Morphometrics of the resting eggs of the fairy shrimp *Branchinella* in Australia (Anostraca: Thamnocephalidae).

BRIAN V. TIMMS¹ AND SUE LINDSAY²

¹Honorary Associate, Australian Museum, 6-9 College Street, Sydney, 2000 and Australian Wetlands and Rivers Centre, BEES, University of New South Wales, Sydney, 2052.

²Microscope Unit, Australian Museum, 6-9 College Street, Sydney, 2000

Published on 14 December 2011 at http://escholarship.library.usyd.edu.au/journals/index.php/LIN

Timms, B.V. and Lindsay, S. (2011). Morphometrics of the resting eggs of the Australian species of the fairy shrimp *Branchinella* (Anostraca: Thamnocephalidae). *Proceedings of the Linnean Society of New South Wales* 133, 53-70.

Branchinella resting eggs are characterised by having surface ridges arranged more or less in polygons and by almost all species having few, if any, spines. The eggs of 33 out of a known possible 40 species (including 4 out of 6 undescribed species) were studied by SEM. A few species are distinctive by being adorned with lighter coloured surface membranes often strengthened by ribs or sparse spines and one (B. longirostris) is regularly spinose. Those species known to be morphological variable also have variable egg morphologies. This makes it difficult to characterise specific egg morphology, but even so in some species eggs are distinct: B. arborea, B. australiensis, B. budjiti, B. compacta, B. complexidigitata, B. hattahensis, B. kadjikadji, B. longirostris, B. lyrifera, B. occidentalis, B. pinderi and B. vosperi. Most of the remainder are easily confused with at least one or more species. Branchinella egg morphology seems of little value in taxonomical studies and of restricted use in distinguishing eggs in dried sediments.

Manuscript received 10 June 2011, accepted for publication 10 December 2011.

KEYWORDS: adnorments, egg size, morphotypes, polygonal surface structure, surface compartments, systematic relationships, value in environmental monitoring.

INTRODUCTION

The resting eggs of fairy shrimps have a tough outer layer, the tertiary envelope, which is often sculptured and may be specifically characteristic (Mura, 1986, 1991a, 1991b, 1992a, 1992b, 2001; Thiéry & Gasc, 1991, Thiéry et al., 2007; Brendonck & Coomans, 1994a,1994b; Hill & Shepard, 1997; Timms et al., 2004). One of the first egg shells to be described was of the Australian Streptocephalus archeri (Sars, 1986) and soon afterwards of Branchinella australiensis Richters, 1876 (as B. eyrensis Daday, 1910). These early descriptions used drawings based on microscopic observations, but in more recent times SEM technology has been used with great success (see references above). For Australia, Timms et al. 2004 provided an SEM study of 31 species, including 22 of Branchinella. This showed that the four genera naturally in Australia (Australobranchipus, Branchinella, Parartemia and Streptocephalus) have distinctive egg shapes and surface patterns and that within Branchinella many species, but not all, have distinctive morphology.

Optimism prevailed in the early 1990s that many species had an immutable unique pattern and hence the presence of a species at a dried site could be detected by examining bottom mud microscopically. Keys were constructed to aid this (eg Thiéry & Gasc, 1991). However further work showed problems in sampling eggs, and far too much variability within many species (Mura 1991b; Mura & Rosetti, 2010) for the widespread use of egg morphology. Moreover, in some species egg sculpturing maybe predator-inducible (Dumont et al., 2002) so masking any morphological relationships. Understandably, enthusiasm for egg studies waned. However environmental evaluations in recent times have needed to assess fauna in dried sites (eg Beladjal & Mertens, 2003; D.C. Rogers, pers. comm. for California; V. Campagna, pers. comm. for Western Australia) so that knowledge on resting eggs is a valuable aid, if the limitations are understood.

The aim of the present study is use detailed morphometrics to evaluate the eggs of as many species of *Branchinella* as possible, with a view to understanding which species have distinctive egg sculpturing, and how much this might be variable

in such widespread species as *B. australiensis* (Geddes, 1981) or species known to be otherwise variable as adults such as *B. longirostris* (Zofkova & Timms, 2009). A subsidiary aim is to gain further insight, within the limits of resting egg variability, of relationships between species as determined by morphology (Geddes, 1981).

MATERIALS AND METHODS

Of the presently 35 described species of *Branchinella*, 29 were available for study. In addition 4 undescribed species were also studied. For most species just one population was examined, but seven species had 2 to 5 populations studied and two species known to be particularly variable (*B. australiensis* and *B. longirostris*) had 7 and 8 populations examined respectively. Locality details for each are given in Appendix 1.

Resting eggs from each collection were removed from the brood pouches of 2 -3 mature ovigerous females which were preserved in 70% alcohol or 4% formalin, and then stored in 90 -95% alcohol for weeks to many months. This variable length of preservation may have affected the degree of hydration of the eggs. If the eggs seemed old or damaged as judged from the breakdown of the tertiary layer they were rejected (e.g. this happened for populations of B. occidentalis and B. frondosa in this study. Also eggs of B. nana had to be rejected as they were immature). Eggs were then air-dried and mounted on carbon tabs on aluminium stubs, gold sputter coated and then 10 per collection were photographed on a Zeiss Evo LS15 SEM using a Robinson Backscatter Detector. For each egg an average egg diameter was determined from three measurements and the character of the ridges and depressions noted. The later were counted on the visible side (whole depressions plus some only partly visible (scored as 3/4 or 1/2 or 1/4 according to how much is readily visible) and then doubled to obtain the total number of depressions per egg. For species with extremely numerous depressions (i.e > 100) only wholly visible depressions were counted. Accuracy was estimated at \pm 3% when less than 100 depressions and at \pm 6% when more than 100. The third quantitative parameter measured was the ratio of wall height to the average width of the depressions (wh:dw).

Some descriptive terms are used to describe the shapes of the depressions. While depression shape is basically polygonal, this term is used loosely; when the sides of the polygon is made up of five almost equal sides then the term pentagonal is used, but

when the shape is irregular and hardly polygonal then the descriptors linear when main axis >2x axis at right angles or constricted when narrower in the middle than at the ends are used. Various terms are used to refer to the shape and thickness of the ridges that make up the walls of the depressions including triangular when the cross section is distinctly angular with straight sides, rounded when the cross section is an inverted U shape, punctuate when pitted, and ropey when uneven with regular bulges like a rope. The floor of the depressions may be flat or mildly, moderately or strongly concave. A mild concavity is one where depth is less than 0.1 of the diameter and a strongly concave surface has a depth greater than 0.5 of the diameter: intermediate values are considered to be moderate concave. However it is believed the degree of concavity is influenced to some degree by the state of hydration of the egg, so this character needs to be assessed with care. The surface of the depressions especially the floor, maybe smooth or dimpled (which may be small/weak, strong or even angled). Weak dimpling is defined as the height being less than the diameter of the dimple, and strong dimples have the height greater than the diameter. All these measurements have to be estimated by eye as there is no vertical scale readily available. Sometimes the depression walls or ridge crests are punctured with pores (simple or complex) or have minute spines. Some species have the crest ridges adorned with membranes or spines of various natures.

RESULTS

Descriptions of the eggs of 33 species are given below and in Tables 1 and 2 and illustrated in Figures 1 -5. For the majority of species, in which only one population was studied, the descriptions are short and may not encompass possible variability in that species. In an attempt to encompass variability, descriptions are longer for those where many populations were studied.

B. affinis Linder 1941 (Fig. 1a,b). Average size 215.3 μm, mean depression number 39.2. Size and number of depressions vary between east and west Australia. Depressions irregular, often invaginated and with dimpled floors and sides. Ridges usually distinct and rounded; if fully hydrated (Fig. 1b) then depression shallow (wall height:depression width ratio <0.2) and floors mildly concave, but if dehydrated (Fig. 1a) then depressions much folded and deep (wh:dw>0.5) and floors markedly concave.

