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A re-evaluation of the *Abarenicola assimilis* group with a new species from the Falkland Islands and key to species

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The new species Abarenicola wellsi *n. sp. from the Falkland Islands is described along with a re-description of* Abarenicola brevior *n. stat. with which it had previously been confused. The two species are distinguished using both morphological and molecular techniques, new characters are described and previously recognized characters are clarified, all of which support the assignment of species status as opposed to subspecies. Both species are part of the* Abarenicola assimilis *'group' within which all taxa were originally described as subspecies. The group as a whole is re-evaluated and all members are elevated from subspecies to species status based mainly on the new characters of shape, distribution and pigmentation of the proboscidial papillae. The group now consists of six species:* Abarenicola assimilis *n. stat.,* A. brevior *n. stat.,* Abarenicola devia *n. stat.,* Abarenicola haswelli *n. stat.,* Abarenicola insularum *n. stat. and* A. wellsi *n. sp.*

Keywords: Cytochrome oxidase I, 16S, taxonomy, Polychaeta, *devia*, *haswelli*, *insularum*, *brevior*, *wellsi*

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INTRODUCTION

Family Arenicolidae Johnston, 1835 contains some of the most well-known polychaete species due to their commercial value and exploitation worldwide. There are four genera, *Arenicola* Lamarck, 1801, *Abarenicola* Wells, 1959, *Arenicolides* Mesnil, 1898 and *Branchiomaldane* Langerhans, 1881, between them containing nearly 30 species. *Arenicola* and *Abarenicola* are the largest, and most widely recognized, genera with seven species in the former group and eight species, three of which are further split into two, four and five subspecies each, in the latter. Few phylogenetic studies have concentrated on the relationships within the family and its genera, the most significant being Bartolomaeus & Meyer (1999), a morphological study using chaetal morphology and development along with other literature and, most recently, a molecular study by Bleidorn *et al.* (2005) in which monophyly of the family as a whole, as well as the genera *Branchiomaldane* and *Abarenicola*, were supported.

Due to their large size and economic importance, species of *Arenicola* and *Abarenicola* have been well-studied historically and therefore few new species of *Abarenicola* have been described since two comprehensive studies in 1959 (Healy & Wells) and 1963 (Wells). Just prior to the former publication, Wells (1959) erected the genus *Abarenicola* for five species: *Abarenicola assimilis* (Ehlers, 1897), *Abarenicola claparedii* Levinsen, 1884, *Abarenicola pacifica* Healy & Wells, 1959, *Abarenicola pusilla* (de Quatrefages, 1866) and *Abarenicola vagabunda* Healy & Wells, 1959. Included within *A. assimilis* was the variety *affinis*, described by Ashworth in 1903. Healy & Wells (1959) then split *A. vagabunda* into two subspecies, *A. v. oceanica* and *A. v. vagabunda*. In 1963, Wells raised *Abarenicola affinis* to a separate species and further split both *A. affinis* and *A. assimilis* into four and five subspecies respectively: *A. affinis affinis*, *A. affinis africana*, *A. affinis clarki*, *A. affinis chiliensis*, *A. assimilis assimilis*, *A. assimilis brevior*, *A. assimilis devia*, *A. assimilis haswelli* and *A. assimilis insularum*. In reference to his reasons for erecting subspecies rather than full species, Wells stated in the paper 'In the circumstances, it has not been difficult to show that various populations differ consistently in their morphological characters, but my estimate of the grade of taxonomic separation between any two forms is necessarily arbitrary, resting largely on analogy with those other Arenicolidae which I have studied in the field'. Indeed, the distinctions between the subspecies of *assimilis* were based primarily on a single internal characteristic, the number of oesophageal caeca, and the geographic separation of the populations. Since that time, no author has challenged the status of these subspecies as to whether they should remain as such or be elevated to full species status.

In 2011, a survey of intertidal polychaetes in the Falkland Islands was initiated. At that time, only a single subspecies of *Abarenicola*, *A. a. brevior*, was known from the islands. During the survey, specimens of *Abarenicola* were collected from a shore close to Stanley where local people thought that more than one species may be present. Samples of the lugworms were taken for molecular analysis which proved, using both 16S and COI genes, that two distinct species were indeed present. A more comprehensive survey of the lugworm populations was then undertaken in 2013, with specimens collected

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from shores around both East and West Falkland and preserved for both morphological and molecular analysis.

In his 1963 paper, Wells used material from the Falkland Islands (some of which had previously been utilized by Ashworth (1903) in his description of *Arenicola assimilis* var. *affinis*), as well as from the Magellan Strait and Beagle Channel, as part of his description of the new subspecies *A. a. brevior*. The specimens he had came from two populations, one on each of the islands of East and West Falkland although he found some differences between the two populations. The specimens from West Falkland were much like those from South America but those from East Falkland differed in some respects such as the occurrence of the first gill and the structure of the nephridiopores. In the paper, he commented on the differences stating that 'it may be that they represent genetically distinct forms'. The differences however did not constitute enough evidence for the establishment of another subspecies, probably because the number of oesophageal caeca in all of the worms were the same and this was the defining character he used in describing all of the different subspecies.

The new collection of specimens from stations all around both East and West Falkland (Figure 1) enabled more detailed observations to be made and additional characters to be brought to light. In conjunction with the molecular analyses, the resulting evidence is hereby used to raise *Abarenicola brevior* n. stat. to full species status and describe *Abarenicola wellsi* n. sp. at species level also.

The holotype and as many paratypes as possible of *A. brevior* were inspected as it was clear that, at least in respect to the Falkland Islands, both species were present within the specimens used by Wells (1963) in his descriptions. However, except for the specimens from the East Falkland population, all of the other type specimens obtained belonged to *A. brevior*.

Additional observations were also made of the other species within the '*assimilis* group', providing new characters on which to separate them and thereby warranting the elevation of these also to species status as *A. assimilis* n. stat., *A. devia* n. stat., *A. haswelli* n. stat. and *A. insularum* n. stat.

MATERIALS AND METHODS

Specimen collection and examination

Specimens were collected by hand from the shore by digging with a fork or spade. Some juvenile specimens were collected by sieving sediment through a 0.5 mm sieve. Most samples were relaxed with 7% magnesium chloride solution and then fixed with 4% formaldehyde in seawater. Prior to fixing, a small sample of tissue was removed from a wide selection of specimens and preserved in 100% ethanol for molecular analysis with the remainder of the animal fixed in formaldehyde. After a period of at least 2 days, fixed animals were rinsed with fresh water and preserved in 80% industrial methylated spirits with 2% propylene glycol added.

Morphological examinations, measurements and drawings were made using a Nikon Eclipse E400 binocular microscope and a Nikon Labophot-2 compound microscope. Microscope photographs were taken using AutoMontage™ software.

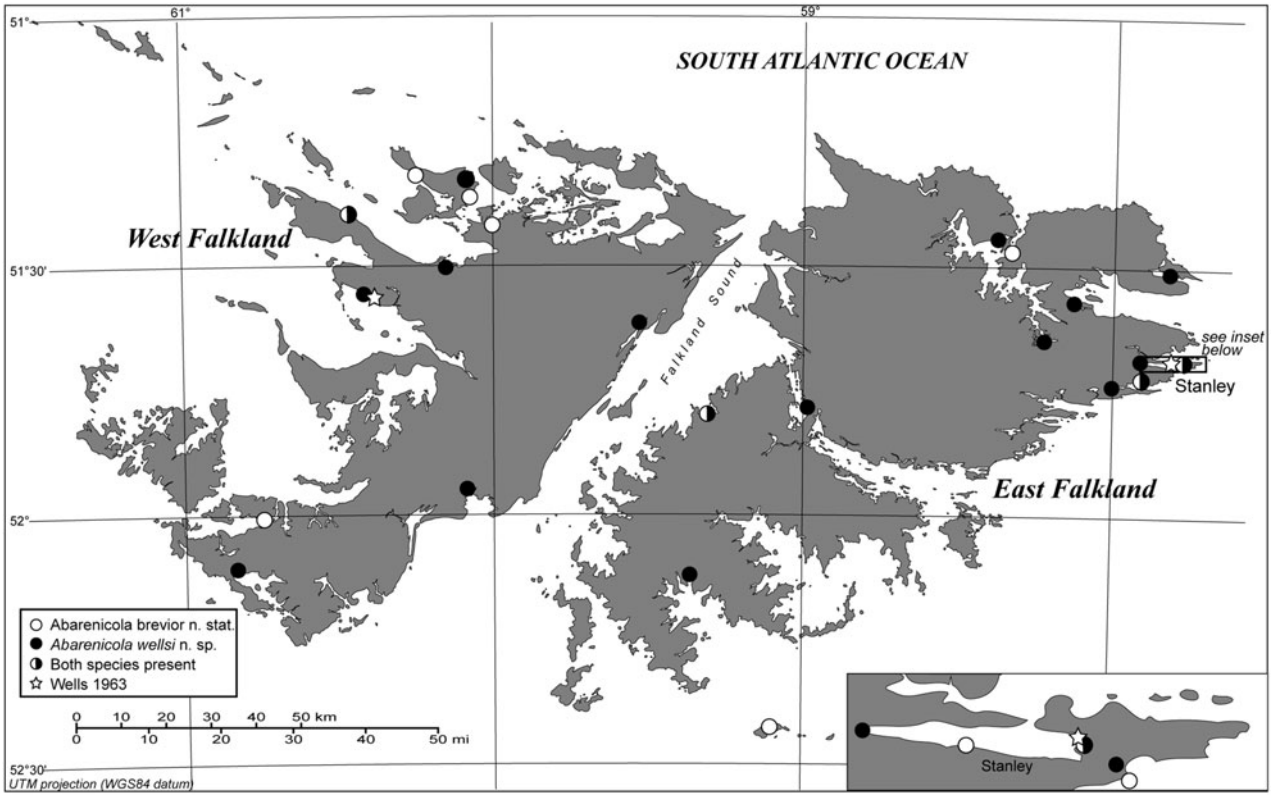

Fig. 1. Map showing the occurrence of *Abarenicola brevior* n. stat. and *Abarenicola wellsi* n. sp. around the Falkland Islands at the different sites sampled. Locations of specimens described by Wells in 1963 are also marked.

