

Monograph

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On the *Bennelongia barangaroo* lineage (Crustacea, Ostracoda) in Western Australia, with the description of seven new species

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Abstract. The ostracod genus Bennelongia De Deckker & McKenzie, 1981 is endemic to Australia and New Zealand. Extensive sampling in Western Australia (WA) revealed a high specific and largely undescribed diversity. Here, we describe seven new species belonging to the *B. barangaroo* lineage: B. timmsi sp. nov., B. gnamma sp. nov., B. hirsuta sp. nov., B. ivanae sp. nov., B. mcraeae sp. nov., B. scanloni sp. nov. and B. calei sp. nov., and confirm the presence of an additional species, B. dedeckkeri, in WA. For five of these eight species, we could construct molecular phylogenies and parsimonious networks based on COI sequences. We also tested for cryptic diversity and specific status of clusters with a statistical method based on the evolutionary genetic species concept, namely Birky's 4 theta rule. The analyses support the existence of these five species and a further three cryptic species in the WA B. barangaroo lineage. The molecular evidence was particularly relevant because most species described herein have very similar morphologies and can be distinguished from each other only by the shape, size and position of the antero-ventral lapel on the right valve, and, in sexual populations, by the small differences in shape of the hemipenes and the prehensile palps in males. Four species of the WA B. barangaroo lineage occur in small temporary rock pools (gnammas) on rocky outcrops. The other four species are mainly found in soft bottomed seasonal water bodies. One of the latter species, B. scanloni sp. nov., occurs in both claypans and deeper rock pools (pit gnammas). All species, except for B. dedeckkeri, originally described from Queensland, have quite clearly delimited distributions in WA. With the seven new species described here, the genus Bennelongia now comprises 25 nominal species but several more await formal description.

Keywords. Taxonomy, evolution, cryptic species, biodiversity, Western Australia.

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Introduction

The ostracod genus *Bennelongia* is endemic to Australia and New Zealand. Extensive sampling in Western Australia (WA) revealed a high specific and largely undescribed diversity of *Bennelongia* (Halse 2002), leading to the taxonomic research reported here. The present paper is the fourth in a recent series of taxonomic contributions on Australian *Bennelongia*. Martens *et al.* (2012) described nine new species in three different lineages within the genus, all based on collections from WA. Shearn *et al.* (2012) redescribed several extant species and described three new species, all from Eastern Australia (mostly from Queensland). In addition, De Deckker & Martens (2013) described the unusually strong morphological changes in valve morphology during the last 3-4 ontogenetic stages in several *Bennelongia*-species, and showed that these changes can be different between the various lineages within the genus. The first two papers, together with the earlier work by De Deckker (1981a,b, 1982) and De Deckker & McKenzie (1981), brought the number of nominal species in the genus to 18, but both recent papers also recognised that some additional cryptic species exist, as determined by molecular methods.

Here, we describe seven new species of *Bennelongia* from WA and report on the occurrence of an additional described species in WA, namely *B. dedeckkeri* Shearn *et al.*, 2012. All of these eight species belong to the *B. barangaroo* lineage within the genus and for five of the species, their specific status is confirmed with molecular methods. Two of the new nominal species furthermore comprise several, sometimes sympatric, cryptic species that could not be recognised using either valve or soft part morphologies.

Material and methods

Collections

Ostracods were collected from pans, lakes and rock pools with a hand net with mesh size of 250 µm during several field trips (see below). Material for morphological analyses originated from both these 'new' collections and from earlier collections from all over WA, mostly collected by SH and preserved in a collection housed at the Department of Environment and Conservation, now DPaW (Woodvale, Perth). The molecular analyses were successful only with newly collected material, using either living specimens or specimens sorted directly in the field and preserved in 100% ethanol. Consequently, molecular analyses were limited to five of the eight species (four of the seven new ones). Locations of populations used for the present paper are indicated on the map in Fig. 1. Type material of the new species is deposited in the Western Australian Museum, Perth, WA (WAM numbers) and in the Ostracod Collection of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (OC numbers) (see Table 1).

Morphological analyses

Ostracods were dissected with valves stored dry in micropalaeontological slides and soft parts in glycerine in sealed slides. Drawings of soft parts were made with a *camera lucida* with a compound microscope (Leica, DM 2500 at Bennelongia Environmental Consultants, Perth). Valves were illustrated and measured using scanning electron microscopy (Philips XL30 SEM at RBINS, Brussels).

Molecular analysis

The Qiagen Blood and Tissue extraction kit was used following the manufacturer's protocol to extract DNA from 99 ostracods representing four nominal and 2 cryptic species of the *Bennelongia barangaroo* lineage. Universal PCR primers (Folmer *et al.* 1994) were applied to amplify part of the mitochondrial COI region in a T personal Thermoblock (Biometra) with the following conditions: 25 μ l volumes of the HotStar Master Mix (Qiagen; 1.5 mM MgCl₂, 0.1 μ M primer, 200 μ M dNTP, Tris·Cl, KCl, (NH₄)₂SO₄, 1.25 U Taq) and 15 min at 95°C, 40 cycles of 1 min at 95°C, 1 min at 44° C, 1 min at 72° C, followed

by a final extension step for 10 min at 72° C. Agarose gel electrophoresis and staining of gels with GelredTM was carried out to check for successful PCR amplifications. PCR products were cleaned with the GFXTM PCR DNA and gel band purification kit (GE Healthcare) according to the manufacturer's protocol and sequenced in both directions with the universal COI primers and the Big Dye kit (ABI) on an ABI 3130X following the manufacturer's protocol.



Fig. 1. Map of Western Australia with localities of Bennelongia - species described in the present paper.

No fresh (living) material of three of the seven new nominal species in the *barangaroo* lineage could be obtained (namely *B. gnamma* sp. nov., *B. hirsuta* sp. nov. and *B. mcraeae* sp. nov.); these species are not represented in the molecular phylogenetic tree and network.

Analyses of sequence data

Sequence chromatograms were visualised with BioEdit (Hall 2007). For each individual, the forward and reverse strand were aligned with ClustalX (Larkin *et al.* 2007), subsequently checked by eye for ambiguities, corrected and finally trimmed to obtain sequences of equal lengths. BLAST searches (Altschul *et al.* 1990) were used to confirm the identity of the obtained sequences in Genbank. We applied jModeltest 2.1.1 (Darriba *et al.* 2012) to identify the optimal model of molecular COI evolution using 88 or 24 models and the AICc criterion. Two different methods were used for phylogenetic reconstructions, Bayesian Inference (BI) in Mr Bayes 3.2 (Ronquist *et al.* 2011; with 4 million generations, sampling every 100th generation, a burn-in of 25% and the parameters identified by jModeltest for 24 different models) and the Maximum-Likelihood method in PhyML (Guindon & Gascuel 2003; with 1000 bootstrap replicates and the parameters of jModeltest for all 88 models). We also constructed a parsimonious network at the 95% probability limit with TCS 1.21 (Clement *et al.* 2000) to connect different sequences (or haplotypes) and to illustrate genetic diversities and genetic relationships within and between populations. Selected sequences of all species have been submitted to Genbank (accession numbers KF724982-KF725015; see Table 1).

Testing for cryptic diversity

We applied the 4 theta rule (Birky *et al.* 2010; Birky 2011) based on the evolutionary genetic species concept (Birky & Barraclough 2009) to identify species boundaries and unravel cryptic diversities. This technique has been successfully used in bdelloid rotifers (Fontaneo *et al.* 2007, 2009; Birky & Barraclough 2009; Birky *et al.* 2011), asexual ostracods (Schön *et al.* 2012) including other *Bennelongia* ostracod species (Martens *et al.* 2012; Shearn *et al.* 2012), and a wide range of asexual prokaryotes (Birky *et al.* 2010).

We used the COI phylogenetic tree to identify statistically supported clades, which could potentially be different species according to the evolutionary genetic species concept. We then estimated sequence diversities within and between these phylogenetic clades with MEGA 5.0 (Tamura *et al.* 2011) using the number of differences (p) and the Tamura-3 parameter model with gamma distribution (allowing for multiple hits, different transition and transversion rates and GC bias) and 1000 bootstrap replicates. Following the procedure by Birky *et al.* (2010), sequence diversities were subsequently corrected for sample size and sequence lengths. In order to fulfill the criteria of the 4 theta rule, the sequence diversity between two sister clades must be at least 4 to 4.3 times larger than within the two clades, depending on the number of samples per clade (Birky *et al.* 2010).

Abbreviations used in text and figures

Ср	=	carapace
CpD/V	=	carapace in dorsal/ventral view
CpRL	=	carapace in right lateral view
F	=	female in Table 1
Н	=	height of valves
il	=	inner list
KMWA	=	original working numbers given to specimens dissected and illustrated by the first author (KM)
K25	=	electrical conductivity standardised to a water temperature of 25°C
L	=	length of valves
Lpp	=	left prehensile palp

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_	lateral shield of heminenis
=	left valve/left valve exterior/left valve exterior
=	external views of valves of males/females
=	medial shield of hemipenis
=	male in Table 1
=	Northern Territory
=	Ostracod Collection in the Royal Belgian Institute of Natural Sciences (Brussels,
	Belgium)
=	ostracod slide dissected by Stuart Halse, retrieved from the voucher collection of
	DEC, now DPaW (Woodvale, Perth)
=	right prehensile palp
=	right valve/right valve exterior/right valve interior
=	South Australia
=	Queensland
=	Western Australia
=	Western Australian Museum, Crustacean Collection (Perth, WA)

Specimens in bold in Table 1 are the holotypes of the species.

Chaetotaxy of the limbs follows the model proposed by Broodbakker & Danielopol (1982), revised for A2 by Martens (1987). The higher taxonomy of the Ostracoda follows the synopsis by Horne *et al.* (2002).

Results

Results of molecular screening

We obtained 680 nucleotide-long sequences for part of the mitochondrial COI region for all 99 ostracods subjected to DNA extraction. jModeltest selected the TPM1uf+I+G model with the following parameters among 88 models: freqA = 0.33; freqC = 0.19; freqG = 0.13; freqT = 0.35; [AC] = 1.00; [AG] = 24.84, [AT] = 2.91; [CG] = 2.91; [CT] = 24.84; [GT] = 1.00; p-inv = 0.56; gamma shape = 1.26. For Bayesian Inference, the HKY+I+G model was selected among 24 models.

The phylogenetic tree (Fig. 2) had a similar topology with ML and BI methods for tree construction. The two clades F1 and F2 (belonging to *B. ivanae* sp. nov. and *B.* sp. nov. F2 respectively) group together with high statistical support. They form the most basal branch and are separated from all other *Bennelongia* specimens. Within the phylogenetic cluster containing the other species, *B. dedeckkeri* forms the most basal branch, followed by clade B1 morphologically forming the new species *B. calei* sp. nov. The remaining specimens can be divided into three subgroups with strong statistical support for the tips but less support for the basal nodes of the subgroups themselves. Subgroup one consists of clade B2 as well as E1 and TST, which form sister clades and all belong morphologically to the new species *B. scanloni* sp. nov. The second subgroup is composed of clade E2, which is morphospecies *B.* sp. nov. E2 and sister clades A3 and A1, while the third subgroup contains clades A4, A2 and A5. According to their morphology, all A clades belong to the new species *B. timmsi* sp. nov.

We then used the topology of the phylogenetic tree (Fig. 2) to test whether clades phylogenetically closest to each other are in fact different genetic species by applying the 4 theta rule (see Table 2). Most sister clades represent different genetic species according to the criteria of the 4 theta rule (Birky *et al.* 2010), with the majority of these genetic species matching the morphologically identified species. This is the case for sister clades F1 and F2 representing *B. ivanae* sp. nov. and *B.* sp. nov. F2, respectively, and for B1 corresponding to *B. calei* sp. nov.. Likewise, morphology and genetics are concordant for the described species *B. dedeckkeri* and clade E2 (*B.* spec nov.). However, within *B. timmsi* sp. nov. and

B. scanloni sp. nov., there are phylogenetic clades representing different genetic species that cannot be distinguished morphologically. Of the five clades within *B. timmsi* sp.nov, only 3 are genetically distinct species (clades A1 and A3 on the one hand and A2+A4+A5 on the other). Within *B. scanloni* sp. nov., the cryptic clades E1/TST and B2 are good genetic species (see below for further remarks on species *B. timmsi* sp. nov. and *B. scanloni* sp. nov.).

If the topology of the tree in Fig. 2 is correct, then *B. timmsi* sp. nov. may be a polyphyletic species, as clades A1 and A3 on the one hand, and clades A2+A4+A5 on the other belong to different clusters of the tree. However, the support of the bifurcation between the two groups of *B. timmsi* clades is doubtful (bootstrap value of 77), so the apparent polyphyly of the species may be an artifact. As also no clear morphological characters, distinctive of the clusters, could be found (see below), we decided not to describe these two groups as distinct nominal species. If at some stage in the future this decision is to be reverted, then *B. timmsi* sp. nov. is defined by clade A5.