	Table 1. Co	mparison	s of eg	g sizes and	depressior	numb	ers	
			egg siz	es in ųm			depression	numbers
	Timms	et al 2004	1	pres	ent study		present	study
Species	range	mean	n	mean	±SD	n	mean	±SD
B. affinis	95 - 134	113.2	20	215.3	44.21	30	39.2	7.29
B. arborea	183 - 201	191.6	10	214.9	4.65	10	20.7	1.64
B. australiensis	197 -222	213.5	20	310.1	50.58	70	21.2	8.11
B. basispina	225 - 275	253.5	10	248.3	8.20	10	31.8	1.48
B. buchananensis	204 -232	217.9	10	244.2	8.78	10	36.2	3.71
B. budjiti	141 -155	144.7	23	225.9	6.71	10	20.8	1.70
B. campbelli	162 - 194	172.3	20	191.6	4.95	10	29.2	2.53
B. clandestina				143.1	8.87	10	32.1	2.33
B. compacta				381.4	83.86	30	20.6	6.64
B. complexidigitata	211 - 307	251.0	40	268.0	5.54	10	127.8	4.05
B. denticulata				175.4	10.28	10	39.6	2.66
B. dubia	187 - 215	187.1	32	222.6	4.93	10	30.2	2.17
B. frondosa	185 - 211	191.1	10	202.0	8.29	10	32.7	2.54
B. halsei				189.9	12.07	50	44.2	22.67
B. hattahensis	254 - 289	268.9	20	257.9	6.20	10	44.4	4.41
B. kadjikadji	254	254.0	2	297.8	6.34	10	102.5	13.29
B. lamellata	124 - 180	147.6	31	182.0	12.94	10	47.1	1.90
B. longirostris	264 - 300	276.9	29	276.4	28.64	80	164.1	64.55
B. lyrifera	158 -183	171.5	20	213.4	14.52	20	615	88.68
B. mcraeae				175.0	7.63	10	35.4	2.20
B. nana	144 -158	152.3	19					
B. nichollsi	187 -247	202.3	30	295.3	5.72	10	34.4	1.84
B. occidentalis	550 - 571	565.3	20	492.2	24.46	20	53.1	17.69
B. papillata				293.9	7.80	10	33.0	2.47
B. pinderi				292.1	6.92	10	95.2	3.40
B. pinnata	173 -190	181.1	10	198.8	13.31	20	27.7	3.69
B. proboscida	158 - 187	174.9	19	220.0	8.32	10	72.7	5.65
B. simplex	144 - 201	176.4	32	301.4	12.32	10	41	3.93
B. vosperi				433.4	7.80	10	111.2	5.31
B. wellardi	158 - 176	168.4	30	181.1	8.44	10	47.2	4.69
B. new species K				160.1	8.45	10	41	3.27
B. new species M				223.7	6.08	10	41.5	4.16
B. new species S				169.5	10.43	10	50.4	6.18
B. new species Y				174.7	9.43	10	35.3	3.30

Proc. Linn. Soc. N.S.W., 133, 2011

Table 2 Measurements from populations of some highly variable species; measurements of 10 eggs from each site.

Location	egg size	e in ųm	depre	essions
	mean	±SD	mean	±SD
B. affinis				
Bloodwood Station, NSW	156.3	4.54	33.8	3.82
near Emu Rock, WA	258.3	6.38	47.7	4.74
Grass Patch, WA	231.3	9.11	36.0	2.75
B. australiensis				
L. Hutchinson, Qld	213.0	6.50	15.8	1.23
The Gums, Qld	332.4	7.06	15.0	1.00
Lake Goran, NSW	321.0	5.62	19.2	1.81
Snowleigh Station, NSW	377.8	8.27	17.1	1.62
Poodina, SA	274.3	12.03	39.1	2.88
Kau NR, Esperance WA	318.5	11.88	23.5	2.17
Laverton, WA	338.6	8.35	18.0	1.87
B. compacta				
Avon Lake, NSW	290.0	10.39	28.2	2.31
Little Unicup Lake, WA	485.2	14.32	13.4	2.70
Marchagee Rd, WA	369.1	4.85	20.2	1.00
B. halsei				
L. Hutchinson, Qld	199.3	4.36	31.4	1.85
Bloodwood Station, NSW	198.8	6.53	36.8	3.55
Ilparpa Claypan, NT	188.0	7.50	85.3	6.48
Lake Cronin, WA	173.9	8.66	22.6	1.33
Mundabullagana Station, WA	189.1	12.48	44.0	3.27
B. longirostris				
Walga Rock, WA	271.5	8.43	273.2	14.62
Wardagga Rocks, WA	311.7	11.25	144.0	10.09
Yorkrakine Rocks, WA	308.6	12.00	125.6	8.29
Elachbutting Rocks, WA	296.1	15.28	178.0	17.53
Andersons Rocks, WA	308.3	5.10	246.0	12.94
Mt Madden, WA	256.1	14.33	106.0	11.94
McDermid Rocks, WA	240.8	14.78	162.0	13.04
Yendeng Rocks, WA	218.4	9.47	78.0	11.94
B. occidentalis				
Rockwell Station, Qld	503.6	24.56	69.6	4.81
east of Carnarvon, WA	478.8	19.22	36.6	6.00
B. pinnata				
Lake Dunn, Qld	193.4	5.43	30.6	1.54
Bloodwood Station, NSW	204.3	14.49	24.8	1.76

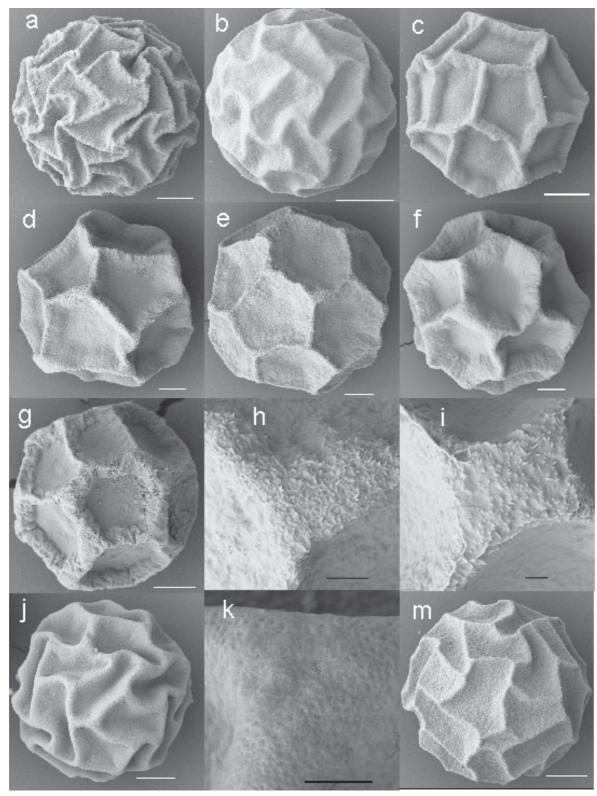


Figure 1. **a**, *B. affinis* Emu Rock; **b**, *B. affinis* Bloodwood; **c**, *B. arborea*, Yantabulla; **d**, *B. australiensis*, The Gums; **e**, *B. australiensis*, L. Goran; **f**, *B. australiensis* Bungarby; **g**, *B. australiensis*, Lake Hutchinson; **h**, *B. australiensis*, L. Goran, details of surface morphology; **i**, *B. australiensis*, Bungarby, details of surface morphology; **j**, *B. basispina*, Balladonia; **k**, *B. basispina*, Balladonia, details of surface morphology;; **m**, *B. buchananensis*, Bloodwood. Scales: white bar 50 µm, black bar 10 µm.

B. arborea Geddes 1981 (Fig. 1c). Average size 214.9 μm, mean depression number 20.7. Most depressions polygonal, some near pentagonal, regularly sized, with distinct triangular ridges with rounded ridgetops and irregularly punctuate. Depressions shallow (wh: dw ratio <0.2) with floors subplanar, with small dimples.

B. australiensis (Richters 1876) (Figs 1d-i). Average size 310.8 µm, mean depression number 21.2. Most populations with eggs larger than 318 μm , and depressions fewer than 20 (Table 2). Depressions largely pentagonal, certainly polygonal (Fig. 1d,e,f,g). Ridges triangular (Fig. 1e) sometimes ropey (Fig. 1g), often with small spines (Fig. 1h,i). Depressions usually dimpled (Fig. 1d,e,f), sometimes smooth (Fig. 1g). Depressions range from shallow (wh:dw ratio <0.2) to moderately deep (wh:dw ratio >0.5) and associated floors vary in degree of concavity. Eastern (L. Hutchinson, The Gums, L. Goran, Bungarby) and western (near Esperance, Laverton) Australian populations with somewhat similar eggs, but the single population from Lake Poodina South Australia different (not illustrated, but see Table 2).

