Org Divers Evol (2010) 10:351–371
DOI 10.1007/s13127-010-0033-z

ORIGINAL ARTICLE

ORGANISMS DIVERSITY & EVOLUTION

Establishing species and species boundaries in *Sabellastarte* Krøyer, 1856 (Annelida: Sabellidae): an integrative approach

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Received: 29 March 2010 / Accepted: 20 September 2010 / Published online: 6 October 2010

Abstract *Sabellastarte* Krøyer, 1856 (Sabellidae), a morphologically homogeneous group distributed in warm and temperate coasts of the Indo-Pacific and Caribbean Sea, is characterized by the presence of a unique combination of features. To date, the genus comprises eight species, but morphological characters traditionally used in diagnostics have shown intra-specific variability, making species boundaries and distributions unclear. The present study constitutes the first attempt to test the monophyly of *Sabellastarte* and its relationships to other sabellid genera by combining molecular (COI and 16S) and morphological data. Results include placement of a clade containing *Stylomma, Sabella, Branchiomma* and *Bispira* as the sister group to *Sabellastarte*. Phylogenetic analyses and genetic divergence among specimens from several localities around the world indicate the presence of at least six lineages within *Sabellastarte*. In the context of a discussion of species boundaries and diagnostic features, the distribution of some of those lineages can be explained by the presence of cryptic species and potential introductions.

Keywords *Sabellastarte* · Sabellidae · Annelida · Integrative taxonomy · Morphology · Mitochondrial DNA

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Introduction

The genus *Sabellastarte* Krøyer, 1856 is a morphologically homogeneous group of fan worms (Sabellidae) distributed in warm and temperate coasts of the Indo-Pacific and Caribbean Sea. These worms are well known among divers and aquarists due to their attractive and colorful branchial crown, which can measure up to 10 cm in diameter (Fig. 1e–g). They are economically important as ornamental species and are heavily collected in some areas such as Oahu, Hawaii (Division of Land and Natural Resources 2008; Walsh et al. 2003). Most individuals settle deep in the cracks and crevices of corals, which making successful collecting difficult and often destructive to corals, as it causes collateral damage to much of the reef community (pers. obs. M.C. and D.R.B.).

In a recent review of the genus, the number of species considered as valid was reduced to eight (Knight-Jones and Mackie 2003). Additional species names previously regarded as belonging to *Sabellastarte* have been synonymised with others in the genus or transferred to *Pseudobranchiomma* Jones, 1962, *Branchiomma* Kölliker, 1858 or *Perkinsiana* Knight-Jones, 1983 (Knight-Jones 1983, 1994; Knight-Jones and Mackie 2003). *Sabellastarte* is characterized by a combination of features such as presence of dorsal flanges on the base of the crown (Fig. 1g), absence of radiolar flanges or radiolar eyes (Fig. 1g), collar fused to the faecal

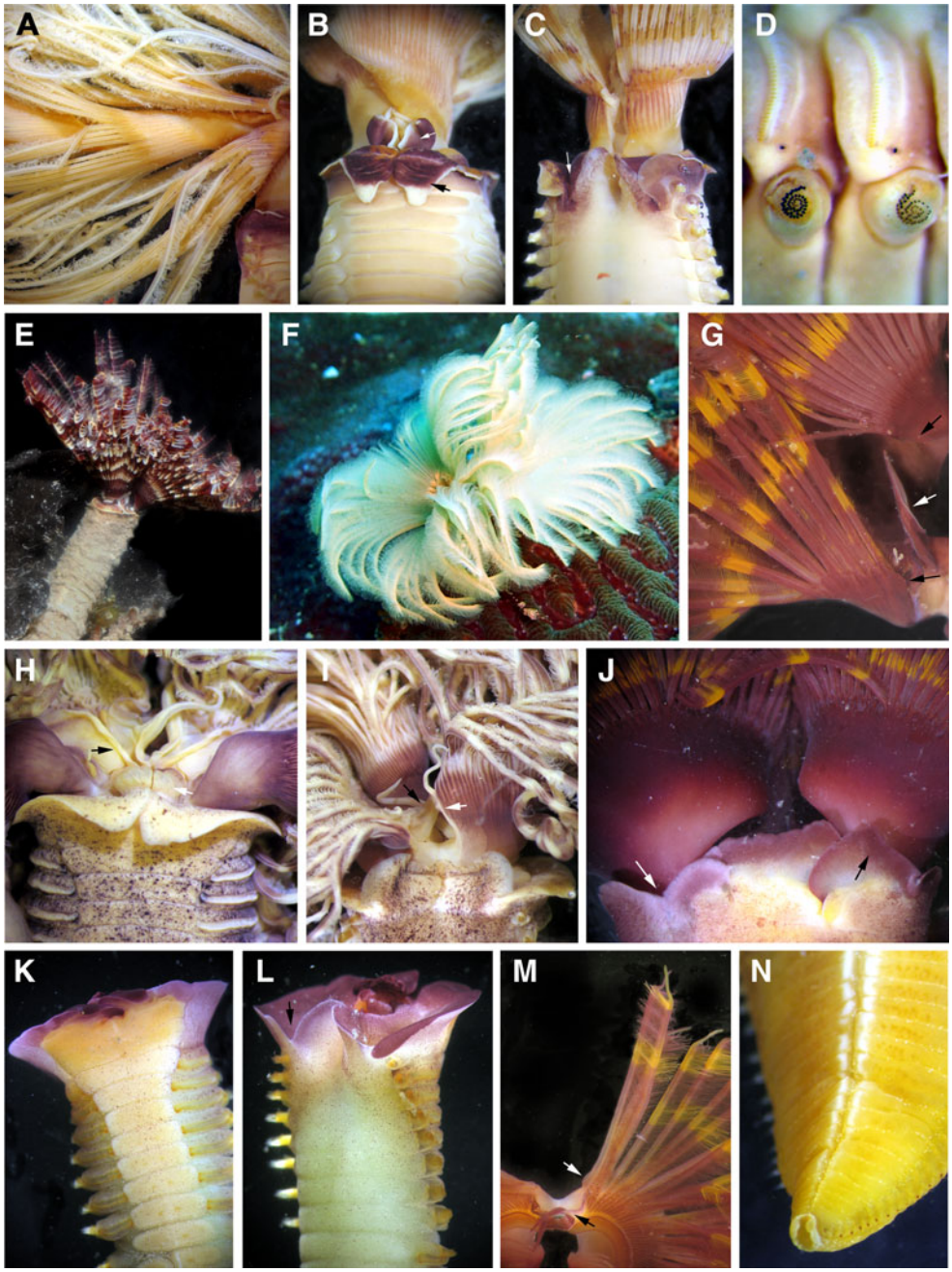


Fig. 1 Photographs of live or preserved specimens. **a**–**d** *Sabella spallanzanii*. **a** Spiral, left branchial lobe. **b** Anterior thoracic segments, ventral view; white arrow: ventral sacs, black arrow: collar ventral lappets. **c** Anterior thoracic segments, dorsal view; white arrow: collar dorsal notches and low pockets. **d** Mid-abdominal chaetigers with tips of chaetae removed, showing spiral arrangement. **e**–**n** *Sabellastarte* spp. **e** Live specimens from Ningaloo Reef, Western Australia. **f** Live specimen from Coffs Harbour, northern New South Wales, Australia. **g**–**n**. Specimens from Port Phillip Bay, Victoria, Australia. **g** Crown of a live specimen, ventral view; black arrows: ventral basal flanges, white arrow, dorsal lips and radiolar appendages. **h** Preserved specimen, anterior thoracic segments, ventral view; black arrow: ventral lips, white arrow: ventral sacs. **i** Specimen as in **h**, dorsal view; black arrow: dorsal lips, white arrow: dorsal basal flanges. **j** Large live specimen, anterior thoracic segments, ventral view; black arrow: collar ventral lappets, white arrow: collar latero-ventral incisions. **k** Juvenile live specimen, anterior thoracic segments, ventral view. **l** Specimen as in **k**, dorsal view; black arrow: collar dorsal notches and pockets. **m** Specimen as in **k**, detail of branchial crown, top view, radioles regenerating; white arrow: dorsal basal flanges, black arrow: dorsal lips and radiolar appendages. **n** Specimen as in **k**, posterior abdominal segments and pygidium

groove forming flanking pockets on both sides (Fig. 1i, l), and presence of parallel lamellae and ventral sacs within the radiolar crown (Fig. 1h, j) (Knight-Jones and Mackie 2003). The monophyly of the group, however, has not been tested in a modern phylogenetic framework, nor have species relationships been clarified. Within *Sabellastarte*, some of the traits traditionally used to distinguish species may change during development and growth or due to regeneration processes (Knight-Jones and Mackie 2003). Because of this, they are mostly likely inappropriate as diagnostic, phylogenetic or systematic characters.

Sabellastarte is currently considered as a member of a group of derived Sabellidae that includes *Bispira* Krøyer, 1856, *Branchiomma, Pseudobranchiomma, Sabella* Linnaeus, 1767, and *Stylomma* Knight-Jones, 1997. This group is characterized by synapomorphies such as the presence of a skeleton of cells supporting the dorsal radiolar appendages and being surrounded by sheath tissue, the presence of inter-ramal eyespots, spinelike chaetae, conical abdominal neuropodia, and abdominal fascicles arranged in a spiral or C-shape (Capa 2008); Fitzhugh 1989, 2003; Fitzhugh and Rouse 1999; Knight-Jones and Mackie 2003). However, there is no consensus concerning the relationships within the group. Several authors have focused on the absence of companion chaetae and hypothesized that *Sabellastarte* is the sister group of *Branchiomma* and *Pseudobranchiomma* (Fitzhugh 2003; Fitzhugh and Rouse 1999; Knight-Jones and Perkins 1998), whereas others have placed *Sabella* as sister to *Sabellastarte* based on the shared presence of dorsolateral notches in the collar and the absence of reproduction by fission (Capa 2008). Recent attempts to assess relationships within Sabellidae incorporated molecular data, but the results were not strongly supported or congruent between studies. For example, some authors proposed *Sabella* as the sister taxon to *Sabellastarte* (Patti et al. 2003, incorporating COI sequence data), whereas others advocated *Branchiomma* in place of *Sabella* (Capa et al. in press, including morphology and data on several DNA fragments; Kupriyanova and Rouse 2008, using various DNA fragments).

The aim of the present study is to apply an integrative approach combining molecular and morphological data to: 1) test the monophyly of *Sabellastarte* and identify its sister taxon, 2) determine the lineages or species in a broad sampling covering a large part of the geographical distribution of *Sabellastarte*, and 3) discuss the usefulness of traditional diagnostic features and propose alternatives. To these ends, phylogenetic analyses were performed using morphological (32 characters) and mitochondrial DNA sequence data (~1.1 kb: 16S rDNA and cytochrome oxidase subunit I) from specimens collected throughout the Indo-Pacific region and the Caribbean.

Material and methods

Material examined

The ingroup contains specimens of *Sabellastarte*, *Branchiomma, Bispira, Pseudobranchiomma, Sabella* and *Stylomma*. These genera form a monophyletic clade based on morphological, molecular or combined data (Fitzhugh 1989; Patti et al. 2003; Capa et al. in press). Throughout this paper, most of the analysed *Sabellastarte* specimens are referred to by collection locality, because species identifications following the existing diagnoses were called into question. The only exceptions from this practice apply to analyses of morphological data in which type specimens were included and scoring was based on the literature (Knight-Jones and Mackie 2003). All analyses were rooted using *Pseudopotamilla* Bush, 1905 as an outgroup.

Tissue samples were obtained by field collecting and loans from various sources (see Table 1, Appendix 1, and Acknowledgements). All specimens were preserved in 95% ethanol. All sequences used in the present study are new, with the exception of *Sabella pavonina* Savigny, 1820, acquired from GenBank. The morphological data matrix consists of 32 characters (Table 2, Appendix 2). To supplement the morphological matrix and adjust characters and states, where necessary, both fresh and preserved material from the following institutions were examined: Australian Museum, Sydney (AM); Brigham Young University-Hawaii Museum of Natural History, Laie (BYUHMNH); Museum and Art Galleries of the Northern Territory, Darwin (MAGNT); Natural History Museum, University of Wroclaw, Wroclaw (MPW); Museum of Victoria, Melbourne (MV).

