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Fixed, free, and fixed: The fickle phylogeny of extant Crinoidea (Echinodermata) and their Permian–Triassic origin

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ABSTRACT

Although the status of Crinoidea (sea lilies and featherstars) as sister group to all other living echinoderms is well-established, relationships among crinoids, particularly extant forms, are debated. All living species are currently placed in Articulata, which is generally accepted as the only crinoid group to survive the Permian–Triassic extinction event. Recent classifications have recognized five major extant taxa: Isocrinida, Hyocrinida, Bourgueticrinina, Comatulidina and Cyrtocrinida, plus several smaller groups with uncertain taxonomic status, e.g., *Guillecrinus*, *Proisocrinus* and *Caledonicrinus*. Here we infer the phylogeny of extant Crinoidea using three mitochondrial genes and two nuclear genes from 59 crinoid terminals that span the majority of extant crinoid diversity. Although there is poor support for some of the more basal nodes, and some tree topologies varied with the data used and mode of analysis, we obtain several robust results. Cyrtocrinida, Hyocrinida, Isocrinida are all recovered as clades, but two stalked crinoid groups, Bourgueticrinina and Guillecrinina, nest among the featherstars, lending support to an argument that they are paedomorphic forms. Hence, they are reduced to families within Comatulida. *Proisocrinus* is clearly shown to be part of Isocrinida, and *Caledonicrinus* may not be a bourgueticrinid. Among comatulids, tree topologies show little congruence with current taxonomy, indicating that much systematic revision is required. Relaxed molecular clock analyses with eight fossil calibration points recover Articulata with a median date to the most recent common ancestor at 231–252 mya in the Middle to Upper Triassic. These analyses tend to support the hypothesis that the group is a radiation from a small clade that passed through the Permian–Triassic extinction event rather than several lineages that survived. Our tree topologies show various scenarios for the evolution of stalks and cirri in Articulata, so it is clear that further data and taxon sampling are needed to recover a more robust phylogeny of the group.

1. Introduction

Crinoid echinoderms were one of the most significant Paleozoic and Mesozoic benthic marine animal groups. Their vast numbers resulted in huge fossil beds that established and controlled sedimentary environments and produced extensive deposits that exist today. Over 6000 fossil crinoid species have been described, some with short stratigraphic ranges that make them important indicators (Hess et al., 1999; Meyer and Ausich, 1983). Almost all crinoid

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lineages went extinct at the end of the Permian (Baumiller and Hagdorn, 1995; Hess, 1999), though the survivors (Articulata) subsequently underwent a series of radiations (Baumiller et al., 2010; Meyer and Macurda, 1977; Roux, 1987; Simms, 1988b; Twitchett and Oji, 2005). Their once spectacular, and largely Paleozoic, diversity means that they are sometimes unfairly regarded as a depauperate group (see Meyer and Macurda, 1977). However, extant crinoids—the sea lilies and featherstars—still constitute important components of a variety of marine assemblages (Bowden et al., 2011; Llewellyn and Messing, 1993; Messing, 1994; Messing et al., 2006). They occur in all modern seas, except the Black and Baltic, from the lower intertidal to over 9000 m (Belyaev, 1966; Oji et al., 2009) and at densities that may exceed 100 per m^2 (Messing, 1994). Almost half of the ~620 nominal extant species occur at depths of 200 m or less; new species in all major groups continue to be described, and deep-sea species are increasingly being recovered by submersibles and remotely operated vehicles (ROVs) (Donovan and Pawson, 2008; Roux, 2004). Still, crinoids remain the least studied of extant echinoderms, and few studies have attempted to assess the phylogenetic relationships among major extant taxa or basic issues such as their monophyly (Cohen et al., 2004; Simms, 1988b). As a result, current understanding of post-Paleozoic crinoid evolution leaves fundamental questions unresolved: (1) What are the relationships among the major groups of extant crinoids, and (2) What is the evolutionary history of today's most diverse taxon, the comatulid featherstars?

Recent classifications have recognized five main groups of crinoids with extant members (Fig. 1)—Isocrinida (Fig. 1A), Hyocrinida (Fig. 1B), Bourgueticrinina (Fig. 1C), Comatulidina (Fig. 1D), and Cyrtocrinida (Fig. 1E)—with various interpretations about how they are related to each other and to fossil groups (e.g., Améziane and Roux, 2005; Hess, 2011a; Simms, 1988b; Simms and Sevastopulo, 1993). The following summary refers to these groups according to the classification used in Hess et al. (2011) and here in Table 1, though other schemes have placed them at different categorical levels with varying terminations (e.g., Bourgueticrinida vs. Bourgueticrinina, Comatulida vs. Comatulidina vs. Comatulidia; Hyocrinida vs. Hyocrinidia) and have included different fossil groups.

Members of Isocrinida (24 extant species) all bear at least rudimentary hook-like cirri along the long stalk, with which they can cling to a variety of substrates (Fig. 1A). They are mainly found at depths of 200–900 m, though *Metacrinus rotundus* occurs as shallow as 100 m off Japan (Oji, 1986). One crown group isocrinid taxon includes fossils dating to the Late Triassic (Hess, 2011i). Hyocrinida (23 extant species) possess a long smooth stalk that cements permanently to hard substrates (Fig. 1B). The stalk attaches proximally to a box-like calyx (a set of skeletal plates that encloses the viscera and supports the five rays) with (usually) five arms. Hyocrinids are found at depths >700 m; there is almost no fossil record for the group, which contributes to considerable debate on their systematic placement (Hess, 2011h).

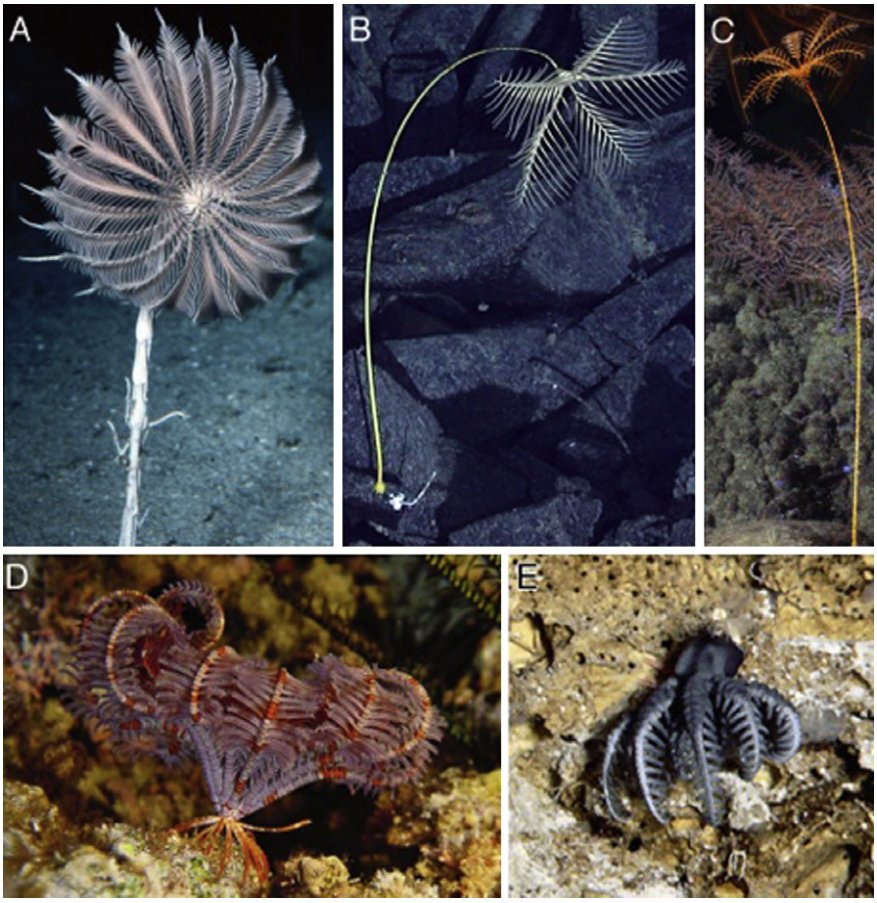

Fig. 1. Representative diversity of the major extant crinoid lineages. (A) *Neocrinus decorus* (Isocrinida), depth 420 m, south of West End, Grand Bahama Island. Note the stalk with cirri. Photo © C.G. Messing. (B) *Laubiericrinus pentagonalis* (Hyocrinidae) from near hydrothermal vents of the North Fiji Basin, western Pacific Ocean, depth ~2000 m. Note columnals without cirri. Photo © Woods Hole Oceanographic Institution. (C) *Bathycrinus* cf. *equatorialis* (Bourgueticrinidae), Davidson Seamount, off California. Note columnals without cirri. Photo © Monterey Bay Aquarium and Research Institute. (D) *Stephanometra indica* (Mariametridae, Comatulida), depth 10 m, Lizard I., Great Barrier Reef, Australia. Note cirri arising from, and obscuring, the centrodorsal. Photo © G.W. Rouse. (E) *Holopus rangii* (Holopodidae, Cyrtocrinida), depth 591 m, Bahamas. The stalk is absent, and the calyx cements directly to the substrate. Photo © Harbor Branch (Florida Atlantic University).

Table 1
Samples and Genbank accession numbers used in this study. New sequences are in bold.

Taxon	Source	COI	16S	CytB	18S	28S
Eleutherozoa						
Arbacia lixula	GB	X80396	X803966	X80396		
Arbacia punctulata	GB				DQ073778	AY026367
Asterias amurensis	GB	AB183559	AB183559	AB183559	D14358	
Asterias forbesii	GB					AF212169
Cucumaria miniata	GB	AY182376	AY182376	AY182376	DQ777082	
Cucumaria salma	GB					AF212170
Ophiopholis aculeata	GB	AF314589	DQ297105	AF314589	DQ060806	DQ029078
Cyrtocrinida						
Holopus rangii	SAM K2143	**GU327836**	**GU327869**	**GU327908**		**GU327942**
Holopus alidis	GB		AY275903		AY275896	
Neogymnocrinus richeri	Genbank/ MNHM EcPh 92	DQ068951	DQ068951	DQ068951	AY275895	**GU327943**
Hyocrinida						
Hyocrinus cf. *biscoiti*[c]	SIO-BIC E4424	**GU327837**	**GU327870**		**GU327930**	**GU327944**
Hyocrinus sp.	SAM K2144	**GU327838**	**GU327871**			**GU327945**
Thalassocrinus? sp.[c]	SIO-BIC E4428	**GU327839**	**GU327872**			**GU327946**
Isocrinida						
Endoxocrinus alternicirrus	SAM K2141		**GU327873**		GQ913341	
Endoxocrinus parrae	GB[a]	GQ913324	**GU327874**		Z80951	
Endoxocrinus cf. *parrae*	SAM K2154	**GU327840**	**GU327875**	**GU327909**		**GU327947**
Endoxocrinus sp.	MNHM Lost (Fiji)	**GU327841**	**GU327876**	**GU327910**		
Hypalocrinus naresianus	SAM K2171	**JX296555**	**JX296556**			
Metacrinus levii	MNHM EcPh 50	GQ913322	**GU327877**			**GU327948**
Metacrinus sp. D1442	GB		AY275904		AY275897	
Metacrinus cf. *rotundus*	GB		AY275905		AY275898	
Proisocrinus ruberrimus	SAM K2151	**GU327842**	**GU327878**			**GU327949**
Saracrinus moosai	MNHM EcPh 10	GQ913323	**GU327879**	**GU327911**		**GU327950**
Comatulida						
Bourgueticrinina						
Bathycrinus cf. *australis*	GB		AY275899		AY275891	
Bathycrinus sp.	SIO-BIC E4432	**GU327843**	**GU327880**	**GU327912**		
Caledonicrinus vaubani	GB		AY275900		AY275892	
Democrinus cf. *brevis*	SAM K2163	**GU327844**	**GU327881**	**GU327913**		**GU327951**
Monachocrinus caribbeus	USMN E42707	**GU327845**	**GU327882**	**GU327914**		**GU327952**
Porphyrocrinus verrucosus	CRECH 144	**GU327846**	**GU327883**	**GU327915**		**GU327953**
Guillecrinina						
Guillecrinus neocaledonicus	GB		AY275901		AY275901	
Vityazicrinus petrachenkoi	SAM K2254	**GU327847**	**GU327884**	**GU327916**	**GU327931**	**GU327954**
Comatulidina						
Antedonidae						
Antedon mediterranea	GB /no voucher	AM404181	AM404181	AM404181	**GU327932**	AF088832
Florometra 'serratissima'	GB /SAM K2140	AF049132	AF049132	AF049132	DQ060789	**GU327955**
Heliometra glacialis form *maxima*	SAM K2145	**GU327848**	**GU327885**	**GU327917**		**GU327956**
Promachocrinus kerguelensis	Pending[b], CAS 159871	DQ823276	**GU327886**	DQ823333	GQ913342	
Aporometridae						
Aporometra wilsoni	SAM K2077	AY669355	**GU327887**			**GU327957**
Charitometridae						
Chondrometra sp.	SAM K2170	**GU327849**	**GU327888**			
Crinometra brevipinna	SAM K2153	**GU327850**	**GU327889**	**GU327918**		**GU327958**
Colobometridae						
Cenometra bella	SAM K2034	**GU327851**	**GU327890**	**GU327920**		**GU327959**
Colobometra perspinosa	GB/IG.31.418	GQ913321	**GU327891**	**GU327919**	GQ913338	**GU327960**
Comasteridae						
Alloeocomatella pectinifera	SAM K2033	**GU327852**	**GU327892**	**GU327921**		**GU327961**
Cenolia trichoptera	SAM K2482	**GU327854**	**GU327894**	**GU327922**		**GU327962**
Clarkcomanthus albinotus	SAM K1978	GQ913312			GQ913328	**GU327964**
Clarkcomanthus littoralis	SAM K1990	GQ913327	**GU327895**	**GU327923**	GQ913344	**GU327963**
Comaster audax	SAM K1979/IG.31.418	**GU327855**			**GU327933**	
Comaster schlegelii	SAM K1966	GQ913317			GQ913333	
Comanthus gisleni	SAM K2004	**GU327856**			**GU327935**	
Comanthus mirabilis	SAM K1945	GQ913313			GQ913329	
Comanthus wahlbergii	SAM K1992	**GU327857**			**GU327934**	
Comatella nigra	SAM K2013	**GU327858**	**GU327896**	**GU327924**		**GU327965**
Comatella stelligera	IG.31.418				**GU327936**	
Oxycomanthus bennetti	IG.31.418	GQ913314			GQ913330	
Oxycomanthus comanthipinna	SAM K2000	GQ913318	**GU327897**		GQ913334	**GU327967**
Oxycomanthus exilis	SAM K1988	**GU327859**				**GU327968**
Oxycomanthus japonicus	SAM K2138	**GU327860**				**GU327966**
Phanogenia gracilis	GB/IG.31.418/SAM K1947	DQ068952	DQ068952	DQ068952	**GU327937**	**GU327969**

