Zootaxa 2504: 1–19 (2010)
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

Phylogenetic relationships in the genus *Astropecten* Gray (Paxillosida: Astropectinidae) on a global scale: molecular evidence for morphological convergence, species-complexes and possible cryptic speciation

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Abstract

With over 150 described species, *Astropecten* Gray (Paxillosida:Astropectinidae) is one of the most species-rich genera among sea stars. This diversity is remarkable, because most species of *Astropecten* have a long-lived planktotrophic larval stage, which would be expected to lead to a low speciation rate. The taxonomy of this genus is complex and not well resolved, and phylogenetic relationships have only been addressed in the beginning of the last century. In order to resolve general taxonomic issues, identify speciation patterns and estimate species diversity within the genus *Astropecten*, we inferred a molecular phylogeny of 117 specimens of *Astropecten* belonging to 40 species from around the world using mitochondrial DNA (mtDNA) sequences of 12S rRNA, 16S rRNA and cytochrome oxidase subunit I (COI). We compared the resulting molecular phylogeny to a previously published morphological one by Döderlein and investigated the possibility of morphological convergence in species from different geographic regions. Finally, we also aimed at identifying potentially problematic descriptions and/or signs of cryptic speciation in *Astropecten.* The global molecular phylogeny exhibited three main clades, each containing specimens of the same geographic region: 1. the Indo-Pacific; 2. the Neotropics; and 3. the eastern Atlantic and Mediterranean. Phylogenetic inferences based on mtDNA indicate that morphological and ecological convergence has taken place in *Astropecten*, resulting in allopatric non-sister taxa with similar morphologies and habitat preferences. The comparison to Döderlein's morphological phylogeny reveals congruence on the whole but many discrepancies on a local scale, indicating that meaningful morphological characters are not easily identified and categorized in *Astropecten.* Our results also reveal that *A. polyacanthus* Müller & Tröschel and *A. indicus* Döderlein are species-complexes*;* cryptic speciation may have occurred within each of these morphospecies. Furthermore, several variants, previously presumed to be conspecific, exhibit genetic distances large enough to justify recognizing them as separate species.

Key words: global phylogeny, Asteroidea, mitochondrial DNA, echinoderm, marine invertebrates

Introduction

Marine organisms with long-lived planktotrophic larvae are thought to rarely undergo speciation due to their enormous capacity for dispersal and large effective population sizes (Palumbi 1992, 1994; Riginos and Victor 2001). Thus, it is unusual to find echinoderm genera with planktonic larvae that contain more than 20 species. However, this trend is not universal among all echinoderms, and estimating probability of speciation from the duration of the larval stage alone can be misleading. Also, as has been shown in several studies using genetic markers, levels of gene flow and extent of species range cannot always be predicted from larval duration (e.g., Sponer and Roy 2002; Waters *et al.* 2004; Paulay and Meyer 2006; Wilson *et al.* 2007).

An exception to the pattern of low species diversity in echinoderms with mostly long-lived planktonic larval stages is the sea star genus *Astropecten* Gray (Paxillosida: Astropectinidae) with approximately 150 species described to date (Say 1825; Gray 1841; Müller and Troschel 1842; Perrier 1875/6; Agassiz 1877; Sladen 1889; Ludwig 1897; Fisher 1906; Koehler 1909, 1910, 1924; Fisher 1911, 1913; Verrill 1914, 1915;

Accepted by M. Eleaume: 22 Apr. 2010; published: 14 Jun. 2010

Döderlein 1917; Clark and Downey 1992). As species of sea stars typically remain defined by morphological characteristics, the high phenotypic variability in *Astropecten* has resulted in the designation of several subspecies, variations and local forms. Many new species have been described based on juvenile or badly preserved specimens, such as *A. exiguus* Ludwig, *A. hermatophilus* Sladen, *A. ibericus* Perrier, *A. progressor* Döderlein and *A. spiniphorus* Madsen. The validity of some of these species is questionable, and extensive revision of this genus is required.

Representatives of *Astropecten* occur worldwide, mainly in shallow waters of tropical and temperate seas. However, several species can also live at greater depths, for instance *A. irregularis* Pennant (> 1000 m; Döderlein 1917; Tortonese 1965) and *A. hermatophilus* Sladen (up to 1500 m; Clark and Downey 1992). *Astropecten* burrow in sandy or muddy substrate where they feed on molluscs and other infaunal invertebrates (Tortonese 1965).

Most species of *Astropecten* seem to have what Mortensen (1921, 1937) considered a typical bipinnaria larva, metamorphosing in the water column and settling as young sea stars (Clark and Downey 1992). Laboratory rearing has shown that the planktonic stage usually lasts for several weeks (Mortensen 1921, 1937; Newth 1925; Hörstadius 1938; Ventura *et al.* 1997).

To date, phylogenetic relationships in *Astropecten* have not been inferred either by a cladistic approach or on the basis of molecular markers. However, in 1917 Döderlein published a monograph on the genus *Astropecten* and its evolutionary history based on morphological characters. Döderlein assigned over 100 species of *Astropecten* to different groups according to their presumed relationships. He stated that there are three major groups of species in *Astropecten* corresponding to the following geographic regions: 1. the East Atlantic and Mediterranean, 2. the east and the west coast of America, and 3. the Indo-West Pacific. According to Döderlein, there is generally no close relationship between the species from these different regions. Between the East Atlantic group and the American group he deduced deep phylogenetic separation. Döderlein thought that there was also a phylogenetic separation between West African and East African *Astropecten*.

Nevertheless, morphologically similar species exist in different geographic regions and occur in similar habitats. Considering the long larval stage and potential dispersal capacity in several *Astropecten* species, the question remains open whether some of them are closely related, or whether they have come to resemble each other due to convergent evolution. For instance, Döderlein suggested that *A. regalis* Gray from the East Pacific and *A. marginatus* Gray from the West Atlantic are sister species, and that a third species from Japan, *A. latespinosus* Meissner, is closely related to these two. Döderlein further proposed that the general direction of species' spread is from east to west and that Hawaii could serve as a stepping stone in the Pacific. Phylogenetic relationships as proposed by Döderlein are shown in Figure 1 and contain by and large the same species included in the present study. The phylogeny suggests that several species with similar morphologies occur allopatrically, whereas other presumed sister species also exist sympatrically, such as *A. articulatus* Say and *A. antillensis* Lütken in the West Atlantic and *A. bispinosus* Otto and *A. platyacanthus* Philippi in the Mediterranean. Whether sympatric speciation has occurred in *Astropecten* has not yet been clarified.

The aim of this study was to resolve general taxonomic issues, identify speciation patterns and estimate species diversity within the genus *Astropecten*. We attempted to resolve these questions by inferring phylogenetic relationships on a global scale using molecular markers of mitochondrial DNA. Given the high diversity and extensive geographic occurrence of *Astropecten*, it was beyond the scope of this study to formally revise all described species of this genus. Nevertheless, we aimed at obtaining data from as many species as possible. In particular, (i) we used a molecular phylogeny to examine the validity of the phylogenetic relationships suggested by Döderlein (1917); (ii) we investigated the possibility of morphological convergence of similar species from different geographic regions; and (iii) we identified potentially problematic species descriptions and/or signs of cryptic speciation in *Astropecten.* A companion paper which dates the splitting of *Astropecten* lineages and correlates them to geological events is in preparation.

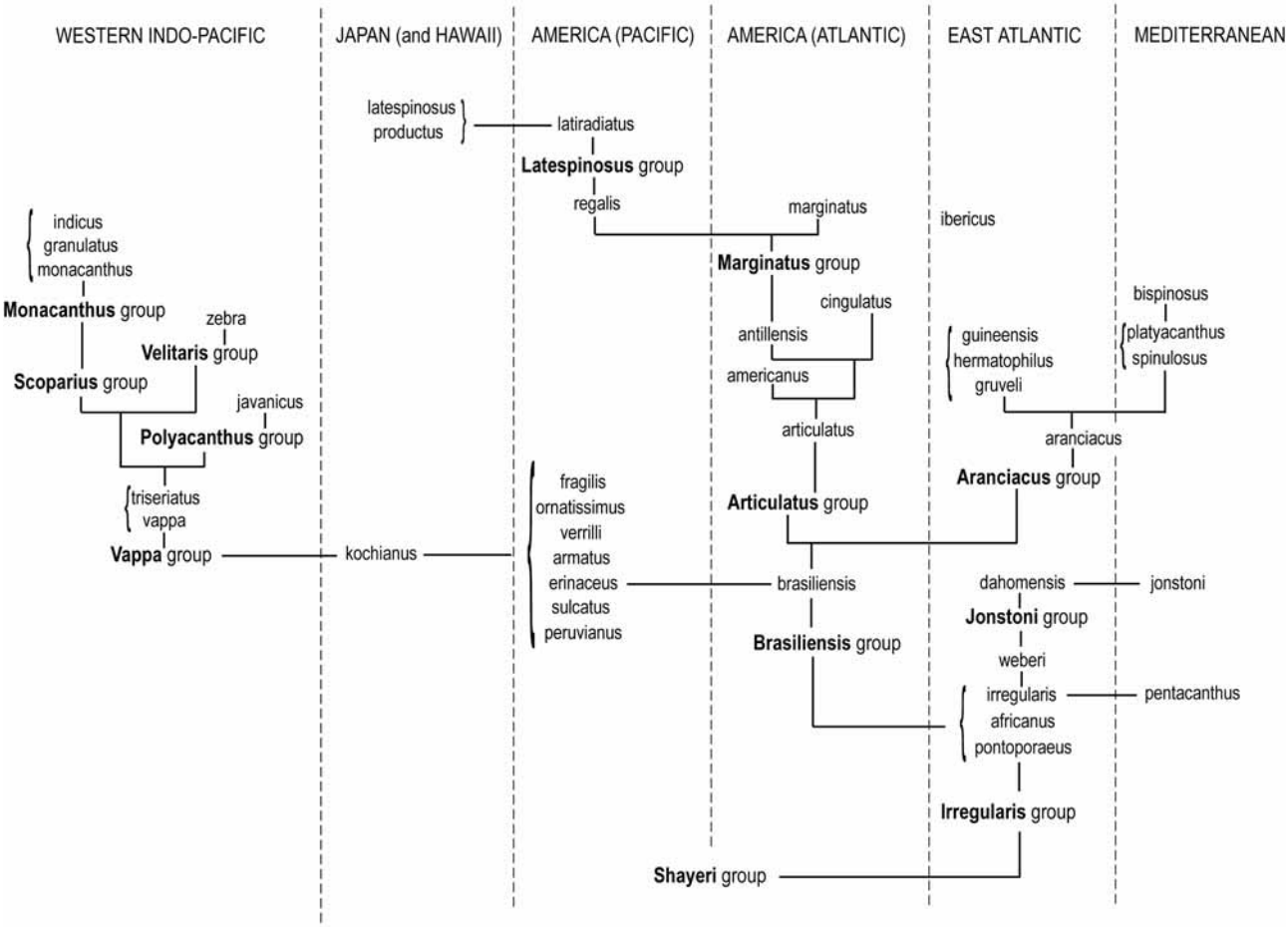

FIGURE 1. Phylogeny of the genus *Astropecten* as suggested by Döderlein (1917) presenting the relationships of species and species groups relevant to this study.

