\mu\text{m}$ , B–D, G and I = 10  $\mu\text{m}$ , E–F, H and J–L = 40  $\mu\text{m}$ .

**Fig. S3** Strict consensus of 1 046 189 most parsimonious trees (tree length = 96) from analysis of the morphology matrix.

**Fig. S4** Majority rule consensus tree from Bayesian analyses of five concatenated genes. Data were partitioned by each gene and first, second and third codon positions. *CytB* third positions were excluded due to saturation. Support is indicated as posterior probabilities from the Bayesian analyses/bootstraps from the parsimony analysis. Asterisks indicate 1.0 or 100% support respectively; dashes indicate unsupported nodes.

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Online-only Supplementary Material for:

**Phylogenetic of Acrocirridae and Flabelligeridae (Annelida, Cirratuliformia)**

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This pdf includes:

Supplemental Materials and Methods – Character discussion

Supplemental References

Supplemental Figure 1 – Character illustrations

Supplemental Figure 2 – Chaetal characteristics

Supplemental Figure 3 – Morphology tree

Supplemental Figure 4 – Five gene molecular tree

Appendix I – Morphology matrix

## Supplemental Materials and Methods

Characters were as follows:

*1. Retractable head:* Head retractable into fold of anterior segments. Flabelligerids can withdraw the anterior portion of their body and head so that they are surrounded by anterior chaetigers (Supplemental Fig. 1E–G, K). No cirratulids have retractile heads, nor does any of the newly discovered swimming species, nor other acrocirrids with a few possible exceptions. Orensanz (1974) suggested that members of *Flabelligella* (Acrocirridae) may be capable of head retraction, but this needs confirmation. *Chauvinelia* spp. and *H. rankini* were originally described as having retractable heads (Laubier 1974; Hartman 1978) but this may not be the case. When describing *Chauvinelia arctica*, Averincev (1980) noted that the expanded anterior part of the body enveloping the preceding segments might have been caused by contraction of the worm during fixation. However, Laubier (1974) noted that prior to fixation, the prostomium of his *C. biscayensis* specimens was hidden inside the cephalic hood and that he was able to evert it with pressure on the anterior body. Salazar-Vallejo *et al.* (2008) suggested that *Chauvinelia* spp., *H. rankini* and possibly *Buskiella* (synonymized with *Flota* Salazar-Vallejo & Zhadan 2007, but maintained as a separate genus here) have a non-retractile anterior but that some anterior chaetigers do form a cephalic hood (see next character). Salazar-Vallejo *et al.*'s (2008) explanation being that these segments are hypertrophied – enlarged due to enlargement of individual cells – and thus expands over parts of the head. This remains to be confirmed by histological examination. No further explanation was given regarding the retractability of anterior segments (Salazar-Vallejo *et al.* 2008). It is unclear if this expansion of non-retractile segments to form a cephalic hood and partially covering the head occurs in life, or just in preserved specimens.

The flabelligerid *Flota* sp. is most often observed *in situ* with its head retracted into the anterior portion of its body (K. J. Osborn *pers. obs.*). Thus the retraction of the head in *Flota* sp. is not a preservation artifact; the worms typically carry the head inverted and can evert it. Relaxation of living specimens does not consistently result in eversion of the head.

Many gelatinous worms wither or contract dramatically when injured or fixed. This occurs as soon as the gelatinous sheath or body is damaged and was directly and repeatedly observed by KJO in *P. meseres* and *Flota* species. The nature of the thin, amorphous membrane surrounding the body of both *Chauvinelia* species (Fig. 1C) indicates that all specimens are considerably shrunken in a way similar to that seen in *Flota* species. Thus *Chauvinelia* spp. may well have thick gelatinous sheathes within which the bodies contract during handling and fixation. This contraction could easily account for the infolding of the anterior region in the specimens of *Chauvinelia* and thus make it impossible to determine if the head is actually retractable until live specimens can be observed. On the other hand, the smooth and featureless nature of the posterior portion of the infolded region, together with the possession of a cephalic hood, leaves the retractability of the anterior end in question. Based on KJO's observations of gelatinous animals and the rest of Acrocirridae lacking retractable heads, we follow the scoring of Salazar-Vallejo *et al.* (2008) and consider the head of *Chauvinelia arctica* non-retractile.

*Helmetophorus rankini* specimens lack the large, loose membrane indicative of both a thick gelatinous sheath and significant contraction of the body away from that sheath during injury or fixation (Fig. 1I). Thus this species was assumed to have only a thin gelatinous sheath, if any, and an unknown amount of contraction. All *H. rankini* heads were covered a similar amount by the cephalic hood, leaving the oral tube and prostomium exposed, possibly suggesting that the head is not retractile. Therefore, with respect to *H. rankini* we follow Salazar-Vallejo *et al.*'s (2008) scoring of this species as "head non-retractile".

2. *Cephalic hood.* Flabelligeridae possess a cephalic hood into which the head is pulled when the head is retracted. In flabelligerids this hood has a smooth, featureless anterior portion that folds into itself leaving the chaetae of the cephalic crown, or first chaetiger, at the anterior-most margin when the head is inverted. The exterior portion of the hood (the part exposed when the head is fully inverted) has a different texture than the interior portion of the hood. Members of Cirratulidae, *Macrochaeta*, *Acrocirrus*, *Flabelliseta* and *Flabelligena* lack any structures that could be construed as cephalic hoods. The nature of a cephalic hood is poorly noted or illustrated for *Flabelligella* by Orensanz (1974) and is scored as absent here.

*Helmetophorus rankini* and *Chauvinelia* spp. have anterior achaetous segments that fold over at least some portion of the peristomium and prostomium in fixed specimens and this feature was called a cephalic hood by previous authors (Laubier 1974; Hartman 1978, Glasby & Fauchald 1991; Salazar-Vallejo *et al.* 2007). As outlined above, Salazar-Vallejo *et al.* (2008) suggested that one or two achaetous anterior segments of *Chauvinelia* spp. and *H. rankini* have enlarged, forming a non-retractile cephalic hood. Both *Chauvinelia* spp. and *H. rankini* have substantial anterior achaetous regions that are as wide or wider than the head and near the width of the widest part of the body. This region appears to be uniform for at least half its length and does fold over anterior segments and parts of the head, but this folding may be a preservation artifact in at least *Chauvinelia* spp., as discussed above. Averincev (1980) described the cephalic hood of *Chauvinelia* as an expanded region that lacks morphological features, is posterior to the achaetous segments supporting the branchiae, and is equal in length to the region supporting branchiae. In *Helmetophorus* the cephalic hood was described as “helmet-like dorsally, low and collar-like ventrally, extending anteriorly to the branchial membrane” by Glasby & Fauchald (1991) and later as “dorsally projecting and ventrally reduced” by Salazar-Vallejo *et al.* (2007). The dorsally projecting shape of the hood is consistent between specimens of *Helmetophorus*, making it a feature distinct from other known acrocirrids. Whether the observed structures are due to contraction during fixation or are actually a feature of the animals remains in question for at least *Chauvinelia* spp. and is here scored as “present” based on historical precedent and the lack of contradictory evidence. *Helmetophorus rankini* certainly has some form of cephalic hood (or “helmet” as the name indicates) based on the consistency of the asymmetrical, dorsally projecting shape of this structure in the types.

