



Research article

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**A review of *Bennelongia* De Deckker & McKenzie, 1981
(Crustacea, Ostracoda) species from eastern Australia
with the description of three new species**

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Abstract. Australia is predicted to have a high number of currently undescribed ostracod taxa. The genus *Bennelongia* De Deckker & McKenzie, 1981 (Crustacea, Ostracoda) occurs in Australia and New Zealand, and has recently shown potential for high speciosity, after the description of nine new species from Western Australia. Here, we focus on *Bennelongia* from eastern Australia, with the objectives of exploring likely habitats for undiscovered species, genetically characterising published morphological species and scanning classical species for cryptic diversity. Two traditional (morphological) species are confirmed to be valid using molecular evidence (*B. harpago* De Deckker & McKenzie, 1981 and *B. pinpi* De Deckker, 1981), while three new species are described using both morphological and molecular evidence. Two of the new species belong to the *B. barangaroo* lineage (*B. dedeckkeri* sp. nov. and *B. mckenziei* sp. nov.), while the third is a member of the *B. nimala* lineage (*B. regina* sp. nov.). Another species was found to be genetically distinct, but is not formally described here owing to a lack of distinguishing morphological features from the existing species *B. cuensis* Martens *et al.*, 2012. Trends in diversity and radiation of the genus are discussed, as well as implications these results have for the conservation of temporary pool microfauna and our understanding of *Bennelongia*'s evolutionary origin.

Keywords. Evolution, cryptic species, biodiversity, Queensland, New South Wales.

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Introduction

Many regions of the earth have been flagged as having poorly documented diversity for inland aquatic crustaceans, such as the Nearctic and Neotropic Amphipoda (Väinölä *et al.* 2008) or the African and South American Ostracoda (Martens *et al.* 2008). For each case this can be due either to a low diversity in the region, or to the fact that the region is understudied. In the latter case, taxonomic explorations and scans for cryptic diversity tend to yield a high number of new taxa. Two of the most remarkable cryptic diversity case studies have come from crustaceans. Over 30 cryptic lineages of the amphipod *Hyaella azteca* (Saussure, 1858) were discovered in desert springs of the USA (Witt *et al.* 2006), while over 35 cryptic species were found in European (Bode *et al.* 2010) and Australian (Koenders *et al.* 2012) populations of the ostracod *Eucypris virens* Jurine, 1820.

With discoveries of cryptic diversity on the rise and a historically low sampling effort, Australia is a region predicted to have a high number of species awaiting description for several crustacean groups (Martens *et al.* 2008; Väinölä *et al.* 2008). Already a high number of copepod (T. Karanovic 2004), branchiopod (Timms 2012) and ostracod (I. Karanovic 2007) species have been found in previously unexplored habitats of Australia, while a high number of cryptic lineages have been identified in desert spring amphipods of inland Australia (Murphy *et al.* 2009) and freshwater shrimps of eastern Australia (Cook *et al.* 2006).

Bennelongia De Deckker & McKenzie, 1981, an ostracod genus occurring in Australia and New Zealand, has shown a similar potential for high speciosity. Recent molecular and morphological work in Western Australia (Martens *et al.* 2012) revealed that some traditional (morphological) species (De Deckker 1981) consisted of species complexes, while unexplored regions yielded several new (morphological) species.

Several *Bennelongia* species were originally described from eastern Australia (De Deckker 1981, 1982; De Deckker & McKenzie 1981). Since then, taxonomically important micro scale characters have been established for the genus (Martens *et al.* 2012). One of these (the anteroventral lapel on the right valve) has enabled the recognition of several visibly discernible types within one traditional eastern Australian species (see figures 7 and 9 in De Deckker 1981) which suggests that described species of this region may in fact consist of larger species complexes. Furthermore, eastern Australia has many habitats with high aquatic crustacean diversity (Timms & Sanders 2002) that have not yet been explored for *Bennelongia*.

The aims of the present study were to sample known and unexplored *Bennelongia* populations of eastern Australia, to test the validity of traditional (morphological) species using molecular techniques, discover new (morphological) species, and determine the potential presence of cryptic species within this genus.

Material and Methods

Sampling

In April 2011, several known *Bennelongia* localities and a range of other wetlands in east Australia, mostly from Queensland, were sampled for ostracods using a 250 µm sweep net. Habitats sampled within the wetlands included littoral vegetation and the sediment-water interface. Contents of the net were emptied into a picking tray and ostracods were picked live using 1 ml pipettes for 30 minutes to one hour. Picked specimens were preserved in 100% ethanol and stored at 4°C. Samples of one *Bennelongia* population from Bloodwood Station in New South Wales were donated by Brian Timms. Locations of the populations used in this study are shown in Fig. 1. All examined material including the type material for new species was deposited in the Queensland Museum, Australia (specimen numbers throughout text with 'W' prefix).

Morphological analysis

Valves were removed and stored dry in micropalaeontological slides. Soft parts were dissected on flat glass slides in Hydromatrix mounting medium, using two 1 μm diameter tip tungsten needles to tease individual appendages from the body of the animal, then sealed with a coverslip. Drawings of soft parts were made using a *camera lucida* on a compound microscope. Valves were illustrated by scanning electron microscopy on a Philips XL30 SEM. Post processing of all images was performed in GIMP v2.6.11 (Kimball *et al.* 1995).

Although all soft parts were examined, only the male hemipenes and prehensile palps were drawn for the species examined here. Other appendages were typical of the genus and illustration was not useful for identification.

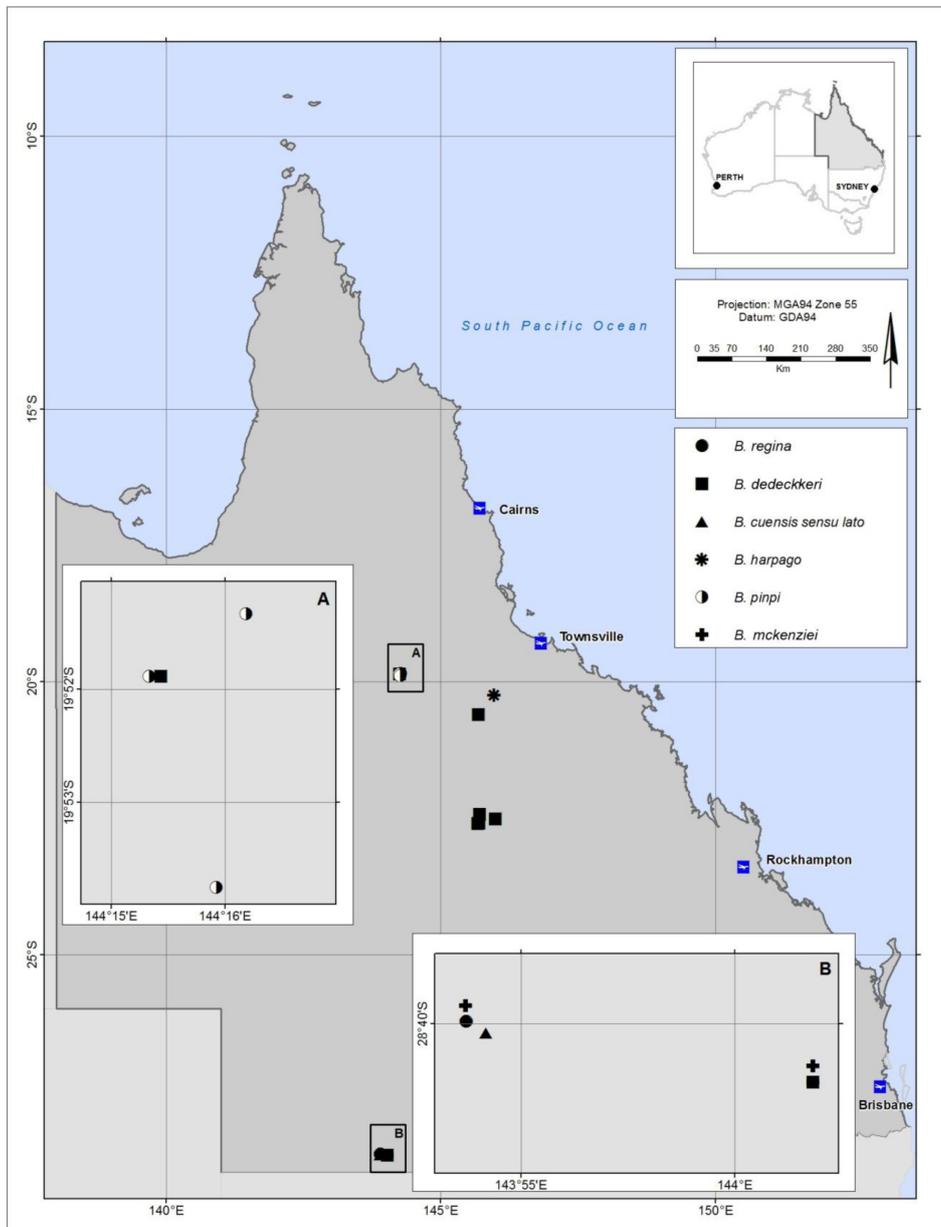


Fig. 1. Map of localities of *Bennelongia* species from eastern Australia recorded in the present study.

Molecular analysis

DNA was extracted from 57 ostracods belonging to 14 populations after their valves were removed and stored in micropalaeontological slides. Soft parts were placed into microcentrifuge tubes with 100% ethanol. The soft parts were rinsed three times in ultrapure water (100 μ l volumes for 10 minutes each rinse), and once in 100 μ l of 1 x PBS for 10 minutes.

The Qiagen DNeasy blood and tissue kit was used to extract DNA following the manufacturer's protocol. Concentrations of extracted DNA were estimated with a nanodrop spectrophotometer. The highest quality DNA eluate was used for PCR amplification of the 'Folmer' region of the mitochondrial COI gene using universal primers (Folmer *et al.* 1994), or where this was unsuccessful, alternative primers for mt COI. For PCR amplification, the Qiagen Hotstar mastermix was used, with 3-5 μ l of DNA template and 0.1 μ M of each primer in a total PCR volume of 25 μ l. Cycling was performed in a BioRad thermocyclers with an initial denaturation temperature of 95°C for 15 min, followed by 40 cycles with one minute at 95°C, one minute at 50°C, two minutes at 72°C and a final 10 minute extension step at 72°C. Success of PCR amplifications was verified by agarose gel electrophoresis and subsequent Gelred or SybrSafe staining. Successful PCR products were purified with the GFX™ PCR DNA and gel band purification kit (GE Healthcare) according to the manufacturer's protocol, then sequenced automatically and directly in both directions with the PCR primers and the Big dye kit (ABI) on an ABI 3130X.

Sequence data analysis

Sequence chromatograms were viewed in FinchTV (Geospiza Inc., n.d.) and, when present, discrepancies were corrected by manually checking sequence chromatograms. The identity of the obtained sequences was confirmed in Genbank using BLAST searches (Altschul *et al.* 1990).

One final consensus sequence per individual was obtained. These sequences were aligned and trimmed to equal length in ClustalX (Larkin *et al.* 2007), together with 26 sequences from already described *Bennelongia* species and one outgroup sequence from the closely related genus *Heterocypris* (identified throughout the text as *Heterocypris* spec.). jModelTest (Posada 2008) was used to identify the optimal model of molecular sequence evolution. Phylogenetic reconstructions were conducted with two different methods, Bayesian Inference (BI) was applied using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; with 2,000,000 generations, a burnin of 10,000 and the Bayesian Information Criterion identified by jModeltest for 24 models), and Maximum Likelihood (ML) in PhyML (Guindon & Gascuel 2003; with 1,000 bootstrap replicates and the Akaike Information Criterion identified by jModeltest for 88 models). Genetic relationships within and between populations were resolved in parsimonious networks constructed at the 95% probability limit using TCS 1.21 (Clement *et al.* 2000). Unconnected networks were considered as different genetic species as suggested by Hart & Sunday (2007). DNA sequences for each species have been submitted to Genbank (accession numbers JX896642-JX896647).

DNA identification of species

The Evolutionary Genetic species concept (Birky & Barraclough 2009) was applied using the 4 x rule (Birky *et al.* 2010) to confirm morphological species boundaries and to detect cryptic species. The 4 x rule was applied as described by Birky *et al.* (2010) and Schön *et al.* (2012) by first identifying well-supported, phylogenetic sister clades and then estimating mean sequence diversities within and pairwise distances between clades with MEGA 5.0 using 1,000 bootstraps (Tamura *et al.* 2011).

The mean pairwise distance D between sister clades was compared to the population estimate of clade nucleotide diversity θ . Where mean pairwise distances between sister clades D were at least 4 to 4.3 times (depending on the sample size of the clades) greater than the population estimate of clade nucleotide diversity θ within these clades, this was considered as evidence that the clades were

different species according to the Evolutionary Genetic species concept (Birky & Barraclough 2009). Correction of pairwise distances D for multiple hits K was not required, as $D: \theta$ ratios were always well over 4. Where sister clades were poorly defined in the tree, D values were first estimated to all other potential sister clades in the polytomy, then the clade of lowest D away was considered the sister clade. Although previous analyses may not have clustered these pairs as sister taxa, for the sake of simplicity and readability, clades that have been identified as having the lowest D away will be referred to as ‘sister taxa’ throughout. Also, in cases where areas of the tree are poorly resolved, it is possible to have many different clades identify a single clade as the shortest pairwise distance D away.

Abbreviations used in text and figures

BI	=	Bayesian Inference
Cp	=	Carapace
CpRI	=	Carapace right lateral view
EtOH	=	Ethanol
H	=	Height of valves
L	=	Length of valves
Lpp	=	Left prehensile palp
ls	=	Lateral shield of hemipenis
LV	=	Left valve
LVi	=	Left valve internal view
ML	=	Maximum Likelihood
ms	=	Medial shield of hemipenis
Rpp	=	Right prehensile palp
RV	=	Right valve
RVi	=	Right valve internal view
W	=	width of valves
AK	=	Annette Koenders
KM	=	Koen Martens
RS	=	Rylan Shearn
NSW	=	New South Wales
QLD	=	Queensland
WA	=	Western Australia

Results

Results of molecular screening

57 COI sequences of 531bp were obtained from 14 *Bennelongia* populations throughout Queensland and New South Wales. BLAST searches identified the COI sequences as closely related to other non-marine ostracods. jModeltest selected TPM1uf+I+G (Kimura 1981) as most appropriate for ML analyses with the following parameters: freqA = 0.3142, freqC = 0.1855, freqG = 0.1122, freqT = 0.3881; [AC] = 1.0000, [AG] = 691.9286, [AT] = 95.0445, [CG] = 95.0445, [CT] = 691.9286, [GT] = 1.0000, p-inv = 0.6140 and gamma shape = 2.4420. The HKY+I+G model (Hasegawa *et al.* 1985) was chosen as the most appropriate amongst 24 models that could be employed in BI with p-inv = 0.6150 and gamma shape = 2.0080.