Material and methods

Sampling and DNA extraction. We obtained 117 specimens belonging to 40 species of *Astropecten*. Seven additional specimens of four other astropectinid genera and one goniasterid genus were sampled as outgroup taxa (Table 1). Specimens were collected by scuba diving, from trawl and gill net operations, from institutional invertebrate collections and private persons at 38 locations around the world (Figure 2).

In the Mediterranean and East Atlantic, we collected 54 specimens belonging to all seven recognized species of *Astropecten* from different sampling locations as listed in Table 1. We did not distinguish between the different varieties of *A. irregularis* when sampling, as many specimens were juveniles and therefore did not exhibit the typical morphological characters necessary for this distinction.

Samples were preserved in 96% ethanol or in 80% ethanol containing dimethyl sulfoxide (DMSO) buffer. DNA from approximately 30 mg of arm tip tissue or tube feet was extracted using a DNeasy Tissue Kit® (QIAGEN) following the manufacturer's instructions for extraction of animal tissue for a final volume of 400 µl and then stored at -20 °C.

DNA sequencing. We amplified fragments of three regions of mitochondrial DNA (mtDNA): approximately 576 bp of the 12S ribosomal RNA (12S), 624 bp of the 16S ribosomal RNA (16S) and 619 bp of the cytochrome oxidase subregion I (COI). The primers used for DNA amplification are listed in Table 2. COI was not easy to amplify in some specimens, especially those stored in museums, and thus often required various combinations of primers. DNA amplifications were performed in a 30 µl-volume reaction with 1.67 U *Taq* DNA *Polymerase*, 3 µl 10x PCR reaction buffer, 0.4 mM dNTPs, 0.2 µM of each primer, 1 mM $MgCl_2$

and 6 µl of template DNA. The PCR protocol consisted of an initial denaturation step at 96 °C for 5 s, 40 amplification cycles (95 °C for 30 s, 50 °C for 45 s and 72 °C for 1 min) and a final elongation step at 72 °C for 10 min performed in a Whatman Biometra T1 Thermocycler. The PCR products were purified with the QIAquick® PCR Purification Kit (QIAGEN) following the supplier's instructions. Forward and reverse sequencing was carried out using the primers marked with an asterisk in Table 1 and using BigDye® Terminator (PE-Applied Biosystems) chemistry. The cycle-sequencing protocol consisted of an initial step at 96 °C for 3 min and 24 sequencing cycles (96 °C for 15 s, 50 °C for 10 s and 60 °C for 3 min). Cycle sequencing products were purified with a DyeEx™ 2.0 Spin Kit (QIAGEN) and subsequently sequenced in an ABI 3730 DNA Analyzer. Sequences were edited using the software SEQUENCHER™ 4.6 (Gene Codes Corporation) and deposited in GenBank under the accession numbers listed in Table 1.

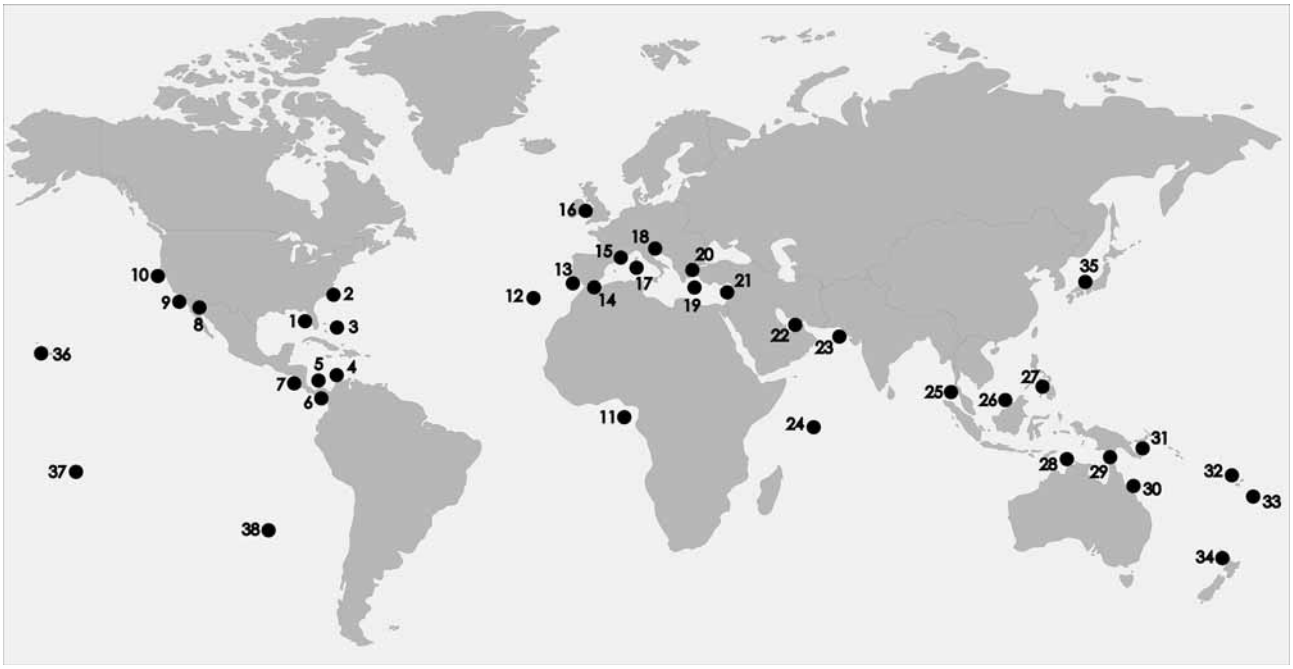

FIGURE 2. Collection sites of *Astropecten* specimens and outgroup taxa.
1. Florida: Sanibel Island and St. Petersburg; 2. South Carolina: Cape Island; 3. Bahamas: Bimini; 4. Colombia: Sta. Marta and Arboletes; 5. Atlantic Panama: Bocas del Toro, San Blas and Colon; 6. Pacific Panama: Gulf of Chiriqui, Bay of Panama; 7. Costa Rica: Guanacaste; 8. Mexico: Puerto Peñasco; 9. California: San Diego; 10. California: Monterrey Bay; 11. São Tomé; 12. Madeira; 13. Portugal: Faro; 14. Spain: La Herradura; 15. France: Toulon; 16. Irish Sea; 17. West Mediterranean: Sardinia and Corsica; 18. Croatia: Cres; 19. Greece: Crete; 20. Greece: Kavala; 21. Cyprus; 22. United Arab Emirates: Dubai; 23. Pakistan: Karachi; 24. Seychelles; 25. Thailand: Phuket; 26. Borneo: Brunei; 27. Philippines: Mindanao and Leyte; 28. Australia: Cobourgh Peninsula; 29. Australia: Torres Strait; 30. Australia: Townsville; 31. SE Papua New Guinea; 32. Fiji; 33. Tonga; 34. Auckland, New Zealand; 35. Toyama Bay, Japan; 36. Hawaii: Maui and. Oahu; 37. Marquesas Islands; 38. Nazca submarine ridge

Alignment. To align the sequences, we used the software CLUSTAL X version 2.0 (Thompson *et al.* 1997; Jeanmougin *et al.* 1998) applying the profile alignment mode. We aligned pairs with lower distances first with a gap opening penalty of 10.0 and extension penalty of 0.2. The alignment software SOAP v. 1.1b1 (Loytynoja and Milinkovitch 2001) was used to find regions that were not well supported when comparing alignments with gap opening penalties ranging from 5.0 to 15.0 in steps of two and gap extension penalties ranging from 0.1 to 10.1 in steps of two. We considered alignment regions that had less than 80% support ambiguous and removed them from the dataset using the software BIOEDIT v. 7.0.9.0 (Hall 1999). The final alignment included 366 bp of 12S, 488 bp of 16S and 546 bp of COI and can be viewed in the Appendix.

Phylogenetic analysis. We tested for phylogenetic congruence between the three mtDNA regions 12S, 16S and COI performing a partition homogeneity test (Farris *et al.* 1995) as implemented in PAUP* v. 4.0b10 (Swofford 2003) and after deleting taxa with missing data. This test produced a *p*-value of 0.01, suggesting

significantly different phylogenetic signals between the three mtDNA regions. Preliminary phylogenetic trees of each region separately were constructed by performing maximum parsimony analysis in PAUP* by heuristic search for 50 random addition replicates using TBR branch swapping option and keeping 100 trees per replicate. These trees showed that two specimens, those of *A. granulatus* and *A. zebra*, appeared in a drastically different position in the COI tree compared to the trees of the other two regions. Removing these specimens from the matrix resulted in a *p*-value > 0.05 in the partition homogeneity test. As the position of *A. granulatus* and *A. zebra* were the only ones conflicting between the regions, we removed the COI sequences of these two specimens from the matrix and performed the phylogenetic analyses using the concatenated dataset of 12S, 16S and COI sequences. The concatenated sequence of the three mtDNA regions amounted to 1390 base pairs, of which 792 were invariant and 508 were potentially phylogenetically informative.

