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Actinia ebhayiensis sp. nov., a new species of sea anemone (Anthozoa: Actiniaria: Actiniidae) from South Africa

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In this work we describe Actinia ebhayiensis *sp. nov. from South Africa. The species is externally similar to the type species of the genus,* Actinia equina, *from which it can be distinguished by nematocyst and allozyme data.* Actinia ebhayiensis *has a smooth, red column, with well-delimited parapet leading to a deep fosse where conspicuous blue acrorhagi can be found. Tentacles and oral disc are crimson red, and the pedal disc has a lighter pink colour. The microbasic* p-*mastigophores and* b-*mastigophores of the mesenterial filaments of the new species are significantly smaller than those of* A. equina. Actinia ebhayiensis *can also be clearly distinguished from other species of the genus in nematocyst measurements, genetic data and muscle morphology.*

Keywords: Anthozoa, genetics, taxonomy, *Actinia ebhayiensis* sp. nov.

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INTRODUCTION

The systematics of *Actinia* sea anemones is controversial, with many morphotypes of *Actinia equina* (Linnaeus, 1758) considered varieties by some authors (Stephenson, 1935; Schmidt, 1971, 1972; Quicke *et al.*, 1983, 1985; Quicke & Brace, 1984; Donoghue *et al.*, 1985) or granted specific status by others (Templeton, 1836; Dalyell, 1848; Cocks, 1850; Tugwell, 1856; Milne-Edwards, 1857; Gosse, 1860; Carter & Thorpe, 1981; Haylor *et al.*, 1984; Monteiro *et al.*, 1997; den Hartog & Ocaña, 2003). Because of this taxonomic confusion, the binomial *Actinia equina* (Linnaeus, 1758) has been used for beadlet anemones found over a large geographical area, from northern Russia and the Baltic Sea to the tropical waters of West Africa and the Red Sea, South Africa, and the Far East (Stephenson, 1935; Carlgren, 1938; Schmidt, 1972; Manuel, 1981; Song & Cha, 2002; Cha *et al.*, 2004).

Since 1981, studies with allozyme electrophoresis have shown that *Actinia equina* comprises a number of reproductively isolated and genetically highly divergent species, usually distinguished by fixed, albeit sometimes subtle, morphological, morphometric and ecological differences, like cryptic or exposed habitat and location in the intertidal zone (Carter & Thorpe, 1981; Haylor *et al.*, 1984; Solé-Cava & Thorpe, 1987, 1992; Monteiro *et al.*, 1997; Schama *et al.*, 2005).

During a genetic study of *Actinia* spp. by Schama *et al.* (2005), it became evident that anemones from South Africa previously identified as *A. equina* by Stephenson (1935) and Carlgren (1938) belong to a new species. The phylogenetic analyses indicated that the species from South Africa formed a sister group with *Actinia tenebrosa* Farquhar, 1898, but were very different from the European *A. equina* and from any other *Actinia* species (Schama *et al.*, 2005). Here we describe this new species, *Actinia ebhayiensis* sp. nov., and compare it genetically and biometrically to *Actinia equina* and to the genetically close *Actinia tenebrosa*. Although externally very similar to *A. equina* (the type species of the genus), *Actinia ebhayiensis* can be readily distinguished from it and from all other *Actinia* species by molecular and morphological differences.

MATERIALS AND METHODS

Six specimens of *Actinia ebhayiensis* were collected from Port Elizabeth (one specimen: 33° 58′S25°40′) and Port Alfred (five specimens: 33°36′S 26°55′E), on the South African coast, in March 1998. The specimens look similar to the red column, pink pedal disc with no blue rim mid-shore morph that Quicke and collaborators examined (Quicke & Brace, 1984; Quicke *et al.*, 1983, 1985; Allcock *et al.*, 1998). The five specimens from Port Alfred are from the same material used in the earlier genetic study of Schama *et al.* (2005). For comparison, specimens of *A. equina* were collected in Fleshwick Bay (Isle of Man, United Kingdom: 54°5′N 4°46′W) and *A. tenebrosa* in Half Moon Bay (Victoria, Australia: 38°08′S 144°43′E). Nematocysts of *A. fragacea* Tugwell, 1856 from the UK (two specimens: 54°5′N 4°46′W) and *A. cari* Delle Chiaje, 1825 from Bay of Piran (North Adriatic Sea, one specimen:

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45°31′N 13°34′E) were also measured. Animals were kept in aquaria (except for *A. tenebrosa*) for up to two weeks and then anaesthetized for four hours in a solution of 7.5% magnesium chloride diluted 1:1 with salt water. Individuals were fixed for 7 days in 4% formaldehyde diluted with salt water and then stored in 70% ethanol.

Nematocysts were analysed from fresh and fixed tissue squashes, mounted with fresh water and without stain, under a compound microscope at 1000 × magnification. The length and width of 20 undischarged capsules within haphazardly selected fields were measured from four tissues (tentacle, acrorhagi, mesenterial filaments and actinopharynx) each; on five individuals of the South African specimens, eight individuals of *A. equina* from the UK and five individuals of *A. tenebrosa* from Australia (Table 1). When two discrete size-classes of the same nematocyst type were present within a tissue, they were counted separately and labelled I and II. The classification of nematocysts follows that of England (1991). Spirocysts were excluded from the analyses as they present large size variations correlated to anemone size as shown in Francis (2004).