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The holotype and most paratypes of *Abarenicola wellsi* n. sp. are accessioned in the zoological collections of National Museum Wales (NMW.Z). Paratypes are also deposited in the Natural History Museum, London (NHMUK), National Museum of Natural History, Smithsonian Institution, Washington DC (USNM) and the Zoological Museum, Hamburg (ZMH). All other specimens of *A. wellsi* n. sp. are accessioned in the National Museum Wales collections.

The holotypes of *Abarenicola brevior* n. stat. and *Abarenicola assimilis* n. stat. were borrowed for comparison from ZMH. Most of the remaining paratypes of *A. brevior* (NHMUK, ZMH, Swedish Museum of Natural History SMNH, Museum für Naturkunde Berlin ZMB) were also inspected to confirm their identification. The paratypes from USNM could not be confirmed. Holotypes of *Abarenicola devia* n. stat., *Abarenicola haswelli* n. stat. and *Abarenicola insularum* n. stat. were borrowed from NHMUK for observation as well as additional syntypes of *A. assimilis* from NHMUK and ZMB.

DNA extraction and sequencing

Universal cytochrome oxidase subunit I (COI) primers (Folmer *et al.*, 1994) were used to amplify an ~675 bp region of the COI 'barcoding' gene of 44 *Abarenicola* specimens (seven *A. brevior* n. stat., 37 *A. wellsi* n.sp.). In addition to COI, an ~544 bp region of the 16S large subunit mitochondrial ribosomal DNA was also sequenced for three specimens (one *A. brevior*, two *A. wellsi*), using the Palumbi (1996) primers 16SarL and 16SbrH.

For 16S, DNA was extracted using a Qiagen DNeasy kit. Between 1–5 µl of extract was used as a template in PCRs using GE Healthcare Illustra PuReTaq PCR beads with 0.25 µl of each primer (10 µM). Each reaction was then made up to 25 µl using ultra-pure water. Cycling conditions (Eppendorf Mastercycler) were as follows: 94°C for 150 s, 35 cycles of 94°C for 45 s, 51°C for 45 s, 72°C for 45 s and finally 72°C for 10 min. For COI, the process was carried out in its entirety by Central Biotechnology Services (CBS), Cardiff University using the same extraction kit and protocols and PCR beads. Cycling conditions were either that published by Pleijel *et al.* (2012): 95°C for 180 s, 5 cycles of 95°C for 40 s, 45°C for 40 s and 72°C for 50 s, 40 cycles of 95°C for 40 s, 51°C for 40 s and 72°C for 50 s, followed by 72°C for 300 s; or 95°C for 120 s, 35 cycles of 95°C for 40 s, 40°C for 45 s and 72°C for 90 s, and finally 72°C for 7 min. Some reactions that failed were re-run successfully with the addition of magnesium chloride to a final concentration of 2 mM. Products were cleaned using Sigma Aldrich GenElute PCR clean up kit, quantified on agarose gels and sequenced by CBS, Cardiff University. Sequences were edited and compiled in ApE v.2.0.38 and all identified haplotypes were submitted to GenBank (Table 1). Edited and aligned sequences were 573 and 463 bp in length for COI and 16S respectively.

DNA datasets and analysis

For 16S, all of the Arenicolidae sequences published by Bleidorn *et al.* (2005), except for *Arenicola marina* Linnaeus, 1758 and *Arenicola defodiens* Cadman & Nelson-Smith, 1993, were downloaded for comparison from GenBank along with two of the outgroup sequences (*Scalibregma inflatum* (Rathke, 1843) and *Clymenura clypeata* (de Saint-Joseph, 1894)) also used by the author. Five sequences of *A. marina* and two of *A. defodiens* were available from the author's personal datasets. For COI, five sequences each of *A. marina* and *A. defodiens* were available from the author's personal datasets but no sequences from any other Arenicolidae species were available on GenBank. The same outgroups were used as for 16S (*S. inflatum*, *C. clypeata*). For those species where sequences from multiple specimens were available (*A. defodiens*, *A. marina*, *A. wellsi* and *A. brevior*), inter- and intraspecific distances were also calculated. For the phylogenetic analyses, a single reference sequence, for both COI and 16S, from each of the latter species was used. Sequences were aligned by CLUSTALW in MEGA v7.0.18 (Kumar *et al.*, 2016) using the default parameters and uncorrected pairwise differences (p-distance) were calculated. Maximum parsimony (MP) analysis with branch and bound search was carried out in MEGA v7.0.18 with clade support assessed via bootstrap values (1000 replicates). Maximum likelihood (ML) analyses were carried out using MEGA v7.0.18. Bootstrap values were calculated from 1000 replicates to provide a measure of clade support. Bayesian Inference (BI) was conducted using MrBayes 3.2 (Ronquist *et al.*, 2012). FindModel v2 (www.hiv.lanl.gov/content/sequence/findmodel/findmodel.html) was used to estimate the appropriate model of sequence evolution (GTR + Γ in each case). Two parallel runs of 1,000,000 generations, sampling trees every 1000 generations with the first 25% of trees discarded as burn-in were implemented in each case. Convergence was reached for both analyses before the burn-in period. The majority-rule consensus tree with posterior probabilities was determined from 751 trees.

RESULTS

SYSTEMATICS

Family ARENICOLIDAE Johnston, 1835
Genus *Abarenicola* Wells, 1959
Type species *Arenicola claparedii* Levinsen, 1884

DIAGNOSIS (Wells, 1959)
Arenicolidae with an achaetous tail. Prostomium non-retractile, in the form of a triangle with lateral extensions of its (anterior) base; with a shallow groove marking the attachment of the brain. Statocysts either present, with a tube to the exterior, or absent. Chaetigers (except the first two or three) subdivided into five annuli. Gills branched, the first (which may be reduced or absent) on chaetiger 7 or 8. None of the neuropodia approaches close to the mid-ventral line. Oesophageal caeca more than one pair. Gular membrane very thin; septal pouches absent. Nephridia five or six pairs, the first opening on chaetiger 4 or 5. Dioecious; gonads on the nephridia.

Abarenicola assimilis (Ehlers, 1897) n. stat.
(Table 2)

Arenicola assimilis Ehlers, 1897: 103–104. – Ehlers, 1900, 1901; Ashworth, 1903: 737–785, Pl. 36–37; *Abarenicola assimilis* Wells, 1959: 307, Pl. 2; *Abarenicola assimilis assimilis* Wells, 1963: 121–159, fig. 2, 3, Pl. 3, Table 1

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Table 1. List of taxa used in this study with source and GenBank accession numbers (newly sequenced taxa in bold).

Taxa	Source	Accession numbers	
		16S	COI
Scalibregma inflatum (Rathke, 1843) (Scalibregmatidae)	GenBank	AY532331	KT307695
Clymenura clypeata (de Saint-Joseph, 1894) (Maldanidae)	GenBank	AY340449	KJ183005
Arenicolidae			
Arenicola cristata Stimpson, 1856	GenBank	AY569682	–
Arenicola loveni Kinberg, 1866	GenBank	AY569683	–
Arenicola marina (Linnaeus, 1758)	West Aberthaw, Wales, UK	**KY652591**	**KY652595**
Arenicola defodiens Cadman & Nelson-Smith, 1993	Porthcawl & Whiteford Burrows, Wales, UK	**KY652590**	**KY652594**
Arenicola defodiens (additional haplotypes)	Whiteford Burrows, Wales, UK	–	**KY661884–5**
Arenicolides ecaudata (Johnston, 1835)	GenBank	AY569688	–
Branchiomaldane vincenti Langerhans, 1881	GenBank	AY569690	–
Branchiomaldane sp.	GenBank	AY569689	–
Abarenicola claparedi (Levinsen, 1884)	GenBank	AY569684	–
Abarenicola pacifica Healy & Wells, 1959	GenBank	AY569685	–
Abarenicola gilchristi Wells, 1963	GenBank	AY569686	–
Abarenicola affinis affinis (Ashworth, 1903)	GenBank	AY568687	–
Abarenicola brevior (Wells, 1963) n. stat.	Falkland Islands	**KY652592**	**KY652596**
Abarenicola brevior (additional haplotypes)	Falkland Islands	–	**KY661886–7**
Abarenicola wellsi n. sp.	Falkland Islands	**KY652593**	**KY652597**
Abarenicola wellsi n. sp. (additional haplotypes)	Falkland Islands	**KY661883**	**KY661888–98**

TYPE MATERIAL EXAMINED

Lectotype: Ushuaia, Beagle Channel (ZMH V 4872a), low shore, coll. W. Michaelsen, 07.12.1892.

