Species delimitation and distribution in *Aporometra* (Crinoidea : Echinodermata): endemic Australian featherstars

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Abstract. *Aporometra* Clark, 1938, which belongs to the monotypic Aporometridae, is a crinoid genus endemic to temperate Australian waters. It has been described as being 'viviparous' and is among the smallest of comatulids. The small size of specimens, and poor morphological justifications for specific diagnoses have created uncertainty over the number of species in the genus and their distributions. This study identified a suite of characters using data from scanning electron microscopy and mtDNA sequencing (*CO1* and *ND2*) to assess the number of species of *Aporometra*. Specimens were obtained from museums and collected from Western Australia, South Australia, Victoria and New South Wales. Type material was also examined when possible. Phylogenetic hypotheses were generated using maximum parsimony-based analyses of the separate and combined datasets. The results support the monophyly of *Aporometra* and the presence of two species, *Aporometra wilsoni* (Bell, 1888) and *Aporometra occidentalis* A. H. Clark, 1938, along the southern Australian coast. The status of the third nominal species, *Aporometra paedophora* (H. L. Clark, 1909), remains to be resolved, but it may be a junior synonym of *A. wilsoni*. Morphological diagnoses are reviewed. *Aporometra occidentalis* was only found in Western Australia, while *A. wilsoni* was found from Western Australia to Victoria. Phylogeographic differentiation between the western and southern populations of *A. wilsoni* is briefly discussed.

Introduction

Crinoid echinoderms are distributed in a great range of habitats across the world, from the shallow intertidal zone to depths of well over two kilometres (Meyer *et al.* 1978). The great majority of comatulid crinoid (featherstar) species are found in shallow-water tropical regions, with species numbers in the Indo-Malayan Archipelago alone comprising 40% of the total described comatulid fauna (Messing *et al.* 2000). Comatulids also extend throughout temperate and polar waters (Messing 1997), albeit with far less diversity. Hence, of the 132 species recorded from Australia, only 24 are known from temperate southern waters (Rowe and Gates 1995). To date there has been no biogeographic or phylogeographic study of any crinoid groups or species.

A. H. Clark, who published a series of monographs over an extended period (1915, 1921, 1931, 1941, 1947, 1950; Clark and Clark 1967), was the dominant voice in the taxonomy and systematics of featherstars during the first half of the twentieth century. Some comatulid families and genera have been revised since (e.g. Messing 1981; Hoggett and Rowe 1986; Rowe *et al.* 1986), though it is only recently that rigorous methods have been used to infer relationships (Messing *et al.* 2000; Messing and White 2001). The present study examines the unusual comatulid genus *Aporometra* Clark 1938, which is endemic to temperate southern

Australian waters (Rowe and Gates 1995). Using a combination of anatomy (including scanning electron microscopy) and mtDNA sequences (*CO1* and *ND2*), we assessed the number of species in *Aporometra*, their distribution and morphology.

Taxonomic status of species within Aporometra

Aporometra are among the smallest of crinoids as adults, and are among the few crinoids observed carrying different stages of larvae in and on the pinnules. The term 'viviparous' has been used to describe this larval brooding (Clark and Clark 1967), but whether there is actual maternal nourishment of embryos and/or larvae has yet to be established. Ovoviviparous may be a better term. Based in part on its unusual reproductive mechanism, H. L. Clark (1938) established the family Aporometridae for *Aporometra* and recognised three species: *Aporometra wilsoni* (Bell, 1888), *Aporometra paedophora* (H. L. Clark, 1909) and *Aporometra occidentalis* H. L. Clark, 1938 (see Fig. 1). The first *Aporometra,* from Port Phillip Bay, Victoria, was described by Bell (1888) as *Antedon wilsoni*. H. L. Clark (1909) described *Himerometra paedophora* from New South Wales and observed attached pentacrinoid postlarvae (Fig. 2). He later postulated that *Antedon wilsoni* and *H. paedophora* represented a single species (H. L. Clark 1916). Initially,

A. H. Clark believed *A. wilsoni* and *H. paedophora* were juveniles of the crinoids *Ptilometra macronema* (Müller, 1846) and *Ptilometra muelleri* A. H. Clark, 1909 respectively (A. H. Clark 1909; A. H. Clark 1911). He examined additional specimens similar to *A. wilsoni* and *H. paedophora* collected from Koombana Bay, Western Australia, and argued that these also represented juvenile *P. macronema* (A. H. Clark 1911). Later, realising that they were actually unusually small adult crinoids, H. L. Clark (1938) reinstated *A. wilsoni* and *H. paedophora* as valid species in a new genus, *Aporometra,* and described the Koombana specimens as a new species, *A. occidentalis* (and designated this as the type species of the genus). The systematics of *Aporometra* was most recently assessed by Clark and Clark (1967) who studied the types of the three nominal species and suggested that they could be 'local races of the same specific type', though they did not formally synonymise them. Rowe and Gates (1995) accepted all three *Aporometra* spp. as valid.

The identification of *Aporometra* species is problematic. H. L. Clark's (1938) key gives cirral length as the sole species-level diagnostic character. However, cirral length is an age-related character; cirri develop around the centrodorsal as comatulids grow, potentially changing in number, size, and proportion over time (Mortensen 1920). Original and subsequent descriptions and illustrations of the nominal *Aporometra* species (Fig. 2) provide few other diagnostic characters.

The known geographic range of *Aporometra* has expanded since Clark (1938) and Clark and Clark (1967) discussed the species boundaries of the genus (Figs 1, 10). Examination of museum collections (see Appendix 1) shows that specimens from the Western Australian coast are commonly attributed to *A. occidentalis*; specimens from the coast of Victoria and South Australia are identified as

A. wilsoni; while specimens from Bass Strait have been identified as *A. paedophora*. Based on these designations, the current bathymetric ranges are: '*A. wilsoni*' 0–20 m, '*A. paedophora*' 20–40 m, and '*A. occidentalis*' 0–18 m (Appendix 1).

Materials and methods

Specimens of *Aporometra* collected for sequencing were deposited in the South Australian Museum, Adelaide (SAM). Specimens were also obtained via loan from collections of the Museum Victoria, Melbourne (MV), Australian Museum, Sydney (AM), Western Australian Museum, Perth (WAM), California Academy of Sciences, San Francisco (CAS), and Museum of Comparative Zoology, Harvard University (MCZ). All specimens are stored in ethanol, apart from the holotype of *A. occidentalis,* which is dry. Appendix 1 lists the origin, locality data, museum registration and GenBank numbers for the 38 specimens examined.

Character states (Table 1) were observed and scored using both light microscopy and scanning electron microscopy (SEM). When possible, partial or complete specimens of *Aporometra* species and *Notocrinus virilis* were observed and photographed using a Philips XL20 scanning electron microscope (Philips, Amsterdam, The Netherlands). Specimens were dissociated in commercial bleach (5% sodium hypochlorite solution); ossicles were rinsed with sterile water, air-dried, and mounted on carbon-covered stubs with conductive silver liquid. Stubs were sputter-coated with gold and palladium or platinum. All other specimens were observed and photographed using a Leica MZ-8 light microscope (Leica Microsystems, Wetzlar, Germany).

Morphological features used to develop characters

Comatulid morphology is described here with attention to features used to develop characters for this cladistic analysis. Table 1 and Appendix 2 list characters and justification for their use; Table 2 shows the morphology matrix. Although many of the characters are not informative in this context owing to the conserved morphology of *Aporometra,* they have potential use in subsequent broader studies. The descriptions below follow the terminology and symbols of Messing *et al.* (2000). Figures in parentheses below refer to numbered characters in Table 1.
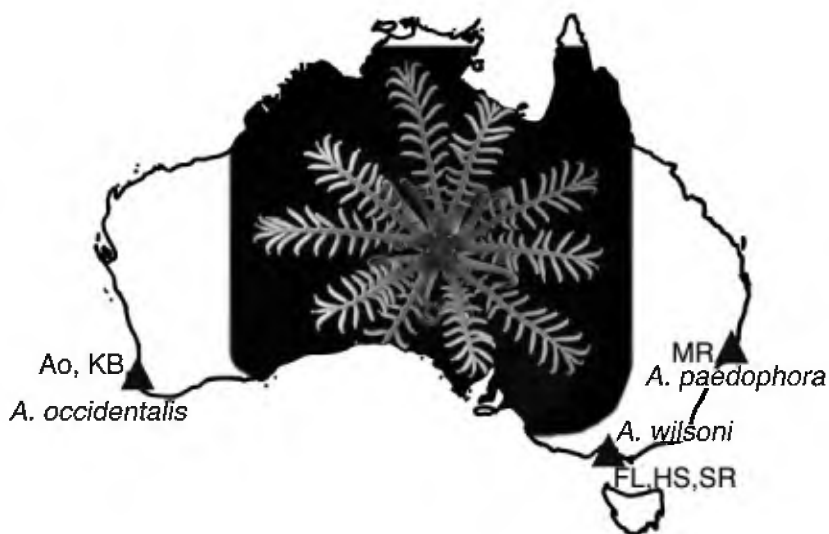

Fig. 1. Map of Australia showing type localities (▲) and corresponding or adjacent sites included in this study. Inset: *Aporometra wilsoni* from Witton Bluff, South Australia.

Centrodorsal

The centrodorsal, the large central aboral ossicle, bears numerous cirrus sockets (Fig. 3*b*, *f*) (20) and may be conical, discoidal or hemispherical (21). As the centrodorsal enlarges, cirri are added to the oral margin and may be situated in columns or crowded across the surface. Flat or raised mid-radial spaces lacking cirrus sockets may be present (22).

Cirri

The hook-like anchoring cirri are made up of ossicles called cirrals. The terminal cirral is a tapered, usually curved claw, and the preceding (penultimate) cirral (8–9) often bears a spine (Fig. 3*a*). Cirral articulations are ligamentary, and the articular facets consist of a central cavity and fulcral tubercle(s) (Figs 3*b*, *f*, 4*d–e*) (1, 10–11). Cirri vary in number, length, and number of cirrals per cirrus (A. H. Clark 1915; Messing *et al.* 2000; Messing and White 2001). Cirral length usually follows a distinct pattern. One or a few proximal cirral(s) are short and similarly shaped in all comatulids. In most cases, at least a few of the following cirrals become longer, with the more distal cirrals decreasing again in length and with most distal cirrals typically quite short. Because the length of the cirrals has been used as the key diagnostic feature of each species (H. L. Clark 1938) this study emphasised cirrus characters and examined in detail one proximal and one distal cirral. The proximal cirral was taken as the first cirral characteristic of the longer proximal cirrals, this ranged between the 3rd to 5th cirral (1–7). The distal cirral was taken as the cirral preceding the penultimate, and was characteristic of the cirrals in the distal half (10–19). Cirral shape (Figs 4, 5) (2–3, 5–6, 12–13, 15–18), surface texture (4, 14) and ornamentation (7–8, 19) also vary and include apomorphic characters (Figs 3*a*, 4, 5). Sometimes cirrals have what can described as a 'keel' in lateral view resulting from an expansion of the aboral surface (Figs 3*a*, 4*a–c*). In this paper the lateral view of an ossicle is also referred to as the profile. Carination is another form of ornamentation that refers to calcareous knobs, nodules or spines present on the surface of the cirrals (Fig. 5*a*, *c*, *d*, *f*, *g*). An effaced cirral surface is characterised by very small holes, or scratches, on the surface of the ossicle.