Nematocyst data were analysed using the program SYSTAT 7.0.1 (© 1997, SPSS) and the R statistical package. A normality test (Kolmogorov–Smirnov) and a homogeneity test were performed to confirm that assumptions required for parametric tests were met. An analysis of variance (ANOVA) and *post hoc* Tukey tests were used to infer significant differences in length, width and length/width ratio among samples.

The length/width ratio, which basically measures how rounded the nematocysts are, was used because it is less influenced by the size of the anemones (Chintiroglou & Simsiridou, 1997). Due to the large intra-specific variation observed, a hierarchical ANOVA was performed to separate the effects of intra- and inter-specific variation (Allcock *et al.*, 1998; Williams, 1998; Watts *et al.*, 2000). A linear discriminant analysis using the length of 1678 nematocysts from 16 individuals was used to summarize the differences found among the species. This type of analysis uses all measurements to discriminate among species (Klecka, 1980). Linear discriminant analysis has already been used in sea anemone nematocyst analysis although in a very different context. Ardelean & Fautin (2004) used this type of analysis to differentiate nematocysts from different regions of the same specimen.

Pedal disc diameter and column height were recorded from live specimens. Photographs of the external morphology were taken using a Nikon CoolPix 5400 digital camera. General morphology was studied under a dissecting microscope. For anatomical and histological analyses samples sections were embedded in paraffin. Serial sections 5–6 μm thick were stained using haematoxylin and eosin and the Mallory triple stain method (Kiernan, 1990). All *A. ebhayiensis* samples were deposited in the cnidarian collection of the National Museum of Rio de Janeiro (MNRJ6386–6391), Brazil and *A. tenebrosa* specimens were deposited in Museum Victoria (MV F112736, MV F112740–112745), Australia.

Genetic data (18 allozyme *loci*) from Schama *et al.* (2005) were re-analysed to compare the different species through a

Table 1. Nematocyst measurements (in micrometres) of *Actinia ebhayiensis* sp. nov., *Actinia tenebrosa* and *Actinia equina*. Mean, range, standard deviation (SD), sample size per individual (N_1) and total sample size (N_2), of measurements of length and width of capsules.

				Length			**Width**				
Tissue	**Type**	**N_1**	**N_2**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**		
A. ebhayiensis											
Acrorhagus	Holotrich	30/20/–/62/43	156	40.41	3.50	33.00–49.00	3.44	0.49	2.47–5.55		
Actinopharynx	Basitrich I	–/21/13/80/20	134	14.53	3.33	6.00–19.84	2.04	0.41	1.16–4.38		
	Basitrich II	–/28/7/37/9	82	21.76	1.48	20.00–27.11	2.25	0.45	1.50–3.44		
Filaments	Microbasic *p*-mastigophore	30/20/20/29/27	127	16.19	1.62	12.11–23.37	3.82	0.66	1.88–5.21		
	Microbasic *b*-mastigophore	30/20/20/11/15	96	18.29	2.80	13.33–25.86	3.60	0.70	2.00–5.45		
	Basitrich	30/18/20/27/10	105	11.18	2.39	5.00–19.09	2.01	0.53	1.33–4.20		
Tentacles	Basitrich	30/16/20/78/43	187	14.87	3.12	5.50–20.00	2.38	0.52	1.50–4.04		
Tissue	**Type**	**N_1**	**N_2**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**		
A. tenebrosa											
Acrorhagus	Holotrich	20/20/20/20/20	100	49.45	4.07	40.00-59.20	3.90	0.44	3.20-4.80		
Actinopharynx	Basitrich I	4/20/20/22/20	86	14.20	2.52	8.00-19.20	2.39	0.42	1.60-3.20		
	Basitrich II	16/20/17/21/12	94	24.47	2.53	20.00-30.40	3.02	0.44	2.40-4.00		
Filaments	Microbasic *p*-mastigophore	20/20/20/20/20	100	19.85	2.12	13.60-28.00	4.61	0.46	3.20-5.60		
	Microbasic *b*-mastigophore	20/20/20/20/20	100	25.64	2.34	18.40-29.60	3.79	0.50	2.40-4.80		
	Basitrich	20/20/20/20/20	100	13.42	1.77	8.80–59.20	2.43	0.32	1.60–4.00		
Tentacles	Basitrich	40/40/40/40/40	200	16.71	4.33	8.00-23.20	2.49	0.57	1.60-3.20		
Tissue	**Type**	**N_1**	**N_2**	**Mean**	**SD**	**Range**	**N_1**	**N_2**	**Mean**	**SD**	**Range**
A. equina											
Acrorhagus	Holotrich	18/18/20/20/17/17/36/30	176	50.37	6.46	35.7–70.0	36/30	66	2.91	0.42	1.94–3.78
Actinopharynx	Basitrich I	20/20/20/20/20/20/29/19	168	13.30	1.98	9.72–19.59	29/19	48	2.01	0.43	1.00–2.98
	Basitrich II	20/20/20/20/20/20/2/42	164	25.37	2.43	20.40–32.20	2/42	44	2.85	0.38	2.03–3.65
Filaments	Microbasic *p*-mastigophore	20/20/20/20/20/20/2/25	147	22.46	2.64	16.50–31.19	2/25	27	3.87	0.77	2.52–6.00
	Microbasic *b*-mastigophore	17/18/15/17/20/19/30/26	162	34.37	4.96	21.18–51.20	30/36	56	3.44	0.42	2.26–4.48
	Basitrich	20/20/20/20/20/20/31/30	181	13.45	2.38	8.35–28.41	31/30	61	1.88	0.41	1.16–2.84
Tentacles	Basitrich	20/20/20/20/20/20/30/63	213	18.22	5.56	8.56–31.80	30/63	93	2.15	0.50	1.20–3.49