The achaetous regions of *Swima bombiviridis* and “Shining” and “Tawi-tawi” Bombers (Fig. 1A, D) are much shorter than those observed in *Chauvinelia* spp. and *H. rankini* and support prominent features such as the pair of nephridiopore papillae and four pairs of elliptical branchiae or, when those are lost (as is often the case), prominent circular scars (Suppl. Fig. 1I, M). The anterior achaetous region of “Horned” and “Tiburón” Bombers (Fig. 1F, G), although similar in relative length to those of *Chauvinelia* spp. and *H. rankini*, are muscular, narrow and never folded over themselves

as is seen in all known specimens of *Chauvinelia* and *H. rankini*. Additionally, the large (length more than half width widest part of the body) elliptical branchiae are prominent throughout the achaetous regions on Horned and Tiburon Bombers. “Juanita” worm possesses a long, broad and relatively featureless achaetous region (Fig. 1H). This achaetous region of Juanita worm is made up of two elongate segments (indicated internally by septa and externally by two rows of clavate papillae in the location parapodia would be expected) just posterior to the branchial membrane and anterior to the first chaetiger. The second, or posterior-most, segment of this region supports the anterior-most pair of gonopores. The body wall in the region, as in the rest of the body, is muscular, lacking a thick gelatinous sheath, and does not extend over anterior segments or the head (Suppl. Fig. 1J). Thus, none of the recently discovered swimming acrocirrids have a cephalic hood.

3. *Achaetous anterior segments*. All cirratulids (except *Ctenodrilus serratus serratus* (Schmidt, 1857)) available for this analysis were scored as possessing a single achaetous anterior segment based on the discussion by Rouse & Pleijel (2001), though the issue is needs further study of the peristomium and the unusual position of the palps in several cirratulids. Acrocirrids have various numbers of achaetous anterior segments. The nature of flabelligerid branchiae and the associated achaetous segments is unknown (see Rouse & Pleijel 2001 and discussion below).

4. *Cephalic cage*. This can be defined as the chaetae of first chaetiger being at least one third longer than those of the midbody. The anterior-most chaetigers of flabelligerids such as *Pherusa* spp., *Flabelligera* spp., and *Piromis* spp. have such a cephalic cage. Cirratulidae and Acrocirridae lack any such chaetae, as do flabelligerids such as *Brada* spp., *P. meseres*, *Flota* spp., and some *Diplocirrus* species.

5. *Externally obvious segment margins on majority of body*. The segments are externally obvious on all cirratulids and on some flabelligerids and acrocirrids. The thickness of the cuticle and of gelatinous sheath, as well as the amount of foreign particles adhered to the gelatinous sheath all impact but do not necessarily determine this state. For example, *Piromis* spp. have thick gelatinous sheathes and a thick layer of adhered particles yet the segment margins are still distinct on the outer surfaces of the animals. Additionally, although a thick gelatinous sheath is present in *S. bombiviridis*, Shining and Tawi-tawi

Bombers, segment margins are externally obvious (Fig. 1A, B, D). On the other hand, species such as *P. meseres* and some *Flabelliderma* spp. have a thick gelatinous sheathes and indistinct segment margins.

6. *Papillae*. Acrocirrids and flabelligerids have small epidermal papillae on at least on some parts of their bodies. Cirratulids lack papillae. Epidermal papillae extend from the epidermis through the gelatinous sheath (when it is present), sometimes extending beyond the outer surface of the sheath, as in *P. meseres* (Suppl. Fig. 1G) and *S. bombiviridis*, Shining and Tawi-tawi Bombers. The papillae can be numerous and closely cover the body as in most *Flabelligera* (Suppl. Fig. 1E) and *Flabelligena* spp. or can be sparse and found only on certain parts of the body such as around the chaetae as in *Acrocirrus validus* (Suppl. Fig. 1A).

7. *Body papillae*. Papillae range from short to long. Longer than wide papillae always have a slightly bulbous distal tip (clavate, Fig. 1B, E) and seem to be associated with thick gelatinous sheaths. Thus this character may be linked to the thickness of the gelatinous sheath since species with long body papillae have thick gelatinous sheaths. The nature of the body papillae has been used extensively in the past (Salazar-Vallejo 2007; Salazar-Vallejo *et al.* 2008).

8. *Interramal papillae*. Some taxa have a concentration of papillae between the rami of the parapodia (Suppl. Fig. 1A–D). Often these papillae are arranged in a row. The papillae can be short (similar width and length; Fig 3A), or long with a narrow stalk and slightly bulbous tip (clavate; Suppl. Fig. 1B). Lollipop and balloon-shaped papillae are unusual and distinct from clavate papillae. Lollipop papillae have a spherical tip and a narrow (less than one quarter the width of the spherical tip) stiff stalk; the slightly “onion-dome”-shaped tip is solid and the surface appears granular as in *Chauvinelia biscayensis* (Suppl. Fig. 1C, but not *C. arctica* Suppl. Fig. 1B) and *Swima* (*e.g.* Suppl. Fig. 1D). Balloon-shaped papillae are known only from *F. incrusta* and have long thread-like stalks and hollow, balloon-like spherical sacs at the distal tips. Hartman (1978) considered *F. incrusta*'s balloon-shaped papillae to be modified notochaetae but Salazar-Vallejo *et al.* (2007) established their identity as papillae.

9. *Sediment adhered to body*. Many flabelligerids adhere particles to their gelatinous sheath, for example *Flabelliderma* spp. (Suppl. Fig. 1H), *Brada* spp., *Pherusa* spp.,

*Piromis* spp. and *Ilyphagus* species. Acrocirrids belonging to *Macrochaeta*, *Flabelligena*, and *Flabelligella* adhere fine particles and *F. incrusta* adheres larger sediment particles to its cuticle. Cirratulids do not adhere particles to their bodies, nor do the acrocirrids *S. bombiviridis*, Shining and Tawi-tawi Bombers, *H. rankini*, *Chauvinelia* spp., nor the flabelligerids *Flota* spp. and *P. meseres*.