Fig. 2. Phylogenetic tree constructed with Bayesian Inference (BI) and Maximum Likelihood (ML) methods from COI sequences of 99 ostracods from the *Bennelongia barangaroo* lineage and with *B. nimala* as outgroup. Numbers above nodes illustrate statistical support for this particular node. Numbers before the hash (/) are % bootstrap values of ML analyses with 1000 replicates, numbers after the hash (/) are Bayesian posterior probabilities (ranging from 0 to 1). Both methods, BI and ML, resulted in the same tree topology. Different phylogenetic clades are indicated by different colours (as in Fig. 3, page 15). The asterisks indicate two nodes (α 1 and α 2) that are weakly supported (see Discussion, p. 55).

sequences are available. Specimens in bold are holotypes.
as whole animals for DNA sequencing, and thus no measurements are available. The present table therefore does not list all 99 specimens for whole animals for DNA sequencing, and thus no measurements are available.
If a molecular sequence was available for the same specimen, the GenBank registration number is also given. However, some specimens were u
Table 1. Individual measurements of specimens used for the present descriptions. All measurements were done using SEM (see Material and method

М		WAN	00	00	WAN	00	WAN	WAM	WAM	WAN	WAN	00	WAN	00	WAM		WAN	WAM						
18 Nr		(C52239	.3317	.3317	IC52240	IC52240	[C52241	IC52242	[C52243	IC52244	IC52245	.3318	[C52246	[C52246	[C52247	[C52248	[C52249	.3319	[C52229	.3314	[C52233	[C52234	[C52235	
KMWA		324	366	366	434	434	982	983	984	985	866	373	435	435	436	980	981	986	987	886	686	066	991	
Genbank accession number							KF725001	KF725002	KF725003		KF725004								KF725005	KF725006	KF725007			
Bennelongia species		timmsi																						
locality		BVT/010/1	BVT/10/02	BVT/10/03	BVT/10/04	BVT/10/05	BVT/10/05	BVT/10/05	BVT/10/05	BVT/10/05														
M/F		F	F	Fext	М	Mext	F	F	F	М	F	F	М	Mext	М	F	Ч	М	Ч	F	F	Ч	Ч	
R	L	1440	1400	1400	1190	1190	1420	1480	1510	1300	1370	1560	1340	1320	1380	1540	1580	1370	1510	1470	1500			
V	Η	882	843	841	730	727	849	903	905	772	843	936	818	801	812	936	960	821	913	883	891			
	L	1520	1480	1460		1240	1490	1530	1580	1340	1440	1630	1410	1380	1440	1650	1660	1430	1600	1530	1550			
LV	Н	907	874	883		752	887	931	942	823	841	686	839	829	841	974	982	854	965	929	925			
Cpl	Γ																						1530	
RL	Н																						891	
Cpl	L																					1580		1
)/V	W																					951		22

Mus Nr	KMWA	Genbank accession	<i>Bennelongia</i> species	locality	M/F	R	V	_	Ŋ	Cpl	RL	CpL)/V
						L	Η	Г	Н	L	Ξ	L	W
OC.3316	993		timmsi	BVT/10/05	М							1370	834
WAMC52236	994		timmsi	BVT/10/05	М					1330	770		
WAMC52237	995		timmsi	BVT/10/05	Μ							1380	840
WAMC52232	966		timmsi	BVT/10/05	М	1283	892						
WAMC52231	997		timmsi	BVT/10/05	М	1300	790	1375	812				
WAMC52228	1073		timmsi	BVT/10/05	Μ	1318	783	1378	817				
OC.3312	1074		timmsi	BVT/10/05	Μ	1320	798	1375	823				
WAMC52230	1075		timmsi	BVT/10/05	Μ	1285	768	1337	800				
OC.3313	379	KF725008	timmsi	BVT/10/05	F	1510	901	1570	950				
OC.3313	379		timmsi	BVT/10/05	Fext	1490	903	1550	943				
WAMC52250	381		timmsi	BVT/10/06	F	1500	892	1560	925				
WAMC52250	381		timmsi	BVT/10/06	Fext	1473	883	1535	919				
WAMC52251	666	KF725009	timmsi	BVT/10/06	F	1510	868	1535	919				
WAMC52252	1000		timmsi	BVT/10/06	М	1329	808	1404	848				
WAMC52253	888	KF725010	timmsi	BVT/10/08	М	/	~	1404	873				
OC.3320	688	KF725011	timmsi	BVT/10/08	Ч	1525	940	1619	981				
WAMC52254	068	KF725012	timmsi	BVT/10/08	Ч	1488	912	1548	948				
WAMC52255	891	KF725013	timmsi	BVT/10/08	М	1344	814	1402	829				
WAMC52256	892		timmsi	BVT/10/08	М							1387	848
WAMC52257	893		timmsi	BVT/10/08	М					1390	852		
WAMC52258	894		timmsi	BVT/10/08	М							1390	868
WAMC52259	895		timmsi	BVT/10/08	F							1613	987
WAMC52260	968		timmsi	BVT/10/08	F							1581	1015
WAMC52261	897		timmsi	BVT/10/08	F					1588	939		
WAMC52262	901		timmsi	BVT/10/08	М	1335	835	1396	840				
OC.3321	902		timmsi	BVT/10/08	М	1327	821	1398	842				
OC.3322	222		gnamma	OSTR012A	F	1450	973	1520	1050				

OC.3328	WAMC52287	WAMC52286	WAMC52286	WAMC52285	WAMC52284	WAMC52282	WAMC52281	OC.3327	OC.3326	OC.3326	WAMC52280	WAMC52280	WAMC52279	OC.3324	WAMC52273	OC.3323	WAMC52269	WAMC52274	WAMC52270	WAMC52272	WAMC52277	WAMC52276	WAMC52275	WAMC52263	WAMC52266	OC.3322		Mus Nr
1076	180	OS007	OS007	904	444	1003	1002	1001	852	852	851	851	903	1102	1103	1106	1105	1104	1101	227	226	225	224	OS178	223	222		KMWA
										KF725015		KF725014																Genbank accession number
mcraeae	mcraeae	mcraeae	mcraeae	spec F2	ivanae	ivanae	ivanae	ivanae	ivanae	ivanae	ivanae	ivanae	hirsuta	hirsuta	hirsuta	hirsuta	hirsuta	hirsuta	hirsuta	hirsuta	hirsuta	hirsuta	hirsuta	gnamma	gnamma	gnamma		Bennelongia species
OSTR014B	OSTR014B	OSTR014B	OSTR014B	BVT/10/09	OSTR013F	DJC/02	DJC/02	DJC/02	DJC/02	DJC/02	DJC/02	DJC/02	BVT/10/09	OSTR012D	OSTR012D	OSTR012D	OSTR012D	OSTR012D	OSTR012D	OSTR012D	OSTR012D	OSTR012D	OSTR012D	OSTR012A	OSTR012A	OSTR012A		locality
М	Fext	Mext	м		ч	ч	н	ъ	Fext	ч	Fext	F	Ъ	ч	М	М	М	ч	ч	М	ч	ч	ч	Ŧ	ч	Fext		M/F
1390	1608	1465	1480		1400				1456	1460	1452	1475	1217			1240	1260	1390	1400					1550		1482	L	R
847	1002	871	878		842				904	910	904	915	719			743	738	813	842					915		960	Н	
1450	1663	1523	1560		1490				1513	1542	1512	1533	1302			1300	1320	1450	1470					1620		1497	Γ	
859	1033	885	968		865				935	947	940	948	719			759	763	824	845					991		1018	Η	Ň
								1488						1410	1300					1450	1430						L	Ср
								931						803	737					837	826						Η	RL
						1452	1498													1447		1500	1430		1560		L	Cpl
						~	1025													855		875	968		1000		W	D/V

Mus Nr	KMWA	Genbank accession	Bennelongia	locality	M/F	RV			V	Cpl	RL	CpI)/V
		нишост				Г	Н	F	H	Г	Ξ	Г	¥
WAMC52289	1078		mcraeae	OSTR014B	Ţ					1650	1000		
WAMC52324	295		scanloni	BRYDE7	F	1460	873	1560	895				
WAMC52324	295		scanloni	BRYDE7	Fext	1448	863	1550	992				
WAMC52325	297		scanloni	BRYDE7	F	1460	863	1550	887				
WAMC52325	297		scanloni	BRYDE7	Fext	1438	098	1533	883				
WAMC52326	299		scanloni	BRYDE7	F					1489	878	1455	858
OC.3339	194		scanloni	BRYDE7	F	1330	807	1044	839				
OC.3339	194		scanloni	BRYDE7	Fext	1322	803	1412	822				
OC.3340	437		scanloni	OSTR013C	F	1310	775	1380	788				
OC.3340	437		scanloni	OSTR013C	Fext	1303	765	1350	782				
WAMC52327	438		scanloni	OSTR013C	Μ	1260	751	1310	761				
WAMC52327	438		scanloni	OSTR013C	Mext	1242	738	1303	749				
WAMC52328	1107		scanloni	OSTR013C	М	1230	889	1320	709				
OC.3338	916		scanloni	TST	Μ	1248	756	1331	769				
	917		scanloni	TST	Ч	1394	810	1487	833				
WAMC52322	918	KF724982	scanloni	TST	Ч	1294	752	1396	711				
WAMC52323	919		scanloni	TST	Ъ							1352	744
OC.3334	855		scanloni	DJC/09	Ч	1438	006	1485	930				
OC.3334	855		scanloni	DJC/09	Fext	1446	068	1490	868				
WAMC52310	856		scanloni	DJC/09	Ч	1465	923	1525	933				
WAMC52310	856		scanloni	DJC/09	Fext	1421	898	1477	910				
WAMC52304	831	KF724983	scanloni	DJC/11	Ъ	1380	797	1463	807				
WAMC52304	831		scanloni	DJC/11	Fext	1360	773	1433	796				
lost	832	KF724984	scanloni	DJC/11	т	1367	803	1458	807				
	832		scanloni	DJC/11	Fext	1352	789	1438	808				
WAMC52297	837		scanloni	DJC/11	F							1497	910
WAMC52298	838		scanloni	DJC/11	Ъ							1448	832

				866	1646	686	1587	ч	BVT/10/10	scanloni		806	WAMC52306
				952	1546	929	1460	ъ	BVT/10/10	scanloni	KF724991	907	OC.3332
		868	1502					ч	DJC/23	scanloni		1021	WAMC52321
948	1608							F	DJC/23	scanloni		1020	WAMC52320
				~	/	919	1498	ч	DJC/23	scanloni	KF724990	1018	WAMC52319
				987	1633	979	1560	Fext	DJC/23	scanloni		797	OC.3337
				1015	1670	973	1573	ч	DJC/23	scanloni		797	OC.3337
		983	1625					ъ	DJC/19	scanloni		1031	OC.3336
1035	1721							ъ	DJC/19	scanloni		1030	WAMC52315
1038	1713							ъ	DJC/19	scanloni		1029	WAMC52314
_	1475							ъ	DJC/19	scanloni		1026	WAMC52313
		887	1515					ъ	DJC/19	scanloni		1025	WAMC52312
				broken	1660	_	~	ъ	DJC/19	scanloni	KF724989	1023	WAMC52311
				906	1519	885	1446	ъ	DJC/19	scanloni	KF724988	1022	OC.3335
				906	1588	883	1485	F	DJC/11	scanloni	KF724987	1013	WAMC52303
				846	1463	827	1383	ъ	DJC/11	scanloni	KF724986	1012	WAMC52302
				688	1562	883	1471	ъ	DJC/11	scanloni		1011	WAMC52301
				919	1583	900	1504	ч	DJC/11	scanloni		1010	WAMC52300
				792	1398	777	1312	ч	DJC/11	scanloni		1009	OC.3331
				775	1356	752	1263	ч	DJC/11	scanloni	KF724985	1008	WAMC52292
				708	1263	696	1190	Μ	DJC/11	scanloni		1007	WAMC52294
				735	1327	723	1244	М	DJC/11	scanloni		1006	WAMC52293
				714	1294	694	1223	Μ	DJC/11	scanloni		1005	WAMC52291
				737	1277	714	1204	Μ	DJC/11	scanloni		1004	OC.3329
745	1222							Μ	DJC/11	scanloni		842	WAMC52296
731	1302	730	1290					М	DJC/11	scanloni		841	WAMC52295
		867	1493					ч	DJC/11	scanloni		839	WAMC52299
W	L	Η	Γ	Η	Γ	Η	L						
D/V	Ср	RL	Срі	LV			R	M/F	locality	<i>Bennelongia</i> species	Genbank accession number	KMWA	Mus Nr

Mus Nr	KMWA	Genbank accession	Bennelongia	locality	M/F	RV	'		LV	Cp	RL	CI	
		number	species	ę									
						L	Н	Г	Н	Γ	Н		Г
WAMC52307	606	KF724992	scanloni	BVT/10/10	Ч	1415	885	1465	868				
WAMC52308	910	KF724993	scanloni	BVT/10/10	Ч	1602	994	1656	1015				
OC.3333	911		scanloni	BVT/10/10	М	1277	/	1350	831				
WAMC52336	822	KF724994	calei	DJC/18	F	1340	802	1432	818				
WAMC52336	822		calei	DJC/18	Fext	broken	792	1419	814				
WAMC52335	823	KF724995	calei	DJC/18	F	1480	857	1555	865				
WAMC52335	823		calei	DJC/18	Fext	1452	844	1552	862				
OC.3344	1014		calei	DJC/18	F	1446	835	1531	848				
WAMC52339	1015		calei	DJC/18	F	1490	864	1588	873				
WAMC52340	1016		calei	DJC/18	Ч	1335	775	1408	864				
WAMC52341	1017	KF724996	calei	DJC/18	Ч	1244	729	1337	748				
OC.3345	828		calei	DJC/18	Ч					1477	815	<u> </u>	
WAMC52337	829		calei	DJC/18	F								1477
WAMC52338	830		calei	DJC/18	F								1518
WAMC52355	236		calei	SPM017B	F	1150	686	1240	711				
WAMC52355	236		calei	SPM017B	Fext	1145	682	1232	703				
WAMC52356	237		calei	SPM017B	F					1290	735		1290
	238		calei	SPM017B	F								1180
WAMC52353	195		calei	SPM017B	F					1330	756		1325
WAMC52354	196		calei	SPM017B	F	1170	699	1260	720				
WAMC52354	196		calei	SPM017B	Fext	1167	692	1252	713				
WAMC52349	870	KF724997	calei	DJC/10	F	1422	833	1522	855				
WAMC52349	870		calei	DJC/10	Fext	1400	837	1508	848			r	
OC.3346	807		calei	BVT/11/04	F	1307	792	1382	797			r	
OC.3347	808		calei	BVT/11/04	F							r —	1427
WAMC52343	608		calei	BVT/11/04	F					1397	783	r	
WAMC52344	810		calei	BVT/11/04	Ч							<u> </u>	1425

Mus Nr	KMWA	Genbank accession	Bennelongia	locality	M/F	R۱	7	l	LV	Cp	RL	Cpl)/V
		number	species	e						-			
						L	Η	L	Η	L	Н	L	W
WAMC52345	813	KF724998	calei	BVT/11/05	F	1383	812	1460	832				
WAMC52345	813		calei	BVT/11/05	Fext	1371	812	1449	832				
WAMC52346	814		calei	BVT/11/05	F							1473	828
WAMC52347	815		calei	BVT/11/05	F							1417	773
WAMC52348	816		calei	BVT/11/05	F					1443	808		
WAMC52329	1080	KF724999	calei	DJC/15	F	/	/	1587	906				
OC.3343	1081		calei	DJC/15	F					1540	688		
WAMC52330	1082		calei	DJC/15	F							1546	842
WAMC52331	1083		calei	DJC/15	F							1506	842
OC.3348	874	KF725000	calei	DJC/36	F	1345	817	1433	833				
OC.3349	879		calei	DJC/36	Ţ							1513	843
WAMC52350	880		calei	DJC/36	Ţ					1497	850		
WAMC52351	881		calei	DJC/36	Ţ							1430	830
WAMC52352	882		calei	DJC/36	Ţ					1410	780		
OC.3350	190		dedeckkeri	KIES10	Ţ					1110	650		
WAMC52359	191		dedeckkeri	KIES10	Ţ							1100	641
WAMC52360	192		dedeckkeri	KIES10	Ţ							1080	651
WAMC52357	193		dedeckkeri	KIES10	F	1110	676	1190	701				
OC.3351	678		dedeckkeri	SIKE9	F	1125	672	1188	715				
OC.3352	679		dedeckkeri	SIKE9	F					1210	717		
WAMC52364	680		dedeckkeri	SIKE9	F							1187	652
WAMC52365	681		dedeckkeri	SIKE9	F							1145	660