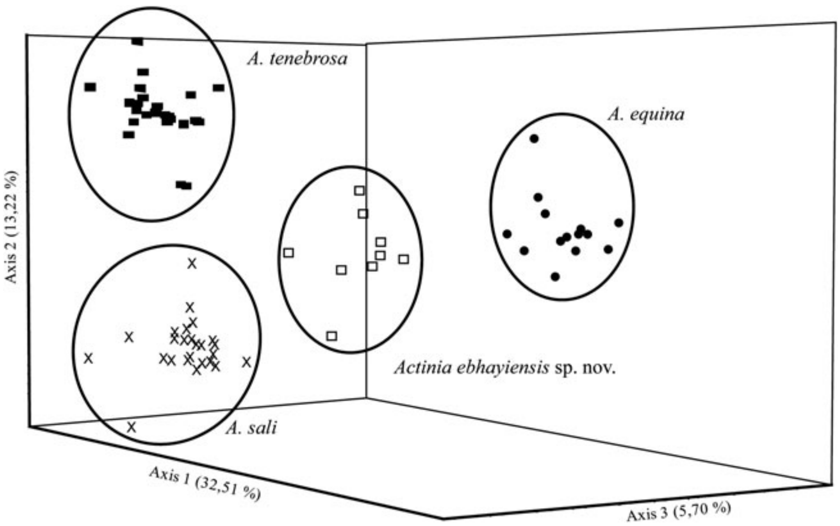

Fig. 1. Factorial correspondence analysis based on 18 allozyme *loci* of *Actinia* species (data from Schama *et al.*, 2005). *A. equina* (●); *A. tenebrosa* (■); *A. sali* (X) and *Actinia ebhayiensis* sp. nov. (☐).

factorial correspondence analysis (FCA), using the program GENETIX 4.05 (Belkhir *et al.*, 1996–2004). This type of analysis is especially useful for estimating associations between multiple qualitative variables, where no *a priori* hypothesis is present (Valentin, 2000). FCA simplifies the analyses of complex data: the multivariate nature of correspondence analysis can reveal relationships that would otherwise not be detected in a series of pair-wise comparisons.

RESULTS

In order to have a clear view of the results on the FCA, only the morphological, genetic and geographically closer species of the genus were re-analysed. The results clearly discriminated the species (Figure 1) and more importantly, *Actinia ebhayiensis* could be readily distinguished in those analyses from European *A. equina* and also from the genetically closer *Actinia tenebrosa* (from Australia) and the morphologically and geographically closer (from Cape Verde) *Actinia sali* Monteiro, Solé-Cava & Thorpe, 1997.

Significant differences (hierarchical ANOVA and *post hoc* Tukey test, after verification of normal distribution of nematocyst measurements) were observed in a number of nematocyst types from different tissues between *Actinia ebhayiensis*, *Actinia equina sensu stricto* and *A. tenebrosa* as follows. Except for the basitrichs of the tentacles, significant differences were found in all nematocyst types among the three species (Table 2). The lengths of the acrorhagial holotrichs were significantly larger in *A. equina* and *A. tenebrosa* when compared to *A. ebhayiensis* and their length/width ratio and width were significantly different among all three species (Table 2). All three species also differed significantly in the length and width of the basitrichs II of the actinopharynx, length of the microbasic *b*-mastigophores and *p*-mastigophores from the mesenterial filaments and width of the basitrichs from the filaments (Table 2). In *A. ebhayiensis*, the basitrichs II of the actinopharynx and the microbasic *b*-mastigophores and *p*-mastigophores of the mesenterial filaments were smaller than in *A. equina* and *A. tenebrosa* (Figure 2) and the basitrichs of the mesenterial filaments, although thinner in *A. ebhayiensis* than in *A. tenebrosa*, were wider than in *A. equina* (Table 1). The length/width ratio of the basitrichs I of the actinopharynx was significantly different between *A. ebhayiensis* and *A. tenebrosa* and all three species differed significantly in the length/width ratio of the microbasic *b*-mastigophores from the filaments, which are more elongated in *A. equina* and *A. tenebrosa*. The basitrichs from the filaments were also significantly smaller in *Actinia ebhayiensis* than in *A. tenebrosa* and *A. equina*. Also the

Table 2. Results of the *post hoc* pairwise Tukey test of species analysed in the analysis of variance of the nematocyst measurements. (L) Length; (W) width; (R) length/width ratio differences found. * $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.

Pharynx/acrorhagus	**Basitrichs I**			**Basitrichs II**			**Holotrichs**		
Species	**L**	**W**	**R**	**L**	**W**	**R**	**L**	**W**	**R**
Actinia equina × *Actinia ebhayiensis*				***	***		***	***	***
Actinia equina × *Actinia tenebrosa*				***	*			***	***
Actinia ebhayiensis × *Actinia tenebrosa*			***	***	***		***	***	**
Mesenterial filaments	***p*-mastigophores**			**Basitrichs**			***b*-mastigophores**		
Species	**L**	**W**	**R**	**L**	**W**	**R**	**L**	**W**	**R**
Actinia equina × *Actinia ebhayiensis*	***		***	***	**	***	***		***
Actinia equina × *Actinia tenebrosa*	***	***	***		***	***	***		***
Actinia ebhayiensis × *Actinia tenebrosa*	***	***		***	***		***		***