Morphological data

Characters used in previous cladistic analyses of sabellids (Capa 2008; Fitzhugh 1989; 2003; Fitzhugh and Rouse 1999) were included here, omitting those that show no variation in the taxa selected for this study. Other characters traditionally used to distinguish the six ingroup genera (Knight-Jones 1994; Knight-Jones and Giangrande 2003; Knight-Jones and Perkins 1998; Nogueira and Knight-Jones 2002; Tovar-Hernández and Knight-Jones 2006) or the *Sabellastarte* species (Knight-Jones and Mackie 2003) were also scored. Additional characters of potential use to diagnosing *Sabellastarte* species were also included to test for homology and synapomorphic status (see Appendix 2).

For examinations of chaetae, parapodia were removed with forceps from the first and second-to-last thoracic chaetigers and from a mid-abdominal chaetiger, and slide mounted. The internal structure of the radioles and lips was studied by slide-mounting semi-thin transverse sections of

Table 1 Taxa used for molecular phylogenetic analyses, collection data, voucher and accession numbers

Taxon	Voucher	Collection site	GenBank accession numbers	
			COI	16s
Pseudopotamilla sp. 1	AM W.30009	Darwin, NT, Australia	–	HQ015106
Pseudopotamilla sp. 2	AM W.30496	QLD, Australia	–	HQ015107
Bispira manicata	AM W.30006	Heron Island, QLD, Australia	HQ015124	–
Bispira porifera	AM W.30007	Darwin, NT, Australia	HQ015123	–
Branchiomma sp. 1	AM W.29050	Foster, NSW, Australia	HQ015125	–
Branchiomma sp. 2	BYUHMNH E.001	Kaneohe Bay, Oahu, Hawaii	HQ015126	HQ015092
Sabella pavonina	–	–	–	AY340482
Sabella spallanzanii	AM W.30505	Adelaide, SA, Australia	–	HQ015113
Stylomma palmatum 1	AM W.30008	Darwin, NT, Australia	HQ015146	HQ015114
Stylomma palmatum 2	AM W.31817	Goodes Island, QLD, Australia	HQ015147	HQ015115
Sabellastarte Hawaii1	BYUHMNH E.002	Kaneohe Bay, Oahu, Hawaii	HQ015127	HQ015093
Sabellastarte Hawaii2	AM W.31843	Kaneohe Bay, Oahu, Hawaii	HQ015128	HQ015094
Sabellastarte Japan1	BYUHMNH E.006	Sagami Bay, Japan	HQ015132	HQ015098
Sabellastarte Japan2	AM W.31845	Sagami Bay, Japan	HQ015133	HQ015099
Sabellastarte Japan3	BYUHMNH E.003	Sagami Bay, Japan	HQ015134	HQ015100
Sabellastarte Japan4	AM W.32020	Sagami Bay, Japan	HQ015135	–
Sabellastarte Japan6	BYUHMNH E.005	Sagami Bay, Japan	HQ015136	–
Sabellastarte Malaysia1.	AM W.30001	Kota Kinabalu, Malaysia	HQ015129	HQ015095
Sabellastarte Malaysia2	AM W.30384	Kota Kinabalu, Malaysia	HQ015130	HQ015096
Sabellastarte Malaysia3	AM W.30386	Kota Kinabalu, Malaysia	HQ015131	HQ015097
Sabellastarte Mexico1	BYUHMNH E.007	Veracruz, Mexico	HQ015140	–
Sabellastarte NSW1	AM W.29659	Port Jackson, NSW, Australia	–	HQ015102
Sabellastarte NSW2	AM W.30383	Port Stephens NSW, Australia	–	HQ015103
Sabellastarte NSW4	AM W.29657	Solitary Island, NSW, Australia	–	HQ015104
Sabellastarte NT1	AM W.29656	Darwin, NT, Australia	HQ015138	HQ015101
Sabellastarte NT2	AM W.31844	Darwin, NT, Australia	HQ015139	–
Sabellastarte Pakistan1	AM W.31823	Karachi, Pakistan	HQ015141	–
Sabellastarte Pakistan2	AM W.31824	Karachi, Pakistan	HQ015142	HQ015105
Sabellastarte Pakistan3	AM W.31825	Karachi, Pakistan	–	HQ015106
Sabellastarte Saipan1	AM W.31847	Saipan, Northern Mariana Islands	HQ015143	HQ015109
Sabellastarte Saipan2	BYUHMNH E.008	Saipan, Northern Mariana Islands	HQ015144	HQ015110
Sabellastarte Samoa1	AM W.32478	Samoa, Samoa	–	HQ015111
Sabellastarte Samoa2	AM W.32479	Samoa, Samoa	HQ015145	HQ015112
Sabellastarte VIC1	MV F.108866	Melbourne, VIC, Australia	–	HQ015116
Sabellastarte VIC2	MV F.108864	Melbourne, VIC, Australia	HQ015148	–
Sabellastarte VIC4	MV F.108865	Melbourne, VIC, Australia	HQ015149	HQ015117
Sabellastarte WA1	AM W.31826	Kimberleys, WA, Australia	HQ015150	HQ015118
Sabellastarte WA2	AM W.31827	Kimberleys, WA, Australia	HQ015151	HQ015119
Sabellastarte WA3	AM W.31828	Kimberleys, WA, Australia	HQ015152	HQ015120
Sabellastarte WA4	AM W.31829	Kimberleys, WA, Australia	HQ015153	HQ015121
Sabellastarte WA5	AM W.31830	Kimberleys, WA, Australia	HQ015154	HQ015122

AM Australian Museum; *BYUHMNH* Brigham Young University-Hawaii Museum of Natural History; *MV* Museum Victoria. Geographic acronyms: *NSW* New South Wales; *NT* Northern Territory; *SA* South Australia; *VIC* Victoria; *WA* Western Australia

Table 2 Matrix of morphological character states ('–', inapplicable; '?', uncertain/unknown); for descriptions, see Appendix 2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Pseudopotamilla sp. 1	0	0	1	3	0	2	1	1	0	1	0	0	1	1	1	0	0	0	0	2	1	1	2	0	0	0	0	0	0	0	0	0
Pseudopotamilla sp. 2	0	0	1	3	0	2	1	1	0	1	0	0	1	1	1	0	0	0	0	2	1	1	2	0	0	0	0	0	0	0	0	0
Stylomma palmatum 1	0	0	1	1	0	1	0	1	1	0	0	1	0	0	0	0	1	0	2	0	1	1	0	1	1	1	0	0	1	0	0	1
Stylomma palmatum 2	0	0	1	1	0	1	0	1	1	0	0	1	0	0	0	0	1	0	2	0	1	1	0	1	1	1	0	0	1	0	0	1
Bispira porifera	0	0	0	–	0	2	1	0	1	1	1	1	0	0	1	0	1	1	1	2	0	0	1	1	1	1	0	0	1	0	1	1
Bispira manicata	1	0	1	2	0	0	0	0	1	0	1	1	0	0	1	0	1	0	1	2	0	1	1	1	1	1	0	0	1	0	1	1
Branchiomma sp. 1	0	0	1	2	1	0	0	0	1	1	1	1	0	0	0	0	1	0	2	0	0	0	0	0	–	1	0	0	1	0	1	0
Branchiomma sp. 2	0	0	1	2	1	0	0	0	1	1	1	1	0	0	0	0	1	0	2	0	0	0	0	0	–	1	0	0	1	0	1	0
Sabella spallanzanii	2	0	0	–	0	0	0	0	1	0	1	0	1	0	0	1	1	2	2	1	1	1	1	1	1	1	?	?	1	0	1	1
Sabella pavonina	2	0	0	–	0	0	0	0	1	0	1	0	1	0	0	1	1	2	2	1	1	1	1	1	1	1	?	?	1	0	1	1
Sabellastarte spectabilis	0	1	0	–	0	1	1	0	1	0	0	0	1	1	0	2	1	1	0	0	1	1	0	0	–	1	0	0	1	?	?	1
Sabellastarte magnifica	0	1	0	–	0	1	1	0	1	0	0	0	1	1	0	1	1	1	0	0	1	0	0	0	–	1	1	?	1	2	?	1
Sabellastarte pectoralis	0	1	0	–	0	1	1	1	1	?	0	0	1	1	0	2	1	1	2	0	1	0	?	0	–	1	0	1	1	0	?	1
Sabellastarte australiaensis	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	0	0	1	0	1	0	–	1	0	0	1	1	1	1
Sabellastarte japonica	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	0	0	1	1	1	0	–	1	0	?	1	2	?	1
Sabellastarte samoensis	0	0	0	–	0	1	1	0	1	?	0	0	1	1	0	2	1	1	0	0	1	1	0	0	–	1	?	?	1	1	?	1
Sabellastarte sanctijosephi	0	0	0	–	0	1	1	1	1	1	0	0	1	1	0	2	0	1	2	0	1	1	0	0	–	1	0	?	1	1	?	1
Sabellastarte Hawaii1	1	1	0	–	0	?	1	1	1	0	0	0	1	1	0	?	1	1	1	0	1	1	1	0	–	1	?	?	1	?	?	1
Sabellastarte Hawaii2	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	1	1	0	–	1	1	1	1	1	0	1
Sabellastarte Japan1	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	?	1	1	1	0	1	1	1	0	–	1	0	1	1	2	1	1
Sabellastarte Japan2	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	1	0	0	–	1	0	1	1	1	1	1
Sabellastarte Japan3	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	?	1	1	1	0	1	1	1	0	–	1	0	1	1	2	1	1
Sabellastarte Japan4	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	1	1	0	–	1	0	1	1	2	1	1
Sabellastarte Japan6	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	?	1	1	1	0	1	1	1	0	–	1	0	1	1	2	1	1
Sabellastarte Malaysia1	1	1	0	–	0	?	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	0	1	0	–	1	1	1	1	1	1	1
Sabellastarte Malaysia2	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	0	1	0	–	1	1	0	1	1	1	1
Sabellastarte Malaysia3	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	1	1	1	1	?	1
Sabellastarte Mexico1	1	0	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	1	1	0	–	1	1	1	1	1	?	1
Sabellastarte NSW1	1	?	0	–	0	?	1	1	1	0	0	0	1	1	0	2	1	1	1	0	1	1	0	0	–	1	0	0	1	1	?	1
Sabellastarte NSW2	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	0	1	0	–	1	1	0	1	2	1	1
Sabellastarte NSW3	1	1	0	–	0	2	1	1	1	0	0	0	1	1	0	2	1	1	2	0	1	0	1	0	–	1	0	1	1	2	1	1
Sabellastarte NT1	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	1	0	1	1	?	1
Sabellastarte NT2	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	1	0	1	1	?	1
Sabellastarte Pakistan1	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	0	1	0	–	1	0	1	1	1	?	1
Sabellastarte Pakistan2	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	1	0	1	0	1	0	–	1	0	1	1	1	?	1
Sabellastarte Pakistan3	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	2	1	1	1	0	1	0	1	0	–	1	0	1	1	1	?	1
Sabellastarte Saipan1	1	1	0	–	0	2	1	1	1	0	0	0	1	1	1	2	1	1	1	0	1	1	0	0	–	1	1	1	1	1	?	1

Table 2 (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Sabellastarte Saipan2	1	1	0	–	0	?	1	1	1	0	0	0	1	1	1	?	1	1	1	0	1	1	0	0	–	1	?	?	1	?	?	1
Sabellastarte Samoa1	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	1	0	0	–	1	0	0	1	0	?	1
Sabellastarte Samoa2	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	1	0	0	–	1	0	0	1	0	1	1
Sabellastarte VIC1	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	0	1	1	1	0	1
Sabellastarte VIC2	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	0	1	1	1	0	1
Sabellastarte VIC3	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	0	1	1	1	0	1
Sabellastarte VIC4	1	1	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	0	1	1	1	0	1
Sabellastarte WA1	1	0	0	–	0	2	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	1	0	1	1	?	1
Sabellastarte WA2	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	1	0	1	1	?	1
Sabellastarte WA3	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	1	0	1	1	?	1
Sabellastarte WA4	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	1	1	1	2	0	1	0	1	0	–	1	1	0	1	0	?	1
Sabellastarte WA5	1	0	0	–	0	1	1	1	1	0	0	0	1	1	0	0	1	1	2	0	1	0	1	0	–	1	1	0	1	1	?	1

the bases of these structures. Sections were examined unstained, using a compound microscope. Line drawings to scale were made with drawing tubes attached to the stereo and light microscopes, and digitalised in Adobe Illustrator. In the laboratory, photographs were taken with an AM413ZT Dino-Lite polarizing handheld microscope camera.