Phylogenetic sister	Spacios	max. θ	D (between	Datia D/A	$n^1 n^2$
clades	Species	(within clades)	clades)	Katio D/0	п,п
A1-A3	D timmai	0.0076	0.114	15.00	07
	D. timmst	0.0078	0.139	17.82	0, /
A2-A4	D timmai	0.0138	0.052	3.77	0.2
	D. timmst	0.0141	0.058	4.11	9, 5
A2-A5	D timmai	0.0138	0.048	3.48	0 0
	D. IIMMISI	0.0141	0.052	3.69	0, 0
A4-A5	D timmai	0.0138	0.049	3.55	25
	B. limmsi	0.0141	0.054	3.83	3, 3
A1-E1	B. timmsi –	0.0133	0.131	9.85	0 12
	B. scanloni	0.0138	0.169	12.25	8, 15
A3-E1	B. timmsi –	0.0133	0.140	10.53	7 12
	B. scanloni	0.0138	0.182	13.19	/, 13
B1-B. dedeckkeri	B. calei -	0.004	0.126	31.5	23,
	B. dedeckkeri	0.004	0.161	40.25	25
B2-E1	D. goguloui	0.020	0.113	5.65	15,
	D. scantoni	0.021	0.141	6.71	13
B2-DJC11	D. a a malani	0.050	0.040	0.80	15 0
	B. scanioni	0.053	0.044	0.83	15, 2
E1-TST	Density	0.0133	0.032	2.41	12.2
	B. scanioni	0.0138	0.034	2.46	13, 2
B2-E2	B. scanloni –	0.020	0.103	5.15	15 2
	<i>B</i> . sp. nov. E2	0.020	0.127	6.35	15, 2
	B. scanloni –	0.0015	0.109	72.67	2.2
E2-151	<i>B</i> . sp. nov. E2	0.0015	0.135	90.00	Ζ, Ζ
F1-F2	B. ivanae –	0.0058	0.061	10.52	2 4
	<i>B</i> . sp. nov. F2	0.0059	0.067	11.36	3,4

Table 2. Results of tests for genetic species boundaries using the 4 theta method for six species of the *Bennelongia barangaroo* lineage.

 θ = population genetic parameter theta, indicating genetic variability within populations. D = genetic distance between sister clades. n¹, n² = number of sequences for each sister clade. θ and D were either calculated as p distance or with the Tamura-3 parameter model (in italics). Nearest neighbours or sister clades were defined from the COI tree constructed with Bayesian Inference and Maximum Likelihood methods (see Fig. 2). In order to fulfill the criteria of the 4 theta rule for species status, the ratio of the mean sequence diversity within as compared to between the two nearest neighbours of one sister clade needs to be 4 or more, depending on the number of specimens per clade (Birky *et al.* 2010). Comparisons, for which these criteria are fulfilled, are printed in bold.



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The structure of the most parsimonious networks in Fig. 3 also reflects the higher genetic than morphological diversity. However, there are two species with a single haplotype each, *B. dedeckkeri* and *B. spec*. E2 sp. nov. (clade E2). For the latter, we obtained COI sequences of two individuals only while the same (identical) haplotype is found in 11 specimens of *B. dedeckkeri*. The three individuals in clade F2 (and *B. spec*. F2 sp. nov.) share two haplotypes, while the five specimens of *B. ivanae* sp. nov. possess 4 haplotypes, of which one remains unconnected. The remaining three species display two (*B. calei* sp. nov.) to five (*B. scanloni* sp. nov.) and six (*B. timmsi* sp. nov.) unconnected haplotype networks, respectively. The different genetic species within *B. timmsi* sp. nov. and *B. scanloni* sp. nov. form unconnected haplotype networks.

Taxonomic descriptions

Class Ostracoda Latreille, 1806 Subclass Podocopa G.O. Sars, 1866 Order Podocopida G.O. Sars, 1866 Suborder Cypridocopina Baird, 1845 Superfamily Cypridoidea Baird, 1845 Family Cyprididae Baird, 1845 Subfamily Bennelongiinae Martens *et al.*, 2012

Genus Bennelongia De Deckker & McKenzie, 1981

Diagnosis

See Martens et al. (2012)

Bennelongia barangaroo lineage

Remarks

De Deckker (1981a) described *B. barangaroo* from Lake Buchanan (QLD – Type locality), but also reported the same species from other localities in QLD, NSW, SA, WA and New Zealand. However, as in De Deckker's (1981a) re-description of *B. australis* (Brady, 1886) (see Martens *et al.* 2012), at least two different species within this lineage were illustrated under the same name. The (type) specimens of *B. barangaroo* in De Deckker's (1981a) figure 7 from Lake Buchanan have a short, sub-quadrate, slightly ventrally pointed lapel on the antero-ventral side of the RV. The specimens from a pool 25 km N of Cue (WA) (De Deckker 1981a: fig. 9), however, appear to have an elongated lapel, much as is the case in *B. calei* sp. nov. (see below). We thus decided previously (Shearn *et al.* 2012) that the true *B. barangaroo* needs to be established based on new material from the type locality. Fortunately, De Deckker (1981a) illustrated the valves and soft parts of the holotype male, which facilitated identification, and allowed Shearn *et al.* (2012) to confidently describe *B. dedeckkeri* as a different species within the *B. barangaroo* lineage. Shearn *et al.* (2012) also described *B. mckenziei* as a second new species from QLD, characterised by a total absence of the lapel on the RV.

Diagnosis of the *B. barangaroo* lineage

All species of the *B. barangaroo* lineage (re-)described here share a number of features: all have relatively elongated and wide (in dorsal view) carapaces, mostly green in colour, relatively smooth (but hirsute) in adults. The RV has an internal eyelet at the posteroventral internal side, mostly situated directly internally of the lapel. This eyelet is best visible with transparent light; although in most species it is also visible on SEM micrographs (see various illustrations below).

Bennelongia timmsi sp. nov. Figs 4-11 <u>urn:lsid:zoobank.org:act:4F6A6E8F-5636-4290-85A4-B234D5DA4466</u>

Diagnosis

Valves in inner view (Fig. 4A-B, D-E) relatively high, with greatest height situated well in front of the middle; ventral margin anteriorly with well-pronounced mandibular curve. LV (Fig. 4A, D) with anterior il not overlapping. RV (Fig. 4B, E) with antero-ventral lapel subtriangular, asymmetrically produced with a ventral point (Fig. 4K-M). Carapace in dorsal and ventral views (Fig. 4G-J) with greatest width in the middle, hirsute, anteriorly with a mild rostrum; in lateral views (Fig. 4C, F) showing a clear anterior LV>RV overlap.

Hemipenes (holotype: Fig. 8F) mostly symmetrical, with ls protruding well beyond ventral tip of ms, ls with broad base, ventrally bluntly beak-shaped. Right prehensile palp (holotype: Fig. 8D) with distal segment elongated, with dorsal margin evenly rounded. Left prehensile palp (holotype: Fig. 8E) with distal segment elongated, reaching beyond ventro-apical margin of proximal segment with at least half of its length.

Etymology

This species is named after Prof. Brian V. Timms (Newcastle, Australia), in recognition of his vast contribution to the knowledge of Australian non-marine crustaceans in general, and of phyllopods from temporary pools in particular. Prof. Timms also collected the material of the present species from a series of pools on various rocky outcrops in WA.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

Holotype \bigcirc (WAMC52228): RV: L = 1318, H = 783. LV: L = 1378, H = 817. Allotype \bigcirc (WAMC52229): RV: L = 1510, H = 913. LV: L = 1600, H = 965.

Type locality

Rock pools on Wave Rock, WA, ca. 2 km E of Hyden. Approximate coordinates: 32° 27'S 118° 54' E (WGS 84). Material handpicked from pools by B.V. Timms on 23 Jul. 2010 (sample BVT/10/05).

Type material

Holotype

 \Diamond (WAMC52228), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide.

Allotype

 \bigcirc (WAMC52229), with soft parts dissected in a sealed slide, and valves stored dry in a micropalaeontological slide.

Paratypes

Numerous males and females from the type locality, either dissected and stored as the holotype, or as carapaces used for SEM (WAMC52230-52237, OC.3312-3316). Ca. 60 $\Im \Im$ and $\Im \Im$ in EtOH as bulk paratypes (WAMC52238).

Other material investigated

All material from WA, collected by B.V. Timms.



Fig. 4. *Bennelongia timmsi* sp. nov., all represent paratypes from Wave Rock (BVT/10/05 – type locality). **A.** \bigcirc , LVi (OC.3313). **B.** \bigcirc , RVi (idem). **C.** \bigcirc , CpRL (WAMC52235). **D**. \bigcirc holotype, LVi (WAMC52228). **E**. \bigcirc holotype, RVi (idem). **F**. \bigcirc , CpRL (WAMC52236). **G**. \bigcirc , CpD (WAMC52234). **H**. \bigcirc , CpV (OC.3315). **I**. \bigcirc , CpV (OC.3316). **J**. \bigcirc , CpD (WAMC52237). **K**. \bigcirc holotype, RVi, detail anterior (WAMC52228). **L**. \bigcirc holotype, RVi, detail anterior, tilted (OC.3313). Scales: A-J = 1 mm; K-M = 200 µm.



Fig. 5. *Bennelongia timmsi* sp. nov., all males with hemipenes and prehensiles palps illustrated (see Figs 8-10). A-L = paratypes from Wave Rock (BVT/10/05), M-O = non-types from King Rocks (BVT/10/06). — A-C. WAMC52232: A. RVi. B. RVi, detail anterior, tilted. C. RVi, detail anterior. — D-F. WAMC52231: D. LVi. E. RVi, detail anterior, tilted. F. RVi. — G-I. OC.3312: G. LVi. H. RVi, detail anterior, tilted. I. RVi. — J-L. WAMC52230: J. LVi. K. RVi, detail anterior, tilted. L. RVi. — M-O. WAMC52252: M. LVi. N. RVi, detail anterior, tilted. O. RVi. Scales: A, D, F-G, I-J, L-M, O = 1 mm; B-C, E, H, K, N = 200 µm.



Fig. 6. *Bennelongia timmsi* sp. nov., all represent females with molecular data available. — A-C. Grahams Rock (BVT/10/02 – WAMC52243 – cryptic species A1): A. LVi. B. RVi, detail anterior, tilted. C. RVi. — D-F. Grahams Rock (BVT/10/02 – WAMC52241 – cryptic species A2): D. LVi. E. RVi, detail anterior, tilted. F. RVi. — G-I. King Rocks (BVT/10/06 – WAMC52251 – cryptic species A2): G. LVi. H. RVi, detail anterior, tilted. I. RVi. — J-L. Wave Rock (BVT/10/05 – allotype WAMC52229 – cryptic species A3): J. LVi. K. RVi, detail anterior, tilted. L. RVi. — M-O. Mt Madden Rocks (BVT/10/08 – WAMC52254 – cryptic species A5): M. LVi. N. RVi, detail anterior, tilted. O. RVi. Scales: A, C-D, F-G, I-J, L-M, O = 1 mm; B, E, H, K, N = 200 μ m.



Fig. 7. *Bennelongia timmsi* sp. nov. — **A-C**. Mt Madden Rocks (BVT/10/08 – \mathcal{J} , WAMC52255 – cryptic species A5). **A**. LVi. **B**. RVi, detail anterior, tilted. **C**. RVi. — **D-F**. Anderson Rock (BVT/10/03 – \mathcal{Q} , WAMC52245). **D**. LVi. **E**. RVi, detail anterior, tilted. **F**. RVi. — **G-I**. Paynes Find Rock (BVT/10/01 – \mathcal{Q} , WAMC52239). **G**. LVi. **H**. RVi, detail anterior, tilted. **I**. RVi. — **J-L**. Grahams Rock (BVT/10/02 – \mathcal{Q} , OC.3317). **J**. LVi. **K**. RVi, detail anterior, tilted. **L**. RVi. — **M**. Burracopin Rock (BVT/10/04 – \mathcal{Q} , OC.3318), RVi, detail anterior, tilted. — **N**. King Rocks (BVT/10/06 – \mathcal{Q} , WAMC52250), RVi, detail anterior, tilted. Scales = 1 mm unless otherwise indicated.



Fig. 8. *Bennelongia timmsi* sp. nov., male type specimens from type locality (Wave Rock, BVT/10/05). — A-C. Paratype M (OC.3312): A. Hemipenis (both hemipenes symmetrical in this specimen). B. Right prehensile palp. C. Left prehensile palp. — D-F. Holotype $\stackrel{\circ}{\circ}$ (WAMC52228): D. Right prehensile palp. E. Left prehensile palp. F. Hemipenis (both hemipenes symmetrical in this specimen). Scale: A-F = 92 µm.



Fig. 9. *Bennelongia timmsi* sp. nov., paratypes from type locality (Wave Rock, BVT/10/05). — A-C. Paratype $\overset{\circ}{\bigcirc}$ (WAMC52231): A. Hemipenis (both hemipenes symmetrical in this specimen). B. Right prehensile palp. C. Left prehensile palp. — D-F. Paratype $\overset{\circ}{\bigcirc}$ (WAMC52230): D. Left prehensile palp. E. Right prehensile palp. F. Hemipenis (both hemipenes symmetrical in this specimen). Scale: A-F= 92 µm.



Fig. 10. *Bennelongia timmsi* sp. nov., male paratype (WAMC52232) from type locality (Wave Rock, BVT/10/05) Aberrant specimen. **A**. Hemipenis. **B**. Hemipenis. **C**. Left prehensile palp. **C'**. Idem, detail of ventroapical part of first segment, showing two lobes and a sensory organ. **C''**. Idem, detail of distal part of second segment, showing aberrant, bilobed morphology. **D**. Right prehensile palp. Scale: $A-D = 92 \mu m$; C', C'' = 37 μm .