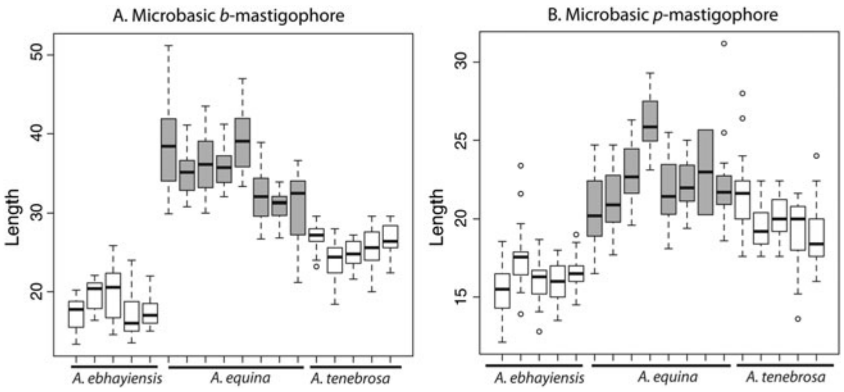

Fig. 2. Box and whiskers plot of nematocyst length of *Actinia ebhayiensis* sp. nov., *Actinia equina* and *Actinia tenebrosa*. (A) Microbasic *b*-mastigophores of the mesenterial filaments; (B) microbasic *p*-mastigophores from the mesenterial filaments. Horizontal line inside the box shows that the median, upper and bottom part of the box are the first and third quartiles. The whiskers show two standard deviations; points either above the third quartile or below the first quartile are plotted individually.

width of the microbasic *p*-mastigophores from the filaments was significantly larger in *A. tenebrosa* than in *A. ebhayiensis* and *A. equina*. The three species could also be clearly distinguished in the discriminant analysis, with high posterior probabilities, in 100% of the cases (Figure 3).

SYSTEMATICS
Order ACTINIARIA
Family ACTINIIDAE Rafinesque, 1815
Genus *Actinia* Linnaeus, 1767
Actinia ebhayiensis sp. nov.
(Figures 4–7)

Synonymy: *Actinia equina*: Stephenson 1935; Carlgren 1938; Kruger & Griffiths 1998; Acuña & Griffiths 2004; *Actinia* sp. 1: Schama 2001; Schama *et al.*, 2005.

TYPE MATERIAL
Holotype: MNRJ-6386—South Africa, Port Elizabeth, 25 March 1998, Claudia Russo col. Paratypes: MNRJ-6387, MNRJ-6388, MNRJ-6389, MNRJ-6390, MNRJ-6391—South Africa, Port Alfred, 26 March 1998, Claudia Russo col.

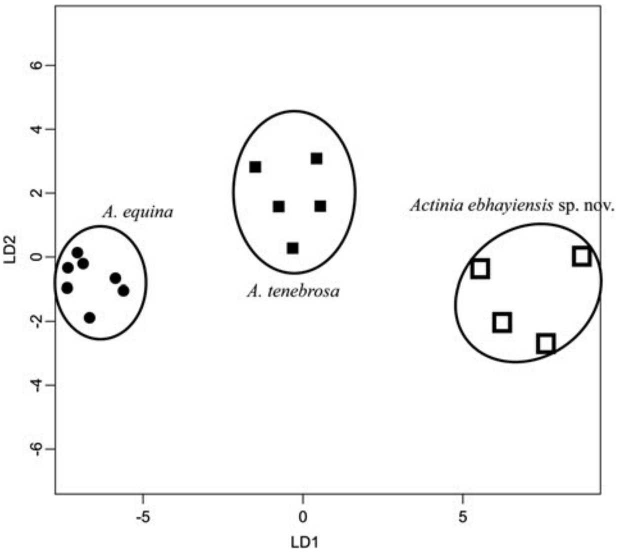

Fig. 3. Discriminant analysis based on length measurements of all nematocysts types. *Actinia ebhayiensis* (□); *Actinia equina* (•) and *Actinia tenebrosa* (■).

DESCRIPTION
Oral disc smooth, circular, uniformly red, usually more transparent than column; mesenterial insertions visible. Mouth central, usually elevated in hypostome.

Tentacles smooth, red, retractile, cone shaped, one-third the length of column, arranged in five cycles, approximately 90 in number. Tentacles fully enclosed by column when animal contracted.

Column smooth, entirely red. Height of column 0.4–1.4 cm (N = 6; mean 0.8 cm), dome shaped in contracted specimens. Parapet and fosse well delimited. Conspicuous blue acrorhagi in fosse, simple or compound, up to 24 in single cycle. Mid-column narrower than oral and pedal disc in live specimens. Margin smooth (Figure 4).

Pedal disc pink, circular, adherent, usually broader than oral disc, diameter 0.8–1.8 cm (N = 6; mean 1.1 cm).

Four cycles of mesenteries arranged hexamerously: 6, 6, 12 and 24. First three cycles perfect and fourth imperfect. No gametic material observed, juveniles found in ceolenteron of some specimens. A ribbed actinopharynx extends half the length of column. Two siphonoglyphs, directive mesenteries attached (Figure 5C). Sphincter endodermal, diffuse (Figure 6). Tentacles with longitudinal ectodermal musculature. Retractor muscles diffuse (Figure 5A), long, usually ending close to parietobasilar muscles. Parietobasilar muscles diffuse, weak, with a short broad mesogleal pennon (Figure 5A).