10. *Gelatinous sheath*. All Flabelligeridae (Salazar-Vallejo 2007 as mucus-sheath or tunic) and many Acrocirridae possess a gelatinous sheath covering the epidermis. The gelatinous sheath can be nearly distinct from the body as in *Flabelligera affinis* Sars, 1829 (Suppl. Fig. 1F), tough and capsule-like as in *Piromis* spp. or *Flabelliderma* spp. (Suppl. Fig. 1H), or an integral part of the body like in *Flota* spp., *P. meseres* (Suppl. Fig. 1G) *S. bombiviridis* and Shining and Tawi-tawi Bombers (Fig. 1A). The gelatinous sheath can be difficult to observe when it is thin as in *Flabelligera commensalis* Moore, 1909, “Squidworm” and Horned and Tiburon Bombers. Cirratulids lack a gelatinous sheath. In all Cirratuliformia that have a gelatinous sheath the body papillae project from the body through the gelatinous sheath (Suppl. Fig. 1G) and may be responsible for secreting it, although this requires further investigation.

11. *Prostomium shape*. The prostomium of cirratulids is terminal and cone-shaped while it is dorsally located and plate-like in flabelligerids (Suppl. Fig. 1K) and acrocirrids (Suppl. Fig. 1I, J, L, N). Flabelligerid prostomiums differ from that of acrocirrids in that the nuchal organs project posteriorly between the branchial membranes (see discussion for characters 14 and 15 below). The margins of the prostomium are difficult to define as evidenced by the various definitions used in the past (Spies 1975; Hutchings 2000; Salazar-Vallejo *et al.* 2008) but are here assumed to consist of the tissue immediately posterior and dorsal to the palps, supporting the nuchal organs, and extending to the anterior margin of the main or first segmental branchiae.

12. *Grooved palps*. Many polychaete groups have grooved palps, for example Spionidae have a single pair on the peristomium and Terebellidae have multiple pairs on the prostomium. A single pair is found in Acrocirridae (Suppl. Fig. 1I, L, N), Flabelligeridae (Suppl. Fig. 1K), and some Cirratulidae such as *Dodecaceria*. Cirratulids such as *Cirratulus* spp. and *Cirriformia* spp. have multiple peristomial palps (though derived from an initial single pair, Rouse & Pleijel 2001). Grooved palps are lacking in *C. s.*

*serratus*. Flabelligerid and acrocirrid palps are considered here to be peristomial, but this needs confirmation by development studies.

*13. Eyes.* Eyes are present and often variable on flabelligerids (Suppl. Fig. 1K) and acrocirrids (Suppl. Fig. 1L). All cirratulids considered here have eyes except *C. s. serratus*. *Poeobius meseres*, *Flota* spp., all recently discovered swimming acrocirrids, *Chauvinelia* spp. and *H. rankini* all lack eyes.

*14 and 15. Nuchal organs.* Nuchal organs are considered to be chemosensory organs consisting of a cluster of ciliated cells on the prostomium. Cirratulids have simple nuchal organs consisting of a pit. Spies (1975) considered only the ciliated pit next to the palps of flabelligerids the nuchal organs but not the two pairs of ciliary tracts that extend out from them along the prostomial ridge, thus contradicting Orrhage (1966) and Schliepers' (1927) interpretations that included the ciliary tracts running parallel to the body axis. We have here considered the ciliary tracts connected to the pits and running along the prostomial ridge (extends dorsally between the branchial membranes) as the nuchal organs of flabelligerids. Acrocirrids have variable nuchal structures ranging from short, relatively straight ciliated ridges as in *S. bombiviridis* and Shining and Tawi-tawi Bombers (Suppl. Fig. 1I arrow) to long, convoluted ridges with many 180° bends as seen in *A. validus* (Suppl. Fig. 1L), or a combination of a long ridge with the ciliated tracts extending onto free-standing structures such as the oppositely branched appendages and spiral structures seen in Juanita worm (Suppl. Fig. 1J). Incidentally, the portion of the head across which the ciliary tracts or ridges extend is here considered the extent of the prostomium until developmental studies can provide further information.

*16. Head branchiae.* Unusual structures are found on the prostomium of *S. bombiviridis* (Suppl. Fig. 1I), Shining, Tawi-tawi, Horned and Tiburon Bombers (Figs 1B, D, F, G), *Chauvinelia* spp. and *H. rankini* that are here considered branchiae, based on the presence of large afferent and efferent vessels within the structures. This follows previous authors (Laubier 1974; Averincev 1980; Glasby & Fauchald 1991; Salazar-Vallejo *et al.* 2007). These structures have broad bases and taper evenly to their tips (Suppl. Fig. 1I). Unlike typical acrocirrid palps and branchiae these are not easily lost and leave a ragged scar if they are. These branchiae occur in one or more rows and the bases are surrounded by the convoluted, ciliated ridges of the nuchal organs of *Chauvinelia* spp., *H. rankini*, and



Horned and Tiburon Bombers (Fig. 1G). The identity of these structures puzzled Hartman (1978) when she originally allied *H. rankini* with Hesionidae, suggesting they were anterior dorsal cirri. The location of these structures surrounded by the nuchal organs would suggest that they are prostomial, but they are always medial to the nuchal organs instead of completely enclosed by the ciliated ridges. Hence it may be that they are segmental in origin and have migrated to their antero-dorsal position. Glasby & Fauchald (1991) concluded the two types of branchiae seen in *H. rankini* were not homologous because they arose from different areas. This type of branchia is not found in Cirratulidae, Flabelligeridae, previously known acrocirrids other than *Chauvinelia* spp. and *H. rankini*, nor Squidworm and Juanita worm.

*17. Main branchiae.* Branchial filaments show great variety within the groups considered here. *Ctenodrilus s. serratus* lacks branchial filaments and *Dodecaceria* spp. have four pairs restricted to anterior segments, though most cirratulids have numerous pairs of long digitiform branchiae along the body. Flabelligerids have various forms of branchiae that are typically concentrated just behind the prostomium on what has often been referred to as the branchial membrane. The branchial membrane can be flat on the body as in *Flabelligera* spp., *Flabelliderma* spp., *Pherusa* spp. and *Diplocirrus* spp., or it can be a projecting structure or structures as in members of *Coppingeria* sp., *Diversibranchus nicolaji* Buzhinskaja, 1993 and *Piromis* species. The branchial membranes may support attachment of four to over 80 pairs of branchial filaments. Acrocirrid branchial filaments are found in pairs on two to usually four anterior segments. In *S. bombiviridis*, Shining, Tawi-tawi, Horned, and Tiburon Bombers and *H. rankini*, these branchiae are short, elliptical bulbs (Fig. 1A, F, G) (see character 19).