Syntypes: Ushuaia, Beagle Channel (NHMUK 1912.5.25.1–2), coll. W. Michaelsen; Stewart Island, Beagle Channel (NHMUK 1912.5.25.3), coll. W. Michaelsen; Ushuaia, Beagle Channel (ZMB 6762), coll. W. Michaelsen, 27.10.1892.

ADDITIONAL OBSERVATIONS

Eversible proboscis covered in papillae with some pigmentation. Proximal section with large, low, rounded, darkly pigmented papillae; median section initially with large, wide, conical and small, narrow, conical papillae, then small papillae only. Small papillae with some pigmentation.

REMARKS

General observations on the holotype and syntypes agreed with the original description of Ehlers (1897), supplemented by Wells (1963). New observations are based on the syntypes from NHMUK, two of which had been dissected to reveal the oesophageal caeca and one of which had an everted proboscis. The holotype (ZMH) also had a partially everted proboscis.

Abarenicola brevior (Wells, 1963) n. stat.
(Figures 1, 2 & 4; Tables 1 & 2)

Arenicola assimilis Ehlers, 1897: 103–104. – Ehlers, 1900, 1901. – Ashworth, 1910, 1912. – *Abarenicola assimilis brevior* Wells, 1963: 133–140, Table 1, Pl. 3.

TYPE MATERIAL EXAMINED

Holotype: Lapataia Nueva, Beagle Channel (V. 4871a).

Paratypes: (ZMH V.4871), 11.1892; Ushuaia, Beagle Channel, paratype (ZMH V.4874), 10.1892; Ushuaia, Beagle Channel, paratype (ZMH V.4872), 12.1892; Ushuaia, Beagle Channel, paratype (SMNH 1414), 05.1896; Puerto Robalo, Beagle Channel, 6 paratypes (NHMUK 1961.12.11–16), 01.1959; Susanna Cove, Magellan Strait, 2 paratypes (ZMB 3629), 1893/5; Roy Cove, West Falkland, Falkland Islands, 2 paratypes (NHMUK 1912.4.9.3–4), 05.09.1910.

COMPARATIVE MATERIAL EXAMINED

East Falkland. Stanley foreshore, station 1a (51°41.454′S 057°51.870′W), under rocks in coarse sand, midshore, 1 specimen (NMW.Z.2011.039.0189), 15.11.2011; Hookers Point, station 6d (51°41.994′S 057°46.747′W), gravel in rock pool, low shore, 1 specimen (NMW.Z.2011.039.0178), 21.11.2011; Whalebone Cove, station 9a (51°41.330′S 057°48.092′W), medium-coarse sand, low shore, 4 specimens (NMW.Z.2011.039.0179–0181), 23.11.2011; Whalebone Cove, station 9b (51°41.318′S 057°48.011′W), medium-coarse sand, midshore, 5 specimens (4–NMW.Z.2011.039.0182; 1– ZMH P-27826), 23.11.2011; Kelp Harbour, by stone corral, station 28 (51°48.597′S 059°19.433′W), muddy sand, midshore, 2 specimens (NMW.Z.2011.039.0183), 04.12.2011; Kelp Harbour, off causeway, station 29a (51°47.715′S 059°18.400′W), coralline coarse sand, mid-low shore, 2 specimens (NMW.Z.2011.039.0184), 04.12.2011; Whalebone Cove, station 31a (51°41.307′S 057°47.985′W), in sand under rocks, high-midshore, 1 specimen (NMW.Z.2011.039.0185), 05.12.2011; Whalebone Cove, station 31b (51°41.308′S 057°48.005′W), medium-fine sand, mid-low shore, 6 specimens (NMW.Z.2011.039.0186), 05.12.2011; Whalebone Cove, station 31c (51°41.325′S 057°48.037′W), medium-fine sand, low shore, 1 specimen (NMW.Z.2011.039.0187), 05.12.2011; Mullet Creek, station 33b (51°43.121′S 057°54.833′W), rocks with coarse gravelly sand, high-mid shore, 1 specimen (NMW.Z.2011.039.0188), 07.12.2011; Hookers Point, station 40 (51°41.994′S 057°46.747′W), rock pool sediment, midshore, 8 specimens (NMW.Z.2012.082.0070–72), 05.12.2011; Whalebone Cove, station 41a (51°41.324′S 057°48.000′W), medium-coarse sand, high shore, 2 specimens (NMW.Z. 2012.082.0073–74), 15.01.2013; Whalebone Cove, station 41b (51°41.322′S 057°48.030′W), medium-coarse sand, midshore, 2 specimens (NMW.Z. 2012.082.0075–76), 15.01.2013; Rincon Grande, station 63a (51°28.241′S

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Table 2. Comparison of morphological characters across all *Abarenicola* species.

Species and type locality	No. of chaetigers	Max. stems per gill	Statocysts (present/absent)	Chaetiger of 1st gill	Nephridia	Oesophageal caeca	Proboscidial papillae
Abarenicola assimilis (Ehlers, 1897) n. stat. Ushuaia, Beagle Channel	20	9–12	P	viii	iv–ix, naked	1 + (4–7)	Some pigmentation, sizes intermix at transition; large – wide, conical; small – narrow, conical
Abarenicola brevior (Wells, 1963) n. stat. Lapataia Nueva, Beagle Channel	19	10–14	P	viii	iv–ix, naked	1 + (9–14)	Unpigmented, sizes intermix at transition; large – triangular; small – conical
Abarenicola insularum (Wells, 1963) n. stat. Campbell Island, New Zealand	19	10–14	P	viii	iv–ix, naked	1 + (4–6)	Some pigmentation, sizes intermix at transition; large – mushroom-like; small – conical
Abarenicola devia (Wells, 1963) n. stat. Shoreham, Victoria, Australia	19	13–18	P	vii	iv–ix, hooded	1 + (4–6)	Some pigmentation, transition between sizes abrupt, no intermixing; large – triangular; small – rounded
Abarenicola haswelli (Wells, 1963) n. stat. Burnie, Tasmania	19	10–13	P	ix	iv–ix, naked	1 + (7–9)	Some pigmentation, transition between sizes abrupt, no intermixing; large – trapezoidal, wider at base, arranged on ridges; small – conical
Abarenicola wellsi n. sp. Falkland Islands	19	12–17	P	vii	iv–ix, hooded	1 + (11–15)	Some pigmentation, sizes intermix at transition; large – triangular; small – conical
Abarenicola affinis affinis (Ashworth, 1903) Otago Harbour, New Zealand	19	No information	P	vii	iv–ix, hooded	1 + (6–9)	No information
Abarenicola affinis clarki Wells, 1963 Ralph's Bay, Hobart, Tasmania	19	–	P	vii	iv–ix, hooded	1 + 4	–
Abarenicola affinis africana Wells, 1963 Luderitz Bay, Southwest Africa	19	–	P	vii	v–ix, hooded	1 + (7–9)	–
Abarenicola affinis chiliensis Wells, 1963 West Chiloe, Chile	19	–	P	vii	iv–ix, hooded	1 + (8–11)	–
Abarenicola gilchristi Wells, 1963 Buffels Bay, Cape Peninsula, South Africa	19	–	P	viii	v–ix, hooded	1 + (16–20)	–
Abarenicola pusilla (de Quatrefages, 1866) Coquimbo, Chile	19	No information	A	viii	iv–ix, naked	1 + 8	No information
Abarenicola claparedii claparedii (Levinsen, 1884) Naples, Mediterranean Sea	19	–	A	vii	v–ix, hooded	1 + (3–4)	–
Abarenicola claparedii vagabunda (Healy & Wells, 1959) False Bay, Washington, USA	19	–	A	vii	v–ix, hooded	1 + (11–18)	–
Abarenicola claparedii oceanica (Healy & Wells, 1959) Dutch Harbour, Alaska, USA	19	–	A	vii	v–ix, hooded	1 + (7–9)	–
Abarenicola pacifica Healy & Wells, 1959 False Bay, Washington, USA	19	–	A	vii	v–ix, naked	1 + (3–6)	–