Fig. 11. *Bennelongia timmsi* sp. nov., non-type males. — **A-D**. Grahams Rock (WAMC52240, BVT/10/02): **A**. Hemipenis. **B**. Hemipenis. **C**. Right prehensile palp. **D**. Left prehensile palp. **D**'. Idem, detail of ventro-apical part of first segment. — **E-F**. King Rocks (WAMC52252, BVT/10/06): **E**. Hemipenis. **F**. Left prehensile palp. **G**. Right prehensile palp. Scale: $A-G = 92 \mu m$; $D' = 37 \mu m$.

Paynes Find Rocks. Approximate coordinates: 29°10' S, 117°40' E (sample BVT/10/01), collected by B.V. Timms on 23 Jul. 2010 (one \bigcirc - WAMC52239).

Grahams Rock. 32°28' S, 119°03' E (sample BVT/10/02), collected by B.V. Timms on 23 Jul. 2010 (six ♂♂ and ♀♀, WAMC52240-52244; OC.3317).

Anderson Rock. 32°10' S, 118°51' E (sample BVT/10/03), collected by B.V. Timms on 23 Jul. 2010 (one ♂, WAMC52245).

Burracopin Rock. 31°24' S, 118°27' E (sample BVT/10/04), collected by B.V. Timms on 26 Jul. 2010 (six ♂♂ and ♀♀, WAMC52246-52249; OC.3318-3319).

King Rocks. 32°19' S, 119°09' E (sample BVT/10/06), collected by B.V. Timms on 23 Jul. 2010 (one 3° and two 9° , WAMC52250-52252).

Yorkrakine Rocks. 31°25' S, 117°30' E (sample BVT/10/07), collected by B.V. Timms on 27 Jul. 2010. *Mt Madden Rock.* 33°14' 22" S, 119°50' 33" E (sample BVT/10/08), collected by B.V. Timms on 01 Aug. 2010 (11 $\bigcirc \bigcirc$ and $\bigcirc \bigcirc$, WAMC52253-52262; OC.332-3321).

Differential diagnosis

Bennelongia timmsi sp. nov. can be distinguished from most species of the *B. barangaroo* lineage by the triangular and ventrally pointed lapel on the RV and the strongly sinuous ventral valve margins. The lapel of *B. scanloni* sp. nov. is also subtriangular and ventrally protruding, but it is rounded, thus looking drop-shaped in internal (non-tilted) view.

Bennelongia timmsi sp. nov. can moreover be distinguished from *B. gnamma* sp. nov. by the less high and less rounded valves and by the ls of the hemipenes, which protrudes well beyond the ms (subequal in *B. gnamma* sp. nov.).

Additional notes on cryptic species

As was described above, five genetic clusters are recognised in this species (A1-5, Fig. 2). According to the calculations of the 4 theta rule, three cryptic species were found in *B. timmsi* sp. nov. with molecular methods, but no morphological diagnostic features could be found. Cryptic species A1 occurred in BVT/10/02, 03 and 07. Cryptic species A3 was found in BVT/10/02, 04, 06 and 08. Cryptic species A2+A4+A5 occurred in BVT/10/03 and 05 and is used here to characterize *B. timmsi* sp. nov. with BVT/10/05 (Wave Rock) as type locality. Note that BVT/10/02 (Grahams Rock) and BVT/10/03 (Anderson Rock) hold at least two sympatric clades/cryptic species each. In order to establish beyond reasonable doubt that the specimens belonging to these clusters and cryptic species are indeed morphologically indistinguishable, long series of specimens are illustrated.

Sample BVT/10/05 from pools on Wave Rock appeared to contain only one genetic cluster and cryptic species and, for this reason, Wave Rock was chosen as type locality. We then proceeded with two different approaches: (1) to dissect a series of males from this sample to test whether male reproductive organs (hemipenes, prehensile palps) showed uniformity within one cluster/cryptic species; (2) we checked for potential differences in the morphology of the valves of specimens belonging to different populations and/or shown to belong to different clusters/cryptic species.

Type specimens

Valves and carapaces of males and females of the type population (in sample BVT/10/05) were illustrated (Fig. 4) and this morphology defines the species. We then dissected several males from the same sample and population and illustrated the soft part and valve morphology. Shape of valves and size and shape of the antero-ventral lapel on the RV were most similar and indeed almost indistinguishable (Fig. 5). In all male specimens the valves have the shape described in the diagnosis above. The lapels are all elongated subtriangular, with a more or less serrated distal margin. In tilted perspective some lapels appear to be

shorter than others (e.g., the lapel in Fig. 5B appears shorter than in 5E), but this is almost entirely a matter of distorted perspective depending on how the valves were positioned when the photographs were taken (the same lapels appear almost equally long in non-tilted views - Fig. 5A and 5F, respectively).

However, there are significant differences in soft part morphology. Whereas the shapes of the hemipenisoutline and of the left prehensile palps are fairly uniform in the different specimens (Figs 8A, C, E-F; 9A, C-D, F), the second segment of the right prehensile palps ranges from elongated sub-triangular with almost equally rounded distal margin (Fig. 8B, D), to sub-rectangular with a clear blunt corner in this margin (Fig. 9B, E). It is not clear to what extend these differences are a biological reality, or whether the differences are distortions of the limbs caused by different positions in the slides. The differences are sufficiently small to be accepted as part of intra-specific variability, yet future investigations should take this variability into account. The morphology in the holotype (WAMC52228 – right prehensile palp in Fig. 8D) determines the specific morphology.

One male (WAMC52232 – Fig. 10A-D) had an aberrant morphology, with the terminal segment of the right prehensile palp (Fig. 10D) being even more elongated and with especially the terminal segment of the left prehensile palp being distally bilobed (Fig. 10C, C"), a morphology never before encountered in Cyprididae. Nevertheless the valves of this male show no differences with other type specimens (Fig. 5A-C).

Morphology within different clades/cryptic species

Valve morphology of specimens for which molecular clades are known (A1: Fig. 6A-C; A2: Fig. 6D-I; A3: Fig. 6J-L; A5: Fig. 6M-O, 7A-C) and for specimens from different populations for which no molecular data were available (Fig. 7D-N) again show no constant differences that could be used as identifying characters. There is some variability in size, shape and degree of crenulation of the lapel, but insufficiently so to use such features to characterise different clades/cryptic species.

One male specimen from BVT/10/02 and thus belonging to either cryptic species A1 or A3, had a right prehensile palp with a terminal segment clearly showing a blunt angle on the distal margin (Fig. 11C), while a male from BVT/10/06, and thus most likely belonging to clade A2, had a more elongated segment there with a more rounded distal margin (Fig. 11G) as in the holotype. In both of these specimens, the terminal segment of the left prehensile palp is slightly shorter than in the type specimens (Fig. 11D, F). Hemipenis outlines (Fig. 11A-B, E) are indistinguishable from those in the types.

Ecology and distribution

Bennelongia timmsi sp. nov. is a typical rock pool species and occurs in fresh water in gnammas on various rocky outcrops in the south/central part of western Australia. Although it appears to be limited to this restricted area, it seems to be quite common there.

Bennelongia gnamma sp. nov. Figs 12, 13A-E urn:lsid:zoobank.org:act:6931D617-1443-4776-891B-C02752E6C0BE

Diagnosis

Valves in internal view (Fig. 12A-B, E-F) high, with greatest height situated in front of the middle; ventral margin almost evenly curved except for middle third. LV (Fig. 12A, E) with anterior il slightly overlapping. RV (Fig. 12B, F) with antero-ventral lapel subtriangular, asymmetrically produced with a dorsal point (Fig. 12H-K).



Fig. 12. *Bennelongia gnamma* sp. nov., type material from type locality (Cairn Rock, OSTR012A). **A.** \bigcirc paratype, LVi (OC.3322). **B.** \bigcirc paratype, RVi (idem). **C.** \bigcirc paratype, LVe (idem). **D.** \bigcirc paratype, RVe (idem). **E.** \bigcirc holotype, LVi (WAMC52263). **F.** \bigcirc holotype, RVi (idem). **G.** \bigcirc paratype, CpD (WAMC52266). **H.** \bigcirc paratype, RVi, detail anterior (OC.3322). **I.** \bigcirc paratype, RVi, detail anterior, tilted (idem). **J.** \bigcirc holotype, RVi, detail anterior (WAMC52263). **K.** \bigcirc holotype, RVi, detail anterior, tilted (idem). Scales: A-G = 1 mm; H-K = 200 µm.



Fig. 13. *Bennelongia gnamma* sp. nov. (A-E, type specimens from type locality, Cairn Rock, OSTR012A) and *Bennelongia hirsuta* sp. nov. (F-I type specimens from type locality, Styles Rock, OSTR012D). Males. — A-E. *B. gnamma* sp. nov.: A. Hemipenis (both hemipenes symmetrical in this specimen, WAMC52264). B. Apical part of hemipenis (WAMC52265). C. Right prehensile palp (WAMC52264). D. Left prehensile palp (WAMC52264). E. Idem, detail of ventro-apical part of first segment (different specimen, WAMC52265). — F-I. *B. hirsuta* sp. nov. (WAMC52271): F. Hemipenis. G. Right prehensile palp. H'. Idem, detail of ventro-apical part of first segment. I. Hemipenis. Scale: A-D, F-I = 92 μ m; E, H' = 37 μ m.

Valves in external lateral view (Fig. 12C-D) high and rounded on all sides, even ventrally to some extent; hirsute and weakly pitted. Carapace in dorsal view (Fig. 12G) anteriorly with a mild rostrum.

Hemipenes (Fig. 13A) mostly symmetrical, with length of ls subequal to that of ms, in one specimen tip of ms even extending beyond that of ls (Fig. 13B); ls with broad base, ventrally bluntly beak-shaped. Right prehensile palp (Fig. 13C) with distal segment broad, with anterior margin straight, distal margin bilobed. Left prehensile palp (Fig. 13D-E) with distal segment short, reaching beyond ventro-apical margin of proximal segment with less than half its length.

Etymology

The present species is named after the Australian term for small to middle-sized rock pools, namely gnammas, in which it occurs.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

Holotype $\stackrel{\bigcirc}{_+}$ (WAMC52264): RV: L = 1550, H = 915. LV: L = 1620, H = 991.

Type locality

Rock pools (gnammas) on Cairn Rock, WA, ca. 67 km SE of Merredin. Approximate coordinates: 31°51'31" S, 118°50'39" E (WGS 84). All material (voucher sample OSTR012A; locality code SPS059) collected by J. McRae & A. Pinder on 24 Sep. 1997 with a sweep net.

Type material

Holotype

 \bigcirc (WAMC52263), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide.

Allotype

 \Diamond (WAMC52264), with soft parts dissected in a sealed slide, and valves stored dry in a micropalaeon-tological slide.

Paratypes

Other material investigated

? 1 \bigcirc (WAMC52268) from Yanneymooning Rocks (identification uncertain).

Differential diagnosis

Bennelonga gnamma sp. nov. can be distinguished from all other species in the lineage by the high and rounded shape of the valves and especially by the subequal ls and ms in the hemipenes.

Ecology and distribution

This species is thus far known with certainty only from its type locality, a set of rock pools on Cairn Rock.

Bennelongia hirsuta sp. nov. Figs 13F-I, 14 <u>urn:lsid:zoobank.org:act:CDE0D1E7-52E6-4F18-B6FF-B3AD48C6E4B4</u>

Diagnosis

Valves elongated, with greatest height situated well in front of the middle, dorsal margin evenly sloping towards the posterior side; ventral margin anteriorly with pronounced mandibular curve. LV (Fig. 14A, D) with antero-ventral inner list large, well-overlapping the dorsal il. RV (Fig. 14B, E) with antero-ventral lapel long, narrow and weakly crenulated (Fig. 14J-M).

Carapace in dorsal and ventral view (Fig. 14G-I) with greatest width in the middle, most hirsute, anteriorly with a clear rostrum; in lateral views (Fig. 14C, F) anteriorly with a clear LV>RV overlap.

Hemipenes asymmetrical (Fig. 13F, I), ls with slender base, ventrally sharply beak-shaped and pointed, only slightly protruding beyond ventral tip of ms. Right prehensile palp (Fig. 13G) with distal segment stout and subquadrate, anterior margin straight, dorsal margin sinuous. Left prehensile palp (Fig. 13H, H') with distal segment short and sickle-shaped, reaching beyond ventro-apical margin of proximal segment with less than a third of its length.

Etymology

Named after the hirsute ('hairy') nature of this species. All species of the *B. barangaroo* lineage are hirsute to some extent, but the present species is more so, with the entire carapace set with long and stiff setae.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

Holotype \Diamond (WAMC52269): RV: L = 1260, H = 738. LV: L = 1320, H = 763. Allotype \heartsuit (WAMC52270): RV: L = 1400, H= 842. LV: L = 1470, H = 845.

Type locality

Rock pools (gnammas) on Styles Rock, WA, ca. 80 km N of Esperance. Approximate coordinates: 33°07'35" S, 121°48'02" E (WGS 84). All specimens (voucher sample OSTR012D; locality code SPS139), collected on 07 Sep. 1998 by J. McRae & A. Pinder with a sweep net.

Type material

Holotype

 \Diamond (WAMC52269), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeon-tological slide.

Allotype

 \bigcirc (WAMC52270), with soft parts dissected in a sealed slide, and valves stored dry in a micropalaeon-tological slide.

Paratypes

Numerous $\Im \Im$ and $\Im \Im$, as values or carapaces (WAMC52271-52277; OC.3323-3325). Nine $\Im \Im$ and $\Im \Im$ in bulk in EtOH (WAMC52278).

Other material investigated

? One $\stackrel{\bigcirc}{_+}$ (WAMC52279) from Lilian Stokes Rocks (BVT/10/09) (identification uncertain).



Fig. 14. *Bennelongia hirsuta* sp. nov., type specimens from type locality (Styles Rock, OSTR012D). A. \bigcirc allotype, LVi (WAMC52270). **B**. \bigcirc allotype, RVi (idem). **C**. \bigcirc paratype, CpRL (WAMC52277). **D**. \bigcirc holotype, LVi (WAMC52269). **E**. \bigcirc holotype, RVi (idem). **F**. \bigcirc paratype, CpRL (WAMC52272). **G**. \bigcirc paratype, CpD (WAMC52275). **H**. \bigcirc paratype, CpV (WAMC52276). **I**. \bigcirc paratype, CpD (WAMC52272). **J**. \bigcirc allotype, RVi, detail anterior (WAMC52270). **K**. \bigcirc allotype, RVi, detail anterior, tilted (idem). **L**. \bigcirc holotype, RVi, detail anterior (WAMC52269). **M**. \bigcirc holotype, RVi, detail anterior, tilted (idem). Scales: A-I = 1 mm; J-M = 300 μ m.