*18. Main branchiae (number of pairs).* Among cirratulids and flabelligerids the number of branchial pairs varies widely. Acrocirrids have four pairs with the possible exception of *Flabelligella* spp. that is reported to have only two pairs (Kolmer 1985) and *Chauvinelia* spp. that were reported to have only three pairs (0–3 in *C. arctica*, Averincev 1980). However, the branchiae referred to by Averincev are the same here referred to as “head branchiae” and we suggest the indistinct swellings on the achaetous segments are branchial scars based on similar structures observed in *S. bombiviridis*, Shining, Tawi-tawi, Horned and Tiburon Bombers and *H. rankini*. Only two pairs of these scars were

drawn (Averincev 1980), thus they are scored here as having just two pairs but it is possible with the contraction, distortion and the poor condition of the specimens that others are present but indistinguishable. Additionally, the other acrocirrids scored as having just two pairs of main branchiae (*F. incrusta*, *Flabelligella* sp. and *H. rankini*) may be the result of missing information as well, since all accounts are unconfirmed or incomplete with respect to this character.

*19. Main branchiae (shape).* The main branchiae of cirratulids are long and digitiform. Branchial filaments in most flabelligerids are also digitiform (Suppl. Figs 1G, K) with the exception of the unusual forms found in *D. nicolaji* and *Flota* species. Acrocirrids such as *A. validus* (Suppl. Fig. 2L), *Macrochaeta* spp., *Flabelligella* spp., *Flabelligena* spp., and *F. incrusta* have digitiform branchial filaments like those of cirratulids, however some acrocirrids have an unusual form. The unusual branchial form was first found in *H. rankini* by Glasby & Fauchald (1991) who referred to them as “foliose”. This branchial form is short, being less than twice as long as wide, or elliptical. These elliptical branchiae are also found in *S. bombiviridis*, Shining, Tawi-tawi, Horned and Tiburon Bombers. These branchiae are the structures autotomized by *S. bombiviridis* and Shining Bomber that bioluminesce brilliant green. Digitiform branchiae that were shorter than normal have also been observed in a possibly undescribed *Flabelligena* species (Osborn *et al.* 2009 suppl. Fig. S2D).

*20. Nephridiopores as papillae.* A pair of large anterior nephridia is found in all adequately investigated Cirratuliformia and the nephridiopores for these nephridia are likewise found in anterior segments. In cirratulids and some flabelligerids these nephridiopores are simple pores. However, other flabelligerids, such as *Pherusa plumosa* CA and *Diplocirrus longisetosus* ((Marenzeller, 1890) Filippova *et al.* 2003), and several acrocirrids, such as *Flabelligena* spp., *Flabelligella* spp. and some species of *Acrocirrus* and *Macrochaeta*, have projecting papillae as the nephridiopores. Banse (1969) was the first to suggest that these structures may be nephridiopores and their presence has been noted or illustrated by other authors (Orensanz 1974; Rouse & Pleijel 2001; Kolmer 1985; Kudenov 1976; Santos & Silva 1993; Aguirrezabalaga & Ceberio 2006).

*21. Nephridiopores (location).* The precise location of the nephridiopore is unknown in cirratulids other than *C. s. serratus*, where it is found on the peristomium (Rouse &

Pleijel 2001). In all recently discovered swimming acrocirrids the large anterior nephridia connect to the papillae on segment 2, confirming Banse's suggestion (1969). In flabelligerids such as *P. plumosa* CA, *D. longisetosus* and *Flabelligera* spp. the nephridiopore is on the branchial membrane. In those flabelligerids with four pairs of branchiae, the nephridiopore papillae are consistently located at the bases of the second pair of branchiae (the most lateral branchia was here considered the first) suggesting that the branchiae of flabelligerids are homologous to the segmental branchiae of acrocirrids.

22. *Parapodia distinct from body wall.* Parapodia are simple structures when present in the groups considered here. Cirratulids generally have no protruding tissue forming parapodia and those of flabelligerids and acrocirrids, when present, are broad, smooth projections.

23. *Uniramous anterior chaetigers.* A few taxa considered here have one or more uniramous anterior chaetigers, for example *Acrocirrus* spp., *Macrochaeta* spp., *Flabelligena* spp. and *H. rankini* have at least one pair of parapodia lacking neurochaetae, *F. incrusta* lacks notochaetae throughout, and all other taxa have only biramous anterior chaetigers.

24. *Chaetae.* Cirratulid chaetae are smooth, while flabelligerid chaetae are unusual in having distinctive cross-barring, referred to as barred (Rouse & Pleijel 2001), or multiarticulate (Salazar-Vallejo *et al.* 2007). Salazar-Vallejo *et al.* (2008) argued that this feature distinguished flabelligerids from other taxa because the articulations are found along the length of each chaeta (articulations may be indistinct basally) and are created by many cone shaped layers stacked on top of each other with complete internal septa running through the external hyaline cortex. With scanning electron microscopy of air-dried chaetae, this is seen as a plate around which the exterior collapses in *Flota* sp. (Fig. 3I). In *P. plumosa* CA (Fig. 3J–K) and *Flabelligera mundata* Gravier, 1906 (Suppl. Fig. 2L) scanning electron microscopy shows the internal layers and external hyaline layer as described by Salazar-Vallejo *et al.* (2008). Acrocirrids on the other hand have what are typically referred to as spinous chaetae, described by Salazar-Vallejo *et al.* (2007) as a dehiscent crust of successive rings of tiny denticles over a smooth, non-annulated core. While this appears to be the case under light microscopy, scanning electron microscopy reveals a slightly different arrangement. The exterior is not smooth as in flabelligerids;

there are always fine spines or fibers on the exterior of the chaetae (Suppl. Figs 2A, D), and these spines are typically most pronounced distally (Suppl. Fig. 2B). In some species the rings of denticles appear to be cones of various lengths whose edges exhibit varying degrees of raggedness (Suppl. Figs 2A–C, F–G). In others there are long layers ending distally in ragged rows (Suppl. Figs 2E, H), or simply denticles randomly dispersed over the surface (Suppl. Fig. 2D). The difference between flabelligerid and acrocirrid chaetae seems to be that flabelligerids appear to have internal segments as stacked cylinders and these are covered by a thin, smooth, outer layer that never has denticles on the surface. Acrocirrid chaetae on the other hand, may have smooth shafts (Suppl. Fig. 2E) but always have denticles on at least the distal tips (Suppl. Figs 2A–H). In some acrocirrids the whorls of spines seem to be the edges of cup-shaped segments of unknown depth through the chaetae (Suppl. Figs 2B–C, F–G). The cups may form an outer sheath as suggested by Salazar-Vallejo *et al.* (2007) or penetrate through the shaft; no evidence was obtained to suggest the cups are only an outer sheath.

25. *Compound chaetae.* Cirratulidae have only simple (lacking a joint) chaetae.