B. basispina Geddes 1981 (Fig. 1j). Average size 248.3 μ m, mean depression number 31.8. Depressions polygonal, irregularly sized, with rounded punctuate ridges merging with floors (Fig. 1k). Depressions deep (wh: dw >0.5) and floors markedly concave.

B. buchananensis Geddes 1981 (Fig. 1m). Average size 244.2 μ m, mean depression number 36.2. Depressions polygonal, irregularly sized, with triangular dimpled walls distinct from weakly concave, strongly dimpled floors. Depressions shallow (wh:dw <0.2).

B. budjiti Timms 2001 (Fig. 2a). Average size 225.9 μm, mean depression number 20.8. Depressions polygonal, fairly regularly sized, and shallow (wh: dw <0.2). Walls rounded and meeting the floors at a distinct break of slope. Ridges with transverse raised areas. Floors weakly concave and strongly dimpled.

 $B.\ campbelli$ Timms 2001 (Fig. 2b). Average size 191.6 µm, mean depression number 29.2. Depressions polygonal, somewhat irregularly sized. Ridges wide, with rounded crests and sloping walls into concave floors. Ridge crests often minutely pitted and walls and depression floors concave; ridge sides and floor strongly dimpled. Depressions shallow (wh:dw about 0.2).

B. clandestina Timms 2005 (Fig. 2c). Average size $143.1 \,\mu\text{m}$, mean depression number 32.1. Depressions irregular, with dimpled floors and sides and thick ridges with rounded crests. Depressions deep (wh:dh >0.5) and floors markedly concave.

B. compacta Linder 1941 (Fig. 2d,e). Average size 381.4 μm, mean depression number 20.6. Size and depression number variable between locations, especially between east (L. Avon) and west (Little Unicup L., Coomberdale) Australia (Table 2). Depressions polygonal, often pentagonal. Ridge crest rounded in Avon Lake site (Fig. 2d), but sharp and ridge triangular in cross section in western sites (Fig. 2e). Floor concave and dimpled in both. Depressions shallow in all populations (wh:dw <0.2).

 $B.\ complexidigitata$ Timms 2002 (Fig. 2f,g). Average size 268.0 μ m, mean depression number 127.8. Most depressions polygonal, similarly sized and shallow (wh:dw <0.2). Ridges narrow, steep sized and with a light coloured fringe midline extended irregularly into sharp points (Fig. 2g). No dimples on walls or on flat floors of depressions.

B. denticulata Linder 1941 (Fig. 2h). Average size 175.4 μ m, mean depression number 39.6. Depressions irregular, often linear with steep-sided ridges and deep (wh:dw >0.5). Floors markedly concave to U-shaped. Ridges and depression floors weakly dimpled.

B. dubia (Schwartz 1917) (Fig.2i). Average size 222.6 μm, mean depression number 30.2. Depressions polygonal, many almost pentagonal. Ridges triangular in cross section, but with rounded crests. Floors of depressions flat; ridge walls and floors with elongated and angular dimples. Depressions shallow (wh:dw<0.2).

B. frondosa Henry 1924 (Fig. 2j). Average size 202.0 μm, mean depression number 32.7. Depressions roughly polygonal, irregularly sized. Ridges with rounded crests and steep sides, though slightly sloping at base. Ridges and depression floors weakly dimpled. Depressions moderately deep (wh:dw ca 0.3 -0.5) and floor almost flat though slightly concave slopping near ridge bases.

B. halsei Timms 2002 (Fig. 3a-d). Average size $189.9 \mu m$, mean depression number 44.2. Depression numbers variable between sites, the Ilparpa claypan

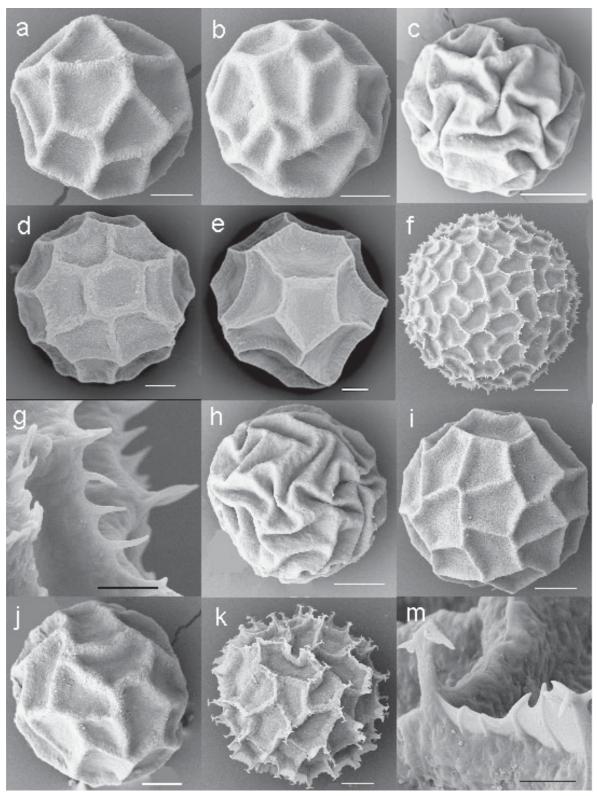


Figure 2. **a**, *B. budjiti*, Rockwell; **b**, *B. campbelli*, Bloodwood; **c**, *B. clandestina*, Yantabulla; **d**, *B. compacta*, Avon Lake; **e**, *B. compacta*, Moora; **f**, *B. complexidigitata*, L. Logue; **g**, *B. complexidigitata*, L. Logue, details of surface morphology; **h**, *B. denticulata*, Carnarvon; **i**, *B. dubia*, Derby; **j**, *B. frondosa*, Clifton Downs; **k**, *B. hattahensis*, Currawinya; **m**, *B. hattahensis*, Currawinya, details of surface morphology. Scales: white bar 50 µm, black bar 10 µm.

near Alice Springs, NT, being the most atypical (Table 2); most have fewer than 40 depressions. Depressions irregular, often linear, with steep sided ridges and deep (wh:dw >0.5), but the Ilparpa eggs with shallow depressions (Fig. 3c). Floor and ridge sides dimpled and ridge crests with minute elongated pits (Fig. 3d).

B. hattahensis Geddes 1981 (Fig 2k,m). Average size 257.9 μm, mean depression 44.4. Depressions polygonal with triangular ridges and flat floors. Ridges often with white membranous extensions supported with spines slightly longer than the membrane, and often with three pronged anchorlike spines usually protruding at ridge wall junctions (Fig. 2m). Depressions tend to be moderate in depth (wh:dw 0.2-0.5) but some shallow (wh:dw <0.2). Depression floor and walls dimpled and sometimes adjacent depressions amalgamated.

B. kadjikadji Timms 2002 (Fig. 3e,f). Average size 297.8 μm, mean depression number 102.5. Depressions polygonal, with adjacent ones sometimes amalgamated. Ridges rounded with a marked break of slope to the flat floor. Crests of ridges extended into white membranes supported regularly with thickened spines slightly longer than the membranes (Fig. 3f). Floor and ridge walls slightly dimpled. Depressions shallow (wh:dw <0.2) if only the basic ridge is considered, but if its membrane is included then the ratio is increased to 0.2 – 0.4.

B. lamellata Timms and Geddes 2003 (Fig. 3g). Average size 182.0 μm, mean depression number 47.1. Depressions irregularly polygonal, with triangular ridges merging with depression floors to give moderate concavities. Ridge crests rounded, floors and walls slightly dimpled. Depressions moderately deep with wh:dw ratio 0.3 -0.5.