Differential diagnosis

Bennelongia hirsuta sp. nov. can be distinguished from all other species in the *B. barangaroo* lineage by the pointed shape of the ls in the hemipenis. Also the shapes of the distal segments of the prehensile palps are distinctive. In valve morphology, the species is easily recognisable by the long and narrow lapel on the RV (which is nevertheless very difficult to see with a normal binocular microscope) and the large antero-ventral il.

Ecology and distribution

The species is thus far only known with certainty from its type locality, a set of rock pools on Styles Rock.

Bennelongia ivanae sp. nov. Fig. 15 urn:lsid:zoobank.org:act:2D60FC82-0938-475A-AF4E-E2DBE24D1D8D

Diagnosis

Valves (Fig. 15A-B) high and rounded, with greatest height situated well in front of the middle; dorsal margin with blunt angle towards the posterior side; ventral margin almost straight, without pronounced mandibular curve. LV (Fig. 15A) with antero-ventral il well-developed in lower third of the valve, dorsal il descending almost to ventral side, thus clearly overlapping with ventral il. RV (Fig. 15B) without antero-ventral lapel (Fig. 15H-J). Carapace in dorsal and ventral views (Fig. 15D-F) with greatest width in the middle, medium hirsute and slightly pitted, anteriorly with a hint of a rostrum; in lateral view (Fig. 15C, G) anteriorly with a clear LV>RV overlap.

Male unknown.

Etymology

This species is named after Dr Ivana Karanovic (South Korea), in recognition of her contributions to the knowledge of the subterranean candonids of the Pilbara area.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

Holotype $\stackrel{\bigcirc}{\downarrow}$ (WAMC52280): RV: L = 1475, H = 915. LV: L = 1533, H = 948.

Type locality

Rock pools (gnammas) on Holland Rocks, WA. Approximate coordinates: 33°21'35.66" S, 118°44'48.55" E (WGS 84) (sample DJC/02). All specimens collected and handpicked by D.J. Cale on 30 Aug. 2011.

Type material

Holotype

 \bigcirc valves stored dry (WAMC52280).

Allotype

As males are unknown, no allotype is designated.

Paratypes

Four $\bigcirc \bigcirc \bigcirc$ with valves or carapaces stored dry (WAMC52281-52282; OC.3326-3327); *ca.* 25 $\bigcirc \bigcirc \bigcirc$ stored dry as bulk in one micropalaeontological slide.

Other material investigated

? $\bigcirc \bigcirc \bigcirc$ valves stored dry (WAMC52284) from Yanneymooning Rocks (OSTR013F).

Differential diagnosis

Bennelongia ivanae sp. nov. differs from all other WA species within the *B. barangaroo* lineage in the total absence of an antero-ventral lapel on the RV and in the fact that the antero-dorsal il in the LV runs almost entirely to the ventral margin. *Bennelongia mckenziei* Shearn *et al.*, 2012 from Queensland also lacks the antero-ventral lapel on the RV completely, but it has a notably different valve and carapace



Fig. 15. *Bennelongia ivanae* sp. nov., type specimens from type locality (Holland Rocks, DJC/02). **A.** \bigcirc holotype, LVi (WAMC52280). **B**. \bigcirc holotype, RVi (idem). **C**. \bigcirc paratype, CpRL (OC.3327). **D**. \bigcirc paratype CpD (WAMC52281). **E**. \bigcirc paratype, CpV (WAMC52282). **F**. \bigcirc paratype, CpV, detail anterior (idem). **G**. \bigcirc paratype, CpRL, detail anterior (OC.3327). **H**. \bigcirc holotype, RVi, detail anterior (WAMC52280). **I**. \bigcirc holotype, RVi, detail anterior, tilted (idem). **J**. \bigcirc paratype, RVi, detail anterior, tilted (OC.3326). Scales: A-E = 1 mm; F = 400 µm; G-J = 200 µm.

shape, with a more pointed caudal margin and an evenly sloping dorsal margin in both valves, a shorter antero-dorsal il in the LV and an anterior LV>RV overlap in a carapace in right lateral view which is twice as large as in *B. ivanae* sp. nov.

Remark

Bennelongia ivanae sp. nov. and *B. mckenziei* have pronounced molecular differences, when the present sequences of *B. ivanae* sp. nov. are compared with those of Shearn *et al.* (2012). Because of the shorter lengths of the sequences provided by Shearn *et al.* (2012), the alignment of COI sequences from *B. ivanae* sp. nov. and *B. mckenziei* are not shown in the present paper.

Ecology and distribution

The species has thus far been found with certainty only from rockpools at Holland Rocks. Two tentatively identified females also originated from rock pools on another outcrop.

Bennelongia sp. nov. F2

Material investigated

Two $\bigcirc \bigcirc \bigcirc$ (KMWA.905, 906) *in toto* used for molecular screening, one \bigcirc with soft parts used for molecular screening and with broken RV stored dry in micropalaeontological cavity slide (WAMC52285 = KMWA.904).

Locality

Lilian Stokes Rocks (eastern Wheatbelt – BVT/10/09), coordinates: 33°4'06" S, 120°05'49" E. Collected on 25 Aug. 2010 by B.V. Timms.

Remarks

The specimens of the F2-group cluster close to those of *B. ivanae* sp. nov. in the phylogenetic tree (Fig. 2), but still constitute a separate genetic species (Table 2). A broken RV could be saved from only one of these specimens (KMWA.904), and it could be ascertained that the antero-ventral lapel on the RV is also fully absent (not shown). It is possible that cluster F2 will turn out to be a cryptic species within *B. ivanae* sp. nov., but this remains to be tested with new material. Note that the same sample (BVT/10/09) also contained one putative female of *B. hirsuta* sp. nov. (see above).

Bennelongia mcraeae sp. nov. Figs 16-17 urn:lsid:zoobank.org:act:DFD1A720-E602-46CC-AABF-C6EDD63CD70E

Diagnosis

Valves (Fig. 16A-B, E-F) high and rounded, with greatest height situated on or close to the middle, dorsal margins almost evenly rounded; ventral margin weakly sinuous. LV (Fig. 16A, E) with anteroventral il large, reaching over half of the anterior margin; dorsal il descending along ca. 4/5 of anterior margin, both lists thus clearly overlapping. RV (Fig. 16B, F) with anteroventral lapel fairly ventrally inserted, large and pronounced, with rounded, weakly crenulated margin (Fig. 16D, I-L). Carapace in lateral view (Fig. 16C- D) pitted, especially along anterior and posterior margins, and set with few setae.

Hemipenes (Fig. 17A, F) largely symmetrical, ls with broad base, ventrally bluntly beak-shaped, only protruding significantly beyond ventral tip of ms. Right prehensile palp (Fig. 17C, E) with distal segment stout, but of somewhat variable shape, either strongly subquadrate or with rounded dorsal and anterior



Fig. 16. *Bennelongia mcraeae* sp. nov., type specimens from type locality (Arro Lake, OSTR014). A. \bigcirc allotype, LVi (WAMC52287). B. \bigcirc allotype, RVi (idem). C. \bigcirc paratype, CpRL (WAMC52289). D. \bigcirc paratype, CpRL, detail anterior (idem). E. \bigcirc holotype, LVi (WAMC52286). F. \bigcirc holotype, RVi (Idem). G. \bigcirc holotype, LVe (idem). H. \bigcirc holotype, RVe (idem). I. \bigcirc holotype, RVe, detail anterior (idem). J. \bigcirc holotype, RVi, detail anterior (idem). K. \bigcirc holotype, RVi, detail anterior, tilted (idem). L. \bigcirc allotype, RVi, detail anterior, tilted (WAMC52287). Scales = 1 mm unless otherwise indicated.



Fig. 17. *Bennelongia mcraeae* sp. nov., type males from type locality (Arro Lake, OSTR014B). — A-C. Holotype \Diamond (WAMC52286). A. Hemipenis. B. Left prehensile palp. C. Right prehensile palp. — D-F. Paratype \Diamond (OC.3328). D. Left prehensile palp. E. Right prehensile palp. F. Hemipenis. Scale: A-F = 92 µm.

margins. Left prehensile palp (Fig. 17B, D) with distal segment long and slender, reaching beyond ventro-apical margin of proximal segment with at least half of its length.

Etymology

The species is named in honour of Jane McRae (Perth, WA) in acknowledgement of her vast knowledge of the taxonomy and morphology of many invertebrate groups of Western Australia. She also collected the type material of the present species and has unrelentingly provided technical help towards the present revision of *Bennelongia* since 2006.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

Holotype \bigcirc (WAMC52286): RV: L = 1480, H = 878. LV: L = 1560, H = 896. Allotype \bigcirc (WAMC52287): RV: L = 1608, H = 1002. LV: L = 1663, H = 1033.

Type locality

Arro Lake, *ca*. 11 km NW of Eneabba, WA. Approximate coordinates: 29°44'11" S, 115°09'58" E (WGS 84). All specimens collected by J. McRae & A. Pinder (voucher OST14B; locality code SPS182) on 23 Sep. 1999 with a sweep net. Arro Lake is an open lake with a *Melaleuca/Casuarina* fringe. Water chemistry at the time of collecting: Salinity = 0.15 g/l, pH = 7.32. Nutrient levels were fairly high: total N = 1700 μ g/l; total P = 220 μ g/l. The milky-white colour of the water equates to a high level of turbidity (2200 NTU).

Type material

Holotype

 \Diamond (WAMC52286), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeon-tological slide.

Allotype

 $\stackrel{\circ}{_{+}}$ (WAMC52287), with soft parts dissected in a sealed slide, and valves stored dry in a micropalaeontological slide.

Paratypes

Two 3328; valves: WAMC52288) and one 2 carapace (WAMC52289). Three females in bulk in EtOH (WAMC52290).

Differential diagnosis

The species is characterised especially by the large and stout lapel on the RV but also by the large anteroventral il on the LV.

Ecology and distribution

Lake Arro (*ca.* 300 km N of Perth) is a large flat-bottomed body of water with episodic inflow that holds water for about 4-24 months after inflow. The lake has a clay base and sediment-driven turbidity. This species is known only from the type locality. From the same sample as the one that yielded *B. macraeae* sp. nov., Timms (2002) described a new species of Anostraca, *Branchinella complexidigitata* Timms, 2002.

Bennelongia scanloni sp. nov. Figs 18-20 <u>urn:lsid:zoobank.org:act:B41BF127-BEEC-47CE-A687-FACD6CBCF028</u>

Diagnosis

Valves (Fig. 18A-B, D-E) high, with greatest height situated close to the middle, dorsal margin evenly sloping towards the posterior side; ventral margin sinuous. LV (Fig. 18A, D) with antero-ventral il of medium size, covering lower third of valve, antero-dorsal il descending to about halfway along antero-ventral il. RV (Fig. 18B, E) with antero-ventral lapel tear-shaped in untilted lateral view; in tilted view, lapel subtriangular with rounded ventral point (Fig. 18K-N). Carapace in dorsal and ventral views (Fig. 18G-J) with greatest width in the middle, hirsute and pitted, anteriorly with a clear rostrum; in right lateral view (Fig. 18C, F) with large anterior LV>RV overlap, anterior margins of RV and LV not parallel.

Hemipenes (holotype: Fig. 20F) asymmetrical, Is with broad base, ventrally bluntly pointed (more so in one hemipenis than in the other), largely protruding beyond ventral tip of ms. Right prehensile palp (holotype: Fig. 20D) with distal segment stout and subquadrate, with sharp angle between anterior and dorsal margins, both of these margins almost straight. Left prehensile palp (holotype: Fig. 20E) with distal segment sickle-shaped and of intermediate length, reaching beyond ventro-apical margin of proximal segment with about half of its length.

Etymology

The species is named in honour of Mike Scanlon (Perth, WA) in acknowledgement of his unrelenting technical help since 2006 towards the present revision of *Bennelongia*.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

Holotype \bigcirc (WAMC52291): RV: L = 1223, H = 694. LV: L = 1294, H = 714. Allotype \bigcirc (WAMC52292): RV: L = 1263, H = 752. LV: L = 1356, H = 775.

Type locality

One Tree Hill Creek, *ca.* 62 km SE of Dongara, WA. Approximate coordinates: 29°35'19.0" S, 115°26'31.0" E (WGS 84). All specimens (sample DJC/11; locality code SPS180) collected by D.J. Cale on 10 Sep. 2011 with a sweep net. Water chemistry at time of collecting: K25 5.62 mS/cm, pH 6.68, water temperature 20.8 °C.

Type material

Holotype

 \Diamond (WAMC52291), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeon-tological slide.

Allotype

 \bigcirc (WAMC52292) valves stored dry in a micropalaeontological slide.

Paratypes

Numerous males and females either as dissection, or as valves or carapaces stored dry (WAMC52293-52304; OC.3329-3331). *Ca.* 30 $\Diamond \Diamond$ and $\bigcirc \bigcirc$ stored as bulk in EtOH (WAMC52305).



Fig. 18. *Bennelongia scanloni* sp. nov., type specimens from type locality (One Tree Hill Creek, DJC/11). **A.** \bigcirc allotype, LVi (WAMC52292). **B**. \bigcirc allotype, RVi (idem). **C**. \bigcirc paratype, CpRL (WAMC52299). **D**. \bigcirc paratype, LVi (OC.3329). **E**. \bigcirc paratype, RVi (idem). **F**. \bigcirc paratype, CpRL (WAMC52295). **G**. \bigcirc paratype, CpD (WAMC52297). **H**. \bigcirc paratype, CpV (WAMC52298). **I**. \bigcirc paratype, CpV (WAMC52295). **J**. \bigcirc paratype, CpD (WAMC52296). **K**. \bigcirc allotype, RVi, detail anterior (WAMC52292). **L**. \bigcirc allotype, RVi, detail anterior, tilted (idem). **M**. \bigcirc paratype, RVi, detail anterior (OC.3329). **N**. \bigcirc paratype, RVi, detail anterior, tilted (idem). Scales: A-J = 1 mm; K-N = 200 µm.



Fig. 19. *Bennelongia scanloni* sp. nov., non-type specimens. — **A-D**. Pool at Latham-Coorow Rd (BVT/10/10, \Im , WAMC52307)). **A**. LVi. **B**. RVi. **C**. RVi, detail anterior. **D**. RVi, detail anterior, tilted. — **E-H**. Tin Dog Creek (DJC/19, \Im , OC.3335). **E**. LVi. **F**. RVi. **G**. RVi, detail anterior. **H**. RVi, detail anterior, tilted. — **I-O**. Pool at Brookton Hwy (Warrine Park) (DJC/23, \Im , OC.3337). **I**. LVi. **J**. RVi. **K**. RVi, detail anterior. **L**. RVi, detail anterior, tilted. **M**. LVe, detail anterior. **N**. RVe. **O**. RVe, detail anterior. Scales: A-B, E-F, I-J, N = 1 mm; C-D, G-H, K-M, O = 200 µm.