After removing 11 redundant haplotypes from the matrix, the program MODELTEST v. 3.7 (Posada and Crandall 1998) was used to perform hierarchical likelihood ratio tests (hLRTs) to select the best model of DNA evolution. The general time-reversible model with a proportion of invariable sites (I) and gamma distribution (Γ) (GTR+I+ Γ) was selected as the model of evolution that best fits the data (−lnL = 16783.8; α value of Γ = 0.746; pinvar = 0.5100). Uncorrected genetic distances, as well as corrected distances using this model were calculated in PAUP*.

Global phylogeny. We performed maximum parsimony (MP) analysis in PAUP* by heuristic search for 50 random addition replicates using TBR branch swapping option and keeping 100 trees per replicate. Gaps were treated as missing data. We estimated clade support in PAUP* performing 1,000 bootstrap resampling replicates and computing decay indices (Bremer 1988).

Bayesian inference (BI) was performed in MRBAYES v. 3.1.2 (Ronquist and Huelsenbeck 2003) assuming the GTR+I+Γ model and unlinking the partitions so that parameters were estimated for each partition separately. Two runs of four chains were performed for 5,000,000 generations sampling every 500 generations and with a temperature for the heated chains of T = 0.02. After discarding the first 500 trees as burn-in, we used the remaining trees to estimate posterior probabilities indicating clade credibility.

Results

Specimen and sequence data

We obtained sequence data of the mitochondrial DNA regions 12S rRNA, 16S rRNA and cytochrome oxidase subregion I (COI) of 117 *Astropecten* specimens and seven outgroup specimens. Of the roughly 40 species of *Astropecten* collected for this study, it was not possible to reliably identify 10 of them either by morphological characters or by mtDNA sequences. Unidentifiable specimens were either juveniles lacking morphological characteristics crucial for identification or adults that did not match any of the species descriptions in the literature. The latter was particularly the case in deep sea specimens of the South Pacific. COI amplification was not successful for 33 specimens and 16S amplification for six (see Table 1 for missing GenBank accession numbers). Although we were not able to obtain the COI sequence for several specimens and, in a few cases, the 16S sequence, we included all taxa in the analysis. Adding taxa with missing data presumably affects phylogenetic resolution more positively than excluding them (Philippe *et al.* 2004; Wiens 2006, Wiens and Moen 2008), especially when more than 500 characters are being analyzed and missing data are not distributed randomly across the dataset but mostly affect the same regions (Wiens 2003). While in model-based analysis, such as BI, even taxa with highly incomplete sequences (> 75% missing data) improve phylogenetic accuracy, in MP more than 25% of the data per sequence are required to rescue the analysis from long branch attraction (Wiens 2005, 2006). As the 12S region amounts to more than 25% of the included base pairs and is present in all specimens, we are confident that there were no deleterious effects from missing data in any of the analyses.

TABLE 1. Species, identification code, localities and sampling date, voucher and GenBank accession codes of *Astropecten* and outgroup specimens.

Species	ID	Location/Date	Voucher	Genbank accession codes		
				12S rRNA	16S rRNA	COI
A. africanus	Aafri - STo1	EA - São Tomé/ Feb 2006		FJ171765	FJ177591	FJ195695
A. alligator	Aalli - Col1	WA - Santa Maria, Colombia; 300 m/ 2001	INV. EQU01809	FJ171787	FJ177548	
A. americanus	Aamer - Flo1	WA - Tampa Bay, Florida, USA; 271 m/ Mar 2003	UF 3471	FJ171786	FJ177547	
A. antillensis	Aanti - Pan1	WA - San Blas, Panama/ Feb 2002		FJ171794	FJ177541	FJ195654
	Aanti - Col1	WA - Arboletes, Colombia; 21 m/ 2001	INV. EQU01719	FJ171793	FJ177541	FJ195714
A. aranciacus	Aaran - Sar1	WM - Costa Colostrai, Sardinia; 17 m/ Aug 2002		FJ171773	FJ177596	FJ195679
	Aaran - Sar2	WM - Costa Colostrai, Sardinia; 17 m/ Aug 2002		FJ171774	FJ177598	FJ195681
	Aaran - Sar3	WM - Costa Colostrai, Sardinia; 17 m/ Aug 2002		FJ171775	FJ177599	FJ195683
	Aaran - Kav1	EM - Kavala, Greece; 8-18 m/ Mar 2006		FJ171770	FJ177592	FJ195669
	Aaran - Kav2	EM - Kavala, Greece; 8-18 m/ Mar 2006		FJ171766	FJ177592	FJ195668
	Aaran - Kav3	EM - Kavala, Greece; 8-18 m/ Mar 2006		FJ171767	FJ177593	FJ195669
	Aaran - Far1	EA - Lagos, Faro, Portugal; 34-42 m/ May 2005		FJ171771	FJ177597	FJ195670
	Aaran - Far2	EA - Lagos, Faro, Portugal; 34-42 m/ May 2005		FJ171772	FJ177596	FJ195671
	Aaran - Far3	EA - Faro, Faro Portugal; 30 m/ Jun 2005		FJ171770	FJ177596	FJ195669
	Aaran - Mad1	EA - Quinta do Lorde, Madeira; 15-25 m/ Oct 2005		FJ171768	FJ177594	
	Aaran - Mad2	EA - Quinta do Lorde, Madeira; 15-25 m/ Oct 2005		FJ171769	FJ177595	
	Aaran - Mad3	EA - Quinta do Lorde, Madeira; 15-25 m/ Oct 2005		FJ171777	FJ177601	FJ195729
	Aaran - Cre1	EM - Croatia, Cres; 5 m/ Oct 2002		FJ171776	FJ177600	FJ195687
	Aaran - Cre2	EM - Croatia, Cres; 5 m/ Oct 2002		FJ171739	FJ177599	FJ195674
	Aaran - Cre3	EM - Croatia, Cres; 5 m/ Oct 2002		FJ171770	FJ177596	FJ195675
A. armatus	Aarma - Mex1	EP - Puerto Peñasco, Northern Sea of Cortez, Mexico; 69 m/ Mar 1985	UNAM 4190	FJ171785	FJ177563	
A. articulatus	Aarti - Pan1	WA - Bocas del Toro, Panama/ 1996		FJ171791	FJ177543	FJ195658
	Aarti - Pan2	WA - Bocas del Toro, Panama/ 1996		FJ171792	FJ177544	FJ195659
	Aarti - Pan3	WA - Bocas del Toro, Panama/ 1996		FJ171792	FJ177544	FJ195660
	Aarti - SCa1	WA - Cape Island, South Carolina, USA; 12-13 m	USC S713	FJ171795	FJ177545	
	Aarti - SCa2	WA - Cape Island, South Carolina, USA; 12-13 m	USC S713	FJ171796	FJ177546	
A. bispinosus	Abisp - Her1	EM - Hersonissos, Crete, Greece; 3-5 m/ Mar 2006		FJ171743	FJ177568	
	Abisp - Her2	EM - Hersonissos, Crete, Greece; 3-5 m/ Mar 2006		FJ171743	FJ177568	
	Abisp - Her3	EM - Hersonissos, Crete, Greece; 3-5 m/ Mar 2006		FJ171743	FJ177568	
	Abisp - Cre1	EM - Cres, Croatia; 20 m/ Oct 2002		FJ171742	FJ177567	FJ195682
	Abisp - Cre2	EM - Cres, Croatia; 20 m/ Oct 2002		FJ171739	FJ177564	FJ195676
	Abisp - Cre3	EM - Cres, Croatia; 20 m/ Oct 2002		FJ171740	FJ177565	
	Abisp - Sar1	WM - Costa Colostrai, Sardinia; 17 m/ Aug 2002		FJ171745	FJ177570	FJ195733
	Abisp - Sar2	WM - Costa Colostrai, Sardinia; 17 m/ Aug 2002		FJ171741	FJ177566	FJ195680
	Abisp - Sar3	WM - Costa Colostrai, Sardinia; 17 m/ Aug 2002		FJ171742	FJ177567	FJ195682
	Abisp - Cor1	WM - St. Florent, Corsica; 1.5 m/ Apr 2005		FJ171744	FJ177569	
A. cingulatus	Acing - Pan1	WA - Isla Escuda de Veraguas, Panama; 42-39 m/ Aug 2004		FJ171802	FJ177552	FJ195664
A. comptus	Acomp - Flo1	WA - Gulf of Mexico, off St. Petersburg, Florida; 116 m/ Nov 2004	UF 3249	FJ171788	FJ177551	

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TABLE 1. (continued)