DNA extraction, amplification and sequencing

Mitochondrial DNA was extracted from specimens using the Qiagen Dneasy protocol for animal tissue (Valencia, CA). Muscle tissue was dissected from the thoracic or abdominal region. DNA vouchers and specimens have been deposited at AM and BYUHMNH. The molecular dataset comprises two gene fragments: 16S ribosomal DNA (16S rDNA; ~470 bp) and the protein-coding cytochrome oxidase subunit I (COI; ~670 bp). Primers for 16S rDNA were 16Sa: 5′-CGC CTG TTT ATC AAA AAC AT-3′; 16Sb: 5′-CTC CGG TTT GAA CTC AGA TCA-3′ (Simon et al. 1994). Primers for COI were: LCO1490: 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′; HCO2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′ (Folmer et al. 1994). All primers were purchased from Integrated DNA Technologies, Inc. The DNA was amplified using a 3-step PCR with 40 cycles at an annealing temperature of 45°C. All PCR products were visualized via agarose gel electrophoresis to assure proper amplification and detect possible contamination using negative controls. Products were purified using Montage PCR Cleanup Kit (Millipore), and cycle-sequenced using BigDye Terminator chemistry (ABI). Sequences were generated using an ABI 3100 capillary sequencer. Complementary strands were sequenced with sufficient fragment overlap to reduce sequencing errors. Sequences were assembled in Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI) and subjected to a BLAST search in GenBank to ensure that no contamination became part of the analysis.

DNA sequence alignment

Sequence data from 16S rDNA was initially aligned in Sequencher 4.8 to ensure that the gene ends were homologues. Once homologous ends had been identified, 16S was trimmed and exported as a single contig for alignment in MUSCLE (Edgar 2004). COI was also aligned in Sequencher 4.8 according to its protein-coding reading frame, and was devoid of gaps. Both alignments were concatenated into a single dataset that included morphological data for further analyses.

Cladistic analysis

Multistate characters of all datasets were treated with equal weights and as unordered. In the morphological dataset,

unknown characters were coded with '?', inapplicable ones with '–', and polymorphic characters with all possible states. The matrix was assembled in Nexus Data Editor, (NDE; Page 1998). Parsimony analyses were conducted in TNT 1.1 (Goloboff et al. 2003, 2008), using the default settings under the new technologies search strategy. Bootstrap (BS; Felsenstein 1985) values were generated via PAUP* 4.0 (Swofford 2002) from 1,000 replicates and using the default settings. For the morphological analyses, jackknife (JK; Farris et al. 1996) values were generated in PAUP* 4.0 (Swofford 2002). Bayesian analyses (BI) were performed via MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). An appropriate substitution model for the molecular data was identified via ModelTest (GTR+I+G; Posada and Crandall 1998). A model for morphological data was identified as in Ronquist et al. (2005). Bayesian analyses involving morphology were conducted as in Bybee et al. (2008), under the Mk model for morphology (Nylander et al. 2004). Bayesian analyses were run for 20 million, 12.8 million, and 12.8 million generations for the morphological, molecular, and combined datasets, respectively. The burn-in for each analysis was identified via Tracer v1.4.1 (http://tree.bio.ed.ac.uk/software/tracer/) and was determined to be approximately the first 10% of trees for each of the tree output files generated from MrBayes. From these files posterior probabilities were visualized via a majority rule consensus in PAUP*. A maximum likelihood (ML) analysis of the molecular data was conducted in RAxML v. 7.0.4 (Stamatakis 2006), with mixed models (GTR+G) representing 16S and COI. Bootstraps were also estimated in RAxML with a quick bootstrap analysis composed of 1,000 replicates (Stamatakis et al. 2008). Matrices and phylogenetic trees and the associated data were archived with TreeBASE (www.treebase.org).

Molecular divergence

Pairwise genetic distances were estimated using Kimura's two-parameter (K2P) method (Nei and Kumar 2000) in MEGA 4.0 (Tamura et al. 2007). All sites were initially included, and the third codon position was deleted for COI. Rates were considered as uniform among sites.

Results

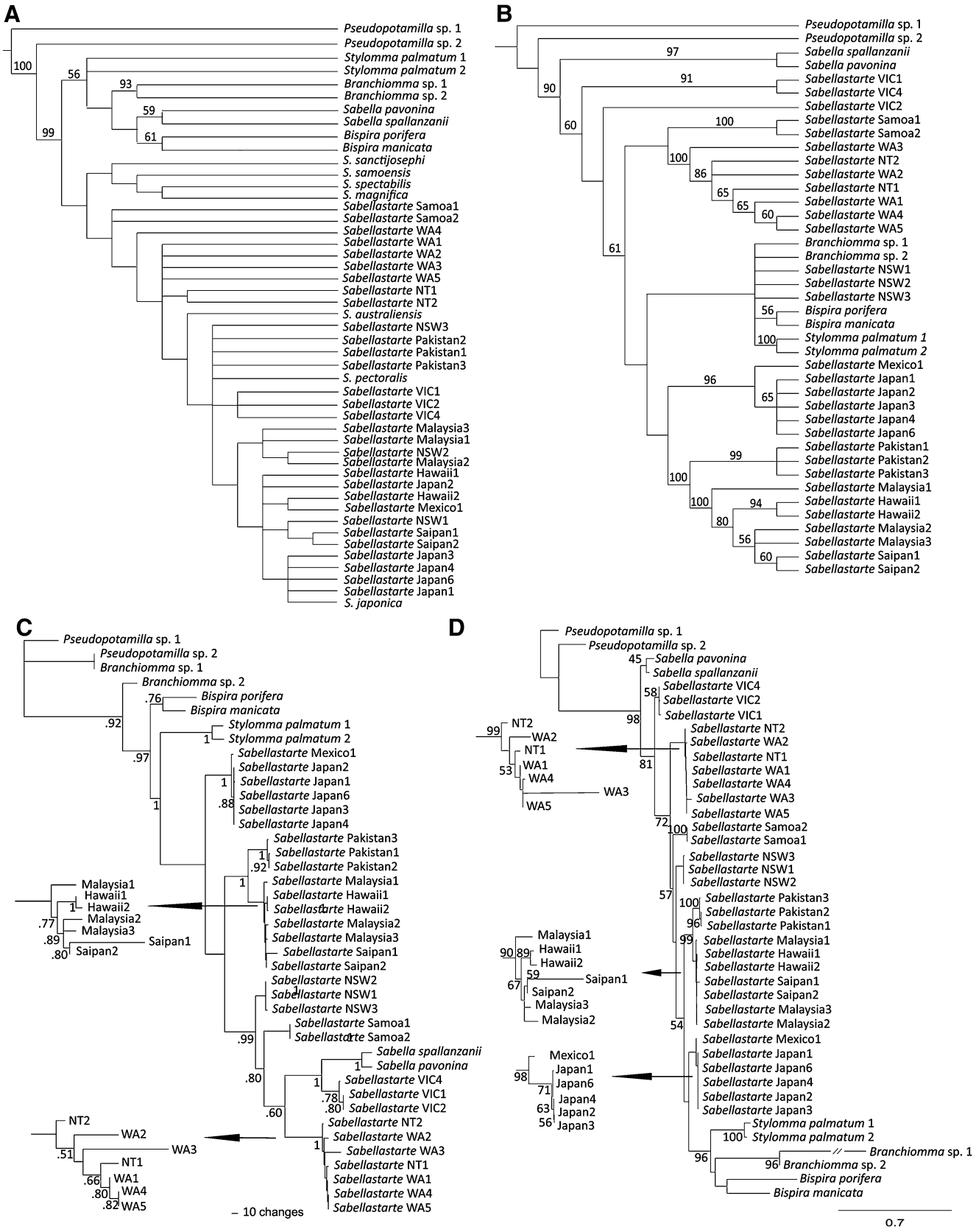
Morphological data

All characters coded in the dataset were parsimony-informative. The cladistic analysis of the morphological data yielded eight most parsimonious trees (MPT) of 102 steps, with a consistency index (CI) of 0.41 and a retention index (RI) of 0.81. These values indicate a high amount of homoplasies in the dataset. According to these hypotheses (Fig. 2a), the genus *Sabellastarte* is monophyletic, though not significantly supported (JK 35), and characterized by four homoplastic character states (radiolar lobes involuted ventrally in more than one whorl, absence of radiolar eyes, presence of skeleton in dorsal radiolar appendages, and thoracic ventral shields decreasing in size posteriorly). The sister-group relationship of *Sabellastarte* to the clade containing *Stylomma, Sabella, Branchiomma* and *Bispira* is well supported (JK 99). This latter clade has *Stylomma* in the basal position and *Sabella, Branchiomma* and *Bispira* collapsed into a polytomy of monophyletic species groups. The clade (JK 56) is supported by five synapomorphies (dorsal basal flanges absent, collar dorsal margins separated, dorsal collar notches absent, dorsal collar pockets absent, and companion chaetae present). Moreover, in the analysis of the 32 morphological characters, including those used in *Sabellastarte* taxonomic accounts, specimens from the same geographic areas (including the types of the currently recognized species) did not cluster as expected.

Molecular data

The combined COI and 16S aligned sequences resulted in 1,102 bp with 418 parsimony-informative characters. The MP analysis recovered four trees 1,378 steps in length (CI 0.65, RI 0.80). The strict consensus MP topology (Fig. 2b) retrieves *Branchiomma*, *Bispira* and *Stylomma* nested within *Sabellastarte* (low support). *Sabella* is the sister taxon to this clade (BS 90). Contrary to this result, Bayesian inference (BI) shows *Sabella* as incorporated within *Sabellastarte* with high support (Fig. 2c; PP 1.0). Groupings of taxa with high support are recovered in both the MP and BI topologies. Six clades are constructed, grouping specimens from Samoa, Japan, Pakistan and northern Australia (including individuals from the Northern Territory and Western Australia), respectively, a clade with a larger central Pacific distribution (including material from Malaysia, Hawaii and Saipan), and another group of specimens from Japan together with the single specimen from the Caribbean. There is a slight difference in the outcome of specimens collected in Victoria, Australia, which were recovered as paraphyletic in the MP analyses but as monophyletic though with low support by BI (PP 0.78). Well supported clades from the ML analyses (Fig. 2d) are congruent with those in the BI and MP trees. Similar to the MP molecular topology, *Sabella* is sister to *Sabellastarte*, and the latter includes *Bispira*, *Stylomma* and *Branchiomma*.