058°19.943′W), muddy with some gravel, midshore, 1 specimen (NMW.Z.2015.002.0001), 19.01.2015; *Saunders Island*. The Neck south, station 42a (51°18.515′S 060°14.396′W), sand, midshore, 5 specimens (NMW.Z.2012.082.0077), 17.01.2013; The Neck south, station 42b (51°18.473′S 060°14.481′W), sand, midshore, 6 specimens (NMW.Z.2012.082.0078), 17.01.2013; The Neck south, station 42c (51°18.472′S 060°14.492′W), sand under stones, midshore, 1 specimen (NMW.Z.2012.082.0079), 17.01.2013; The Neck south, station 42e (51°18.485′S 060°14.488′W), sand, low shore, 6 specimens (NMW.Z.2012.082.0080–81), 17.01.2013; Sealer Cove harbour, station 44a (51°21.739′S 060°04.910′W), mud & rocks, midshore, 1 specimen (NMW.Z.2012.082.0082), 18.01.2013; bay below settlement, station 45 (51°21.923′S 060°04.964′W), sand, low shore, 5 specimens (NMW.Z.2012.082.0083–84), 18.01.2013; *Sea Lion Island*. Cow Point, station 71 (52°25.287′S 059°04.596′W), fine sand in rock pool, low shore, 1 specimen (NMW.Z.2015.002.0002), 26.01.2015; *West Falkland*. South Harbour, station 52a (52°00.201′S 060°44.791′W), sand under rocks, high-mid shore, 2 specimens (NMW.Z.2012.082.0085–86), 27.01.2013; South Harbour, station 52b (52°00.201′S 060°44.791′W), sand, midshore, 4 specimens (NMW.Z.2012.0087–88), 27.01.2013; South Harbour, station 52d (52°00.201′S 060°44.791′W), silty sand, low shore, 1 specimen (NMW.Z.2012.082.0089), 27.01.2013; Hot Stone Cove Creek, Dunbar, station 54b (51°23.078′S 060°30.919′W), sand, high shore, 1 specimen (NMW.Z.2012.082.0090), 29.01.2013; Hot Stone Cove Creek, Dunbar, station 54c (51°22.999′S 060°30.909′W), fine sand, midshore, 2 specimens (NMW.Z.2012.082.0091), 29.01.2013; Hot Stone Cove Creek, Dunbar, station 54d (51°22.895′S 060°30.892′W), under stones in fine sand, low shore, 1 specimen, (NMW.Z.2012.082.0092), 29.01.2013; Hot Stone Cove Creek, Dunbar, station 54f (51°22.883′S 060°30.886′W), fine sand, low shore, 5 specimens (3– NMW.Z.2012.082.0093; 1– NHMUK 2017.87; 1– USNM 1422117), 29.01.2013; Shallow Bay, station 57d (51°25.255′S 059°59.857′W), shell/

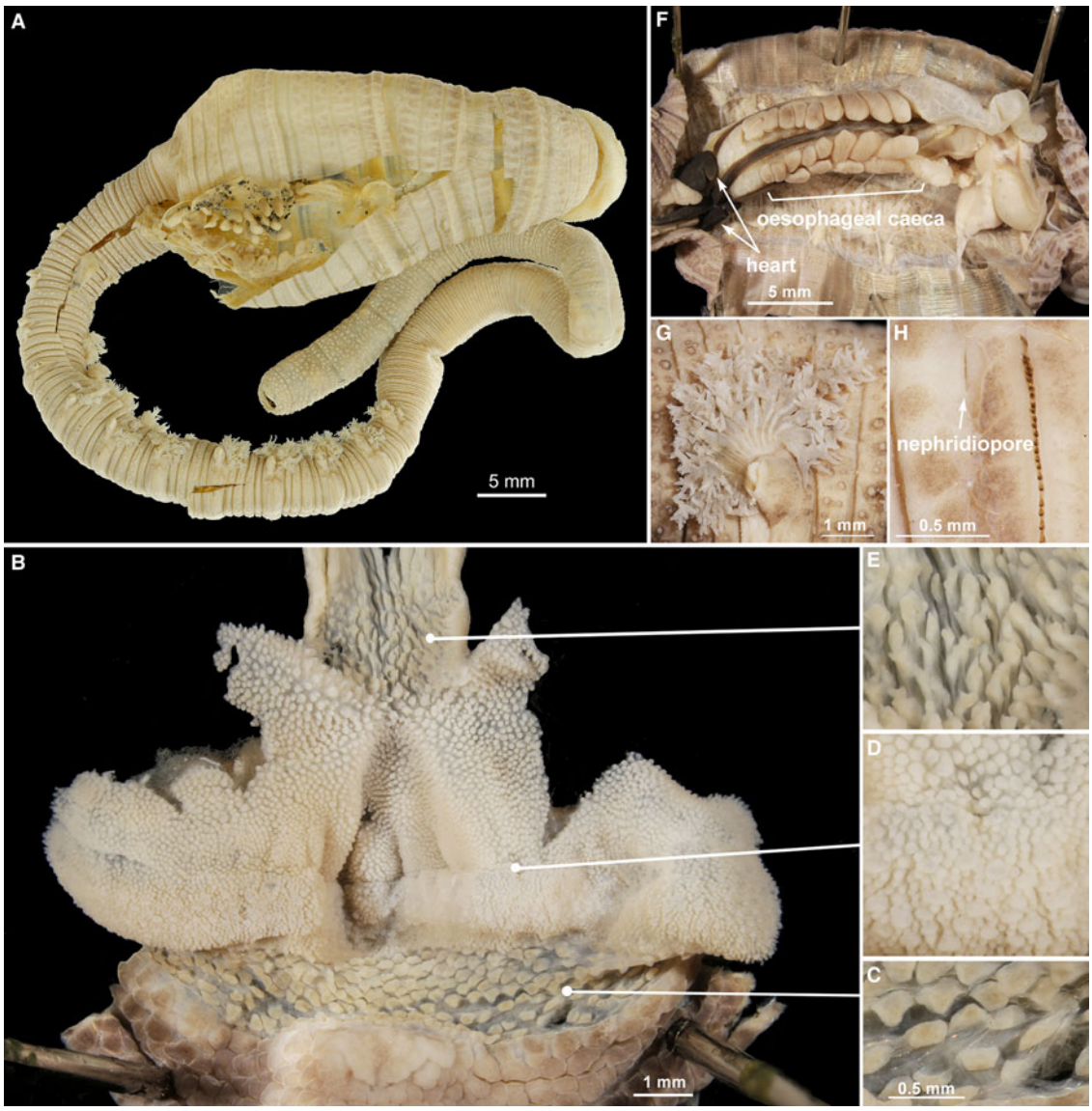

Fig. 2. *Abarenicola brevior* (Wells, 1963) n. stat. (A: HZM V.4871a; B–H: NMW.Z.2011.039.0181). A. holotype, whole body, dorsal view; B. everted proboscis, dorsal view; C. proximal papillae; D. median papillae; E. distal papillae; F. oesophageal caeca; G. chaetiger 14 gill, notopodium and notochaetae; H. chaetiger 7, nephridiopore and neurochaetae.

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gravel/stones, midshore, 3 specimens (NMW.Z.2012.082.0094–95), 01.02.2013.

DESCRIPTION (incorporating Wells, 1963)

Holotype complete (Figure 2A), 85 mm long (tip of prostomium to end of final chaetiger), 9 mm wide at 1st chaetiger, 19 chaetigers. Paratypes 28–63 mm long, additional non-type specimens 3.5–184 mm long. Description based on holotype except for statolith form.

Body cylindrical, divided externally into three distinct regions: anterior ('head'), thorax ('body') and posterior caudal region ('tail'). Body widest over first few anterior chaetigers, tapering towards end of chaetigers, tail narrow, tubular. Colour pale brown (preserved). Epidermis tessellate to chaetiger 6, papillate from chaetiger 6 onward. All segments with distinct annulation. First 3 chaetigerous annuli slightly swollen, intervening annuli not reduced; number of annuli between first 4 chaetigers 2–3–4, thereafter 4.

Anterior region consisting of prostomium and 2 achaetigerous segments. Prostomium trilobate, non-retractile. Nuchal groove and statocyst with open-ended duct on either side. Statoliths small, numerous, evenly shaped, amber. Eyes absent. Eversible proboscis covered in unpigmented papillae (Figure 2A–E). Proximal section with large, flat, triangular papillae, more sparsely distributed than in following sections (Figure 2B, C). Median section densely covered initially with large, rounded and small conical papillae, then small papillae only (Figure 2B, D). Distal section papillae elongate, conical, irregular in size (Figure 2B, E).

Oesophageal caeca with 1 elongate and 10 or 11 smaller caeca (Figure 2F).

Thorax with 19 chaetigers (Figure 2A). Each segment with one enlarged annulation bearing noto- and neuropodia and, on chaetigers 8–19, branchiae (Figure 2A).

Notopodium trapezoidal outer torus with inner retractile, rounded lobe (Figure 2G) bearing 2 parallel rows of up to 25 simple capillaries. Neuropodia raised, elliptically shaped tori containing single row of 22–49 unidentate hooks, minutely serrated on the upper edge.

Branchiae large, branched, highly vascularized, 12 pairs (Figure 2G). First 2 pairs reduced in size. Median branchiae with 13 gill stems with multiple lateral branches and gill filaments off each stem. Gill stems fused together over lower third portion of length.

Six pairs of nephridia on chaetigers 4–9. Nephridiopores naked dorsi-ventral clefts located posterior to dorsal end of neuropodium (Figure 2H).

Achaetous tail papillate tube (Figure 2A), easily lost. Anus terminal.