Species	ID	Location/Date	Voucher	Genbank accession codes		
				12S rRNA	16S rRNA	COI
	Acomp - Flo2	WA - Gulf of Mexico, off St. Petersburg, Florida; 116 m/ Nov 2004	UF 3249	FJ171788	FJ177551	
	Acomp - Flo3	WA- Off Sanibel Island, Florida, USA; 184 m/ 2001	UF 490	FJ171790	FJ177549	
A. duplicatus	Adupl - SCa1	WA - Cape Island, South Carolina, USA; 10 m	USC S1097	FJ171800	FJ177539	
	Adupl - SCa2	WA - Cape Island, South Carolina, USA; 10 m	USC S1098	FJ171801	FJ177540	
	Adupl - Bim1	WA - Bimini, Bahamas; 0 m/ Feb 2003		FJ171797	FJ177537	FJ195717
	Adupl - Bim2	WA - Bimini, Bahamas; 0 m/ Feb 2003		FJ171798	FJ177538	FJ195721
	Adupl - Flo1	WA - Sanibel Island, Florida, USA, 15-16m, 2001	UF 115, *A. forbesi*	FJ171799	FJ177542	
A. erinaceus	Aerin - Pan1	EP - Isla Montuosa, Golf of Chiriqui, Panama; 42.6 m/ May 2004		FJ171778	FJ177556	FJ195656
A. granulatus	Agran - Aus1	IP - Cobourg Peninsula, Northern Territory, Australia; 13 m/ Sep 1985	SI NMNH E38949	FJ171825	FJ177620	FJ195685
A. indicus	Aindi - Pak1	IO - Clifton, Karachi, Pakistan/ 2005		FJ171820	FJ177617	FJ195690
	Aindi - Pak2	IO - Clifton, Karachi, Pakistan/ 2005		FJ171820	FJ177617	FJ195691
	Aindi - Bru1	IP - Serasa, Brunei/ Dec 2004		FJ171821	FJ177618	FJ195726
	Aindi - Tha1	IP - Phuket, Thailand/ Apr 1997	PMBC 19223, *A. monacanthus*	FJ171819	FJ177619	FJ195692
A. irregularis	Airre - Sar1	WM - Costa Colostrai, Sardinia; 0-3 0m/ Jun 2001		FJ171749	FJ177574	FJ195677
	Airre - Sar2	WM - Costa Colostrai, Sardinia; 0-30m/ Jun 2001		FJ171751	FJ177576	FJ195693
	Airre - Sar3	WM - Costa Colostrai, Sardinia; 0-30m/ Aug 2002		FJ171750	FJ177575	FJ195678
	Airre - Far1	EA - off Faro, Portugal; 530-540 m/ May 2005		FJ171758	FJ177583	FJ195665
	Airre - Far2	EA - off Faro, Portugal; 290 m/ May 2005		FJ171758	FJ177584	FJ195666
	Airre - Far3	EA - off Faro, Portugal; 106-128 m/ May 2005		FJ171758	FJ177584	FJ195667
	Airre - Kav1	EM - Kavala, Greece; > 50 m/ Mar 2006		FJ171752	FJ177577	FJ195696
	Airre - Kav2	EM - Kavala, Greece; > 50 m/ Mar 2006		FJ171753	FJ177578	FJ195697
	Airre - Kav3	EM - Kavala, Greece; > 50 m/ Mar 2006		FJ171754	FJ177579	FJ195698
	Airre - Mad1	EA - Quinta do Lorde, Madeira; 15-25m/ Oct 2005		FJ171755	FJ177580	FJ195732
A. javanicus	Ajava - Bru1	IP - Serasa, Brunei/ Dec 2004		FJ171824	FJ177616	FJ195725
A. jonstoni	Ajons - Tso1	EM - Tsoutsouras, Greece; 4 m/ Mar 2006		FJ171761	FJ177587	FJ195701
	Ajons - Tso2	EM - Tsoutsouras, Greece; 4 m/ Mar 2006		FJ171763	FJ177589	FJ195701
	Ajons - Tso3	EM - Tsoutsouras, Greece; 4 m/ Mar 2006		FJ171764	FJ177589	FJ195701
	Ajons - Sar1	WM - Costa Colostrai, Sardinia/ Aug 2000		FJ171762	FJ177588	FJ195703
	Ajons - Sar2	WM - Costa Colostrai, Sardinia; 1-30 m/ Aug 2002		FJ171762	FJ177590	FJ195703
	Ajons - Sar3	WM - Costa Colostrai, Sardinia, 1-30 m / Jun 2001		FJ171759	FJ177585	FJ195699
	Ajons - Sar4	WM - Costa Colostrai, Sardinia, 1-30 m / Jun 2001		FJ171760	FJ177586	FJ195700
A. latespinosus	Alate - Jap1	WP - Toyama Bay, Japan/ 2004		FJ171829	FJ177623	FJ195722
A. marginatus	Amarg - Pan1	WA - Colon, Panama; 10 m/ 2004		FJ171803	FJ177553	FJ195662
	Amarg - Pan2	WA - Colon, Panama; 10 m/ 2004		FJ171804	FJ177554	FJ195663
	Amarg - Col1	WA - Santa Marta, Colombia/ 2003	INV EQU02562	FJ171805	FJ177555	FJ195715

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TABLE 1. (continued)

Species	ID	Location/Date	Voucher	Genbank accession codes		
				12S rRNA	16S rRNA	COI
A. monacanthus	Amona - Aus1	IP - Torres Strait, Queensland, Australia; 0 m/ Jun 1979	SI NMNH E35296	FJ171826	FJ177621	FJ195735
A. nitidus	Aniti - Col1	WA - Santa Marta, Colombia; 153 m/ 2001	INV EQU01841	FJ171789	FJ177550	FJ195716
A. oerstedii	Aoers - Pan1	WA - San Blas, Panama/ Feb 2002		FJ171782	FJ177561	FJ195655
A. platyacanthus	Aplat - Cyp1	EM - Cap Greco, Cyprus; 10 m/ Oct 2004		FJ171748	FJ177573	FJ195724
	Aplat - Cyp2	EM - De Capo Bay, Cyprus; 3 m/ Oct 2004		FJ171748	FJ177573	FJ195724
	Aplat - Cyp3	EM - Cap Greco, Cyprus; 10 m/ Oct 2004		FJ171748	FJ177573	FJ195724
	Aplat - Sar1	WM - Sardinia; 1-30m/ 2002		FJ171747	FJ177572	FJ195684
	Aplat - Sar2	WM - Sardinia; 1-30m / 2002		FJ171747	FJ177572	FJ195684
	Aplat - Sar3	WM - Cannigione, Sardinia, 1-30m/ Sep 2003		FJ171747	FJ177572	FJ195684
	Aplat - LaH1	WM - La Herradura, Spain; 5 m/ Aug 2005		FJ171746	FJ177571	
	Aplat - Tou1	WM - Toulon, France/ May 2000		FJ171747	FJ177572	
A. polyacanthus	Apoly - Mau1	CP - Maui, Hawaiian Islands; 10 m/ Apr 2005	LACM 2005-64.1	FJ171807	FJ177603	
	Apoly - Mau2	CP - Kanaio, Maui, Hawaii; 10 m/ Feb 2006		FJ171808	FJ177602	FJ195694
	Apoly - Sey1	IO - Picard Island, Aldabra Islands, Seychelles; reef flat/ Mar 1987	SI NMNH E35107	FJ171810	FJ177607	FJ195686
	Apoly - Sey2	IO - Picard Island, Aldabra Islands, Seychelles; reef flat/ Mar 1987	SI NMNH E35107	FJ171811	FJ177608	FJ195688
	Apoly - Fij1	SP - Bligh Water, Fiji; 143-173 m/ Aug 1998	MNHN EcAh 4734	FJ171827		
	Apoly - NZ1	SP - Auckland, New Zealand/ 2003		FJ171813	FJ177610	FJ195711
	Apoly - NZ2	SP - Auckland, New Zealand/ 2003		FJ171814	FJ177611	FJ195712
	Apoly - Jap1	WP - Toyama Bay, Japan/ Nov 2003		FJ171806	FJ177605	FJ195719
	Apoly - Jap2	WP - Toyama Bay, Japan/ Nov 2003		FJ171806	FJ177606	FJ195720
	Apoly - Phi1	IP - Aligbay, Mindanao, Philippines; 1.5 m/ May 1979	SI NMNH E48900	FJ171812	FJ177609	FJ195736
	Apoly - Dub1	IO - Dugass Beach, Dubai, U.E.A./ Feb 1981	SI NMNH E35065	FJ195652	FJ177633	
A. regalis	Arega - CRi1	EA - Coco, Guanacaste, Costa Rica; 4 m/ 1933	LACM 1933-123	FJ171780	FJ177558	FJ195653
A. scoparius	Ascop - Jap1	WP - Toyama Bay, Japan/ Nov 2003		FJ171818	FJ177613	FJ195718
A. siderealis	Aside - Pan1	EP - Coiba Island, Panama; 58 m/ May 2004		FJ171784	FJ177562	FJ195657
A. sp. 1	Asp1 - Fij1	SP - Malolo, Viti Levu, Fiji; 39 m/ Oct 1998	MNHN EcAh 4730	FJ171817		
A. sp. 2	Asp2 - Fij1	SP - SE Viti Levu, Fiji; 244-252 m/ Aug 1998	MNHN EcAh 4725	FJ171836	FJ177627	FJ195702
A. sp. 3	Asp3 - Fij1	SP - NW Taveuni Island, Fiji; 327-420 m/ Mar 1999	MNHN EcAh 4729	FJ171830	FJ177624	FJ195704
	Asp3 - Fij2	SP - off Suva, Fiji; 478-500m/ Mar 1999	MNHN EcAh 4735	FJ171832	FJ177625	FJ195705
	Asp3 - Fij3	SP - Bligh Water, N Viti Levu, Fiji; 471-475 m/ Aug 1998	MNHN EcAh 4759	FJ171831		FJ195710
A. sp. 4 (possibly *A. tasmanicus* or *A. eremicus*)	Asp4 - Fij1	SP - S Namenalala, Fiji; 364-369 m/ Mar 1999	MNHN EcAh 4738	FJ171833	FJ177626	FJ195707
	Asp4 - Fij2	SP - S Namenalala, Fiji; 364-369 m; Mar 1999	MNHN EcAh 4739	FJ171834	FJ177626	

continued next page

TABLE 1. (continued)