In both phylogenies, the outgroup (*Heterocypris spec.*) clustered outside of the *Bennelongia* lineage (Fig. 2). Both phylogenetic trees contained 12 different *Bennelongia* clusters with high posterior probabilities and bootstrap support (Fig. 2). Six of these clusters represented *Bennelongia* species with published COI sequences, namely *B. gwelupensis*, *B. coondinerensis*, *B. lata*, *B. cuensis*, *B. strellyensis*

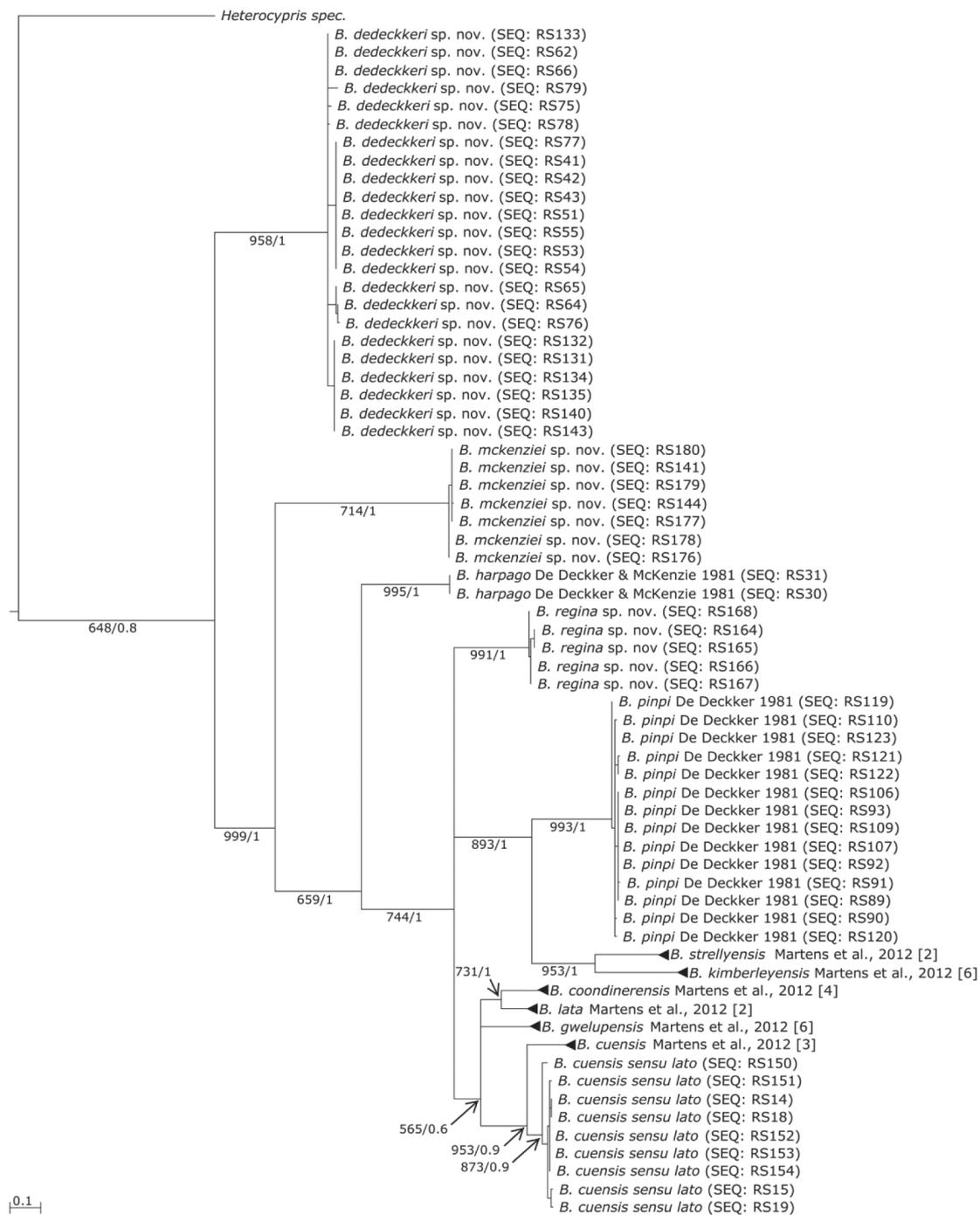


Fig. 2. Phylogenetic tree constructed with 57 novel COI sequences of *Bennelongia*, 26 published *Bennelongia* sequences and one *Heterocypris spec.* as outgroup (sequence names are given in brackets at the end of species names). This tree represents two trees of identical topology inferred by ML and BI. Bootstrap values (for 1000 bootstrap replicates) from ML analyses and Bayesian posterior probabilities (ranging from 0 to 1) are shown for each node (in the format: ‘Bootstrap Support/Posterior Probability’). Branch lengths are proportional to the genetic distance scale at the bottom left. Clades with published sequences have been collapsed; the number of sequences in these clades is included in brackets after the species name. Nodes with less than 50% bootstrap support and a posterior probability of less than 0.5 have been collapsed. The tree shows six strongly supported clades that correspond to the species presented in this study.

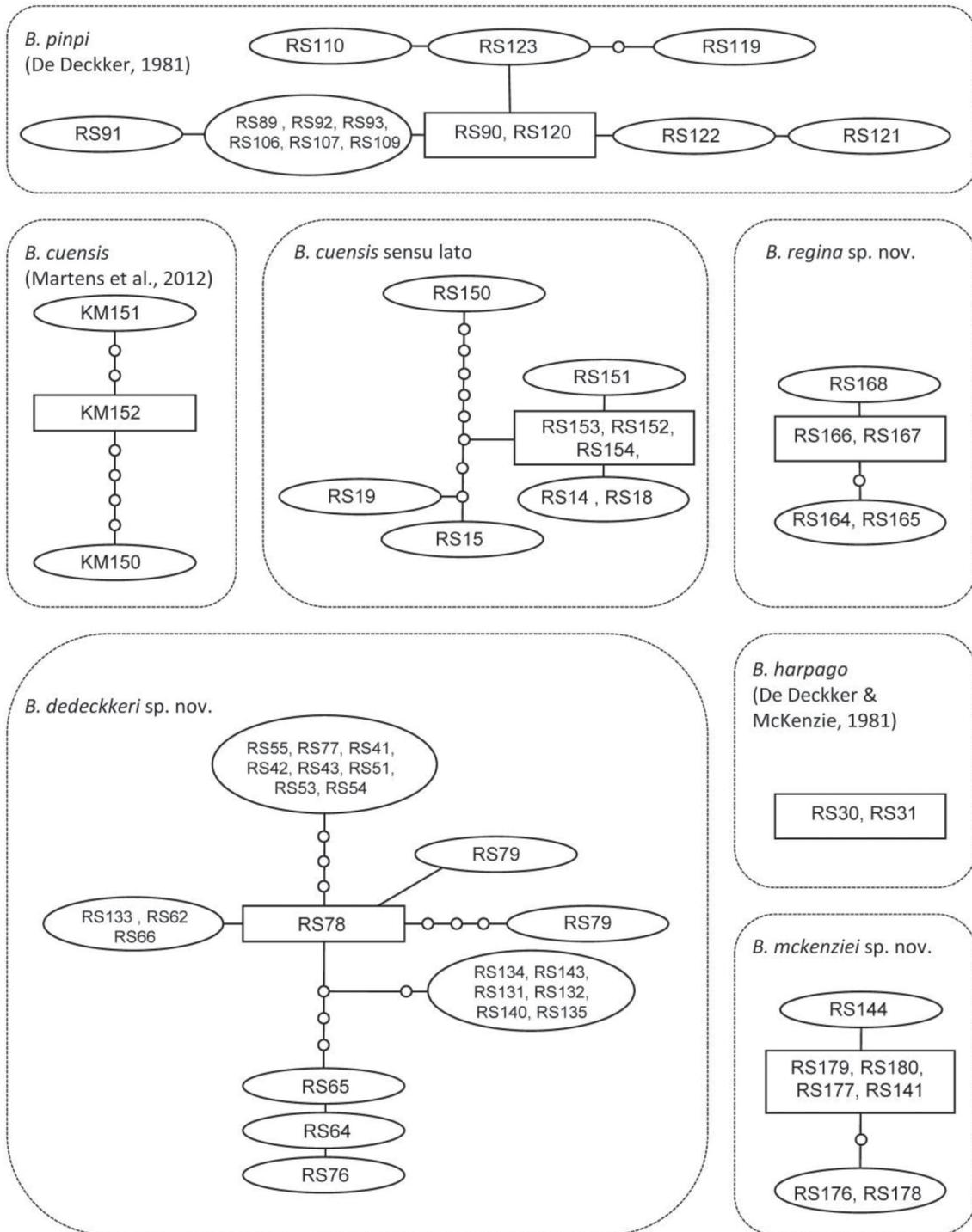


Fig. 3. Parsimonious network constructed at the 95% probability limit with COI sequences from *Bennelongia* specimens. For each haplotype, sequence names are given. Squares indicate ancestral haplotypes, small circles missing haplotypes and dotted lines connection boundaries. Only seven of the 12 unconnected networks are shown here, the remaining five were clearly separated from the sequences investigated here and corresponded to the published networks in Martens *et al.* (2012). Six of the unconnected networks shown here are congruent with the newly described species. One network with previously published sequences (*B. cuensis* Martens *et al.*, 2012) was included to illustrate its separation to the cryptic species *B. cuensis sensu lato*.

and *B. kimberleyensis* (all from Martens *et al.* 2012, see Fig. 2). Specimens identified in the current study as *B. pinpi* De Deckker, 1981, *B. harpago* De Deckker & McKenzie, 1981 and *B. cuensis sensu lato* formed their own distinct phylogenetic clusters, as did *B. dedeckkeri* sp. nov., *B. mckenziei* sp. nov. and *B. regina* sp. nov. described here as new species (Fig. 2). *Bennelongia pinpi* formed one larger phylogenetic clade together with *B. kimberleyensis* Martens *et al.*, 2012 and *B. strellyensis* Martens *et al.*, 2012 (the *B. pinpi* lineage) while *B. cuensis sensu lato* grouped with *B. cuensis* Martens *et al.*, 2012 (Fig. 2). The *Bennelongia regina* sp. nov. clade (of the *B. nimala* lineage) was formed outside of the *B. pinpi* lineage cluster and the cluster representative of the *B. australis* lineage (*B. coondinerensis* Martens *et al.*, 2012, *B. lata* Martens *et al.*, 2012, *B. gwelupensis* Martens *et al.*, 2012 and *B. cuensis* Martens *et al.*, 2012). Although the phylogenetic relationships at the higher taxonomic level between the *B. pinpi*, *B. australis* and *B. nimala* lineages could not be resolved, they still belong to the same, distinctive phylogenetic clade with high support (Fig. 2). On the other hand, *Bennelongia dedeckkeri* sp. nov., *B. mckenziei* sp. nov. and *B. harpago* De Deckker & McKenzie, 1981 formed their own distinct clusters (Fig. 2).

Twelve unconnected parsimonious networks were obtained (Fig. 3), although five of these are not shown as they corresponded to the published networks of Martens *et al.* (2012). One corresponded to the published network of *B. cuensis* (Martens *et al.* 2012) and is shown, as it formed a separate network to the morphologically similar *B. cuensis sensu lato* sp. nov. The two specimens of *B. harpago* De Deckker & McKenzie, 1981 formed one separate network, as did all specimens of *B. pinpi* De Deckker, 1981 and the three novel species described below, *B. regina* sp. nov., *B. dedeckkeri* sp. nov., and *B. mckenziei* sp. nov., respectively.

Eight different haplotypes were found in the 14 specimens of *B. pinpi* De Deckker, 1981 (Fig. 3) while the two analysed *B. harpago* De Deckker & McKenzie, 1981 specimens belonged to one haplotype. *Bennelongia regina* sp. nov. and *B. mckenziei* sp. nov. were represented by three closely related haplotypes from the five and seven individuals, respectively, that had been investigated. *Bennelongia dedeckkeri* sp. nov. was the most sampled group with 23 specimens, and also showed the most haplotypes. Of the ten haplotypes, two were especially common. The cryptic *B. cuensis sensu lato* was shown as unconnected to *B. cuensis* Martens *et al.*, 2012. In the nine sequences of *B. cuensis sensu lato*, one distant and five closely related haplotypes were found.

For the 4 x rule, the *B. cuensis sensu lato* clade and *B. cuensis* Martens *et al.*, 2012 clade were identified as sister clades of one another based on the tree topology in Fig. 2. The sister clades for all other novel genetic clades were poorly defined in the tree topology in Fig. 2, so the following sister clades were identified after searching for the lowest *D* and *K* to other clades in the polytomy: *B. dedeckkeri* sp. nov. – *B. regina* sp. nov., *B. mckenziei* sp. nov. – *B. harpago* De Deckker & McKenzie, 1981, *B. regina* sp. nov. – *B. gwelupensis* Martens *et al.*, 2012, *B. pinpi* De Deckker, 1981 – *B. strellyensis* Martens *et al.*, 2012 and *B. harpago* De Deckker & McKenzie, 1981 – *B. regina* sp. nov. For all pairs of sister clades, mean pairwise distances *D* between them were more than four times higher than the population estimate for clade nucleotide diversity θ within these clades (Table 1) and ranged from 5 (*B. cuensis sensu lato* - *B. cuensis* Martens *et al.*, 2012) to 57.3 times higher (*B. mckenziei* sp. nov. – *B. harpago* De Deckker & McKenzie, 1981).

Table 1. Results from calculations for the 4x rule.

Following the procedure of Birky *et al.* (2010), ratios of D to θ are shown for phylogenetic sister clades or the two clades with the lowest mean pairwise distance. In order to fulfil the criteria for the Evolutionary Genetic species concept, this ratio must be equal to or greater than 4.

n_1 = number of sequences in first sister clade; n_2 = number of sequences in second sister clade; Max. θ = maximum population nucleotide diversity of the two clades; D = mean pairwise distance between each sister clade pair.