(*continued on next page*)

Table 1 (*continued*)

Taxon	Source	COI	16S	CytB	18S	28S
Himerometridae						
Himerometra robustipinna	SAM K1950	GQ913326	**GU327898**	**GU327925**	GQ913343	**GU327970**
Mariametridae						
Lamprometra palmata	SAM K1995	**GU327861**	**GU327899**	**GU327926**	**GU327938**	**GU327971**
Liparometra articulata	SAM K1966	GQ913319	**GU327900**	**GU327927**	GQ913335	**GU327972**
Stephanometra indica	SAM K1967	GQ913320			GQ913336-7	**GU327973**
Notocrinidae						
Notocrinus virilis	CAS 160475	AY669365	**GU327901**			**GU327974**
Pentametracrinidae						
Pentametracrinus sp.	CAS 160484	**GU327864**	**GU327902**			**GU327975**
Ptilometridae						
Ptilometra macronema	SAM K2481	**GU327866**	**GU327903**			**GU327976**
Thalassometridae						
Cosmiometra aster	SAM K2142	**GU327863**	**GU327904**			**GU327977**
Tropiometridae						
Tropiometra afra	Lost (Japan)	**GU327867**	**GU327906**	**GU327928**		**GU327978**
Tropiometra carinata	Eeckhaut (Madagascar)				**GU327941**	
Zenometridae						
Psathyrometra fragilis	SIO-BIC E4433	**GU327865**	**GU327905**			**GU327979**
Zygometridae						
Zygometra microdiscus	SAM K2054	**GU327868**	**GU327907**	**GU327929**		**GU327980**

CAS, California Academy of Sciences (Invertebrate Zoology); **CRECH**, Coral Reef Research Foundation, Koror, Palau; **USMN**, National Museum of Natural History, Smithsonian; **MNHM**, Museum national d'Histoire naturelle, Paris; **GB**, Genbank; **IG**, Institut Royal des Sciences de Belgique; **SAM**, South Australian Museum; **SIO-BIC**, Scripps Institution of Oceanography, Benthic Invertebrate Collection.

[a] COI and 16S sequences for *E. parrae* were from the same specimen as in Littlewood et al. (1997).

[b] Specimen for COI, 16S and Cytb; catalogue number pending from USNM. The specimen has the number Ci43fa in the supplementary information for Wilson et al. (2007).

[c] The terminals referred to as *Hyocrinus* cf. *biscoiti* and *Thalassocrinus*? sp. are being described as a new species and a new genus and species respectively (Roux and Messing, in preparation).

Bourgueticrinina (~40 extant species: Fig. 1C) show five or 10 arms (up to 40 in one species) and occur at depths from ~150 m to >9000 m (Oji et al., 2009). The long stalk lacks cirri and terminates in a disk that cements to hard substrates, or in a branching radix that roots in sediments; fossils placed in extant genera date to ~100 mya (Salamon, 2007).

Comatulidina (featherstars: Fig. 1D) represent the most diverse and widely distributed extant crinoid taxon (>500 extant species) and includes the only crinoids now found in shallow water, though some reach a depth of 5220 m (Clark and Clark, 1967). The oldest accepted comatulid fossils date to the Lower Jurassic (~196 mya), although the oldest paracomatulid—a putative comatulid stem lineage with a short segmented stalk—dates to the Upper Triassic (Hagdorn and Campbell, 1993). Cyrtocrinids cement to hard substrates either via a stalk composed of one or a few columnals or, when the stalk is absent, directly via an expanded calyx. Eight extant species in four genera are found at depths of 90–900 m; *Holopus* (Fig. 1E) is an example of a stalkless form (Donovan and Pawson, 2008). Arguable fossils of crown group cyrtocrinids date to the Middle Jurassic, although the origins of the group remain obscure (Hess, 2011d).

With respect to interrelationships, hyocrinids and cyrtocrinids have previously been placed within Millericrinida, a taxon that otherwise only includes Mesozoic taxa (Rasmussen, 1978a; Simms, 1988b; Simms and Sevastopulo, 1993). However, the most recent classification (Hess, 2011d,j), which is not overtly phylogenetic, treats these three taxa as separate orders (although acknowledging a possible relationship between Millericrinida and cyrtocrinids). The cirrus-bearing Articulata, which include Isocrinida, Comatulidina and extinct Triassic Holocrinida (Baumiller and Hagdorn, 1995), are generally accepted as being closely related. Simms (1988b) placed comatulids and isocrinids in suborders within Isocrinida, and Heinzeller (1998) united the two as a taxon "Cirrata" based on the neuroanatomy of extant species. However, they are currently placed in separate groups at the ordinal level (Hess, 2011i; Hess and Messing, 2011; Simms and Sevastopulo, 1993).

While some authors have treated Bourgueticrinina as a suborder within Millericrinida based on similarities among stalk articulations (Gislén, 1938; Pisera and Dzik, 1979; Roux, 1977, 1987), others have proposed that they are derived from, or are very close to, comatulids (Gislén, 1924; Rasmussen, 1978b; Simms, 1988b, 1989). Bourgueticrinina have been treated as a suborder within Isocrinida (Simms, 1988a,b,c), or within Comatulida (Hess, 2011i) as they are treated here, or even as a family of Comatulidina by Simms et al. (1993).

Comatulidina abandon their stalk following a postlarval stage (Haig and Rouse, 2008), except for the uppermost ossicle, the cirrus-bearing centrodorsal (Fig. 1D). Their apparent 'evolutionary success', relative to taxa that retain the stalk, has been argued as deriving from key features (e.g., mobility and distastefulness) that have allowed them to tolerate or escape shallow-water durophagous predators (McClintock et al., 1999; Meyer and Macurda, 1977; Oji and Okamaoto, 1994). However, Roux (1987) doubted that 'stalked' crinoids previously occupied many of the shallow-water niches now occupied by comatulids, suggesting instead that shallow comatulid success resulted more from a greater tolerance of higher-energy conditions. Comatulidina contains nine superfamilies, six with extant and fossil taxa and three with fossil representatives only (Hess and Messing, 2011). No phylogenetic analysis of the group as a whole has been undertaken, and relationships among and within families are poorly understood (Hess and Messing, 2011; Rowe and Gates, 1995).

Several stalked crinoids, such as *Guillecrinus* (2 species), the monotypic *Vityazicrinus petrachenkoi* and the monotypic *Proisocrinus ruberrimus*, have not had a stable placement as part of a major stalked crinoid taxon. *Guillecrinus* was originally thought to be a relict of the otherwise extinct Inadunata (Bourseau et al., 1991) (an obsolete taxon that included the Cladida: Simms and

Sevastopulo, 1993), but was subsequently placed, with the then newly discovered *Vityazicrinus*, in the suborder Guillecrinina within Hyocrinida (Mironov and Sorokina, 1998). Améziane and Roux (2005) noted that both of these genera share features with Hyocrinida (e.g., sometimes fewer than five basal ossicles, a homeomorphic adult stalk having symplexies with crenular units and often greater than five-part articular facet symmetry) and Bourgueticrinina (e.g., highly differentiated columnal synarthries in some bourgueticrinins and juvenile *Guillecrinus*). By contrast, Hess and Messing (2011) tentatively treated Guillecrinina as a suborder of Comatulida, based on the molecular phylogeny of Cohen et al. (2004). *Proisocrinus* has been considered as the only extant representative of Millericrinidae (Roux, 1980), a crinoid with "Jurassic affinity" (Roux, 1994), or as part of Isocrinida (Oji and Kitazawa, 2008; Rasmussen, 1978a). Attribution to the former has led to its treatment as a bathyal relict (e.g., Cecca, 2002; Zezina, 1997).

All the groups discussed above, as well as other extinct post-Paleozoic crinoids, belong to the taxon Articulata (Hagdorn, 2011; Hess, 2011a; Simms and Sevastopulo, 1993). Articulata was generally recognized as monophyletic, but it remains under debate which Late Paleozoic taxon constitutes the sister group (Simms and Sevastopulo, 1993; Webster and Jell, 1999; Webster and Lane, 2007; Webster et al., 2004). The most recent classification treats only the post-Paleozoic taxa as Articulata (Hess, 2011a). In addition, no unambiguous apomorphy supports the taxon, which has typically been diagnosed by a combination of characters, none unique to the clade or easily recognizable in fossil material (Hess, 2011b; Simms, 1988b; Simms and Sevastopulo, 1993). Simms and Sevastopulo (1993) argued that the extant articulates date to "a common ancestor of probable late Permian or early Triassic age", a view also supported by Hess (2011a). Others have discussed the possibility that Articulata represents a polyphyletic assemblage from different Permian lineages of crinoids (Rasmussen, 1978b; Roux, 1997; Ubaghs, 1978). The use of molecular data from extant crinoid taxa to assess the origin of the extant Articulata can address whether the lineages that exist today converge to a most recent common ancestor near or after the Permian–Triassic boundary (~252 mya: Shen et al., 2011), or sometime before that. The former would arguably refute the polyphyletic Articulata hypothesis, while the latter would be consistent with either.

To address questions concerning the broad phylogenetic relationships among extant crinoids and the origin of Articulata, nuclear (18S ribosomal DNA (18S), 28S ribosomal DNA (28S)) and mitochondrial (Cytochrome Oxidase subunit I (COI), Cytochrome b (Cytb) and 16S ribosomal DNA (16S)) sequence data was gathered from a suite of extant Crinoidea that spans the extant diversity of the group. Available sequence data from previous studies was also incorporated (Cohen et al., 2004; Helgen and Rouse, 2006; Janies and Mooi, 1999; Janies et al., 2011; Lanterbecq et al., 2010; Perseke et al., 2008; Scouras and Smith, 2001, 2006; Wilson et al., 2007) to examine a total of 59 crinoid terminals.

2. Materials and methods

2.1. Specimen collection

Specimens were gathered by snorkeling, SCUBA, dredging, trawling, submersible or ROV. A few specimens were obtained from museum collections, but DNA extractions from numerous samples showed that most specimens preserved for over 10 years yielded little viable DNA. Table 1 lists details of voucher specimens; when possible, institutional acronyms follow those registered at www.biorepositories.org. Most are deposited at the South Australian Museum, Adelaide, Australia (prefix SAM K); others are deposited at the Benthic Invertebrate Collection, Scripps Institution of Oceanography, La Jolla, CA (SIO-BIC); California Academy of Sciences, San Francisco, CA (CAS); Coral Reef Research Foundation, Koror, Palau (CRECH); Muséum national d'Histoire naturelle, Paris (MNHM); Institut Royal des Sciences de Belgique, Brussels (IG), or the National Museum of Natural History, Smithsonian Institution, Washington, DC (USNM). The terminals representing Hyocrinida, referred to in this paper as *Hyocrinus* cf. *biscoiti* and *Thalassocrinus*? sp., are being described as a new species and a new genus and as a new species, respectively (Roux and Messing, in preparation).

2.2. DNA extraction, amplification and sequencing

All tissues, usually pinnules with gonads, were stored in ethanol; total genomic DNA (gDNA) was extracted, according to the Qiagen DNeasy protocol for animal tissues, using proteinase K and isolated using spin filter followed by ethanol precipitation. The gDNA was then cleaned using the CTAB method of Scouras and Smith (2001) and the samples were stored at 4 °C. Three mitochondrial gene fragments (COI, up to ~1200 bp; Cytb, ~800 bp; 16S, ~350 bp) and two nuclear gene fragments (18S, ~1750 bp; 18S, ~1750 bp) were amplified and sequenced. Primers used for COI amplification can be found in Helgen and Rouse (2006); for 16S and Cytb we used primers kindly provided by Andrea Scouras (5′–3′ 16S forward ACG TAG ATA GAA ACT GAC CTG, reverse GAC GAG AAG ACC CTG TGG AGC; Cytb forward TTT TAT TTC TTT ACC TTG TC, reverse AAA CGT AAA ACA CCA CCT AAC). Primers listed in Cohen et al. (2004) for 18S and in Hillis and Dixon (1991) for 28S were used. Polymerase chain reaction (PCR) was generally carried out under the following conditions: denaturation at 94 °C for 45 s, annealing at 48 °C and 55 °C for 45 s, and extension at 72 °C for 1 min; this was repeated for 35 cycles. The PCR reactions included 0.2 μl TaqGold (5 units/μl), 2 μl per primer (5 μM), 4 μl dNTPs (10 μM), 8 μl $MgCl_2$ (25 μM), 5 μl TGold Buffer, 5 μl gDNA, and 23.8 μl sterile water.

PCR products were cleaned using a MoBio spin clean kit. If more than one product was present, the desired product was isolated using agarose gel (1.5%) electrophoresis and cleaned using the Qiagen QIAquick gel extraction kit. PCR products were generally amplified before sequencing using a mixture of 5 μl of PCR product, 1 μl of primer (5 μM), 2 μl Big Dye 5× Buffer, 4 μl Big Dye version 3, and 8 μl sterile water. The PCR reactions were generally run using a thermocycler with denaturation at 96 °C for 30 s, annealing at 50 °C for 15 s, and extension at 60 °C for 4 min; repeated for 25 cycles. PCR products were cleaned using 70% isopropanol before sequencing with automated sequencers (Applied Biosystems, Inc.). Sequence data was edited using SeqEd v1.0.3 (Applied Biosystems, Inc.) and deposited with GenBank (Table 1).

2.3. Terminals

One terminal was included from each of the other four major echinoderm lineages (Asteroidea, Echinoidea, Holothuroidea and Ophiuroidea) to act as outgroups. Sequences were not available for all five genes from most of these taxa, so chimeras were created from sequences of closely related species. For Asteroidea, the mitochondrial genes and the 18S gene were available for *Asterias amurensis*; the 28S gene was obtained from *Asterias forbesii*. For Echinoidea, *Arbacia lixula* provided the mitochondrial genes while *Arbacia punctulata* provided the nuclear genes. For Holothuroidea, the mitochondrial genes and the 18S gene were available for *Cucumaria miniata*; these were combined with the 28S gene from *Cucumaria salma*. For Ophiuroidea, all five genes were available for *Ophiopholis aculeata*.