The relationships among *Sabellastarte* clusters are incongruent between the MP and ML and the BI analyses, with the two approaches suggesting different hypotheses of relationships between clades. For example, MrBayes


A
Pseudopotamilla sp. 1
Pseudopotamilla sp. 2
Stylomma palmatum 1
Stylomma palmatum 2
Branchiomma sp. 1
Branchiomma sp. 2
Sabella pavonina
Sabella spallanzanii
Bispira porifera
Bispira manicata
S. sanctijosephi
S. samoensis
S. spectabilis
S. magnifica
Sabellastarte Samoa1
Sabellastarte Samoa2
Sabellastarte WA4
Sabellastarte WA1
Sabellastarte WA2
Sabellastarte WA3
Sabellastarte WA5
Sabellastarte NT1
Sabellastarte NT2
S. australiensis
Sabellastarte NSW3
Sabellastarte Pakistan2
Sabellastarte Pakistan1
Sabellastarte Pakistan3
S. pectoralis
Sabellastarte VIC1
Sabellastarte VIC2
Sabellastarte VIC4
Sabellastarte Malaysia3
Sabellastarte Malaysia1
Sabellastarte NSW2
Sabellastarte Malaysia2
Sabellastarte Hawaii1
Sabellastarte Japan2
Sabellastarte Hawaii2
Sabellastarte Mexico1
Sabellastarte NSW1
Sabellastarte Saipan1
Sabellastarte Saipan2
Sabellastarte Japan3
Sabellastarte Japan4
Sabellastarte Japan6
Sabellastarte Japan1
S. japonica
B
Pseudopotamilla sp. 1
Pseudopotamilla sp. 2
Sabella spallanzanii
Sabella pavonina
Sabellastarte VIC1
Sabellastarte VIC4
Sabellastarte VIC2
Sabellastarte Samoa1
Sabellastarte Samoa2
Sabellastarte WA3
Sabellastarte NT2
Sabellastarte WA2
Sabellastarte NT1
Sabellastarte WA1
Sabellastarte WA4
Sabellastarte WA5
Branchiomma sp. 1
Branchiomma sp. 2
Sabellastarte NSW1
Sabellastarte NSW2
Sabellastarte NSW3
Bispira porifera
Bispira manicata
Stylomma palmatum 1
Stylomma palmatum 2
Sabellastarte Mexico1
Sabellastarte Japan1
Sabellastarte Japan2
Sabellastarte Japan3
Sabellastarte Japan4
Sabellastarte Japan6
Sabellastarte Pakistan1
Sabellastarte Pakistan2
Sabellastarte Pakistan3
Sabellastarte Malaysia1
Sabellastarte Hawaii1
Sabellastarte Hawaii2
Sabellastarte Malaysia2
Sabellastarte Malaysia3
Sabellastarte Saipan1
Sabellastarte Saipan2
C
Pseudopotamilla sp. 1
Pseudopotamilla sp. 2
Branchiomma sp. 1
Branchiomma sp. 2
Bispira porifera
Bispira manicata
Stylomma palmatum 1
Stylomma palmatum 2
Sabellastarte Mexico1
Sabellastarte Japan2
Sabellastarte Japan1
Sabellastarte Japan6
Sabellastarte Japan3
Sabellastarte Japan4
Sabellastarte Pakistan3
Sabellastarte Pakistan1
Sabellastarte Pakistan2
Sabellastarte Malaysia1
Sabellastarte Hawaii1
Sabellastarte Hawaii2
Sabellastarte Malaysia2
Sabellastarte Malaysia3
Sabellastarte Saipan1
Sabellastarte Saipan2
Sabellastarte NSW2
Sabellastarte NSW1
Sabellastarte NSW3
Sabellastarte Samoa1
Sabellastarte Samoa2
Sabella spallanzanii
Sabella pavonina
Sabellastarte VIC4
Sabellastarte VIC1
Sabellastarte VIC2
Sabellastarte NT2
Sabellastarte WA2
Sabellastarte WA3
Sabellastarte NT1
Sabellastarte WA1
Sabellastarte WA4
Sabellastarte WA5
Malaysia1
Hawaii1
Hawaii2
Malaysia2
Malaysia3
Saipan1
Saipan2
NT2
WA2
WA3
NT1
WA1
WA4
WA5
10 changes
D
Pseudopotamilla sp. 1
Pseudopotamilla sp. 2
Sabella pavonina
Sabella spallanzanii
Sabellastarte VIC4
Sabellastarte VIC2
Sabellastarte VIC1
NT2
WA2
NT1
WA1
WA4
WA3
WA5
Sabellastarte NT2
Sabellastarte WA2
Sabellastarte NT1
Sabellastarte WA1
Sabellastarte WA4
Sabellastarte WA3
Sabellastarte WA5
Sabellastarte Samoa2
Sabellastarte Samoa1
Sabellastarte NSW3
Sabellastarte NSW1
Sabellastarte NSW2
Sabellastarte Pakistan3
Sabellastarte Pakistan2
Sabellastarte Pakistan1
Sabellastarte Malaysia1
Sabellastarte Hawaii1
Sabellastarte Hawaii2
Sabellastarte Saipan1
Sabellastarte Saipan2
Sabellastarte Malaysia3
Sabellastarte Malaysia2
Malaysia1
Hawaii1
Hawaii2
Saipan1
Saipan2
Malaysia3
Malaysia2
Mexico1
Japan1
Japan6
Japan4
Japan2
Japan3
Sabellastarte Mexico1
Sabellastarte Japan1
Sabellastarte Japan6
Sabellastarte Japan4
Sabellastarte Japan2
Sabellastarte Japan3
Stylomma palmatum 1
Stylomma palmatum 2
Branchiomma sp. 1
Branchiomma sp. 2
Bispira porifera
Bispira manicata
0.7

◀ **Fig. 2** Phylogeny reconstructions. **a** Strict consensus of eight most parsimonious trees (MPT) based on morphological data (L: 102 steps, Ci: 0.41, Ri: 0.81); jackknife support values shown above branches. **b** Strict consensus of four MPT based on sequences of mitochondrial 16S ribosomal DNA and cytochrome oxidase subunit I (L: 1,378 steps, Ci: 0.65, Ri: 0.80); bootstrap support values shown above branches. **c** Bayesian topology based on combined mitochondrial DNA data; branch length represents number of changes, posterior probabilities shown at nodes. **d** Maximum likelihood phylogram based on mitochondrial DNA sequences; branch length represents number of changes, bootstrap values shown at nodes

retrieves the specimens from Mexico and Japan as sister to the remaining *Sabellastarte*, and those from North Australia as derived. In contrast, MP recovers the specimens from Victoria, Australia as sister to all other *Sabellastarte*, and those from Hawaii, Saipan and Malaysia as derived.

Combined data

Analyses of the combined data (morphology, 16S and COI) produced more resolved topologies than molecular data alone, and also defined clades not retrieved in the morphological or molecular data partitions. Parsimony analysis yielded three MPT, in which *Sabellastarte* is monophyletic, with a clade composed of *Sabella, Bispira, Branchiomma* and *Stylomma* as the sister group (strict consensus in Fig. 3a). Bayesian inference recovers a paraphyletic *Sabellastarte*, with *Sabella* in a sister-group relationship to *Sabellastarte* specimens collected in Victoria (PP 1; Figs. 2d, and 3b).

The MP and BI analyses were in agreement (also with analyses of the molecular partition) with regard to the clustering of *Sabellastarte* specimens. Thereby, the 31 specimens clustered into eight well supported terminal clades similar to those retrieved after analyses of molecular data, with specimens from Victoria now forming a clade (BS 81, PP 0.98).

However, the relationships between these eight *Sabellastarte* clades vary among the two approaches. Parsimony analysis retrieved three main clades within *Sabellastarte* (Fig. 3a). A clade with the specimens from Victoria as sister to the remaining *Sabellastarte*, a second clade that includes specimens from the Northern Territory and north Western Australia (BS 100), and a third clade with the remainder of the terminals (BS <50). Within this latter group four clades are well supported, including one with the Samoan specimens (BS 100), another with specimens from New South Wales (BS 93), and a third with specimens from Japan as sister to the only specimen from Mexico (BS 91). The fourth clade includes the specimens from Pakistan as sister group to a clade with those from Hawaii, Malaysia and Saipan (BS 100; Fig. 3a).

Bayesian analyses recovered three main clades, with specimens from Mexico and Japan being basal (PP 0.99), a clade grouping the specimens from Pakistan with those from Malaysia, Hawaii and Saipan (PP 0.99), and a clade with specimens from Australia and Samoa, with the two species of *Sabella* as sister to the specimens from Victoria (PP 0.98; Fig. 3b).

The character transformation of morphological features along these topologies (not shown) indicates that terminal clades are not supported by the traditional diagnostic characters of the currently recognized species, denoting their inaccuracy as diagnostic features.

Molecular divergence

Genetic divergence among (D) and within (π) the eight *Sabellastarte* clades is shown in Tables 3 and 4. The maximum distance value between clades for 16S was found between the Japan and New South Wales clusters, and between the Victoria and Pakistan clusters (D 17% in both cases), whereas the minimum distance was found between the Pakistan clade and the clade clustering the specimens from Hawaii, Saipan and Malaysia (D 6%). The maximum divergence found within clades was in the Northern Territory and Western Australia cluster (π 6%). The remaining clades showed distances equal to or below 1% (Table 3).

The maximum sequence divergence measured between clades for mitochondrial COI occurred between the Victoria and the Hawaii + Malaysia + Saipan clades, as well as between the Victoria and the Pakistan clades (D 20% in both cases). The minimum distance between clades was found between the specimens from Japan and the single specimen collected in Mexico (D 1%). Values measured when the third codon had been omitted were different (D range 2–28%) but the order of pairwise distances was the same (Table 4).

Integrating results—Systematics of *Sabellastarte* and species boundaries

Some of the resulting terminal clades, congruent in all analyses, gathered specimens from a narrow range distribution, e.g. those collected in Victoria, Samoa, Pakistan, New South Wales and Japan, respectively, while others gathered specimens from wider geographic areas, such as those from the central Pacific, including Malaysia, Hawaii and Saipan, and those from northern Australia (Northern Territory and north Western Australia). The sequence divergences measured between individuals and clades complemented the phylogenetic results and allowed us to decide which clades could be interpreted as potentially isolated populations or species. This was the case with the specimens from Victoria, New South Wales and Samoa which displayed minor intra-lineage genetic distance compared to other clades.

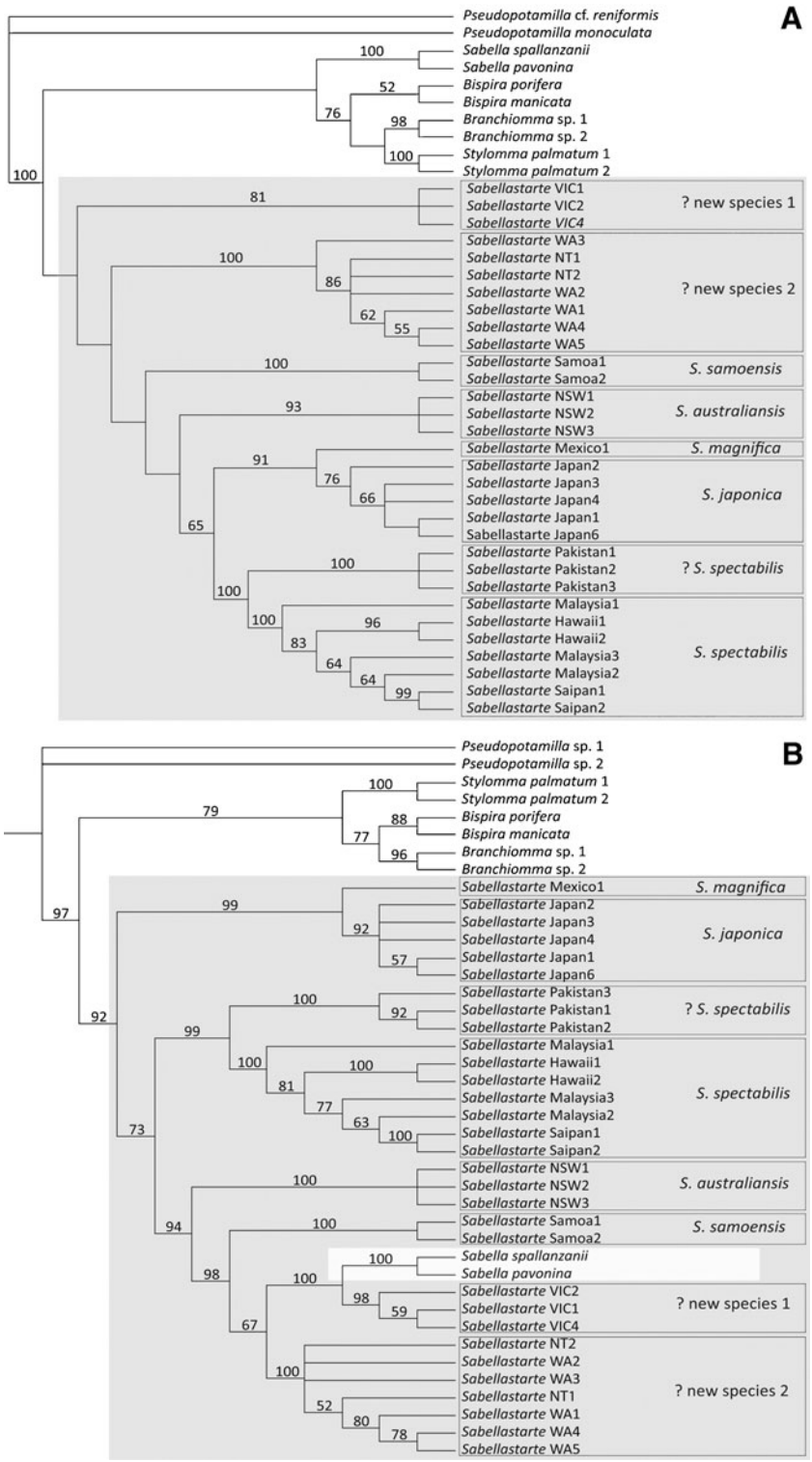


Fig. 3 **a** Strict consensus of three MPT (L: 1,481; Ci: 0.63; Ri: 0.80) based on combined morphological and mitochondrial DNA data; jacknife support values shown above branches. **b** Bayesian interference topology based on combined COI, 16S and morphological data; posterior probabilities shown at nodes

At least two different lineages (not closely related and with genetic distance over 16%) are present in the south of Australia and could be legitimately considered as separate species, even though re-examination of specimens after our analyses showed no link between certain variable morphological features and a particular distribution. The clade clustering the specimens collected in northern Australia showed a within-clade divergence of 6% (16S), representing the largest pairwise distance between specimens collected at the same site of the Kimberleys, Western Australia.