Sister clades	($n_1 n_2$)	Max. θ	D	$D : \theta$
<i>B. dedeckkeri</i> sp. nov. – <i>B. regina</i> sp. nov.	(23 5)	0.009	0.140	15.0:1 ($D > 49$)
<i>B. mckenziei</i> sp. nov. – <i>B. harpago</i>	(7 2)	0.003	0.159	57.3:1 ($D > 49$)
<i>B. regina</i> sp. nov. – <i>B. gwelupensis</i>	(5 6)	0.005	0.090	17.3:1 ($D > 49$)
<i>B. pinpi</i> – <i>B. strellyensis</i>	(14 5)	0.004	0.140	35:1 ($D > 46$)
<i>B. harpago</i> – <i>B. regina</i> sp. nov.	(2 5)	0.004	0.128	33.3:1 ($D > 49$)
<i>B. cuensis sensu lato</i> – <i>B. cuensis</i>	(9 3)	0.011	0.061	5.4:1 ($D > 46$)

Taxonomic descriptions

Class Ostracoda Latreille, 1806
 Subclass Podocopa G. W. Müller, 1894
 Order Podocopida G.O. Sars, 1866
 Suborder Cypridocopina Baird, 1845
 Superfamily Cypridoidea Baird, 1845
 Family Cyprididae Baird, 1845
 Subfamily Bennelongiinae Martens, Halse & Schön, 2012
 Genus *Bennelongia* De Deckker & McKenzie, 1981

Bennelongia barangaroo lineage

Remarks

Bennelongia barangaroo De Deckker, 1981 was originally described from Cauckingburra Swamp (near Lake Buchanan, QLD) and was diagnosed by valve and hemipenes structure (De Deckker 1981). The description included illustrations of specimens from other populations, additional to the type locality, one of which was from a creek bed near Cue in Western Australia (De Deckker 1981). The SEM plates show the type specimen with an obvious anteroventral lapel on the RV, and the specimen from Cue with a reduced or non-existent anteroventral lapel (De Deckker 1981). More recently, a revision of the Western Australian species of the genus *Bennelongia* established this lapel as an important morphological character at the species level (Martens *et al.* 2012). Thus, it has become apparent that the population from Cue (De Deckker 1981) was likely to be a different species from the true *B. barangaroo* of Cauckingburra Swamp.

Although this finding regarding the specimens from Cue did not change the specific status of *B. barangaroo*, it did bring into question whether the other populations identified in the original description were actually *B. barangaroo*, or whether they were part of a more diverse species complex. Here further evidence is provided for this *B. barangaroo* species complex through the description of two new species within the lineage, both of which are clearly different from *B. barangaroo* and the specimen from Cue illustrated in De Deckker (1981).

The *Bennelongia barangaroo* lineage consists of three species, *B. barangaroo* De Deckker, 1981, *B. dedeckkeri* sp. nov. and *B. mckenziei* sp. nov. They are characterized by a small carapace ($L \approx 1000 \mu\text{m}$, the smallest of all described *Bennelongia* species). They can be distinguished further from the *B. cygnus* and *B. tunta* lineages by the rounded dorsal margin of the Cp (triangular in *B. cygnus*, rectangular in *B. tunta*), from the *B. australis* lineage by the less pronounced anterior rostrum in dorsal view, from the *B. pinpi* lineage by the less pronounced beak structure of the LV, from the *B. nimala* lineage by the small anteroventral overlap of LV over RV (large overlap in *B. nimala*) and less elaborate external valve ornamentation and from the *B. harpago* lineage by the absence of an anterodorsal hump-like thickening of the LV.

Bennelongia dedeckkeri sp. nov.

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Figs 4-5

Diagnosis

Cp relatively small ($L \approx 1000 \mu\text{m}$), with similar size and appearance between sexes; in dorsal view with reduced anterior rostrum, blunt posterior and reduced anteroventral overlap of LV over RV; in lateral view with rounded dorsal margin; LV with less developed anteroventral beak-like structure, rounded dorsal margin; RV with straight posterodorsal margin (hinge), greatest height slightly anterior of the middle, short and ventrally pointed anteroventral lapel present; hemipenes with ls protruding well beyond ms, tips of ls and ms separated, ls distally pointed; distal Rpp segment with pointed dorsal margin, proximal segment with rounded ventral margin and nearly straight dorsal margin; Lpp proximal segment elongated with subapical sensory organ, distal segment sickle shaped, shorter than proximal segment and sharply curved over 180° .

Etymology

The species is named after Dr P. De Deckker, who described the *Bennelongia* genus with Dr K. McKenzie in 1981.

Type material

Holotype

♂ (W40075), with soft parts dissected in a sealed slide, RV and LV stored dry in a micropalaeontological slide.

Allotype

♀ (W40018), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide.

Paratypes

Three ♂ carapaces stored in a micropalaeontological slide (W40021-W40023), 3 ♀ carapaces stored in a micropalaeontological slide (W40024-W40026), 2 ♂♂ with soft parts dissected in a sealed slide and valves stored in a micropalaeontological slide (W40016-W40017), ♀ LV stored in a micropalaeontological slide (W40020) and ♀ RV stored in a micropalaeontological slide (W40019).

Several *in toto* specimens in EtOH (W40076).

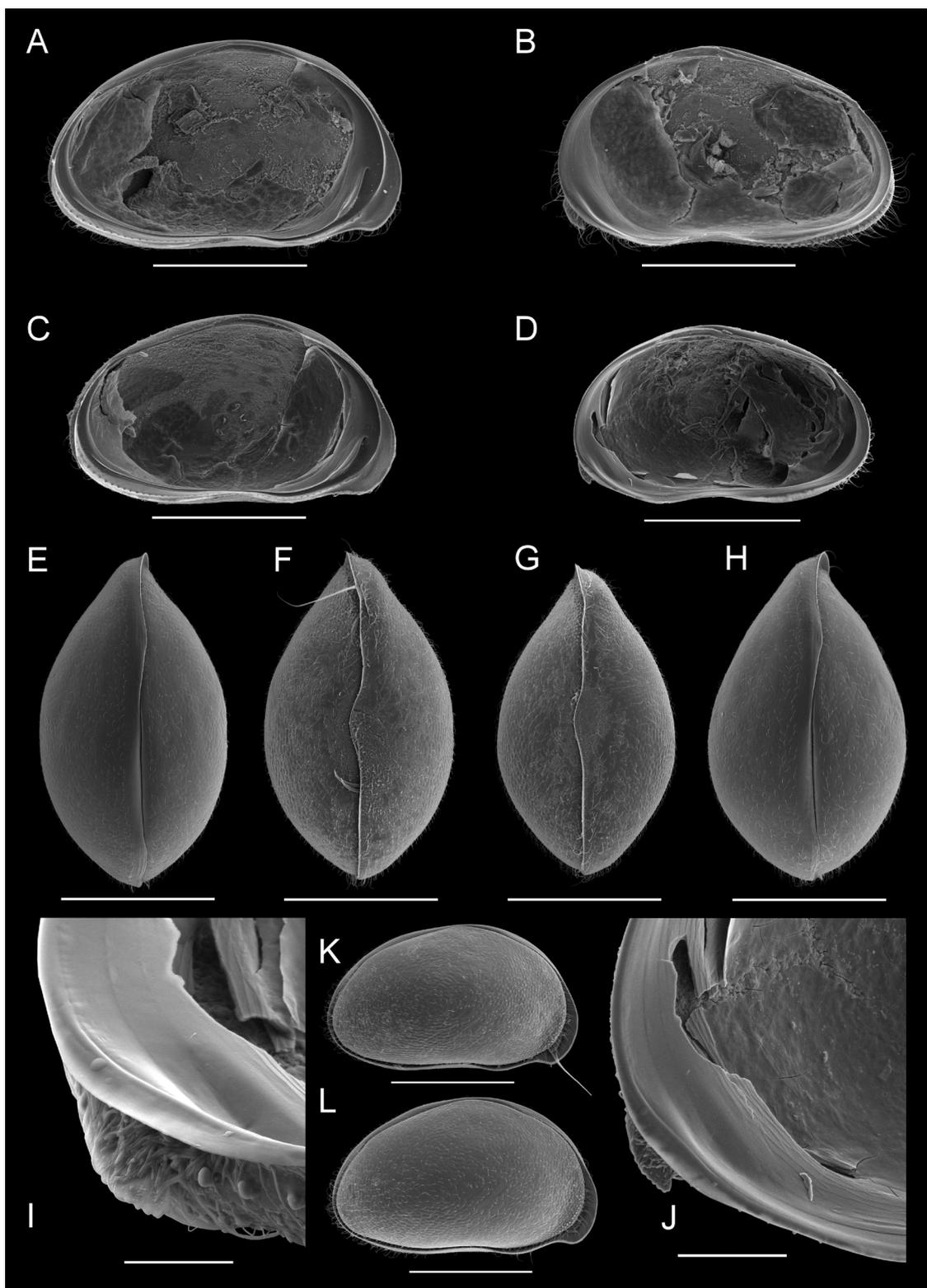


Fig. 4. *Bennelongia dedeckkeri* sp. nov., all from Lake Galilee, QLD (Type locality). **A.** ♀, LVi (W40020). **B.** ♀, RVi (W40019). **C.** ♂, LVi (W40016). **D.** Holotype ♂, RVi (W40075). **E.** ♀, Cp dorsal (W40025). **F.** ♀, Cp ventral (W40026). **G.** ♂, Cp (ventral W40023). **H.** ♂, Cp dorsal (W40022). **I.** Holotype ♂, RVi detail anteriorly (W40075). **J.** ♂, RVi detail anteriorly (W40019). **K.** ♂, CpR1 (W40021). **L.** ♀, CpR1 (W40024). Scales: A-H, K-L = 500 µm, I-J = 100 µm.

Type locality

Lake Galilee, south bank, approximately 70 km north-east of Aramac QLD, coordinates: 22°25'55.4" S - 145°42'27.2" E. Material used for the present description was collected on 07 Jun. 2011 by RS and AK (Sample QLDP9A).

Measurements (in μm)

♂: RV: L = 985-1007 ($n=2$), H = 572-597 ($n=2$). LV: L = 1053-1067 ($n=2$), H = 604-625 ($n=2$). Cp: L = 1010-1082 ($n=4$), W = 581-644, H = 600-603. ($n=2$).

♀: RV: L = 1099-1150 ($n=5$), H = 663-706 ($n=5$). LV: L = 1157-1222 ($n=5$), H = 685-738 ($n=5$). Cp: L = 1078-1224 ($n=9$), W = 613-738 ($n=4$), H = 724-733 ($n=4$).

Other material investigated

Dam near Lake Dunn, north east of Aramac QLD collected by RS and AK on 7 Jun. 2011, coordinates: 22°36'15.7" S - 145°40'21.3" E (Sample QLDP10A).

Stagnant Channel, near Lake Dunn north east of Aramac QLD collected by RS and AK on 7 Jun. 2011, no ♂♂ were found, coordinates: 22°35'42.3" S - 145°41'58" E (Sample QLDP8A).

Flooded field, near Lake Dunn north east of Aramac QLD collected by RS and AK on 6 Jun. 2011, no ♂♂ were found, coordinates: 20°36'10.3" S - 145°41'14" E (Sample QLDP6A).

Pelican Lake, north of Hughenden QLD collected by RS and AK on 9 Jun. 2011, no ♂♂ were found, coordinates: 19°51'53.1" S - 144°15'20" E (Sample QLDP13B).

Cardenyabba Lagoon, Kilcowera Station, south of Thargomindah QLD collected by AK on 14 Jun. 2011, no ♂♂ were found, coordinates: 28°40'13.2" S - 143°54'10.4" E (Sample QLDP18A).

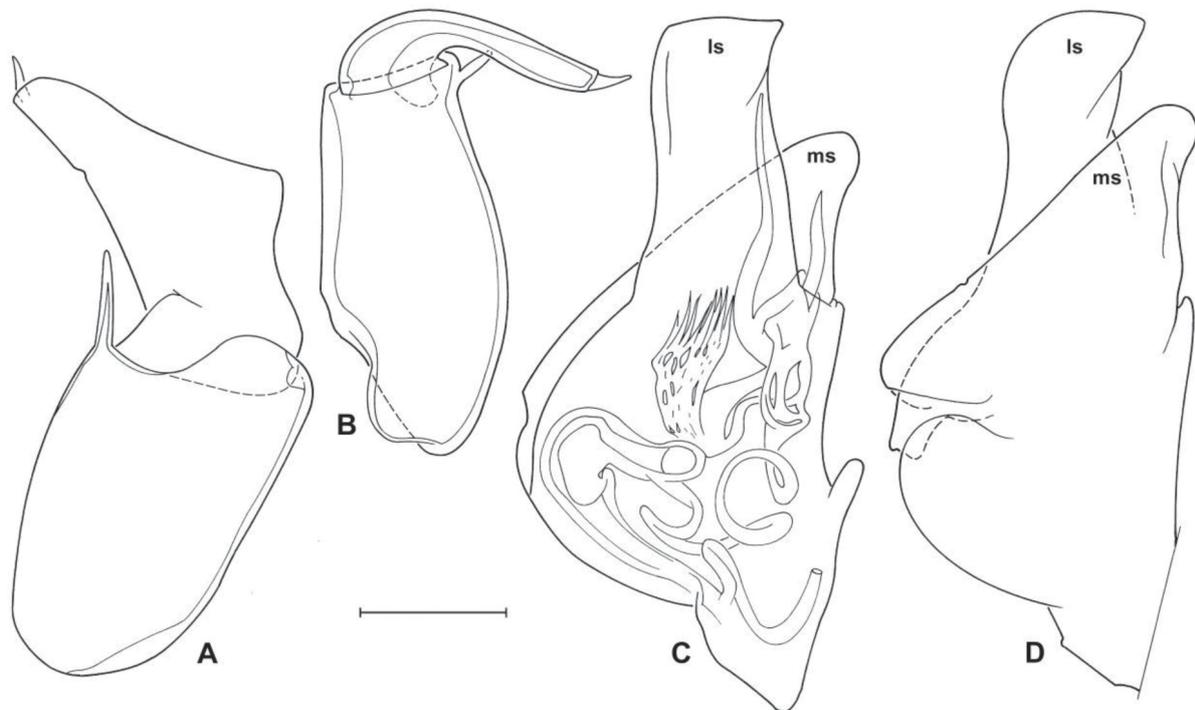


Fig. 5. *Bennelongia dedeckkeri* sp. nov. (A = W40017 & B-D = W40016 ♂). **A.** Right prehensile palp. **B.** Left prehensile palp. **C.** Hemipenis showing inner anatomy. **D.** Outline of other hemipenis. Scales: A-B = 30.9 μm , C-D = 89.3 μm .