Calyx

The calyx is made up of two rings of ossicles: basals and radials. The radials are wedge-shaped ossicles that form a circlet or pentagon adjacent to the centrodorsal, support the disk and give rise to the rays. In all comatulids except atelecrinids, the basals are absent or reduced to a small internal rosette that covers the oral centrodorsal cavity, usually with interradial basal rays (23) that may appear between the aboral corners of the radials (Fig. 3*f*).

Disk

The disk is the crinoid's visceral mass, which bears both the mouth and anal cone on its upper (oral) surface (Fig. 3*e*) (26). The disk is covered by a tissue layer, the tegmen, across which the ambulacral grooves (25) extend from the mouth to the arms and pinnules. The tegmen may also contain calcium carbonate plates (24) or nodules.

Arms/Rays

Rays extend from the calyx and are made up of individual ossicles called brachials. The rays bifurcate at axils. An arm is the terminal branch of a ray, after the last axil. Although many crinoids have numerous arms, the rays of all *Aporometra* species bifurcate once, giving rise to 10 arms. The two brachials following the radial (the second is the axil) constitute a brachial (designated 1Br2). Individual brachitaxis ossicles are numbered in sequence designated by a subscript Arabic numeral (i.e. $1br_1$ and $1br_2$) and the following brachials of the undivided arm are numbered in sequence br_1, br_2, etc. Brachials vary in size and shape (28), and can be smooth, keeled, ridged, spined or sculpted (Fig. 3*c*, *d*).

Pinnules

Pinnules arise from alternating sides of successive brachials and are made up of pinnular ossicles joined by muscular articulations. Pinnulars may be smooth or carinate and may have side and cover plates and/or spicules along the ambulacral groove (Fig. 6). Pinnules are denoted by 'P' followed by an Arabic subscript on the outer side of the
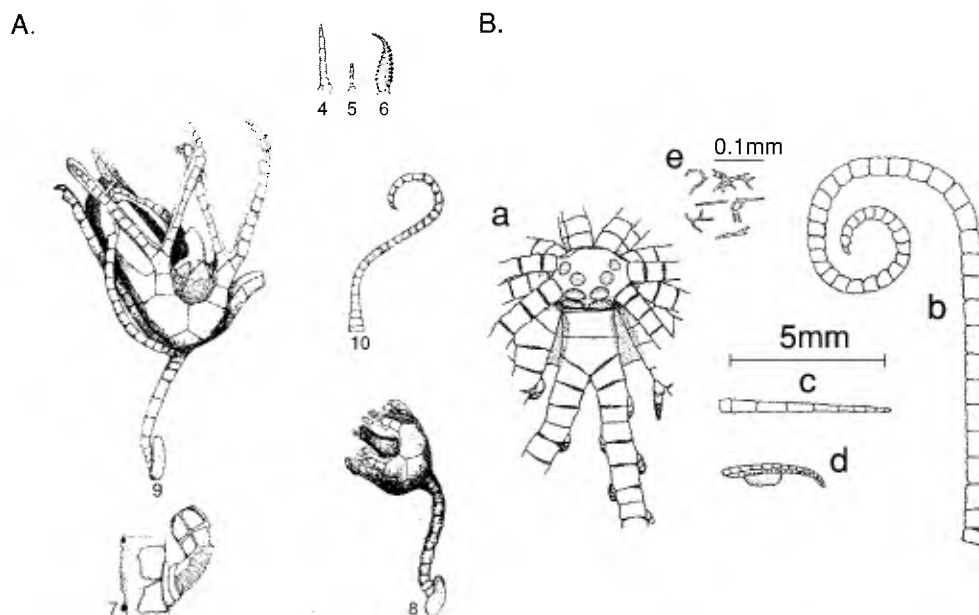

Fig. 2. The only published illustrations of *Aporometra* (*A*) *Aporometra paedophora*: *4–6*, pinnules; *7–9*, larvae; *10*, cirrus (H. L. Clark 1909). (*B*) *Aporometra wilsoni*: *a*, diagonal view of the centrodorsal; *b*, cirrus; *c–d*, pinnules; *e*, pinnular spicules (A. H. Clark and A.M. Clark 1967).

arm (e.g. P_1 on br_2 is the most proximal pinnule) and a letter subscript for the inner side of the arm (e.g. P_a follows P_1 but is the most proximal inner pinnule). The most proximal few pinnules are referred to as the oral pinnules (Fig. 6*a–d*) (29–31); they usually differ from succeeding pinnules in being more robust and spiny, longer and more flexible, or in lacking an ambulacral groove (Messing *et al.* 2000). A series of one or more genital (or middle) pinnules (Fig. 6*e–h*) follow the oral pinnules

and rarely differ from the more distal pinnules (Fig. 6*i, j*) that are primarily used for food collection. The gonads (27) are usually found along the pinnules in crinoids but are also located in the arms and more rarely in the tegmen (Holland 1991). Free spawning is the most common form of reproduction in crinoids; however, brooding, in which embryos develop to varying degrees in the maternal pinnules, also occurs (Holland 1991).

Table 1.   List of characters and character states

Proximal cirral cross section
    (*1*) *Fulcral ridge encloses central cavity:* (0) fully; (1) partly.
    (*2*) *Shape of cirral cross section:* (0) taller than wide; (1) round; (2) wider than tall.
Proximal cirral oral surface
    (*3*) *Shape of lateral edges:* (0) concave; (1) straight.
Proximal cirral aboral surface
    (*4*) *Texture of surface:* (0) smooth; (1) effaced; (2) carinate.
    (*5*) *Shape of lateral edges:* (0) straight; (1) concave.
Proximal cirral profile
    (*6*) *Shape of lateral edges:* (0) straight; (1) concave.
    (*7*) *Aboral edge:* (0) 'smooth'; (1) keeled.
Terminal ossicles
    (*8*) *Aboral edge of terminal cirral:* (0) 'smooth'; (1) with opposing spine.
    (9) Aboral margin of terminal claw: (0) straight; (1) curved.
Distal cirral cross section
    (*10*) *Shape of fulcral ridge:* (0) ovoid and elongate; (1) crescent.
    (*11*) *Fulcral ridge encloses central cavity:* (0) fully; (1) partly.
    (*12*) *Shape of cirral cross section:* (0) taller than wide; (1) wider than tall; (2) round.
Distal cirral oral surface
    (*13*) *Shape of distal edge:* (0) convex; (1) concave.
Distal cirral aboral surface
    (*14*) *Texture of surface:* (0) smooth; (1) carinate.
    (*15*) *Shape of aboral surface:* (0) rounded; (1) flattened.
Distal cirral profile
    (*16*) *Shape of proximal edge:* (0) convex; (1) s-shaped.
    (*17*) *Shape of distal edge:* (0) concave; (1) s-shaped.
    (*18*) *Shape of surface:* (0) flattened; (1) rounded.
    (*19*) *Aboral edge:* (0) keeled; (1) 'smooth'
Cirrus sockets
    (*20*) *Shape of fulcral ridge:* (0) ovoid and elongate; (1) crescentic.
Centrodorsal
    (*21*) *Shape:* (0) conical; (1) hemispherical; (2) discoidal.
    (*22*) *Mid-radial area:* (0) crowded with sockets; (1) clear.
    (*23*) *Distal, aboral corners of radial ossicles:* (0) adjacent; (1) interrupted by basal ray.
    (*24*) *Tegmen:* (0) naked; (1) plated.
    (*25*) *First ambulacral division:* (0) on disk; (1) at edge of disk; (2) distal to disk edge.
    (*26*) *Disk border at the:* (0) br_{10}; (1) br_4; (2) br_2 (3) br_6
Rays
    (*27*) *Location of gonads:* (0) arm; (1) pinnule.
    (*28*) *Cross section of Ibr$_1$:* (0) rounded; (1) triangular.
Oral pinnule (P_1)
    (*29*) *Aboral surface:* (0) smooth; (1) carinate.
    (*30*) *Other surfaces:* (0) smooth; (1) carinate.
    (*31*) *Adambulacral edges of pinnulars:* (0) smooth; (1) with distally directed spines.
Genital/middle pinnule
    (*32*) *Oral edges of pinnulars:* (0) smooth; (1) with distal knob.
    (*33*) *Coverage of ambulacral groove:* (0) no plates; (1) cover and side plates.
    (*34*) *Tissue along ambulacral groove:* (0) empty; (1) contains spicules.
    (*35*) *Terminal pinnular relative to preceding pinnular:* (0) equally spinose; (1) more spinose.
Distal pinnules
    (*36*) *Coverage of ambulacral groove:* (0) no plates; (1) cover and side plates.
    (*37*) *Terminal pinnular relative to preceding pinnular:* (0) equally spinose; (1) more spinose.
Reproduction
    (*38*) *Larvae develop: (0) away from adult; (1) on the adult.*

Characters not used

Taxonomic descriptions of crinoid morphology often include configurations of the centrodorsal cavity and radial articulations. These were observed in only a few *Aporometra* specimens using SEM because complete disassociation of the animal is required. Given that these features were the same across the specimens observed, and that many specimens could not be disassociated, character scores were not included for these features. Characters of the rays were also excluded owing to a lack of variation in carination and shape across all specimens examined. Some pinnule characters of shape and carination were excluded from the final analyses as they were similarly uniform.

DNA extraction

All tissues were stored in ethanol; total genomic DNA was extracted according to the QIAGEN DNeasy protocol (QIAGEN, Hilden, Germany) for animal tissues using proteinase K and was isolated using spin filter followed by ethanol precipitation. The gDNA was then cleaned using the cetyltrimethylammonium bromide (CTAB) method of Scouras and Smith (2001) and the samples stored at 4°C.

Amplification

Sequences were initially amplified for *Aporometra* species and *Notocrinus virilis* using primers designed by Andrea Scouras and Teena Browning (personal communication). Specific primers were then developed for amplifying sequence from these taxa (Table 3). Polymerase chain reaction (PCR) was carried out in a thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions, repeated for 35 cycles: denaturation at 94°C for 45 s, annealing at minimum 48°C and maximum 55°C for 45 s and extension at 72°C for 1 min. The PCR reagents in a 50-μL reaction were 0.2-μL TaqGold (5 units μL^{-1}, Applied Biosystems, Foster City, CA, USA), 2 μL per primer (5 μM), 4-μL dNTPs (10 μM), 8 μL MgCl$_2$ (25 μM), 5 μL TGold Buffer (Applied Biosystems), 5 μL gDNA and 23.8 μL sterile water.