B. longirostris Wolf 1911 (Fig 4a-f). Average size 276.4 μm, mean depression number 164.1. Size and number of depressions variable between localities (Table 2). Depressions generally polygonal, but sometimes with some lateral amalgamations (Fig 4a), thin walled and with spines at junctions of walls. These spines generally bi-hooked (Fig 4a,c) but sometimes hookless (Fig. 4d), usually long and numerous (Fig 4a,f), but sometimes sparse, short and stumpy (Fig.4d). Sometimes walls between compartments almost absent (Fig. 4b), or triangular in cross section, so that compartments have markedly concave floors (Fig. 4e), rather than typically flat floors (Fig. 4a). Depression floors typically strongly dimpled (Fig 4a,c,d,e), but may be smooth (Fig. 4b).

Depressions generally moderately or markedly deep (wh:dw >0.5, often >0.8).

B. lyrifera Linder 1941 (Fig 3h,i). Average size 213.4 μm, mean depression number 615. Numerous small rounded pinnacles arranged around polygonal hollows, some amalgamated and many joined by low ridges. Each pinnacle with a few clumped white hair like structures thinning and largely absent from floor of depressions (Fig 3i). Height of pinnacles about half width of depressions. Depression floors concave and lumpy in the Bokeen claypan population (Fig 3h), but flat and smooth in the Plover pan population (not illustrated).

B. mcraeae Timms 2005 (Fig. 3j,k). Average size 175.0 μm, mean number of depressions 35.4. Depressions broadly polygonal, many constricted and linear. Ridges triangular, base merging into floor of depressions so that floor markedly concave. Floor and side walls and wall crest strongly dimpled; wall crest also with pores (Fig. 3k). Depressions moderately deep, (wh:dw 0.3-0.5).

B. nichollsi Linder 1941 (Fig. 3m). Average size 295.3 μm, mean number of depressions 34.4. Depressions polygonal, some pentagonal. Walls triangular and floors weakly concave. Walls and floors strongly dimpled. Depressions shallow, (wh:dw <0.2).

B. occidentalis Dakin 1914 (Fig. 4g,h). Average size 492.2 μm, mean number of depressions 53.1. Depressions irregular polygonal with steep walls and ridgetop with a fringe of short spines (Fig. 4h). Compound pores on ridge walls (Fig. 4h). Depressions deep (wh;Dw >0.5) and floors flat and dimpled (Fig. 4g) or markedly concave (Bulla claypan population). Depressions more numerous and deeper in east Australian population (Bulla claypan) than in population from the west (Carnarvon claypan).

B. papillata Timms 2008 (Fig. 4i). Average size 293.9 μ m, mean number of depressions 33.0. Depressions polygonal, with narrow wall crests and wide bases merging with the floors so that floors weakly concave. Floors and walls moderately dimpled. Depressions shallow, wh: dw <0.2.

B. pinderi Timms 2008 (Fig. 4j,k). Average size 292.1 μm, mean number of depressions 95.2. Depressions regularly polygonal with some amalgamations. Thick walls with base merging with flat floors. Walls and depression floor covered with long hairs, more concentrated on the walls, otherwise almost smooth (Fig. 4k).

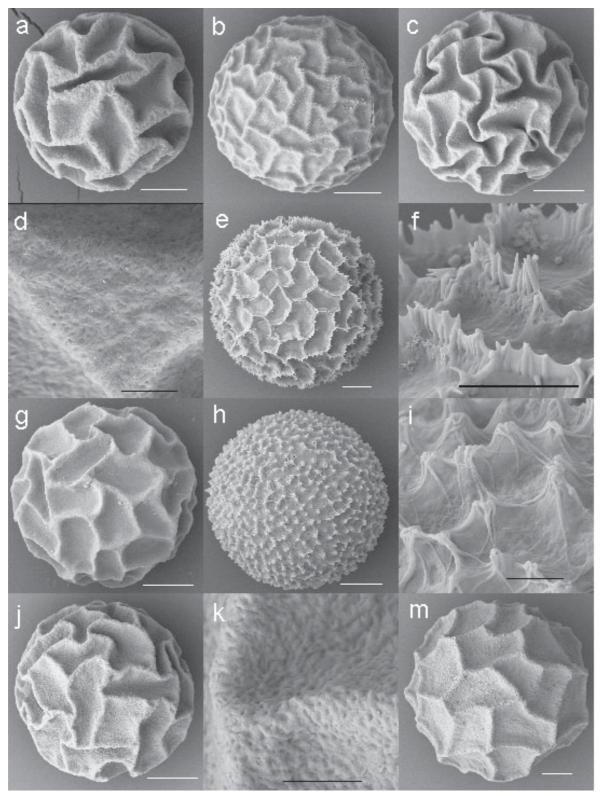


Figure 3. **a**, *B. halsei*, L. Hutchinson; **b**, *B. halsei*, Alice Springs; **c**, *B. halsei*, Pilbara; **d**, *B. halsei*, L. Hutchinson, details of surface morphology; **e**, *B. kadjikadji*, Wyalkatchem; **f**, *B. kadjikadji*, L. Hutchinson, details of surface morphology; **g**, *B. lamellata*, Thargomindah; **h**, *B. lyrifera*, Bokeen claypan, Currawinya; **i**, *B. lyrifera*, Currawinya, details of surface morphology; **j**, *B. mcraeae*, Onslow; **k**, *B. mcraeae*, Onslow, details of surface morphology; **m**, *B. nichollsi*, Kalgoorlie. Scales: white bar 50 µm, black bar 10 µm.

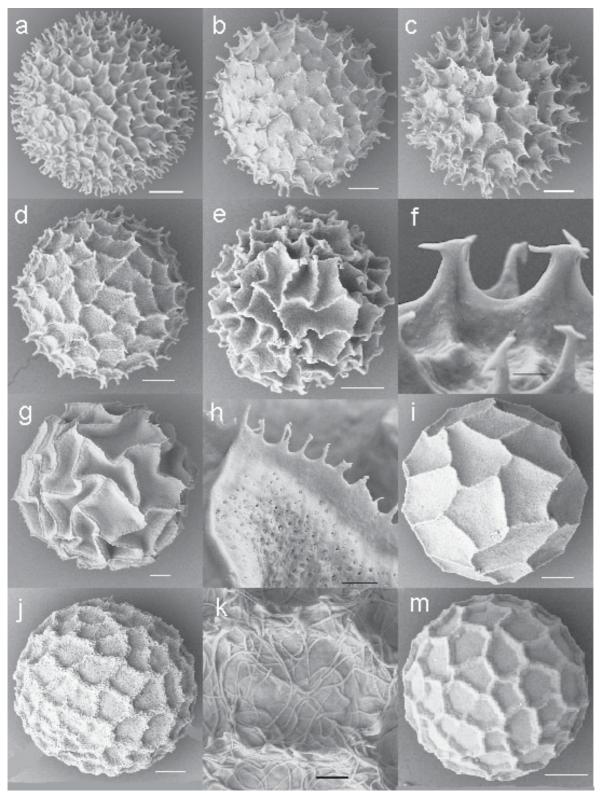


Figure 4. **a**, *B. longirostris*, Walga Rock; **b**, *B. longirostris*, Wardagga Rock; **c**, *B. longirostris*, Yorkrakine Rock; **d**, *B. longirostris*, Mt Madden; **e**, *B. longirostris*, Yendang Rock; **f**, *B. longirostris* Walga Rock, details of surface morphology; **g**, *B. occidentalis*, Carnarvon; **h**, *B. occidentalis*, Carnarvon, details of surface morphology; **i**, *B. papillata*, Esperance; **j**, *B. pinderi*, Onslow; **k** *B. pinderi*, Onslow, surface details; **m**. *B. proboscida*, Bloodwood. Scales: white bar 50 µm, black bar 10 µm.

B. pinnata Geddes 1981 (Fig. 5a,b). Average size 198.8 μ m, mean number of depressions 27.7. Depressions bold, polygonal with some constricted and all bordered by wide walls. Walls merge basally into almost flat floors. Floors and walls dimpled and ridge crests with numerous minute grooves (Fig 5b). Depressions moderately deep (wh:dw 0.3 – 0.5).

B. proboscida Henry 1924 (Fig. 4m). Average size 220.0 μm, mean depression numbers 72.7. Depressions regularly polygonal, some pentagonal and a few amalgamations. Walls triangular on a flat, slightly dimpled floor. Depressions shallow (wh:dw <0.2).