Table 3 16S sequence divergences (K2P corrected) within each *Sabellastarte* terminal clade (π values, on table diagonal) and among clades (*D* values, below diagonal)

Terminal clades		1	2	3	4	5	6	7
1	Hawaii + Malaysia + Saipan	0.01						
2	Japan	0.10	0.00					
3	NSW	0.15	0.12	0.00				
4	WA + NT	0.15	0.17	0.14	0.06			
5	Pakistan	0.06	0.11	0.16	0.15	0.01		
6	Samoa	0.14	0.14	0.11	0.12	0.15	0.00	
7	Victoria	0.15	0.15	0.16	0.15	0.17	0.14	n/c

NSW New South Wales; *NT* Northern Territory; *WA* Western Australia

Specimens from Japan and the Caribbean coast of Mexico were recovered as sister taxa in all analyses (excluding morphology) with significant support (Figs. 2b–d and 3a, b). Sequence distances between specimens from Japan (π 0–1%) and Mexico are low (D 1%) (Tables 3, and 4), indicating the possibility of all of them belonging to a single lineage. The Pakistan clade is retrieved as sister to the clade clustering specimens from Malaysia, Hawaii and Saipan, with high support values. Intra-clade sequence divergence is low (π≤1%) in both groups; between the two clades the difference is larger (D 6–7%) but not as much as is seen in other clades (D 10–17%) (Tables 3, and 4). The morphological variability, including colour, among these specimens is low (Fig. 6).

Discussion

Monophyly of *Sabellastarte* and relationships among other genera

This research represents the first attempt to test the monophyly of *Sabellastarte* and its relationships to other sabellid genera. The question of monophyly remains open, as only our MP analyses incorporating morphological data resulted in a monophyletic *Sabellastarte* characterized against the sister clade (containing *Stylomma, Sabella, Branchiomma* and *Bispira*) by a unique combination of features. In the past, the genus had been characterized by a combination of morphological features (Knight-Jones and Mackie 2003) partially shared with some of those related taxa. These were the presence of dorsal flanges on the base of the crown (also present in *Stylomma* and some species of *Bispira*), absence of radiolar flanges or radiolar eyes (as in *Sabella* and some *Pseudobranchiomma* and *Bispira* species), collar fused to the faecal groove forming flanking pockets on both sides (as in *Sabella*), and presence of parallel lamellae and ventral sacs within the radiolar crown (as in *Stylomma*) (Knight-Jones and Mackie 2003). Only one of the earlier-used features, the absence of radiolar eyes, supports the *Sabellastarte* clade in the phylogenetic hypotheses presented here, together with another set of homoplasious charcater states: radiolar lobes involuted ventrally in more than one whorl, presence of skeleton in dorsal radiolar appendages, and thoracic ventral shields decreasing in size posteriorly. In any case, these features should be taken with caution until the relationships within this clade are resolved and character transformation is understood.

The sister group to *Sabellastarte* is also still elusive, as the results from our different datasets and methods of

Table 4 COI sequence divergences (K2P corrected) within each *Sabellastarte* terminal clade (π values, on table diagonal) and among clades (*D* values, below diagonal)

Terminal clades		1	2	3	4	5	6	7
1	Hawaii + Malaysia + Saipan	0.01						
2	Japan	0.16	0.00					
3	Mexico	0.12	0.01	n/c				
4	WA + NT	0.18	0.19	0.19	0.01			
5	Pakistan	0.07	0.16	0.16	0.19	0.00		
6	Samoa	0.17	0.18	0.19	0.17	0.16	n/c	
7	Victoria	0.20	0.19	0.18	0.17	0.20	0.16	0.01

NT Northern Territory; *WA* Western Australia

analysis are not congruent and do not show significant support. Previous studies have suggested *Branchiomma* and *Pseudobranchiomma* (Fitzhugh and Rouse 1999) or *Sabella* (Capa 2008; Patti et al. 2003) as sister to *Sabellastarte*, but support for these relationships was not significant.

Relationships within *Sabellastarte*

The analyses performed here are congruent concerning the number and content of the terminal clades recovered, though the different datasets and analytical methods provide different sets of relationships within the terminal clades. These clades group individuals from discrete geographic areas. The large gap, of approximately an order of magnitude, in genetic sequence distance measured within and between clades suggests that these clades could correspond to isolated lineages. Considering the intra- and inter-specific divergences in these particular mitochondrial genes observed in other annelids (e.g. Erséus and Kvist 2007; Hilario et al. 2010; Osborn et al. 2007; Virgilio et al. 2009, Vrijenhoek et al. 2009, Wirchansky and Shain 2010), we could conclude that it is justifiable to assign species rank to these lineages.

However, there are two ambiguous results in this pattern in which the genetic divergence measured is intermediate between the two groups evident for inter- and intra-lineage values. One is the case found among specimens from northern Western Australia (π 6%). The other is found between the specimens from Pakistan and those from Hawaii, Saipan and Malaysia, with a maximum genetic divergence of 6% between members of the two sister clades. We have not found any morphological differentiation between members of the respective clades, thus are unable to determine whether these cases represent two widely distributed species or groups of different (potentially sympatric) species with more restricted distributions (see Fig. 4). It would be interesting to conduct an in-depth study of these cases to determine whether they involve single species with wide distribution areas, and which mechanisms might explain that fact, or whether we are dealing with species complexes instead.

Another open issue concerns the specimens from Japan and the Mexican Caribbean as members of the same species due to their close relationship and the low genetic distance. The small sample size (only one individual from the Caribbean, and only COI amplified) does not allow great confidence in that solution. Furthermore, some differences in body color have been noted between specimens from these two distant localities. There are distinct spots on the Japanese specimens which were not observed in Mexico (Fig. 5). Our findings are consistent with other records of specimens from those areas (Knight-Jones and Mackie 2003; Tovar-Hernández and Salazar-Vallejo 2006).

The present study does not allow us to determine the number of species in the genus. We have not covered the whole distribution range of *Sabellastarte*, as material from some areas has been inaccessible (including the west coast of Africa, the eastern Tropical Pacific, the Red Sea and several localities in the Indo-Pacific, such as India, South East Asia, south Western Australia, and many of the Pacific islands) The impossibility of amplifying DNA from types (fixed in formalin) precluded inclusion of the name-bearing specimens in the analyses; therefore the terminal clades or operational taxonomic units (OTUs) resulting from this study do not necessarily correspond to nominal species,

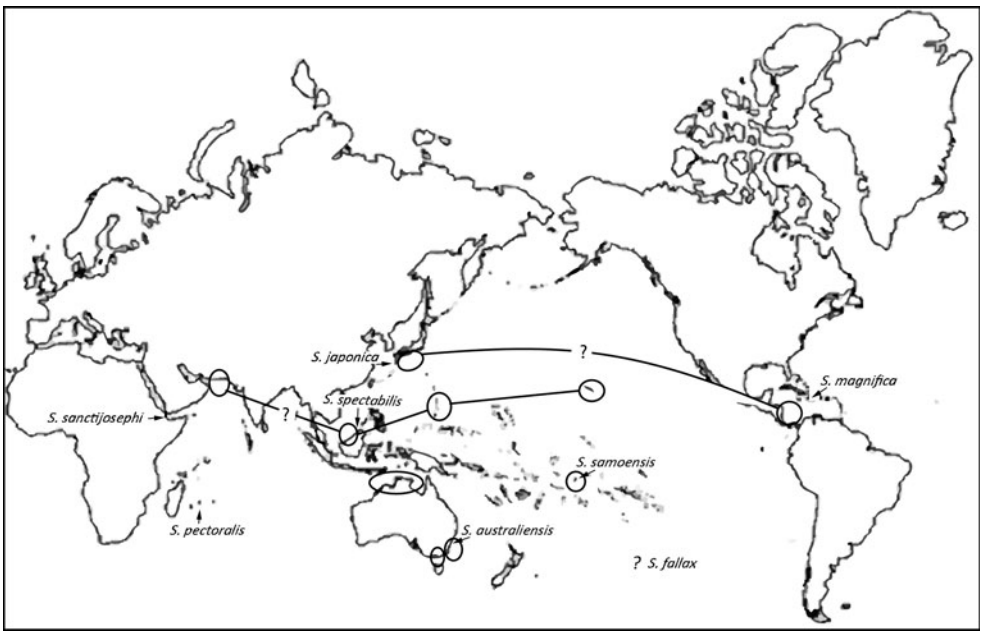


Fig. 4 World map indicating type localities of the eight currently recognized *Sabellastarte* species (*black arrows*; after, e. g., Knight-Jones and Mackie 2003), and distribution of lineages resulting in present study (*encircled*)

even though some of the material was collected close to type localities. However, the results showed that at least six isolated lineages could be considered, some of which have a distribution inconsistent with previous records (as in Knight-Jones and Mackie 2003).

On these grounds, we find it reasonable to assign a currently recognized *Sabellastarte* species name to a well delineated lineage, considered herein as a species, if the type locality of a nominal species falls within the geographic distribution of a clade resulting from the present analyses and if no other species has been found in the same area (see Fig. 4). Consequently, we have assigned names to clades (as in Fig. 4) for *S. spectabilis* (Grube, 1878), *S. magnifica* (Shaw, 1800), *S. samoensis* (Grube, 1870), *S. japonica* (Marenzeller, 1885), and *S. australiensis* (Haswell, 1884), and additionally assume two potentially new cryptic species.

Regardless of the apparently continuous distribution of *Sabellastarte* along the coast of Australia, the specimens from New South Wales, Victoria and northern Australia belong to different lineages, considered herein as different species. Prior to the present study only a single species had been recorded in that area, *S. australiensis* (Haswell), described from Sydney and recorded from Victoria to southern Queensland. In our results, however, the specimens collected in Victoria were not related to those from New South Wales. Recent studies have shown a phylogeographic break in the vicinity of the Bass Strait, Victoria, Australia (e.g. Dawson 2005; Halt et al. 2009; O'Hara and Poore 2000; Waters 2008). This biogeographical boundary should be studied to investigate its effects on members of *Sabellastarte*.

Another process to be taken into account when discussing *Sabellastarte* distribution is the accidental introduction through ballast water and ship hull fouling. At least some species of *Sabellastarte* have larvae that spend 7–8 days in the water column before settlement and metamorphosis (Bybee et al. 2006a). Although this lecitotrophic stage is not as long as in the related species, *Sabella spallanzanii*—known to have been accidentally introduced into Australia and New Zealand from the Mediterranean (Giangrande et al. 2000; Giangrande and Petraroli 1994; www.biosecurity.govt.nz), and with larval development lasting around 21 days (Giangrande et al. 2000)—it might be sufficient to travel significant distances in ballast water. For example, *Sabellastarte spectabilis* is thought to have been accidentally introduced into Hawaii (Coles et al. 1999; Coles and Eldredge 2002); although this assumption relies on morphological features demonstrated here to be invalid, there is some evidence for a wide distribution of this species according to our analyses including molecular data.