Sequencing

Polymerase chain reaction products were cleaned using MoBio spin clean kit (MoBio, Carisbad, CA, USA). If more than one product was present, the desired product was isolated using agarose gel (1.5%) electrophoresis and cleaned using the QIAGEN QIAquick gel extraction kit. Polymerase chain reaction products were amplified for sequencing using a 20 μL reaction mixture of 5 μL of PCR product, 1 μL of primer (5 μM), 2 μL Big Dye 5× Buffer (Applied Biosystems), 4 μL Big Dye version 3 (Applied Biosystems) and 8 μL sterile water. The reaction was run using a thermal cycler (Eppendorf) with denaturation at 96°C for 30 s, annealing at 50°C for 15 s and extension at 60°C for 4 min.; this was repeated for 25 cycles. The product was cleaned using 70% isopropanol and sequenced at the Institute of Medical and

Table 2. Matrix of morphology characters for all *Aporometra*, *Notocrinus virilis* and *F. serratissima*

|	Characters			
	0000000001 1234567890	1111111112 1234567890	2222222223 1234567890	33333333 12345678
Fs1	?00110111	0111111111	1001111110	00011011
Ao3	02?0100111	011111111?	1001111110	00011011
KB1	110110011?	?111111111	1001111110	11011011
KB2	0111100111	0111111111	1001A11110	11011011
KB3	??11100??1	011?111111	1001111110	11011011
DU1	0112000111	111111111?	2001111110	1?011011
DU2	?112000011?	?111111111?	2001221110	1?011011
DU3	011000011?	?111111111?	2001221110	1?011011
AL1	0211000111	011111111?	1001111110	11011011
AL2	0101100111	0111111111	1001A11110	11011011
AL3	0111100111	0111111111	2001111110	11011011
ES1	1201100110	1111111111	1001111110	11011011
RA1	0100110111	0111111111	1001131110	00011011
RA2	0100110111	0111111111	10?1A11110	00011011
RA3	0100110111	0111111111	1001111110	00011011
AD1	1201100111	1111111111	1001111110	10011011
AD2	1201100111	1111111111	1001111110	10011011
AD3	1201100111	1111111111	?001111110	10011011
AD4	1201100111	1111111111	1001111110	10011011
FL1	1201100111	0111111111	0001111110	10011011
FL2	1201100111	0111111111	1001111110	10011011
SR1	?10110011?	?21?111111	2001221110	10011011
SR2	?10110011?	?211111111	2001221110	?0011011
Gl1	1101110111	1111111111	1001111110	10011011
Gl2	1101110111	1111111111	1001111110	10011011
Gl3	1101110111	1111111111	1001111110	10011011
Gl4	1101110111	1111111111	1001111110	10011011
MR1	1201110111	011111111?	20?1121?10	10011011
MR2	120111011?	011111111?	20?1221?10	00011011
MR3	120111011?	011111111?	20?1221?10	00011011
MR4	120111011?	0111111111	20?1221?10	10011011