Fig. 20. *Bennelongia scanloni* sp. nov., males. — **A-C'**. Non-type (OC.3338, Three Springs Tumulus Stream - TST): **A**. Hemipenis (both hemipenes symmetrical in this specimen). **B**. Right prehensile palp. **C**. Left prehensile palp. **C'**. Idem, detail of ventro-apical part of first segment. — **D-F**. Holotype (WAMC52291, One Tree Hill Creek – DJC/11): **D**. Right prehensile palp. **E**. Left prehensile palp. **F**. Hemipenis (both hemipenes symmetrical in this specimen). — **G-J**. Non-type (WAMC52327, from OSTR013C): **G**. Hemipenis. **H**. Right prehensile palp. **I**. Left prehensile palp. **I'**. Idem, detail of ventro-apical part of first segment. J. Hemipenis. Scale: A-J = 92 µm; C', I' = 37 µm.

Other material investigated

One Tree Hill Creek. 29°35'19.0" S, 115°26'31.0" E, collected by S. Halse and A. Pinder on 11 Aug. 1999 (OSTR013C), see Fig. 20G-J (WAMC52327). Water chemistry at time of collecting: K25 3.12 mS/ cm, pH 7.65, water temperature 19.4 °C.

Pools at Latham-Coorow Rd. 29°51'S, 116°16' E (sample BVT/10/10), collected by B.V. Timms on 10 Sep. 2010 (WAMC52306-52308; OC.3332-3333).

Dam at Solomons Well. Approximate coordinates: $31^{\circ}11'58.8"$ S, $116^{\circ}21'47.7"$ E (sample DJC/04), collected by D.J.Cale on 09 Sep. 2011 (4 \bigcirc \bigcirc : WAMC52332-52334; OC.3343). Water chemistry at time of collecting: K25 0.12 mS/cm, pH 7.85, water temperature 14.4 °C.

Three Pools along Eneabba-Carnamah Rd. Approximate coordinates: $34^{\circ}18'32.80"$ S, $115^{\circ}39'16.44"$ E (sample DJC/09), collected by D.J. Cale on 10 Sep. 2011 (2 \bigcirc \bigcirc : WAM52310; OC.3334). Water chemistry at time of collecting: K25 8.7 mS/cm, pH 7.2, water temperature 20.3 °C.

Second pool along Carnamah-Eneabba Road on south side (Eneabba Springs). Approximate coordinates: 29°48'23.62" S, 115°25'6.11" E (sample DJC/10), collected by D.J. Cale on 10 Sep. 2011. Water chemistry at time of collecting: K25 3.19 mS/cm, pH 6.6, water temperature 20.9 °C.

Petruder Dam. Approximate coordinates: $30^{\circ}25'20.87''$ S, $116^{\circ}57'39.43''$ E (sample DJC/15), collected by D.J. Cale on 11 Sep. 2011 (5 \bigcirc \bigcirc : WAMC52329-52331, OC.3341-3342). Water chemistry at time of collecting: K25 0.16 mS/cm, pH 7.85, water temperature 22.0 °C.

Tin Dog Creek. Approximate coordinates: $31^{\circ}11'53.5''$ S, $117^{\circ}01'41.4''$ E (sample DJC/19), collected by D.J. Cale on 23 Sep. 2011 ($\bigcirc \bigcirc$: WAMC52311-52315, OC.3335-3336. Juveniles: WAMC52316-52318). Water chemistry at time of collecting: K25 1.56 mS/cm, pH 7.06, water temperature 24.2 °C.

Pools near Brookton Hwy (in Warrine Park). Approximate coordinates: $32^{\circ}23'50.4"$ S, $116^{\circ}48'00.4"$ E (sample DJC/23), collected by D.J. Cale on 01 Oct. 2011 ($4 \bigcirc \bigcirc$: WAMC52319-52321, OC.3337). Water chemistry at time of collecting: K25 0.44 mS/cm, pH 8.1, water temperature 21.6 °C.

Three Springs Tumulus Stream. 29°35'31" S, 115°27'1" E, collected by A. Pinder on 29 Sep. 2010 (1 male: OC.3338; 2 ♀♀: WAMC52322-52323).

East Lake Bryde. 33°21' S, 118°49' E (sample BRYDE7), collected by D.J. Cale on 22 Mar. 2006 (4 \bigcirc \bigcirc : WAMC52324-52326; OC.3339). Water chemistry at time of collecting: K25 0.17 mS/cm, pH 6.91, water temperature 25.8 °C.

Lake Cronin. Episodically filled waterbody with extensive shrub and *Melaleuca* fringe, collected by S. Halse and A. Pinder on 25 Sep. 1997. Approximate coordinates: $32^{\circ}23'02''$ S, $119^{\circ}45'51''$ E. Water chemistry at time of collecting: K25 0.23 mS/cm, pH 9.48, water temperature 18.0 °C. *Material investigated:* one dissected Q (nr OS.544), with soft parts in a sealed slide and valves stored dry in micropalaeontological cavity slide (illustrated: Fig. 24E-H).

Reserve Esperance 26140 near Munglinup. Seasonally filled lake with trees across most of flooded area, collected 27 Oct. 1986 by S. Halse. Approximate coordinates: $33^{\circ}26'24''$ S, $120^{\circ}31'48''$ E. Water chemistry: salinity 0.27 mg/L TDS, pH 6.93. *Material investigated:* one dissected \bigcirc (nr OS.604), with soft parts in a sealed slide and valves stored dry in a micropalaeontological cavity slide (illustrated: Fig. 25I-L).

Remarks on the latter two localities: the lapels of both specimens are slightly larger than in most specimens of *B. scanloni* sp. nov. and as (1) no males are at hand to check for the morphology of the hemipenes and the prehensile palps in these populations and (2) no molecular data are available, the identifications of these two specimens are tentative.

Additional notes on cryptic species

Specimens from the type locality (One Tree Hill Creek, sample DJC/11) all belong to cryptic species E1, which is thus the true *B. scanloni* sp. nov. *s.s.* Also specimens from sample DJC/23 (pools near Brookton Hwy in Warrine Park) belong to this lineage (Fig. 19I-O). Two specimens from cryptic species

B2 are also illustrated here, one female from pools beside Latham-Coorow Rd (western Wheatbelt) (BVT/10/10) (Fig. 19A-D) and one female from Tin Dog Creek (DJC/19) (Fig. 19E-H). There are no clear differences between the two cryptic species in valve morphology.

The soft parts of the male from Three Springs Tumulus Stream (TST) (Fig. 20A-C) are slightly different from those of the holotype (Fig. 20D-F). Yet, within the molecular phylogeny the TST specimens cluster closely with the cryptic species E1, which is the same as for the type specimens. The hemipenis outline and the prehensile palps of the male from OSTR013C (Fig. 20G-J) are almost identical to those of the holotype, though no molecular data on this population are available, and it is thus also not clear to which of the two cryptic species within *B scanloni* sp. nov. this specimen belongs.

Thus far, the two molecular species cannot be distinguished morphologically. Interestingly, these two cryptic lineages occur sympatrically in no less than 4 localities (DJC/09, DJC/11, DJC/19 and BVT/10/10).

Differential diagnosis

The drop-shaped lapel on the RV and the sharp angle on the distal margin of the terminal segment of the right prehensile palp distinguish *B. scanloni* sp. nov. from all other species within the *B. barangaroo* lineage.

Ecology and distribution

This is arguably the most common species in this lineage in the south-western part of WA. It typically occurs in pools, dams and lakes with soft sediments.

Bennelongia calei sp. nov. Figs 21-22 urn:lsid:zoobank.org:act:DBD2498B-9E05-4E2A-9597-67844A85653E

Bennelongia barangaroo - De Deckker 1981a: 104, fig. 9 (partim).

Diagnosis (based on type specimens)

Valves (Fig. 21A-B, E-F) elongated, with greatest height situated close to the middle, dorsal margin evenly sloping towards the posterior side; ventral margin almost straight. LV (Fig. 21A, E) with anteroventral il large and reaching beyond middle of valve, antero-dorsal il descending to about halfway along antero-ventral il. RV (Fig. 21B, F) with antero-ventral lapel large, elongated and wide, with crenulated edge (Fig. 21C-D, G-H). Carapace in dorsal and ventral views (Fig. 21K-L) with greatest width in the middle, most slender of all species described here, external surface rather smooth to weakly pitted, set with only few short setae; anteriorly with a clearly delimited rostrum. Carapace in right lateral view (Fig. 21I-J) with greatest height in the middle, dorsal margin evenly sloping to bluntly rounded posterior margin; anteriorly with the widest LV>RV overlap of all species described here.

Males unknown.

Etymology

This species is named after D.J. Cale (Woodvale, WA) in honour of his longstanding contribution to the knowledge of freshwater invertebrates in WA, including at Fraser Lake which is the type locality of the present species (Cale *et al.* 2004), and also in recognition of the fact that he has collected so many of the samples used for the present revision of the *Bennelongia barangaroo* lineage.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

Holotype $\stackrel{\bigcirc}{\downarrow}$ (WAMC52335): RV: L = 1480, H = 857. LV: L = 1555, H = 865.

Type locality

Fraser Lake, *ca*. 8 km SE of Dowerin, WA. Approximate coordinates: 31°15'18.0" S, 117°4'22.0" E (WGS 84). All material (sample code DJC/18) collected on 23 Sep. 2011 by D.J. Cale with a sweep net. Water chemistry at time of collecting: K25 1.76 mS/cm, pH 8.1, water temperature 23.9 °C.

Type material

Holotype

 \bigcirc (WAMC52335) valves stored dry in a micropalaeontological slide.

Allotype

As males are unknown, no allotype is designated.

Paratypes

Eight $\bigcirc \bigcirc \bigcirc$, either as dried valves or carapaces (WAMC52337-52341; OC.3344-3345). *Ca.* 45 females stored as bulk in EtOH (WAMC52342).

Other material investigated

Fraser Lake (type locality). Four $\bigcirc \bigcirc \bigcirc$ valves and carapaces stored dry (WAMC52353-52356) from the same locality, but collected on another date (sample nr SPM017B, collected by D.J. Cale, 24 Nov. 2000), were also used during the present assessment of this species but are not considered as type material here. *Second pool along Carnamah-Eneabba Road on south side (Eneabba Springs).* Approximate coordinates: 29°48'23.62" S, 115°25'6.11" E (sample DJC/10), collected by D.J. Cale on 10 Sep. 2011 (one \bigcirc WAMC52349). Water chemistry at time of collecting: K25 3.19 mS/cm, pH 6.6, water temperature 20.9 °C.

Jerramungup West. Approximate coordinates: $33^{\circ}59'16.03''$ S, $118^{\circ}56'28.15''$ E (sample DJC/36), collected by D.J. Cale on 21 Oct. 2011 (five QQ valves and carapaces stored dry WAMC52350-52352; OC.3348-3349). Water chemistry at time of collecting: K25 0.73 mS/cm, pH 8.74, water temperature 25.8 °C

Oak Flat W pit gnamma via Goomalling. Approximate coordinates $31^{\circ}08'21''$ S, $116^{\circ}52'46''$ E (sample BVT/11/04), collected by B.V. Timms on 16 Aug. 2011 (four 99 valves and carapaces stored dry WAMC52343-52344; OC.3346-3347).

Horse Collar gnamma, *on Magee Rd via Kulin*. Approximate coordinates: $32^{\circ}48'04''$ S, $118^{\circ}23'34''$ E (sample BVT/11/05), collected by B.V. Timms on 4 Sep. 2011 (four $\bigcirc \bigcirc$ valves and carapaces stored dry WAMC52345-52348).

Additional illustrations

Several other populations of this species were found and for four of these (listed above), valves of female specimens are also illustrated here (Fig. 22). All of these specimens comply with the above diagnosis, and where specimens were available for molecular analyses, they also fell into the *B. calei* sp. nov. – cluster. No cryptic species were identified in this species.

Differential diagnosis

Bennelongia calei sp. nov. can easily be distinguished from all other species in the *B. barangaroo* lineage by the elongated and stout antero-ventral lapel on the RV, which is slightly rounded and has a



Fig. 21. *Bennelongia calei* sp. nov. — A-H, J-L. Fraser Lake (type locality, females, DJC/18): A. Paratype, LVi (OC.3344). **B**. Paratype, RVi (idem). **C**. Paratype, RVI, detail anterior (idem). **D**. Paratype, RVI, detail anterior, tilted (idem). **E**. Holotype, LVi (WAMC52335). **F**. Holotype, RVI (idem). **G**. Holotype, RVi, detail anterior (idem). **H**. Holotype, RVi, detail anterior, tilted (idem). J. Paratype, CpRL (OC.3345). **K**. Paratype, CpD (WAMC52338). **L**. Paratype, CpV (WAMC52337). — I. Oak Flat W pit gnamma, via Goomalling (BVT/11/04, non-type female, WAMC52343). CpRL. Scales: A-B, E-F, I-L = 1 mm; C-D, G-H = 200 μ m.



Fig. 22. *Bennelongia calei* sp. nov., non-type specimens. — **A-D**. Oak Flat W pit gnamma, via Goomalling (BVT/11/04, \bigcirc , OC.3346). **A**. LVi. **B**. RVi. **C**. RVi, detail anterior. **D**. RVi, detail anterior, tilted. — **E-H**. Horse Collar gnamma, on Magee Rd via Kulin (BVT/11/05, \bigcirc , WAMC52345). **E**. LVi. **F**. RVi. **G**. RVi, detail anterior. **H**. RVi, detail anterior, tilted. — **I-L**. Second pool along Carnamah-Eneabba Road on south side, Eneabba Springs (DJC/10, \bigcirc , WAMC52349). **I**. LVi. **J**. RVi. **K**. RVi, detail anterior. **L**. RVi, detail anterior, tilted. — **M-O**. Pool at Jerramungup West (DJC/36, \bigcirc , OC.3348). **M**. LVi. **N**. RVi. **O**. RVi, detail anterior, tilted. Scales: A-B, E-F, I-J, M-N = 1 mm; C-D, G-H, K-L, O = 200 µm.

crenulated distal margin, the large anterior LV>RV overlap, the stout antero-ventral il on the LV and the clear anterior rostrum on the carapace in dorsal or ventral view.