Flabelligerids have only simple capillaries in their notopodia and capillaries, hooks, and compound or pseudocompound hooks in their neuropodia. Acrocirrid notochoetae are capillary and their stout neurochaetae can be simple, pseudocompound or compound. The term pseudocompound has been inconsistently applied to intermediates between simple and compound chaetae, typically defined as having a fold instead of a true hinge, having an incomplete socket, or having a double ligament restricting the free movement of the second element within the socket (Merz & Woodin 2006). The stout chaetae found in members of *Acrocirrus*, *Macrochaeta*, *Flabelligena*, *Flabelligella*, *Flabelliderma*, *Diplocirrus*, *Piromis*, *Therochaeta*, *Flabelligera* (Suppl. Fig. 2L) and in Juanita worm have been referred to as pseudocompound because of the rigidity of the joint and incomplete socket, however these are not differentiated for this project from the truly compound chaetae (Suppl. Fig. 2F) found in *Chauvinelia* spp., Tawi-tawi (Suppl. Fig. 2F), Horned and Tiburon Bombers.

26. *Broad, flattened chaetae.* Tawi-tawi Bomber and Squidworm have broad, flattened chaetae (Suppl. Figs 2E–H). This feature is unique among the taxa considered here

although several flabelligerids and acrocirrids have chaetae whose shafts are not cylindrical (Suppl. Fig. 2L).

27. *Neurochaetae (second element)*. The distal element of the compound chaetae when present can be straight (Suppl. Fig. 2F), slightly curved along its length, or distinctly hooked at the tip (Suppl. Fig. 2L).

28. *Discrete gonads in few segments*. Cirratulids produce gametes in multiple abdominal segments, as do previously studied acrocirrids (Banse 1969). Flabelligerids generally have gonads in a few anterior segments (*e.g. Pherusa* spp. Schlieper 1927 and Amor 1994; *F. commensalis* Spies 1977; *P. meseres* Robbins 1965; *Flota flabelligera* Hartman 1967). All recently discovered swimming acrocirrids have gonads in three or fewer anterior segments (Fig. 1A).

29. Like the majority of polychaetes (Rouse & Fauchald 1997), cirratulid guts are straight, as are those of members of *Acrocirrus*, *Macrochaeta* and *Flabelligena*. The guts of all recently discovered swimming acrocirrids have at least one loop (Fig. 1A–B), similar to all examined flabelligerids (Fig. 2G).

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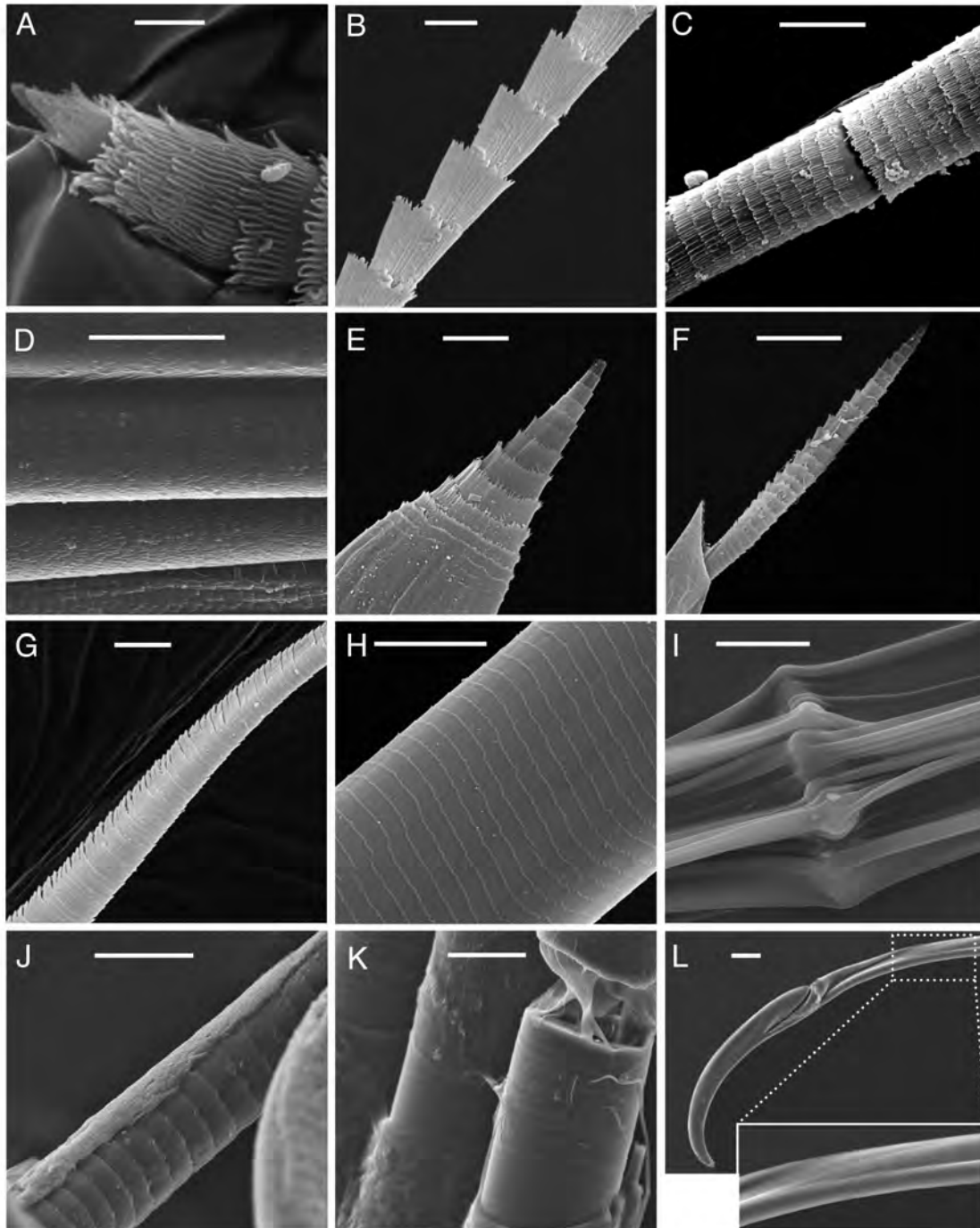
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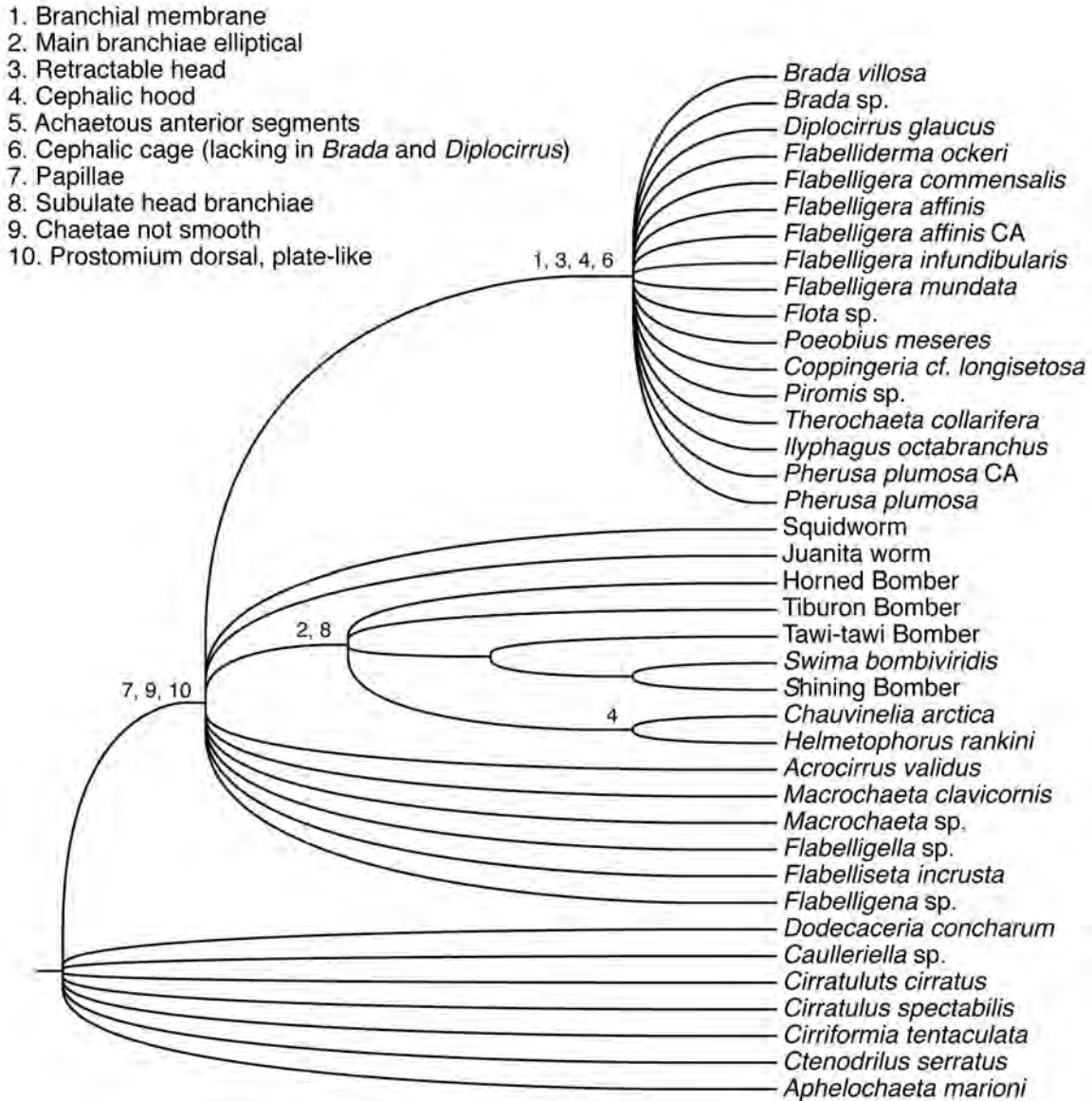