A, Polymorphism 0&1.
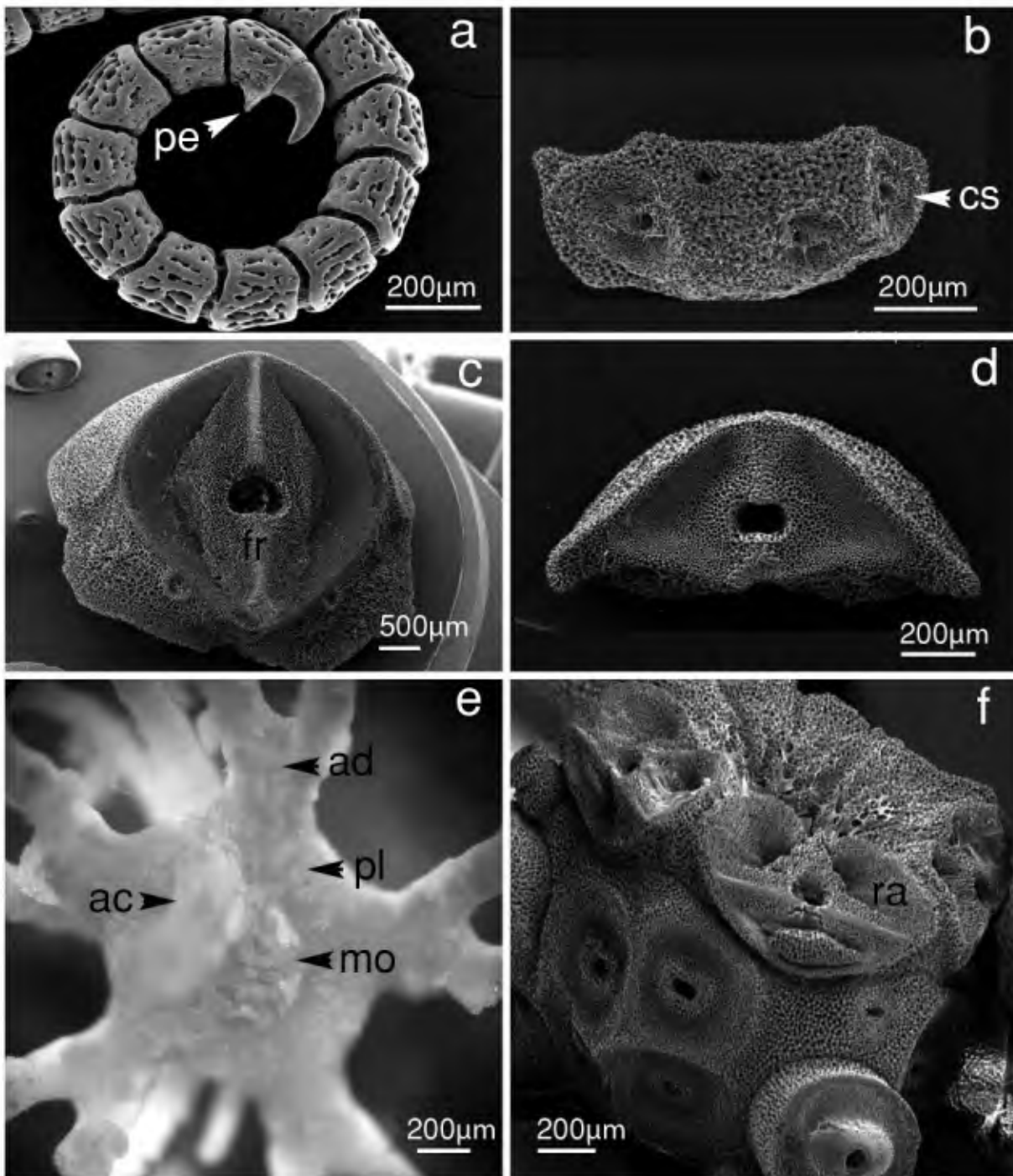
Fig. 3. Morphology of *Aporometra wilsoni* (*a*, *b*, *d–f*) and *Notocrinus virilis* (*c*). *a*, cirrus profile from Adelaide (AD4); *b*, centrodorsal from San Remo (SR1); *c*, Ibr_1 distal articulation showing rounded shape: *N. virilis*; *d*, Ibr_1 distal articulation showing triangular shape: Gabo Island (GI3); *e*, partially dissolved disk showing ambulacral division beyond edge of disk: San Remo (SR1); *f*, calyx with socket fulcral ridge crescentic and ends of basal rays not visible: Gabo Island (GI4). Abbreviations: ac, anal cone; ad, ambulacral division; cs, cirrus socket; fr, fulcral ridge; mo, mouth; pe, opposing spine on penultimate cirral; pl, plate; ra, radial.
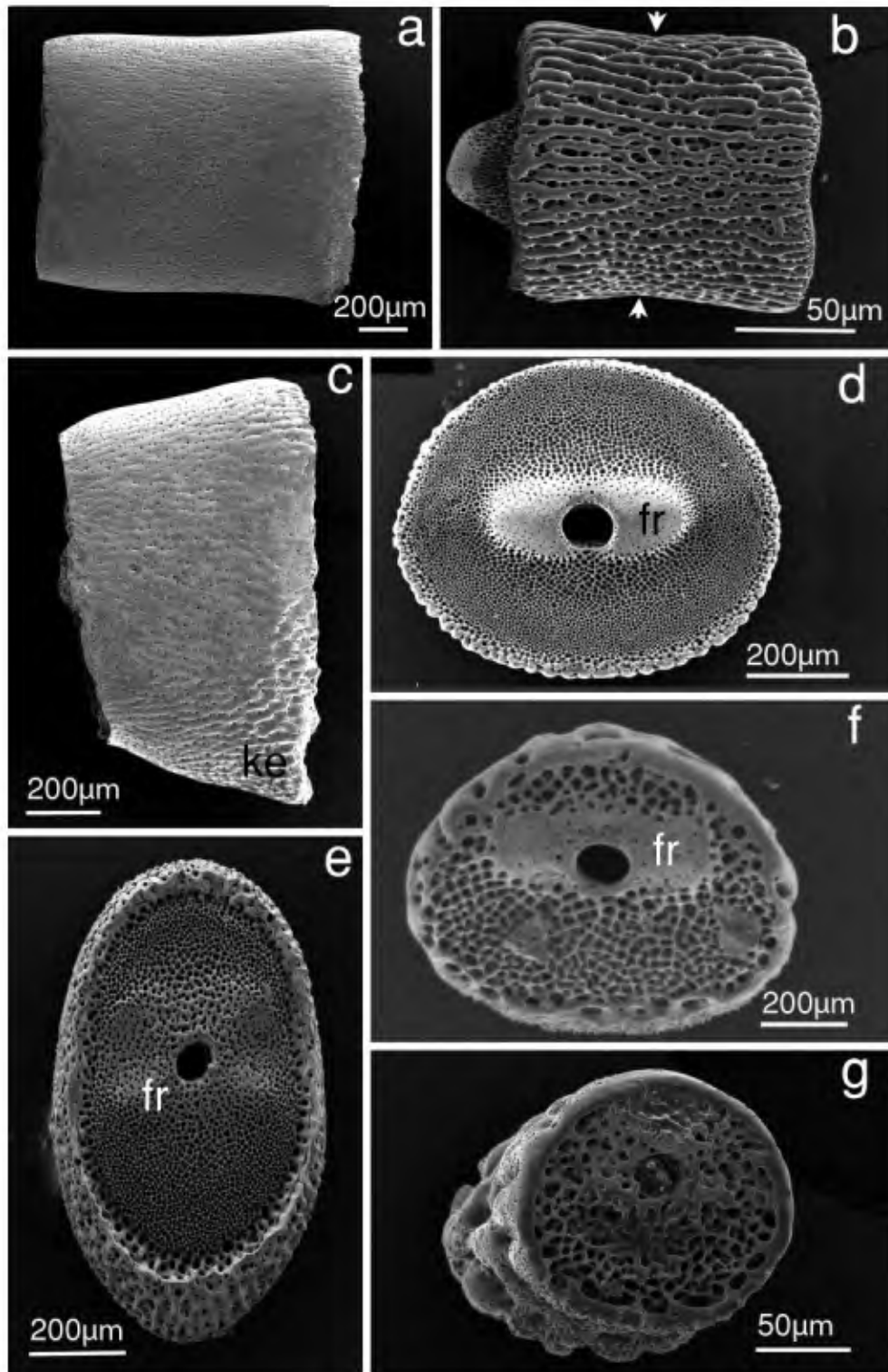
Fig. 4. Cirral morphology. *a*, proximal cirral profile of *Notocrinus virilis*; *b*, proximal cirral profile showing concave lateral edges (arrows) and smooth aboral surface: *Aporometra occidentalis* from Recherche Archipelago (RA1); *c*, distal cirral profile showing aboral keel: *N. virilis*; *d*, proximal cirral wider than tall in cross section and fulcral ridge partly surrounds lumen: *A. wilsoni* from Flinders (FL1); *e*, distal cirral taller than wide in cross section and fulcral ridge surrounds lumen: *N.* virilis; *f*, distal cirral wider than tall in cross section and fulcral ridge partly surrounds lumen: *A. wilsoni* from Perth (PE4), and *g*, distal cirral round in cross section: *A. wilsoni* from San Remo (SR1). Abbreviations: fr, fulcral ridge; ke, keel.
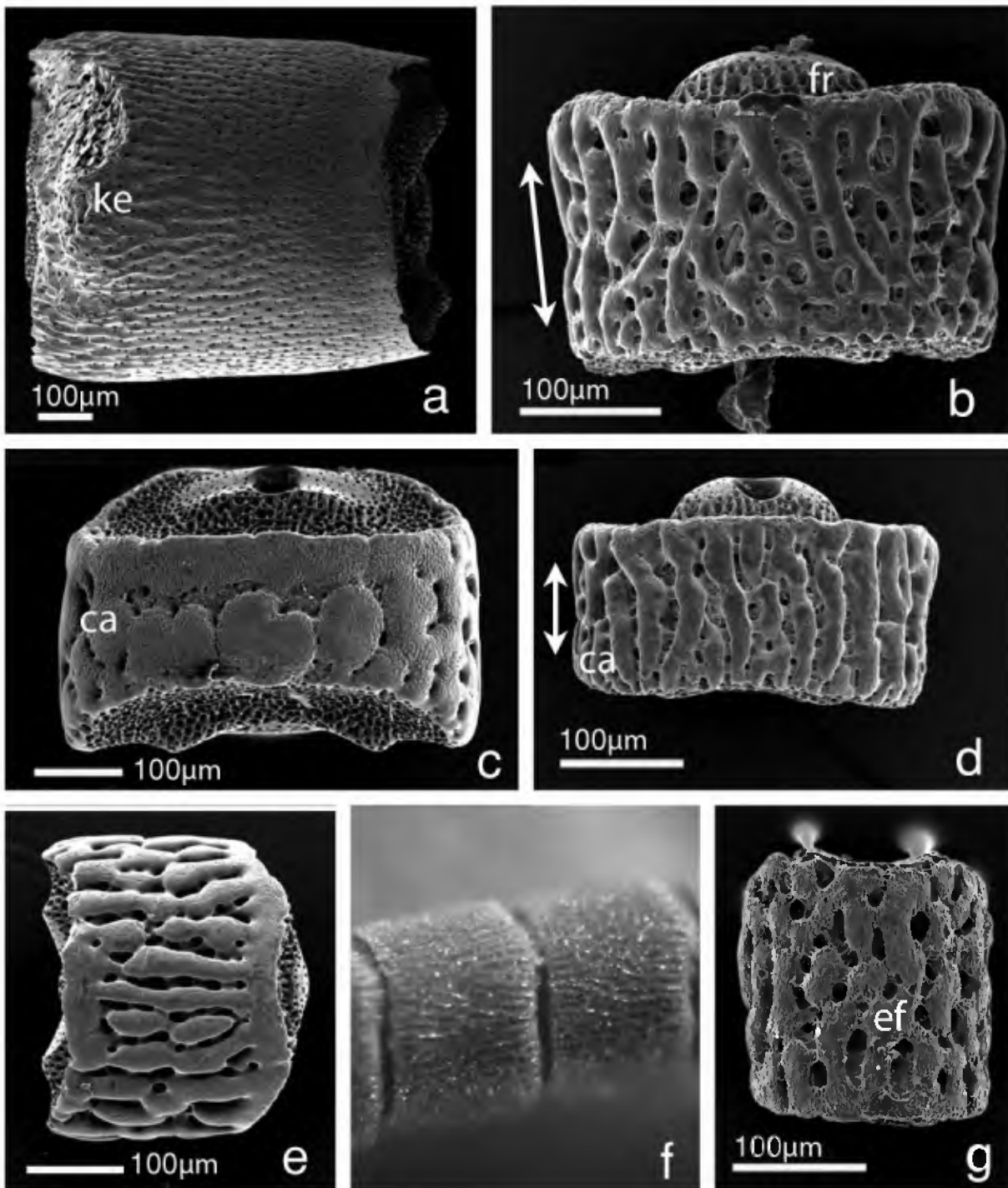
Fig. 5. Cirral morphology: *a*, distal cirral aboral surface smooth and rounded: *Notocrinus virilis*; *b*, proximal cirral with effaced oral surface and straight lateral edges (line with arrows): *Aporometra wilsoni* from Dunsborough (DU1); *c*, distal cirral with carinate aboral surface of *A. wilsoni* from Esperance (ES1); *d*, proximal cirral with carinate aboral surface and straight lateral edges (line with arrows): *A. wilsoni* from Dunsborough (DU1); *e*, distal cirral oral surface with concave distal edge: *A. wilsoni* from Gabo Island (GI3); *f*, proximal cirral with smooth aboral surface: *A. occidentalis* from the type collection (Ao1); *g*, proximal cirral with effaced aboral surface: *A. wilsoni* from San Remo (SR1). Abbreviations: ca, carinate; ef, effaced.
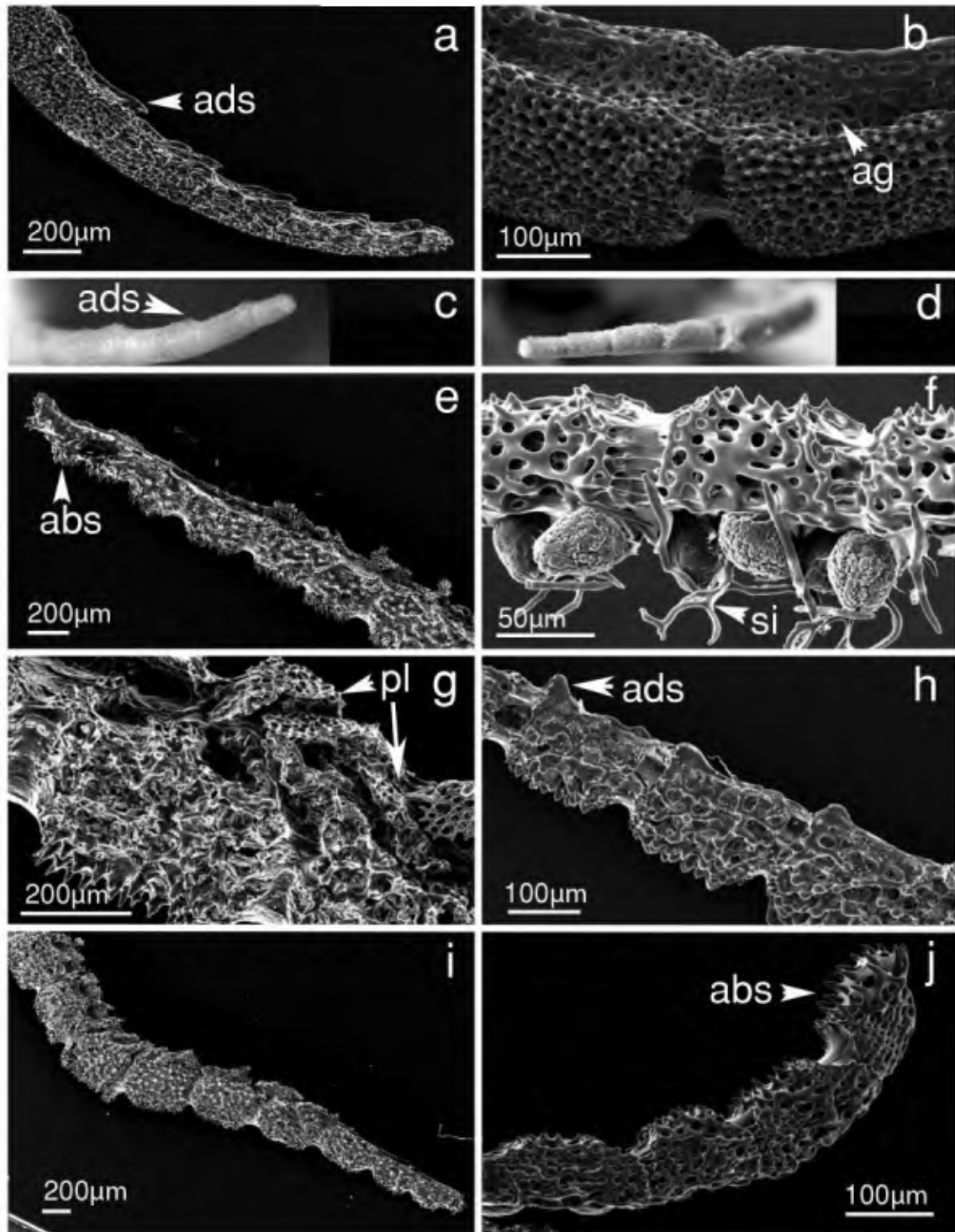
Fig. 6. Pinnule morphology. *a*, distal portion of P$_1$ showing distally directed spines (ads): *Aporometra wilsoni* from Esperance (ES1); *b*, portions of two successive middle pinnulars of P$_1$ showing smooth adambulacral edges: *A. occidentalis* from Recherche Archipelago (RA1); *c*, light microscope image of P$_1$ showing distally directed spines (ads): *A. wilsoni* from Honeysuckle Point; *d*, light microscope image of P$_1$ showing smooth adambulacral edges of *A. occidentalis* from the type collection (Ao1). *e*; distal portion of middle pinnule: *Notocrinus virilis*; *f*, three pinnulars of the middle pinnule showing spicules: *A. wilsoni* from Adelaide (AD4); *g*, pinnular from middle pinnule showing side plates: *N. virilis*; *h*, three distal pinnulars of the middle pinnule showing distal knobs: *A. wilsoni* from Esperance (ES1); *i*, distal portion of distal pinnule showing lack of strong spines on terminal pinnulars: *N. virilis*; *j*, distal portion of distal pinnule showing terminal pinnular more strongly spinose than preceding pinnulars: *A. wilsoni* from Adelaide (AD4). Abbreviations: abs, aboral spines; ads, adambulacral spines; ag, ambulacral groove; pl; plates; si, spicules.

Veterinary Science, Adelaide, SA, Australia using an automated sequencer (Applied Biosystems).

Editing and alignment

Sequence data was edited using SeqEd version 1.0.3 (Applied Biosystems) and aligned manually using SeAl version 2.0a11 (Rambaut 1996). Owing to sequence length variation 1273 bp of *CO1* (*Florometra serratissima* mtDNA nucleotide positions 7382 to 8655) and 332 bp of *ND2* (*F. serratissima* mtDNA nucleotide positions 5303 to 5634) were retained for analyses. Morphological data was entered into MacClade 4.6 (Maddison and Maddison 2003) with molecular data added as an extension of the morphological data for corresponding specimens, where relevant. All characters were translated into numeric code (A = 0; C = 1; G = 2; T = 3) for the combined analysis. Sequences are deposited in GenBank (www.ncbi.nih.gov/Genbank/index.html, verified April 2006) under the accession numbers listed in Appendix 1.

Analysis

We had no sequence data from type material and needed to assess where these terminals fell in relation to those with sequence data available. In order to assess congruence of the various datasets, the following analyses were performed:

Morphology only

All specimens (38 terminals). Specimen SR3 (Appendix 1) was in poor condition, and morphology scores could not be determined for it.

Reduced morphology

One *Aporometra* specimen from each locality from which *ND2* sequence data was also obtained (nine terminals) and included terminals for which the most complete data was available. Hence the analysis excluded type material and specimens from Esperance or Koombana Bay (both Western Australia). This sampling of terminals was thus for those where the most complete data was available.

CO1

All specimens from which *CO1* data was obtained (26 terminals).