Species	ID	Location/Date	Voucher	Genbank accession codes		
				12S rRNA	16S rRNA	COI
A. sp. 5	Asp5 - Ton1	SP - SW Tongatapu, Tonga; 319-333 m/ Jun 2000	MNHN EcAh 4741	FJ171835		FJ195708
A. sp. 6	Asp6 - Phi1	IP - Leyte Island, Philippines; 76 m/ Nov 1979	SI NMNH E53739	FJ171828	FJ177622	FJ195737
A. sp. 7	Asp7 - Aus1	SP - Pallarenda Beach, Townsville, Australia; 0 m/ May 2004		FJ171823	FJ177615	FJ195728
A. spinulosus	Aspin - Cre1	EM - Cres, Croatia; 5 m/ Oct 2002		FJ171756	FJ177581	FJ195706
	Aspin - Cor1	WM - St. Florent, Corsica; 1.5m/ Apr 2005		FJ171757	FJ177582	FJ195723
A. triseriatus	Atris - Oah1	EP - Kailua, Oahu, Hawaiian Islands; 22m/ 1980	BM 1980.536	FJ171809	FJ177604	
A. vappa	Avapp - Bru1	IP - Serasa, Brunei/ Dec 2004		FJ171816	FJ177612	FJ195727
A. verilli	Averr - Pan1	EP - Panama/ 1996?		FJ171779	FJ177557	FJ195661
	Averr - Cal1	EP - Monterrey Bay, California, USA/ May 1996	Calacad 105628	FJ171783	FJ177560	FJ195713
	Averr - Cal2	EP - Point Loma, San Diego, USA; 220 m/ Nov 2002	SIO BIC E3481	FJ171781	FJ177559	FJ195730
	Averr - Cal3	EP - Point Loma, San Diego, USA; 220 m/ Nov 2002	SIO BIC E3481	FJ171781	FJ177559	FJ195731
A. zebra	Azebr - PNG1	SP - Deboin Mission, SE Papua New Guinea,/ Jun 1979	SI NMNH E50681	FJ171822	FJ177614	FJ195689
Tethyaster sp. 1	Tethy - Ton1	SP - Eua, Tonga; 463-464m/ Jun 2000	MNHN EcAh 4758	FJ171840		FJ195709
Tethyaster sp. 2	Tethy - Naz1	EP - Nazca submarine ridge; 230-280 m/ May 1987		FJ171841	FJ177630	FJ195734
Ctenophoraster sp.	Cphor - Mar1	SP - Fatu Hiva, Marquesas Islands; 85-130m/ Sep 1997	MNHN EcAh 4749	FJ171837		
Ctenopleura sp.	Cpleu - Fij1	SP - Bligh Water, N Viti Levu, Fiji; 143-173 m/ Aug 1998	MNHN EcAh 4732	FJ171843	FJ177632	
Pseudarchaster parelii	Ppare - Pan1	WA - San Blas, Panama/ Feb 2003		FJ171838	FJ177628	FJ195738
Tethyaster subinermis	Tsubi - Far1	EA - off Portimão, Faro, Portugal; 120-131m/ May 2005		FJ171839	FJ177629	FJ195672
Thrissacanthias penicillatas	Tpeni - SDi1	EP - off San Diego, California, USA; 1215 m/ Oct 2005	SIO BIC E3857	FJ171842	FJ177631	FJ195673
Total	124					

EA = East Atlantic; WM = West Mediterranean; EM = East Mediterranean; WA = West Atlantic; EP = East Pacific; CP = Central Pacific; SP = South Pacific; WP = West Pacific; IP = Indo-Pacific; IO = Indian Ocean. BM = Bishop Museum, Honolulu, Hawaii; Calacad = California Academy of Science; INV = INVEMAR, Instituto de Investigaciones Marinas y Costeras, Colombia; LACM = Los Angeles County Museum; MNHN = Musée nationale de la histoire naturelle, Paris, Echinoderm Collection; PMBC = Phuket Marine Biological Center; SI NMNH = Smithsonian National Museum of Natural History, Invertebrate Collection; SIO BIC = Scripps Institution of Oceanography - Benthic Invertebrate Collection; UF = Florida Museum of Natural History; UNAM = Universidad Nacional Autonoma de Mexico, Colleccion Nacional de Equinodermos; USC = University of South Carolina.

Global phylogeny

The maximum parsimony (MP) analysis on a global scale for all mtDNA regions combined resulted in 2600 most parsimonious trees (length = 3447), from which we constructed a strict consensus tree. This total evidence tree with bootstrap support values (BSP) and decay indices (DI) is shown in Figures 3a and 4a. Ingroup taxa were monophyletic (BSP = 95; DI = 16) and clustered into three main clades, each clade consisting of taxa from the same geographic region. The three clades corresponded to the following regions: 1. Indian Ocean and Pacific, excluding the West American coast (clade A); 2. east and west coast of America (clade B); and 3. East Atlantic and Mediterranean (clade C).

Bayesian inference (BI) resulted in 10,000 trees of which the first 500 were discarded as burn-in. The remaining trees were used to construct a consensus tree following the 50% majority rule. BI produced a tree

topology similar to the maximum parsimony (MP) tree and was not in conflict with respect to any of the major clades (Figures 3b and 4b). Posterior probability (PP) supporting the monophyly of the ingroup equalled 0.99, and the three main geographic regions within the ingroup were again distinct; however, *A. aranciacus* specimens appeared as a separate, fourth clade in the phylogeny and did not group with the remaining Mediterranean and East Atlantic species.

In general, deeper nodes received lower nodal support, indicating that relationships at this level were not as well resolved. To obtain better resolution and higher statistical support at this level, sequences of a slower evolving gene could be analyzed using additional resources. During this study, the nuclear 18S rRNA and Histone 3 genes were tested for this purpose on distantly and closely related *Astropecten* species but did not exhibit any variation.

Clade A: Indo-Pacific

Although the Indo-Pacific clade (Figures 3a and 3b) did not receive high node support in the MP reconstruction (BSP < 50, DI = 3), this clade appeared in both the MP and BI trees, with considerable support in the latter (PP = 0.93). Within this clade, three main groups formed: the first group contained all *A. polyacanthus* specimens, except for one from Fiji (clade D; BSP = 96; DI = 8; PP = 1.00); the second group comprised a mix of species from various geographic regions ranging from the North Arabian Sea to the South Pacific (clade E; BSP < 50; DI = 1; PP = 0.99); and the third group included specimens exclusively from the South Pacific Islands (clade F; BSP = 52; DI = 2, PP = 0.91). Clades E and F were not well supported by BSP and DI but were again present both in the MP and BI topologies. Within clade D, *A. scoparius* Müller & Troschel from Japan and an unidentified specimen (*A.* sp. 1) from Fiji were sister to the clade comprising mainly *A. polyacanthus* (clade G). *A. polyacanthus*, however, was paraphyletic, as two other species, *A. triseriatus* Müller & Troschel from Hawaii and *A. vappa* Müller & Troschel from Brunei, grouped within this clade. *A. triseriatus* grouped with *A. polyacanthus* from Hawaii, Japan and Dubai (BSP = 88; DI = 3; PP = 1.00) and *A. vappa* with *A. polyacanthus* from New Zealand (BSP = 93; DI = 8; PP = 1.00). Between specimens of *A. polyacanthus*, uncorrected genetic distances ranged from 0.002 to 0.106 substitutions per site. Corrected genetic distances using the parameters estimated in MODELTEST were between 0.002 and 0.144 substitutions per site.

Clade E included various species from different locations and also one *A. polyacanthus* specimen from Fiji. Specimens of *A. indicus* from Brunei, Pakistan and Thailand formed a monophyletic clade (BSP = 100; DI = 24; PP = 1.00). Among specimens of *A. indicus* uncorrected and corrected genetic distances ranged from 0.001 to 0.056 and from 0.001 to 0.065 substitutions per site, respectively.

Specimens grouping into clade F were exclusively from the South Pacific and were collected from deeper waters. Specimen Asp2-Fij1 was obtained at 250 m, while the others were collected below 320 m. Two other specimens from Fiji, *A. polyacanthus* (Apoly-Fij1) and an unidentified specimen (Asp1-Fij1) were collected above 180m and did not appear in this clade but grouped in clade E.

Clade B: east and west coast of America

All specimens from the East and the West coast of America grouped into one clade with high node support (BSP = 98; DI = 15; PP = 1.00). Within this clade, all specimens from the Pacific coast firmly grouped together (clade H; BSP = 99; DI = 12; PP = 1.00). Specimens of *A. verrilli* de Loriol were included in this clade but were not monophyletic. Between *A. armatus* Gray and *A. siderealis* Verrill uncorrected and corrected genetic distances both equalled 0.003 substitutions per site, indicating that these specimens very likely belong to the same species. Species from the Atlantic coast were paraphyletic; they grouped into three clades according to the MP tree in Figure 3a (clades I, J and K). Clade I included *A. alligator* Perrier, *A. americanus* Verrill, *A. comptus* Verrill and *A. nitidus* Verrill and also appeared in the BI phylogeny although with low support (BSP = 51; DI = 2; PP = 0.93). Specimens of *A. alligator* and *A. americanus* probably belong to the same species, as genetic distance between the two equalled 0.001 substitutions per site (uncorrected and corrected). *A. antillensis* Lütken, *A. duplicatus* Gray and three specimens of *A. articulatus* Say grouped into clade J (BSP = 98; DI = 6; PP = 1.00), but *A. articulatus* was not monophyletic because two

additional specimens formed a clade sister to clade J. The third group, clade K, consisting of *A. marginatus* Gray and *A. cingulatus* Sladen, only appeared in MP and was poorly supported (BSP < 50; DI = 1).