Cnidom: spirocysts, holotrichs, basitrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores (Figure 7). See Table 1 for size and distribution.

HABITAT
Rocky shores, usually in crevices or under rocks, from high to low intertidal zone. More common in the supralittoral zone, in high energy and low suspension areas. Individuals tend to form aggregations on rocks with 2–3 cm spacing among them.

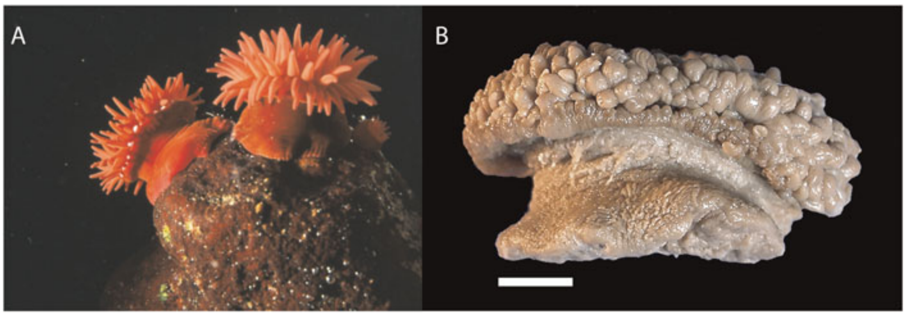

Fig. 4. External morphology of *Actinia ebhayiensis* sp. nov. (A) Live specimens, from Port Alfred (photography by Dr Toufiek Samaai); (B) preserved holotype (scale bar = 0.5 cm).

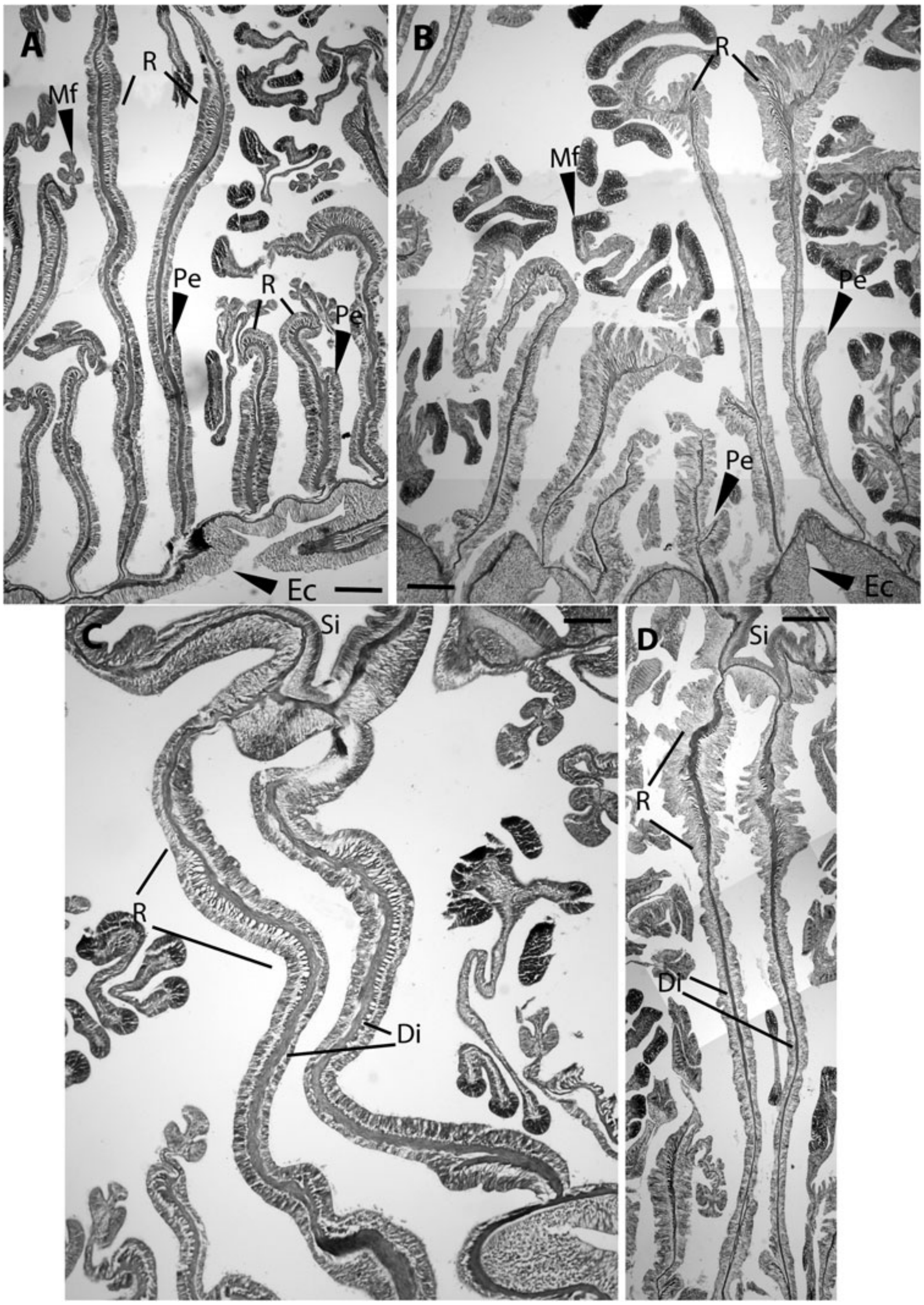

Fig. 5. Histology of *Actinia ebhayiensis* sp. nov. (A & C) and *A. equina* (B & D). Actinopharynx (Ph); siphonoglyph (Si); directive mesenteries (Di); retractor muscle (R); parietobasilar muscle pennon (Pe); mesenterial filaments (Mf). Scale bar = 200 µm.