Diagnostic morphological features

After close examination of specimens of *Sabellastarte* collected from different localities around the world and cladistic analyses based on morphological features, we confirm that *Sabellastarte* is homogeneous regarding morphological features and that some of the character states traditionally considered as diagnostic vary within populations. The low support values of the clades within *Sabellastarte* together with the partial lack of resolution of part of the topology of the strict consensus (Fig. 2a) demonstrate the difficulty of making a phylogenetic

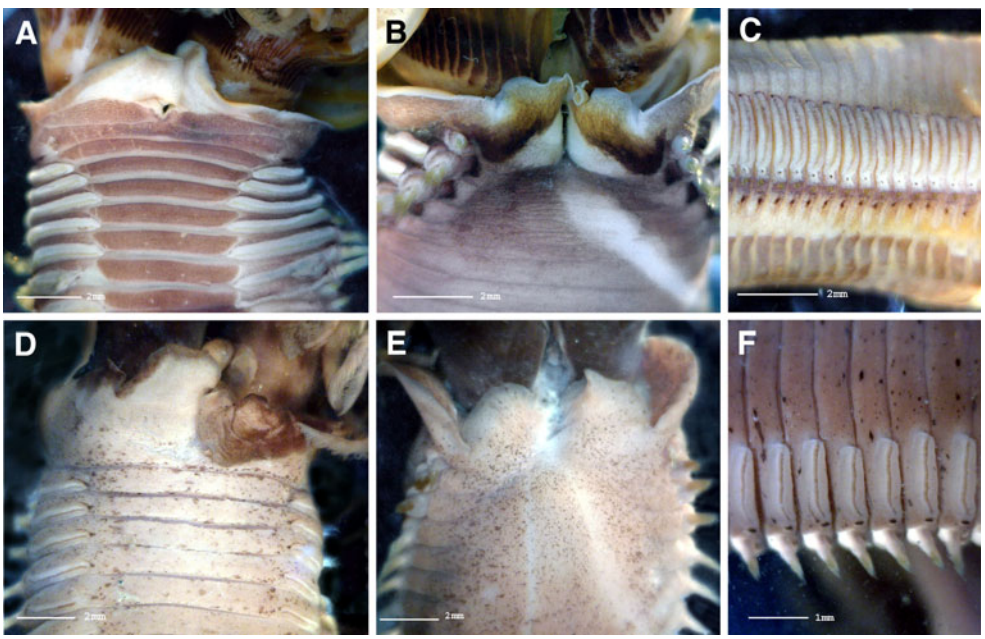


Fig. 5 **a**–**c** *Sabellastarte* from Veracruz, Mexico; AM W.31846. **a** Anterior thoracic segments, ventral view. **b** Anterior thoracic segments, dorsal view. **c** Mid abdominal segments, lateral view. **d**–**f** *Sabellastarte* from Sagami Bay, Japan; AM W.32320. **d** Anterior thoracic segments, ventral view. (E) Anterior thoracic segments, dorsal view. **f** Mid abdominal segments, lateral view

estimate for the group based solely on morphological data. Some of the characters that have traditionally been used to distinguish species change during development, growth and regeneration, and should not be considered as diagnostic. For example, the number of chaetae in a fascicle, the presence of interdigitating radioles, and spiralling of the crown lobes are all known to vary with age (Knight-Jones and Mackie 2003; authors' pers. obs.). Other features, such as the relative length of the branchial crown (Fig. 1 m) or the number of thoracic or abdominal chaetigers, are also known to be insufficient for species identification as they can change due to regeneration (Knight-Jones and Bowden 1984; Knight-Jones and Mackie 2003). Moreover, some other characters previously used in diagnoses refer to form or measurements of soft structures, and have been demonstrated to be inconsistent among populations and to show deformation due to fixation and preservation (Costa-Paiva et al. 2007). For this reason, the following features were not included in the analyses: relative lengths of radiolar crown and body, shape of the outer margin of radioles, length of radiolar tips, length of basal membrane, relative width and length of thorax, length of the first thoracic segment compared to the remaining segments, and length of the protruding parts of thoracic chaetae.

In addition, characters such as the shape of chaetae, normally a useful feature in sabellid taxonomy, showed overlap between lineages in this case. The shape of uncini also displayed variability even within individual specimens, making the establishment of intra- and inter-specific variation problematic. There is one exception to this pattern: the shape of uncini with long necks is constant among specimens of *S. japonica* (for which species the feature was indicated as unique by Knight-Jones and Mackie 2003), *S. magnifica* and the lineage considered here as a new species from north Australia.

Despite painstaking examination of all specimens included in the present study we have been unsuccessful in finding any new diagnostic features of external morphology. Surprisingly, it appears that certain color patterns, such as the dark body specks or the number of rows of eyespots in the abdominal chaetigers, could be consistent in certain species and might reveal diagnostic synapomorphies for these species, as has been found in other taxa (e.g. Pleijel et al. 2009). This needs to be examined further across a wider range of specimens and species, and to be verified by DNA data.

Proposed goals for future research

This study highlights the benefit of combining approaches for the recognition of species boundaries. But we also realise that increasing sample sizes (concerning numbers of specimens and geographic areas) will be essential. Including more taxa will probably help in finding morphological synapomorphies and assessing potentially cryptic or introduced species, and thus increase our understanding of relationships within the genus and better define its lineages. Specifically, material from the Red Sea, east coast of Africa, Tropical Eastern Pacific, Pacific Islands, South East Asia, China and from several localities in Australia is needed to provide greater resolution in order to address these questions.

Reproductive strategy and life history characters may also help elucidate these issues. Currently, there is a minimal amount of information for only a few of the recognized species in the literature, and even some of that is conflicting (Bybee et al. 2006b). For example, *S. spectabilis* from Hawaii is a protandrous hermaphrodite (Bybee et al. 2006b), while the same species is reported to be gonochoric in Micronesia (Rouse and Fitzhugh 1994). Similar conflicting observations exist for *S. magnifica* (Rouse and Fitzhugh 1994). Gametogenic patterns, gamete morphology and larval development are only known from Hawaiian populations (Bybee et al. 2007) in which broadcast spawners produce lecithotrophic larvae that spend 7–8 days in the water column before settlement and metamorphosis (Bybee et al. 2006a). These observations along with the efforts put forth by this research (which represents a first estimate of *Sabellastarte* phylogenetics) indicate that a stable taxonomy for the group may be taking shape but is far from complete, and that further efforts to characterize the natural history, morphology and molecular phylogenetics of the group will likely be a fruitful endeavor.

Acknowledgements We would like to thank numerous colleagues that have made this study possible by helping us collect or sending specimens from various parts of the world. These are Pat Hutchings, Kate Attwood, Anna Murray and Steve Keable (Australian Museum, Australia), Chris Glasby and Belinda Álvarez de Glasby (Museum and Art Galleries of the Northern Territory, Australia), Robin Wilson (Museum Victoria, Australia), Roger Goodwill (Brigham Young University-Hawaii, USA), Ana Rubio (Fisheries, Australia), Javed Mustaquim (University of Pakistan, Pakistan), María Ana Tovar-Hernández (Universidad Nacional Autónoma de México, Mexico), Tetsuya Kato and Mitsuru Ohta (Shirahama Aquarium at Kyoto University, Japan), Eijiroh Nishi (Yokohama National University, Japan), Douglas Fenner (Department of Marine and Wildlife Resources, American Samoa), and Greg Rouse (Scripps Institution of Oceanography, USA). We are also grateful to Brigham Young University Provo and particularly to Michael Whiting and the Whiting Laboratory of Molecular Systematics for the use of their facilities and lab space. Rebecca Johnson (Australian Museum) assisted in the molecular laboratory. We wish to thank the Willi Hennig Society for allowing free access to TNT software. Finally, we would like to thank two anonymous reviewers and the editors, Martin V. Sørensen and Olaf Bininda-Emonds, for useful comments and suggestions which helped improve the manuscript.

Appendices

Appendix 1 Material examined

Mexico

Veracruz: AM W31846 (1 specimen), BYUHMNH E.007.

Samoa

Samoa: AM W.32478 (1 spec.), AM W.32479 (1 spec.).

Philippines

Bohol Islands: MPW 374. Lectotype.

Malaysia

Kota Kinabalu: AM W.30001 (1 spec.), AM W30384 (1 spec.), AM W30386 (1 spec.).

Hawaii

Oahu, Kaneohe: AM W.31843 (1 spec.).

Coconut Island: BYUHMNH E.002 (1 spec.).

Saipan

Northern Marianas Islands: AM W.31847 (1 spec.), BYUHMNH.E.008 (1 spec.).

Pakistan

Karachi, Hawkes Bay: AM W.31823 (1 spec.), AM W.31824 (1 spec.), AM W.31825 (1 spec.).

Japan

Sagami Bay: AM W.81845 (1 spec.), AM W.320202 (1 spec.), BYUHMNH E.003 (1 spec.), BYUHMNH E.004 (1 spec.), BYUHMNH E.005 (1 spec.), BYUHMNH E.006 (1 spec.).

Australia, New South Wales

Port Jackson: AM G.2045 (1 spec.), AM G.11203 (2 spec.), AM G.11204 (1 spec.), AM W.24905 (1 spec.), AM W.20625 (1 spec.), AM W.29659 (1 spec.), AM W.29658 (1 spec.), AM W.32007 (1 spec.), AM W.4280 (1 spec.), AM W.6194 (2 spec.), AM W.4408 (1 spec.), AM W.6538 (1 spec.), AM W.1763 (1 spec.), AM W.24279 (1 spec.).

Solitary Islands: AM W.29657 (1 spec.).

Newcastle: AM W.34243 (1 spec.), AM W.34771 (3 spec.), AM W.17844 (1 spec.).

North Sydney: AM W.6193 (1 spec.), AM W.6195 (1 spec.), AM W.24904 (1 spec.), AM W.5706 (1 spec.).

Port Stephens: AM W.33910 (1 spec.), AM W.30383 (1 spec.), AM W.30383 (1 spec.).

Port Hacking: AM W.24899 (3 spec.).

Botany Bay: AM W.34770 (1 spec.).

Port Kembla: AM W.34244 (1 spec.), AM W.34245 (1 spec.), AM W.34246 (1 spec.), AM W.34772 (1 spec.), AM W.32012 (1 spec.).

Batemans Bay: AM W.31105 (4 spec.).

Eden: AM W.34242 (1 spec.).

Twofold Bay: AM W.199766 (1 spec.), AM W.199769 (3 spec.), AM W.199765 (3 spec.), AM W.199767 (1 spec.), AM W.199768 (1 spec.), AM W.199770 (1 spec.), AM W.199771 (1 spec.), AM W.199772 (1 spec.).

Victoria

Port Phillip Bay: AM W. 11967 (1 spec.), AM W. 14129 (1 spec.).

Northern Territory

Darwin: AM W.29656 (1 spec.), NTM W.18990 (2 spec.), NTM W.53 (1 spec.), NTM W.54 (1 spec.), NTM W.55 (1 spec.), NTM W.57 (1 spec.), NTM W.58 (1 spec.), NTM W.59 (1 spec.), NTM W.17359 (1 spec.), AM W.31844 (1 spec.).

Western Australia

Dampier Archipelago: AM W.30024.

Kimberleys: AM W.31826 (1 spec.), AM W.31827 (1 spec.), AM W.31828 (1 spec.), AM W.31829 (1 spec.), AM W.31830 (1 spec.).

Appendix 2 List of morphological characters and character states

Char. 1–11 Branchial crown and other prostomial appendages

1 Crown lobe shape: (0) two semicircles or slightly involuted ventrally, never forming one whorl; (1) both halves involuted ventrally in more than one whorl; (2) only one half spiraled.
2 Inter-digitating radioles: (0) absent; (1) present.
3 Radiolar eyes: (0) absent; (1) present.
4 Type of radiolar eyes: (0) simple eyespots; (1) unpaired distal compound eyes; (2) paired compound eyes; (3) unpaired compound eyes.
5 Stylodes: (0) absent; (1) present.
6 Number of cells in radiolar 'skeleton': (0) four; (1) four to ten; (2) more than ten.
7 Dorsal basal flange: (0) absent; (1) present.
8 Dorsal radiolar appendages: (0) short, i.e. less than 1/4 of crown; (1) long, i.e. more than 1/4 of crown.
9 Skeleton of dorsal radiolar appendages: (0) absent; (1) present.
10 Dorsal pinnular appendages: (0) absent; (1) present.
11 Ventral sacs: (0) within crown base; (1) outside of crown; (2) absent.