REMARKS

Abarenicola brevior n. stat. is part of the 'cysted' group of *Abarenicola* species that possess statocysts with ducts to the exterior. This group comprises *Abarenicola assimilis* n. stat., *Abarenicola devia* n. stat., *Abarenicola haswelli* n. stat., *Abarenicola insularum* n. stat., *Abarenicola wellsi* n. sp., *Abarenicola affinis affinis*, *Abarenicola affinis africana*, *Abarenicola affinis clarki*, *Abarenicola affinis chiliensis* and *Abarenicola gilchristi*. Of these species, *A. brevior* is most similar to *A. assimilis*, *A. gilchristi* and *A. insularum* in having the first gill occurring on chaetiger 8 as opposed to chaetiger 7 (this is a correction to Wells' original description in which he stated that the first gill could occur on either chaetiger 7 or 8). However, *A. brevior* is easily distinguished from *A. assimilis*, which has 20 chaetigers instead of the usual 19 possessed by *A. brevior* and the rest of the genus, and from *A. gilchristi* which has only 5 pairs of nephridia, from chaetigers 5–9, and hooded nephridiopores as opposed to 6 pairs of nephridia, on chaetigers 4–9, and naked nephridiopores. *Abarenicola brevior* can finally be distinguished from *A. insularum* using both the oesophageal caeca count and the appearance of the proboscidial papillae. In *A. brevior* the oesophageal caeca formula is 1 + (9–12) while in *A. insularum* the formula is 1 + (4–6). Additionally, the proboscidial papillae in the two species are quite different in form. *Abarenicola brevior* has a short sparse region of large, flat, triangular papillae followed by a densely papillated median region that initially has a small number of larger papillae intermixed with smaller conical papillae transitioning to small papillae only. *Abarenicola insularum*, however, has a long region of large, mushroom-shaped papillae (short but wide 'stalk' with rounded 'head'). Small numbers of small conical papillae gradually intermix with the larger papillae before increasing in number.

HABITAT

Sand of most grades from high to low shore and occasionally in the sand of rock pools.

DISTRIBUTION

Beagle Channel & Magellan Strait; Falkland Islands

Abarenicola devia (Wells, 1963) n. stat. (Table 2)

Arenicola assimilis var. *affinis* Ashworth, 1911: 22–23. – Stach, 1944: 272.
Abarenicola assimilis devia Wells, 1963: 134, 140–141, fig. 3, Pl. 4, Table 1.

TYPE MATERIAL EXAMINED

Holotype: Shoreham, Victoria, Australia (NHMUK 1961.12.2); coll. F.H. Drummond, 1954.

ADDITIONAL OBSERVATIONS

Eversible proboscis covered in papillae with some pigmentation. Proximal section long with large, triangular, widely distributed papillae, slight pigmentation only; median section densely populated with very small, unpigmented, rounded papillae; distal section with larger, conical papillae. Abrupt transition from proximal to median section with distinct narrow division between.

Abarenicola haswelli (Wells, 1963) n. stat. (Table 2)

Arenicola assimilis var. *affinis* Ashworth, 1911: 22–23. – *Abarenicola assimilis haswelli* Wells, 1963: 134, 141, fig. 3, Table 1.

TYPE MATERIAL EXAMINED

Holotype: Burnie, Tasmania (NHMUK 1912.4.9.31); coll. W.A. Haswell.

ADDITIONAL OBSERVATIONS

Eversible proboscis covered in papillae with some pigmentation. Proximal section with dense, large, trapezoidal papillae,

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appearing to be arranged on transverse 'ridges'; median section densely populated with small, conical papillae. Abrupt transition from proximal to median section with distinct narrow division between.

Abarenicola insularum (Wells, 1963) n. stat.
(Table 2)

Arenicola assimilis var. *affinis* Ashworth, 1903: 754–764, 777–780, Pl. 36, figs 2, 7, 8, 11, 15, 20, Pl. 37, fig. 23. – Ashworth, 1911. – Ehlers, 1912. – Benham, 1921: 108. – Monro, 1939: 133. – Fauvel, 1952.
Abarenicola assimilis insularum Wells, 1963: 134, 140, figs 3, 10e, Pl. 4, Table 1.

TYPE MATERIAL EXAMINED
Holotype: Tucker Cove, Campbell Island, New Zealand (NHMUK 1961.12.1); coll. P.M. Johns, 01.1960

ADDITIONAL OBSERVATIONS
Eversible proboscis covered in papillae with some pigmentation. Proximal section long with large, unpigmented, 'mushroom-shaped' papillae; median section initially with mostly large papillae intermixed with some small, conical papillae, transitioning gradually to denser, small papillae only.

Abarenicola wellsi n. sp.
(Figures 1, 3 & 4; Tables 1 & 2)

Arenicola assimilis var. *affinis* Ashworth, 1903: 768–772. – *Abarenicola assimilis brevior* Wells, 1963 (in part): 133–140, figs 2, 3, Table 1.

TYPE MATERIAL. Holotype: *East Falkland*. Whalebone Cove, station 9a (51°41.330′S 057°48.092′W), medium-coarse sand, low shore (NMW.Z.2011.039.0190), 23.11.2011.

Paratypes: *East Falkland*. (previously identified as *A. a. brevior*) Whale Sound, Stanley Harbour, 5 specimens (NHMUK 1961.12.17–21), Spring 1902; Whalebone Cove, station 9a (51°41.330′S 057°48.092′W), medium-coarse sand, low shore, 5 specimens (2– NMW.Z.2011.039.191–192; 1– NHMUK 2017.85; 1– ZMH P-27824; 1– USNM 1422115), 23.11.2011; Whalebone Cove, station 41a (51°41.324′S 057°48.000′W), medium-coarse sand, high shore, 1 specimen (NMW.Z. 2012.082.0096), 15.01.2013; Whalebone Cove, station 41c (51°41.327′S 057°48.081′W), medium-coarse sand, low shore, 1 specimen (NMW.Z. 2012.082.0097), 15.01.2013; *West Falkland*. Fox Bay West, station 50c (51°56.199′S 060°04.725′W), fine sand, mid-low shore, 2 specimens (NMW.Z.2012.082.0098–99), 25.01.2013; The Creek, Hill Cove, station 56a (51°30.094′S 060°07.447′W), medium sand, high shore, 2 specimens (NMW.Z.2012.082.0100–0101), 31.01.2013; The Creek, Hill Cove, station 56b (51°30.067′S 060°07.520′W), medium sand, midshore, 1 specimen (NMW.Z.2012.082.0102), 31.01.2013; Port Howard, station 59a (51°36.983′S 059°31.250′W), medium sand & shell, midshore, 2 specimens (NMW.Z.2012.082.0103–104), 03.02.2013.

COMPARATIVE MATERIAL EXAMINED.
East Falkland. The Canache, station 2a (51°41.680′S 057°46.967′W), medium sand, high shore, 5 specimens (NMW.Z.2011.039.0193), 16.11.2011; The Canache, station 2b (51°41.708′S 057°46.996′W), medium sand, midshore, 1 specimen (NMW.Z.2011.039.0194), 16.11.2011; Moody Brook, station 3b (51°41.201′S 057°55.099′W), filamentous algae over muddy fine sand, low shore, 1 specimen (NMW.Z.2011.039.0195), 17.11.2011; Volunteer Point lagoon, station 5b (51°28.752′S 057°50.437′W), fine sand, just below low water, 4 specimens (NMW.Z.2011.039.0196), 20.11.2011; Mount Kent, station 7c (51°34.069′S 058°08.615′W), sandy mud, midshore, 4 specimens (NMW.Z.2011.039.0197), 22.11.2011; Coral Creek, Estancia, station 8a (51°39.036′S 058°13.036′W), soft sand over gravel, high shore, 1 specimen (NMW.Z.2011.039.0198), 22.11.2011; Whalebone Cove, station 9a (51°41.330′S 057°48.092′W), medium-coarse sand, low shore, 1 specimen (NMW.Z.2011.039.0199), 23.11.2011; Kelp Harbour, by stone corral, station 28 (51°48.597′S 059°19.433′W), muddy sand, midshore, 1 specimen (NMW.Z.2011.039.0200), 04.12.2011; Whalebone Cove, station 31c (51°41.325′S 057°48.037′W), medium-fine sand, low shore, 2 specimens (NMW.Z.2011.039.0201), 05.12.2011; Mullet Creek, station 33b (51°43.121′S 057°54.833′W), coarse gravelly/pebbly sand, high-midshore, 6 specimens (NMW.Z.2011.039.0202), 07.12.2011; Mullet Creek, station 33c (51°43.150′S 057°54.545′W), medium sand, mid-low shore, 4 specimens (NMW.Z.2011.039.0203), 07.12.2011; Sand Bay, Port Harriet, station 34c (51°44.169′S 058°00.610′W), fine sand over clay, high-midshore, 1 specimen (NMW.Z.2011.039.0204), 08.12.2011; Camilla Creek, station 36b (51°46.668′S 058°57.760′W), soft mud, midshore, 2 specimens (NMW.Z.2011.039.0205), 09.12.2011; Camilla Creek, station 36d (51°46.680′S 058°57.760′W), muddy sand, midshore, 1 specimen (NMW.Z.2011.039.0206), 09.12.2011; Port Salvador, station 39c (51°26.509′S 058°22.230′W), fine sand, high-midshore, 4 specimens (NMW.Z. 2012.082.0105–108), 14.01.2013; Whalebone Cove, station 41b (51°41.322′S 057°48.030′W), medium-coarse sand, midshore, 4 specimens (NMW.Z. 2012.082.0109–112), 15.01.2013; Whalebone Cove, station 41c (51°41.327′S 057°48.081′W), medium-coarse sand, low shore, 16 specimens (NMW.Z. 2012.082.0113–124), 15.01.2013; North Arm, station 47b (52°06.835′S 059°22.224′W), soft mud over sand and gravel, midshore, 1 specimen (NMW.Z. 2012.082.0126), 21.01.2013; North Arm, station 47c (52°06.835′S 059°22.224′W), soft mud over sand and gravel, low shore, 3 specimens (1– NMW.Z. 2012.082.0127; 1– ZMH P-27825; 1– NHMUK 2017.86), 21.01.2013; *Saunders Island*. above East Point, station 43 (51°19.679′S 060°05.527′W), muddy sand, midshore, 1 specimen (NMW.Z.2012.082.0125), 18.01.2013; *West Falkland*. Fox Bay West, station 50b (51°56.182′S 060°04.746′W), fine sand, midshore, 3 specimens (2– NMW.Z.2012.082.0128; 1– USNM 1422116), 25.01.2013; Fox Bay West, station 50d (51°56.238′S 060°04.612′W), fine sand, low shore, 7 specimens (NMW.Z.2012.082.0129–132), 25.01.2013; Fox Bay West, station 50e (51°56.235′S 060°04.673′W), fine sand, low shore, 42 specimens, (NMW.Z.2012.082.0133–134), 25.01.2013; Moonlight Bay, station 51a (52°06.211′S 060°50.364′W), coarse sand, high-midshore, 2 specimens (NMW.Z.2012.082.0135), 26.01.2013; Moonlight Bay, station 51b (52°06.227′S 060°50.361′W), coarse sand, midshore, 1 specimen (NMW.Z.2012.082.0136), 26.01.2013; Moonlight Bay, station 51f (52°06.269′S 060°50.305′W), medium-coarse sand, extreme low shore, 3 specimens (NMW.Z.2012.