Reduced CO1

One *Aporometra* from each locality, i.e. the same taxon selection as analyses 2, 5 and 6 (nine terminals).

ND2

One *Aporometra* from each locality, as in analyses 2, 4, and 6 (nine terminals).

Reduced molecular

Combined *ND2* and *CO1* sequences from the same taxon set as the Reduced Morphology, *ND2* and Reduced *CO1* analyses (analyses 2, 4, 5) (nine terminals).

Total molecular

Genetic data from all taxa for which *CO1* and/or *ND2* sequence was available-the same terminals used in the *CO1* analysis (analysis 3) (26 terminals).

Total combined

Morphology scores, *CO1* and *ND2* sequence. Only those *Aporometra* with two or more datasets were included to minimize gaps (25 terminals).

Outgroups

Outgroups used in the analyses were the featherstars *Notocrinus virilis* (Notocrinidae) and *Florometra serratissima* (Antedonidae). We used *N. virilis* in the morphological and combined analyses as a representative of the monogeneric Notocrinidae because this family and the Aporometridae constitute the Notocrinida in the most current classification of comatulids (Clark and Clark 1967). Sequence for *ND2* could not be obtained for *N. virilis* so another outgroup, *F. serratissima* was used in all analyses since sequence data for both *CO1* and *ND2* was available.

Maximum parsimony analyses were conducted using PAUP* 4.0b10 (Swofford 2003) for the morphological, molecular and combined data analyses. All characters were unordered and equally weighted. Heuristic searches were conducted using random stepwise addition for 100 replicates. Tree bisection reconnection (TBR) branch swapping algorithm with maximum zero-length branches collapsed. Accelerated transformation (ACCTRAN) was used for character transformations. MacClade was used to observe the character changes in the trees generated in PAUP*. Kimura two-parameter distances were also calculated in PAUP* for the molecular datasets. Bootstrap and Jackknife support values were calculated for all trees with molecular data, and decay values for the trees with morphological data, using the

Table 3. Primers used for amplification and sequencing of cytochrome c oxidase subunit 1 and NADH dehydrogenase subunit 2

Primer ID	Sequence (5 –3)	Direction	Mitochondrial map position (Florometra Serratissima)	Designer
Fsco1 F	AGT CGT TGG TTG TTT TCT AC	F	7263 – 7282	A. Scouras
Co1er	GCT CGT GTR TCT ACR TCC AT	R	8142 – 8161	A. Scouras
Co1 820f	ATG GTT TAT GCT ATG GTT GCT AT	F	8082 – 8105	T. Browning
Co1 3 r	CAA TGA GTA AAA CCA GAA	R	8423 – 8440	T. Browning
awArg	AAG CAC ACG AGC CGA AAT	R	8834 – 8852	L. Helgen
Awco1f	GTG CTT GAT CTG GAA TGG TTG	F	7334 – 7355	L. Helgen
Co1 635r	GGA TCA CCA CCW CCA GCW GGA TC	R	7898 – 7949	T. Browning
Awco1f2	GAT CGA CTW CCT TTA TTT GT	F	7714 – 7723	L. Helgen
Awco1r2	AAT TAC TAA ACG ACT TGA GAT	R	8577 – 8597	L. Helgen
fstyr*	TTA GTC TTT CAG CCA TTT TG	F	5072 – 5091	A. Scouras
Fsile	TCA AAT CCT CAC AAA CTC TAT G	R	6130 – 6200	A. Scouras
awnd2f	ATT MAA GCA MTA GCW GCA GC	F	5353 – 5372	L. Helgen
awnd2r	TCA TCC WAT RTG TTT TAT TG	R	5641 – 5660	L. Helgen

* Minor modification by L. Helgen

heuristic search method, with 10 to 100 random stepwise additions, for 100 to 1000 replicates. For the Jackknife support 37% character deletion was used according to Farris, Albert *et al.* (1996).

Results

Morphology

Nineteen of the 38 characters were uninformative, but may prove useful in more comprehensive crinoid studies. Maximum parsimony analysis of the morphology dataset

resulted in 42 847 most parsimonious trees with a length of 71 (consistency index, $CI = 0.68$; rescaled consistency index, $RC = 0.56$ for informative characters only), and the strict consensus tree is shown in Fig. 7*a*. The two specimens of *Aporometra* from closest to the type locality of *A. wilsoni* (San Remo, Victoria) and the four specimens in the type series of *A. paedophora* from Manning River (MR), New South Wales consistently formed a clade with specimens from Perth (PE, four specimens), Koombana Bay (KB, 3),
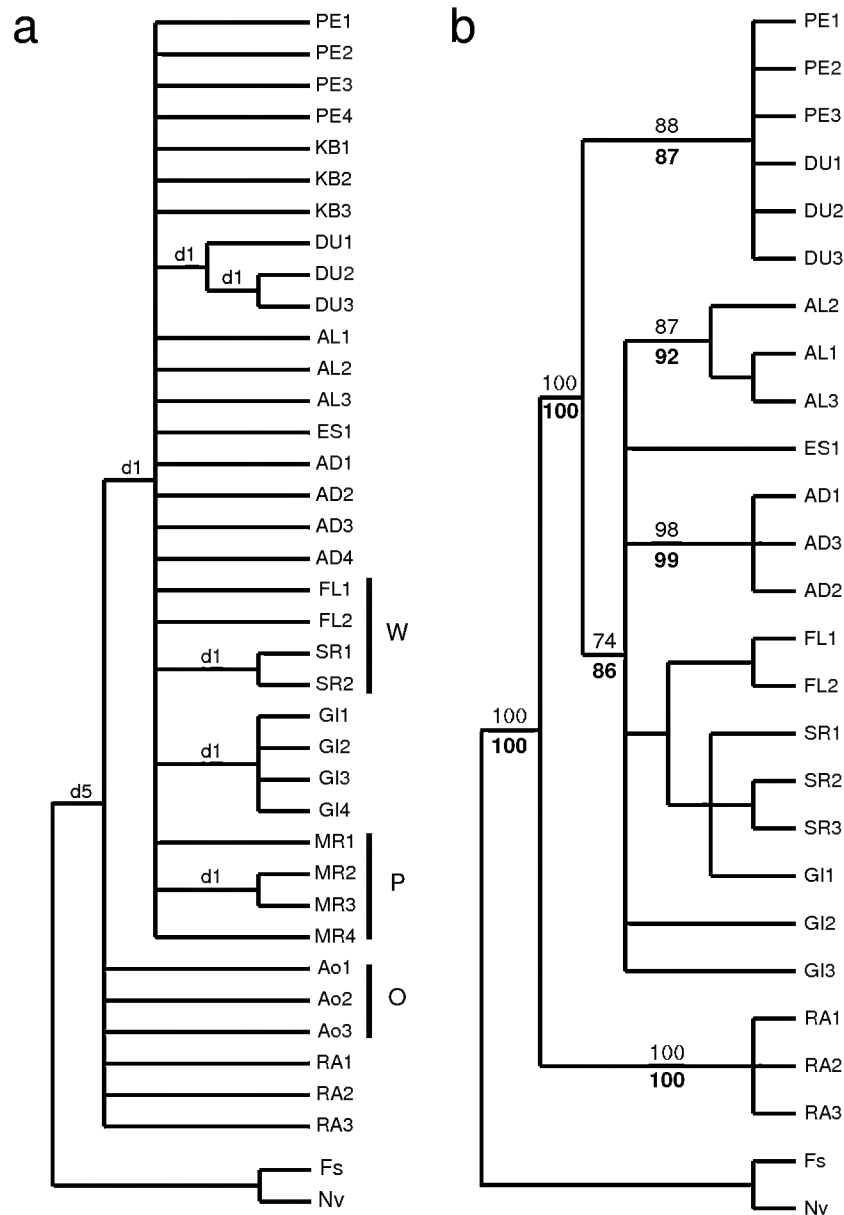

Fig. 7. *a*, Strict consensus of 42 847 most parsimonious trees (length 71) from morphology data showing decay indices. The labels P = *Aporometra paedophora* and O = *A. occidentalis* are based on type material and W = *A. wilsoni* specimens collected in proximity to the type locality. *b*, Strict consensus of 521 most parsimonious trees (length 324) from *CO1* data showing support values for bootstrap (top) and jackknife (bottom, bold) >70%

Dunsborough (DU, 3) and Esperance (ES, 1), all Western Australia; Adelaide (AD, 4) South Australia, Flinders (FL, 2) and Gabo Island (GI, 4), both Victoria, and with a decay value of 1. The remaining six *Aporometra* specimens (the three types of *A. occidentalis* from Koombana Bay (Ao) and three specimens from the Recherche Archipelago (RA) both Western Australia) formed a polytomy with the preceding clade.

Reduced morphology

Twenty-eight of the 38 characters were uninformative, and the analysis produced 24 trees with a length of 33 (consistency index (*CI*) = 0.82, rescaled consistency (*RC*) = 0.51 for informative characters only). The consensus tree is not shown but is similar to the morphology tree, in that the RA specimens are sister to a clade of the remaining *Aporometra* specimens (decay value = 1). Within this clade, only PE1 and SR1 consistently formed a clade (decay value = 2).

CO1

Analysis of the *CO1* sequence data, which had 122 parsimony informative sites, produced 521 shortest trees of length 324 (*CI* = 0.85; *RC* = 0.77 for informative characters only). Figure 7*b* shows the strict consensus tree with values from bootstrap and jackknife analyses. Here, the three RA specimens were sister group to a well supported clade of all remaining *Aporometra* specimens. Within this clade, the Western Australia specimens from Perth and Dunsborough consistently formed a clade that was sister to the remaining terminals.

Reduced CO1

This analysis had 33 informative sites and produced a single most parsimonious tree (Fig. 8*a*) with a length of 241 (*CI* = 0.80; *RC* = 0.79 for informative characters only). This pruned sample of terminals resulted in the same basic topology as in the broader analysis of *CO1* data (Fig. 7*b*).

ND2 *only*

The *ND2* data, with only six informative sites, produced 163 trees with a length of 92 (*CI* = 0.86; *RC* = 0.71); the consensus tree is shown in Fig. 8*b*. As with the *CO1* data, the specimen from the Recherche Archipelago (RA1) was the sister group to the remaining *Aporometra*.

Reduced molecular

The restricted terminal analysis of combined *CO1* and *ND2* sequence data (39 informative sites) resulted in a single most parsimonious tree (Fig. 9*a*) of length 333 (*CI* = 0.97; *RC* = 0.77 for informative characters only) and was identical to the Reduced *CO1* analysis.

Total molecular

Analysis of terminals for which any sequence data was available (131 informative sites) produced 5957 (for majority of reps) trees of length 416 (*CI* = 0.80; *RC* = 0.65); the consensus tree (not shown) has a very similar topology to the Total combined tree (Fig. 9*b*).