Sabellastarte and some members of *Bispira* present ventrally involuted or spiraled crown lobes (Fig. 1i), whereas *Sabella spallanzanii* (Gmelin, 1791) has only one side of the crown that is spiralled (Fig. 1a). The remaining terminal taxa show semicircular or slightly involuted, brown lobes. Some individuals of *Sabellastarte* have inter-digitating radioles appearing as a second internal row (Knight-Jones and Mackie 2003). *Sabella* and *Sabellastarte* typically lack radiolar eyes, similar to some members of

Bispira and *Pseudobranchiomma,* while members of *Branchiomma* characteristically have several pairs of compound eyes along radioles, and *Stylomma* has unpaired subterminal eyes or some randomly distributed eyespots (e.g. Capa 2008). Other radiolar structures found in the ingroup are the stylodes, unique to members of *Branchiomma*, and the radiolar flanges, present in *Bispira*, *Pseudobranchiomma* and *Stylomma*. The number of longitudinal rows of cells forming the 'radiolar skeleton' has traditionally been used as a diagnostic generic feature among sabellids (Fitzhugh 1989; Fitzhugh and Rouse 1999; Rouse and Fitzhugh 1994). The character states have been modified from previous studies to accommodate the variability shown in the taxa considered for the present study. Members of *Sabellastarte* have a dorsal basal flange on the dorsal margin of the crown (Fig. 1g), shared with members of *Stylomma* (Capa 2008) and with some species of *Pseudobranchiomma* (Nogueira and Knight-Jones 2002) and *Pseudopotamilla* (Capa 2007). All ingroup terminals except one show dorsal lips with dorsal radiolar appendages (Fig. 1h, i) supported by a radiolar 'skeleton' (Fitzhugh 2003); the exception is *Stylomma* (authors' pers. obs.), but the attribute is also absent in *Pseudopotamilla*. Members of *Sabellastarte* and *Stylomma* have ventral sacs located inside the crown lobes (Fig. 1h, j), as in the outgroup, while in the remaining ingroup terminals the sacs are located outside the radiolar crown (Fig. 1b) (Capa 2008; Knight-Jones and Perkins 1998).

Char. 12–15 Peristomium and collar

12 Collar dorsal margins: (0) fused to the faecal groove; (1) margins of collar free, dorsal.
13 Dorsal notches: (0) absent; (1) present.
14 Dorsal pockets: (0) absent; (1) present.
15 Ventro-lateral notches: (0) absent; (1) present.

The posterior peristomial ring collar dorsal margins are fused to the faecal groove in *Sabellastarte* (Fig. 1i, l), *Sabella* (Fig. 1c), and in some members of *Bispira* (Capa 2008) and *Branchiomma* (e.g. Knight-Jones 1994; Licciano and Giangrande 2008). Although *Sabella* has always been considered as having dorsal collar margins separated by a distinct gap, it should be treated as having them fused to the faecal groove, with conspicuous dorsal notches and no pockets (Capa 2008; Fig. c). *Sabellastarte, Sabella* and *Pseudopotamilla* typically have latero-dorsal notches and pockets present (Fig. 1i, l), whereas *Bispira*, *Branchiomma* and *Stylomma* (included herein) have smooth dorsal margins that are widely separated.

Char. 16–20 Thorax

16 Thoracic chaetiger number: (0) fixed to eight; (1) lower than eight; (2) higher than eight.
17 Inter-ramal eyespots: (0) absent; (1) present.
18 Thoracic ventral shields: (0) all of equal width; (1) decreasing in size posteriorly; (2) first noticeably longer than others.
19 Shape of anterior margin of first ventral shield: (0) M-shaped; (1) W-shaped; (2) straight.
20 Thoracic neuropodial tori and ventral shields: (0) all in contact; (1) the anterior separated, the posterior in contact; (2) all separated.

Most sabellids have eight thoracic chaetigers, but in some taxa that reproduce by scissiparity this number can vary due to inconsistencies during the regeneration of segments (Capa 2007, 2008; Knight-Jones and Bowden 1984; Knight-Jones and Giangrande 2003; Tovar-Hernández and Knight-Jones 2006). In the ingroup, interramal eyespots are present on both thorax and abdomen (Fig. 1d, n), a synapomorphy of the clade (Fitzhugh 1989; Fitzhugh and Rouse 1999). The ventral shields, easily distinguished in the terminals included, can be similar in size along the thorax as in *Branchiomma*, *Pseudobranchiomma* and *Pseudopotamilla*, or decrease posteriorly as in *Sabella or Sabellastarte* (Fig. 1b, h, k). The anterior margin of the first ventral shield can vary in shape, herein classified as straight, M-shaped (when the midline notch dips in the margin) or W-shaped (when the border of the ventral shield extends upwards in the middle to contact the notch) (Capa 2008). In members of *Sabellastarte* all thoracic tori are in contact with the ventral shields (Fig. 1h, k), whereas they are all separated in *Pseudopotamilla*. This feature shows intra-generic variation in other members of the ingroup, and some species have separated or joint tori and ventral shields (Capa 2007, 2008; Knight-Jones 1994; Nogueira and Knight-Jones 2002).

Char. 21–25 Thoracic chaetae

21 Rows of teeth over the main fang: (0) maximally five rows; (1) many rows.
22 Base of thoracic uncini: (0) concave; (1) straight line.
23 Length of thoracic uncini handle: (0) short; (1) medium; (2) long.
24 Companion chaetae: (0) absent; (1) present.
25 Shape of companion chaetae: (0) with symmetrical membrane; (1) with asymmetrical membrane.

Traditionally the chaetae of sabellids, especially the inferior thoracic notochaetae, have been used as a diagnostic feature for genera and species (Fitzhugh 1989; Knight-Jones and Perkins 1998). Spine-like inferior thoracic notochaetae are characteristic of the ingroup, whereas in the outgroup the chaetae are paleate. These chaetae are homogeneous among species of *Sabellastarte*. Similarly, the shape of uncini is commonly used as a diagnostic feature. They show variability in the number of rows and

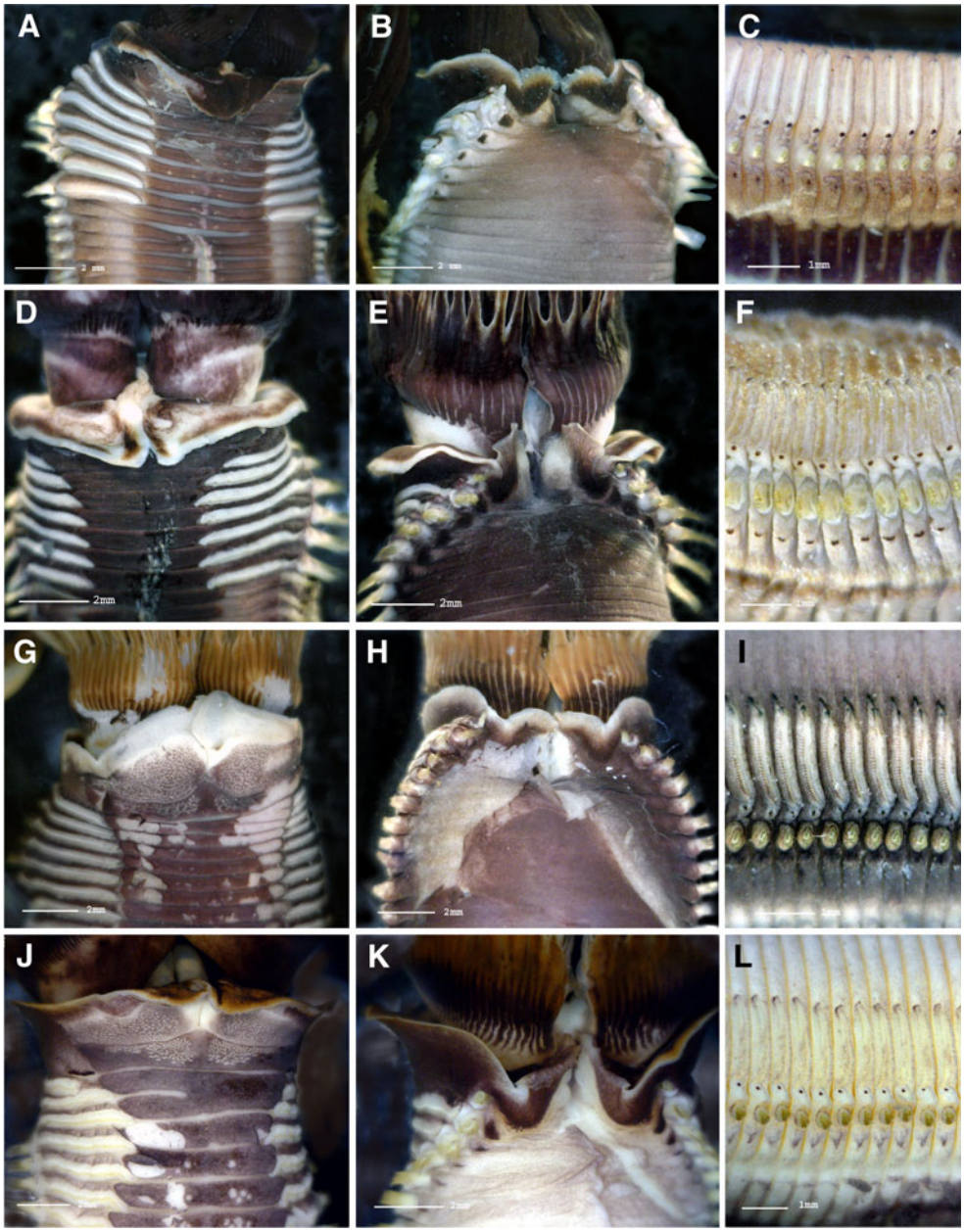


Fig. 6 **a**–**c** *Sabellastarte* from Kota Kinabalu, Malaysia; AM W.32025. **a** Anterior thoracic segments, ventral view. **b** Anterior thoracic segments, dorsal view. **c** Mid abdominal segments, lateral view. **d**–**f** *Sabellastarte* from Oahu, Hawaii; AM W.31843. **d** Anterior thoracic segments, ventral view. **e** Anterior thoracic segments, dorsal view. **f** Mid abdominal segments, lateral view. **g**–**i** *Sabellastarte* from Saipan, Northern Marianas Islands; AM W.31847. **g** Anterior thoracic segments, ventral view. **h** Anterior thoracic segments, dorsal view. **i** Mid abdominal segments, lateral view. **j**–**l** *Sabellastarte* from Karachi, Pakistan; AM W.31824. **j** Anterior thoracic segments, ventral view. (K) Anterior thoracic segments, dorsal view. **l** Mid abdominal segments, lateral view

size of teeth above the main fang in *Bispira*, *Branchiomma* and *Pseudobranchiomma*. These teeth are large and arranged in a few rows (five or fewer), while *Sabella* and *Sabellastarte* show smaller teeth and numerous rows. The development of the breast of thoracic uncini has been incorporated in previous phylogenetic analyses of Sabellidae (Capa 2007, 2008; Fitzhugh 1989; Fitzhugh and Rouse 1999; Rouse and Fitzhugh 1994), which differentiated a plesiomorphic narrow breast condition from an apomorphic well developed breast. Handle length has also been considered as informative (e.g. Capa 2007; 2008; Fitzhugh 1989; Fitzhugh and Rouse 1999), being classified as short when the handles are shorter than the distance between the tip of the main fang and the base of the breast, as medium when the length is anywhere up to double that distance, and as long when it is even longer. The base of the uncini and handle can be straight, concave or occasionally convex. Members of *Branchiomma* and *Pseudobranchiomma* have a concave base, whereas *Bispira* show a straight base. In members of *Sabellastarte*, however, this character can be inconsistent, even among members of the same population (Fig. 8). The uncini of members of *Pseudopotamilla* have longer handles than in the ingroup, and the base is straight.