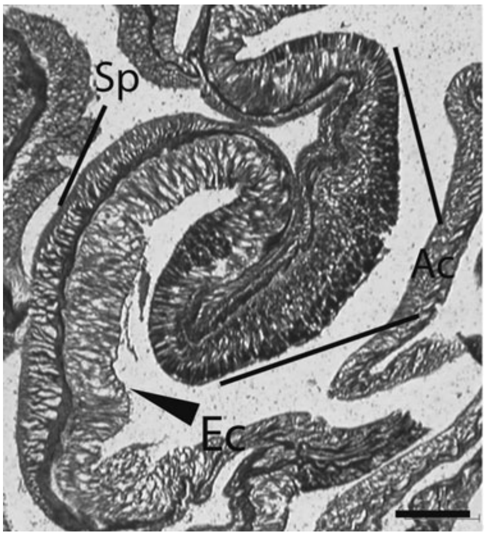

Fig. 6. Longitudinal section through the sphincter muscle (Sp) and acrorhagus (Ac), showing ectodermis (Ec) of *Actinia ebhayiensis*. Scale bar = 200 μm.

DISTRIBUTION

Port Elizabeth and Port Alfred, South Africa. It may also occur on the Indian and Atlantic coasts of South Africa, since individuals identified as *Actinia equina* were found on the east and west coasts of the Cape Peninsula (Carlgren, 1938; Kruger & Griffiths, 1998; Acuña & Griffiths, 2004; Branch *et al.*, 2007). Its occurrence in other parts of Africa is unknown as all occurrences of red *Actinia* along the African coast to date were identified as *A. equina* (Kruger & Griffiths, 1998; Acuña & Griffiths, 2004; Branch *et al.*, 2007). The presence of more than one species of the genus in the same locality is well documented for a number of places and cannot be discarded, but they usually present clear morphological differences between them (Ocaña *et al.*, 2005; see also Perrin *et al.*, 1999 for a review). To better understand the species distribution along the South African coast, more extensive sampling needs to be conducted. It is unlikely that *A. equina* occurs on the South African coast since Ocaña and collaborators extensively sampled the Macaronesia islands and never found the species, indicating that it does not disperse this far. Many studies have shown that phylogeographical breaks seem to play an important role in the distribution of benthic marine animals (Hellberg, 2009) further emphasizing the importance of a continuous distribution for the dispersal of these animals.

ETYMOLOGY

The species is named after Port Elizabeth, the city where the holotype has been collected. In Xhosa, the major ethnic group in that region, the city is called Ebhayi.

DIFFERENTIAL DIAGNOSIS

Although very similar to the type species of the genus, *A. equina, Actinia ebhayiensis* can be distinguished from it by the presence of 9 diagnostic genetic *loci* (*mdh-1, mdh-2, got-1, pep-1, pep-2, xod, odh, pgi-1* and *pgi-2*: see Schama *et al.*, 2005) and by significant nematocyst differences. Of the 18 nematocyst measurements, *A. ebhayiensis* differs significantly from *A. equina* in 12. The most striking differences are the smaller size, in *A. ebhayiensis*, of the acrorhagial holotrichs and the microbasic *b*-mastigophores and *p*-mastigophores from the mesenterial filaments (Figure 2). The two species also differ in muscle morphology: in *A. ebhayiensis* the retractor muscles are weaker and the parietobasilar muscles have a smaller, non-detached pennon than in *A. equina* (Figure 5A–D). Another difference between these two species is the relative size of two different types of nematocysts. The microbasic *b*-mastigophores of the mesenterial filaments are smaller than the basitrichs II of the actinopharynx in *A. ebhayiensis*, a characteristic previously observed only in *A. schmidti* (Schmidti, 1971, 1972; Chintiroglou & Simsiridou, 1997; Monteiro *et al.*, 1997), which differs from *A. ebhayiensis* by its much larger size, nematocyst differences (Table 3) and the apparent absence of broods (Monteiro *et al.*, 1997). Similar to *A. tenebrosa* (Ayre, 1984) *Actinia ebhayiensis* may reproduce asexually, as indicated by broods found in the coelenteron of many specimens (Carlgren, 1938; Griffiths, 1977; this study). In all brooding *Actinia* species genetically studied to date (*A. equina*, *A. tenebrosa* and *Actinia bermudensis* (McMurrich, 1889)), the broods were produced exclusively through asexual reproduction (Black & Johnson, 1979; Orr *et al.*, 1982; Monteiro *et al.*, 1998).