Clade C: Mediterranean and East Atlantic

MP analysis (Figure 4a) and BI (Figure 4b) were consistent in considering *A. aranciacus* as a reciprocally monophyletic clade with respect to the other Mediterranean and East Atlantic species. The two methods also did not differ significantly with regard to the relation of the *A. aranciacus* clade to the rest of the Mediterranean and East Atlantic species. While BI indicated that *A. aranciacus* is a completely independent clade (clade C2), MP grouped *A. aranciacus* with the other species from this region into clade C (Figures 3a and 4a) but with weak support (BSP < 50; DI = 1). Mediterranean and East Atlantic species were monophyletic with the exception of *A. irregularis*. This species was paraphyletic as Portuguese specimens (clade L; Far1−3) appeared separated from the rest of the species (clade M). Non-Portuguese *A. irregularis* were more closely related to *A. spinulosus* (clade N; BSP = 100; DI = 18; PP = 1.00) than to the Portuguese *A. irregularis*. Between Portuguese and non-Portuguese *A. irregularis* genetic distances ranged from 0.063 to 0.089 (uncorr.) and from 0.073 to 0.112 (corr.) substitutions per site. Within non-Portuguese *A. irregularis* (clade M), genetic distances between one specimen (Sar3) and the other non-Portuguese *A. irregularis* were between 0.021 and 0.039 (uncorr.) and between 0.022 and 0.042 (corr.) substitutions per site.

While clade credibility for *A. bispinosus* and *A. platyacanthus* being sister species was high (BSP = 100; DI = 16; PP = 1.00), there was only low support for their grouping within the *irregularis-spinulosus* clade (BSP = 81; DI = 4; PP = 0.66). *A. jonstoni* and *A. africanus* Koehler each formed separate clades and were more distantly related to the rest of the Mediterranean and East Atlantic species.

TABLE 2. Primers used for PCR-amplification of three mitochondrial DNA regions in *Astropecten* species.

Region	ID	Direction	Sequence 5'-3'	Reference
12S	12Saf*	forward	CTT AGC AAC CGA TTT GGT CCT AGT CC	this study
	12Sar	reverse	GCT GGT AAG GTT TTT CGT GGG TTA TCG	this study
	12Sa2r *	reverse	CCG CCA AGT CCT TTG AG	this study
16S	16Sbr*	forward	CCG GTC T(C/G)A (GA/AC)T CAG ATC ACG	Palumbi *et al.* 1996
	16Sar *	reverse	CGC CTG TTT ACC (A/T)AA AAC AT	Palumbi *et al.* 1996
	16Sa3r*	reverse	GTT AAA CGG CCG CGG TAT TTT GAC CG	this study
COI	cHCOf *	forward	(TGA/GAT) TTT TTG GTC ACC CTG AAG TTT A	Folmer *et al.* 1994
	AstroCOImf	forward	TAC TAT GTT GTA GCA CAC TT	this study
	ECOIa	forward	ACC ATG CAA CTA AGA CGA TGA	Smith *et al.* 1993
	AstroCOI2r*	reverse	TCT GAG TAT CGT CGT GGC ATT CC	this study
	COIe*	reverse	CCA GAG AAG AGG GGA AAC CAG TG	Palumbi 1996
	AstroCOImr*	reverse	AAG TGT GCT ACA ACA TAG TA	this study
	ECOIb*	reverse	GGT AGT CTG AGT ATC GTC G(AT)G	Knott *et al.* 2000

* = primers used for cycle sequencing reaction

In summary, our phylogenetic analyses resulted in a clustering of species from three geographic regions: 1. Indo-Pacific; 2. Neotropics; and 3. Mediterranean and East Atlantic. The total evidence molecular phylogenies resulting from maximum parsimony analysis (MP) and Bayesian inference (BI) were mostly in agreement and did not support any close relationship among species of these three different regions. The Mediterranean and East Atlantic species *A. aranciacus*, however, did not confidently cluster together with other species of the Atlanto-Mediterranean region or with species of any other region. Furthermore, several species were not monophyletic according to molecular data, including the Mediterranean and East Atlantic species *A. irregularis*.

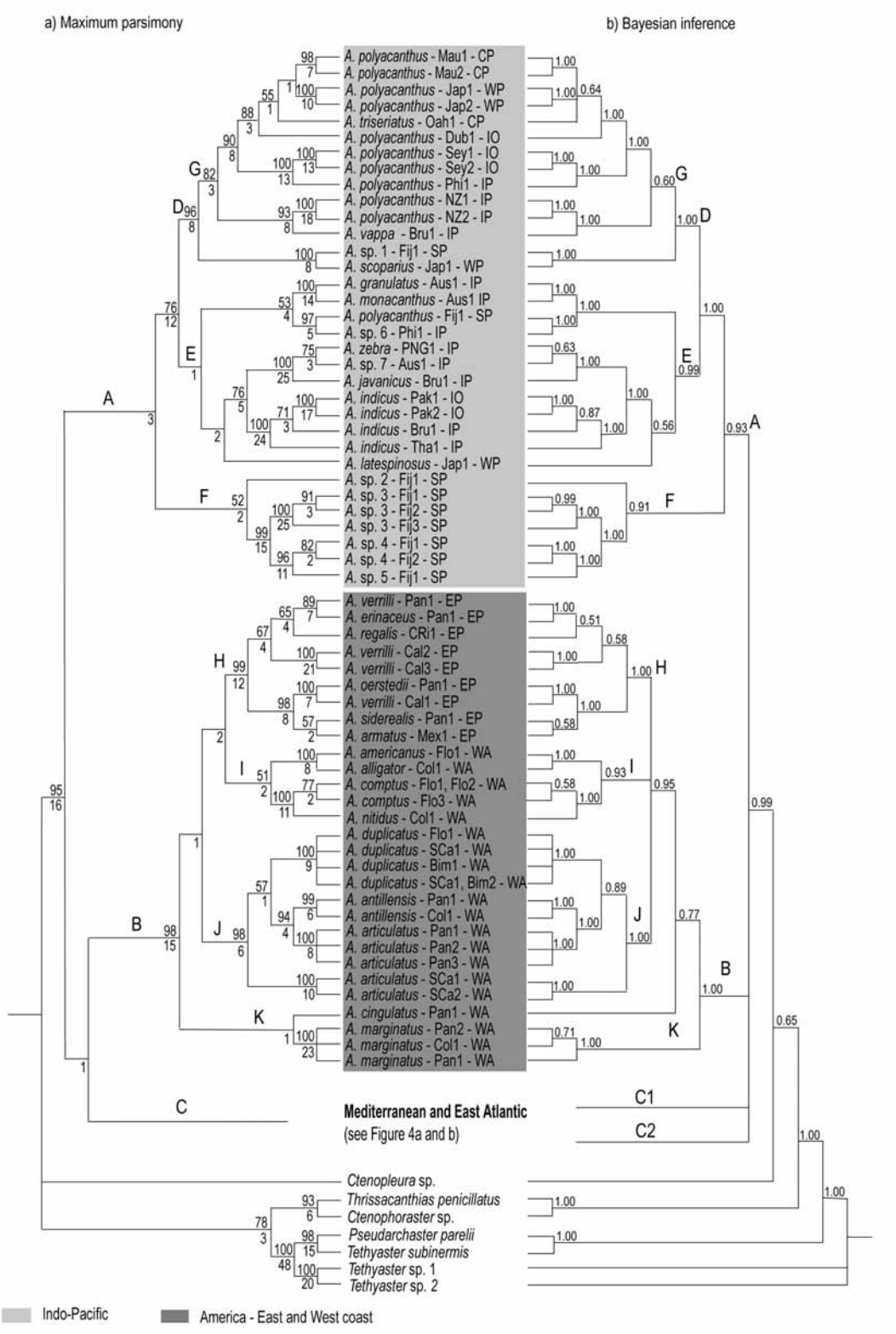

FIGURE 3. Total evidence molecular phylogeny of 117 specimens of *Astropecten* and seven outgroup taxa based on combined 12S, 16S and COI sequences of the mitochondrial DNA. (a) Maximum parsimony strict consensus tree of 2600 most parsimonious trees (*length* = 3447). Above nodes: bootstrap support values above 50% from 1,000 replicates. Below nodes: decay indices. (b) Bayesian inference tree resulting from 9,500 trees based on the GTR+I+Γ model. Posterior probabilities are indicated above nodes. See Table 1 for collection locality of specimens. EP = East Pacific, WA = West Atlantic; CP = Central Pacific; WP = West Pacific; IP = Indo-Pacific; IO = Indian Ocean; EA = East Atlantic, EM = East Mediterranean, WM = West Mediterranean