Ecology and distribution

The species is most common in pools and lakes in the southwest of WA. However, *B. calei* sp. nov. was also recovered from a totally different kind of habitat, namely pit gnammas near Goomalling (BVT/11/04) and Kulin (BVT/11/05). Whereas the other species in this lineage apparently occur either in rock pools or in soft bottomed pools and lakes, *B. calei* sp. nov. can apparently survive in both (very different) types of habitats. *Bennelongia calei* sp. nov. is, together with *B. timmsi* sp. nov. and *B. scanloni* sp. nov., one of the more common species in its area.

Bennelongia dedeckkeri Shearn et al., 2012 Figs 23, 24I-L

Bennelongia dedeckkeri sp. nov. - Shearn et al., 2012: 10-14, figs 4-5.

Material investigated

Dam at Kylena Well (Pilbara). Approximate coordinates: 22°06'00" S, 119°39'00" E (sample KIES10). Collected on 23 Apr. 2006 by the authors.

Unnamed saline billabong N of Coolcalaya Rd (Murchinson, Gascoyne). Approximate coordinates: 27°48'28" S, 114°48'18" E (sample SIKE2). Collected on 5 Jul. 2011 by the authors. Water chemistry at time of collecting: K25 8.8 mS/cm, pH 8.8, water temperature 11.0 °C.

McNeil Claypan, *Carnarvon* (Murchinson, Gascoyne). Approximate coordinates: 24°52'06" S, 113°42'56" E (sample SIKE9). Collected on 6 Jul. 2011 by the authors. Water chemistry at time of collecting: K25 0.19 mS/cm, pH 9.4, water temperature 10.8 °C.

Roadside ditch 1, North-West Coastal Hwy, Minilya Station (Murchinson, Gascoyne). Approximate coordinates: 23°54'25'' S, 114°01'45'' E (sample SIKE18). Collected on 7 Jul. 2011 by the authors. Water chemistry at time of collecting: K25 0.66 mS/cm, pH 7.4, water temperature 17.3 °C.

Roadside ditch 2, *North-West Coastal Hwy*, (Murchinson, Gascoyne). Approximate coordinates: 23°54'25'' S, 114°01'47'' E (sample SIKE19). Collected on 7 Jul. 2011 by the authors. Water chemistry at time of collecting: K25 0.69 mS/cm, pH 7.3, water temperature 17.3 °C.

Lake Gregory, *south of Halls Creek.* Approximate coordinates: 20°12' S, 127°27' E. Collected by S. Halse on 29 May 1991 in fresh water (see Halse *et al.* 1998). One dissected Q (nr OS.260), with soft parts in a sealed slide and valves stored dry in micropalaeontological cavity slide (illustrated in Fig. 24I-L).

Brief redescription

Smallest of the species described here, with females being only slightly longer than 1 mm.

Valves (Fig. 23A-B, E-F) high, with greatest height situated well in front of the middle, dorsal margin evenly sloping towards the posterior side; ventral margin nearly straight. LV (Fig. 23A, E) with anteroventral il of medium size in lower half of valve, antero-dorsal il descending to about halfway along the antero-ventral il. RV (Fig. 23B, F) with antero-ventral lapel almost rectangular, but slightly skewed and bluntly pointed towards the ventral side (Fig. 23C-D, G-H).

Carapace in dorsal and ventral views (Fig. 23K-L) with greatest width in the middle, hirsute and heavily pitted, anteriorly without a rostrum.

Males not yet found in WA.

Measurements (all measurements in μ m – see Table 1 for measurements of all specimens illustrated with SEM)

- ♀ (Pilbara, KIES10, WAMC52357): RV: L= 1110, H= 676. LV: L= 1190, H= 701.
- ♀ (Murchinson/Gascoyne, SIKE9, OC.3351): RV: L= 1125, H= 672. LV: L= 1188, H= 715.



Fig. 23. *Bennelongia dedeckkeri*, non-type females. — **A-D**, **I**, **K-L**. Dam at Kijlena Well, Pilbara (KIES/10). **A**. LVi (WAMC52357). **B**. RVi (idem). **C**. RVi, detail anterior (idem). **D**. RVi, detail anterior, slightly tilted (idem). **I**. CpRL (OC.3350). **K**. CpD (WAMC52360). **L**. CpV (WAMC52359). — **E-H**, **J**. McNeil Claypan, Murchinson/Gascoyne (SIKE9). **E**. LVi (OC.3351). **F**. RVi (idem). **G**. RVi, detail anterior (idem). **H**. RVi, detail anterior, tilted (idem). **J**. CpRL (OC.3352). Scales: A-B, E-F, I-L = 1 mm; C-D, G-H = 200 μm.

Ecology and distribution

Bennelongia dedeckkeri Shearn *et al*, 2012 was first described from Queensland, from a sexual population. In WA it is not uncommon, but thus far only asexual populations have been found. To date *B. dedeckkeri* is the only species of the *B. barangaroo* group found in both the eastern and the western parts of Australia.



Fig. 24. *Bennelongia* spp. (no museum nrs), females. — **A-D**. *Bennelongia* sp. X1. Crane Pan (OS.255). **A**. LVi. **B**. RVi. **C**. RVi, detail anterior. **D**. RVi, detail anterior, tilted. — **E-H**. ? *Bennelongia scanloni* sp. nov., Lake Cronin (OS.544). **E**. LVi. **F**. RVi. **G**. RVi, detail anterior. **H**. RVi, detail anterior, tilted. — **I-L**. *Bennelongia dedeckkeri*, Lake Gregory (OS.260). **I**. LVi. **J**. RVi. **K**. RVi, detail anterior. **L**. RVi, detail anterior, tilted. Scales: A-B, E-F, I-J = 1 mm; C-D, G-H, K-L = 200 μm.

Bennelongia sp. indet.

There are several single specimens, mostly female, from various (older) collections that could not be identified with certainty. Some of these are here illustrated to allow for future reference, in case new collections will become available. It is entirely possible that some of these specimens represent hybrid clades within the *B. barangaroo*-group. As none of these specimens have as yet been identified with certainty, no museum numbers have been allocated to them.

Bennelongia sp. X1 Fig. 24A-D

Material investigated

One dissected \mathcal{Q} (nr OS.255), with soft parts in a sealed slide and valves stored dry in a micropalaeontological cavity slide.

Locality

Canegrass covered claypan beside North-West Coastal Highway on Wooramel Station (CB35a), 25°40'52" S, 114°13'14" E, collected by S. Halse and A. Clarke on 24 Aug. 1994.

Morphology and affinities

The specimen is characterised by (1) a large antero-ventral lapel on the RV, subquadrate and only weakly crenulated, i.e., a shape unlike that of any of the other species (re-) described in the present paper, (2) a relatively small antero-ventral il on the LV and, (3) the bluntly pointed posterior margin of the LV, with a pronounced flange. The latter character is unique within the *B. barangaroo* lineage. If this character is stable and also occurs in other specimens, it could be indicative at a specific level.

Bennelongia sp. X2 Fig. 25A-D

Material investigated

One dissected \mathcal{Q} (nr KMWA.917), with soft parts in a sealed slide and valves stored dry in a micropalaeontological cavity slide.

Locality

Three Springs Tumulus stream, 29°35'31"S, 115°27'1" E, collected by A. Pinder on 29 Sep. 2010.

Morphology and affinities

The specimen has some affinity with *B. scanloni* sp. nov., but the antero-ventral il on the LV is smaller and the antero-ventral lapel of the RV is of a different shape, being larger and almost rectangular.

Remarks

Several other specimens from the same sample belong to cryptic species E1 of *B. scanloni* sp. nov., as shown by morphological and molecular evidence (see above).

Bennelongia sp. nov. E2 Fig. 25E-H

Material investigated

One dissected $\stackrel{\bigcirc}{\rightarrow}$ (nr KMWA.806), with soft parts used for molecular analysis and valves stored dry in a micropalaeontological cavity slide.



Fig. 25. *Bennelongia* spp. (no museum nrs). — **A-D**. *Bennelongia* sp. X2, Three Springs Tumulus Stream (\bigcirc , KMWA.917). **A**. LVi. **B**. RVi. **C**. RVi, detail anterior. **D**. RVi, detail anterior, tilted. — **E-H**. *Bennelongia* sp. nov. E2, BYK (\bigcirc , KMWA.806) = Species E2 in Figs 2-3. **E**. LVi. **F**. RVi. **G**. RVi, detail anterior. **H**. RVi, detail anterior, tilted. — **I-L**. *? Bennelongia scanloni* sp. nov., pool near Esperance (\bigcirc , OS.004). **I**. LVi. **J**. RVi. **K**. RVi, detail anterior. **L**. RVi, detail anterior, tilted. Scales: A-B, E-F, I-J = 1 mm; C-D, G-H, K-L = 200 µm.

Locality

Yakabindie Claypan, approximately 27°34' S, 120°31' E (sample LN3006), collected by Outback Ecology on 24 Mar. 2011.

Morphology and affinities

Although general valve appearance again shows some affinities to *B. scanloni* sp. nov., the different shape of the antero-ventral il on the LV (less pronounced and evenly rounded) and the large and subquadrate anteroventral lapel on the RV distinguishes this specimen from all other species (re-)described here. Molecular analysis of the soft parts of the same specimen shows that it clusters outside of the *B. scanloni* sp. nov. group (including cryptic species B2 and E1), and constitutes a different genetic species. Lack of additional material and males prevents us from formally describing this new species here.

General Discussion

De Deckker (1981a) reported two species of Bennelongia from WA: B. australis (Brady, 1886) and B. barangaroo De Deckker, 1981. Meanwhile, Martens et al. (2012) described nine new species from three separate lineages within this genus from WA (the B. australis lineage, the B. cvgnus lineage and the B. *pinpi* lineage) and showed that *B. australis* is actually a species group with at least seven nominal species and potentially more. Shearn et al. (2012) contributed to the knowledge of the genus Bennelongia in eastern Australia by confirming the validity of B. pinpi De Deckker, 1981 and B. harpago De Deckker & McKenzie, 1981, using genetics to identify the occurrence of a cryptic lineage within a species of the B. australis lineage (B. cuensis Martens et al., 2012), and by describing two new species in the B. barangaroo lineage (B. dedeckkeri Shearn et al., 2012 and B. mckenziei Shearn et al., 2012) and one new species within the *B. nimala* lineage (*B. regina* Shearn *et al.*, 2012). The previous work of De Deckker (1981a,b, 1982) and De Deckker & McKenzie (1981) as well as these two new papers (Martens et al, 2012; Shearn et al., 2012) show that the genus Bennelongia has extensive radiations in both eastern and western Australia. The present paper formally describes seven new species within the *B. barangaroo* lineage, redescribes *B. dedeckkeri* and indicates the putative presence of several other species within the B. barangaroo lineage, all from WA. With the seven new species described here, the genus Bennelongia now comprises 25 nominal species (Table 3) but several more await formal description.

Morphological features

Once again, the size and shape, and in some cases the sheer presence or absence, of the antero-ventral lapel on the RV has proven to be indispensable to characterize species of *Bennelongia*. The plasticity of this feature within the *B. barangaroo* lineage is amazing and ranges from being fully absent through being small and triangular, to large and drop-like and to a large, elongated and heavily serrated structure. Whereas Martens et al. (2012) hypothesized that the function of the lapel is to lock the sulcus in the LV when valves need to be closed tightly (e.g., when attacked by predators, or in cases where habitat is rapidly desiccating), one could ask why such a wide range of morphologies of the lapel is necessary for highly similar functions in the different lineages and species. When homologous structures have widely different morphologies in closely related species, sexual selection is often invoked as causality. Several authors have indeed already mentioned the potential of sexual selection in ostracod radiations (for example Tsukagoshi 1988; Martens 2000). However, such structures must usually (1) display sexual dimorphism and be most common in males, (2) be accessible during pre-copulation by putative partners, i.e., females, to determine whether or not to accept the male as a partner for reproduction, and (3) occur only in the adult stage, i.e., after the final moult. Only the latter of these three conditions is fulfilled in Bennelongia because there is no apparent sexual dimorphism in lapel-shape and lapels are in general not easily available for inspection by females during the pre-copulatory stage. It is therefore unlikely that lapel morphology has evolved through sexual selection.

Table 3. Species presently described in *Bennelongia* and their distribution (species in bold are newly described here). Only certain distributions, based on type localities and documented range extensions, are given here. * indicates the type species.

Bennelongia australis (Brady, 1886): SA Bennelongia barangaroo De Deckker, 1981: WA Bennelongia bidgelangensis Martens et al., 2012: WA, Gascoyne Bennelongia calei sp. nov.: WA Bennelongia coondinerensis Martens et al., 2012: WA, Pilbara Bennelongia cuensis Martens et al., 2012: WA, Yilgarn Bennelongia cygnus Martens et al., 2012: WA, Swan Valley Bennelongia dedeckkeri Shearn et al., 2012: QLD, WA Bennelongia frumenta Martens et al., 2012: WA, Wheatbelt Bennelongia gnamma sp. nov.: WA Bennelongia gwelupensis Martens et al., 2012: WA, Perth, southwest coast *Bennelongia harpago De Deckker & McKenzie, 1981: QLD Bennelongia hirsuta sp. nov.: WA Bennelongia ivanae sp. nov.: WA Bennelongia kimberleyensis Martens et al., 2012: WA, Kimberley Bennelongia lata Martens et al., 2012: WA, Gascoyne-Murchinson region Bennelongia mckenziei Shearn et al., 2012: QLD Bennelongia mcraeae sp. nov.: WA Bennelongia nimala De Deckker, 1981: NT Bennelongia pinpi De Deckker, 1981: QLD Bennelongia regina Shearn et al., 2012: QLD Bennelongia scanloni sp. nov.: WA Bennelongia strellyensis Martens et al., 2012: WA, Pilbara Bennelongia timmsi sp. nov.: WA Bennelongia tunta De Deckker, 1982: QLD

This leaves the possibility that lapels have evolved by chance (not a very parsimonious solution) or that natural selection is acting on the evolution of this morphological feature and that selection pressures are quite stringent. The morphological differences between species living in similar environments (pools, lakes) can be either substantial, as is the case for *B. macraeae* sp. nov., *B. scanloni* sp. nov. and *B. calei* sp. nov., or almost non-existent as in the case of the rock pool dwelling species, which are either cryptic species without morphological differences or have very small differences (*B. timmsi* sp. nov. with 3 cryptic species and *B. gnamma* sp. nov.).

Bennelongia timmsi sp. nov. as a biological reality?