Although no nematocyst or histological analyses were made, in this study, on the geographically closer species *A. sali* from Cape Verde or *A. nigropunctata* den Hartog & Ocaña, 2003 from the island of Madeira, they are

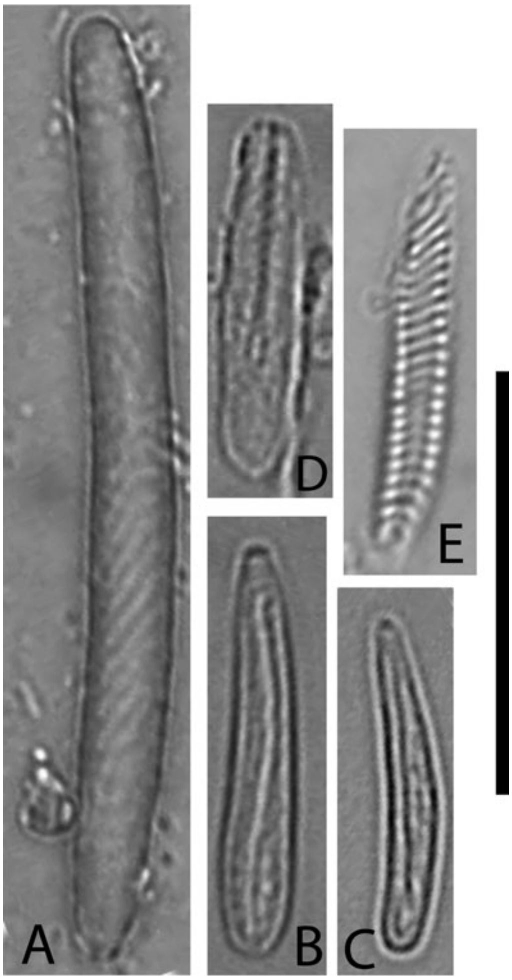

Fig. 7. Representative cnidae from *Actinia ebhayiensis* sp. nov. (A) Holotrich; (B) basitrich; (C) microbasic *b*-mastigophore; (D) microbasic *p*-mastigohpore; (E) spirocyst. Scale bar = 20 μm.

Table 3. Nematocysts of *Actinia* spp. Length measurements in micrometres. Mean or range taken from this study (1) and from the literature: (2) Monteiro *et al.* (1997); (3) Schmidt (1972); (4) Carlgren (1950); (5) Carlgren (1938) (as *A. equina*); (6) Chintiroglou & Stefanidou (1996); (7) Chintiroglou & den Hartog (1995); and (8) Allcock *et al.* (1998).

	Acrorhagus	Actinopharynx		Filaments			Tentacles
***Actinia* spp.**	**Holotrich**	**Basitrich I**	**Basitrich II**	**Microbasic *p*-mastigophore**	**Microbasic *b*-mastigophore**	**Basitrich**	**Basitrich**
A. ebhayiensis [1]	40.41	14.53	21.76	16.19	18.29	11.18	14.87
A. ebhayiensis [5]	36.0–60.0	–	14.0–29.0	17.0–24.0	–	10.0–24.0	17.0–24.0
A. striata[6]	–	15.80	–	18.30	23.04	12.06	16.03
A. cari[1]	48.70	12.18	19.91	18.68	25.09	11.87	18.11
A. cari[7]	53.10	–	21.30	20.30	26.10	12.40	18.20
A. equina[1]	50.37	13.30	25.37	22.65	34.37	13.45	18.22
A. equina[2]	53.89	14.53	29.81	24.28	32.05	15.16	19.06
A. equina red/pink[8]	56.30	–	–	24.90	34.90	15.40	20.80*
A. equina green/grey[8]	49.10	–	–	21.80	33.10	13.70	23.40*
A. fragacea[1]	33.97	14.88	–	13.97	19.00	8.27	11.51
A. prasina[2]	51.03	12.76	27.81	23.05	33.00	13.66	16.99
A. schmidti[2]	51.76	14.97	28.47	20.55	23.76	14.71	23.49
A. schmidti[3]	55.50	–	28.30	21.40	25.20	14.70	23.20
A. sali[2]	47.97	12.94	21.46	19.41	24.14	13.26	15.78
A. tenebrosa[1]	49.45	14.20	24.47	19.85	25.64	13.42	16.71
A. tenebrosa[4]	43.7–56.4	–	19.7–26.8	14.1–22.6	22.6–28.0	12.7–15.5	18.3–21.0

*, Allcock *et al.* (1998) was the only one to differentiate two size-classes, the table contains the bigger one, and the smaller one has a mean of 15.00 for the red/pink morph and 2.80 for the green/grey morph.

clearly distinct from *A. ebhayiensis* both genetically (Figure 1; Schama *et al.*, 2005) and morphometrically (Table 3). *Actinia sali* is externally similar to *A. ebhayiensis*, but, among other nematocysts differences, its microbasic *b*-mastigophores from the mesenterial filaments are larger than the basitrichs II of the actinopharynx (Carlgren, 1950; Monteiro *et al.*, 1997; Table 3). *Actinia nigropunctata* differs from *A. ebhayiensis* by the presence of numerous black spots on its column, the tendency towards restriction of its retractor muscles and the distinctiveness of its holotrichs (den Hartog & Ocaña, 2003). In *A. ebhayiensis* the holotrichs have spirally arranged tubes, like most *Actinia* species, whereas in *A. nigropunctata* the tubes are erratically arranged (Ocaña *et al.*, 2005).

In a genetic analysis of some *Actinia* species, *A. tenebrosa* appears as a sister species of *A. ebhayiensis*, although two allozyme *loci* are fixed for different alleles in each species, clearly separating these species (Schama *et al.*, 2005). Morphologically these species can be distinguished by a greater number of tentacles in *A. tenebrosa* (121–144, against 88–100 in *A. ebhayiensis*) and *A. tenebrosa* also has weaker longitudinal muscles with no pennons (Carlgren, 1924). Also in *A. tenebrosa* the sphincter, is well developed with a tendency to form humps (Carlgren, 1924), whereas in *A. ebhayiensis*, the sphincter is weak and, diffuse (Figure 6). Significant nematocysts differences were also found: the two species differ significantly in 12 out of 18 measurements analysed (Table 2). *Actinia ebhayiensis* has significantly smaller acrorhagial holotrichs, bastitrichs II of the actinopharynx and microbasic *b*-mastigophores and *p*-mastigophores from the filaments than *A. tenebrosa* (Table 1).