As well as the new data generated for this study, effectively all relevant data available on GenBank for Crinoidea was also included. The whole mitochondrial genome of *Antedon mediterranea*, generated by Perseke et al. (2008), provided the three mitochondrial gene sequences, which were concatenated with the partial 28S gene sequence from Janies and Mooi (1999). The whole mitochondrial genome of *Neogymnocrinus richeri*, from Scouras and Smith (2006), provided the 16S, COI, and Cytb sequences, which were concatenated with the 18S gene sequence of the same species from Cohen et al. (2004). The 28S sequence was obtained from another *N. richeri* specimen obtained for this study. The COI, Cytb and 16S sequences from the mitochondrial genome of *Phanogenia gracilis*, sequenced by Scouras and Smith (2001), were combined with the 18S and 28S sequences from other conspecific specimens collected for this study. Data from the mitochondrial genome of *Florometra serratissima*, sequenced by Scouras and Smith (2006), was combined with a 28S sequence from another specimen nominally of the same species collected for this study (from Japan) and an 18S sequence available from GenBank (Janies et al., 2011), so these sequences may not represent a single species.

A series of crinoid terminals, for which only 16S and 18S sequences were available, were used here. These sequences were derived from the study by Cohen et al. (2004). Similarly, a study by Lanterbecq et al. (2010) generated numerous COI and 18S sequences of crinoids that were also used here. In a number of cases (e.g., *Clarkcomanthus littoralis*, *Himerometra robustipinna*), the same specimens, or specimens identified as the same species but from a different locality (e.g., *Colobometra perspinosa*), were sequenced for the additional genes used here. The 18S sequence of *Saracrinus nobilis* (GQ913340), published by Lanterbecq et al. (2010), was not used here, as it was clearly of poor quality and gave a misleading indication of the informativeness of this gene for crinoid phylogenetics. One of the COI sequences published by Helgen and Rouse (2006) for *Aporometra wilsoni* was concatenated with 16S and 28S sequences generated for this study from the same specimen. Similarly, Wilson et al. (2007) published a large number of COI and Cytb sequences for *Promachocrinus kerguelensis*, which were used for one individual for which 16S was also sequenced. These sequences were concatenated with the 18S sequence from a specimen collected from the Bransfield Strait, Antarctica (GQ913342) published in Lanterbecq et al. (2010).

2.4. Alignment, assessment of saturation, and site selection

The COI and Cytb sequences were aligned manually using SeaView 4.3 (Gouy et al., 2010). Notably the ophiuroid *Ophiopholis aculeata* showed a 9-base insert in COI compared to all crinoids and other echinoderms. The 16S, 18S, and 28S sequences were aligned using the L-INS-i option of MAFFT 6.864b (Katoh, 2008). The alignments thus inferred were then improved manually using SeaView. These master alignments are available from Treebase (www.treebase.org/).

The 18S sequences for crinoids were particularly conserved but some areas of alignment ambiguity were noted with reference to the outgroup; this overall conservation with some hypervariable areas was also noted for 28S, which had large indel areas. The 16S dataset showed high variability across the terminals. The 18S and 16S datasets were run through the Gblocks server (Castresana, 2000) allowing for the least stringent exclusion options to remove the dubious areas of alignment. The master alignment of 28S also contained dubiously aligned sections and large indels, which were identified and excluded using SeaView (the GBlocks server was tried but this led to the removal of too many of the well-aligned sites in the middle of the alignment). All sites selected for removal are marked as such in the alignments at Treebase.

To assess whether COI and Cytb were saturated, tests of substitution saturation were conducted using DAMBE 4.1.33 (Xia and Xie, 2001; Xia et al., 2003). When all terminals, including the outgroup taxa, were tested for COI and Cytb, there was significant saturation, with index of substitution saturation (Iss) values significantly higher than the critical parameter values IssSym and IssAsym, even with third positions excluded. However, when the tests were run on the ingroup terminals only, the Iss values were significantly lower than the IssSym and IssAsym values, even with third positions included. This suggests that the COI and Cytb sequences were not significantly saturated, as could be seen by more traditional plots of transitions and transversions against distance (not shown). The only risk that including all the data then posed, with reference to the outgroup, was that the root position might be wrongly placed. Phylogenetic analyses of the individual gene partitions were also conducted to assess if the root was being placed "incorrectly", compared to the concatenated dataset.

Given that most commonly used phylogenetic methods assume that the data have evolved under globally stationary, reversible, and homogeneous (SRH) conditions (Jayaswal et al., 2011) and may generate biased phylogenetic estimates when these assumptions are violated by the data (Ho and Jermiin, 2004; Jermiin et al., 2004), the data was surveyed using the matched-pairs test of symmetry (Ababneh et al., 2006). The data was divided into nine partitions (i.e., 16S, 18S, 28S, COI 1st codon site, COI 2nd codon site, COI 3rd codon site, Cytb 1st codon site, Cytb 2nd codon site, and Cytb 3rd codon site) and assessed with Homo 1.0, a program written by LSJ (http://www.bioinformatics.csiro.au). Homo implements the matched-pairs test of symmetry for pairs of sequences and produces a table with *p*-values (i.e., probabilities of obtaining the corresponding χ^2 values by chance), one for each sequence pair. Under the null model (i.e., assuming evolution under globally SRH conditions), the distribution of *p*-values is uniform; in other words, assuming evolution under these conditions, a linear relationship between the observed and expected *p*-values should be found, with 5% of the tests producing *p*-values less than 0.05. Following the above assessment three datasets were generated from the alignments: one with all data included (=complete), one with the 3rd codon sites for COI and Cytb excluded (=3rd excluded), and one with the 3rd codon sites for COI and Cytb excluded and some of the sequences for outgroups excluded (18S = *Asterias* and *Arbacia*; 28S *Asterias* and *Arbacia*; COI *Ophiopholis* and *Cucumaria*; Cytb *Cucumaria*. This dataset is referred to as 3rd + excluded). The three final (i.e., concatenated) alignments are available from Treebase (www.treebase.org/).

2.5. Phylogenetic analyses

The datasets were analyzed using maximum parsimony (MP), Bayesian inference (BI) and maximum likelihood (ML) methods. In all cases gaps and missing data were treated in the same way (i.e., as missing data). MP analyses were carried out using PAUP* 4.0a122 (Swofford, 2002), with all characters unordered and equally weighted. Heuristic searches were conducted using random stepwise addition of the terminals for 1000 replicates with the tree bisection re-connection (TBR) permutation algorithm and with maximum zero-length branches collapsed. The resulting trees were summarized via strict consensus. Clade support was assessed using jackknifing of sites (Farris et al., 1996) on 1000 replicates with 10 random additions per iteration.

Prior to the BI-based phylogenetic analyses, jModeltest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008) was used to assess the most suitable model of sequence evolution for each gene partition via the Akaike Information Criterion (AIC). GTR+I+Γ was the model used for each partition. The data was partitioned by gene and further by codon position for the protein coding genes

(resulting in nine or seven partitions). BI was carried out in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003); default priors were used and the data partitions were unlinked for parameter estimations (i.e., unlink statefreq=(all) revmat=(all) shape=(all) pinvar=(all), with GTR + I + Γ applied to each partition). Several iterations of ten million generations were run with six chains, sampling a tree every 1000 generations. Convergence of the Markov chain Monte Carlo (MCMC) runs was assessed using AWTY (Nylander et al., 2008) and Tracer 1.5 (Rambaut and Drummond, 2007). A majority rule consensus tree was made from the trees remaining after an appropriate set (~25%) was discarded as burn-in.

The ML-based phylogenetic analyses were conducted using RAxML 7.2.3 (Stamatakis, 2006; Stamatakis et al., 2008) under the GTR + Γ model of substitution on the CIPRES Portal 2.2 (Cyberinfrastructure for Phylogenetic Research at the UC San Diego Supercomputer Center (Miller et al., 2010)). The shape parameter of the Γ distribution for the variable sites was estimated during the analyses (for reasons given in the RaxML 7.2.3 manual: http://www.phylo.org/tools/raxmlhpc2.html). The data was partitioned by gene and by codon position for the protein coding genes (i.e., nine or seven partitions). Non-parametric bootstrapping was carried out with 1000 bootstrap pseudo-replicates under the same model conditions.

A null hypothesis of monophyly for Comatulidina (featherstars), which was never recovered in any of the analyses, was assessed for the complete dataset using the Approximately Unbiased (AU) test (Shimodaira, 2002), as implemented in program PAUP*. Monophyly of Comatulidina was constrained, and the ML tree then inferred was compared with the unconstrained ML tree; both trees were generated in RAxML 7.2.3 with the same parameters as outlined above. The ML tree and ML-constrained tree were then imported into PAUP*, and subsequently the RELL method (Kishino et al., 1990), also implemented in PAUP*, was utilized with 100,000 bootstrap replicates.

2.6. Divergence dating

The complete, 3rd excluded and 3rd + excluded datasets were used to assess the divergence times for crinoid lineages and the origin of Articulata. Minimum age estimates were applied for the Echinodermata, Eleutherozoa and Echinozoa nodes and to six nodes of the crinoid phylogeny that were recovered in the analyses. These were all constrained to be monophyletic. The age for the Articulata node (i.e., all extant Crinoidea) was left free. The phylogeny (given the constraints) and divergence times for the complete dataset were then estimated using the Beast program package 1.6.2 (Drummond and Rambaut, 2007), which implements a Bayesian relaxed molecular clock method (Drummond et al., 2006). The data partitions and conditions assumed above for the BI analysis were used. The following was assumed and included in the Beast.xml file: uncorrelated log-normal prior model of rate change, a Yule prior process to model divergences, and fixed divergence dates based on fossils (see below) with normal distributions. A random starting tree was used for each Beast analysis, and 10 separate MCMC analyses with 50 million generations were run to provide independent samples of parameter estimates, which were checked using Tracer (Nylander et al., 2008) for convergence, stationarity, and effective sample sizes. The last 5000 trees of the runs that achieved stationarity at the highest level of likelihood were combined using LogCombiner from the Beast package. These trees were then analyzed using TreeAnnotator from the Beast package to give the maximum-clade credibility majority rule consensus tree, which was then used to estimate divergence times and corresponding 95% confidence intervals for the mean (and median) ages for free nodes. As recommended by Heled and Drummond (2011), discordance was assessed between the specified prior calibration densities and resulting posterior marginal densities after the Beast analyses; this was done by running analyses with the priors, but without sequence data. The marginal distributions for the calibrated nodes against the specified calibration densities were then examined.

In general, the median of the upper and lower boundary ages of the geological stage interval was used as the age of the most recent common ancestor at a node. Stratigraphic divisions are as used in Hess et al. (2011) and the Subcommission for Stratigraphic Information (2010). Dates were set with a normal distribution and standard deviation covering 95% of the time estimate (indicated by a '±' in brackets after the date). The dates are summarized in Table 2 but are discussed here. The earliest accepted echinoderm fossils (Smith, 1988) are from the Lower Cambrian (Tereneuvian, ~530–524 mya); the group then diversified into a stunning variety of forms in the Upper Cambrian (Sumrall and Wray, 2007). Most of these subsequently went extinct, leaving Crinoidea and Eleutherozoa as the two clades that have descendants today, though they may not be particularly closely related (see Mooi, 2001). There has also been considerable debate over the origin of Crinoidea (Ausich, 1998; Mooi, 2001). The earliest currently accepted fossils of Crinoidea appear to be *Aethocrinus* or *Apektocrinus* (Guensburg, 2012; Guensburg and Sprinkle, 2001, 2009) from the Lower Ordovician (Lower Ibexian = Tremadoc, ~479–488 mya). Blake and Guensburg (2005) argue that *Eriaster ibexensis,* also from the Lower Ibexian is part of Asteroidea, with even older fossils such as *Asteriacites* representing stem Eleutherozoa. Furthermore, several authors (Paul and Smith, 1984; Simms et al., 1993; Smith and Savill, 2001) place *Stromatocystites*, which dates to the Middle Cambrian (513–524 mya: Paul and Smith, 1984), as a stem eleutherozoan genus (but see David et al., 2000). As this suggests that Eleutherozoa and Crinoidea diverged between ~485 and ~515 mya, 500 mya was used with a normal distribution (±15 mya) for these analyses (Table 2, A).

The minimum age for divergence at the eleutherozoan node was set to 484 mya, also with a normal distribution (±4 mya), based on *Eriaster* being an asteroid (Blake and Guensburg, 2005) that had already diverged from other Eleutherozoa (Table 2, B). Within Echinozoa, the timing of the split of holothuroids from echinoids is poorly understood (Smith and Savill, 2001). Based on Gilliland (1993), Simms et al. (1993) suggested that the earliest known holothuroid is the ophiocistoid *Volchovia* from the Lower Ordovician (Arenigian, ~472–479 mya). However, the first unequivocal holothuroid (Reich, 2010) appears to be *Paleocucumaria* from the Llanvirnian (461–468) with others dating to the Silurian (Wenlock, 423–428 mya) (Gilliland, 1993; Reich and Kutscher, 2001; Simms et al., 1993). Problems exist in identifying the origin of Echinoidea (Smith and Savill, 2001), with Simms et al. (1993) accepting that the Ordovician *Aulechinus* (Ashgill, ~444–449 mya) is a crown group echinoid. Based on the appearance of holothuroids, we set the minimum age for the split between Holothuroidea and Echinoidea (Echinozoa node) earlier than this at 464 (±3) mya (Table 2, C).

With regard to fossil date divergences within Articulata, our taxon sampling allowed us to use six dates, all with truncated normal distributions: Cyrtocrinida, Isselicrinidae, Comatulidina (Hess and Messing, 2011; = Comatulidia of Simms and Sevastopulo, 1993), Mariametroidea, Comasteridae and "Antedonidae". Sources for the dating are chiefly from Simms et al. (1993), Hess and Messing (2011) and Hess (2011d,h,i). Cyrtocrinida has fossils dating to the Lower Jurassic (Sinemurian, ~189–196 mya), but these may represent stem group fossils. Sequence data was available for two cyrtocrinid genera (Table 1): *Holopus* (Holopodidae) and *Neogymnocrinus* (Sclerocrinidae) (see Hess, 2006 regarding the change of name *Gymnocrinus* to *Neogymnocrinus*). *Cyrtocrinus newtans,*

Table 2
Summary of node age constraints (minimum ages) used in divergence time estimation. Node H was not constrained in two analyses, and two dates were used for node F. See text for details.