These populations were the same Evolutionary Genetic species (Birky & Barraclough 2009) as the type population, which confirmed morphological evidence that they belong to the same species as the type population.

Differential diagnosis

Bennelongia dedeckkeri sp. nov. belongs to the *B. barangaroo* lineage, because of its relatively small carapace size ($L \approx 1000 \mu\text{m}$), rounded dorsal margin, less pronounced anterior rostrum in dorsal view, less pronounced beak structure of the LV and reduced anteroventral overlapping of LV over RV. It can be distinguished from the other species in this lineage (*B. barangaroo* De Deckker, 1981 and *B. mckenziei* sp. nov.) by the pointed dorsal margin of the second Rpp segment (more rounded in *B. barangaroo*), the straight posterodorsal margin (hinge) of the RV (more rounded in *B. barangaroo* and *B. mckenziei* sp. nov.), the beak point of the carapace being positioned well above the ventral plane (positioned on the ventral plane in *B. barangaroo*) and the presence of an anteroventral and ventrally-pointed lapel on the RV (absent in *B. mckenziei* sp. nov.).

Additional description

Male and female Cp (Fig. 4E-H, K-L) similar in size and appearance with blunt anterior rostrum and blunt posterior side; in lateral view with rounded dorsal margin, LV overlapping RV on all sides, overlap narrow at middle of dorsal margin, greatest height slightly anterior of the middle; external valve surface hirsute with setae approximately $50 \mu\text{m}$ in length.

Male and female LV (Fig. 4A, C) with rounded dorsal margin, greatest height situated slightly in front of the middle; anteroventral beak-like expansion small and blunt, point of beak well above ventral plane; ventral margin straight for approximately half its length; anterior inner list reaching well beyond the middle of the valve.

Male and female RV (Fig. 4B, D) with rounded dorsal margin anteriorly but straight posteriorly from the middle onwards (dorsal hinge); greatest height slightly anterior of the middle, outer list of RV with ventral row of tubercles; posterior list submarginal, but merging with selvage at beginning of straight dorsal margin (hinge); selvage at height of lapel expanded, lapel (Fig. 4I, J) broad, but running along short length of selvage with light serration sometimes visible.

Most appendages as typical of the genus and without special features.

First segment of Rpp (Fig. 5A) with rounded ventral margin and nearly straight dorsal margin, elongated, approximately 2 x as long as central width, apically with one long slender sensory organ; second palp segment with margins diverging distally, distally pointed with sensory organ and also pointed on dorsal margin.

First segment of Lpp (Fig. 5B) with rounded ventral margin and nearly straight dorsal margin, narrowing distally; elongated, approximately 2 x central width; sub-apically with sensory organ; second palp segment sickle shaped, approximately 0.75 x as long as first segment and sharply curved asymmetrically over 180° , with distal sensory organ.

Hemipenes (Fig. 5C-D) with symmetrical outlines, ls protruding well beyond ms, tips of ls and ms well separated, ls distally pointed and ms with pointed ventral lobe.

Ecology and distribution

This species has been found in both permanent and ephemeral wetlands, occurring sympatrically with *B. pinpi* in Pelican Lake QLD and *B. mckenziei* sp. nov. in Cardenyabba Lagoon QLD (both species are

similar in appearance). Males have been found in only some of these populations, suggesting that the others may be parthenogenetic, particularly in those localities in which other species of *Bennelongia* occur (*B. pinpi* in Pelican Lake and *B. mckenziei* in Cardenyabba Lagoon).

Bennelongia mckenziei sp. nov.

urn:lsid:zoobank.org:act:321592CE-0627-4962-96CB-F1C7D26F6D8A

Fig. 6

Diagnosis

Cp small ($L \approx 1000 \mu\text{m}$), in dorsal view with reduced anterior rostrum, blunt posterior and reduced anteroventral overlap of LV over RV; in lateral view with rounded dorsal margin, no visible anteroventral lapel and greatest height at the middle; LV with greatest height at the middle, anteroventral beak-like expansion not pointed, instead expressed as a linear horizontal extension of the LV ventral margin; RV with rounded margin near posterodorsal hinge, greatest height slightly anterior to the middle, anteroventral area without lapel, but with an expansion of the selvage.

Males unknown.

Etymology

This species is named after Dr K. McKenzie, who described the *Bennelongia* genus together with Dr P. De Deckker in 1981.

Type material

Holotype

♀ (W40071), with soft parts dissected in a sealed slide, RV and LV stored dry in a micropalaeontological slide.

Paratypes

♀ (W40068) with soft parts dissected in a sealed slide, two ♀ carapaces stored in separate micropalaeontological slides (W40072-W40073), ♀ LV (W40069) and ♀ RV (W40070) stored dry in a micropalaeontological slide.

Type locality

Horseshoe Lagoon, Kilcowera Station, south of Thargomindah QLD, coordinates: 22°25'55.4" S - 145°42'27.2" E. Material used for the present description was collected on 16 Jun. 2011 by AK (Sample QLDP21C).

Measurements (in μm)

♀: RV: L = 1082, H = 660. LV: L = 1078, H = 626. Cp: L = 1008-1060 ($n=2$), W = 618-647 ($n=2$), H = 622.

Other material investigated

Cardenyabba Lagoon, Kilcowera Station, south of Thargomindah QLD, collected by AK on 14 Jun. 2011, coordinates: 28°41'23.2" S - 143°56'35" E. This population was the same Evolutionary Genetic species (Birky & Barraclough 2009) as the type population, which confirmed morphological evidence that they belong to the same species as the type population.

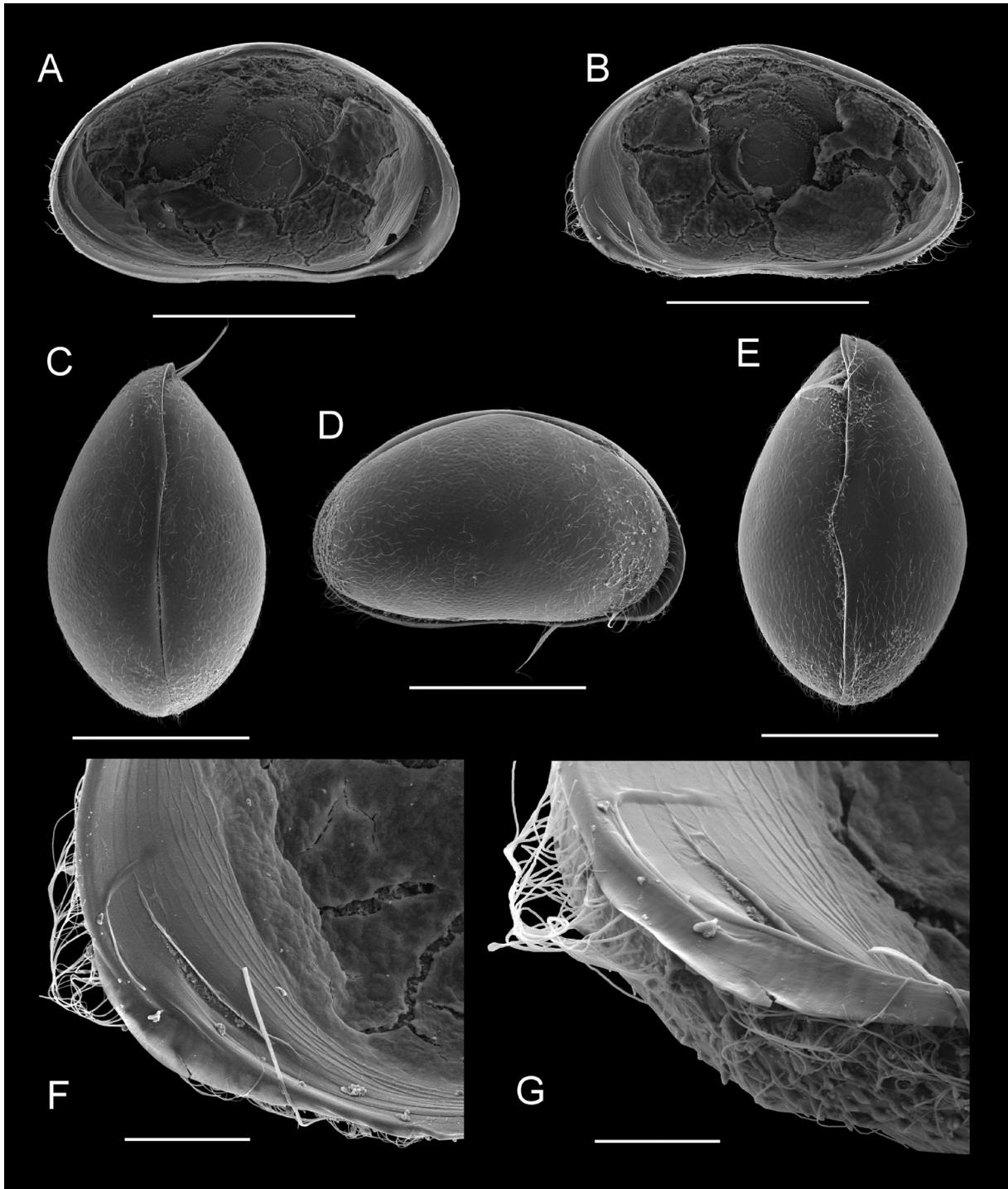


Fig. 6. *Bennelongia mckenziei* sp. nov., all from Horseshoe Lagoon, QLD (Type locality). **A.** Holotype ♀, LVi (W40071). **B.** Holotype ♀, RVi (W40071). **C.** ♀, Cp dorsal (W40072). **D.** ♀, CpRI (W40073). **E.** Holotype ♀, Cp ventral (W40071). **F.** Holotype ♀, RVi detail anteriorly (W40071). **G.** Holotype ♀, RVi detail anteriorly (W40071). Scales: A-E = 500 µm, F-G = 100 µm.

Differential diagnosis

Bennelongia mckenziei sp.nov. belongs to the *B. barangaroo* lineage, because of its relatively small carapace size ($L \approx 1000 \mu\text{m}$), rounded dorsal margin, less pronounced anterior rostrum in dorsal view, less pronounced beak structure of the LV and reduced anteroventral overlapping of LV over RV. It can be distinguished from the other species in this lineage (*B. dedeckkeri* sp. nov. and *B. barangaroo* De Deckker, 1981) by the fact that the greatest height of the Cp is at the middle (slightly anterior in *B. dedeckkeri* sp. nov.), the rounded posterodorsal margin of the RV hinge (straight in *B. dedeckkeri* sp. nov.), the absent anteroventral lapel of the RV (present in *B. barangaroo* and *B. dedeckkeri* sp. nov.), the rounded anteroventral margin of the LV (pointed in *B. dedeckkeri* sp. nov. and *B. barangaroo*), and the straight ventral margin of the LV extending to the distal point of the beak (sinuous ventral margin and more pronounced anteroventral beak structure in *B. dedeckkeri* sp. nov. and *B. barangaroo*). As no males are known for this species, we cannot compare shapes of prehensile palps and of hemipenes.

Additional description of female

Cp in dorsal and ventral view egg shaped (Fig. 6C, E), with blunt anterior and posterior ends; in lateral view (Fig. 6D) with rounded dorsal margin, greatest height in the middle, LV overlapping RV on all sides, overlap narrow at middle of dorsal margin; external valve surface hirsute with setae approximately $50 \mu\text{m}$ in length.

LV (Fig. 6A) with rounded dorsal margin, greatest height at the middle, anteroventral beak-like expansion not pointed, instead expressed as a linear horizontal extension of the LV ventral margin; ventral margin straight over *c.* two thirds of its length.

RV (Fig. 6B, F-G) with rounded dorsal margin, rounded margin near posterodorsal hinge, greatest height situated slightly anterior of the middle; outer list with ventral row of tubercles; posterior inner list submarginal, but merging with selvage at beginning of dorsal hinge; anteroventral area without lapel or bulbous expansion of the valve margin, instead only an expansion of the selvage in this area visible.

Female appendages as typical of the genus and without special features.

Male unknown.

Ecology and distribution

The species has been recorded in a limited distribution, namely two localities on Kilcowera Station, south of Thargomindah QLD. No males were found in either of the two recorded localities, which suggested the populations were parthenogenetic, particularly in Cardenyabba Lagoon where it was recorded sympatrically with *B. dedeckkeri* sp. nov.

Bennelongia nimala lineage

Remarks

The *B. nimala* lineage consists of two nominal species, *B. nimala* De Deckker, 1981 and the here described *B. regina* sp. nov. The lineage is characterized by oval carapaces in dorsal view with hirsute and pustulose shell surfaces and a pronounced overlap of the RV by the LV anteroventrally, more so than in other species of *Bennelongia*.

Species in this lineage can be distinguished from all other lineages of *Bennelongia* by their heavily hirsute and pustulose shell surfaces. They can also be distinguished from the *B. cygnus* lineage by the serrated margin of the anteroventral lapel on the RV, from the *B. tunta* lineage by the rounded dorsal margin in lateral view (rectangular in *B. tunta*), from the *B. pinpi* lineage it differs by the presence of an

anteroventral lapel in the RV (absent in *B. pinpi* lineage), from the *B. australis* lineage by the smaller male adult carapace size ($L \approx 1400 \mu\text{m}$) and serrated margin of the RV anteroventral lapel (smooth in *B. australis* lineage), and from the *B. harpago* lineage by the absence of a dorsal hump-like thickening of the LV (present in *B. harpago*).

Bennelongia regina sp. nov.