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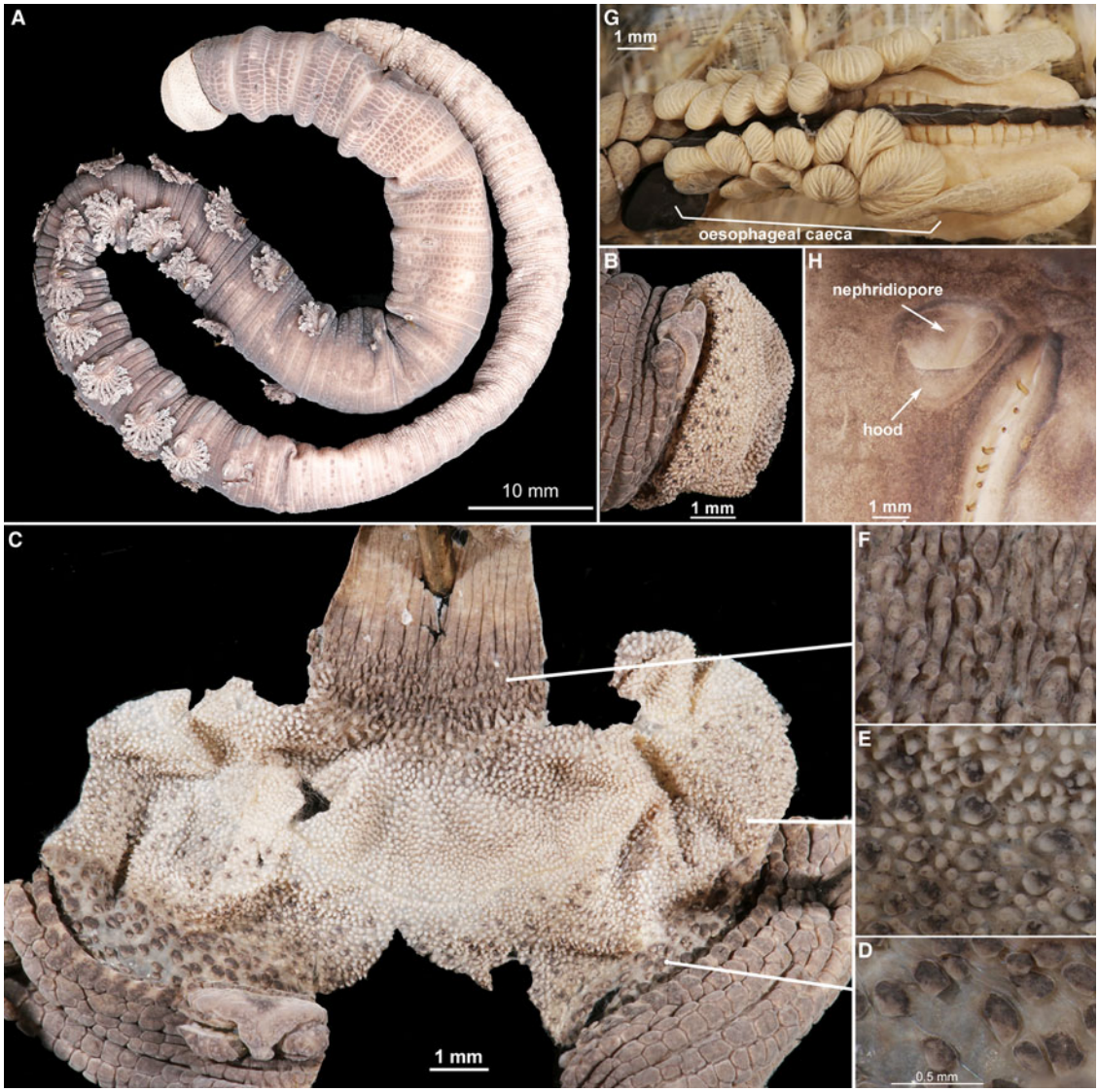

Fig. 3. *Abarenicola wellsi* n. sp. (A: NMW.Z.2011.039.0190; B: NMW.Z.2011.039.0192, paratype). A. holotype, whole body, dorsal/lateral view; B. prostomium & everted proboscis, dorsolateral view; C. everted proboscis, dorsal view; D. proximal papillae; E. median papillae; F. distal papillae; G. oesophageal caeca; H. chaetiger 7, nephridiopore and neurochaetae.

082.0143–144), 26.01.2013; Hot Stone Cove Creek, Dunbar, station 54c (51°22.999′S 060°30.909′W), fine sand, midshore, 3 specimens (NMW.Z.2012.082.0137), 29.01.2013; Crooked Inlet, Roy Cove, station 55a (51°32.521′S 060°20.810′W), soft black fine sand, high shore, 1 specimen (NMW.Z.2012.082.0138), 30.01.2013; The Creek, Hill Cove, station 56c (51°30.058′S 060°07.568′W), medium sand, midshore, 2 specimens (NMW.Z.2012.082.0139), 31.01.2013; The Creek, Hill Cove, station 56f (51°30.040′S 060°07.726′W), fine sand, low shore, 1 specimen (NMW.Z.2012.082.0140), 31.01.2013; Port Howard, station 59a (51°36.983′S 059°31.250′W), medium sand & shell, midshore, 2 specimens (NMW.Z.2012.082.0141), 03.02.2013.

DESCRIPTION. Holotype complete (Figure 3A), 90 mm long (tip of prostomium to end of final chaetiger), 19 chaetigers. Paratypes 15–130 mm long, additional non-type specimens 2–225 mm long. Description based on holotype except for internal characters.

Body cylindrical (Figure 3A), divided externally into three distinct regions: anterior ('head'), thorax ('body') and posterior caudal region ('tail'). Body widest over first few anterior chaetigers, tapering toward end of chaetigers, tail narrow. Colour both alive and fixed dark brown (paratypes and non-types vary in shade from brown to black). Epidermis tessellated up to chaetiger 7, thereafter more papillated. First 3 chaetigerous annuli slightly swollen, intervening annuli not reduced; number of annuli between first 4 chaetigers 2–3–4, thereafter 4.

Anterior region consisting of prostomium and 2 achaetigerous segments. Prostomium trilobate (Figure 3B), non-retractile. Nuchal groove and statocyst with open-ended duct on either side. Statoliths small, numerous, evenly shaped, amber. Eyes absent. Eversible proboscis covered in papillae (Figure 3A–F). Proximal section with widely distributed large, flat, triangular papillae (Figure 3C, D); darkly pigmented on the body, pale tips. Median section with large, pigmented and small, conical, unpigmented papillae intermixed initially (Figure 3C, E), transitioning to small papillae

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only (Figure 3C). Distal section more darkly pigmented, papillae conical, irregular in size (Figure 3C, F).

Oesophageal caeca with one elongate and 11–15 smaller caeca (Figure 3G) on either side of midline. Elongate pair less than twice length of other caeca.

Thorax with 19 chaetigers (Figure 3A). Each segment with one enlarged annulation bearing noto- and neuropodia and, on chaetigers 7–19, branchiae (Figure 3A).

Notopodia rounded, retractable lobes within oval torus. Lobe darkly pigmented, outer edge pale. Notochaetae up to 25 capillaries, single line.

Neuropodia elliptically shaped tori containing a single row of unidentate hooks, minutely serrated on the upper edge, up to 37.

Branchiae large, branched, highly vascularized, 13 pairs (Figure 3A). First pair reduced in size. Up to 13 main gill stems; multiple lateral branches and gill filaments off each stem. Gill stems fused together over lower third portion of length.