Node	Age mya	Node	Source (others in text)
A	500 (±15)	Split for Crinoidea/Eleutherozoa (Cambrian)	Guensburg and Sprinkle (2009) and Smith and Savill (2001)
B	484 (±4)	Crown Eleutherozoa (Lower Ordovician)	Blake and Guensburg (2005)
C	464 (±3)	Crown Echinozoa (Lower Ordovician)	Reich (2010)
D	165 (±2)	Crown Cyrtocrinida (Middle Jurassic)	Hess (2011d)
E	165 (±2)	Crown Comatulidina (Middle Jurassic)	Hess and Messing (2011)
F	78 (±6)	Crown Isselicrinidae (Upper Cretaceous)	Baumiller and Gazdzicki (1996) and Hess (2011i)
F′	132 (±2)	Crown Isocrinida (Lower Cretaceous)	Baumiller and Gazdzicki (1996) and Roux et al. (2009)
G	53 (±3)	Crown Mariametroidea (early Eocene)	Hess and Messing (2011)
H	22 (±1)	Crown Comasteridae (late Miocene)	Simms et al. (1993)

from the Middle Jurassic (Bathonian, ~164–167 mya), is the oldest known sclerocrinid, whereas holopodids are somewhat younger (Tithonian, ~146–151 mya) (Hess, 2011d). Both cyrtocrinid suborders, Cyrtocrinina and Holopodina, include extant species. Assuming the two suborders are reciprocally monophyletic, the minimum age for divergence at the Cyrtocrinida node was set at 165 (±2) mya (Table 2, D).

Fossils of Isocrinida are argued to date to the early Middle Triassic (Hagdorn, 2011; Hess, 2011i; Simms et al., 1993; Stiller, 2011), but divergences among crown group taxa are not well established and conflicting views on the taxonomy of the group exist. Some authors (Bourseau et al., 1991; David et al., 2006; Roux et al., 2009) place all extant Isocrinida in four subfamilies of the family Pentacrinitidae: Balanocrininae (extant = *Hypalocrinus*, *Neocrinus*), Diplocrininae (extant = *Endoxocrinus, Teliocrinus*), Isocrininae (extant = *Cenocrinus*) and Metacrininae (extant = *Metacrinus* and *Saracrinus*). On the other hand, Hess (2011i), following Simms (1988b) in part, placed the extant forms in four families: Isocrinidae (extant = Isocrininae, *Hypalocrinus*, *Neocrinus*), Cainocrinidae (extant = *Teliocrinus*), Isselicrinidae (extant = Diplocrininae, *Cenocrinus* and *Endoxocrinus*; Metacrininae, *Metacrinus* and *Saracrinus*) and Proisocrinidae (extant = *Proisocrinus)*. Simms et al. (1993) did not subdivide Isocrinida (referred to incorrectly therein as order Isocrinina), or provide any dates for divergences within the group, though Hess (2011i) did so. We follow here the classification of Hess (2011i), though as becomes apparent in our results below, no current classification is satisfactory. *Endoxocrinus, Metacrinus* and *Saracrinus* are currently placed in two of the three subfamilies of Isselicrinidae (Hess, 2011i; Klikushin, 1977), with *Endoxocrinus* in Diplocrininae and *Metacrinus* and *Saracrinus* in Metacrininae. The oldest known fossils of Metacrininae (*Eometacrinus*) date to the early Eocene (Baumiller and Gazdzicki, 1996) around 54 mya, and the oldest diplocrinin is thought by Hess (2011i) to be from the Upper Cretaceous (Campanian, 71–84 mya), though Roux et al. (2009) suggest that the oldest fossil Diplocrininae date to the Lower Cretaceous (Hauterivian, 130–134 mya). For some analyses the date from Hess (2011i) was accepted, assuming the monophyly of these subfamilies, and a date of 78 (±6) mya was used for the minimum age of their divergence (Table 2, F). In other analyses the Lower Cretaceous date of 132 (±2) mya was used (Table 2, F′). The enigmatic *Proisocrinus* (Prosiocrinidae) has no fossil record, and this is also the case for *Hypalocrinus* (Isocrinidae). Fossils of Isocrininae, the isocrinid subfamily in which *Hypalocrinus* is currently placed, are thought to date to the middle Triassic (Hess, 2011i). However, our results (see below) consistently showed *Hypalocrinus* (and *Proisocrinus*) to be nested among the isselicrinids terminals, so we chose not to use any other constraints beyond those chosen for Isselicrinidae. Further assessment of the morphology and systematics of isocrinid fossils is clearly warranted.

Among Comatulidina (featherstars), fossil taxa such as *Paracomatula triadica* date to the Upper Triassic (Norian, ~204–217 mya: Hagdorn and Campbell, 1993), and are currently placed in their own superfamily, Paracomatuloidea (Hess and Messing, 2011), but these may not form part of comatulid crown group. The same case can be made for the supposed first "true" comatulids, *Palaeocomaster* spp. and *Procomaster pentadactylus*, which date to the Lower Jurassic (Hettangian, ~196–199 mya: Hagdorn and Campbell, 1993; Hess and Messing, 2011; Simms, 1988a). These taxa are currently placed in the entirely extinct superfamily Solanocrinitoidea, or as *incertae sedis* (Hess and Messing, 2011). Arguably the oldest crown group fossil comatulid is the Middle Jurassic *Semiometra abnormis* (Bathonian, ~164–167 mya), currently assigned to Notocrinidae in Notocrinoidea (Hess and Messing, 2011; Simms et al., 1993). We therefore used a date of 165 (±2) mya for the minimum age of crown Comatulidina (Table 2, E).

One of the major taxa in Comatulidina, in terms of extant species richness, is Antedonidae within Antedonoidea; the oldest known fossil attributed to this group is a Lower Cretaceous (Albian, ~100–112 mya) fossil of *Roiometra columbiana*. However, Antedonidae was recovered as paraphyletic or polyphyletic in the current study and there was no clear place within the phylogeny for this fossil calibration point. *Cosmiometra* (Thalassometridae), *Chondrometra* and *Crinometra* (Charitometridae), *Ptilometra* (Ptilometridae), *Tropiometra* (Tropiometridae) and currently fall within superfamily Tropiometroidea, which, according to Rasmussen (1978b) and Simms et al. (1993), includes the long extinct fossil family Pterocomidae, which dates to the Jurassic (Tithonian, ~146–151 mya). However, Tropiometroidea did not form a clade in any of our analyses and fossil Pterocomidae lack the prismatic pinnules (triangular in section with an aboral keel) that is supposedly diagnostic of Tropiometroidea (Rasmussen, 1978b); as a result, no real evidence exists for using a Jurassic date for the most recent common ancestor for a clade including tropiometrids and other crinoids. Mariametroidea comprises several extant families, but only Himerometridae includes any fossils (Simms et al., 1993). These date to the early Eocene (Ypresian, ~49–56 mya), so we used earliest appearance of 53 (±3) mya for Mariametroidea (Table 2, H).

Although including ~100 nominal species today, the earliest known fossil Comasteridae, *Comaster formae*, dates only to the early Miocene (Aquitanian, ~20–23 mya: Simms et al., 1993), so a minimum age of 22 (±1) mya was used for the family (Table 2, I). Reports of a comasterid fossil in the genus *Nemaster* from the Lower Eocene in Hess and Messing (2011) are not supported by any data in the literature cited (Howe, 1942). The unusual Upper Cretaceous genera *Uintacrinus* and *Marsupites* have been treated as a family that is sister to Comasteridae (Milsom et al., 1994). However, little exists to associate them except the eccentric mouth and more or less central anus found in *Unitacrinus* and many living comasterids (both features undetermined in *Marsupites*). Hess and Messing (2011) place *Uintacrinus* and *Marsupites* in separate families in their own comatulid superfamily. Given the relatively recent date for the oldest comasterid fossil, we also ran Beast analyses

with the date for the most recent common ancestor of Comasteridae left as free to vary.

With respect to the stalked Bourgueticrinina, the oldest known fossil is *Bourgueticrinus* sp., which dates to the Cenomanian (~94–100 mya) in the Upper Cretaceous (Salamon, 2007; Salamon et al., 2010). Simms et al. (1993) reduced all four families known at the time (Bourgueticrinidae, Bathycrinidae, Phrynocrinidae and Porphyrocrinidae) to a single family Bourgueticrinidae, a practice not generally followed (e.g., Hess and Messing, 2011; Roux et al., 2002). The results found below consistently showed that Bourgueticrinina is not monophyletic, with *Monachocrinus* and *Caledonicrinus* (both Bathycrinidae) consistently falling separately outside a clade composed of *Democrinus* (Bourgueticrinidae), *Porphyrocrinus* (Porphyrocrinidae), *Bathycrinus* sp. and *Bathycrinus* cf. *australis* (Bathycrinidae). Rather than applying a deeper age for the first appearance of Bourgueticrinina, we left this group without a calibration point. We also did not use calibration points for Hyocrinida, for which we had specimens of *Hyocrinus* and *Thalassocrinus*, both in the subfamily Hyocrininae (Mironov and Sorokina, 1998). All extant taxa are placed within Hyocrinidae. Although Hess (2011h) lists no fossils for this group, Roux and Plaziat (1978) identified hyocrinid fossils dating to the Paleocene (Thanetian, 55.8–58.7 mya). However, the fossils were not placed into any taxon, and there is no way of establishing the minimum age for the clade consisting of *Hyocrinus* and *Thalassocrinus*.

2.7. Transformations: stalks and Cirri

We assessed the evolution of two key features in crinoid anatomy; the stalk and cirri (Heinzeller and Welsch, 1994). All crinoids have a calyx that contains or supports the viscera, and many have a series of columnal ossicles that form an aboral stalk, which anchors them to a substrate (Fig. 1A–C). Extant featherstars (Comatulidina) are notable in that, at some point in their early postlarval development, they detach from most of this stalk (Grimmer et al., 1984; Kohtsuka and Nakano, 2005). They retain a solitary columnal, the centrodorsal, from which arise usually hook-like cirri (Hess and Messing, 2011). Hence, as adults they are 'stalkless' and are free to move from one location to another—via arm-crawling or swimming—and anchor with their cirri (Fig. 1D). Some cyrtocrinids, such as *Holopus* (Fig. 1E), are truly stalkless and cement the calyx directly to the substrate (Hess, 2011d). Cirri are only found on extant crinoids belonging to Isocrinida or Comatulidina (Hess, 2011i; Hess and Messing, 2011), though purportedly stem articulate groups from the Early Triassic (Holocrinida and Encrinida: Baumiller and Hagdorn, 1995; Hagdorn, 2011; Hess, 2011e,g) also had cirri, at least on the proximal portion of the stalk.

The transformations for stalks and cirri were assessed on the tree topology generated by RAxML for the complete dataset. For stalks, an unordered multistate character was used, with the four eleutherozoan terminals, which have a fundamentally different body organization compared to the crinoids, having their own state (0). Crinoid terminals were then scored for either having a stalk as adults (1), lacking a stalk as adults (2), or having a stalk limited to a centrodorsal (3). For cirri an unordered multistate character was used, with the eleutherozoan terminals again having their own state (0). Crinoid terminals were then scored as either having no columnals (1), having columnals but none with cirri (2), or having one or more columnals with cirri (3). The transformations were then generated using Mesquite 2.75 (Maddison and Maddison, 2011) via two methods. One approach used the tree topologies as well as the branch lengths generated by the respective analyses and a Markov k-state 1 parameter (Mk1) model that corresponded to Lewis' (2001) likelihood method of character transformation for morphological characters. The other approach used the most parsimonious reconstructions (MPRs) (Swofford and Maddison, 1987, 1992) across the tree topologies alone with no consideration of branch lengths.

3. Results

Sequence data for the five genes (18S, 28S, 16S, COI and Cytb) totaled 7059 bp when aligned, Gblocked (where necessary) and concatenated. Each partition was analyzed separately, but these results are not shown here, as they were largely congruent. Likewise, the analyses of the datasets with 3rd codon sites removed (3rd excluded) and 3rd codon sites plus some outgroup sequences removed (3rd + excluded) showed minor differences (see below) from the complete dataset analyses except as outlined below. The eleutherozoan terminals, designated as a monophyletic outgroup, showed varied topological relationships according to the analysis used. In some of the complete and 3rd excluded analyses *Arbacia* and *Asterias* were sister taxa and *Cucumaria* and *Ophiopholis* formed a clade or basal grade (Fig. 2, Suppl. Figs. 4 and 5). However the MP (Suppl. Fig. 1) and BI analysis (topology not shown) of the complete dataset and the ML analysis of the 3rd + excluded dataset (Suppl. Fig. 6) recovered a topology with Echinozoa (*Arbacia* and *Cucumaria*) as a clade and Asterozoa (*Ophiopholis* and *Asterias*) forming a basal grade.

The complete dataset contained 978 variable but uninformative sites and 1848 parsimony informative sites, mostly derived from the mitochondrial partitions. Hence, even though these partitions provided only 37.4% (i.e., 2629 sites) of the complete alignment, they provided 65% (i.e., 1203 sites) of the phylogenetically informative sites for the phylogenetic analyses. Of the nuclear genes, 531 of the 2784 bp in 28S were parsimony informative, and many of them provided information on the relationships within Articulata. However, most of the 115 parsimony informative sites in 18S (totaling 1646 bp) varied among the outgroup taxa, or established the monophyly of Articulata. With the outgroup taxa excluded, there were only 53 variable sites and 26 informative sites across crinoids. This resulted in very low uncorrected pairwise distances among the crinoids, with the greatest value being 1.6% (*Antedon* vs. *Hyocrinus*).

For the complete dataset, the monophyly of extant Articulata was highly supported, regardless of the analytical method, as were the major taxa Cyrtocrinida, Hyocrinida and Isocrinida (Fig. 2). Isocrinida was recovered as the sister group to the remaining Articulata in the BI and ML analyses, though it formed a clade with Cyrtocrinida and Hyocrinida in the MP analysis (Suppl. Fig. 1). Of the three represented families of Isocrinida, Isselicrinidae, Isocrinidae and Proisocrinidae, the first was always paraphyletic, with both *Proisocrinus* and *Hypalocrinus* forming a clade or grade and grouping, with strong support, with *Endoxocrinus*. *Metacrinus* and *Saracrinus* formed a clade consistent with their classification as Metacrininae within Isselicrinidae, though *Metacrinus,* with three terminals, was always paraphyletic with *M. levii* appearing as a sister group to *S. moosai* (Figs. 2 and 3, Suppl. Fig. 1). *Endoxocrinus* was well supported as a monophyletic clade, but also formed a strongly supported clade with *Proisocrinus* and *Hypalocrinus* rather than being a sister group to Metacrininae, as would be expected if Isselicrinidae were a clade (Fig. 2). *Neogymnocrinus* and two species of *Holopus*, the latter forming a well-supported clade, represented Cyrtocrinida. Three terminals represented Hyocrinida, and the two *Hyocrinus* terminals formed a well-supported clade. Cyrtocrinida and Hyocrinida formed a clade in the analyses (Fig. 2), though with low support in the ML and MP analyses.