[urn:lsid:zoobank.org:act:EC592D84-3E90-4F5A-BB64-9C0CCF5DA2CA](https://doi.org/10.21203/rs.3.rs-1000000)

Figs 7-8

Diagnosis

Cp with well-developed anterior rostrum in dorsal view, LV heavily overlapping RV anteroventrally, posteriorly blunt; in lateral view with rounded dorsal margin, LV overlapping RV on all sides. LV with broadly rounded anteroventral beak and rounded dorsal margin. RV with rounded dorsal margin, greatest height situated slightly anterior of the middle, anteroventral lapel broad, serrated and with its distal margin running parallel to selvage. Hemipenes with ls protruding well beyond ms, tips of ls and ms separated; ls distally pointed and ms dorsally with in-fold. Rpp with first segment with rounded ventral margin, long subapical sensory organ and broad second segment. Lpp with proximal segment elongated with nearly parallel margins over two thirds of the segment length, distal segment sickle shaped, much shorter than proximal segment.

Etymology

The Latin “Regina” means Queen; the species has been named as such due to its location within Queensland, but also for its splendour in appearance.

Type material

Holotype

♂ (W40060), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide.

Allotype

♀ (W40074), with carapace stored dry in a micropalaeontological slide.

Paratypes

Three ♂ carapaces stored in a micropalaeontological slide (W40062-W40064), three ♀ carapaces stored in a micropalaeontological slide (W40065-W40067), ♀ RV and LV (W40061) stored in a micropalaeontological slide.

Several *in toto* specimens in EtOH (W40077).

Type locality

Horseshoe Lagoon, Kilcowera Station, south of Thargomindah QLD; coordinates: 28°39'57" S - 143°53'42.4" E. Material used for the present description was collected on 16 Jun. 2011 by AK (Sample QLD21A).

Measurements (in μm)

♂: RV: L = 1381, H = 825. LV: L = 1467, H = 889. Cp: L = 1431-1471($n=2$), W = 852-906.

♀: RV: L = 1667, H = 1012. LV: L = 1769, H = 1079. Cp: L = 1687-1817($n=2$), W = 1002-1102($n=2$), H = 1079.

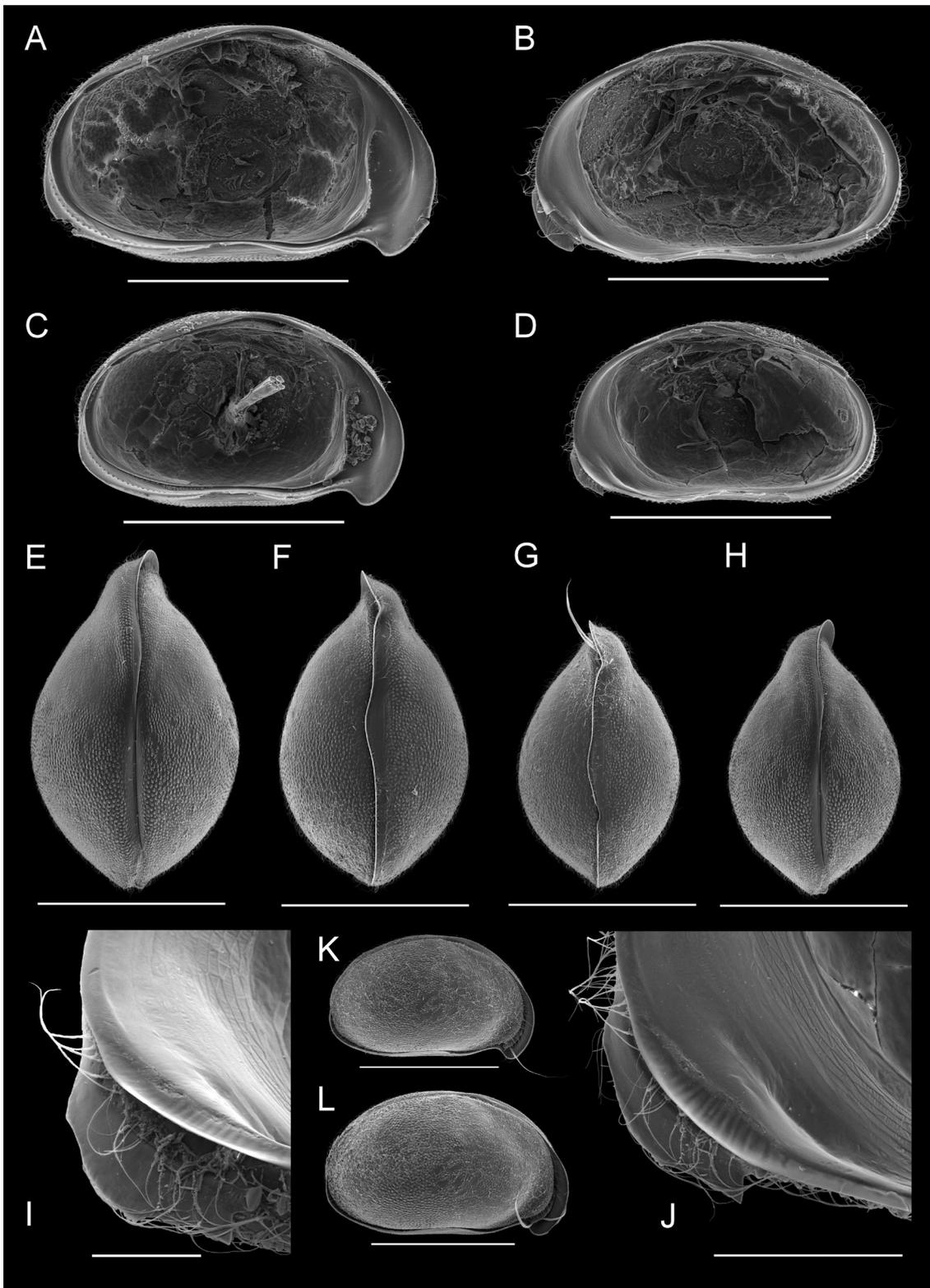


Fig. 7. *Bennelongia regina* sp. nov., all from Horseshoe Lagoon, QLD (Type locality). **A.** ♀, LVi (W40061). **B.** ♀, RVi (W40061). **C.** Holotype ♂, LVi (W40060). **D.** Holotype ♂, RVi (W40060). **E.** ♀, Cp dorsal (W40067). **F.** ♀, Cp ventral (W40066). **G.** ♂, Cp ventral (W40063). **H.** ♂, Cp dorsal (W40062). **I.** Holotype ♂, RVi detail anteriorly (W40060). **J.** Holotype ♂, RVi detail anteriorly (W40060). **K.** ♂, CpRI (W40064). **L.** Allotype ♀, CpRI (W40074). Scales: A-H, K-L = 1000 µm, J = 200 µm, I = 100 µm.

Other material investigated

The species is thus far known only from its type locality.

Differential diagnosis

Bennelongia regina sp. nov. belongs to the *B. nimala* lineage because of its hirsute and pustulose valve surface and the large anteroventral overlap of the RV by the LV. It can be distinguished from the other species in this lineage (*B. nimala* De Deckker, 1981) by the reduced anterodorsal hump-like thickening of the LV in lateral view, the shorter and less abundant setae on the valve surface, the slender ls and the absence of a circular lump shaped copulatory sheath of the hemipenis, the rounded ventral margin of the first Rpp segment, the long subapical sensory organ on the first Rpp segment and the parallel margins of the first Lpp segment.

Additional description

Male and female carapaces in dorsal and ventral view oval shaped with rostrum well-developed, and with large anteroventral overlap of the RV by the LV (Fig. 7E-H, K-L); greatest width situated slightly posterior to the middle (Fig. 7E-H). Carapace in lateral view with rounded dorsal margin and LV overlapping RV on all sides (Fig. 7K-L); greatest height situated near the middle; anterior rostrum (in dorsal view) well-developed, posteriorly bluntly pointed. External valve surface pustulose and hirsute, with setae approximately 50 μm in length. Male valves smaller than female valves, but otherwise similar in appearance.

Male and female LV (Fig. 7A, C) with rounded dorsal margin, greatest height situated slightly in front of the middle; anteroventral beak-like expansion large and broadly rounded; ventral margin almost straight over approximately half its length; anterior inner list reaching well beyond the middle of the valve.

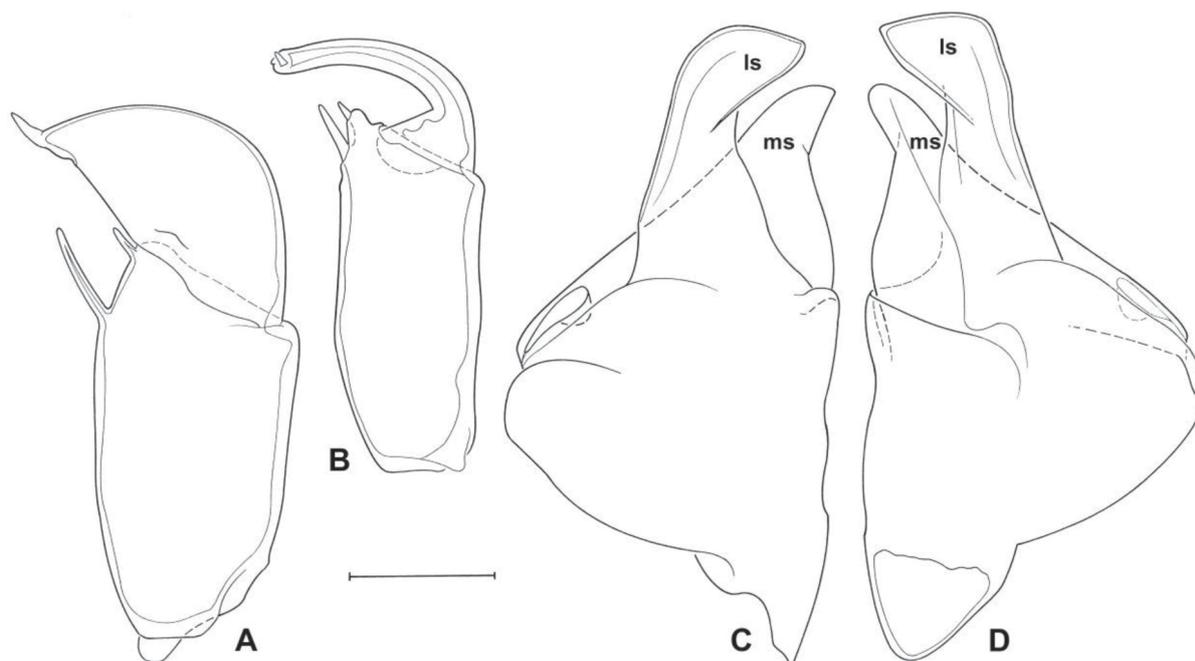


Fig. 8. *Bennelongia regina* sp. nov. (all from holotype ♂ = W40060). **A.** Right prehensile palp. **B.** Left prehensile palp. **C.** Hemipenis. **D.** Outline of other hemipenis. Scales: A-B = 89.3 μm , C-D = 104.2 μm .

Male and female RV (Fig. 7B, D) with rounded dorsal margin, greatest height situated slightly anterior of the middle; outer list of right valves with ventral row of tubercles (Fig. 7B, D); posterior selvage submarginal, posterior list merging with selvage at dorsal hinge; selvage at height of lapel expanded, striate; lapel (Fig. 7I-J) broad, serrated and with distal margin running parallel to selvage.

Most appendages as typical of the genus and without special features.

First segment of Rpp (Fig. 8A) with rounded ventral margin and straight dorsal margin, elongated, approximately 2 x as long as central width, subapically with one long slender sensory organ, apically with shorter sensory organ, both situated quite far apart; second palp segment sub-triangular, broad and with rounded distal margin; apically with slender sensory organ.

First segment of Lpp (Fig. 8B) with parallel margins over two thirds of the length, but narrowing in proximal third, elongated, more than 2.7 x as long as central width, subapically with one long slender sensory organ, apically with shorter sensory organ on elongated outgrowth; second palp segment sickle-shaped, approximately 0.75 x as long as first segment and asymmetrically curved over *c.* 180°, with small distal sensory organ.

Hemipenes (Fig. 8C-D) outlines asymmetrical, one hemipenis with ms having two lobes that embrace the ls on inner and outer sides (Fig. 8D), one lobe distal and embracing the ls as a three dimensional structure and the other lobe proximal and incomplete. The other hemipenis (Fig. 8C) with ms having a single lobe only embracing the ls on the outer side. Both hemipenes with ls protruding well beyond ms, tips of ls and ms well-separated, ls distally pointed ventrally; ms with pointed ventral lobe, dorsally with in-folding of ms.

Ecology and distribution

The species has thus far been found only in its type locality, a turbid temporary freshwater pool with small macrophytes. The species co-existed with another *Bennelongia* species (*B. mckenziei* sp. nov. – assumed to be parthenogenetic) and several macroinvertebrates. No further environmental data are available.

Bennelongia pinpi lineage

Remarks

The combination of a pronounced anteroventral beak-like structure of the LV with the absence of an anteroventral lapel of the RV and bulbous expansion in its place will enable the diagnosis of this lineage amongst all currently described *Bennelongia* species as detailed in Martens *et al.* (2012).