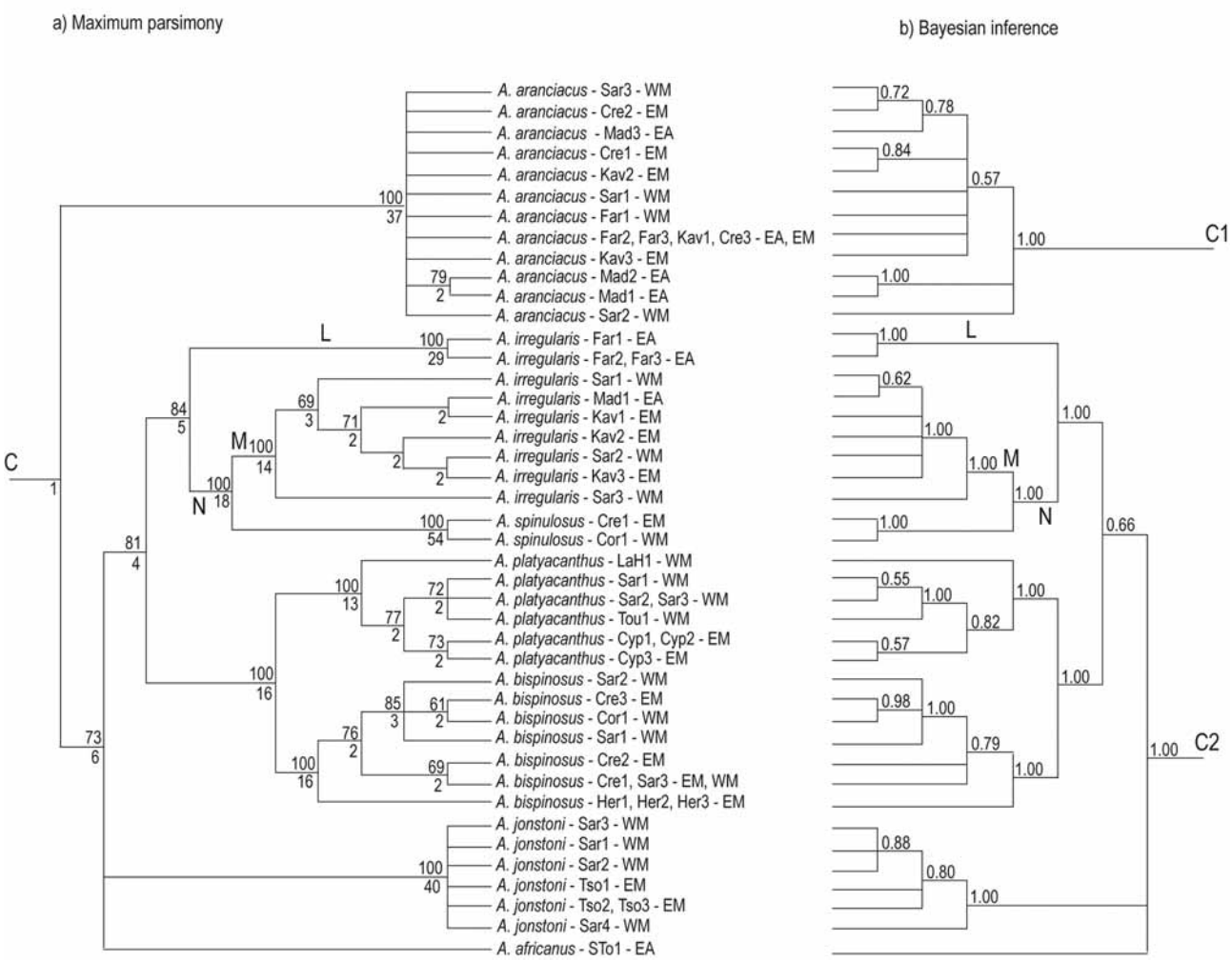

FIGURE 4. Mediterranean and East Atlantic (see caption of Figure 3 for further explanations)

Discussion

Molecular phylogeny vs. the phylogeny of Döderlein, 1917

Both the MP and BI methods of phylogenetic reconstruction produced tree topologies that displayed a phylogenetic gap between *Astropecten* species of three large geographic regions: 1. Indo-Pacific; 2. Neotropics; and 3. East Atlantic and Mediterranean. On the basis of morphological data, Döderlein (1917) also suggested a phylogenetic gap between species that inhabit these three regions. However, Döderlein further assumed that some extant species can be seen as connected between these regions, as for example *A. marginatus* from the Atlantic and *A. regalis* from the Pacific coast of America. Döderlein proposed that these two species are sisters and are closely related to *A. latespinosus* from Japan, thus acting as a "phylogenetic connection" between these regions. The molecular phylogeny does not support this hypothesis as each of these three species clearly groups with other species of the respective geographic region. Thus, molecular data indicate that the similarities of these species in morphology and habitat preference are likely to have evolved independently.

While the general grouping of the three large geographic regions as Döderlein suggested is in agreement with molecular data, there are several differences on a more local scale:

Indo-Pacific region:

Within the Indo-Pacific clade the only agreement of the molecular phylogeny with Döderlein's grouping is that most *A. polyacanthus* specimens cluster into the same clade, and that *A. monacanthus* and *A. granulatus*

are closely related. However, *A. indicus* is not in the same group with the latter two species as Döderlein had suggested (see Figure 1). Moreover, although *A. vappa* and *A. triseriatus* both clustered within the *Polyacanthus* clade, they are phylogenetically not as closely related as Döderlein proposed. Also, according to Döderlein, *A. javanicus* Lütken would be in the same group as *A. polyacanthus*, but molecular data placed this species with *A. zebra* Sladen, which Döderlein considered as belonging to the *Velitaris* group.

American region:

MtDNA shows evidence of a phylogenetic separation between East Pacific and West Atlantic species. This generally agrees with Döderlein's assumptions, except that molecular data do not show evidence of a close relationship between the West Atlantic *A. marginatus* and the East Pacific *A. regalis* as Döderlein had proposed. Among the West Atlantic species there are similarities between Döderlein's *Articulatus* group and the clade comprised of *A. antillensis*, *A. articulatus* and *A. duplicatus* in the molecular phylogeny. However, Döderlein's inclusion of *A. cingulatus* in this group disagrees with the molecular data, which either grouped *A. cingulatus* with *A. marginatus* or placed it in a separate clade. Similarly, Döderlein assigned *A. americanus* to the *Articulatus* group, but molecular data placed this species in a different clade together with *A. alligator*, *A. nitidus* and *A. comptus*, although with low support. Döderlein did not include the latter three species in his monograph.

Döderlein also suggested that Hawaii is a stepping stone for species spreading from East to West, which he believed to be the general direction of species colonization around the globe. The molecular data show no evidence of this connection, as all Hawaiian specimens clearly group within the Indo-Pacific clade. Similar results have been found in other marine taxa, such as sea urchins and fish (Colborn *et al.* 2001; Lessios *et al.* 2001). It is more probable that the *Astropecten* phylogeny was mainly influenced by geological events, such as the closure of the Tethys, the appearance of the Benguela upwelling, and the rise of the Isthmus of Panama.

Mediterranean and East Atlantic:

The molecular and Döderlein's approaches agree that *A. bispinosus* and *A. platyacanthus* are sister species and placed *A. irregularis* in a separate clade. On the other hand, the molecular phylogeny includes *A. spinulosus* in the *Irregularis* clade, whereas Döderlein considered *A. spinulosus* to be more closely related to *A. bispinosus* and *A. platyacanthus* within the *Aranciacus* group. The molecular data support Döderlein's assumption that *A. jonstoni* forms a group separate from the other Mediterranean species. The main difference to Döderlein's phylogeny is that mtDNA sequence data placed *A. aranciacus* at the base or even outside of the Mediterranean and East Atlantic group and not in the same group as *A. bispinosus* and *A. platyacanthus*. According to the molecular phylogeny, *A. aranciacus* was the first species to split off from the other Mediterranean species and would deserve a group of its own in Döderlein's system. Furthermore, mtDNA did not place *A. africanus* within the *Irregularis* group as Döderlein proposed but rather suggests that this species diverged earlier from the other species of the *Irregularis* group. Based on molecular data it is therefore not justified to consider *A. africanus* a subspecies of *A. irregularis*.

Contrary to Döderlein's view, the molecular phylogeny neither supports a phylogenetic relationship between the *Irregularis* group (clades L and M) and the *Braziliensis* group (clade H) nor suggests any relationship between the *Braziliensis* group and the *Aranciacus* group (clade C). Döderlein considered *A. braziliensis* from the Southwest Atlantic as being closely related to East Pacific species in the *Braziliensis* group. We were not able to obtain mtDNA sequence data from this species; its inclusion in the analysis could perhaps reveal a different view on the relationship of these Atlanto-Mediterranean and transisthmian groups.

In summary, although the comparison between the molecular phylogeny and Döderlein's morphological relationships reveals a great deal in common on the large scale relationships of geographic groups, many discrepancies emerge on a local level. Assessing morphological characters and then using a cladistic approach could perhaps result in a more adequate comparison of molecular and morphological phylogenies. However, morphological diversity is very high in *Astropecten*, and characters are often continuous rather than discrete. Also, many characters are only expressed in fully grown adults and not in juvenile specimens. For these reasons, building a matrix of morphological characters is not easy and would probably lead to many

ambiguities. Considering the morphological complexity, we believe that until meaningful characters and character categories have been determined, molecular data are a more reliable approach to resolve phylogenetic relationships in *Astropecten* than morphological characters.

Taxonomic issues

Although it is beyond the scope of this study to revise the genus *Astropecten*, our results suggest several reassessments of the current taxonomy:

Astropecten polyacanthus Müller & Troschel

Given the genetic variation within this clade, it would be more appropriate to speak of a *Polyacanthus* species-complex. While the genetic distances of > 10% substitutions per site, particularly between geographically distant specimens, suggest separate species, the morphology is very similar, and current descriptions of *Astropecten* do not permit delineation of species. By comparison, the sister taxa *A. articulatus* and *A. antillensis*, which are morphologically clearly distinct, show a genetic distance of around 2% substitutions per site for the studied mtDNA regions. In COI, the average intraspecies divergence in echinoderms was estimated to be approximately 0.6% (Ward *et al.* 2008). The specimen from Dubai that is morphologically most similar to *A. polyacanthus* actually lacks the typical character of missing spines on the 2. (−4.) supero-marginal plate. Nevertheless, according to molecular data, it qualifies for inclusion in the *Polyacanthus* species-complex. On the other hand, a specimen from Fiji expressing this morphological character did not group within this complex in the molecular phylogeny. Our results show that current morphological descriptions are not sufficient to distinguish between the species, and that cryptic speciation is most likely present in this group.

Astropecten indicus Döderlein

This species appears monophyletic in the molecular phylogeny; however, specimens from Brunei, Pakistan and Thailand are genetically clearly distinct from each other with distances over 5% substitutions per site. Therefore, our data suggest that specimens of this clade are again part of a species-complex and morphological descriptions need to be refined because cryptic speciation might also have taken place in *A. indicus*.

Astropecten verrilli de Loriol

Although *A. verrilli* is mainly described from the western coast of Central America and *A. californicus* (Fisher 1906) from California, Döderlein (1917) synonymised the two as there are no apparent morphological differences between these two species. Some of our specimens from the Pacific coast of Panama also meet the descriptions of *A. verrilli*, but in the molecular phylogeny they appear in different clades than the Californian specimens. Therefore, our data suggest that *A. verrilli* and *A. californicus* are not synonyms and that *A. californicus* should be used for the specimens that were collected in San Diego, California (Cal2, Cal3). This implies that the specimen from Monterrey (Cal1) belongs to yet another species, but its small size prevented us from using morphological criteria for assigning it to a known species.