The basal nodes among the main lineages of Articulata generally were poorly supported, as was also the case for some comatulid subgroups (Fig. 2). The MP analysis (five most parsimonious trees of length 10,374), disclosed marked topological incongruities

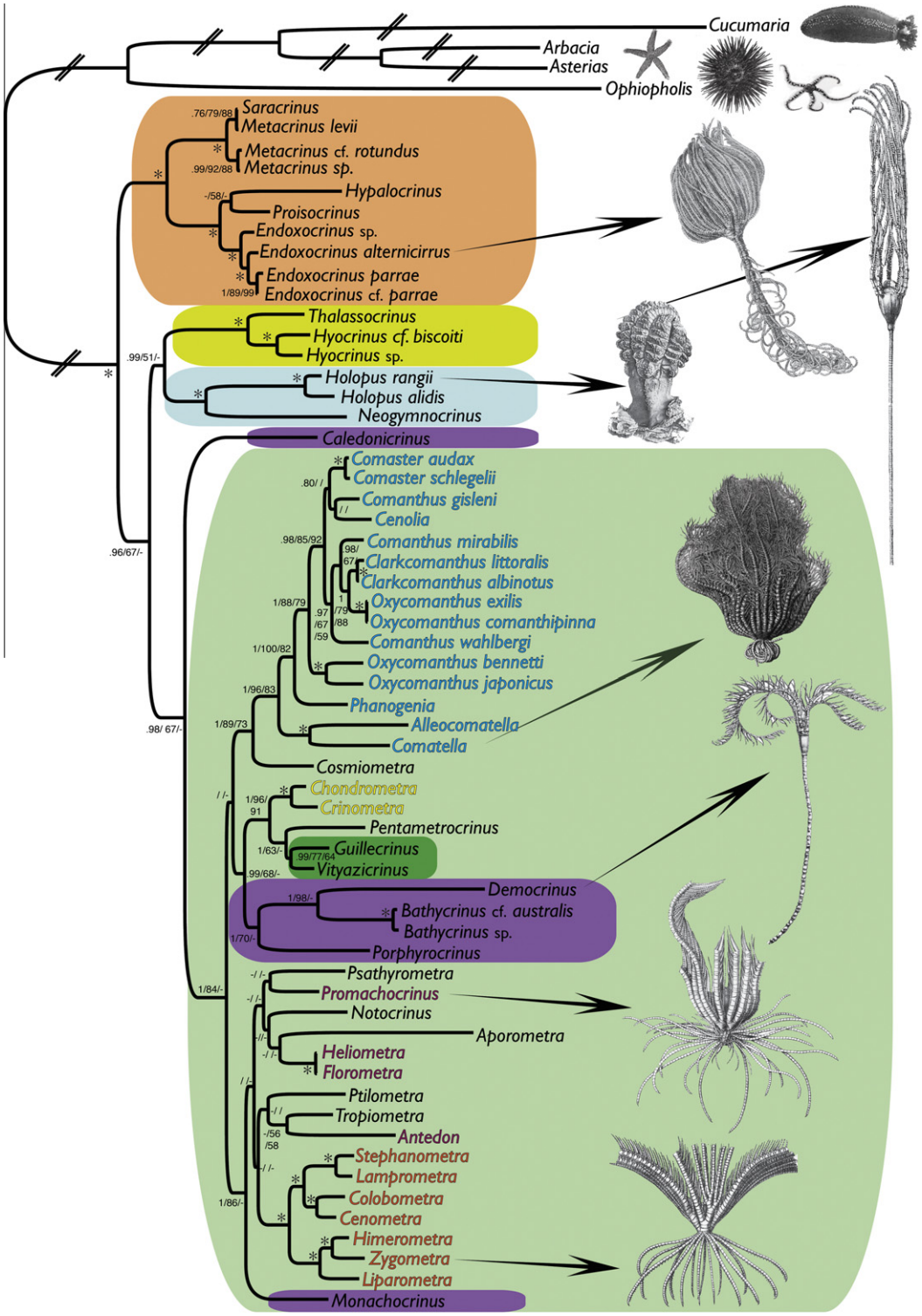

Fig. 2. Maximum likelihood (ML) tree (lnL −52786.972333) inferred from the concatenated five-gene complete dataset (nine partitions). Branch lengths for Eleutherozoa terminals and to the ingroup are shortened (//) for clarity. Symbols above, or otherwise adjacent to, nodes refer to posterior probability (PP), Bootstrap Scores (BS) and Parsimony Jackknife (JK) majority rule consensus tree values. Values are in the following order: PP/BS/JK. Values <50% for BS or JK or <0.70 for PP are indicated by a space. A hyphen (-) indicates the node was not found in the MP analysis. An asterisk (*) indicates >90 for BS or JK and >0.95 for PP for the node. Boxes surround members of the following taxa: Light Blue = Cyrtocrinida; Yellow = Hyocrinida; Orange = Isocrinida; Purple = 'Bourgueticrinina'; Light green = Comatulida, Dark green = Guillecrinidae. Blue taxon names = Comasteridae, Yellow = Charitometridae, Orange = Mariametroidea, Purple = Antedonidae. Images of *Ophiopholis aculeata* (Ophiuroidea), *Asterias forbesii* (Asteroidea), *Arbacia punctulata* (Echinoidea), *Cucumaria frondosa* (Holothuroidea), all from Clark (1904), and *Holopus rangii* (Holopodidae, Cyrtocrinida), *Endoxocrinus parrae* (Isselicrinidae, Isocrinida), *Hyocrinus bethellianus* (Hyocrinidae, Hyocrinida), *Comatella stelligera* (Comasteridae, Comatulida), *Democrinus rawsoni* (Bourgueticrinidae, Comatulida), *Promachocrinus kerguelensis* (Antedonidae, Comatulida) and *Zygometra elegans* (Zygometridae, Comatulida) all from Carpenter (1884, 1888).

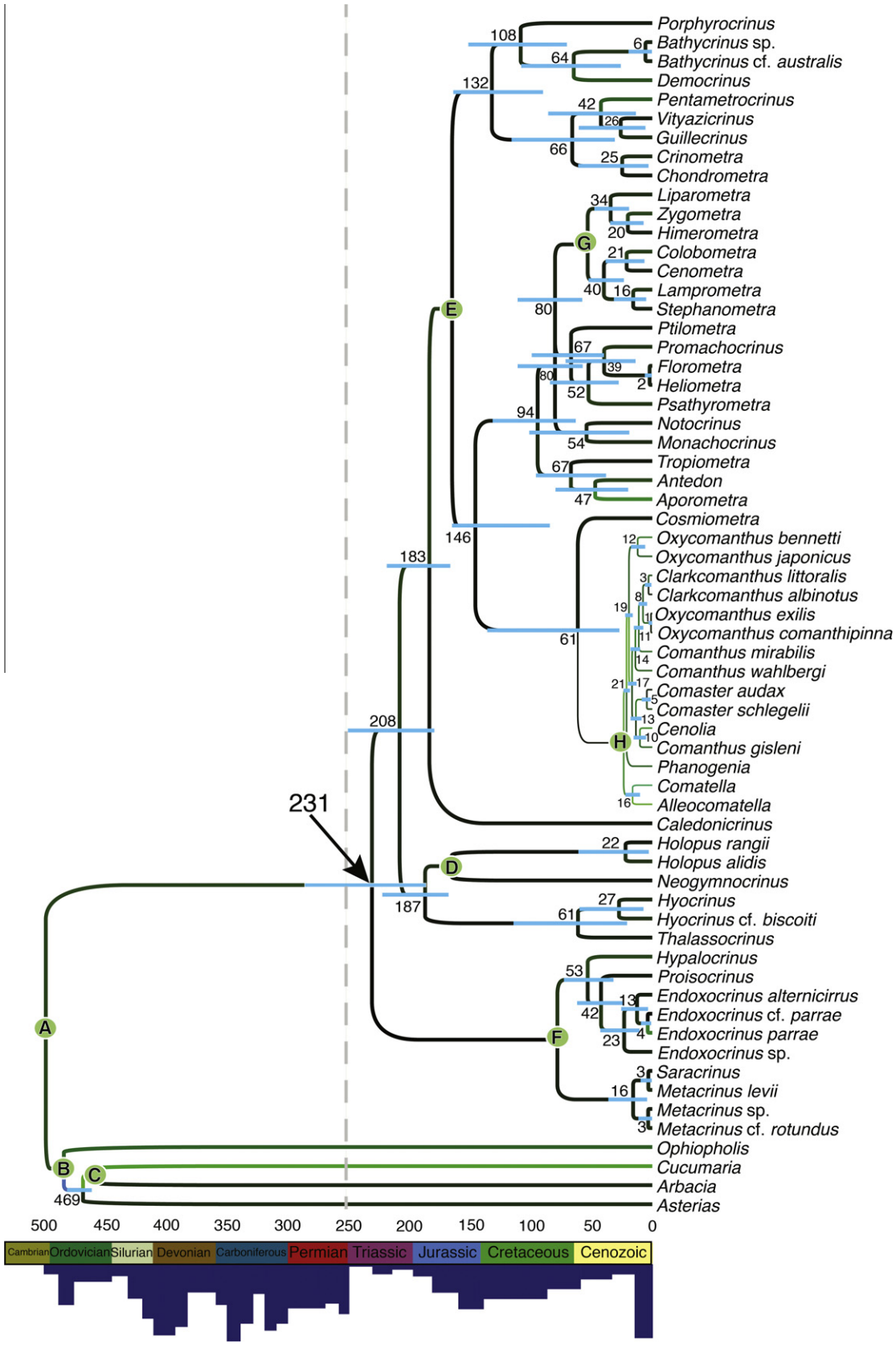

Fig. 3. Divergence times for Articulata. Mean divergence times adjacent to nodes were estimated via Beast (Drummond and Rambaut, 2007) with partitioned Bayesian MCMC analyses using a relaxed molecular clock model on the complete dataset. Blue lines across nodes indicate 95% confidence intervals for dates. Eight nodes (circles with letters) were used for fossil-based time constraints (see text and Table 2). The mean time estimate to the MRCA for Articulata was 231 mya, derived via the other parameters. The tree branch colors reflect the relative rate of overall substitutions per site per mya, with black branches having the slowest rates, and green intermediate and blue fastest. Note that Comasteridae (Node H) has an overall faster rate than other parts of the tree, as do branches leading to terminals such as *Aporometra, Democrinus* and *Endoxocrinus parrae* and the outgroups *Cucumaria* and *Ophiopholis*. Dark blue bars beneath the time scale indicate generic diversity of Crinoidea through time (from Hess et al. (1999)), clearly showing the mass extinction that affected the group at the end of the Permian.

with the model-based approaches (Suppl. Fig. 1). Most notably, the featherstar *Aporometra wilsoni* appeared as sister group to the remaining Articulata in the five most parsimonious trees and the bourgueticrinids *Democrinus* and *Bathycrinus* spp. formed a basal grade with respect to the remaining crinoids (Suppl. Fig. 1). All these terminals were clearly nested well within Comatulidina in the BI and ML analyses (Fig. 2). The topology of the MP analysis (Suppl. Fig. 1) was otherwise largely similar to model-based approaches, except that the non-comatulid taxa Isocrinida, Hyocrinida, Cyrtocrinida and *Caledonicrinus* formed a poorly supported clade in the MP analysis (Suppl. Fig. 1).

The featherstar taxon Comatulidina was generally paraphyletic, with stalked taxa from Bourgueticrinina and Guillecrinina nested inside the taxon in the ML and BI analyses (Figs. 2 and 3). Guillecrinina, represented by *Guillecrinus* and *Vityazicrinus*, formed a strongly supported clade that was a sister group, in the ML and BI analyses, to the five-armed featherstar *Pentametrocrinus* (Pentametrocrinidae), and this clade was in turn a sister group to the featherstar family Charitometridae (*Crinometra* and *Chondrometra*). Charitometridae is currently classified within superfamily Tropiometroidea, while Pentametrocrinidae is part of Antedonoidea (Hess and Messing, 2011). Bourgueticrinina was consistently polyphyletic: *Bathycrinus* spp., *Democrinus* and *Porphyrocrinus* formed a well-supported sister group to a Pentametrocrinidae + Charitometridae + Guillecrinina clade in the MP and BI analyses, though *Bathycrinus* spp. and *Democrinus* formed a basal grade with respect to most crinoids in the MP analysis (Suppl. Fig. 1). *Monachocrinus* was always found as part of a separate featherstar clade, and *Caledonicrinus* was always found outside of Comatulidina (Fig. 2, Suppl. Fig. 1). To assess the possibility that *Aporometra, Democrinus* and *Bathycrinus* spp. were subject to long branch attraction (Bergsten, 2005; Smith, 1994), based on the observation that they all had relatively long branches and that the outgroup taxa also had long branches (see Fig. 2), we ran an MP analysis with the four eleutherozoan terminals excluded. The resulting tree topologies (Suppl. Fig. 2) placed *Aporometra, Democrinus* and *Bathycrinus* spp. in the same positions as in the trees inferred in the MP and BI analyses with the outgroups included, nested well among the other featherstars (Fig. 2).

Using ML analysis, a tree enforcing monophyly of Comatulidina (i.e., containing only featherstars and excluding Bourgueticrinina and Guillecrinina, but otherwise analyzed in the same fashion) had significantly ($P < 0.05$) worse likelihood (lnL −52915.897440) than the unconstrained ML tree (lnL −52785.384527). The AU test thus rejected the monophyly of Comatulidina as currently accepted.

Of the main taxa of featherstars, Comasteridae (the only family in the superfamily Comasteroidea), represented by 16 terminals, was a well-supported clade in all analyses. Comasteridae was the sister group to *Cosmiometra*, the single representative of Thalassometridae, which is part of superfamily Tropiometroidea. Within Comasteridae, two of the genera with multiple terminals, *Comanthus* and *Oxycomanthus*, were not recovered as monophyletic, while others such as *Clarkcomanthus* and *Comaster* formed clades. Comasteridae is currently divided into Comasterinae (including *Comaster*, *Cenolia*, *Clarkcomanthus*, *Comanthus*, and *Oxycomanthus*, all represented here), Comatulinae (*Comatula*, not included), Capillasterinae (several genera, not included) and Phanogeniinae (*Alleocomatella*, *Comatella* and *Phanogenia* included) (Hess and Messing, 2011). In all analyses, Phanogeniinae formed a basal grade of Comasteridae, with *Comatella* and *Alleocomatella* forming a clade that was sister to the remaining comasterids. Comasterinae was always recovered as a clade, generally with good support (Fig. 2, Suppl. Figs. 1, 2, 4, 5, 6).