Bennelongia pinpi De Deckker, 1981

Figs 9-10

Remarks

Bennelongia pinpi De Deckker, 1981 was described from Pine Tree Creek Lagoon, north of Hughenden in Queensland, Australia. The species was characterized by its strongly pronounced beak-like expansion of the LV and the outline of the hemipenes (De Deckker 1981). At the time of the description these characters were unique and enabled a simple diagnosis of the species. Since then, two more species from within the same lineage have been described (Martens *et al.* 2012) that are closely related to *B. pinpi* with respect to the absence of anteroventral lapel on the RV, the formation of a bulbous expansion in that place, and the hemipenis outline. Furthermore, some characters were found to be variable within *B. pinpi* upon examination of non-type populations, making the diagnosis of *B. pinpi* from its lineage sister taxa less obvious than is indicated in its description. Illustrations showing the variability within this

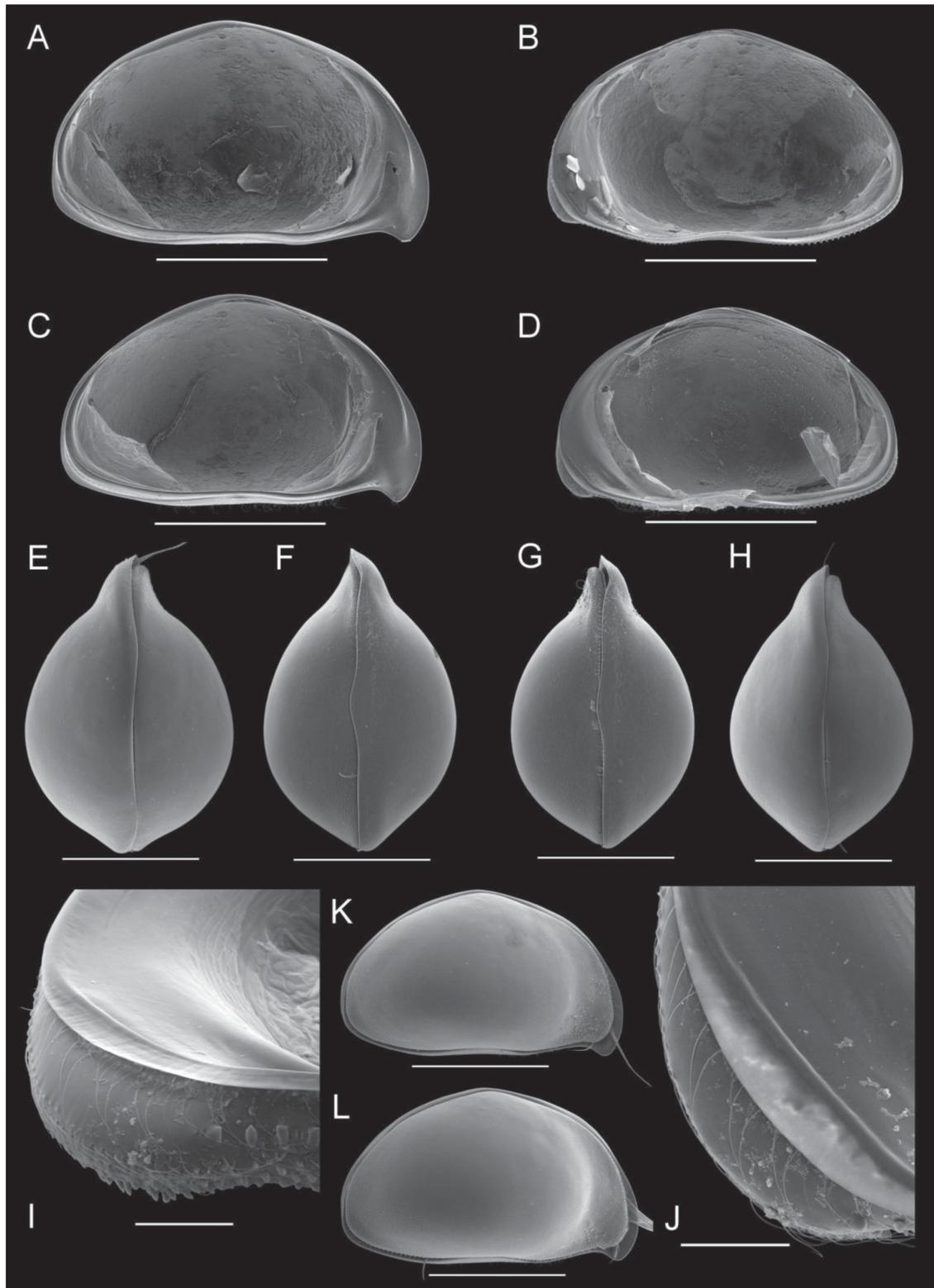


Fig. 9. *Bennelongia pinpi* De Deckker, 1981, all from Salt Lake, north of Hughenden, QLD. **A.** ♀, LVi (W40028). **B.** ♀, RVi (W40028). **C.** ♂, LVi (W40027). **D.** ♂, RVi (W40027). **E.** ♀, Cp dorsal (W40030). **F.** ♀, Cp ventral (W40031). **G.** ♂, Cp ventral (W40032). **H.** ♂, Cp dorsal (W40034). **I.** ♂, RVi detail anteriorly (W40027). **J.** ♂, RVi detail anteriorly (W40027). **K.** ♂, CpR1 (W40033). **L.** ♀, CpR1 (W40029). Scales: A-H, K-L = 1000 µm, J-I = 100 µm.

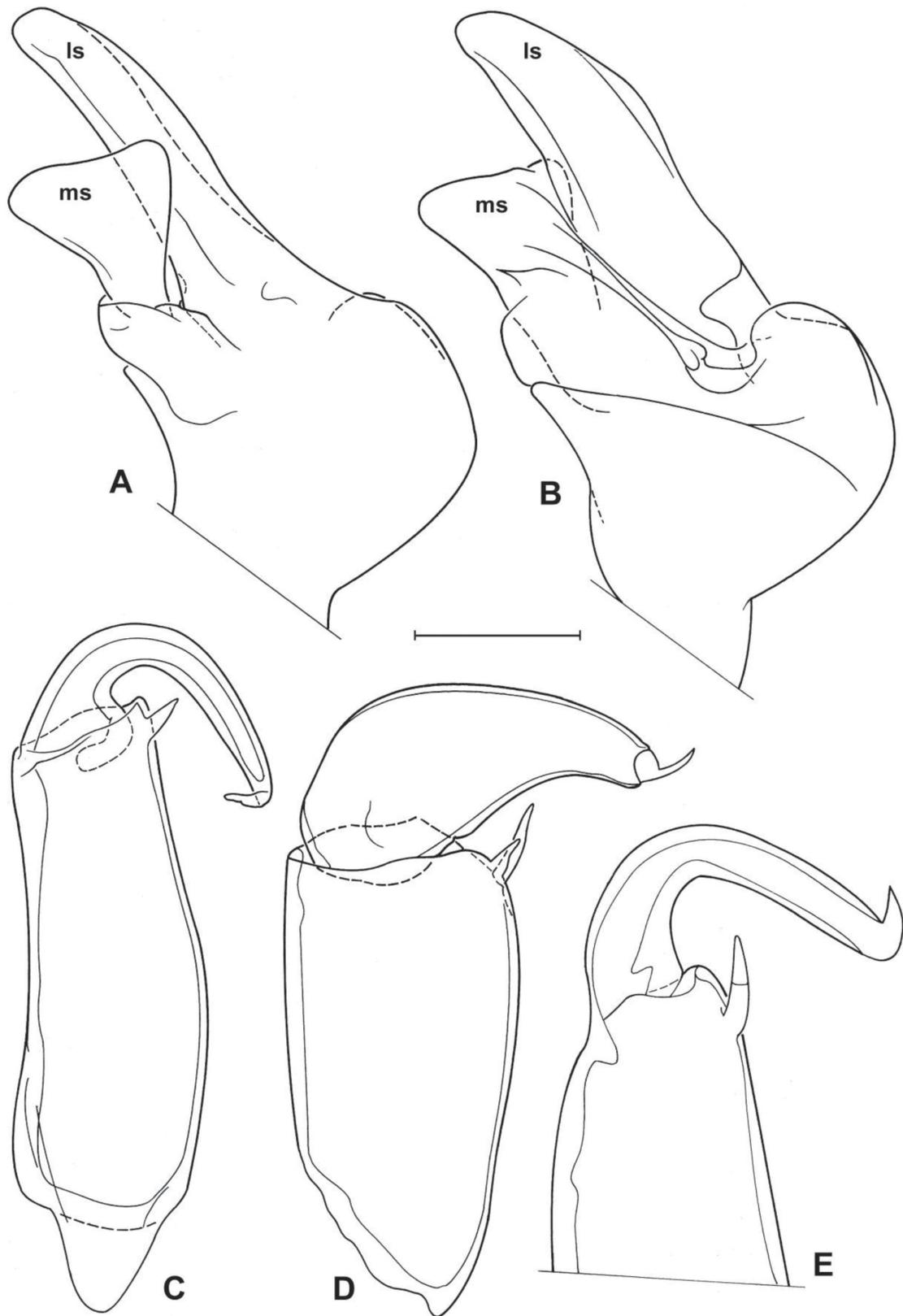


Fig. 10. *Bennelongia pinpi* De Deckker, 1981. (A-D = W40043 from Pelican Lake QLD, E = W40035 from Lake Louisa QLD). **A-B.** Outlines of hemipenes. **C.** Left prehensile palp. **D.** Right prehensile palp. **E.** Left prehensile palp. Scales: A-B = 156.3 μ m, C-D = 89.3 μ m, E = 42.6 μ m.

species were needed to help elucidate the diagnostic characters that can be used for their identification. Thus, detailed drawings of the hemipenes and prehensile palps from non-type localities are provided, which alongside the *B. pinpi* description (De Deckker 1981) show the expected variability within the species and the characters that allow their diagnosis from the more recently described *B. strellyensis* Martens *et al.*, 2012 and *B. kimberleyensis* Martens *et al.*, 2012.

Diagnosis (modified after De Deckker 1981)

Cp with pronounced anterior rostrum but bluntly pointed posterior; sub-triangular in right lateral view, circular in dorsal/ventral view (more so in females), LV overlapping RV on all sides, external valve surface smooth to pseudopunctate with sparsely distributed setae restricted to ventral and anteroventral region. LV with pronounced anteroventral beak-like expansion often extending below plane of horizontal ventral margin. RV with anteroventral flange or lapel absent, but with bulbous expansion of outer list in its place; anterior selvage with flange-like expansion and additional inner submarginal list. Rpp with proximal segment approximately two times as long as central width, distal palp segment short and triangular with rounded distal margin and apical sensory organ. Lpp with first segment over three times as long as central width; second palp segment sickle-shaped, short, and variably rounded, sometimes by over 180 degrees. Hemipenis with ls protruding well beyond ms, more so than any other described species in this lineage; ms forming a three dimensional structure, embracing ls on three sides, dorsal lobe with rounded distal margin.

Measurements (in μm)

♂: RV: L = 1998-2095 ($n=3$), H = 1175-1218 ($n=3$). LV: L = 2208-2223 ($n=3$), H = 1293-1320 ($n=3$). Cp: L = 2063-2232 ($n=9$), W = 1285-1523 ($n=3$), H = 1258-1368 ($n=3$).

♀: RV: L = 2080-2103 ($n=3$), H = 1233-1273 ($n=2$). LV: L = 2200-2280 ($n=2$), H = 1288-1335 ($n=2$). Cp: L = 2055-2300 ($n=7$), W = 1195-1265 ($n=5$), H = 1218-1300 ($n=2$).

Localities investigated

Salt Lake, freshwater lake north of Hughenden on Kennedy Developmental Highway QLD, collected on 9 Jun. 2011 by RS and AK, coordinates: 19°51'19.9" S - 144°16'11.3" E (Sample QLDP11A).

Pelican Lake, north of Hughenden on Kennedy Developmental Highway QLD, collected on 9 Jun. 2011 by RS and AK, coordinates: 19°51'53.1" S - 144°15'20" E (Sample QLDP13A).

Lake Louisa, north of Hughenden on Kennedy Developmental Highway QLD, collected on 9 Jun. 2011 by RS and AK, coordinates: 19°53'45.6" S - 144°15'55.5" E (Sample QLDP12A).

Material investigated or illustrated

♂♂ from Pelican Lake (W40043), Salt Lake (W40027) and Lake Louisa (W40035), with soft parts dissected in sealed slide, valves stored dry in a micropalaeontological slide. Three ♂ carapaces from Pelican Lake (W40045-W40047), Salt Lake (W40029-W40031) and Lake Louisa (W40037-W40039) stored in a micropalaeontological slide.

♀♀ from Pelican Lake (W40044), Salt Lake (W40028) and Lake Louisa (W40036) with soft parts dissected in sealed slide, valves stored dry in a micropalaeontological slide. Three ♀ carapaces from Pelican Lake (W40048-W40050), Salt Lake (W40032-W40034) and Lake Louisa (W40040-W40042) stored in a micropalaeontological slide.

Several *in toto* specimens in EtOH from Pelican Lake (W40080).

Differential diagnosis

Bennelongia pinpi can be diagnosed as within the *B. pinpi* lineage due to its large carapace, well developed anterior rostrum, blunt posterior, the bulbous expansion of the valve in place of the absent anteroventral lappet of the RV, the well-developed anteroventral beak-like expansion of the LV and the well sclerotized hemipenes with a three dimensional ms enveloping the ls.

It can be distinguished from the other species in this lineage by the elongated first Lpp segment (shorter in *B. strellyensis* Martens *et al.*, 2012 and *B. kimberleyensis* Martens *et al.*, 2012), the rounded dorsal margin of the Rpp second segment (triangular-pointed in *B. kimberleyensis* Martens *et al.*, 2012), the narrow ls of the hemipenis (broader in *B. strellyensis* Martens *et al.*, 2012 and *B. kimberleyensis* Martens *et al.*, 2012) and the extension of the ls far beyond the ms (much less extended in *B. strellyensis* Martens *et al.*, 2012 and *B. kimberleyensis* Martens *et al.*, 2012).

Additional description (modified after De Deckker 1981)

Cp (Fig. 9E-H) with pronounced anterior rostrum but bluntly pointed posterior; sub-triangular in right lateral view (Fig. 9K-L), circular in dorsal/ventral view (more so in females), LV overlapping RV on all sides, external valve surface smooth to pseudopunctate with sparsely distributed setae restricted to ventral and anteroventral region.

LV (Fig. 9A, C) with pronounced anteroventral beak-like expansion, often extending below plane of horizontal ventral margin, dorsal margin highest at valve mid-point.

RV (Fig. 9B, D) in lateral view with dorsal margin highest at valve mid-point; anteroventral flange or lappet absent, but with bulbous expansion of outer list in its place (Fig. 9I-J); anterior selvage with flange-like expansion, and additional inner submarginal list.

Most appendages as typical of the genus and without special features.

Rpp (Fig. 10D) with first segment approximately two times as long as central width, subapically with two slender sensory organs, one long and one short often overlapping; second palp segment short and triangular with rounded distal margin and apical sensory organ.

Lpp (Fig. 10C, D) with first segment over three times as long as central width, subapically with one sensory organ; apically with rounded outgrowth; second palp segment sickle-shaped, short, and variably rounded (Fig. 10C versus Fig. 10E), sometimes by over 180 degrees with distal sensory organ.

Hemipenes (Fig. 10A-B) with width of ls variable depending on slide preparation (Fig. 10a vs Fig. 10B), ls protruding well beyond ms, more so than any other described species in this lineage; ms consisting of three lobes, distal part of ms produced into a three-dimensional lobe embracing the ls on proximal and distal sides, a small additional dorsal lobe with rounded distal margin, and a proximal lobe nearly straight and incomplete.