Total combined

The addition of the morphology dataset for those terminals for which any sequence data was available (150 informative
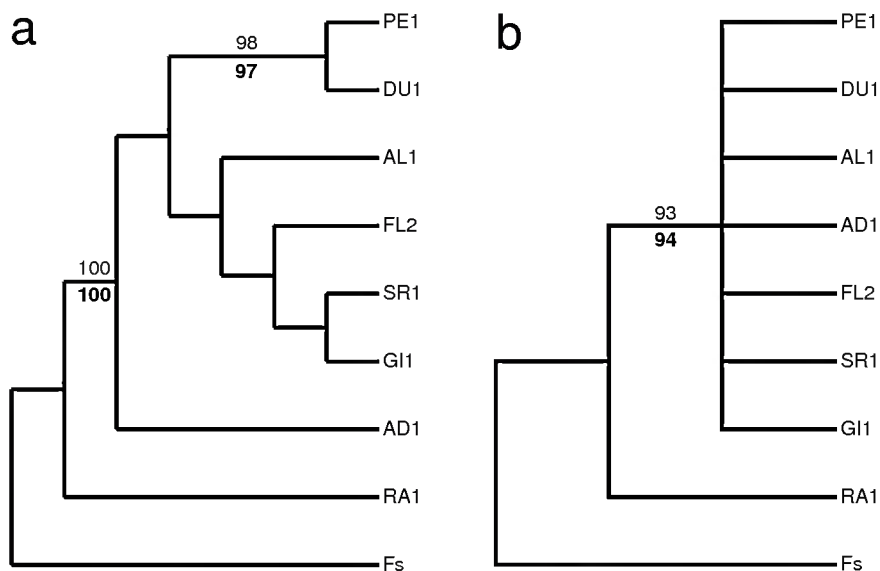

Fig. 8.   *a*, Single most parsimonious tree (length 241) from *CO1* reduced taxon set; *b*, strict consensus of 163 most parsimonious trees (length 92) from *ND2* sequence. Support values for bootstrap (top) and jackknife (bottom, bold) >70% are shown on both trees.

characters) produced 438 shortest trees with a length of 487 (*CI* = 0.90; *RC* = 0.78 for informative characters only). The consensus tree and one of the 438 trees are shown in Fig. 9*b*, *c*, respectively. This total evidence analysis gave a result that was congruent with the other partitioned analyses.

A few clades appeared consistently in the analyses. All analyses returned a well supported clade encompassing

specimens from Perth to Gabo Island (Figs 7–9) and containing individuals collected near the type locality of *A. wilsoni* (Figs 1, 10). This clade is referred to hereafter as *A. wilsoni* (see Discussion). Within the *A. wilsoni* clade there was reciprocal monophyly between the specimens from Perth and Dunsborough in the west and specimens from the remaining populations from southern waters (Figs 7*b*, 8*a*, 9).
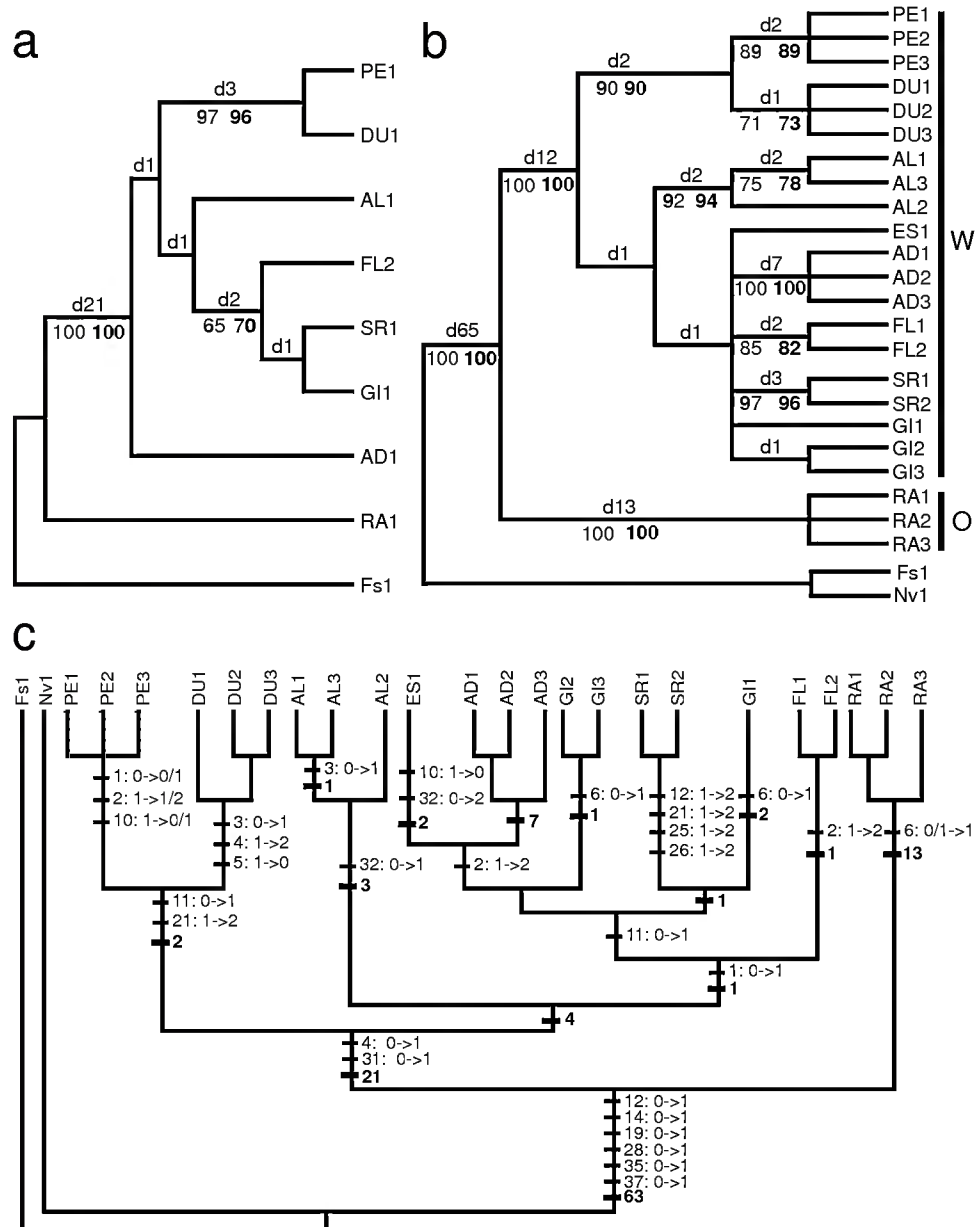

Fig. 9. *a*, Single most parsimonious tree (length 333) from the restricted taxon analysis of combined *CO1* and *ND2* sequence data; *b*, strict consensus of 438 most parsimonious trees (length 487) from the total combined dataset both showing decay indices (top) and support values for bootstrap (bottom left) and jackknife (bottom right) >70%. The labels W = *Aporometra wilsoni* and O = *A. occidentalis* are based on results of this study; *c*, one of the 438 most parsimonious trees from the total combined dataset showing informative transformations in the morphological data (except for the Recherche Archipelago (RA) clade) and the number of informative transformations in the molecular data (bold bars).

These clades occurred in five of the eight analyses, and genetic distances are small (*CO1* distance = 0.93% to 2.07%) relative to those expected for species level differences (Landry *et al.* 2003; Won *et al.* 2003). Hence, we apply the name *A. wilsoni* to the *Aporometra* specimens in this clade as well.

All analyses returned a clade that included the Recherche Archipelago specimens (Figs 7*b*, 9*b*), which formed a polytomy with the *A. occidentalis* holotype and paratypes in the morphology tree (Fig. 7*a*). The Recherche Archipelago specimens shared with the holotype of *A. occidentalis* a uniquely smooth adambulacral border of P_1 (Fig. 6*b*, *d*) (plesiomorphic, see Discussion), and we have applied the name *A. occidentalis* to the Recherche Archipelago specimens (see Discussion). Distally directed spines present along the adambulacral border of P_1 were found in the other *Aporometra* specimens. However, not all specimens in the *A. occidentalis* paratype series shared the pinnule morphology with the holotype or the Recherche Archipelago specimens. These appear to be specimens of *A. wilsoni* (see below and Appendix 2) and were not included in the analyses. The genetic distances between *A. occidentalis* and *A. wilsoni* ranged from 3.64% to 4.24%, using *CO1* data, and between 4.88% and 6.89% based on *ND2* sequence. There was no divergence within either gene for the single *A. occidentalis* population from the Recherche Archipelago. Distances between the populations of *A. wilsoni* varied from 0% to 2.07% for *CO1* sequence and between 0% and 1.29% for the *ND2* data.

Transformations

The tree used to show transformations is one of 428 (length = 484) from the total combined analysis (Fig. 9*c*). It was chosen at random because the key transformations occur in each of the trees. Monophyly of *Aporometra* was supported by 63 molecular and six morphological characters. The morphological apomorphies for *Aporometra* are: the distal cirral is wider than tall in cross section (12) (Fig. 4*f*) (and develops a round state in the San Remo population; Fig. 4*g*); its aboral surface is carinate rather than smooth (14) (Fig. 5*c*), and it does not bear a keel (19) (Figs 4*f*, *g*, 5*c*); Ibr$_1$ is triangular in cross section rather than rounded (28) (Fig. 3*d*) and the terminal pinnular is more spinose than the preceding pinnular on both middle (35) and distal (37) pinnules (Fig. 6*j*).

The Recherche Archipelago, or *A. occidentalis*, clade was supported by 13 molecular apomorphies, but only one homoplastic morphological transformation; the concave shape of the lateral edges of the proximal cirral profile (6) (Fig. 4*b*) (also shown in the Gabo Island *Aporometra* specimens). One of the outgroups, *F. serratissima*, also showed this state while *N. virilis* showed the alternative state (straight lateral edges)
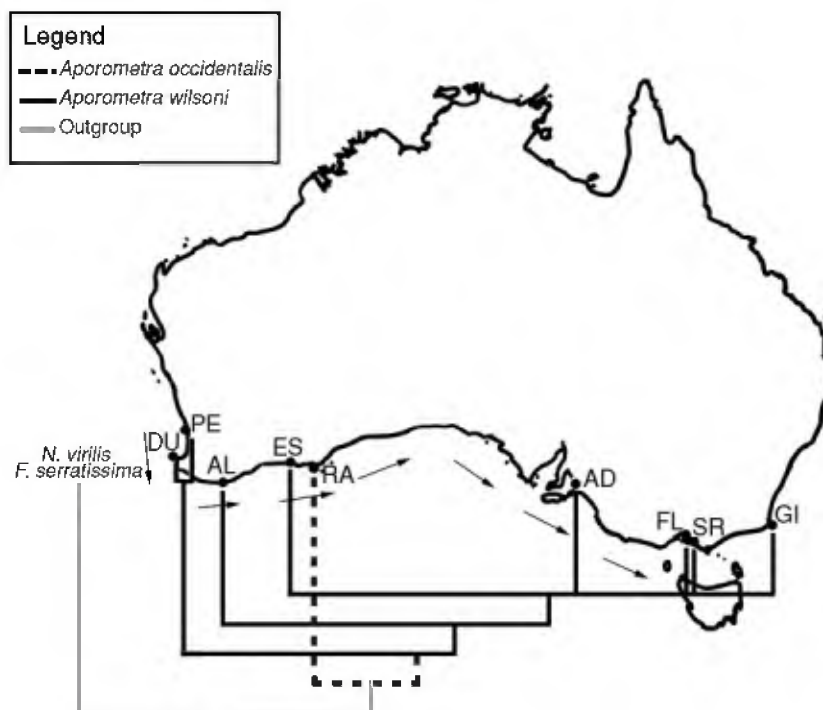

Fig. 10. Map of Australia with ocean currents, showing the distribution of *Aporometra occidentalis* and *A. wilsoni* as well as the relationships within *Aporometra* as shown in Fig. 9*b*. The dotted branch leads to *A. occidentalis*, solid black branches to *A. wilsoni* and the branches leading to the outgroups are grey.