The seven representatives of Mariametroidea formed a strongly supported clade. However, the topology of well-supported internal nodes did not correspond to the currently accepted family-group classification (Figs. 2–4, Suppl. Figs. 1, 2, 4, 5, 6). Colobometridae (*Cenometra*, *Colobometra*) did form a clade, but this was the sister group to two of the Mariametridae terminals (*Lamprometra*, *Stephanometra*), whereas the other mariametrid, *Liparometra*, was a sister taxon to the representatives of Himerometridae (*Himerometra*) and Zygometridae (*Zygometra*). Hence, Mariametridae was paraphyletic. Mariametroidea was nested inside a paraphyletic assemblage of featherstars that comprised representatives of several comatulid superfamilies (Antedonoidea, Notocrinoidea and Tropiometroidea). This relationship was poorly supported, as was the topology among these various terminals. Antedonoidea was not monophyletic; as noted above, *Pentametrocrinus* formed a clade with Charitometridae and Guillecrinina. Other Antedonoidea terminals included here were *Antedon*, *Florometra*, *Heliometra* and *Promachocrinus* (all in Antedonidae) and *Psathyrometra* (Zenometridae). *Florometra* and *Heliometra* formed a well-supported clade, but *Promachocrinus* was closest to *Psathyrometra*, and *Antedon* was sister to *Aporometra* (Notocrinoidea) in the BI, ML and MP analyses, though with low support. In the MP analyses, *Promachocrinus* formed a clade with *Florometra*/*Heliometra* and *Psathyrometra* (Suppl. Figs. 1 and 2) and with *Antedon* as sister to *Tropiometra* (Tropiometroidea) or *Aporometra* Suppl. Figs. 1 and 2); in other words the results always showed Antedonidae as paraphyletic or polyphyletic. The terminals from the four Tropiometroidea families included here also did not form a clade. As noted above, Charitometridae grouped with *Pentametrocrinus* and some Bourgueticrinina; the thalassometrid *Cosmiometra* grouped with Comasteridae, and the other two, *Ptilometra* (Ptilometridae) and *Tropiometra* (Tropiometridae), did not group closely together in any analysis (Fig. 2, Suppl. Figs. 1 and 2). Apart from *Aporometra*, the other representative of Notocrinoidea as currently construed, *Notocrinus*, was consistently the sister group to the bourgueticrinid *Monachocrinus* (Fig. 2, Suppl. Figs. 1, 2, 4, 5, 6).

The results of the matched-pairs test of symmetry are presented in the Suppl. text, the Suppl. Fig. 3 and the Suppl. Table 1. The tests provided a justification for removing 3rd codon sites (leading to the 3rd excluded dataset) and a few sequences (leading to the 3rd + excluded dataset). In contrast to the MP analysis of the complete dataset (Suppl. Fig. 1), the MP analysis of the 3rd excluded dataset (Suppl. Fig. 4) resulted in a topology broadly similar to the model-based topologies inferred from the complete dataset (Fig. 2), though with Hyocrinida and Cyrtocrinida forming a basal grade and Comatulidina showing a basal grade comprised of some of the bourgueticrinids. Analyses run with the 3rd excluded dataset yielded essentially the same tree topologies for ML and BI as with the total dataset (i.e., Fig. 2), though with some minor differences in support values and branch lengths, and topological differences among some comatulid families, and within Comasteridae (Suppl. Fig. 5). The 3rd + excluded dataset yielded the same result for MP (not shown) as the 3rd excluded dataset. The ML analysis of the 3rd + excluded dataset also showed a similar topology to the analyses based on the 3rd excluded and complete datasets (Suppl. Fig. 6), but a marked difference in the Beast analysis of this dataset was that Isocronida, Cyrtocrinida, and Hyocrinida and *Caledonicrinus* formed a (poorly supported) clade that was a sister group to the remaining crinoids (Fig. 4).

3.1. Divergence times

Ten Beast runs of 50 million generations were carried out with the complete dataset and eight fossil calibration points (using the F constraint, Table 2). Five of the runs reached stationarity at maximum likelihood levels (median lnL −53372.52) markedly worse than in the other five runs. No marked discordance between the specified prior calibration densities and resulting marginal

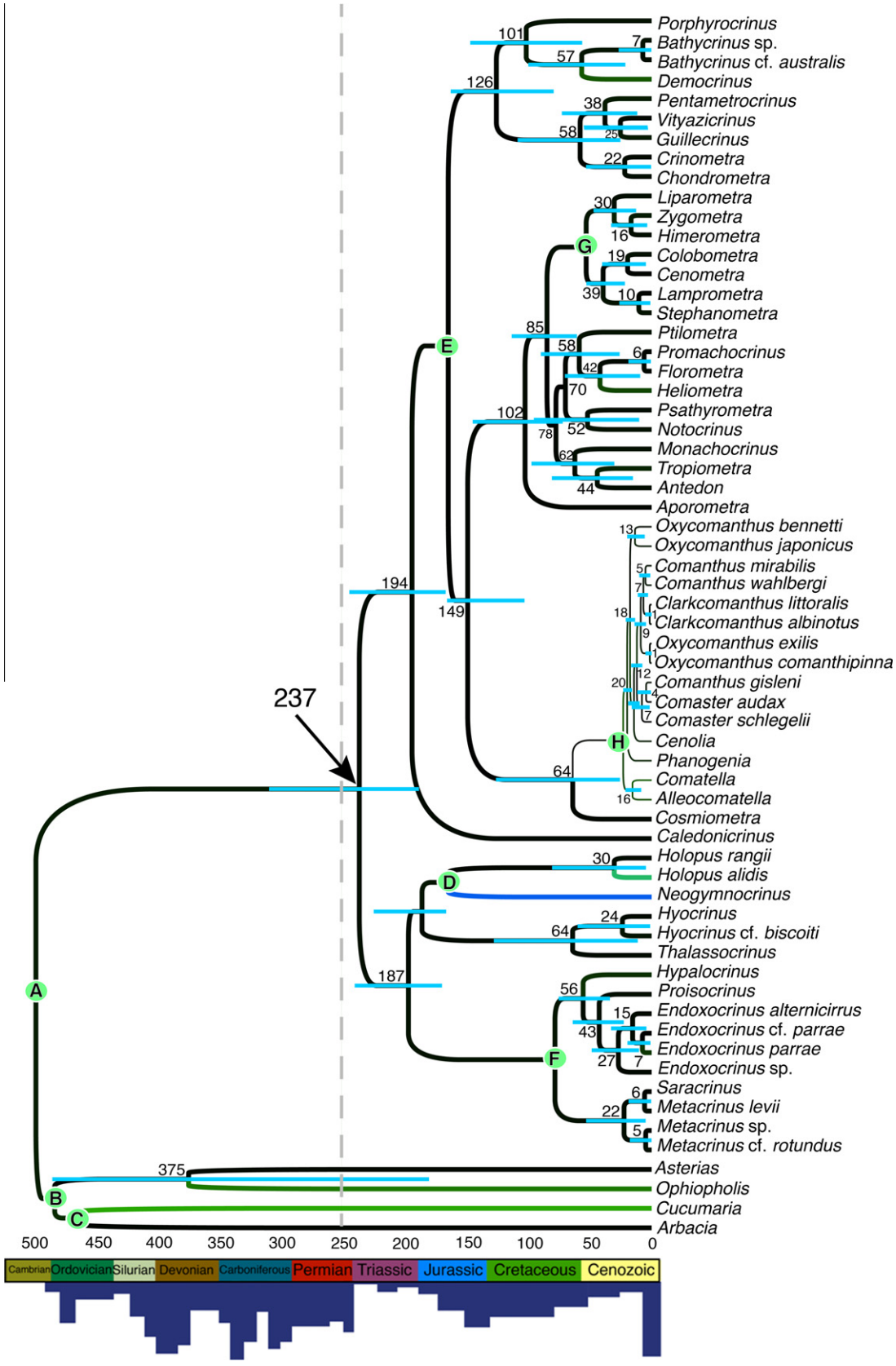

Fig. 4. Divergence times for Articulata. Mean divergence times adjacent to nodes were estimated via Beast (Drummond and Rambaut, 2007) with partitioned Bayesian MCMC analyses using a relaxed molecular clock model on the 3rd + excluded dataset. Parameters were otherwise the same as those seen in Fig. 3. The median estimate for the age of the MRCA for Articulata was 237 mya, derived via the other parameters.

densities was apparent. The five runs with the best likelihood converged at a similar maximum likelihood range (median lnL −53278.05) and sets of the resulting trees were combined (after appropriate burn-in exclusion), while those from the other five

were excluded. The majority rule consensus tree with median ages (rounded to the nearest mya) and 95% confidence limits for nodes is shown in Fig. 3 and was the same as that found in the ML and BI analyses of the complete dataset (Fig. 2).

With respect to the time to the most recent common ancestor (MRCA) for Articulata, a date that was left free to vary, the median age for this node was 231 mya (mean age = 235 mya), after the end-Permian extinction event (Fig. 3). The 95% distribution of dates did however extend into the Permian, though only as a minority of the distribution of ages. The median age for the divergence of Isocrinida from the remaining Articulata was 208 mya. Hyocrinida and Cyrtocrinida were recovered as sister groups that split 187 mya, suggesting that stem Cyrtocrinida extend back to this date. The median age for the Hyocrinida node was 61 mya, which does not conflict with the Paleocene age of the only fossils attributed to this group. The split between Comatulida and *Caledonicrinus* was recovered with an age of 183 mya, suggesting that stem Comatulida date back to around this age or to the split from the Hyocrinida/Cyrtocrinida clade at 208 mya. The 95% confidence interval for this node extended slightly further back into the Permian. The median age of the clade that comprised most of the included Bourgueticrinina (*Porphyrocrinus*, *Democrinus* and two species of *Bathycrinus*) was 108 mya.

Beast analyses were also run with a date of 132 mya as the constrained date of divergence of Metacrininae from Diplocrininae (Table 2, F′). The results were essentially the same (Suppl. Fig. 7) as those outlined above, though the median age for the MRCA for Articulata was slightly older at 235 mya. The rate of evolution (substitutions per site per mya) for Comasteridae was notably higher than those for most of the other of the ingroup lineages, which otherwise were relatively similar (Fig. 3, Suppl. Fig. 7). This suggested either that the constrained date for Comasteridae at 22 mya may have been too recent, or that the rate of evolution of Comasteridae was faster than other crinoids. When the date for Comasteridae was left free to vary and Beast analyses performed with dates otherwise as above (and using either F or F′), the median ages for the time to the MRCAs for Comasteridae and Articulata were 101–103 mya and 235 or 240 mya respectively (Suppl. Figs. 8 and 9).

Beast analyses were also run with the 3rd + excluded datasets and the eight fossil calibration points (using the F constraint, Table 2). The 3rd + excluded dataset showed a median age for the MRCAs for Articulata at 237 mya (Fig. 4), and the Isocrinida, Cyrtocrinida and Hyocrinida clade at 187 mya. Dates for divergences within Comatulida were largely the same as with the complete dataset (Fig. 3). The 3rd excluded dataset showed the oldest age for the MRCA for Articulata, with a median age of 252 mya (Suppl. Fig. 10) and a 95% confidence interval extending well into the Permian for this node. The 95% confidence interval for ages for the MRCA for the two clades comprised of Comatulida plus *Caledonicrinus* and Cyrtocrinida plus Hyocrinida also extended slightly into the Permian. Otherwise dates were largely similar to the complete and 3rd + excluded datasets (Figs. 3 and 4, Suppl. Figs. 7–9).

3.2. Transformations: stalks

Fig. 5A and B shows the inferred character state evolution assuming the phylogeny shown in Fig. 2. Under the Mk1 model and the most parsimonious reconstructions (MPRs), the absence of an adult stalk in *Holopus* appears as loss of a stalk in Fig. 5A. Under the Mk1 model, the plesiomorphic condition for Comatulida as a whole was either stalk present, or having a stalk limited to a centrodorsal. Several taxa within Comatulida have a complete adult stalk, and the topology suggests two alternatives: either as many as three reversals to retaining the complete adult stalk from an ancestor that lost the postlarval stalk (once for *Monachocrinus*, once for the remaining Bourgueticrinina and once for Guillecrininа), or as many as four reductions of stalk to a centrodorsal (once each for the *Cosmiometra*/Comasteridae clade, the clade including Mariametroidea, the *Chondrometra*/*Crinometra* clade and *Pentametrocrinus*). The various scenarios resulted in eight MPRs concerning the multiple gain (Suppl. Fig. 11A) or multiple reduction (Suppl. Fig. 11B) of a complete stalk in Comatulida. The implication for this last transformation (Suppl. Fig. 11B) is that *Monachocrinus* and the remaining Bourgueticrinina and Guillecrinina show an ancestral stalked adult condition.

3.3. Transformations: cirri

The phylogeny shown in Fig. 5B suggests that the most likely plesiomorphic condition for Articulata is the presence of cirri on columnals, with up to four transformations to columnals lacking cirri. Of the 17 MPRs for this tree, some suggested that cirri may have appeared up to five times independently in Isocrinida and Comatulida (Suppl. Fig. 12A) and even that the lack of cirri was plesiomorphic for Articulata. Other MPRs suggested that columnals with cirri was the plesiomorphic condition for Articulata with as many as five losses (Fig. 5B, Suppl. Figs. 12B).

4. Discussion

This study included a broad-scale selection of taxa across Crinoidea, including a series of difficult-to-access deep-sea forms. The sequencing of two nuclear and three mitochondrial genes has clearly resolved the phylogenetic positions of some taxa. However, the low support (Fig. 2) for several key basal nodes makes it clear that further sequence data is required to solve questions about relationships among several extant crinoid groups. Nevertheless, some robust results allow us to recommend changes that should place the classification of Articulata on a more secure phylogenetic basis. The recovery of unusual relationships among the outgroup taxa, such as *Arbacia* and *Asterias* forming a clade (Fig. 2), might suggest to some that our analyses may be recovering incorrect topologies among Articulata as well. However, the MP analysis of the complete dataset (Suppl. Fig. 1) and the ML analysis of the 3rd + excluded dataset (Suppl. Fig. 6) recovered topologies that are more in line with current thinking of relationships among Eleutherozoa (e.g. Janies et al., 2011). For these analyses as well as most of the others shown here, the root position of Articulata and overall relationships among the terminals of crinoids were stable (Fig. 2. Suppl. Figs. 4–6), so we suggest that this is not especially problematic. Nevertheless, future studies may be wise to include further terminals for Eleutherozoa, as two long branches were apparent for these taxa (*Ophiopholis* and *Cucumaria*) relative to all other terminals.

4.1. Systematics and nomenclatural revisions

To date, there have been few explicit phylogenetic analyses of articulate crinoids (Cohen et al., 2004; Simms, 1988b) or their subgroups (Heinzeller et al., 1996; White et al., 2001). However, competing classifications (see Hess, 2011a) and alternative placements of particular taxa such as *Guillecrinus* (Améziane and Roux, 2005; Roux, 1985) and *Proisocrinus* (Oji and Kitazawa, 2008; Roux, 1980, 1997) have been offered, as have discussions about evolutionary scenarios among articulate groups and major subgroups (Gislén, 1924, 1938, 1939; Rasmussen, 1978b; Roux, 1987).