B. simplex Linder 1941 (Fig 5c,d). Average size 301.4 μ m, mean depression numbers 41.0. Depressions regularly polygonal with some pentagonal. Walls ropey and flat floors with strong dimples (Fig. 5d). Depressions shallow (wh:dw < 0.2).

B. vosperi Timms 2008 (Fig. 5e,f,g). Average size 433.4 μm, mean depression numbers 111.2. Depressions polygonal, various sizes and many amalgamations. Walls thin, vertical and with thin extensions anastomosing and forming free filaments (Fig. 5f,g). Even thinner membrane between some of the anastomosing branches and extending to based of the free filaments. Depressions thus unusually deep (wh:dw > 0.8), floors flat and lacking dimples.

B. wellardi Milner 1929 (Fig. 5h). Average size 181.1 μ m, mean depression numbers 47.2. Depressions polygonal, ridges triangular in cross section, floors concave and weakly dimpled. Some elevated flat areas between depressions. Depressions moderately deep (wh:dw 0.3-0.5).

Branchinella new species K (Fig.5i). Average size $160.1 \, \mu m$, mean depression number 41.0. Depressions polygonal with thick walls forming wide rounded ridges. Floors subplanar, except where invaginated, and smooth. Depressions shallow (wh:dw <0.2).

Branchinella new species M (Fig. 5j). Average size 223.7 μm, mean depression number 41.5. Depressions somewhat polygonal, but generally constricted and often linear. Walls thick with ridge crests lumpy facilitated by weak transverse grooves. Floors concave and strongly dimpled. Depressions moderately deep (wh:dw 0.3 -0.5).

Branchinella new species S (Fig. 5k). Average size 169.5 μm, mean depression number 50.4. Depressions

polygonal with thick walls forming wide rounded ridges. Floors flat to slightly concave, moderately dimpled. Depressions shallow to moderately deep (wh:dw 0.2-0.4).

Branchinella new species Y (Fig. 5m). Average size 174.7 μ m, mean depression number 35.3. Depressions irregularly polygonal, many constricted and linear. Walls wide and ridge crests rounded and with minute pores. Floors vary from subplanar in shallow depressions (wh:dw <0.2) to concave in deep depressions (wh:dw >0.5). Floors and ridge walls moderately dimpled.

DISCUSSION

While egg morphology in large branchiopods is not as immutable as it was once thought (Brendonck et al. 1990; Mura & Rossetti, 2010), there is still value in understanding the range of structures seem in the various species. Sometimes morphologies are distinct and invariable enough to be able to construct a key to species (e.g. for Eulimnadia of the world, Rabet, 2010, and pers. comm..), but in many genera there are species with unique morphology and other species which are too variable to choose a morphotype as distinctive. This is the case for Chirocephalus (Anostraca: Chirocephalidae) in Italy (Mura, 2001; Mura and Rossetti, 2010), Branchinecta (Anostraca: Branchinectidae) in North America (Mura, 1991a) and for Branchinella in Australia the situation is imtermediate

In understanding egg morphologies it is important to optimise the chances for studying mature unaltered eggs. Only mature females with full ovisacs of mature eggs should be chosen (Mura, 1991b) and the same author liked to obtain eggs from live females by allowing them to drop their eggs isolated in small containers, and hence avoid contamination. In the present study and many others (Brendonck et al. 1990; Thiery & Gasc, 1991) this was not possible and eggs were carefully removed from the brood pouch of a female isolated from others to prevent contamination. Another factor rarely mentioned by other authors is the effect of dehydration (associated with egg age or environment or preservation?) on egg surface morphology. Many eggs, mainly those without strong polygonal morphology, appeared shrivelled, and if they could have been expanded then similarities in structure would have been more apparent. In the case of Tanymastix stagnalis (Linneaus) variability in egg shape was shown to be due to variable embryo volumes (Thiéry et al., 2007). In the present study

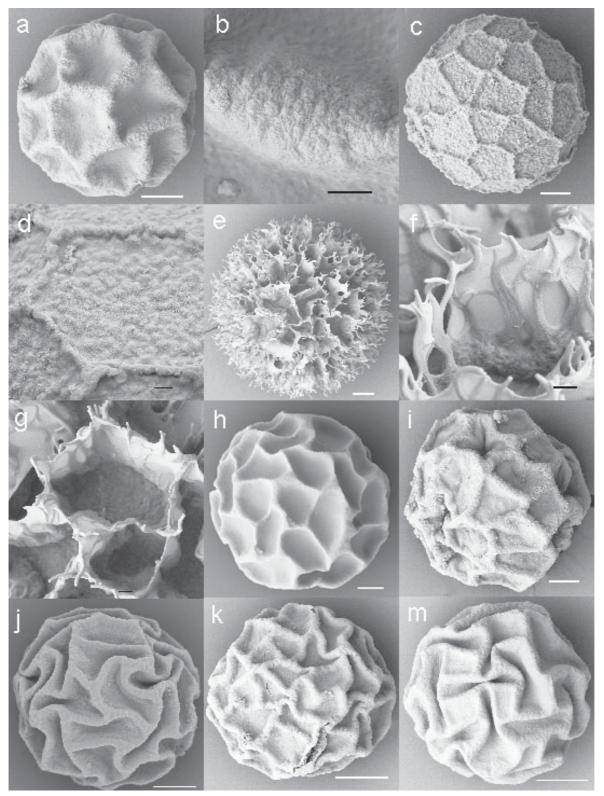


Figure 5. **a**, *B. pinnata*, Bloodwood; **b**, *B. pinnata*, Bloodwood, details of surface morphology; **c**, *B. simplex*, Lake Carey; **d**, *B. simplex*, Lake Carey, details of surface morphology; **e**, *B. vosperi*, Esperance; **f**, *B. vosperi*, Esperance, details of surface morphology; **g**, *B. vosperi*, Esperance, details of surface morphology; **h**, *B. wellardi*, Bloodwood; **i**, *Branchinella* n. sp. K, Birdsville Track; **j**, *Branchinella* n. sp. M, Moora; **k**, *Branchinella* n. sp. S, Sumana; **m**, *Branchinella* n. sp. Y, Yarromere. Scales: white bar 50 µm, black bar 10 µm.

the simple explanation of variable dehydration associated with preservation is more likely. This shrivelling would have been another factor affecting egg size so that standard deviations for egg size need to be interpreted with care. Presumedly dehydration also occurs in nature, so that eggs in sediments could change shape and size as they age, but this has not been investigated.

All species in which more than one population was studied exhibited variation in egg morphology (Table 2), though usually within acceptable limits. For the widespread B. australiensis at least three morphotypes were observed (Fig. 1, Table 2) I Bloodwood, II The Gums, L Goran, Laverton, Snowleigh and III Poodina). For B. longirostris isolated on numerous inselbergs in Western Australia, at least six morphotypes were evident (Fig. 4, I = Andersons, Elachbutting, Walga; II = Madden; III =McDermid; IV = Yorkrakine; V = Wardagga; VI = Yendang). In the case of B. longirostris where morphologies of adult males are known (Zofkova and Timms, 2009), there is no relationship between egg type and adult features, as is also the case for Chirocephalus (Mura and Rossetti, 2010). For B. affinis, shrivelling associated with dehydration would explain most of the differences observed between populations (Fig. 1), though in B. halsei, the L Cronin and Ilparpa populations could represent different morphotypes (Fig. 3). In B. compacta there are either two morphotypes (Fig.2, I = L Avon; II = Little Unicup, Marchagee) or perhaps the difference between east and west Australia suggest the two groups may be separate species. In this case the two groups exhibit adult morphological differences, to be examined elsewhere. Though only one morphotype was noted in the present study for B. occidentalis, two apparently different ones were recorded by Timms et al. (2004). This situation has not been resolved, and indeed all the morphotypes mentioned above are enigmatic, much like the situation in Chirocephlaus ruffoi (Mura & Rossetti, 2010).