Ecology and distribution

The distribution of *B. pinpi* appears to be isolated to an area north of Hughenden. At the time of its description, the species had been found in Pine Tree Creek Lagoon, Agnes Lake, Salt Lake and Louisa Lake (De Deckker 1981). It appears that most of these populations still exist. Here, an additional locality (Pelican Lake) is recorded.

Bennelongia harpago lineage**Remarks**

This lineage only consists of one species (*B. harpago* De Deckker, 1981), which can be diagnosed from the other described lineages of *Bennelongia* by using the description below.

Bennelongia harpago De Deckker, 1981

Fig. 11

Diagnosis (modified after De Deckker 1981)

Cp in dorsal view with blunt anterior rostrum and rounded posterior, oval to sub-rectangular in lateral view, LV overlapping RV on all sides but more so antero-dorsally with hump-like thickening. External valve surface pseudopunctate with sparse, short setae. LV with dorsal margin highest at one third from the anterior where hump-like thickening occurs. RV with anteroventral flange absent and greatest height slightly anterior of the middle.

Measurements (in μm)

♀: RV: L = 1193, H = 736. LV: L = 1261, H = 792. Cp: L = 1256-1282 ($n=3$), W = 813, H = 765-788 ($n=2$).

Locality investigated

Lake Powlathanga, west of Charters Towers QLD, coordinates: 20°14'42.2" S - 145°58'8.2" E. Material was collected on 5 Jun. 2011 by RS and AK (Sample QLDP1A).

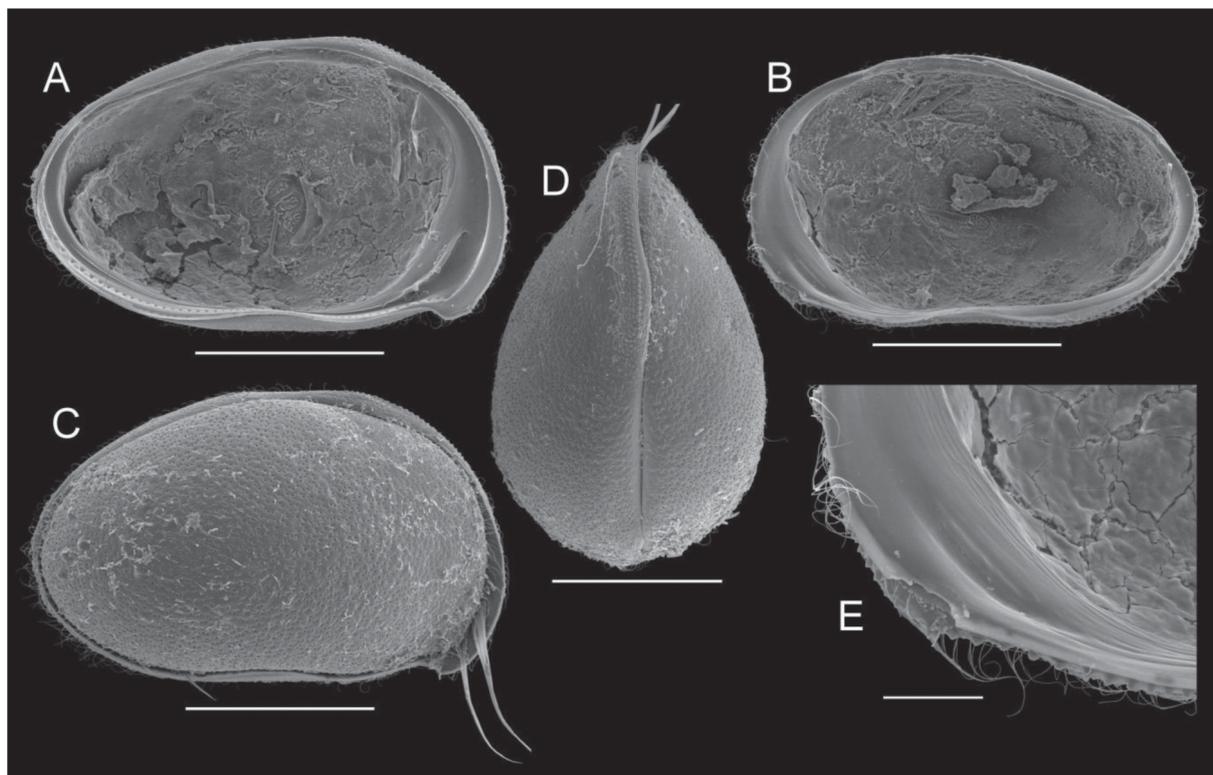


Fig. 11. *Bennelongia harpago* De Deckker & McKenzie, 1981, all from Lake Powlathanga, QLD. **A.** ♀, LVi (W40014). **B.** ♀, RVi (W40014). **C.** ♀, CpRl (W40015). **D.** ♀, Cp dorsal (RS35). **E.** ♀, RVi detail anteriorly (W40014). Scales: A-D = 500 μm , E = 100 μm .

Material investigated

Two ♀♀ (W40013-W40014) from Lake Powlathanga, with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide. One carapace stored in a micropalaeontological slide (W40015).

Differential Diagnosis

Bennelongia harpago can be diagnosed from the other *Bennelongia* lineages by the anterodorsal hump-like thickening of the LV (absent in all other described lineages). Identification can be confirmed further by the small carapace ($L \approx 1.2$ mm, larger in *B. cygnus*, *B. australis*, *B. pinpi*, *B. tunta* and *B. nimala* lineages), the absence of an anteroventral lapel in the RV (present in *B. cygnus*, *B. australis* and *B. nimala* lineages) and the blunt anterior rostrum in dorsal view (more pronounced in *B. australis*, *B. pinpi* and *B. tunta* lineages).

Additional Description (modified after De Deckker & McKenzie 1981)

Cp (Fig. 11C-D) oval in dorsal view with rounded posterior and blunt anterior rostrum; oval to subrectangular in lateral view, LV overlapping RV on all sides (more so in the first one third from anterior, external valve surface pseudopunctate with sparse short setae).

LV (Fig. 11A) with pointed anteroventral beak-like expansion, dorsal margin highest at approximately one third from anterior where thickening of the valve forms a hump-like appearance.

RV (Fig. 11C) in lateral view with dorsal margin highest at slightly anterior to the middle. Anteroventral lapel very reduced to non-existent.

Remarks

Bennelongia harpago was described from a dam near Mt Teddy in Queensland, Australia (De Deckker & McKenzie 1981). This study has provided molecular evidence from specimens in Lake Powlathanga that confirmed their species status. As there have since been nine additional species described from Western Australia and three described here, notes on the additional characters that now diagnose *B. harpago* from all described *Bennelongia* are provided below.

Ecology and distribution

Bennelongia harpago was originally recorded at a number of localities throughout Queensland (De Deckker & McKenzie 1981). One of these populations (Lake Powlathanga) was sampled in this study showing that they still exist there. At present, males have not been recorded for this species.

Bennelongia australis lineage

Remarks

The diagnostic characters provided for this lineage in Martens *et al.* (2012) are still valid after the revision of eastern Australian *Bennelongia* species. The combination of a large carapace, intermediate anteroventral beak-like structure of the LV, presence of an anteroventral lapel on the RV, rounded dorsal margin and pronounced anterior rostrum in dorsal view will still enable the diagnosis of this lineage amongst all described *Bennelongia* species.

Bennelongia cuensis sensu lato

Figs 12-13

Diagnosis

Cp large ($L \approx 1800 \mu\text{m}$) with strong anterior rostrum and bluntly pointed posterior in dorsal view. LV strongly overlapping RV on all sides, LV with straight dorsal margin and bluntly pointed anteroventral beak. RV with broad and elongated anteroventral lappet running parallel to the selvage. Hemipenis with distal margin of ms straight and broad, producing a pointed ventral and a rounded dorsal lobe; ls asymmetrical in the two hemipenes: one with distal thumb-like expansion, the other without; tips of ls and ms in both hemipenes well separated; ls extending well beyond ms. Rpp with distal segment sub-triangular, rounded on dorsal margin, elongated and with narrow base. Lpp with proximal segment apically with small triangular outgrowth; distal segment sickle shaped, of equal length to proximal segment, distal half with parallel margins. Ratio for maximum length of Lpp distal segment to length of hemipenis ms distal margin 1:1.7. Ratio for maximum length Rpp distal segment to length of hemipenis ms distal margin approximately 1:1.3.

Remarks and Etymology

Two populations from the Paroo region of eastern Australia constitute a molecular lineage that is morphologically similar, but genetically distinct from *B. cuensis* Martens *et al.*, 2012. Although some minor morphological differences have been found for Western Australian animals (provided in the description below), these do not appear to constitute reliable differences. Analysis of additional populations may enable the identification of more reliable diagnostic characters in the future. Therefore, this lineage is here not formally described as a different species from *B. cuensis* Martens *et al.*, 2012.

Measurements (in μm)

♂: RV: L = 1815-1863 ($n=2$), H = 1043-1090 ($n=2$). LV: L = 1955-1978 ($n=2$), H = 1155-1165 ($n=2$). Cp: L = 1865-1892 ($n=5$), W = 1000-1027 ($n=3$), H = 1113-1123 ($n=2$).

♀: RV: L = 2053-2113 ($n=2$), H = 1198-1270 ($n=2$). LV: L = 2200-2280 ($n=2$), H = 1288-1335 ($n=2$). Cp: L = 2055-2300 ($n=7$), W = 1195-1265 ($n=5$), H = 1218-1300 ($n=2$).

Localities investigated

Unnamed pool, Kilcowera Station, south of Thargomindah QLD, coordinates: 28°40'13.2" S - 143°54'10.4" E. Material used for the present illustration was collected on 14 Jun. 2011 by AK (Sample QLDP19A).

Island claypan, on Bloodwood Station, north west of Bourke NSW, collected 28 Sep. 2010 by Brian Timms, coordinates: 29°29' S - 144°49' E (Sample NSWB1).

Material investigated

♂ from unnamed pool, Kilcowera Station QLD (W40052) and four ♂♂ from island claypan, Bloodwood Station NSW (W40001-W40004), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide.

Two ♀♀ from island claypan, Bloodwood Station NSW (W40011-W40012), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide.

♀ from unnamed pool, Kilcowera Station QLD (W40051), with soft parts dissected in a sealed slide and valves stored dry in a micropalaeontological slide.

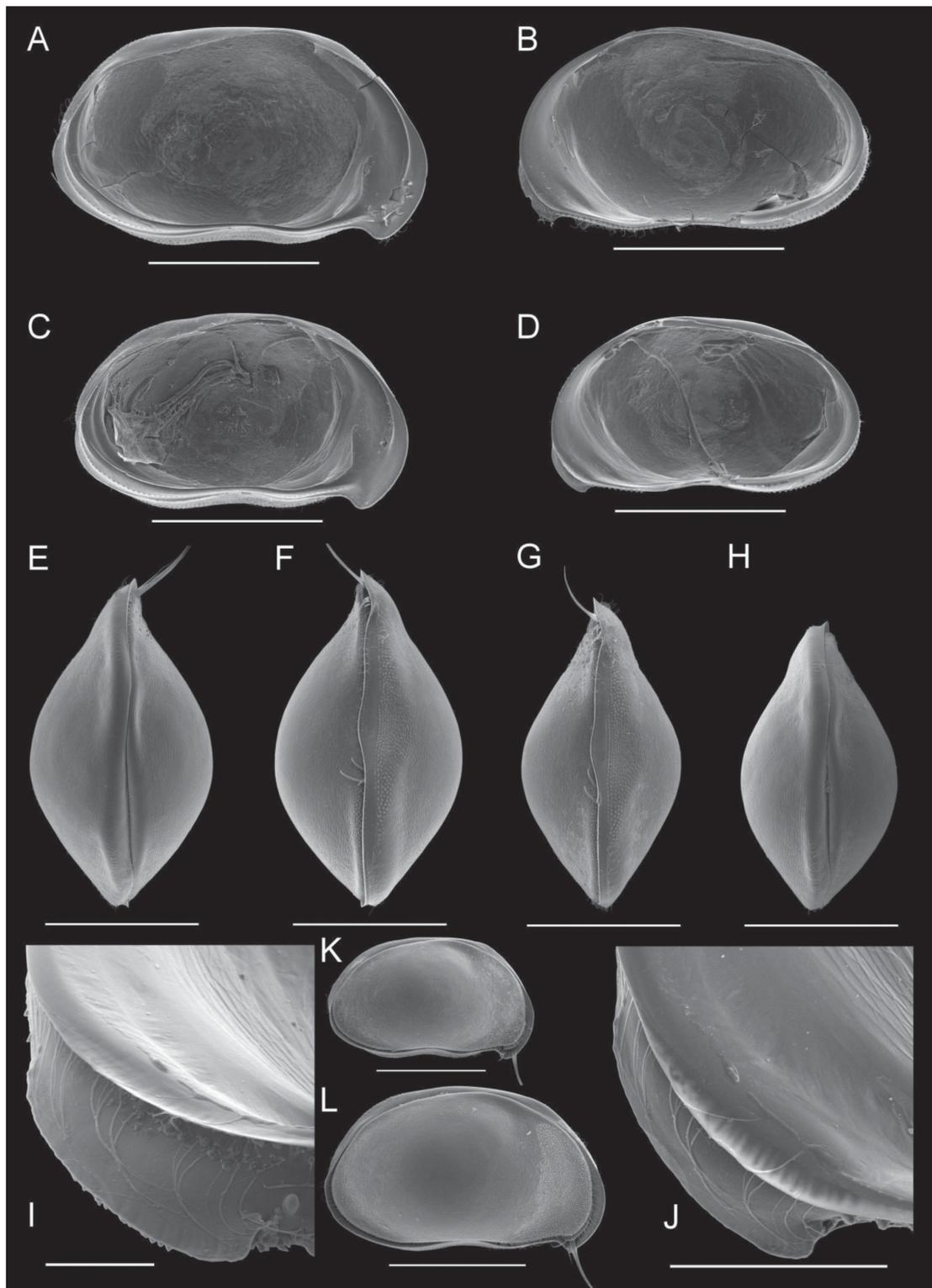


Fig. 12. *Bennelongia cuensis sensu lato* A-B, D-L from unnamed pool near Kilcowera Station, QLD; C from island claypan on Bloodwood Station NSW. **A.** ♀, LVi (W40053). **B.** ♀, RVi (W40053). **C.** ♂, LVi (W40002). **D.** ♂, RVi (W40052). **E.** ♀, Cp dorsal (W40059). **F.** ♀, Cp ventral (W40058). **G.** ♂, Cp ventral (W40056). **H.** ♂, Cp dorsal (W40055). **I.** ♂, RVi detail anteriorly (W40052). **J.** ♂, RVi detail anteriorly (W40052). **K.** ♂, CpRl (W40054). **L.** ♀, CpRl (W40057). Scales: A-H, K-L = 1000 µm, J = 200 µm, I = 100 µm.