Recent research has shown that cryptic species are not uncommon in non-marine ostracods, as Shearn *et al.* (2012) found a genetically distinct eastern Australian lineage within the otherwise western Australian *B. cuensis* Martens *et al.*, 2012, and confirmed that both clades within this species are morphologically indistinguishable. Schön *et al.* (2012) found several cryptic species within putative ancient asexual darwinulid ostracods, while Bode *et al.* (2010) revealed no less than 40 cryptic species within the Palaearctic ostracod species *Eucypris virens* (Jurine, 1820).

In the *B. barangaroo* lineage, both *B. timmsi* sp. nov. and *B. scanloni* sp. nov. comprise cryptic species as identified by molecular phylogenies based on the mitochondrial COI gene (Fig. 2) and the 4 theta rule (Table 2). *Bennelongia scanloni* sp. nov. comprises three clusters and three unconnected genetic networks (Fig. 3) of which two are identified as separate genetic species by the 4 theta rule, and the two clusters together form a monophyletic clade within the tree. The smaller TST-clade is phylogenetically slightly separate from the E1 clade, forms an additional network, but does not constitute a separate genetic species. The situation in *B. scanloni* sp. nov. is thus a classic case of a monophyletic species consisting of diverged, but morphologically unrecognisable, clades.

In *B. timmsi* sp. nov., however, the situation is less straightforward. Five clades are recognised in the phylogenetic tree (Fig. 2 - A1-5) and there are six unconnected networks (Fig. 3), of which three are considered valid genetic species (A1, A3, A2+4+5). However, unlike the situation in *B. scanloni* sp. nov., *B. timmsi* sp. nov. does not appear as a monophyletic taxon in the phylogenetic analysis, because A1 and A3 cluster together in a different clade than A2+4+5. Extensive morphological comparisons (Figs 4-11) could not reveal any specific differences in valve or soft part morphology between the clusters (though see below), excluding the possibility that even clades A1+A3 on the one hand and clades A2+4+5 on the other could be described as different monophyletic species. We considered it of little use to describe two different species when they cannot be identified, except with molecular techniques.

Adding complexity to the phylogenetic uncertainty described above, the morphology of the anteroventral lapel on the RV shows some variability in length and position on the valve among specimens within the *B. timmsi* sp. nov. clades, but this limited variability could not be linked to the phylogenetic position of the specimens. While the shape of the terminal segment of the right prehensile palp was variable within what is assumed to be the same cryptic species (in clade A5), it differed little between cryptic species. Whether or not the differences observed in clade A5 specimens are real or artefacts remains to be seen. The morphologies of the hemipenis-outlines and of the left prehensile palp were fairly constant across all five clades and three cryptic species.

The situation in *B timmsi* sp. nov. is the first case within the revision of the genus *Bennelongia* where morphological and molecular data are incongruent. At this stage, we have chosen to follow the results of the morphological analyses, as it seems that, within the tree, the nodes indicated by an * (Fig. 2) are weakly or not statistically supported, and if those nodes are collapsed into a polytomy, the virtual polyphyletic position of *B. timmsi* sp. nov. would disappear. As soon as we have described and screened all new species of *Bennelongia* from our collections, the molecular phylogeny of the genus as a whole will be reconstructed and it is hoped that this more complete analysis will shed light on the presumed polyphyletic status of *B. timmsi* sp. nov. In the meantime, *Bennelongia timmsi* sp. nov. is proposed here as a valid biological species.

Distribution and Ecology

The continental-scale distribution of *Bennelongia* as a whole and phylogeography of selected species will be dealt with elsewhere. At this stage, however, it is useful to point out that almost all species in this genus have fairly restricted distributions: this appears to be so for the species within the *B. australis* and *B. cygnus* lineages (Martens *et al.* 2012), as well as in the *B. pinpi* and the *B. barangaroo* lineages (Martens *et al.* 2012). One notable exception appears to be *B. dedeckkeri*, which has meanwhile been reported from Queensland in eastern Australia (Shearn *et al.* 2012) and from both northern and central Western Australia (present paper - Fig. 1). Molecular screening has shown that species must have a very efficient means of dispersal compared to its congeners. So far, only parthenogenetic populations have been found in WA, which would be one way to explain a potentially recent expansion from the east (where sexual populations do appear to exist) to the west. Indeed, parthenogens are assumed to be more

efficient dispersers, as one egg is potentially enough to establish a viable population, whereas sexual groups must have both genders colonising the same habitat, and these moreover must find each other in a spatially and temporally diluted environment (Horne & Martens 1999). All screened specimens from WA had identical COI sequences (Fig. 3), and the apparent absence of genetic diversity between localities more than 1000 km apart supports this hypothesis of parthenogens.

Bennelongia dedeckkeri was recovered from both ephemeral lakes and pans (*e.g.*, the McNeil Claypan in Carnarvon), as well as semi-permanent lakes (e.g., the remote Lake Gregory, in the Tanami Desert, but see Halse *et al.* 1998), and thus seems to be able to thrive in different types of environments. The species might very well have a General Purpose Genotype (Van Doninck *et al.* 2003). The remainder of the species of the *B. barangaroo* lineage are distributed in more particular habitat types: *B. timmsi* sp. nov., *B. gnamma* sp. nov., *B. hirsuta* sp. nov. and *B. ivanae* sp. nov. occur only in rock pools, and this diversity within a single lineage confirms the rock pools on the granite outcrops in southwestern Australia as foci of diversification of aquatic animals (Pinder *et al.* 2000). *Bennelongia mcraeae* sp. nov. and *B. scanloni* sp. nov. were sampled in seasonal or episodic soft-sediment lakes and pools only. *Bennelongia calei* sp. nov. occurs mainly in the latter types of habitats, but was also found in at least two localities in pit-gnammas, which are deep and narrow rock pools. Populations from both types of habitats were also shown to belong to one and the same genetic species (Fig. 2), and this species thus has a puzzling autecology.

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References

Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403-410. <u>http://dx.doi.org/10.1016/S0022-2836(05)80360-2</u>

Birky Jr. C.W. 2011. Species detection and identification in sexual organisms using population genetic theory and DNA sequences. *PLoS ONE* 8: e52544. <u>http://dx.doi.org/10.1371/journal.pone.0052544</u>

Birky Jr. C.W. & Barraclough T.G. 2009. Asexual speciation. *In*: Schön I., Martens K. & van Dijk P. (eds) *Lost Sex*. Springer Scientific Publishers, Dordrecht: 201-216.

Birky Jr. C.W., Adams J., Gemmel M. & Perry J. 2010. Using Population Genetic Theory and DNA sequences for species detection and identification in asexual organisms. *PLoS ONE* 5: e10609. <u>http://</u><u>dx.doi.org/10.1371/journal.pone.0010609</u>

Birky Jr. C.W., Ricci C., Melone G. & Fontaneto D. 2011. Integrating DNA and morphological taxonomy to describe diversity in poorly studied microscopic animals: new species of the genus *Abrochtha* Bryce, 1910 (Rotifera: Bdelloidea: Philodinavidae). *Zoological Journal of the Linnean Society* 161: 723-734. http://dx.doi.org/10.1111/j.1096-3642.2010.00674.x

Bode S.N.S., Lamatsch D.K., Martins M.J.F., Schmit O., Vandekerkhove J., Mezquita F., Namiotko T., Rossetti G., Schön I., Butlin R.K. & Martens K. 2010. Exceptional cryptic diversity and multiple origins of parthenogenesis in a freshwater ostracod. *Molecular Phylogeny and Evolution* 54: 542-552. <u>http://dx.doi.org/10.1016/j.ympev.2009.08.022</u>

Broodbakker N.W. & Danielopol D.L. 1982. The chaetotaxy of Cypridacea (Crustacea, Ostracoda) limbs: proposals for a descriptive model. *Bijdragen tot de Dierkunde* 52: 103-120.

Cale D.J., Halse S.A. & Walker C.D. 2004. Wetland monitoring in the wheatbelt of south-west Western Australia: site descriptions, waterbird, aquatic invertebrate and groundwater data. *Conservation Science Western Australia* 5: 20-135.

Clement M., Posada D. & Crandall K. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1660. <u>http://dx.doi.org/10.1046/j.1365-294x.2000.01020.x</u>

Darriba D., Taboada G.L., Doallo R. & Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* 9: 772. <u>http://dx.doi.org/10.1038/nmeth.2109</u>

De Deckker P. 1981a. Taxonomy and ecology notes of some ostracods from Australian inland waters. *Transactions of the Royal Society of South Australia* 105 (3): 91-138.

De Deckker P. 1981b. Ostracoda from Australian inland waters – notes on taxonomy and ecology. *Transactions of the Royal Society of Victoria* 93 (1): 43-85.

De Deckker P. 1982. On *Bennelongia tunta* De Deckker sp. nov. A Stereo-Atlas of Ostracod Shells 9 (21): 117-124.

De Deckker P. & McKenzie K.G. 1981. *Bennelongia*, a new cyprididid ostracod genus from Australasia. *Transactions of the Royal Society of South Australia* 105 (2): 53-58.

De Deckker P. & Martens K. 2013. Extraordinary morphological changes in valve morphology during the ontogeny of several species of the Australian ostracod genus *Bennelongia* (Crustacea, Ostracoda). *European Journal of Taxonomy* 36: 1-37. <u>http://dx.doi.org/10.5852/ejt.2013.36</u>

Folmer O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Fontaneto D., Herniou E.A., Boschetti C., Caprioli M., Melone G., Ricci C. & Barraclough T.G. 2007. Independently evolving species in asexual bdelloid rotifers. *PLoS ONE* 5: e87. <u>http://dx.doi.org/10.1371/journal.pbio.0050087</u>

Fontaneto D., Kaya M., Herniou E.A. & Barraclough T.G. 2009. Extreme levels of hidden diversity in microscopic animals (Rotifera) revealed by DNA taxonomy. *Molecular Phylogenetics and Evolution* 53: 182-189. <u>http://dx.doi.org/10.1016/j.ympev.2009.04.011</u>

Guindon S. & Gascuel O. 2003. PhyML – a simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52: 696-704. <u>http://dx.doi.org/10.1080/10635150390235520</u>

Hall T. 2007. BioEdit: Biological sequence alignment editor for Win95/98/NT/2K/XP [Online]. Website last modified on June 27, 2007 (accessed on September 13, 2011). Available at <u>http://www.mbio.ncsu.edu/BioEdit/bioedit.html</u>

Halse S.A. 2002. Diversity of Ostracoda (Crustacea) in inland waters of Western Australia. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 28: 914-918.

Halse S.A., Shiel R.J. & Williams W.D. 1998. Aquatic invertebrates of Lake Gregory, north-western Australia, in relation to salinity and ionic composition. *Hydrobiologia* 381: 15-29. <u>http://dx.doi.org/10.1023/A:1003263105122</u>

Horne D.J. & Martens K. 1999. Geographical parthenogenesis in European non-marine ostracods: postglacial invasion or Holocene stability? *Hydrobiologia* 391: 1-7. <u>http://dx.doi.org/10.1023/</u> <u>A:1003508210166</u>

Horne D.J., Cohen A. & Martens K. 2002. Taxonomy, Morphology and Biology of Quaternary and Living Ostracoda. *In*: Holmes J.A. & Chivas A.R. (eds) *The Ostracoda: Application in Quaternary Research*. Geophysical Monograph 131. American Geophysical Union, Washington, DC: 5-36.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. & Higgins D.G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947-2948. <u>http://dx.doi.org/10.1093/bioinformatics/btm404</u>

Martens K. 1987. Homology and functional morphology of the sexual dimorphism in the antenna of *Sclerocypris* Sars, 1924 (Crustacea, Ostracoda, Megalocypridinae). *Bijdragen tot de Dierkunde* 57: 183-190.

Martens K. 2000. Factors affecting the divergence of mate recognition systems in the Limnocytherinae (Crustacea, Ostracoda). *In*: Horne D.J. & Martens K. (eds) Proceedings of the XIII International Symposium on Ostracoda. *Hydrobiologia* 419: 83-101. <u>http://dx.doi.org/10.1023/A:1003954513004</u>

Martens K., Halse S. & Schön I. 2012. Nine new species of *Bennelongia* De Deckker & McKenzie, 1981 (Crustacea, Ostracoda) from Western Australia, with the description of a new subfamily. *European Journal of Taxonomy* 8: 1-56. http://dx.doi.org/10.5852/ejt.2012.8

Pinder A.M., Halse S.A., Shiel R.J. & McRae J.M. 2000. Granite outcrop pools in south-western Australia: foci of diversification and refugia for aquatic invertebrates. *Journal of the Royal Society of Western Australia* 83: 149-161.

Ronquist F., Teslenko M., van der Mark P., Ayres D., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539-542. <u>http://dx.doi.org/10.1093/sysbio/sys029</u>

Schön I., Pinto R.L., Halse S.A., Smith A.J., Martens K. & Birky Jr. C.W. 2012. Cryptic species in putative ancient asexual darwinulids (Crustacea, Ostracoda). *PLOS ONE* 7: e39844. <u>http://dx.doi.org/10.1371/journal.pone.0039844</u>

Shearn R., Koenders A., Halse S., Schön I. & Martens K. 2012. A review of *Bennelongia* De Deckker & Mckenzie, 1981 (Crustacea, Ostracoda) species from eastern Australia, with the description of three new species. *European Journal of Taxonomy* 25: 1-35. <u>http://dx.doi.org/10.5852/ejt.2012.25</u>

MARTENS K., HALSE S. & SCHÖN I., The Bennelongia barangaroo lineage in Western Australia

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Likelihood, Distance, and Parsimony methods. *Molecular Biology and Evolution* 28: 2731-2739. <u>http://dx.doi.org/10.1093/molbev/msr121</u>

Timms B.V. 2002. The fairy shrimp *Branchinella* Sayce (Crustacea: Anostraca: Thamnocephalidae) in Western Australia, including a description of four new species. *Hydrobiologia* 486: 71-89. <u>http://dx.doi.org/10.1023/A:1021330230369</u>

Tsukagoshi A. 1988. Reproductive character displacement in the ostracod genus *Cythere*. *Journal of Crustacean Biology* 8: 563-575. <u>http://dx.doi.org/10.2307/1548693</u>

Van Doninck K., Schön I., Maes F., De Bruyn L. & Martens K. 2003. Ecological strategies in the ancient asexual animal group Darwinulidae (Crustacea, Ostracoda). *Freshwater Biology* 48: 1285-1294. <u>http://dx.doi.org/10.1046/j.1365-2427.2003.01078.x</u>

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