In general, there is a great deal of confusion regarding the taxonomy of *Astropecten* from the West American coast (Fisher 1906; Verrill 1914; Döderlein 1917; Ziesenhenne 1939). Several characters that have been used to describe species in this area (such as the presence of supero-marginal spines and number of paxillae per plate) are not always expressed in juveniles. Descriptions are often based on preserved specimens alone—thus not including any indication of color when alive. They are generally not sufficient to identify specimens reliably.

Astropecten articulatus Say

A. articulatus is not monophyletic in the molecular phylogeny. Specimens obtained from the University of South Carolina have only very small spines on the supero-marginal plates and can therefore be assigned to the variation *A. articulatus* var. *valenciennii* Döderlein. As these specimens are not sister to *A. articulatus* from

Panama and the genetic distance between the two is above 3.5 % substitutions per site (uncorr. and corr.), it would be appropriate to consider them as a separate species rather than a variation.

Astropecten bispinosus Otto and *Astropecten platyacanthus* Philippi

The molecular phylogeny and the genetic distances between specimens clearly indicate that *A. bispinosus* and *A. platyacanthus* are not just variations as Ludwig (1897) and Koehler (1924) thought but should be considered as separate species. Although these two species occur sympatrically, they are morphologically clearly distinguishable. However, some intermediate forms have been found (G. Ribi *et al.* unpublished), suggesting that occasional hybridization between the two sister species is a possibility.

Astropecten irregularis Pennant and *Astropecten pentacanthus* Delle Chiaje

Among *A. irregularis* specimens genetic variability is high. Our data suggest that at least three species can be distinguished. Specimens from Portugal (Far1, 2 and 3), collected at a much greater depth (100−540 m), are genetically clearly distinct from the other *A. irregularis*. Although the Portuguese specimens meet the description of *A. irregularis pentacanthus* Delle Chiaje, this description may apply to more than one species. *A. irregularis* has been recorded from depths of over 900 m, but it is not clear whether specimens from the deep belong to the same species as the ones collected in Portugal. Genetic data of *Astropecten* from deeper waters of the Mediterranean and Northeast Atlantic could provide valuable information on diversity within *Astropecten*. *A. ibericus* is another species recorded from the Iberian Peninsula from depths reaching 130 m, and is similar in morphology to the specimens from Portugal. However, as only small specimens of *A. ibericus* have been collected so far, Clark and Downey (1992) considered this species doubtful. Genetic data of *A. ibericus* could clarify the validity of this species and would resolve the relation to the Portuguese specimens used in this study. Within non-Portuguese *A. irregularis*, there is evidence for two species, as the genetic distances between one specimen from Sardinia (Sar3) and the other non-Portuguese specimens are above 2% substitutions per site. In *A. irregularis*, several subspecies have been described (Döderlein 1917; Koehler 1924; Tortonese 1965; Clark and Downey 1992): the typical Mediterranean form *A. irregularis pentacanthus* Delle Chiaje, the South African form *A. irregularis pontoporaeus* Sladen and the East Atlantic form *A. irregularis irregularis* Pennant, occurring from Norway to Cape Verde. Some authors consider *A. irregularis irregularis* and *A. irregularis pentacanthus* as plain variations of *A. irregularis* (Döderlein 1917; Koehler 1924; Clark and Downey 1992). Whether the specimen from Sardinia (Sar3) can be considered as one of these variants needs to be more thoroughly investigated. Furthermore, the specimen from Madeira (Mad1) indicates that *A. irregularis pentacanthus* is not endemic to the Mediterranean as Clark and Downey (1992) believed but also occurs in the Atlantic as suggested by Ludwig (1897) and Döderlein (1917).

Conclusions

Phylogenetic inferences based on mtDNA indicate that morphological convergence has taken place in *Astropecten* resulting in allopatric non-sister taxa with similar morphologies and habitat preferences. Although morphology suggests several close relationships between species in geographically distant areas, molecular data showed evidence of a clear phylogenetic separation of these regions. The comparison to Döderlein's morphological phylogeny reveals many discrepancies, particularly on a local scale, indicating that informative morphological characters are not easily identified and categorized in *Astropecten*.

Döderlein assumed, based on morphological characters, that *A. marginatus* and *A. regalis* on either side of the Isthmus of Panama were closely related. The molecular phylogeny presents a different reconstruction of evolutionary history. *A. marginalis* is, in fact, distantly related to *A. regalis*. It is possible that the rise of the Isthmus of Panama separated populations of the common ancestor of clades I (West Atlantic) and H (East Pacific), which then split into *A. duplicatus, A. antillensis,* and *A. articulatus* in the Atlantic and into *A. verilli, A. erinaceus* Gray, *A. regalis, A. oerstedi* Lütken, *A. siderealis,* and *A. armatus* in the eastern Pacific. For the entire concatenated sequence of 12S, 16S and COI, the uncorrected divergence between clades I and H in

Astropecten is 6.1% and the corrected divergence is 7.5%. For separate genes, the uncorrected genetic distance was 4.8% in 12S, 5.2% in 16S and 12.3% in COI. Other echinoderms likely to have been split by the completion of the Isthmus approximately 3 mya show divergences of 5.6−6.1% in 12S, 6.5−12.8% in 16S, and 8.7−13.5% in COI (Lessios 2008). It is, therefore, quite likely that clades I and H are geminate, having been split not much more than 3 mya. If so, the generation of so many species on either side of the Isthmus within this relatively short period of time indicates a remarkable rate of speciation, unparalleled by any other echinoderm with the same presumed phylogenetic history relative to the Isthmus. Among other echinoderms with known transisthmian clades, only the echinoid *Lytechinus* comes close, with three post-isthmian species in the eastern Pacific and three (or possibly four) in the Atlantic (Zigler and Lessios 2004).

The molecular data indicated that it is appropriate to consider several widely distributed taxa, such as *A. polyacanthus* and *A. indicus*, as species complexes. Many formerly presumed within species morphological variants exhibit genetic distances large enough to raise them to the species level, as for instance in *A. articulatus* and *A. irregularis*. In several other cases, such as *A. polyacanthus* and *A. indicus*, the possibility of cryptic speciation within each of these species can not be ruled out and requires additional investigation.

While genetic data support the view that a few described species should be synonymized, many new species still remain to be described, such as several deep-sea populations of the South Pacific. It is therefore possible that even though the genus *Astropecten* is known for its species-richness, the diversity in this genus might still be underestimated. In *A. indicus* and *A. polyacanthus* sibling species occur allopatrically and most likely have speciated by vicariance. Based on current distributions of sister species though, sympatric speciation in *Astropecten* cannot be ruled out. The possibility of sympatric speciation has also been considered for sea urchins, such as *Diadema* Gray (Lessios *et al.* 2001) and *Lytechinus* Agassiz (Zigler and Lessios 2004), and although it has been rejected in *Diadema,* it is not clear whether all *Lytechinus* species are the result of allopatric speciation. In the Indo-Pacific region, molecular data reveal a few close relationships between supposedly conspecific populations of *Astropecten* from geographically distant locations, such as between *A. polyacanthus* specimens from Hawaii and from Dubai. Waters *et al.* (2004) suggested that large distance genetic connections throughout the Indo-Pacific even occur in some asterinids lacking planktonic larval stages. To what extent ocean currents or other mechanisms have lead to this pattern in South Pacific *Astropecten* remains to be determined.

Many difficulties were encountered in this study while attempting to identify species morphologically based on species descriptions, and it became evident that a taxonomic revision of this genus is needed. The present study has shown that molecular markers provide a valuable complement to our predecessors' morphological work and help to clarify unresolved issues of evolutionary history and systematics.

Acknowledgements

For providing samples and hosting museum visits we thank the following people and institutions: The late C. Ahearn and the Smithsonian National Museum of Natural History, N. Améziane and the Muséum National d'Histoire Naturelle in Paris, the Zoological Museum Amsterdam, G. Borrero and INVEMAR in Colombia, M. Brogger, A. Cabrinovich and the London Natural History Museum, T. Cerveira Borges and the BIOPESCAS team (CCMAR, University of Algarve), the California Academy of Science Collections, R. Collin, J. Collins, T. Danckwart, S. Diliberto, A. Gebruk and the Shirshov Institute, L. Granek, S. Godwin and the Bishop Museum, G. Hendler and the Natural History Museum of Los Angeles County, J. Hoover, M. Huber, M. Komatsu, D. Lane, L. Lovell and the Scripps Institution of Oceanography, C. Lüter and the Zoological Museum Berlin, H. Maag, M. Matsubara, P. Morais, C. Nielsen and the Zoological Museum of the University of Copenhagen, G. Pauley and the Florida Museum of Natural History, the Phuket Marine Biological Center, C. Ruckstuhl, H. Sekiguchi, M. Sewell, F. Solis-Marin and the Colleccion National de Equinodermos de la Universidad Nacional Autonoma de Mexico, the collections of the University of South Carolina, Q. Tahera and the Marine reference collection & Resource centre in Karachi, S. Tanner, S. Tassis, M. von Arx, K. Wakabayashi and P. Wirtz. We thank T. Bucher, A. and L. Calderon, S. Sbilordo and S. Tanner

for technical and lab assistance. We received valuable advice on data analysis from M. Bernasconi, A. Faucci, A. Hiller-Galvis, R. Sponer and T. Wilson, for which we are very grateful. Further, we would like to thank D. Blake, A. Gale and C. Mah for their help with species identification and general advice and two anonymous reviewers for their useful comments. We are also greatly indebted to the late G. Ribi who helped outlining the project and provided assistance in many ways. This project was partially funded by the Smithsonian Tropical Research Institute and the Swiss National Science Foundation.

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