Simms (1988b) used 25 morphological characters in a cladistic analysis of the major lineages of Articulata, including fossil groups. He found the chiefly fossil Millericrinina (including extant Hyocrinidae) as the sister group to Cyrtocrinina and united both as Millericrinida, a classification followed in Simms et al. (1993). Cohen

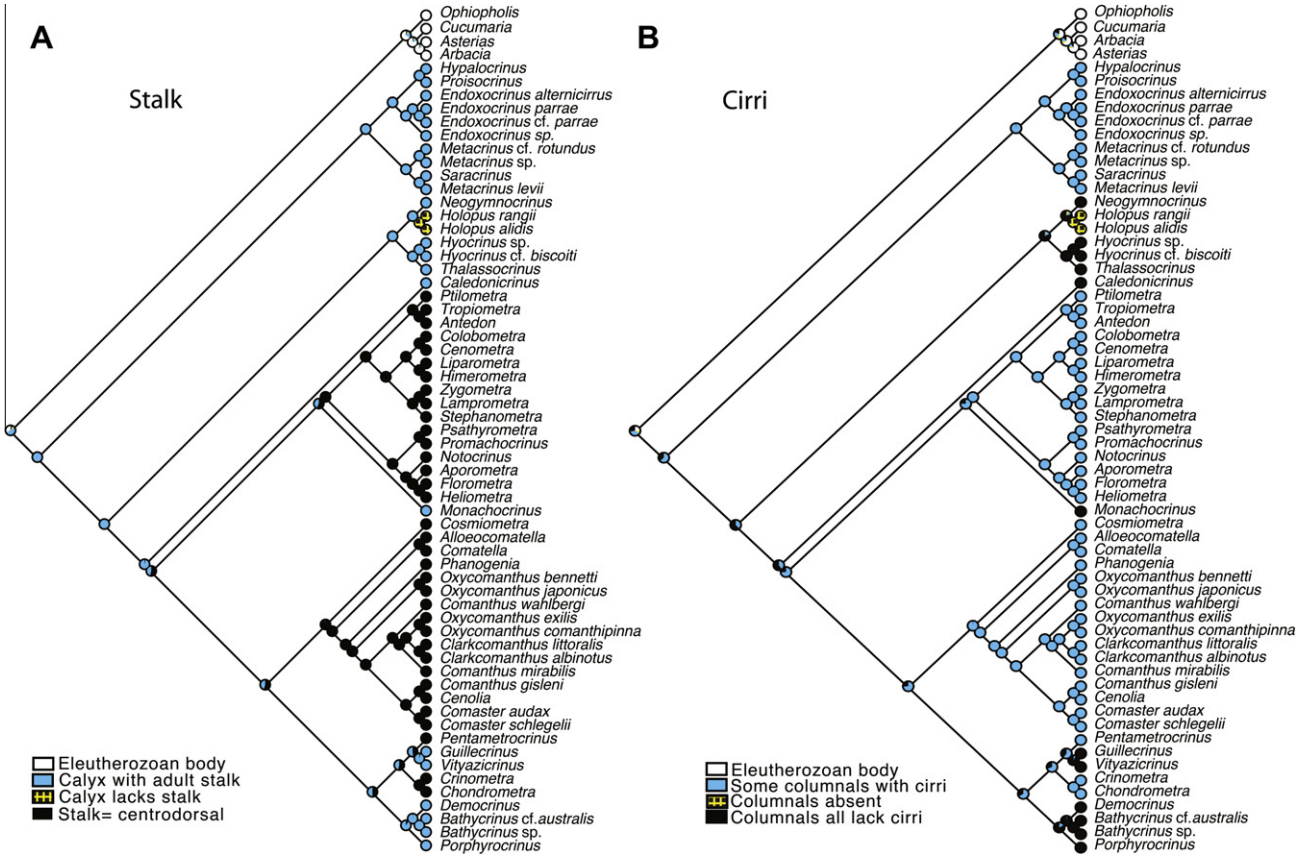

Fig. 5. Maximum likelihood transformations for the stalk (A) and cirri (B). The ancestral character states were reconstructed using the MK1 model implemented in Mesquite 2.75 (Maddison and Maddison, 2011) whilst assuming the phylogeny shown in Fig. 2. Most parsimonious reconstructions (MPRs) were also inferred (Suppl. Figs. 11 and 12). (A) The reconstruction suggests the plesiomorphic condition for Comatulida is either an adult stalk present or a stalk as a centrodorsal. It is unequivocal that *Monachocrinus* is a reversal to retaining the adult stalk but this is not clear for the remaining Bourgueticrinina (= Bourgueticrinidae) and Guillecrinidae. It is also possible that the centrodorsal condition may have evolved several times. There were eight MPRs for this character, some of which suggest the complete adult stalk has reappeared several times in Comatulida, though others suggest that the stalk of Bourgueticrinidae and Guillecrinidae is a retained plesiomorphic state and the centrodorsal has appeared several times. (B) The reconstruction suggests the most likely plesiomorphic condition for Articulata is cirri present, with subsequent transformations to columnals lacking cirri or cirri reappearing, as well as the complete loss of columnals in *Holopus*. Of the 17 MPRs, some of which suggest cirri are plesiomorphic for Articulata, while others suggest that cirri may have appeared independently in Isocrinida and Comatulida.

et al. (2004), using nuclear 18S and ITS sequences and mitochondrial 16S sequences, inferred the phylogeny of crinoids based on three representatives of Cyrtocrinida (*Neogymnocrinus richeri*, *Holopus alidis* and *Cyathidium foresti*, though the latter turned out not to have been sequenced (Cohen et al., 2004, p. 614)), three Isocrinida (*Endoxocrinus parrae*, *Metacrinus* cf. *rotundus*, *Metacrinus* sp.), two Bourgueticrinina (*Bathycrinus* cf. *australis* and *Caledonicrinus vaubani*), and the enigmatic *Guillecrinus neocaledonicus*. However, they included only a single (chimeric) terminal representing Comatulidina and no Hyocrinida. In their results, the cyrtocrinids *Neogymnocrinus richeri* and *Holopus alidis* did not form a clade unless *Caledonicrinus* was included, in contrast to our results in which *Holopus* spp. and *Neogymnocrinus* always formed a clade with reasonable levels of support.

Hess et al. (2011) treated Cyrtocrinida and Hyocrinida as separate orders, as did Rasmussen and Sieverts-Doreck (1978). Simms (1988b) treated Cyrtocrinida and Hyocrinida as a clade, a weakly supported arrangement recovered with nearly all analyses here (Figs. 2–4, Suppl. Figs. 1, 2, 5, 6, 7, 8, 9 and 10). However, the MP analysis for complete, 3rd excluded and 3rd + excluded datasets showed Cyrtocrinida and Hyocrinida as a grade of Articulata (Suppl. Figs. 1, 2, and 4). Given the variable placement and weak support, we make no recommendations regarding classification of Hyocrinida and Cyrtocrinida. Additional sequence data is required to resolve their placements relative to each other and to other articulate groups.

Simms' (1988b) cladistic results presented Isocrinida as an order that included (a) most isocrinids as suborder Isocrinina, (b) Comatulida as suborder Comatulidina, (c) bourgueticrinids as suborder Bourgueticrinina and (d) several fossil taxa. However, Simms et al. (1993) subsequently listed Isocrinina [*sic*] and Comatulidina as separate orders with no discussion. Hess et al. (2011) followed Rasmussen and Sieverts-Doreck (1978) in treating them at the ordinal level as Isocrinida and Comatulida. Cohen et al. (2004) found that their three isocrinid terminals, *Endoxocrinus parrae*, *Metacrinus* cf. *rotundus*, and *Metacrinus* sp., formed a well-supported clade, which was either the sister group to all other crinoids (their MP result) or formed a clade with the cyrtocrinids and *Caledonicrinus*, which was then the sister group to a *Bathycrinus*/*Guillecrinus*/comatulid clade (their ML result). Most analyses described here did not recover Simms' (1988b) sister group relationship between (his) extant Isocrinina and comatulids; instead Isocrinida was either the sister group to the remaining crinoids (Figs. 2 and 3, Suppl. Figs. 5, 7, 8, 9, 10), or it formed a clade with Cyrtocrinida, Hyocrinida and *Caledonicrinus*, though with low support (Fig. 4, Suppl. Figs. 1). Again, additional sequence data is clearly required to resolve the relationships among these major lineages.

With the exception of Cainocrinidae, our sampling from Isocrinida represented all the currently accepted families of Isocrinida with extant members, namely Isocrinidae, Isselicrinidae, and Proisocrinidae (Hess, 2011i). Although Bourseau et al. (1991) and Roux et al. (2002) placed *Proisocrinus ruberrimus* within the otherwise

extinct Millericrinidae, it has generally been regarded as part of Isocrinida (Hess and Messing, 2011; Oji and Kitazawa, 2008; Rasmussen, 1978a). Oji and Kitazawa (2008) included *P. ruberrimus* within Isocrinidae (*sensu lato*), but argued that its synostosial articulation between primibrachials supported a derivation from a form with a cryptosyzygy or synostosis in that location, that is, either Cainocrinidae or Isselicrinidae (Hess, 2011i). Our results consistently recovered *P. ruberrimus* as forming a clade with *Endoxocrinus* with strong support (Fig. 2). The latter genus is currently in Isselicrinidae (subfamily Diplocrininae) according to Hess (2011i). Although further representatives of Isocrinida, including extant Cainocrinidae, need to be sequenced, our current data would place *Proisocrinus* within Isselicrinidae, potentially as part of Diplocrininae with *Endoxocrinus*.

Similarly, our placement of *Hypalocrinus* naresianus, currently regarded as part of Isocrinidae as part of a clade with *Endoxocrinus*, further rendered Isselicrinidae a paraphyletic taxon. Our results are therefore inconsistent with both the classification of Hess (2011i) and the classifications that favor placing all extant Isocrinida within the otherwise exclusively fossil Pentacrinitidae (Bourseau et al., 1991; David et al., 2006; Roux et al., 2009). This suggests that the current classification of extant Isocrinidae and hence potentially also fossil taxa needs revision. *Metacrinus* and *Saracrinus*, are also isselicrinids (Metacrininae) according to Hess (2011i). Our results suggest that *Metacrinus* may be paraphyletic with respect to *Saracrinus*, though more taxon sampling, particularly of the respective type species, is required to resolve their relationships. Overall then, the current classification of extant Isocrinida, including its fossil taxa, needs revision.

Gislén (1924) noted that the stalks of larval Bourgueticrinina and Comatulidina share similar synarthrial articulations between columnals. He first suggested that bourgueticrinins were derived from the extinct Thiollericrinidae, now included within the extinct comatulid superfamily Solanocrinitoidea (Hess and Messing, 2011; Simms et al., 1993), but subsequently (Gislén, 1938) provided an alternative hypothesis that they were derived from Apiocrinitidae, which is part of the extinct Millericrinida. Rasmussen (1978b) raised again the issue of the striking similarity between the stalks of larval comatulids and bourgueticrinins and suggested a proterogenetic (=paedomorphic) origin of the latter from the former. He removed bourgueticrinins from Millericrinida but placed them in their own articulate order: Bourgueticrinida. Simms (1988b) also supported a "neotenous derivation from some group within the Isocrinida, either Isocrinina or Comatulidia". In that paper, he treated Bourgueticrinina as a suborder of Isocrinida, but later Simms et al. (1993) reduced it to a family, Bourgueticrinidae, within the comatulid superfamily Solanocrinitacea (=Solanocrinitoidea). Subsequently, Simms (1999) called bourgueticrinins a "neotenous offshoot of the comatulids" and reinforced this conclusion with a comparative study of the stereom of extant crinoids (Simms, 2011). Cohen et al. (2004) found a well supported *Bathycrinus*/*Guillecrinus*/comatulid clade under both MP and ML analyses but rejected the argument that bourgueticrinins were derived 'neotenously' from comatulids. They instead inferred from their tree that comatulids originated from a bourgueticrinid-like ancestor. Our study, which included several comatulids and bourgueticrinins, place most of the latter group firmly within Comatulidina and offer a variety of scenarios regarding the evolution of extant stalked articulates and the possible paedomorphic origin of bourgueticrinins (see below in Section 4.4).

In the current classification, Hess and Messing (2011) divided the order Comatulida into three suborders: Guillecrinina, Comatulidina and Bourgueticrinina. Mironov and Sorokina (1998) erected Guillecrinidae for *Guillecrinus reunionensis* and *G. neocaledonicus*, Vityazicrinidae for *Vityazicrinus petrachenkoi*, and the new suborder, Guillecrinina, for both within Hyocrinida. Hess (2011f) synonymized Vityazicrinidae under Guillecrinidae, and Hess and Messing (2011) moved the family and its suborder to Comatulida. Our results show that *Vityazicrinus* is the sister group to *Guillecrinus*, so we maintain the synonymy of the two families. Our results also show that both Guillecrinina and Bourgueticrinina clearly arose from within Comatulidina, rendering the latter group paraphyletic and its subordinal name redundant. We therefore suggest that the name Comatulida be used for the clade as a whole. Within Comatulida, we recommend elimination of both Guillecrinina and Bourgueticrinina. We maintain Guillecrinidae for *Guillecrinus* and *Vityazicrinus* but without superfamilial assignment. However, the taxonomic fate of the various bourgueticrinins remains unclear, because our results consistently scattered the included taxa across the trees in at least three places: *Monachocrinus, Porphyrocrinus*/*Bathycrinus*/*Democrinus* and *Caledonicrinus* (Figs. 2–4, Suppl. Figs. 1, 2, 4, 5, 6, 7, 8, 9, and 10). *Bathycrinus, Caledonicrinus* and *Monachocrinus* are currently placed together in Bathycrinidae by Roux et al. (2002) and Hess (2011c), though Mironov (2008) placed *Caledonicrinus*, along with *Naumachocrinus*, in the family Caledonicrinidae based on morphological differences. *Democrinus* is currently placed in Bourgueticrinidae, and *Porphyrocrinus* in Phrynocrinidae. We recommend following Simms et al. (1993) and use the family-group name Bourgueticrinidae for the *Porphyrocrinus*/*Bathycrinus*/*Democrinus* clade (without superfamilial assignment), thus synonymizing Bathycrinidae and Phrynocrinidae under Bourgueticrinidae. The familial diagnosis remains the same as for Bourgueticrinina in Hess (2011c), despite the omission of *Caledonicrinus*. The specimen of *Monachocrinus caribbeus* that we used was a museum specimen (USNM E42707) collected in 1957, and, although the sequences we obtained were unique, we recommend sequencing another specimen from this genus before offering any firm conclusion about its status.