Char. 26–28 Abdomen

26 Abdominal neuropodia: (0) transverse ridges; (1) conical lobes.

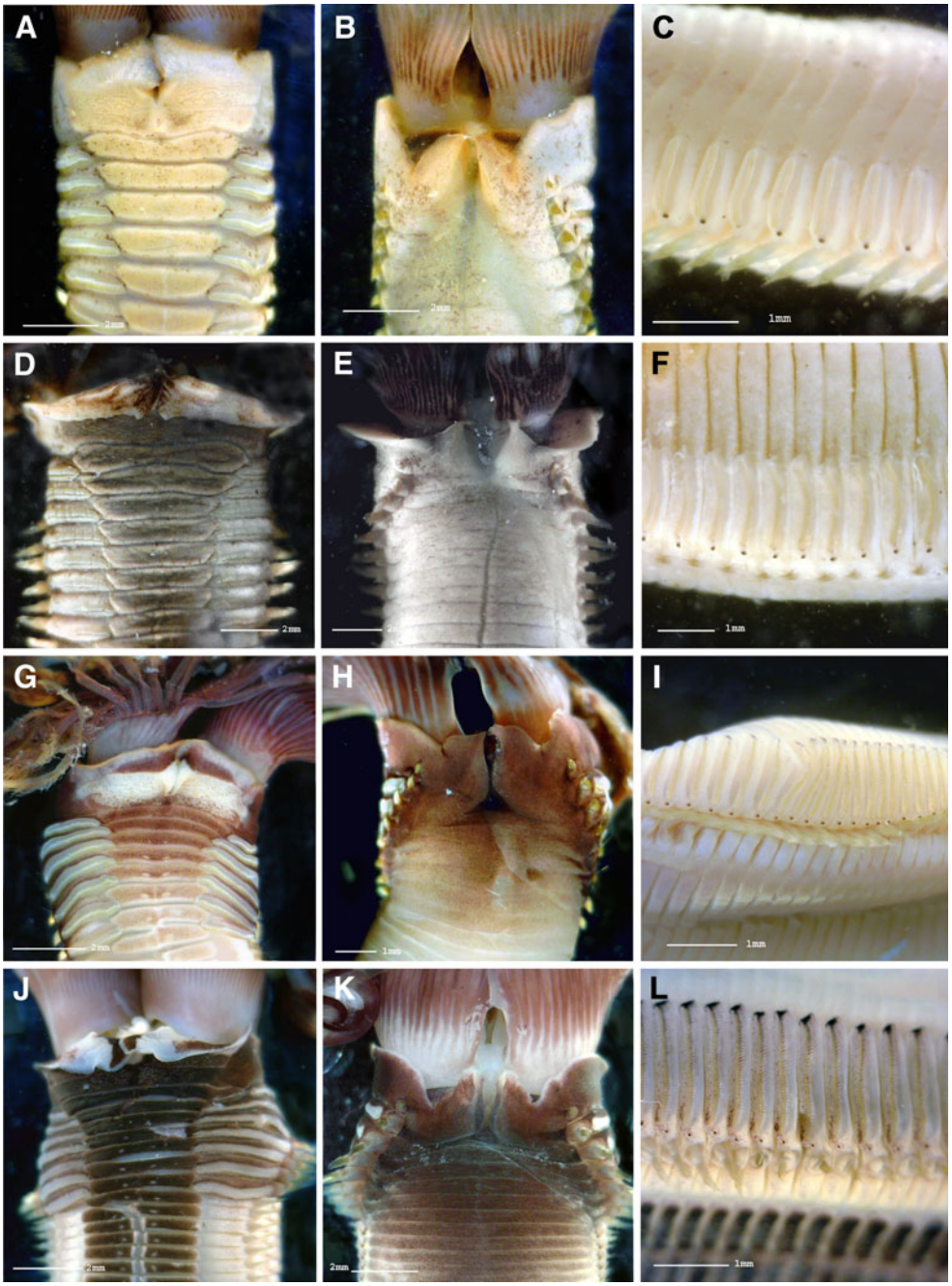


Fig. 7 **a**–**c** *Sabellastarte australiensis* from Sydney, New South Wales, Australia. **a** Anterior thoracic segments, ventral view. **b** Anterior thoracic segments, dorsal view. **c** Mid abdominal segments, lateral view. **d**–**f** *Sabellastarte* from Mallacoota, Victoria, Australia, MV F.108859. **d** Anterior thoracic segments, ventral view. **e** Anterior thoracic segments, dorsal view. **f** Mid abdominal segments, lateral view.**g**–**i** *Sabellastarte* from Kimberleys, Western Australia. **g** Anterior thoracic segments, ventral view. **h** Anterior thoracic segments, dorsal view. **i** Mid abdominal segments, lateral view. **j**–**l** *Sabellastarte* from Darwin, Northern Territory, Australia, AM W.31844, **j** Anterior thoracic segments, ventral view. **k** Anterior thoracic segments, dorsal view. **l** Mid abdominal segments, lateral view

27 Dorsal notopodial eyes in abdominal chaetigers: (0) absent; (1) present.
28 Neuropodial eyespots in abdominal chaetigers: (0) absent; (1) present.

The neuropodium in abdominal chaetigers is a transverse ridge in *Pseudopotamilla* and a conical lobe in the ingroup (Fig. 1d). As described for the thorax, the genera included in the ingroup show interramal eyespots in the abdomen (Capa 2008; Fitzhugh 1989; Fitzhugh and Rouse 1999). Some individuals of *Sabellastarte* have spots on the dorsum of the abdominal notopodia and on the venter of neuropodia; although no photoreceptor has been observed, the spots seem to be similar to those found between noto- and neuropodia, meaning that there could be three eyespots on each parapodium (Figs. 5c, f, 6c, f, i, k, 7 l and 8).

Char. 29, 30 Abdominal chaetae

29 Superior abdominal chaetae: (0) in straight line; (1) in C-shaped arrangement.
30 Number or rows of inferior abdominal chaetae: (0) one; (1) two; (2) three; (3) higher than three.

The ingroup taxa are characterized by having the superior abdominal chaetae displayed in a C-shape or a

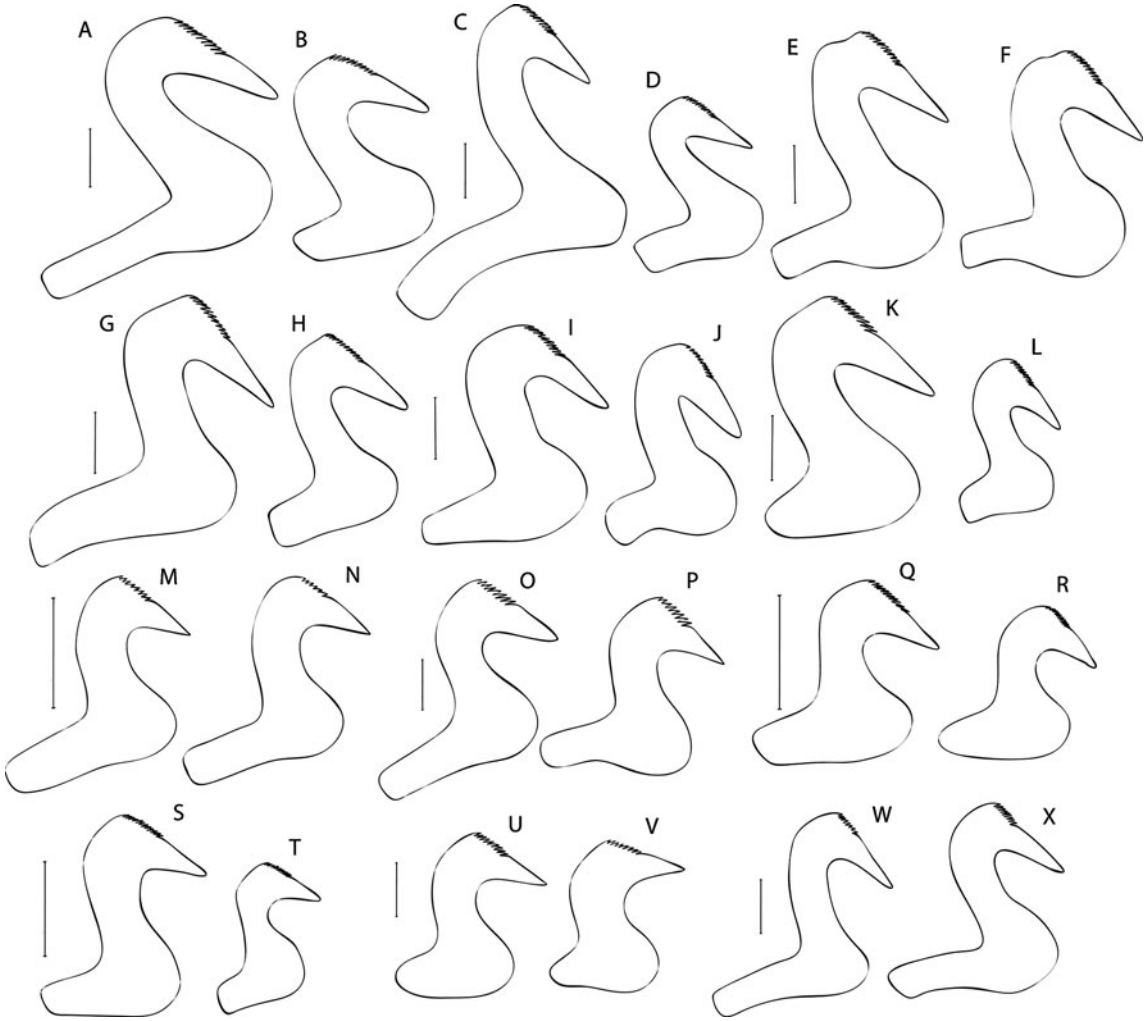


Fig. 8 Line drawings of pairs of uncini from various specimens; respective left drawing: uncinus from seventh thoracic chaetiger, right drawing: uncinus from a mid-abdominal chaetiger. **a**, **b** Veracruz, Mexico; AM W.31846. **c**, **d** Sagami Bay, Japan; AM W.31845. **e**, **f** Karachi, Pakistan; AM W.31823. **g**, **h** Kota Kinabalu, Malaysia; AM W.30385. **i**, **j** Saipan, Northern Marianas Islands; AM W.31847. **k**, **l** Pago Pago, American Samoa; AM W.32479. **m**, **n** Port Jackson, New South Wales, Australia; AM G.2045 (?type). **o**, **p** Port Jackson, New South Wales, Australia; AM W.4280. **q**, **r** Port Phillip Bay, Victoria, Australia; AM W.11967. **s**, **t** Port Phillip Bay, Victoria, Australia; MV.F.108866. **u**, **v** Kimberleys, Western Australia; AM Ex W.202945. **w**, **x** Darwin, Northern Territory, Australia; NTM W.17197. Scales: **a**–**l**, **u**–**x**: 1 μm; **m**–**t**: 4 μm

spiral in members of *Sabella* (Fig. 1d; see also Capa 2008). In their revision of the genus, Knight-Jones and Mackie (2003) considered the number of chaetae in mature specimens as diagnostic for some *Sabellastarte* species. Due to our observation that the number is quite variable among the observed specimens, the number of rows of chaetae has been taken into account instead, and it has been counted on the anterior abdominal segments.

Char. 31, 32 Pygidium

31 Pygidial eyes: (0) absent; (1) present.
32 Pygidium: (0) bilobed; (1) rim; (2) papilla.

The shape of the pygidium and the presence of pygidial eyespots (Fig. 1 n) have been included in the present study, but some museum specimens were so damaged that these characters could be scored as question marks only.

Excluded characters

Certain morphological characters that show variation among populations have been excluded from the morphological data matrix even though they were included in species descriptions in the past. For example, the length of the branchial crown relative to body length has been used in *Sabellastarte* species descriptions (Knight-Jones and Mackie 2003), even though it varies during regular growth of the animal as well as during regeneration after damage. The outer margins of radioles have been considered as rounded or subrectangular in a previous cladistic analysis (Fitzhugh 1989), but objectively their shape is hard to

assign to discrete categories, and variation has been observed among radioles from a single specimen or even along their individual length. The radiolar pinnules of most sabellids, especially in large species, are constant in length along the radiole, diminishing in size at the distal end. However, some *Sabellastarte* show different lengths of pinnules along the radioles, giving an undulating appearance to their margins, but this attribute is hard to perceive in fixed specimens with curled pinnules, therefore has not been included. The relation between thorax length and width was used to distinguish species of *Sabellastarte* (Knight-Jones and Mackie 2003), but the corresponding measurement results can depend on fixation procedures (Costa-Paiva et al. 2007). The number of chaetae in *Sabellastarte* thoracic parapodia, a character used to describe new species (Knight-Jones and Mackie 2003), can reach higher numbers than in any other sabellid. However, the number of thoracic chaetae varies during growth and development, thus has been omitted here. The width of the 'knee' or 'hood' relative to the 'shaft' or 'core' of the thoracic chaetae was also used in species descriptions (Knight-Jones and Mackie 2003). This character shows intra-specific variation, even among chaetae on a single parapodium, and as a quasi-cylindrical structure varies with the observation angle.

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