DISCUSSION

This study describes the new species *Actinia ebhayiensis* sp. nov. We confirm that, though morphologically very similar to the type species of the genus, *A. ebhayiensis* is differentiated from *A. equina* on genetic and morphological grounds. The two species completely differ in nine (out of 18) allozyme *loci* (Schama *et al.*, 2005; see below), and significantly differ in several nematocyst measurements (Figures 2 & 3; Tables 1 & 2). Furthermore, the microbasic *p*-mastigophores from the mesenterial filaments and the holotrichs from the acrorhagi are much smaller in *Actinia ebhayiensis* sp. nov. than in other species of the genus (except *A. fragacea*; Table 3).

Although the use of nematocysts as characters in lower taxonomic differentiation is still under debate (Ardelean & Fautin, 2004; Francis, 2004; Fautin, 2009), they have been widely used in sea anemone taxonomy (Stephenson, 1935; Carlgren, 1938, 1950; Schmidt, 1971, 1972; Shick, 1991); including differentiation of intra-specific colour morphs (Chintiroglou *et al.*, 1997; Allcock *et al.*, 1998).

Carlgren (1900) suggested that any description of a sea anemone should not be considered to be complete without nematocyst measurements, whereas Weill (1934) considered that cnidae size had little taxonomic value. It was usually the presence or absence of the different types of cnidae that were deemed important in differentiating species, since most authors argued that cnidae size may vary with nutrition state and size of the anemones (Chintiroglou, 1996; Chintiroglou & Simsiridou, 1997) or were altogether too variable within a single individual (Ardelean & Fautin, 2004).

Nevertheless modern statistical analysis of the cnidae in different species of sea anemones has shown that random samples of cnidae follow a normal distribution and statistical tests that took into account the intraspecific variability could be used to compare different species when proper statistical sampling is conducted (Williams, 1996, 1998; Allcok *et al.*, 1998; Watts *et al.*, 2000).

Although authors have argued that the length and width of nematocysts may vary with body size and weight, the relative proportions of cnidae size (such as length/width ratio) seem to be less influenced by anemone size (Schmidt, 1971, 1972; Chintiroglou & Simsiridou, 1997). In this study, we observed that between species of *Actinia* both length and width can be important characters when based on statistically significant differences. The different species can also be discriminated by the length/width ratio of some nematocyst types, corroborating the other statistical differences found (Table 2).

Many marine invertebrate species that were considered cosmopolitan turned out to be, under genetic scrutiny, complexes of morphologically similar but nevertheless distinct species (Knowlton, 1993, 2000; Thorpe & Solé-Cava, 1994; Klautau *et al.*, 1999). The alleged cosmopolitanism of *A. equina* seems to be a typical case, and what was considered to be one single species of worldwide distribution is actually a group of at least six different species (ordered by discovery date: *A. fragacea*, *A. prasina*, *A. schimidti*, *A. sali*, *A. nigropunctata* and *A. ebhayiensis*).

Certainly, the joint use of genetic, morphology and biometrics characters is necessary for a better understanding of this complex genus. The number of allozyme diagnostic *loci* and the consistent biometric and morphological differences clearly separate *Actinia ebhayiensis* from all other species of the genus. *Actinia ebhayiensis* corresponds to *Actinia* sp. 1 in Schama (2001) and Schama *et al.* (2005), where several diagnostic gene loci (DGL) were found between *Actinia* from South Africa and the supposedly conspecific *A. equina* from the United Kingdom (DGL = 9; Nei's (1978) genetic distance, D = 1.48). Conspecific populations of invertebrate species usually have $D < 0.18$ (Thorpe & Solé-Cava, 1994). In the same study, other species of *Actinia* were found to be highly divergent genetically from the South African samples: *A. prasina* (DGL = 9; D = 1.56), *A. sali* (DGL = 2; D = 0.25), *A. bermudensis* (DGL = 2; D = 0.32), *A. nigropunctata* (DGL = 10; D = 1.89), *A. tenebrosa* (DGL = 2; D = 0.28) and *A. schmidti* (previously *A. equina mediterranea* form I, *sensu* Schmidt; DGL = 3; D = 0.58). The levels of genetic distance were within the range usually found between distinct, congeneric species (Thorpe & Solé-Cava, 1994). The genetic differences between *Actinia* species studied are better represented through a FCA, which clearly shows the genetic differences found between *Actinia ebhayiensis* and the genetically close species *A. tenebrosa* and the geographically closer *A. sali* (Figure 1).

It is evident that the binomial '*Actinia equina*' has been incorrectly used to identify a number of distinct species from several geographical areas (*A. prasina* and *A. fragacea* in the UK; *A. schmidti* in the Mediterranean; *A. sali*, *A. nigropunctata* and, now, *Actinia ebhayiensis* in the eastern Atlantic). Pending a revision of the genus *Actinia*, we propose that the name *Actinia equina* be designated for specimens from the north-east Atlantic and for the asexually reproducing Mediterranean specimens. Specimens from other geographical locations, such as the Indo-Pacific Ocean, the Red Sea, and the Far East, should be re-evaluated using both morphological and molecular characters.

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