Six pairs of nephridia on chaetigers 4–9. Nephridiopores partially hooded dorsi-ventral clefts located posterior to dorsal end of neuropodium (Figure 3H).

Achaetous tail papillate tube (Figure 3A), easily lost. Anus terminal.

ETYMOLOGY. *Abarenicola wellsi* is named after G.P. Wells, who contributed to our knowledge of both the anatomy and taxonomy of the Arenicolidae so significantly, and to whom most of the knowledge of the genus *Abarenicola* can be attributed.

HABITAT. Mostly from midshore down, in sand.

DISTRIBUTION. Falkland Islands

REMARKS. *Abarenicola wellsi* n. sp. is most similar to *Abarenicola devia* n. stat. as both species are the only members of the '*assimilis* group' with both the first gill on chaetiger 7 and hooded nephridiopores (*Abarenicola brevior* n. stat., *Abarenicola haswelli* n. stat. and *Abarenicola insularum* n. stat. all have the first gill on chaetiger 8 and naked nephridiopores). However, the two species can be distinguished using the oesophageal caeca formula and the form of the proboscidial papillae. The formula for *A. wellsi* is 1 + (11–15) and that for *A. devia* is 1 + (4–6). In *A. wellsi*, the proboscis has a short, proximal section of large, triangular, flap-like and widely spaced pigmented papillae followed by a median section of dense, intermixed, small, conical, unpigmented and large pigmented papillae transitioning to small papillae only, and finally a distal section of slightly larger, conical papillae. In *A. devia* the proximal section of large papillae forms a region approximately twice the length of that in *A. wellsi* and the papillae are only slightly pigmented. Additionally, the median region is densely covered with unpigmented small, rounded papillae only before a final distal section of larger conical papillae.

Variation

Body colour of both *Abarenicola brevior* n. stat. and *Abarenicola wellsi* n. sp. varied in life from pale to dark brown, occasionally nearly black, although *A. wellsi* tended to be darker in colour more often. Branchiae may be variably pigmented and extended following preservation. The number of gill stems is generally largest on the median branchiae (chaetigers 12–14). Across the paratypes and non-type specimens of both species, this number varied from 9–17 and roughly correlated to size.

Although levels of pigmentation relating to both the epidermis and branchiae are variable, the pigmentation of the proboscidial papillae appeared to be consistent. The larger proximal papillae of *A. wellsi* are always darkly pigmented along with those larger papillae intermixed with the smaller conical papillae of the median section, resulting in a 'spotted' appearance of the proboscis (Figure 3A–E). In contrast, all of the proboscidial papillae of *A. brevior* are unpigmented (Figure 2B–E). This character appears to remain consistent even after preservation and can be identified on those specimens in the type series of *A. brevior* collected as far back as 1892.

Healy & Wells (1959) introduced the practice of describing the number of oesophageal caeca using the formula $1 + n/m$, where 1 indicated the first pair of long caeca and n the number of subsequent smaller caeca on each side (or n/m where the number on each side differed). Within *A. brevior* the formula for oesophageal caeca is 1 + (9–14) and for *A. wellsi* 1 + (11–15). This does not vary significantly with size. The long caeca are generally less than twice the length of the other caeca, however they can be up to three times the length.

DNA results

For the 16S dataset, maximum parsimony returned two most parsimonious trees (tree length = 521; CI = 0.5208), the single difference between them being whether or not the two pairs of *Arenicola* species (*Arenicola defodiens*–*Arenicola marina* and *Arenicola cristata*–*Arenicola loveni*) grouped together as a clade. Both maximum likelihood and Bayesian inference yielded the same tree topology as the MP tree with the *Arenicola* clade (Figure 4), although this clade itself had no substantial bootstrap support and only weak support from Bayesian posterior probabilities (0.73). An *Arenicola*–*Abarenicola* clade was also unsupported despite it being recovered by all analyses. *Branchiomaldane* was recovered as monophyletic, well supported by maximum parsimony and Bayesian inference but not by maximum likelihood and *Arenicolides* was recovered as sister to *Arenicola-Abarenicola*, although again with only poor support. Monophyly of *Abarenicola* gained high support from all analyses (>85% bootstrap support, 0.97 Bayesian posterior probability) and within that, the clades representing both the cysted and cyst-less *Abarenicola* species were also strongly supported by all analyses (>95% bootstrap support and >0.99 posterior probabilities). The two Falkland Islands species formed a strongly supported group in themselves. The short branch lengths indicate how closely related *Abarenicola brevior* n. stat. and *Abarenicola wellsi* n. sp. are, more so than to the other *Abarenicola* species represented. This is also borne out by the pairwise differences which show a difference of only 0.02 between the two species. Between all of the *Abarenicola* species analysed, the remaining pairwise differences ranged from 0.032 (*A. brevior*–*Abarenicola affinis affinis*) to 0.1 (*Abarenicola gilchristi*–*Abarenicola pacifica*). In comparison, pairwise differences within the four *Arenicola* species analysed ranged from 0.035 (*A. marina*–*A. defodiens*, sympatric species in the UK) to 0.109 (*A. loveni*–*A. defodiens*).

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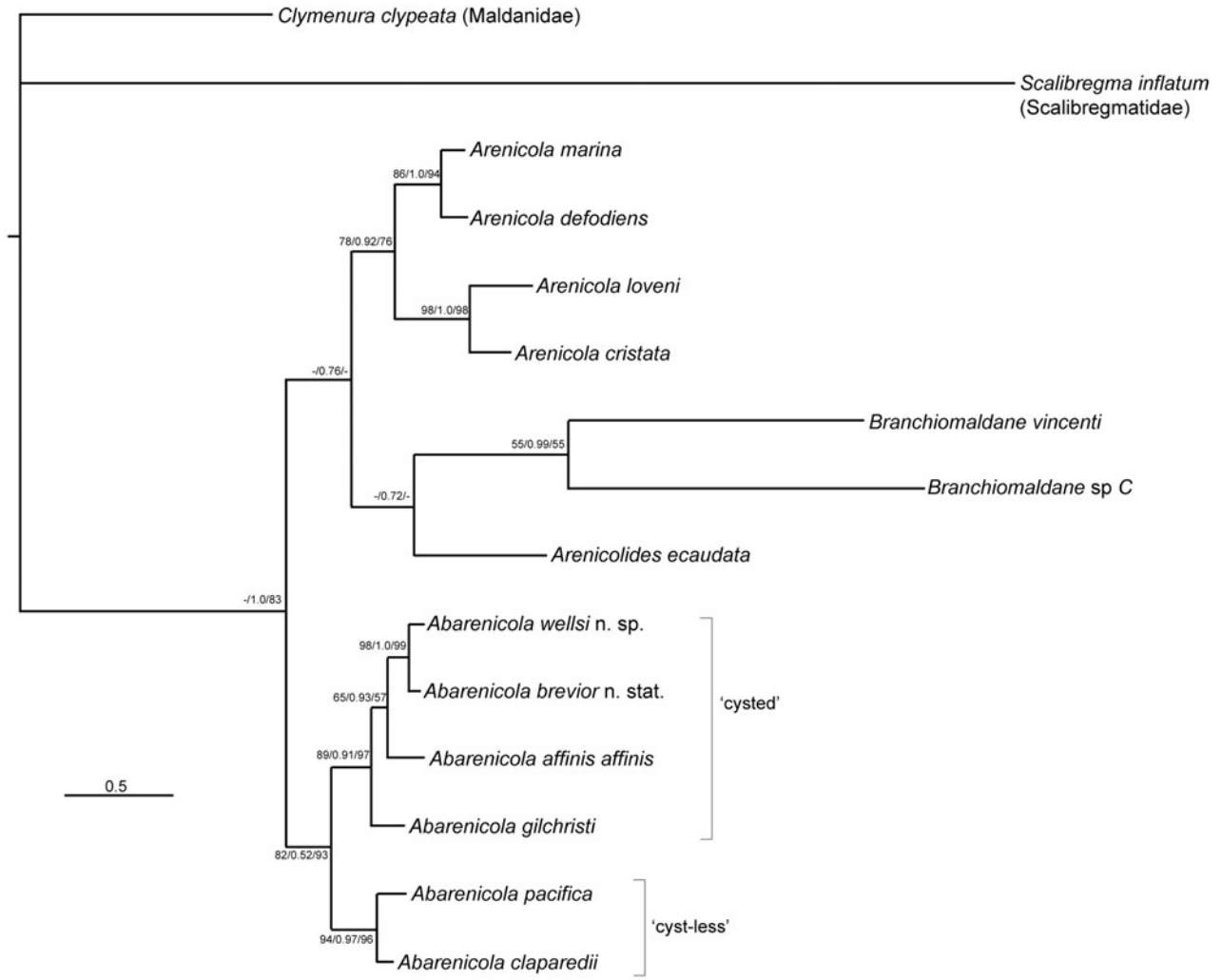

Fig. 4. Bayesian inference tree of the 16S rRNA gene dataset. The first value at each node represents the ML bootstrap support, the second the Bayesian posterior probabilities and the third the MP bootstrap support.