**Supplemental Figure 1.** Photos of various characters. A. *Acrocirrus validus*, left lateral view showing four branchiae scars (numbered), the nephridiopore (white arrow), palp (p) and clusters of short interramal papillae (black arrow). B. *Chauvinelia arctica* (ZMUC POL-0159) clavate interramal papillae. C. *Chauvinelia biscayensis* holotype possible lollipop-shaped interramal papillae. D. Shining Bomber lollipop interramal papillae. E. *Flabelligera affinis* from Edithburgh, Australia with gelatinous sheath removed showing long clavate body papillae. F. *Flabelligera affinis* from same collecting event, with gelatinous sheath intact. G. *Poebobius meseres* showing thick gelatinous sheath (gs) and

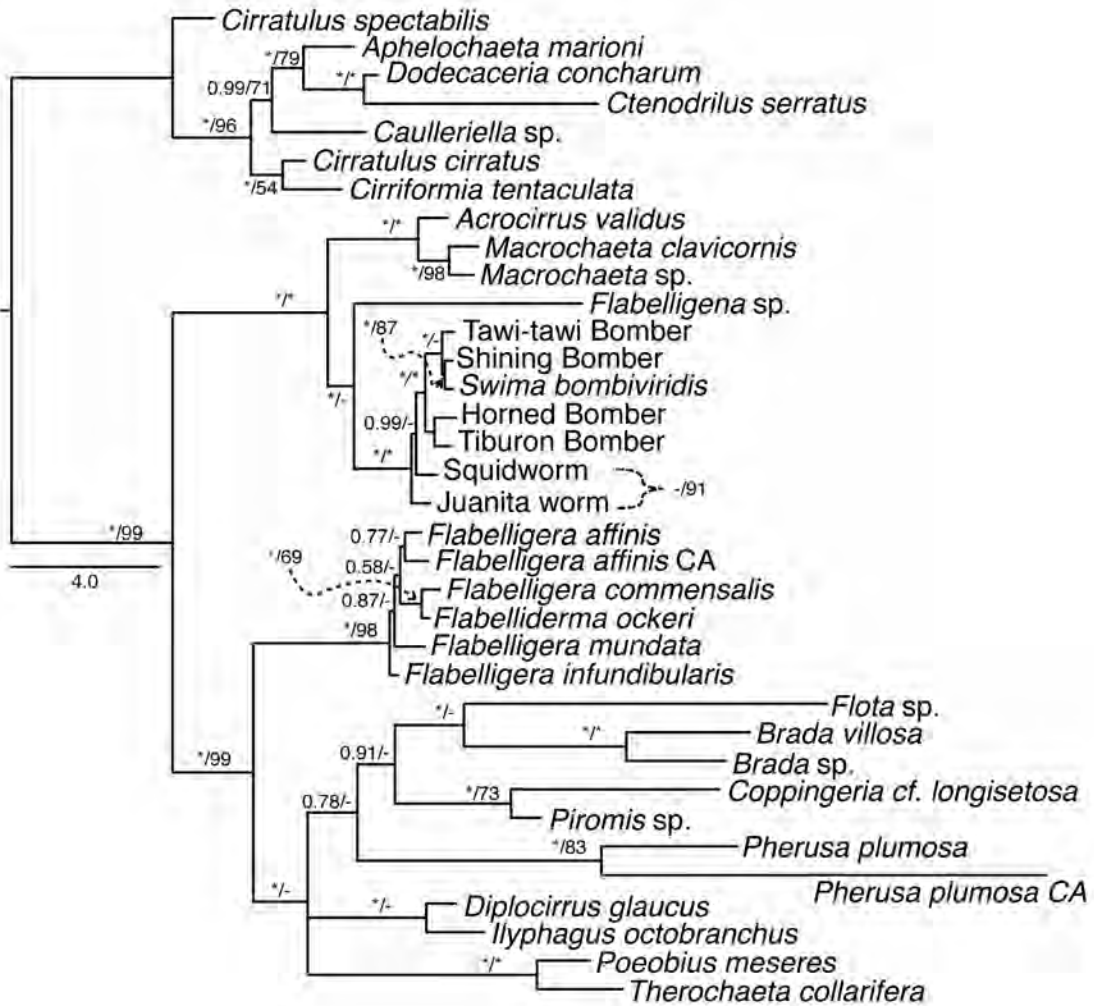
protruding body papillae (arrow). H. Body of *Flabelliderma ockeri* (right) removed from its sediment encrusted gelatinous sheath (left). I. *Swima* sp., dorsal view of head showing palps, medial subulate branchia (sb) and nuchal organ (arrow). J. Juanita worm, dorsal view of head showing palp scar (p) and branchiae scars (numbered), spiral (spn) and oppositely branched (obn) free-standing nuchal organs. K. Head of *Pherusa plumosa* CA showing a palp, branchia, eyes and nephridiopore papillus (arrow). L. *Acrocirrus validus* head in dorsal view showing palps, branchiae, eyes, and convoluted nuchal ridges, photo F. Pleijel. M. *Swima bombiviridis* ventral view of anterior showing nephridiopores (arrow indicates left nephridiopore), lollipop interramal papillae and subulate branchia. N. Antero-dorsal view of head of Squidworm showing palps, branchiae, oppositely branched free-standing nuchal organs and nephridiopores (arrow indicates left nephridiopore), photo L.P. Madin.