Three ♂ carapaces stored in a micropalaeontological slide (W40054-W40056), three ♀ carapaces stored in a micropalaeontological slide (W40057-W40059). ♀ (W40053) with valves stored dry in a micropalaeontological slide, all from unnamed pool, Kilcowera Station QLD.

Three ♂ carapaces from island claypan, Bloodwood Station NSW (W40005-W40007), three ♀ carapaces stored in a micropalaeontological slide (W40008-W40011).

Several *in toto* specimens in EtOH, from unnamed pool, Kilcowera Station QLD (W40078).

Several *in toto* ♀ specimens in EtOH, from island claypan, Bloodwood Station NSW (W40079).

Differential diagnosis

The species belongs to the *B. australis* lineage due to its large size ($L \approx 1800 \mu\text{m}$), the strong anterior rostrum in dorsal view and the presence of an anteroventral lapel on the RV. It can be distinguished from the other species in this lineage by the straight dorsal margin of the LV (less so in *B. gwelupensis* Martens *et al.*, 2012 and *B. coondinerensis* Martens *et al.*, 2012, the well separated tips of the ls and the ms of both hemipenes (close in *B. bidgelangensis* Martens *et al.*, 2012), the considerable overlap of the RV by the LV, sub-quadrate outline of the Cp in lateral view, the narrow lobes of the ms (limited overlap of RV by LV, rounded Cp outline and blunt ms lobes in *B. lata* Martens *et al.*, 2012 and by the smaller Rpp to hemipenis ratio (maximum length of Rpp second segment to distal margin of Hp ms approximately 1:1.3 in *B. cuensis sensu lato*, 1:2.2 in *B. cuensis* Martens *et al.*, 2012).

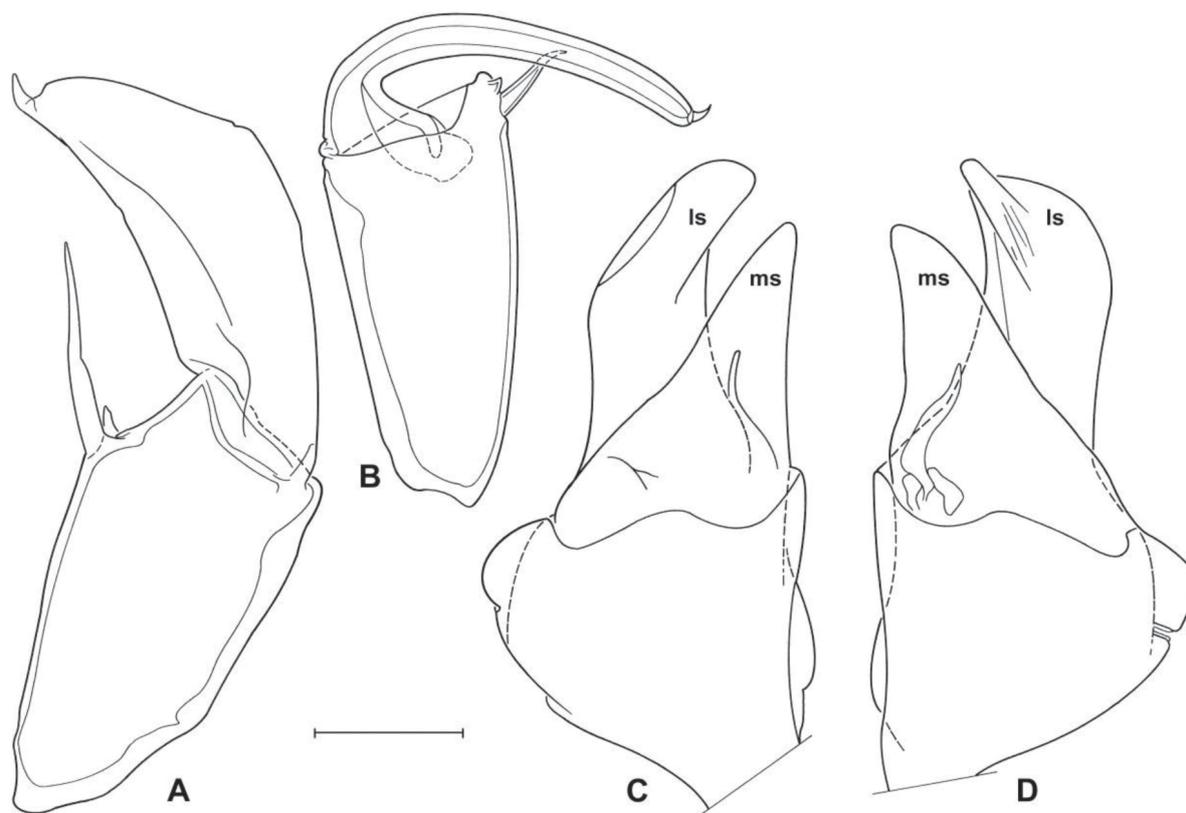


Fig. 13. *Bennelongia cuensis sensu lato*, all from unnamed pool near Kilcowera Station, QLD, ♂ (W40052). **A.** Right prehensile palp. **B.** Left prehensile palp. **C.** Hemipenis. **D.** Outline of other hemipenis. Scales: A-B = $89.3 \mu\text{m}$, C-D = $104.2 \mu\text{m}$.

Additional description

Male and female carapaces (Fig. 12E-H, K-L) in right lateral view with straight dorsal margin, LV overlapping RV on all sides, particularly dorsally, greatest height situated throughout the middle third of the Cp length; greatest Cp width situated near the middle in dorsal and ventral view, anterior side with a rostrum and posterior side pointed; Cp surface set with short setae and weakly pitted. Male carapaces and valves slightly smaller than in the female, but with very similar shape.

Male and female LV (Fig. 12A, C) with straight dorsal margin, greatest height situated throughout the middle third of the valve length, anteroventral beak bluntly pointed.

Male and female RV (Fig. 12B, D) with greatest height situated slightly anterior of middle in lateral view, dorsal margin rounded; posterior selvage sub-marginal, inner list merging with selvage posteriorly; selvage expanded and striate at vicinity of anteroventral lapel (Fig. 12I-J); lapel mildly striate, broad and running parallel to selvage (Fig. 12I-J).

Most appendages as typical of the genus and without special features.

Rpp (Fig. 13A) with first segment length approximately 1.5 times its central width; subapically with two sensory organs, the proximal one long and slender, the distal one short; second palp segment nearly as long as the first one, with a narrow base and bluntly rounded dorsal margin, ratio for maximum length of second segment to length of hemipenis ms distal margin approximately 1:1.3, distally with small sensory organ.

Lpp (Fig. 13B) with first segment approximately two times its central width, subapically with one long proximally based and one short distally based sensory organ, apically with rounded outgrowth; second segment sickle shaped, equal in length to first segment, ratio for maximum length of second segment to length of hemipenis ms distal margin approximately 1:1.7, distal half straight with almost parallel margins, distally with small sensory organ.

Hemipenes (Fig. 13C-D) asymmetrical, one hemipenis with ls distally bluntly pointed (Fig. 13C), the other with ls more sharply pointed with thumb-like expansion (Fig. 13D); bluntly pointed ls of one hemipenis often folding at rounded dorsal margin suggesting the presence of a three dimensional structure; both hemipenes with ls and ms tips well separated, ls extending beyond ms, ms ventrally pointed and dorsally with rounded lobe.

Ecology and distribution

This cryptic lineage appears to have only a limited distribution near the Paroo region of south western Queensland and north western New South Wales. The species was found in high abundance in a turbid pool with grass and small aquatic weeds on Kilcowera Station.

Discussion

Trends in Diversity and Radiation

The *B. barangaroo* lineage now comprises at least the two morphotypes shown in the original description of *B. barangaroo* De Deckker, 1981 and the two new species described here (*B. dedeckkeri* sp. nov. and *B. mckenziei* sp. nov.). Thus, there are potentially four species within this lineage and currently they share a number of characters that obviously set them apart from the other lineages of *Bennelongia*. However, the high genetic distance between *B. dedeckkeri* sp. nov. and *B. mckenziei* sp. nov., and the morphological disparity of their diagnostic characters (anteroventral lapel on RV) suggest that if more

recent radiations have occurred in this group, these species are likely to belong to diverse lineages of their own.

Considering 1) the genetic distance of *B. regina* sp. nov. to all other species, 2) the knowledge of at least one other species existing in the same lineage (*B. nimala*), and 3) the extensive radiation of other lineages in the genus (*B. australis* lineage and *B. pinpi* lineage), it is likely that the *B. nimala* lineage consists of several more species. Further exploration of habitats throughout Australia is likely to yield more species.

Cryptic diversity

The discovery of cryptic lineages throughout all metazoan phyla (Beheregaray & Caccone 2007; Pfenninger & Schwenk 2007) is having profound implications for conservation and management (examples in Brown *et al.* 2007; Elmer *et al.* 2007; Fontaneto *et al.* 2008; Gustafsson *et al.* 2009; Marrone *et al.* 2010). Indeed, if genetic diversity is cryptic, it is difficult to see when it is becoming lost. A case in point is the baffling discovery of close to 40 reproductively isolated lineages (“species”) within a single classical ostracod species, *Eucypris virens* (Bode *et al.* 2010). In some of the investigated ponds no less than 9 cryptic species occurred sympatrically (Bode *et al.* 2010). If one has to rely exclusively on morphological analyses, one could not deduce the fate of these cryptic species over time. Of further concern is that this is not an isolated case, as inland aquatic crustaceans appear to show high frequencies of cryptic diversity (Murphy *et al.* 2009; Witt *et al.* 2006) and the identification of the *B. cuensis sensu lato* cryptic lineage in the present study follows this notion.

Morphological Characters

Molecular methods allow stable, finer scale characters to be identified during the description of species that would otherwise have remained cryptic and unnoticed. Here, evidence for six genetically distinct groups within collections of *Bennelongia* in eastern Australia has been provided from phylogenetic (Maximum Likelihood and Bayesian Inference) and Parsimonious network analyses. 4 x rule tests confirmed their identity as Evolutionary Genetic species following the arguments in Birky *et al.* (2010). These molecular methods were fundamental in the identification of the stable morphological character states defined for the three new species (*B. dedeckeri* sp. nov., *B. regina* sp. nov. and *B. mckenziei* sp. nov.), and in the identification of a truly cryptic lineage of a described species with no reliable morphological characters known at present (*B. cuensis sensu lato*).

The absence of both an anteroventral lapel and a bulbous expansion of the RV evident in *B. mckenziei* sp. nov. is a new character state not seen in this genus until now (absent from descriptions in De Deckker 1981, 1982; De Deckker & McKenzie 1981; Martens *et al.* 2012). Both of these characters have the function of sealing the entrance of the LV sulcus when the valves are tightly closed (Martens *et al.* 2012). Thus the question arises of what this species uses when shutting the valves tightly, for example to survive a certain period of pond desiccation in a torpid state. It is possible that the anteroventral expansion of the selvage in *B. mckenziei* sp. nov. performs the same function.

The genetic clustering of *B. pinpi* De Deckker, 1981 with the other two species of the *B. pinpi* lineage confirmed the taxonomic significance of the bulbous expansion of the RV and the strongly produced anteroventral beak-like structure of the LV in the lineage diagnosis. Yet the position of *B. pinpi* De Deckker, 1981 as a separate clade in the phylogeny and as an unconnected cluster in the parsimonious network, together with the significant result of applying the 4 x rule, strengthened the status of *B. pinpi* as a valid species. In the same way, the genetic identity and specific validity of *B. harpago* De Deckker & McKenzie, 1981 has been shown.

The evolutionary origin of *Bennelongia*

The Cyprididae Baird, 1845 are currently a dominant group that make up approximately half of all extant non-marine ostracods (Martens *et al.* 2008). During the Cretaceous, the *Cypridea* Bosquet, 1852 lineage was the dominant non-marine lineage and our current understanding is that it became extinct around the K/T boundary. *Cypridea* had an internal marginal valve morphology (Horne & Colin 2005) and beak-like feeding apparatus (Martens *et al.* 2012) which was somewhat similar to those in *Bennelongia*. For this reason it has been suggested that *Bennelongia* is not a member of Cyprididae, but in fact a surviving descendant of the ancient *Cypridea* lineage (Horne & Martens 1998). If correct, this would also imply that the Cyprididae are not directly related to *Cypridea*. Instead, a small number of cypridid lineages may have coexisted with *Cypridea* and undergone an explosive radiation following the extinction of the *Cypridea* lineage (hypothesized in Martens *et al.* 2012).

Upon analysis of the results presented here, the presence of an anteroventral beak-like structure in all *Bennelongia* species indicates that this character is ancestral. The characters that function in sealing the sulcus however, originate from different structures in different lineages, suggesting that they have developed more recently. Moreover, two structures used for sealing the sulcus (modification of the outer list as a bulbous expansion and the expansion of the selvage) are congruent with molecular data, which show monophyly for these character states.

The existence of the three sulcus sealing character states provides a potentially interesting case of convergent evolution, whereby selection pressure for prevention of threats like desiccation and predation could have been applied after the development of the beak-like feeding apparatus. If *Bennelongia* is indeed a remnant of *Cypridea*, perhaps it was this pressure that drove selection for sulcus sealing character states and the extinction of the other *Cypridea*.

Conclusion

Speculation aside, the present paper reports on six species of *Bennelongia* from eastern Australia, divided over five different lineages within the genus. The validity of two existing species (*B. pinpi* De Deckker, 1981 and *B. harpago* De Deckker & McKenzie, 1981) was confirmed. Three species were formally described as new (*B. dedeckkeri* sp. nov., *B. mckenziei* sp. nov. and *B. regina* sp. nov.). The final taxon was a true cryptic species (*B. cuensis sensu lato*) that was established with molecular methods, but could not be identified based on morphology and therefore is not formally described here.

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