Egg sizes vary markedly within and between species, not only in this study but for many species shared between Timms et al. (2004) and this study (Table 1). Three of the seven species where multiple populations were studied had egg sizes varying more than 65% (Table 2) and five of the 22 species common to Timms et al. (2004) and this study had sizes varying in excess of 45% (Table 1). Of the many factors affecting egg size (Mura, 1991b) altitude of collecting site and water chemistry are hardly important in this study, but female size could be. Although many studies have shown no such relationship (e.g. Mura, 1991b, Belk, 1977), for the

species studied here (ranging from 8 to 33 mm) there is a positive relationship between female length and the size of eggs (y = 9.485x +100.0; r²= 0.54, P >0.05%, where y is egg size and x is female length when preserved. It is conceivable that as females grow the eggs they produce in each batch could increase in size and so result in a range of sizes for a species. Species with particularly large eggs (> 300 um) include, in order from the largest, *B. occidentalis, B. vosperi, B. compacta, B. australiensis and B. simplex.* At the other extreme, small eggs (< 200 um) are typical in *B. clandestina, Branchinella* new species K, S and Y, *B. denticulata* and *B. mcraeaee*.

The number of depressions on the egg surface is also variable within species. Across all species the mean number of depressions is 69 and median 39. Species with numerous depressions include (from most numerous), *B. lyrifera, B. complexidigitata, B. kadjikadji, B. vosperi* and *B. pinderi* (all > 95) Likewise species with unusually low numbers of depressions include *B. compacta, B. arborea, B. budjiti* and *B. australiensis* (all between 20.6 and 21.2). The number of depressions is unrelated to egg size and can vary between populations (Table 1 and 2). This is seen in *B. affinis, B. australiensis, B. compacta, B. halsei, B. longirostris* and *B. occidentalis* where standard deviations increased markedly when multiple populations were studied (Table 1).

Other morphometric features separating species include the presence of pores, particularly on wall crests, spines and/or transverse ridges on wall crests, the degree of dimple development, on the floors of the depressions, and most importantly the nature of superficial adnornments in the form of membranes and spines on the ridges. The adaptive value of these features is unknown, though for *B. longirostris* the hooked spines may be a deterrent for egg predators such as planarians known to be common in their habitat (Dumont, et al., 2002; Jocqué et al., 2007).

By a combination of all these features the following species have distinctive eggs (with reasons in parenthesis): *B. occidentalis* (egg size, complex pores, secondary frill on ridge crests), *B. longirostris* (numerous hooked spines, numerous depressions), *B. vosperi* (secondary membranes between support struts, numerous depressions, large egg), *B. lyrifera* (extremely numerous depressions, depression walls in form of mounds covered with hairs) *B. pinderi* (depression walls and adjacent floor covered with hairs), *B. kadjikadji* and *B. complexidigitata* (numerous depressions, crests with membranes between spines), *B. hattahensis* (few hooked spines and crest with incomplete membranes and few spines, strong dimpling of depression floors), *B.*

simplex (strong dimpling of depression floors, ropey depression walls, shallow depressions), *B. australiensis*, *B. compacta*, and *B. arborea* (all with few depressions, many tending towards pentagonal, shallow depressions) and *B. budjiti* (few depressions, many tending towards pentagonal, transverse ridges on walls, depression floors strongly dimpled). Species with minute pores in compartment walls and crests include *B. basispina*, *B. halsei* (not all populations), *B. mcraeae*, *B. occidentalis* (complex pores) and *B. pinnata*. Only *B. australiensis* (most populations) have numerous small spines (spikes) on elevated areas.

Because many species have similar eggs, or eggs which vary so that some populations have eggs similar to those of other species, it is difficult to establish a dichotomous key to delineate eggs. Eggs in sediments may also be dehydrated, although probably similarly to those preserved in alcohol. The key provided below does not identify all species, only those with distinctive characters. In the couplets only characters visible at up to 100x magnification are used and care has been exercised in its preparation to allow for greater variation (20%) than that generally observed in this study. By using greater magnification and referring to the descriptions above and Figs. 1-5 and Table 1 and 2, it may be possible to separate species among the groups in some couplets. Generic differentiation is certainly possible (Timms et al., 2004) and with this key many species of Branchinella can now be identified.

It remains to consider if there are any phylogenetic relationships between the egg types and the three systematic groups thought significant by Geddes (1981). While there are four pairs with considerable similarity — B. australiensis and B. compacta in Geddes' Group 1, B. affinis and B. denticulata in his Group II and B. basispina and B. frondosa, also B. dubia and B. arborea in Group III — there are far more dissimilarities than these few similarities (for example B. occidentalis is unique in Group I, B. longirostris is unique in Group II, and B. wellardi is unique in Group III. If species described since 1981 are added to the groups (Timms, 2002, 2005, 2009), then possible interrelationships become even more blurred. Overall though, resting eggs of Branchinella (subgenus Branchinella), which is endemic to Australia (Rogers, 2006) are characterised by having polygonal depressions and few spines. The only species in the subgenus Branchinellites studied (B. kugenumaensis and B. madurai, Brendonck and Belk, 1997) also have polygonal depressions, but the former has smooth ridges and the later dense spinulae, like those in *B. australiensis*.

KEY TO EGGS OF SOME SPECIES OF BRANCHNELLA

[Use carefully. In couplets relying solely on in differences in size and number of depressions, it is possible not enough leeway has been given to possible variation in known values. So check the result against the descriptions and figures. The most reliable delinations are among those species with adornments (couplets 3 to 9).]

1a. Eggs with grooved surface depressionss (i.e.
long deep furrows on surface), either dominating or
sometimes secondary to elongated polygonal
compartments2
1b. Eggs with most surface depressions polygonal with
subequal axes, but perhaps with a few as elongated
polygons; no grooved depressions3
2 The following species are hard to separate largely

2 The following species are hard to separate, largely because their depression characteristics depend largely on the state of hydration. If the eggs are larger than 235 um they are likely to be *B. basispina* and if smaller than 180 um they could be *B. clandestina*, *B. denticulata* or *Branchinella* n. sp. Y. Other species with grooved surface depressions include *B. affinis*, most populations of *B. halsei* and *Branchinella* n. sp. M

3a (1b). Depression wall crests with adornments
adding to their height; adornments aligned along
crests4
3b (1b). Depression wall crests smooth or lumpy or
even slightly ridged, but without protruding spines,
hooks or membranes (B. australiensis may have very
short spines on the crests, but they are unaligned)9

4a (3a). Crest adornments string-like applied to
surface5
4b (3a). Crest adornments in the form of protruding
spines or membranes6