**Suppl. Figure 2.** Acrocirrid (A– H) and flabelligerid (I–L) chaetae. A. Shining Bomber distal tip neurochaeta. B. Juanita worm notochaeta. C. Tiburon Bomber neurochaeta. D. *Acrocirrus validus* notochaetae. E. Tawi-tawi Bomber notochaeta distal tip. F. Tawi-tawi Bomber distal element neurochaeta. G. Squidworm distal portion neurochaeta. H. Squidworm shaft of neurochaeta. I. *Flota* sp. segment joint of notochaetae. J. *Pherusa plumosa* CA medial portion notochaeta. K. *Pherusa plumosa* CA basal portion notochaetae. L. *Flabelligera mundata* neurochaeta. Scale bars, A 2  $\mu\text{m}$ , B–D, G, I 10  $\mu\text{m}$ , E–F, H, J–L 40  $\mu\text{m}$ .



**Suppl. Figure 3.** Strict consensus of 1,046,189 most parsimonious trees (tree length = 96) from analysis of the morphology matrix.



**Suppl. Figure 4.** Majority rule consensus tree from Bayesian analyses of five concatenated genes. Data were partitioned by each gene and first, second and third codon positions. *CytB* third positions were excluded due to saturation. Support is indicated as posterior probabilities from the Bayesian analyses/bootstraps from the parsimony analysis. Asterisks indicate 1.0 or 100 % support respectively; dashes indicate unsupported nodes.

**Appendix I.** Matrix of multistate coding scores of morphology. Unknowns are coded with “?” and inapplicable with “-”.

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<b>Cirratulidae</b>																													
<i>Aphelochaeta marioni</i>	0	0	1	0	1	0	-	-	0	0	0	0	1	0	-	0	0	0	0	0	?	0	0	0	0	0	-	0	0
<i>Cauleriella</i> sp.	0	0	1	0	1	0	-	-	0	0	0	0	1	0	-	0	0	0	0	0	?	0	0	0	0	0	-	0	0
<i>Cirratulus spectabilis</i>	0	0	1	0	1	0	-	-	0	0	0	2	1	0	-	0	0	0	0	0	?	0	0	0	0	0	-	0	0
<i>Cirratulus cirratus</i>	0	0	1	0	1	0	-	-	0	0	0	2	1	0	-	0	0	0	0	0	?	0	0	0	0	0	-	0	0
<i>Cirriformia tentaculata</i>	0	0	1	0	1	0	-	-	0	0	0	2	1	0	-	0	0	0	0	0	?	0	0	0	0	0	-	0	0
<i>Ctenodrilus serratus serratus</i>	0	0	0	0	1	0	-	-	0	0	0	1	0	0	-	0	2	-	-	0	2	0	0	0	0	0	-	0	0
<i>Dodecaceria concharum</i>	0	0	1	0	1	0	-	-	0	0	0	0	1	0	-	0	0	1	0	0	?	0	0	0	0	0	-	0	0
<b>Acrocirridae</b>																													
<i>Acrocirrus validus</i>	0	0	2	0	0	1	0	0	0	0	1	0	1	1	2	0	0	1	0	0	1	1	1	2	1	0	2	0	0
<i>Chauvinelia arctica</i>	0	1	5	0	0	1	0	1	0	2	1	0	0	1	2	1	0	3	?	?	?	1	?	2	1	0	0	1	1
<i>Flabelligella</i> sp.	1	0	1	0	1	1	0	1	1	0	1	0	0	?	?	0	0	3	0	1	1	0	0	1	1	0	2	0	?
<i>Flabelligena</i> sp.	0	0	?	0	1	1	0	1	1	0	1	0	?	?	?	0	0	1	0	1	1	0	1	2	1	0	2	0	0
<i>Flabelliseta incursta</i>	0	0	?	0	1	1	0	3	1	2	1	0	?	?	?	0	0	3	0	?	?	0	1	2	1	0	0	?	?
<i>Helmetophorus rankini</i>	0	1	?	0	0	1	0	1	0	0	1	0	0	1	2	1	0	3	1	?	?	1	1	2	0	0	-	?	?
Horned Bomber	0	0	3	0	0	1	0	2	0	1	1	0	0	1	2	1	0	1	1	1	1	1	0	2	1	0	0	1	1
Juanita worm	0	0	3	0	0	1	0	2	0	0	1	0	0	3	2	0	0	1	0	?	0	1	0	2	1	0	1	1	1
<i>Macrochaeta clavicornis</i>	0	0	2	0	0	1	0	1	1	0	1	0	1	1	?	0	0	1	0	1	1	0	1	2	1	0	2	0	0
<i>Macrochaeta</i> sp.	0	0	2	0	0	1	0	1	1	0	1	0	1	1	?	0	0	1	0	1	1	0	1	2	1	0	2	0	0
Shining Bomber	0	0	3	0	0	1	1	2	0	2	1	0	0	1	0	1	0	1	1	1	1	1	0	2	0	0	-	1	1
Squidworm	0	0	?	0	0	1	0	2	0	1	1	0	0	2	2	0	0	1	0	1	0	1	1	2	0	1	-	1	1
<i>Swima bombiviridis</i>	0	0	3	0	0	1	1	2	0	2	1	0	0	1	0	1	0	1	1	1	1	1	0	2	0	0	-	1	1
Tawi-tawi Bomber	0	0	4	0	0	1	1	2	0	2	1	0	0	1	1	1	0	1	1	1	1	1	0	2	1	1	0	1	1
Tiburon Bomber	0	0	3	0	0	1	0	2	0	1	1	0	0	1	2	1	0	1	1	1	1	1	0	2	1	0	0	1	1
<b>Flabelligeridae</b>																													
<i>Brada</i> sp.	1	1	?	0	0	1	0	?	1	0	2	0	?	1	?	0	1	0	0	?	0	0	0	1	0	0	-	1	1
<i>Brada villosa</i>	1	1	?	0	0	1	0	?	1	0	2	0	?	1	?	0	1	0	0	?	0	0	0	1	0	0	-	1	1
<i>Coppingeria</i> sp.	1	1	?	1	1	1	0	0	1	0	2	0	1	1	0	0	1	0	0	?	0	0	0	1	0	0	-	?	?
<i>Diplocirrus glaucus</i>	1	1	?	0	0	1	0	?	1	0	2	0	0	1	0	0	1	1	0	1	0	0	0	1	1	0	2	1	?