In the COI dataset, the only comparison available to the two Falkland Islands species was with the two UK species of *Arenicola*. All analyses recovered both *Abarenicola* and *Arenicola* as monophyletic clades but although, as with the 16S analysis, the *Abarenicola* clade gained strong support (99% bootstrap support; 0.99 posterior probability) there was once again no significant support for the *Arenicola* clade. Pairwise differences between congeners were higher than for 16S at 0.1 between *A. brevior* and *A. wellsi* compared with 0.14 between *A. marina* and *A. defodiens*. Intraspecific distances were 0.006 (*A. defodiens*), 0 (*A. marina*), 0.002 (*A. brevior*) and 0.002 (*A. wellsi*).

DISCUSSION

At the current time, there are six species described within the genus *Abarenicola*, of which three (*assimilis*, *affinis*, *claparedii*) are further split into multiple subspecies (Wells, 1963, 1964). In general, the subspecies are separated only on the internal character of the number of oesophageal caeca even though Wells did note differences in other morphological characters such as the form of the nephridiopores, position of the first gill and ecological variations in habitat and geographic location. In a much later paper, Wells stated that 'in retrospect I am no longer so sure about my division of *Abarenicola assimilis* into four subspecies' (Wells, 1980). However, he still felt that there were not enough clear morphological characters available in these taxa in order to describe them at anything higher than subspecies level.

In 1963, Wells summarized many of the different characters that he felt existed for lugworms and rated them as to how useful he believed them to be in distinguishing species. The first of these, serial differentiations, included the number of chaetigers, the position of the first gill and the number of nephridia as well as details of the septa and septal vessels. Based on his observations of *Arenicola marina*, showing that considerable anomalies could occur in these differentiations (Wells, 1957), Wells stated that it was 'best not to separate species on the basis of such characters unless their variation was accompanied by differences of other kinds'. In the present study, observations on *Abarenicola brevior* and *Abarenicola wellsi* were made on 81 and 142 individuals respectively from sites located across both East and West Falkland (Figure 1). Although there was some variation in the size and occurrence of the first gill,

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there was always at least one gill of some size present on chaetiger 7 for *A. wellsi*, but never for *A. brevior* on which the first gill always occurred on chaetiger 8. This was true even for juveniles once the branchiae had developed. One aberrant specimen did occur in the paratypes from Puerto Robalo (Chile) whereby the gills started from chaetiger 7 (not 8), however there were also only 18 chaetigers on that specimen instead of 19. It is therefore believed that for these species, the characters of occurrence of the first gill and number of chaetigers are valid in distinguishing them.

In describing the gills and nephridiopores, Wells did not state how useful he felt them to be as characters. In the two species *Arenicola marina* and *Arenicola defodiens*, that are sympatric in the UK, the gills have been shown to be useful characters in respect to the branching pattern (Cadman & Nelson-Smith, 1993; Brind & Darbyshire, 2015). However, in the *Abarenicola* species investigated here, the branching pattern and number of main gill stems do not show significant differences. The nephridiopores however, are consistent characters as to whether they are hooded (to any degree) or naked.

The oesophageal caeca were the main character that Wells used to distinguish his subspecies and the formula for the caeca, as defined by Healy & Wells (1959), is consistent within each species. Between *A. brevior* and *A. wellsi* however, there is no significant difference in the caecal count and other characters must be used. Observations on the other species in the *assimilis* group show that the relative size of the elongate caeca may also be an important character. For example, in *A. assimilis*, the elongate caeca were over three times longer than those of *A. brevior*, and in *Abarenicola insularum* around twice as long. However, at this time only the holotypes of the other species have been inspected and to be considered as a potential character many more individuals need to be investigated in order to determine how relevant this character may be.

The statocysts and statoliths are useful characters to distinguish the larger groups of *Abarenicola* e.g. the *assimilis* group, *affinis* group and *Abarenicola gilchristi*, but within the groups they are generally not distinct enough for use.

Other characters such as length, position of the nerve cord and the chaetae were not considered useful and indeed, between *A. brevior* and *A. wellsi* no discernible difference was found between either the notochaetae or neurochaetae. In general, *A. wellsi* tended to be larger and darker than *A. brevior*, something also noted by Wells (1963), however neither of these characters differed significantly enough to be useful.

Finally, one character that was not considered by Wells that has proven to be relevant in this work is that of the proboscidial papillae. These have been found to differ in their pigmentation, shape and arrangement between the species and remain consistent both with size and, of particular relevance to pigmentation, the length of preservation.

The molecular results add additional detail to the analysis published by Bleidorn *et al.* in 2005, in which no species from the *A. assimilis* group were included. The topology of the tree recovered here is similar to that from the latter study based on their combined dataset of three genes, the main difference being the position of the acaudate genera, *Arenicolides* and *Branchiomaldane*, with respect to *Arenicola*. In both analyses, *Abarenicola* is well-supported as a monophyletic genus, and, within that, both the cysted and cyst-less species are also well-supported as sister-groups. The results do, however, reflect those of Bartolomaeus & Meyer (1999) although this must be viewed with caution as some groups, despite being consistently recovered, did not receive support. The latter analysis, based on chaetal morphology and literature, hypothesized *Arenicola* and *Abarenicola* forming the taxon Caudata (despite being recovered in this analysis there was no support), with *Arenicolides* as sister taxon (as Arenicolinae; also present here but weakly supported) and finally, with *Branchiomaldane*, forming Arenicolidae.

It is unfortunate that only the 16S results are comparable with other sequences and that no other Arenicolidae species, other than *A. marina* and *A. defodiens* (Pires *et al.*, 2015 and this study), have been sequenced for the COI gene at this time. However, using the 16S results, the two Falkland Islands species are shown to be more closely related to each other than to the other *Abarenicola* species analysed and there is strong support for the formation of a clade with the other cysted species (*A. a. affinis*, *A. gilchristi*). The resulting tree also shows them to be grouped with *A. a. affinis*, although this has limited support. The results do, however, agree with those from the morphological analyses whereby greater differences are apparent between *A. gilchristi* and both the *A. assimilis* and *A. affinis* groups than between the latter two groups. The pairwise differences are lower between *A. brevior* and *A. wellsi* than between any of the other Arenicolidae species. The next lowest scores are found between *A. marina* and *A. defodiens*, two other species that show several parallels to the Falkland Islands species in their geographic closeness and taxonomic history but that are universally accepted as being distinct species. Indeed, *A. marina* and *A. defodiens* were also historically only distinguished as separate varieties of *A. marina* (Gamble & Ashworth, 1898; Ashworth, 1912), a distinction that Wells (1957) investigated and refuted, attributing the differences to minor genetic separation between populations. It was only in the 1990s that the two varieties were shown to be distinct both genetically and morphologically (Cadman & Nelson-Smith, 1990, 1993; Cadman, 1992). The intra- and interspecific distances for the Falkland Islands specimens also demonstrate that there is minimal genetic variation within each species compared with between them.

Despite Wells' doubts about the validity of some of the morphological characters, they have been shown to remain consistent within a species from juvenile through to adult and across numerous specimens from multiple populations. Although supporting molecular data are currently lacking for the other *assimilis* subspecies, the morphological characters are clear enough to also warrant the elevation of these taxa to species level as *Abarenicola assimilis* n. stat., *Abarenicola devia* n. stat., *Abarenicola haswelli* n. stat. and *Abarenicola insularum* n. stat. Additional molecular data for these species would however be useful to fully clarify the relationships between them and the other *Abarenicola* species. It is also likely that new investigations of the *affinis* subspecies may well bring to light new morphological characters to distinguish these in the same way as has been found for the *A. assimilis* group and, again, molecular analyses would be highly desirable for comparative purposes.

KEY FOR THE *ABARENICOLA* `*ASSIMILIS* GROUP´ SPECIES

1 Statocysts present (otic grooves absent)... 2 (southern hemisphere cold temperate species)
– Statocysts absent (otic grooves present)... northern hemisphere and *A. pusilla*

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2 First 3 chaetigers strongly developed with reduction of intervening ordinary annuli. . . *Abarenicola affinis* group (4 subspecies)
– First 3 chaetigers normally developed or only slightly thickened with no corresponding reduction of intervening annuli 3
3 20 chaetigers. . . . . *Abarenicola assimilis* n. stat.
– 19 chaetigers 4
4 Nephridiopores naked. 5
– Nephridiopores hooded 7
5 Branchiae from chaetiger 8 6
– Branchiae from chaetiger 9 . . . . . *Abarenicola haswelli* n. stat
6 Oesophageal caeca formula 1 + (4–6); long proximal section of 'mushroom-shaped' proboscidial papillae, median section initially with more large than small papillae . . . . . *Abarenicola insularum* n. stat.
– Oesophageal caeca formula 1 + (9–14); short proximal section of wide, triangular proboscidial papillae, median section initially with more small than large papillae . . . . . *Abarenicola brevior* n. stat.
7 5 pairs of nephridia (chaetigers 5–9); branchiae from chaetiger 8; more than 16 oesophageal caeca . . . . . *Abarenicola gilchristi*
– –6 pairs of nephridia (chaetigers 4–9); branchiae from chaetiger 7; less than 16 oesophageal caeca 8
8 Oesophageal caeca formula 1 + (4–6); large and small proboscidial papillae clearly divided on proboscis. . . . . *Abarenicola devia* n. stat.
– Oesophageal caeca formula 1 + (11–15); large and small proboscidial papillae intermix at beginning of median section . . . . . *Abarenicola wellsi* n. sp.

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