7a (6b). Spines simple	16b (13b) Walls of depressions triangular in cross section so crests sharpish
like ship's anchors8	17a (16a) Depressions relatively shallow, depth/width ratio > 0.25; number of depressions > 75
9. (7h) Eid- <0 damas-isdamas-is11-	
8a (7b). Eggs with <60 depressions; depression walls	17b (16a) Depressions deep, depth/width ratio > 0.3
inverted U-shaped; membrane between anchor-like	number of depressions < 60
spines with supporting struts; eggs from inland eastern	B. frondosa, Branchinella n. sp. K, n. sp. S
Australia	19a (16b) Eas sine > 210 mm
8b (7b). Eggs with >75, often >125 depressions;	18a (16b) Egg size > 210 \(\text{um}\)
depression walls thin sheets or rarely lacking; often	<i>B. dubia</i> 18b (16b) Egg size < 200 ym19
with a short membrane between spines and never with supporting struts; eggs from pan gnammas of	180 (100) Egg Size < 200 till
WA	19a (18b) Floor of depressions smoothB. wellardi
wAb. tonguosu is	19b (20b) Floor of depressions with tumidities
9a(3b). Eggs with short spines on crests of depressions;	
eggs with flat elevated areas between depressions; usually <34 depressions	
9b (3b). Eggs without short spines on depression	ACKNOWLEDGEMENTS
crests; eggs usually without flat elevated areas	TIGILI (O (VEDE GENERAL)
between depressions (of present, then >35	BVT thanks numerous landholders across Australia
depressions)10	for access to their ponds and pans for collecting specimens
•	We are both thankful for Christopher Rogers and two
10a (9b). Eggs large > 450 μm diameter; depression	anonymous referees whose suggestions greatly improved
wall crest ridged and frilled, this being of similar	the manuscript.
material to that of crest and not a white adornment	
B. occidentalis	
10b (9b). Eggs smaller < 400 μm diameter, often	REFERENCES
much smaller; depression wall smooth or wrinked	
transversely11	Beladjal, L., and Mertens, J., (2003). Interstitial remains
	for fauna reconstruction of desert pools using fairy shrimps as example (Anostraca). <i>Journal of</i>
11a (10b) Fewer than 25 depressions <i>B. arborea</i> ,	Crustacean Biology 23: 60-68.
B, australiensis, B. budjiti, B. compacta	Belk, D., (1977). Zoogeography of the Arizona fairy
11b (10b) More than 26 depressions12	shrimps (Crustacea, Anostraca). Journal of the
12 (111) M 4 (0.1 P 1	Arizona-Nevada Academy of Science 12: 70-78.
12a (11b) More than 60 depressions <i>B. proboscida</i>	Brendonck, L., and Belk, D., 1997. Branchinella
12b (11b) Fewer than 50 depressions	maduraiensis Raj (Crustacea, Branchiopoda, Anostraca) shown by new evidence to be a valid
12. (121) F	species. <i>Hydrobiologia</i> 359 : 93-99.
13a (12b) Egg size > 275 um	Brendonck, L., and Coomans, A., (1994a). Egg
13b (12b) Egg size < 270 um16	morphology in African Streptocephalidae (Crustacea,
14. (12.) Demosione com challent death/width	Branchiopoda, Anostraca). Part I. South of Zambezi
14a (13a) Depressions very shallow, depth/width	and Kunene rivers and Madagascar. Archiv für
ratio <0.10; compartment floors with large tumidities; compartment walls very uneven (lumpy)B. simplex	Hydrobiologie 99 : 313-334.
14b (13a) Not as above	Brendonck, L., and Coomans, A., (1994b). Egg
140 (13a) Not as above13	morphology in African Streptocephalidae (Crustacea, Branchiopoda, Anostraca). Part II. North of Zambezi
15a (14b) Fewer than 30 depressions, relatively deep	and Kunene rivers. Archiv für Hydrobiologie 99 : 335-
(depth/width ratio >0.4)	356.
15b (14b) More than 31depressions, moderately deep	Brendonck, L., Thiéry, A., and Coomans, A., (1990).
(depth/width ratio 0.2-0.4) <i>B. nichollsi</i> , <i>B. papillata</i>	Taxonomy and biogeography of the Galapagos
(separ main ratio 0.2 0.1)D. menousi, D. papmata	Branchiopod fauna (Anostraca, Notostraca and
16a (13b) Walls of depressions wide and rounded (i.e.	Spinicaudata). Journal of Crustacean Biology 10:
	676-694.
inverted U-shaped), so crest is rounded17	

- Daday, E., (1910). Monographie systématique des Phyllopodes Anostracés. Annals des Sciences Naturelles, Paris (Zoologie) 9: 91-492.
- Dumont, H.J., Nandini, S., and Sarma, S.S.S., (2002). Cyst ornamentation in aquatic invertebrates: a defence against egg predation. *Hydrobiologia* **486**: 161-167.
- Geddes, M. C., (1981). Revision of the Australian species of Branchinella (Crustacea: Anostraca). Australian Journal of Marine and Freshwater Research 32: 253-295.
- Hill, R.E. and Shepard, W.D., (1997). Observations on the identification of Californian anostracan cysts. *Hydrobiologia* **359**: 113-123.
- Jocqué, M., Timms, B.V., and Brendonck, L., (2007). A contribution on the biodiversity and conservation of the freshwater fauna of rocky outcrops in the central Wheatbelt of Western Australia. *Journal of the Royal* Society of Western Australia 90: 137-142.
- Mura, G., (1986). SEM morphological survey on the egg shell in the Italian Anostracans (Crustacea, Branchiopoda). *Hydrobiologia* **134**: 273-286.
- Mura, G., (1991a). SEM morphology of resting eggs in the species of the genus *Branchinecta* from North America. *Journal of Crustacean Biology* 11: 432-436.
- Mura, G., (1991b). Additional remarks on cyst morphometrics in Anostracans and its significance. Part I: Egg size. *Crustaceana* **61**: 241-252.
- Mura, G. (1992a). Pattern of egg shell morphology in Thamnocephalids and Streptocephalids of the new world (Anostraca). *Crustaceana* **62**: 300-311.
- Mura, G., (1992b). Additional remarks on cyst morphometrics in Anostracans and its significance. Part II: Egg morphology. *Crustaceana* **63**: 225-246
- Mura, G., (2001). Morphological diversity of the resting eggs in the anostracan genus *Chirocephalus* (Crustacea, Branchiopoda). *Hydrobiologia* **450**: 173-185
- Mura, G., and Rossetti, G., (2010). Intraspecific morphological diversity of anostracan resting eggs: *Chirocephalus ruffoi* Cottarelli and Mura, 1984 as a study case. *Journal of Biological Research, Thessaloniki* 14: 137-150.
- Rabet, N., Revision of the egg morphology of *Eulimnadia* (Crustacea, Branchiopoda, Spinicaudata). Zoosystema 32(3): 373-391.
- Rogers, D.C., (2006). A genus level revision of the Thamnocephalidae (Crustacea: Branchiopodidae: Anostraca). *Zootaxa* 1260: 1-25.
- Thiéry, A., and Gasc, C., (1991). Resting eggs of Anostraca, Notostraca and Spinicaudata (Crustacea, Branchiopoda) occurring in France: identification and taxonomical value. *Hydrobiologia* **212**: 245-259.
- Thiéry, A., Rabet, N. and Néve, G., 2007. Modelling intraspecific resting egg shape variation in a freshwater fairy shrimp *Tanymastix stagnalis* (L., 1758)(Crustacea, Branchiopoda). *Biological Journal of the Linnean Society of London* 90: 55-60.
- Timms, B.V., (2002). The Fairy Shrimp Genus *Branchinella* Sayce (Crustacea: Anostraca: Thamnocephalidae) in Western Australia, including a

- description of four new species. *Hydrobiologia* **486**: 71-89
- Timms, B.V., (2005). Two new species of *Branchinella* (Anostraca: Thamnocephalidae) and a reappraisal of the *Branchinella nichollsi* group. *Memoirs of the Queensland Museum* **50:** 441-452.
- Timms, B.V., (2008). Further studies on the fairy shrimp genus *Branchinella* (Crustacea, Anostraca, Thamnocephalidae) in Western Australia, with descriptions of new species. *Records of the Western Australian Museum* **24:** 289-306.
- Timms, B.V., (2004). An Identification Guide to the Fairy Shrimps (Crustacea: Anostraca) of Australia. CRCFC Identification and Ecology guide No 47, Thurgoona NSW, 76pp.
- Zofkova, M. and Timms, B.V. (2009). A conflict of morphological and generic patterns in the Australian anostracan *Branchinella longirostris*. *Hydrobiologia* **635**, 67-80.