(Fig. 4*a*). As *N. virilis* is currently placed in the same super-family as *Aporometra*, while *F. serratissima* is placed in a separate suborder, character 6 has been included in the tree to support the *A. occidentalis* clade.

The *A. wilsoni* clade was supported by 21 molecular characters and two morphological characters. The morphological apomorphies for this clade are the effaced aboral surface of the proximal cirral (4) (Fig. 5*g*) (this becomes carinate in the Dunsborough population; Fig. 5*d*) and the presence of distally directed spines along the adambulacral margins of P$_1$ (Fig. 6*a*, *c*) (31) rather than remaining smooth (Fig. 6*b*). The western clade within *A. wilsoni* was supported by two molecular characters as well as having the central cavity in the distal cirral partly rather than completely surrounded by the fulcral ridge (11), and the centrodorsal discoidal rather than conical or hemispherical (21). The centrodorsal is also discoidal in the San Remo population. The eastern *A. wilsoni* clade was supported by four molecular characters only.

Discussion

Phylogenetic results and taxonomy

This study revealed little morphological (Fig. 7*a*) or molecular diversity among the *Aporometra* exemplars studied here. This lack of morphological variation was anticipated following observations by Clark and Clark (1967) on the similarity of the three nominal species. Although the *Aporometra* specimens varied little across most of the geographic range sampled, the combined morphological and molecular data (Fig. 9*c*) did show two well supported and reciprocally monophyletic groups. Analysis of the partitions of data also were congruent in showing a well supported clade of *Aporometra* found all across southern Australia that was sister group to a much more geographically restricted Western Australian clade (Figs 7*b*, 8*a*, *b*, 9*a*). The exception was analysis 1 (Morphology only, Fig. 7*a*), which returned a clade containing the San Remo and Flinders specimens from near the type locality of *A. wilsoni* (Port Philip Bay, Vic.) as well as the types of *A. paedophora*. This clade formed part of a polytomy with the specimens from Recherche Archipelago, WA and the type material of *A. occidentalis*, also from Western Australia. For the widely distributed clade we decided here to use the name *Aporometra wilsoni*, the oldest available name within the group. The status of *A. paedophora* is discussed below. One morphological apomorphy for *A. wilsoni* is an effaced aboral surface of the proximal cirral (Fig. 5*g*, character 4), though this is transformed within the clade to carinate in the Dunsborough population (Fig. 5*d*). The effaced surface is characterised by very small holes, or scratches, on the surface of the ossicle while the carinate surface has many small raised bumps. The unequivocal morphological apomorphy for *A. wilsoni* is the presence of distally directed spines (Fig. 6*a*, *c*) along the adambulacral edges of P$_1$ in all specimens (character 31). This was

described by Clark and Clark (1967:29): 'the ventrolateral ends of the distal borders of the segments are slightly produced', although not emphasised in their drawings (see Fig. 2). Although differences in P$_1$ morphology were noted several times in descriptions of *Aporometra* (A. H. Clark 1909; Clark 1911; H. L. Clark 1938) they were overlooked as important in distinguishing *A. wilsoni* from *A. occidentalis*.

Sequence data reinforce the distinctiveness of the Recherche Archipelago *Aporometra* specimens from the *A. wilsoni* specimens (Figs 7*b*, 8*a*, *b*, 9*b*), and the name *A. occidentalis* is applied to these terminals (see below). Genetic distances (calculated using the Kimura two-parameter model) between *A. wilsoni* and *A. occidentalis* as delineated here are consistent with levels of inter-specific divergence in other marine invertebrates such as sea urchins (Landry *et al.* 2003) and mussels (Won *et al.* 2003). Divergence of *COI* sequence between populations of *A. wilsoni* across southern Australia is low, ranging from 0 to 2%. However, between *A. wilsoni* and *A. occidentalis* the genetic distance is 3.6 to 4.2%. The Recherche Archipelago, or *A. occidentalis*, clade was well supported by molecular data (Figs 7*b*, 9*b*), but by only one morphological apomorphy; the concave shape of the lateral margins along the proximal cirral profile (Fig. 4*b*, character 6). This was also seen in the Gabo Island *Aporometra* specimens, but not in the holotype of *A. occidentalis* (though it was present in one of the paratypes). The lack of the feature in the holotype suggests that this feature needs further investigation as diagnostic for *A. occidentalis*.

The combined data reveal another distinguishing feature for *A. occidentalis*: the adambulacral border of the oral pinnules (P$_1$) is smooth (Fig. 6*b*, *d*). Clark and Clark (1967: 33) noted this when describing *A. occidentalis*, 'The pinnule is perfectly smooth, the segments joining evenly end to end without any production of the distal ends'. The Recherche Archipelago shared this distinguishing feature with the holotype of *A. occidentalis*, justifying the application of the name *A. occidentalis* to this clade. It should be noted that having a flat adambulacral border of the oral pinnules (P$_1$) is a plesiomorphy and is the outgroup condition. The type material of *A. occidentalis* is from Koombana Bay, further west than the Recherche Archipelago. Some of the numerous paratypes of *A. occidentalis*, however, had the apomorphic P$_1$ morphology of *A. wilsoni* and may actually belong to this species (see Appendix 2). These were not included in the analysis because *A. wilsoni* specimens from the same Koombana Bay trawl as the type series, deposited in SAM, were already represented in the morphology analysis as KB1, KB2 and KB3.

On the basis of analysis 1 (Morphology only, Fig. 7*a*), *A. paedophora* might reasonably be regarded as a junior synonym of *A. wilsoni*, which then extends the latter's range up the NSW coast. However, the tubercles created by basal rays (A. H. Clark and A.M. Clark, 1967) potentially distinguish *A. paedophora* from the other *Aporometra*.

Unfortunately, this feature could not be seen in the types and was scored accordingly (see Appendix 2). Due to a lack of resolution in morphology of the paratypes, and the lack of any other available evidence, particularly molecular data, the status of *A. paedophora* remains unresolved. The status of *A. paedophora* may only be resolved by collecting more specimens from at or near the type locality or by destroying a type specimen for SEM and sequencing, which would likely require ancient DNA methods given our experience with other crinoid specimens of similar age and preservation.

Cirral shape was the only character used to distinguish the species of *Aporometra* (Clark 1946). However, cirral shape appears to differ between populations, or regions, not species. The Western Australia specimens of *A. wilsoni* and *A. occidentalis* have a similar hour-glass shape (Fig. 4*b*) that differs from the straight (Fig. 5*e*, *g*), bell shape and extremely elongate cirral shapes found in the central and eastern populations. This study shows that *A. wilsoni* and *A. occidentalis* can be distinguished reliably by the presence of distally directed spines along the oral pinnules in *A. wilsoni* (see Fig. 6*a*, *c*) and their absence in *A. occidentalis* (see Fig. 6*b*, *d*). This feature is visible to the naked eye in larger individuals or can be seen via stereo microscope.

Distribution of Aporometra *species*

As mentioned above, this study has revealed that *A. wilsoni* and *A. occidentalis* are sympatric in Western Australia. The adambulacral spines along P_1 of *A. wilsoni* specimens are larger in the western populations and this should make separation of this species from *A. occidentalis* fairly easy. The distribution of *A. wilsoni* is from Perth, Western Australia, to Gabo Island, Victoria (Fig. 10). Specimens of *A. wilsoni* were collected for this study at depths of 0–18 m, with most specimens collected in 0–2 m. Available records show *A. wilsoni* was found on brown algae (in some cases identified as *Cystophora*). *Aporometra occidentalis* was found only in Western Australia at two localities: Koombana Bay and the Recherche Archipelago (Fig. 10) at depths of 9–15 m. The types of only *A. occidentalis* were collected on *Cystophora* and the seagrass *Cymodocea* (the only habitat record for the species) (H. L. Clark 1938) though as noted earlier, this material also included *A. wilsoni*. The overlapping depth ranges and lack of any known algal habitat preference make it difficult to infer any potential habitat or niche differences between the two species.

Aporometra wilsoni collected from Perth and Dunsborough (western group) are well supported as a monophyletic group in all but the *ND2* sequence and morphology analyses (Figs 7*a*, 8*b*). The average genetic distance between the Perth and Dunsborough populations for *CO1* is only 0.12%, compared to >0.97% between Perth, Dunsborough and the remaining populations. However, this is still much less than currently applied 'species-level' distances (Hebert *et al*. 2004). The relationships of the remaining populations,

from Albany to Gabo Island (southern group), are unresolved. In half of the analyses tried, the Albany population forms a sister group to a clade of the other southern populations (Figs 8*a*, 9*a*). Greater sampling and other analytical methods such as likelihood and haplotype networks will be required to assess more about population structures of *A. wilsoni*. The primary goal of this project was to determine the number of species in *Aporometra*. However, the distinction between western and southern populations within *A. wilsoni* has interesting biogeographic implications that can be highlighted in this context.

Prevailing currents as well as recent and historic climate are thought to play a role in defining species distributions along Australia's southern coast (Bennett and Pope 1953; O'Hara and Poore 2000). The Leeuwin and Great Australian Bight currents have the potential to aid dispersal down the western coast and across the southern coast of Australia, resulting in a warm temperate zone in south-western Australia. Eastern Australia is also a warm temperate zone hypothesised to result from the East Australian Current, allowing dispersal as far as south-eastern Tasmania during summer (Waters and Roy 2003*a*; Waters and Roy 2003*b*). The waters become cool temperate in south-eastern Australia (including Tasmania); a region proposed as a zone of overlap created by periodical falls in sea level with the formation of a land bridge between Tasmania and the mainland. This may have caused the vicariance of many populations that have since dispersed across Bass Strait in interglacial periods (Dartnall 1974).

Aporometra wilsoni appears to have a biogeographic structure found in several phylogenetic studies of southern Australian marine invertebrates (e.g. Kassahn *et al*. 2003) in that there is a phylogeographic division between populations of the far western and southern coasts of Australia. However, these studies, like several others (Waters and Roy 2003*a*; Waters and Roy 2003*b*; Waters *et al*. 2004) also show a more common east/west division near Bass Strait. However, only very low levels of variation exist among the South Australian and Victorian samples of *A. wilsoni*, with no data to support a vicariant episode across the Bassian isthmus. These low levels of divergence between southern populations suggest that they may not have been reproductively isolated and that dispersal possibly continues along the southern coast. The easterly Great Australian Bight current (Fig. 10) may drive this dispersal. The slightly greater genetic distance between the western and southern populations of *A. wilsoni* is consistent with the southerly direction, and occasional reversal, of the Leeuwin current, perhaps reducing the potential for dispersal around the tip of the west coast. As larval dispersal in *A. wilsoni* is likely to be limited owing to its ovoviviparity (A. H. Clark and A.M. Clark 1967), a likely method of dispersal is by rafting of juveniles or adults on algae. Some species of algae on which *Aporometra* have been recorded are known to shed seasonally as part of their reproductive

strategy (Hotchkiss 1999). These ideas would have to be tested with further sampling, particularly along the coast of Western Australia. Greater sampling may also lead to a better understanding of the distribution of *A. occidentalis* and hopefully the inclusion of *A. paedophora* in future work. This study has shown that the inclusion of molecular data to support morphology is a valuable approach in resolving species-level relationships in crinoids.