Character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>Flabelliderma ockeri</i>	1	1	?	1	0	1	1	1	1	2	2	0	1	1	0	0	1	0	0	0	0	1	0	1	1	0	2	1	1
<i>Flabelligera affinis</i>	1	1	?	1	0	1	1	1	0	2	2	0	1	1	0	0	1	0	0	?	0	1	0	1	1	0	2	1	1
<i>Flabelligera affinis</i> CA	1	1	?	1	0	1	1	1	0	2	2	0	1	1	0	0	1	0	0	?	0	1	0	1	1	0	2	1	1
<i>Flabelligera commensalis</i>	1	1	?	1	0	1	0	0	0	1	2	0	1	1	0	0	1	0	0	?	0	1	0	1	1	0	2	1	1
<i>Flabelligera infundibularis</i>	1	1	?	1	0	1	1	1	0	2	2	0	1	1	0	0	1	0	0	?	0	1	0	1	1	0	2	1	1
<i>Flabelligera mundata</i>	1	1	?	1	0	1	1	1	0	2	2	0	?	1	0	0	1	0	0	?	0	0	0	1	1	0	2	1	1
<i>Flota</i> sp.	1	1	?	0	0	1	1	0	0	2	2	0	0	1	0	0	1	3	0	0	?	1	0	1	0	0	-	1	1
<i>Ilyphagus octobranchus</i>	1	1	?	1	1	1	1	?	1	0	?	0	?	1	?	0	1	0	0	?	0	0	0	1	0	0	-	?	?
<i>Pherusa plumosa</i>	1	1	?	1	0	1	0	?	1	0	2	0	1	1	0	0	1	1	0	?	0	0	0	1	0	0	-	?	1
<i>Pherusa plumosa</i> CA	1	1	?	1	1	1	0	?	1	0	2	0	1	1	0	0	1	1	0	1	0	0	0	1	0	0	-	?	1
<i>Piromis</i> sp.	1	1	?	1	1	1	0	?	1	2	2	0	1	1	0	?	1	0&1	0	?	0	1	0	1	1	0	-	?	1
<i>Poeobius meseres</i>	1	1	-	-	1	1	1	?	0	2	1	0	0	1	0	0	1	0	0	0	0	0	-	3	-	-	-	1	1
<i>Therochaeta collarifera</i>	1	1	?	1	1	1	1	?	1	0	?	0	?	1	?	0	1	1	0	?	0	0	0	1	1	0	2	?	?

### Characters

1. Retractable head: 0. absent or 1. present.
2. Cephalic hood: 0. absent or 1. present.
3. Achaetous anterior segments: 0. absent, 1. possessing 1, 2. possessing 2, 3. possessing 3, 4. possessing 4, or 5. possessing 6.
4. Cephalic cage (chaetae of first chaetiger at least 1/3 longer than those of midbody): 0. absent or 1. present.
5. Externally obvious segment margins on majority of body: 0. present or 1. absent.
6. Papillae: 0. absent or 1. present.
7. Body papillae: 0. short or 1. long, clavate.
8. Interramal papillae: 0. short, 1. clavate, 2. lollipop, or 3. balloon.
9. Sediment adhered to body: 0. absent or 1. present.
10. Gelatinous sheath: 0. absent, 1. thin, doesn't obscure features, or 2. thick, obscures features such as parapodial rami.

11. Prostomium shape: 0. terminal, lobe-like, with projecting anterior margin, 1. dorsal, plate-like, posterior margin smooth, not projecting posteriorly over segments, or 2. dorsal, plate-like, posterior margin projecting posteriorly over segments.
12. Grooved palps: 0. 1 pair, 1. absent, or 2. more than one pair.
13. Eyes: 0. absent or 1. present.
14. Nuchal organs: 0. pit only, 1. ciliate ridge only, 2. at least part of ciliated ridge on a free-standing oppositely branched structure, or 3. at least part of ciliated ridge on free-standing, oppositely branched and spiral structures.
15. Nuchal organs (ridge shape): 0. straight (with less than 180 degree bend), 1. U-shaped (with single 180 degree bend), 2. convoluted (with multiple 180 degrees bends).
16. Head branchiae: 0. absent or 1. subulate, not easily lost.
17. Main branchiae: 0. on one or more segments, not on branchial membrane, 1. on branchial membrane, or 2. absent.
18. Main branchiae (number pairs): 0. more than 4 pairs, 1. 4 pairs, 2. 3 pairs, 3. 2 pairs.
19. Main branchiae (shape): 0. long, tapered or 1. short, elliptical.
20. Nephridiopores as papillae: 0. absent or 1. present.
21. Nephridiopores (location): 0. branchial membrane, 1. segmental, or 2. peristomial.
22. Parapodia distinct from body wall: 0. absent or 1. present.
23. Anterior chaetigers (one or more): 0. biramous or 1. uniramous.
24. Chaetae: 0. smooth, 1. cross-barred, segmented, 2. spinous, or 3. absent.
25. Compound chaetae: 0. absent or 1 present.
26. Broad, flattened chaetae: 0. absent, or 1. present.
27. Neurochaetae (second element): 0. straight, 1. curved, or 2. hooked.
28. Discrete gonads in few segments: 0. absent or 1. present.
29. Gut: 0. straight or 1. looped