The placement of *Caledonicrinus* in particular varied among analyses (see Figs. 2 and 3, Suppl. Figs. 1, 2, 4, 5, and 6), and it never grouped with other taxa of bourgueticrinids. The implication that *Caledonicrinus* may not be a bourgueticrinid is supported by some morphological evidence. In addition to unusual features pointed out by Mironov (2008), Bohn and Heinzeller (1999) found that *C. vaubani* had a different kind of nervous system from bourgueticrinids and other crinoids that they studied. However, it should also be noted that only 18S and 16S data were available for *C. vaubani* (from Cohen et al. (2004)), so additional sequence data are clearly needed to establish its systematic position.

Of the six currently recognized extant comatulid superfamilies, we lacked only Atelecrinoidea (Atelecrinidae). Of the 18 remaining extant families, we lacked representatives of only Eudiocrinidae (Mariametroidea), Asterometridae and Calometridae (both Tropiometroidea) and Atopocrinidae (superfamily unassigned). Among the comatulid taxa that were well sampled, Mariametroidea and Comasteridae were well supported as clades in all analyses. However, the subfamilial classification of Comasteridae clearly needs revision, as do the genera *Oxycomanthus* and *Comanthus*. This is not surprising as, for example, two of our *Oxycomanthus* species, *O. exilis* and *O. comanthipinna*, that recover as sister taxa, have few small cirri on a small discoidal centrodorsal, whereas *O. bennetti* and *O. japonicus*, which form a separate clade, have numerous robust cirri on a large, thick centrodorsal (Clark, 1931; Rowe et al., 1986). Our results for the relationships among Comasteridae reflect a similar need for the revision of the group as shown by White et al.'s (2001) analysis of 16S sequence data. The mariametroid family Mariametridae was paraphyletic in all our analyses with Colobometridae, Himerometridae, and Zygometridae all nested within; additional taxon sampling and sequencing will no doubt revise our understanding of relationships of this group.

Comatulids from the superfamilies Tropiometroidea and Antedonoidea were not well sampled, but neither taxon was recovered

as monophyletic in any analysis, and their representatives were scattered across Comatulida. The four terminals from the family Antedonidae were always recovered as polyphyletic (Fig. 2, Suppl. Figs. 1, 2, 4, 5, and 6), and the superfamily Notocrinoidea, containing *Notocrinus* and *Aporometra*, was not monophyletic.

4.2. Low rates of sequence divergence in crinoid 18S rDNA

Low rates of 18S sequence divergence have been found for Ctenophora (Podar et al., 2001), in which pairwise distances across the group never exceed 5%. Podar et al. (2001) found much greater distances among members of clades such as Porifera, Cnidaria and Platyhelminthes, in which the pairwise distances average 12%, 10% and 17%, respectively. Molluscan groups such as Gastropoda and Scaphopoda also exhibit relatively high divergence among 18S sequences (Steiner and Dreyer, 2003; Weigand et al., 2012; Winnepenninckx et al., 1998). The low variability found here among crinoid 18S sequences shows that this marker is clearly of limited utility in elucidating relationships within the group.

Our taxon sampling arguably covers much of the extant diversity of the group with divergences among these taxa going back into the Triassic, more than 200 mya (see below). However, we found only 53 variable sites (with only 26 parsimony informative) for 18S among crinoids, with a greatest uncorrected pairwise distance of only 1.6%. Our results showed the greatest pairwise distance among the echinoderms sampled here was 14%, for the eleutherozoans *Ophiopholis* and *Cucumaria*. The pairwise distances from the crinoid terminals to *Cucumaria* were also on the order of 12%. However, the uncorrected pairwise distance for all the crinoid terminals to the other eleutherozoan terminals was only 5% to 8%. Holothuroids have a greater 18S divergence than other echinoderms (Raff et al., 1988), and Littlewood et al. (1997) showed that holothuroids had 4.5 times as many changes as their echinoid sister group. Lacey et al. (2005) had a much broader sampling of holothuroids and found 408 polymorphic sites for 18S, of which 291 were parsimoniously informative. It is notable, however, that holothuroids passed through the Permian–Triassic extinction event relatively unscathed compared to Crinoidea and Echinoidea (Twitchett and Oji, 2005). However, Littlewood and Smith (1995) found 279 variable sites in 18S from a suite of 22 echinoid species spanning lineages dating to the Triassic (Smith et al., 2006), a much higher proportion than we found for Crinoidea. It thus appears that the rate of 18S divergence among crinoids is much lower than in other echinoderm and major metazoan groups.

4.3. Articulata: radiation after the Permian–Triassic extinction?

The relaxed molecular clock analyses with eight fossil calibration points recovered Articulata with a median date to the MRCA ranging from ~231 to 252 mya (Figs. 3 and 4, Suppl. Figs. 7, 8, 9, and 10), providing some support for the hypothesis that the group is a radiation from a small clade that passed through the Permian–Triassic extinction event (Hess, 2011a; Simms and Sevastopulo, 1993). This result only suggests that crown Articulata went through a major bottleneck at the end of the Permian; its origins may still extend well into the Permian as argued by several authors (Webster and Jell, 1999; Webster and Lane, 2007) and the 95% confidence limits on the Beast analyses extend into the Permian. In literature summarized by Simms and Sevastopulo (1993), it has been argued that Articulata represents a polyphyletic assemblage derived from multiple Paleozoic crinoid ancestors (see also Roux, 1997). Our results provide no support for such an origin, as our results presumably would have shown a convergence to a most recent common ancestor in the Permian. Members of Articulata are thought to have been present in the late Permian (along with other crinoids), but none of their respective families survived into the lineages that date to the Triassic (Twitchett and Oji, 2005). It should be noted that our trees showed poor support for the relationships among Isocrinida, Comatulida, Cyrtocrinida and Hyocrinida, and further sequence data will be needed to have more confidence in the results.

The earliest crinoid fossils after the Permian extinction are those of Holocrinidae, which date to the Olenekian (246–249.5 mya) (Hagdorn, 2011; Hess, 2011g), or possibly to the Induan stage (249.5–251 mya) of the Lower Triassic (Hagdorn and Baumiller, 1998). They were soon followed by the first Dadocrinidae in the Aegean (Hagdorn, 2011), the earliest substage of the Anisian (237–246 mya). Hess (2011e,g) treats Holocrinidae and Dadocrinidae as members of two distinct (and extinct) articulate orders, Holocrinida and Encrinida, respectively. Hagdorn (2011) considers holocrinids as the stem lineage leading to Isocrinida, whereas the Encrinida went extinct in the Upper Triassic (Carnian, ~217–229 mya).

The Beast analyses (Figs. 3 and 4, Suppl. Figs. 7, 8, 9, and 10) suggest that Isocrinida diverged from other crinoids at 231–252 mya. Of the extant crinoid lineages, Hagdorn (2011) and Stiller (2011) dated Isocrinida to the mid-to-late Anisian (Pelsonian and Illyrian substages, ~242–246 mya) and treated these earliest members as Isocrinidae. Our results suggest that one of the two extant genera currently attributed to this family, *Hypalocrinus* (Hess, 2011i), actually falls within another extant family, Isselicrinidae, which Hess (Hess, 2011i) treated as a relatively recent isocrinid taxon. Given this result, and the placement of *Proisocrinus* among isselicrinid taxa (e.g., Fig. 2), a review of fossil and extant Isocrinida is warranted, with a thorough morphological and cladistic analysis followed by a reclassification.

The earliest accepted comatulid-like fossil is that of *Paracomatula* (Paracomatulidae), which dates to the Upper Triassic (Norian) and has been regarded as the sister to comatulids *sensu stricto* (Simms, 1988b; Simms et al., 1993). Arguable stem-group comatulids belong to the extinct superfamily Solanocrinitoidea (Hess and Messing, 2011), which dates to the Lower Jurassic (Hettangian, ~197–200 mya), suggesting the date of 183–208 mya (Fig. 3) for the split from stalked crinoids such as *Caledonicrinus* and Cyrtocrinida/Hyocrinida, respectively, may be reasonable. The median age for the MRCA for the bourgueticrinids *Democrinus*, *Porphyrocrinus*, *Bathycrinus* sp. and *Bathycrinus* cf. *australis* was 101–131 mya (Figs. 3 and 4, Suppl. Figs. 7, 8, 9, and 10). The oldest known fossil bourgueticrinid is a *Bourgueticrinus* sp. that dates to the Cenomanian (~94–100 mya) in the Upper Cretaceous (Salamon, 2007; Salamon et al., 2010); however, we did not use this date as a constraint in the Beast analyses, because we did not recover a monophyletic Bourgueticrinidae (due to the external placement of *Caledonicrinus* and *Monachocrinus*). The congruence of the dates for the oldest known fossil and the age of the clade with most bourgueticrinids is notable, however.

The colors of the edges in the trees inferred using Beast (Figs. 3 and 4, Suppl. Figs. 7, 8, 9, and 10) reflect the relative rate of substitutions per site per mya, with black branches having the slowest rates, green branches having intermediate rates, and blue branches having the fastest rates. It is obvious that Comasteridae, for which the MRCA in some analyses was constrained to be 22 mya (Node H) (Figs. 3 and 4, Suppl. Figs. 7, and 10), had an overall faster rate than other parts of the tree. However, this result might be an artifact resulting from our use of the 22 mya divergence date Another series of Beast analyses without the constraint for Comasteridae (Suppl. Figs. 8, and 9) showed the median age of the MRCA for Comasteridae to be either 101 or 103 mya and with a similar overall substitution rate similar to other crinoids. One apparent apomorphy that diagnoses Comasteridae is the modification of the distal oral pinnulars as a comb (Clark, 1931), but these are tiny skeletal structures not likely to be fossilized. It is certainly possible

that Comasteridae is an older group than the current fossil record indicates, and the unconstrained dates are old enough to permit the possibility that *Uintacrinus* (and perhaps *Marsupites*) indeed are close to Comasteridae (Milsom et al., 1994).

4.4. Stalk and cirri transformations

It is well known that postlarval featherstars are attached to a substrate through a stalk, although both stalk and terminal attachment disk develop in the larva before settlement (e.g. Haig and Rouse, 2008; Mortensen, 1920). The postlarvae develop cirri on the proximal-most columnal, which becomes the centrodorsal, and then abandon the remainder of the stalk (Grimmer et al., 1984; Kohtsuka and Nakano, 2005). As discussed above, several authors, particularly Rasmussen (1978b) and Simms (1988b,c, 1989, 1999, 2011), have argued for a paedomorphic origin for bourgueticrinids whereby they retain their stalk completely as adults. However, based on their molecular phylogeny, Cohen et al. (2004) rejected this and suggested that "comatulids appear to have arisen from a bourgueticrinid-like ancestor", and this hypothesis is indeed consistent with one of our MP analyses (e.g., Suppl. Fig. 4). In general, however, our results provide support for the hypothesis that some bourgueticrinids may indeed be paedomorphic comatulids, perhaps originating several times, and that Guillecrinidae represents another independently derived paedomorphic group (Figs. 2–4, Suppl. Figs. 1, 2, 4, 5, and 6). Still the Mk1 transformation for the stalk character (Fig. 5A) shows that there are various possibilities for the transformation of the stalk within Comatulida, clearly seen in the MPRs on the same topology (Suppl. Fig. 11). In some cases (e.g., Suppl. Fig. 11A), there have been up to three reversals from the centrodorsal condition to a full stalk. However, other MPRs (e.g., Suppl. Fig. 11B) support the homology of the adult stalk of bourgueticrinids and Guillecrinidae with crinoids such as Isocrinida and Hyocrinida, and that the stalk has been lost below the centrodorsal several times. Narrowing the choice among these scenarios will require more taxon sampling of Comatulida and better resolution of the relationships among the major clades of extant crinoids, in particular *Caledonicrinus*, which consistently appeared as the stalked sister group to Comatulida.

With regard to cirri, Simms (1988b, Fig. 21.1) argued that they are homologous, though plesiomorphic for Isocrinida and Comatulida, with losses occurring for Hyocrinida, Cyrtocrinida and bourgueticrinids. This is in part supported by the observation that stem-groups of Articulata from the early Triassic such as Holocrinidae, Dadocrinidae and Encrinidae all had an adult stalk with cirri on various columnals (Hagdorn, 2011; Simms, 1988b). Cohen et al. (2004) on the other hand suggested that "cirri originated independently in lineages leading to the Isocrinida and to the Comatulida". While the ML tree in Fig. 5B suggests that cirri have been lost several times within Articulata, there is ambiguity at the basal nodes and 17 MPRs for this feature (Suppl. Fig. 12). Eight of the 17 MPRs for the ML tree (e.g., Suppl. Fig. 12A) reconstruct the condition for the Articulata ancestral node as "cirri present", in accordance with the evidence that stem group fossil Articulata had cirri (Hagdorn, 2011; Simms, 1988b); two support the hypothesis that cirri have been lost multiple times in Articulata and that they are plesiomorphic for Isocrinida and Comatulida, while the remaining seven show cirri appearing independently two or more times. Further study on the fine structure of cirri is warranted to assess the primary homology of crinoid cirri. Holland and Grimmer (1981) studied the ultrastructure of cirri of several comatulids and found they were essentially similar. However, they pointed out some discrepancies with their observations and those of studies of isocrinid cirri and argued for the need for examining cirri of stalked crinoids by transmission electron microscopy.

4.5. Conclusions

Our results have taken several important steps toward answering basic questions about post-Paleozoic crinoid phylogeny posed at the beginning of this paper, though further sequence data are clearly needed to resolve some key nodes. With respect to the relationships among the major groups of extant crinoids, we recovered three as clades (Cyrtocrinida, Hyocrinida, Isocrinida), although the details of their relationships remain unresolved, whereas two other major stalked groups (Bourgueticrinina and Guillecrinina) appear to nest among the featherstars, Comatulida. Although some major subgroups of Comatulida (e.g., Comasteridae) are well-supported clades, several others clearly require major revision (e.g., Tropiometroidea, Antedonoidea), reflecting the great deal of research that remains to be done. Our molecular clock analyses with fossil calibration points recovered extant Articulata as dating to a most recent common ancestor around 231–252 mya. This tends to support the hypothesis that the crown group is a radiation following the post-Permian–Triassic extinction event, rather than from several Permian lineages. Our tree topologies show various scenarios for the evolution of stalks and cirri in Articulata. Common among these scenarios are repeated acquisitions or losses of traits; further integration of fossils forms is warranted in future analyses.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.09.018.

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