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Appendix 1. List of specimens scored and sequenced for the present study

Species	Museum label	Identifier	Locality	Depth (m)	Registration number	GenBank Acc. #
Aporometra wilsoni	*Aporometra* sp.	PE1	Elizabeth Reef, Perth, WA	2–3	SAM K2067	AY669341, AY669366
Aporometra wilsoni	*Aporometra* sp.	PE2	Elizabeth Reef, Perth, WA	2–3	SAM K2068	AY669342
Aporometra wilsoni	*Aporometra* sp.	PE3	Elizabeth Reef, Perth, WA	2–3	SAM K2069	AY669343
Aporometra wilsoni	*Aporometra* sp.	PE4	Elizabeth Reef, Perth, WA	2–3	SAM K2127	
Aporometra occidentalis	*Aporometra occidentalis* (Holotype)	Ao1	Koombana Bay, Bunbury, WA	9.1–14.6	MCZ 964	
Aporometra occidentalis	*Aporometra occidentalis* (Paratype)	Ao2	Koombana Bay, Bunbury, WA	9.1–14.6	MCZ 964	
Aporometra occidentalis	*Aporometra occidentalis* (Paratype)	Ao3	Koombana Bay, Bunbury, WA	9.1–14.6	MCZ 964	
Aporometra wilsoni	*Aporometra paedophora*	KB1	Koombana Bay, Bunbury, WA	9.1–14.6	SAM K2086	
Aporometra wilsoni	*Aporometra paedophora*	KB2	Koombana Bay, Bunbury, WA	9.1–14.6	SAM K2086	
Aporometra wilsoni	*Aporometra paedophora*	KB3	Koombana Bay, Bunbury, WA	9.1–14.6	SAM K2086	
Aporometra wilsoni	*Aporometra* sp.	DU1	Bunker Bay, Dunsborough, WA	1–2	SAM K2070	AY669344, AY669367
Aporometra wilsoni	*Aporometra* sp.	DU2	Bunker Bay, Dunsborough, WA	1–2	SAM K2071	AY669345
Aporometra wilsoni	*Aporometra* sp.	DU3	Bunker Bay, Dunsborough, WA	1–2	SAM K2072	AY669346
Aporometra wilsoni	*Aporometra* sp.	AL1	King George Sound, Albany, WA	Intertidal	SAM K2074	AY669348, AY669368
Aporometra wilsoni	*Aporometra* sp.	AL2	King George Sound, Albany, WA	Intertidal	SAM K2075	AY669347
Aporometra wilsoni	*Aporometra* sp.	AL3	King George Sound, Albany, WA	Intertidal	SAM K2082	AY669349
Aporometra wilsoni	*Aporometra wilsoni*	ES1	Ten Mile Lagoon, Esperance, WA	0–2	MV F66247/K2122	AY669350
Aporometra occidentalis	*Aporometra occidentalis*	RA1	Recherche Archipelago, Esperance, WA	10	WAM Z12188	AY669351, AY669369
Aporometra occidentalis	*Aporometra occidentalis*	RA2	Recherche Archipelago, Esperance, WA	10	WAM Z12188	AY669352
Aporometra occidentalis	*Aporometra occidentalis*	RA3	Recherche Archipelago, Esperance, WA	10	WAM Z12188	AY669353
Aporometra wilsoni	*Aporometra* sp.	AD1	Witton Bluff, Adelaide, SA	0–1.5	SAM K2076	AY669354, AY669370
Aporometra wilsoni	*Aporometra* sp.	AD2	Witton Bluff, Adelaide, SA	0–1.5	SAM K2077	AY669355, AY669371
Aporometra wilsoni	*Aporometra* sp.	AD3	Witton Bluff, Adelaide, SA	0–1.5	SAM K2078	AY669356
Aporometra wilsoni	*Aporometra* sp.	AD4	Witton Bluff, Adelaide, SA	0–1.5	SAM 2129	
Aporometra wilsoni	*Aporometra wilsoni*	SR1	San Remo, Westernport Bay, Vic.	Intertidal	SAM K2079/K2136	AY669359, AY669373
Aporometra wilsoni	*Aporometra wilsoni*	SR2	San Remo, Westernport Bay, Vic.	Intertidal	SAM K2080	AY669360
Aporometra wilsoni	*Aporometra wilsoni*	SR3	San Remo, Westernport Bay, Vic.	Intertidal	SAM K2081	AY669361
Aporometra wilsoni	*Aporometra wilsoni*	–	Honeysuckle Point, Westernport Bay, Vic.		MV F84857	
Aporometra wilsoni	*Aporometra wilsoni*	FL1	Mushroom Reef, Flinders, Vic.	0–2	MV F91681/SAM K2128, K2130	AY669357
Aporometra wilsoni	*Aporometra wilsoni*	FL2	Mushroom Reef, Flinders, Vic.	0–2	MV F91681	AY669358, AY669372
Aporometra wilsoni	*Aporometra wilsoni*	G1	Gabo Island, Vic.	18.3	AM J14803	AY669362, AY669374
Aporometra wilsoni	*Aporometra wilsoni*	G2	Gabo Island, Vic.	18.3	AM J14803	AY669363
Aporometra wilsoni	*Aporometra wilsoni*	G3	Gabo Island, Vic.	18.3	AM J14803/SAMK2129, K2131	AY669364
Aporometra wilsoni	*Aporometra wilsoni*	G4	Gabo Island, Vic.	18.3	SAM K2120	
Aporometra paedophora (Paratype)	*Aporometra paedophora* (Paratype)	MR1	Thetis St. 28, off Manning River, NSW	40.2	AM J831	
Aporometra paedophora (Paratype)	*Aporometra paedophora* (Paratype)	MR2	Thetis St. 28, off Manning River, NSW	40.2	AM J831	
Aporometra paedophora (Paratype)	*Aporometra paedophora* (Paratype)	MR3	Thetis St. 28, off Manning River, NSW	40.2	AM J831	
Aporometra paedophora (Paratype)	*Aporometra paedophora* (Paratype)	MR4	Thetis St. 28, off Manning River, NSW	40.2	AM J831	
Notocrinus virilis	*Notocrinus virilis*	Nv	Elephant Island, Antarctica		CAS 160475	DQ186656
Notocrinus virilis	*Notocrinus virilis*	–	BANZARE St. 107, Antarctica		SAM K2132, K2133	
Florometra serratissima	*Florometra serratissima*	Fs	–		–	NC001878

Appendix 2.   Terminals used in the morphology matrix with notes on scoring

The following is list of the populations/specimens (the names used are from museum labels) represented in the morphology matrix (Table 2). The list is presented in geographic order with the outgroup taxa included. Explanation of the scoring for some of the terminal specimens is given where there are multiple unknown character states, polymorphisms or ambiguities in the states observed or described in literature.

'*Aporometra* sp.', Elizabeth Reef, Perth, WA, SAM K2067–9, K2127

'*Aporometra occidentalis*', Koombana Bay, Bunbury, WA, MCZ 964 (type specimens)

Collector: H. L. Clark, Date: 26.x.1929, depth 5–8 fms [9.1–14.6 m], habitat: dredged from *Cystophora* and *Cymodocea* beds.

This lot includes two morphology types: the smooth borders of P_1 found in the Holotype and some paratypes (Fig. 6*b*, *d*), and the distally directed spines along the border of P_1 found in some paratypes. The spines are consistent with the morphology of *Aporometra wilsoni* (Fig. 6*a*, *c*). Hence the specimens with this feature have been isolated in the type lot and were not used to represent *A. occidentalis* in this study. Some cirral, and cirrus socket, character states are scored as unknown in specimens with cirri that were all proximally intact. Variation in the shape of the lateral edges of the proximal cirral, when viewed aborally (5) and in profile (6), reflects the very slight central depression around the cirral being hard to observe from some angles.

'*Aporometra paedophora*', Koombana Bay, Bunbury, WA, SAM K2086

Some cirral character states are scored as unknown in specimens with cirri that were all proximally intact. Some distal cirri were also worn and character states unable to be determined, due to long preservation of the specimen. The fulcral ridge enclosing the central cavity (1) varies within this population and may be influenced by erosion. The state was still scored and included as it may be part of variation in this character observed between the geographically close Perth and Dunsborough specimens. There are five ambulacral divisions (25) and the slightly asymmetrical nature of the disk in KB2 resulted in one of the five divisions occurring on the disk. This was scored as a polymorphism; however, the position of the majority of the ambulacral divisions may be a sufficiently informative character.

'*Aporometra* sp.', Bunker Bay, Dunsborough, WA, SAM K2070–2

Unknown characters in these specimens were due to their extremely small size. The specimens also showed juvenile characteristics of fewer, smaller ossicles in the rays, cirri and pinnules. The proximal cirral aboral surface was scored as carinate (4) due to the observation of very small spines at the distal and proximal edges of the cirrals in DU1 and DU2.

'*Aporometra* sp.', King George Sound, Albany, WA, SAM K2074–5, K2082

AL2 was scored for the same polymorphism in the position of the ambulacral groove as KB2.

'*Aporometra wilsoni*', Ten Mile Lagoon, Esperance, WA, MV F66247, SAM K2122

'*Aporometra occidentalis*', Recherche Archipelago, Esperance, WA, WAM Z12188

The appearance of basal rays (23) is marked unknown for RA2 due to obstruction by the arms and cirri from viewing this character, basal rays are not evident in the other two specimens hence their presence in this specimen is extremely unlikely. RA2 also shows the same polymorphic state in ambulacral division position (25) as KB2 and AL2.

'*Aporometra* sp.' Witton Bluff, Adelaide, SA, SAM K2076–8, K2129

The shape of the centrodorsal is unknown as AD3 was damaged during collection however as sequence data was generated from this specimen it was also scored for morphology.

'*Aporometra wilsoni*', Mushroom Reef, Flinders, Vic., MV F91681, SAM K2128, K2130

'*Aporometra wilsoni*', San Remo, Melbourne, Vic., SAM K2079–81, K2136

Like the Dunsborough specimens, these specimens were very small with juvenile features. Hence, several character states are unknown. The presence of distally directed spines on P_1 (31) is scored for SR1 but was not able to be photographed as the pinnules were so small they became too degraded during processing for SEM Hence Fig. 6*c* shows the state in a specimen from nearby Honeysuckle Point, Western Port Bay, Vic., MV F84857.

'*Aporometra wilsoni*', Gabo Island, Vic., AM J14803, SAM K2120, K2129, K2131

'*Aporometra paedophora*', off Manning River, NSW, AM J831 (Type specimens)

Date: 5.iii.1898, depth: 22 fms [40.2 m], habitat: dredged from fine grey sand.

Owing to the age and poor condition of these small specimens, some character states are unknown. The appearance of basal rays (23) that were noted in Clark and Clark (1967: 24) as forming tubercles in the angle of the calyx are no longer visible in these specimens, and so they have been scored as unknown.

'*Notocrinus virilis*', Elephant Island, Antarctica, CAS 160475; BANZARE St. 107, Antarctica, SAM K2132, K2133

'*Florometra serratissima*' (Clark 1915, fig. 391; Clark 1921, fig. 95, pl. 5, figs 11, 13; Clark and Clark 1967; Mladenov and Chia 1983)

Several character states were not discussed in the text and are marked unknown. The cirrals are noted as becoming more laterally compressed in the distal half. This is interpreted to indicate that the proximal cirrals are taller than wide in cross section and the distal cirrals become narrower (2, 12).