Appendix 1. Localities for the species studied

Species	Locality	Coordinates	Date	Collector*
B. affinis	Turkey Pan, Bloodwood Station, 130 km NW of Bourke, NSW	29° 33′ 23″S; 144° 50′ 15″E	31-iii-1999	BVT
B. affinis	a rock pool near Emu Rock, 51 km E of Hyden, WA	32° 26' 54S; 119° 24' 34'E	21-ix-2004	BVT
B. affinis	unnamed lake on Guest Rd, Grass Patch, WA	33° 07' 56"S; 121° 48' 10"E	19-i-2007	BVT
B. arborea	roadside pool, N Yantabulla, 125 km NW of Bourke, NSW	29° 19' 5"S; 144° 00' 32"E	18-ii-2010	BVT
B. australiensis	L. Hutchinson, 39 km E of Thargomindah, Qld	27° 55' 32"S; 144° 12' 47"E	9-vi-1998	BVT
B. australiensis	a gilgai 22 km W of The Gums, Qld	27° 23′ 20″S; 149° 58′ 7″E	9-vi-2008	BVT
B. australiensis	Lake Goran, 20 km S of Curlewis, NSW	31° 17' 15"S; 150° 11' 28"E	8-iv-1996	BVT
B. australiensis	East Snowleigh Lake, Bungarby, 51 km S of Cooma, NSW	36° 40' 51"S; 148° 59' 59"E	13-iii-2010	BVT
B. australiensis	Lake Poodina, Gawler Ranges, SA	31° 55′ 30″S; 135° 13′ 10″E	30-i-2007	PH & GT
B. australiensis	pool on Mt Nev Track, 56 km NW of Esperance, WA	33° 28' 26S; 122° 21' 25"E	30-i-2007	BVT
B. australiensis	pool 20 km W of Laverton, WA	28° 35' 39"S; 122° 13' 3"E	22-i-2007	BVT
B. basispina	Balladonia Rock, 200 km E of Norseman, WA	32° 27' 40"S; 123° 51' 30'E	18-i-2007	BVT
B. buchananensis	Gidgee Lake, Bloodwood Station, 130 km NW of Bourke, NSW	29° 32′ 50″S; 144° 50′ 6″E	20-ii-2010	BVT
B. budjiti	clay pan on Rockwell Station, 180 km SW of Cunnamulla, Qld	28° 54' 4"S; 144° 57' 12"E	9-vi-2007	BVT
B. compacta	Avon Lake, 45 km S of Cooma, NSW	36° 37′ 04″S; 149° 02′ 58″E	14-iii-2010	BVT
B. compacta	Little Unicup Lake, 13 km NNE of L. Muir, WA	34° 19' 54"S; 116° 42' 43'E	18-viii-2009	BVT
B. compacta	Coomberdale West Rd, 20 km N of Moora, WA	30° 28' 03"S; 115° 59' 21"E	6-ix-2009	BVT
B. campbelli	Muella Lake, Bloodwood Station, 130 km NW of Bourke, NSW	29° 30' 26"S; 144° 53' 9"E	1-v-1998	BVT
B. clandestina	swamp at Yantabulla, 125 km NW of Bourke, NSW	29° 19' 36"S; 145° 00' 14"E	20-i-2010	BVT
B. complexidigitata	pool near Lake Logue, 13 km SW of Eneabba, WA	29° 59' 0"S; 115° 07' 43"E	17-ix-2009	BVT
B. denticulata	unnamed canegrass pan, Carnarvon area, WA	24° 47"S; 114° 09"E	unknown	m SH
B. dubia	pool, 89 km E of Derby on Gibb R Rd, WA	17° 26"S; 124° 26"E	31-i-1985	MT
B. frondosa	pool on Clifton Downs Station, 135 km NW of Bourke, NSW	29° 19' 53"S; 144° 29' 26"E	8-vi-2007	BVT
B. halsei	L. Hutchinson, 39 km E of Thargomindah, Qld	27° 55' 32"S; 144° 12' 47"E	17i-2007	BVT
B. halsei	Crescent Pool, Bloodwood Station, 130 km NW of Bourke, NSW	29° 32' 34"S; 144° 51' 33"E	18-x-2006	BVT
B. halsei	Ilparpa claypan, Alice Springs, NT	23° 45' 14"S; 133° 45' 52"E	13-i-2010	JR
B. halsei	Lake Cronin, 82 km E of Hyden, WA	32° 23' 5"S; 119° 45' 53'E	16-x-2008	BVT
B. halsei	Yarraloola claypan, Mundabullengana Station, Pilbara, WA	21° 25′ 12″S; 145° 41′ 0″E	18-viii-2005	$\mathrm{JM} \ \& \ \mathrm{AP}$
B. hattahensis	Mid Kaponyee Lake, Currawinya Nat. Pk., via Hungerford, Qld	28° 50' 9"S; 144° 20' 1"E	7-xii-1999	BVT

Appendix 1 continued	73			
B. kadjikadji	claypan near Cowcowing Lakes, Wyalkatchem, WA	30° 57' 52"S; 117° 27' 37"E	28-viii-2004	BVT
B. lamellata	claypan, Bindegolly Nat. Pk., 41 km E of Thargomindah, Qld	28° 00' 0"S; 144° 14' 12"E	1-x-2001	BVT
B. longirostris	Walga Rock, 48 km E of Cue, WA	27° 24' 10"S; 117° 27' 52"E	26-viii-2001	BVT
B. longirostris	Wardagga Rocks, 24 km SW of Paynes Find, WA	29° 23' 21"S; 117° 30' 1."E	25-viii-2001	BVT
B. longirostris	Yorkrakine Rock, 25 km N of Tammin, WA	31° 25' 15"S; 117° 30' 53"E	2-viii-2003	BVT
B. longirostris	Elachbutting Rock, 53 km NE of Mukinbudin, WA	30°35'30"S; 118°36'43"E	2-viii-2003	BVT
B. longirostris	Anderson Rock, 32 km N of Hyden, WA	32° 10' 5"S; 118° 51' 23"E	28-viii-2001	BVT
B. longirostris	Mt Madden, 42 km N of Ravensthorpe, WA	33° 14' 23"S; 119° 50' 32"E	5-ix-2010	BVT
B. longirostris	McDermid Rock, 180 km E of Hyden, WA	32° 01' 16"S; 120° 44' 13"E	19-i-2009	BVT
B. longirostris	Yendang Rk 155 km W of Menzies, WA	29° 18' 29"S; 120° 18' 16"E	1-ix-2004	BVT
B. lyrifera	Bokeen claypan, Currawinya Nat. Pk. Via Hungerford, Qld	28° 49' 50"S; 144° 20' 57"E	26-vi-2000	BVT
B. lyrifera	Plover claypan, Bloodwood Station, 130 km NW of Bourke, NSW	29° 31' 1S; 144° 49' 39"E	7-viii-1998	BVT
B. mcraeae	a claypan near Onslow, WA	21° 47' 36"S; 115° 06' 1"E	15-ii-2009	BVT
B. nichollsi	swamp on Yarri Rd, 19 km NE of Kalgoorlie, WA	30°37'3"S; 121°36'0"E	24-i-2007	BVT
B. occidentalis	N Bulla claypan, Rockwell Station, 180 km SW of Cunnamulla, Qld	28° 53' 2"S; 144° 56' 1"E	9-vi-2007	BVT
B. occidentalis	a claypan inland of Carnarvon, WA	unknown	23-viii-1994	SH
B. papillata	pool near Kau Rock, 59 km NE of Esperance, WA	33° 24' 32"S; 122° 19' 47"E	29-i-2007	BVT
B. pinderi	a claypan near Onslow, WA	21° 48' 13"S; 115° 06' 1"E	15-ii-2009	BVT
B. pinnata	pool near Lake Dunn, 68 km NE of Aramac, Qld	22° 39' 00"S; 145° 43' 01"E	12-ii-2010	BVT
B. pinnata	Crescent Pool, Bloodwood Station, 130 km NW of Bourke, NSW	29° 32′ 34″S; 144° 51′ 33″E	19-i-2010	BVT
B. proboscida	Dead Ram Pan, Bloodwood Station, 130 km NW of Bourke. NSW	29° 31' 46"S; 144° 52' 2"E	2-vi-2001	BVT
B. simplex	Standpipe Ck, Lake Carey, 30 km S of Laverton, WA	28° 56' 49"S; 122° 23' 32"E	24-iv-2004	BD
B. vosperi	pool on Mt Nev Track, 56 km NW of Esperance, WA	33° 28' 29"S; 122° 21' 24"E	30-i-2007	BVT
B. wellardi	Marsilea Pond, Bloodwood Station, 130 km NW of Bourke, NSW	29° 32' 13"S; 144° 52' 26"E	7-vi-2007	BVT
Branchinella n. sp. K	Branchinella n. sp. K dam on Kulamurina Station 205 km N of Marree, SA	unknown	15 -vi-2000	unknown
Branchinella n. sp. M	Branchinella n. sp. M pool on Coorow-Green Head Rd, 20 km SW of Coorow, WA	29° 57' 58"S; 115° 55' 1"E	5-ix-2009	BVT
Branchinella n. sp. S	Branchinella n. sp. S claypan on Sumana Station, 100 km N of Aramac, Qld	22° 18' 38"S; 145° 52' 57"E	2-iv-2009	BVT
Branchinella n. sp. Y	pool on nw beach of Lake Buchanan, Yarromere Station, Qld	21° 32′ 14″S; 145° 48′ 51″E	26-ii-2008	BVT

* BD = Bindy Datson; PH & GT =Peter Hudson & Graeme Thomason; SH = Stuart Halse; JM & AP = Jane Mcrae and Adrian Pinder; JR = Jochem van der Reijden; BVT = Brian Timms; MT = Mike Tyler.