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Biancolina japonica Ishimaru 1996: first record of this burrowing amphipod from Australia and a review of host use in the genus *Biancolina* (Amphipoda: Peracarida: Crustacea)

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Abstract

Background: *Biancolina* Della Valle (Monograpie 20:1893) are minute amphipods that bore tunnels into algal tissue. Their global distribution and diversity are poorly known due to their small size and concealed habit.

Results: *Biancolina japonica* Ishimaru (J Crust Biol 16:395–405, 1996) is reported from Bare Island, Botany Bay, New South Wales, Australia and is only the second species of *Biancolina* recorded in Australian waters following *B. australis* Nicholls (Rec South Aust Mus 6:309–334, 1939). *B. japonica* is found exclusively on species of *Sargassum* where it burrows into algal tissues to feed.

Conclusions: We report *Biancolina japonica* Ishimaru (J Crust Biol 16:395–405, 1996), previously known only from Japan, for the first time in Australian waters. A review of the known algal hosts of all eight species of *Biancolina* indicates high levels of feeding specialisation on brown algae from the order Fucales.

Keywords: *Biancolina japonica*, Amphipods, Australia, Burrowing

Background

Biancolina Della Valle, 1893 are minute amphipods, usually less than 2 mm as adults, that bore tunnels into algal tissue. Eight species of *Biancolina* have been recorded world-wide, from the Sea of Japan, Hawaii, Caribbean Sea, Mediterranean, Sargasso Sea and southern Australia. Diversity in the genus *Biancolina* is most likely underrepresented owing to their small size and concealed habit. Here, we report *Biancolina japonica* Ishimaru, 1996, previously known only from Japan, for the first time in Australian waters.

Biancolina japonica was found exclusively on two species of *Sargassum*, *S. linearifolium* (Turner) C. Agardh 1820 and *S. vestitum* (Turner) C. Agardh 1820 as part of a wider survey of algal associated amphipods (Poore *et al.*, 2000). While feeding on algal material is common across many amphipod taxa, few are known to burrow into algal tissues and feed internally (Mejaes *et al.* 2015). Apart from *Biancolina*, these include members of the Eophliantidae (Lorz et al. 2009), *Najna* Derzhavin, 1937 and *Carinonajna* Bousfield & Marcoux, 2004 from the Najnidae (Bousfield and Marcoux 2004) and *Amphitholina cuniculus* (Stebbing, 1874), *Peramphithoe stypotrupetes* Conlan & Chess, 1992 and *P. lessionophila* Conlan & Bousfield, 1982 from the Ampithoidae (Conlan and Chess 1992; Gestoso *et al.* 2014). These burrowing amphipods have convergent morphologies, sharing a subglobular head, small and compact bodies and stout pereopods (Mejaes *et al.* 2015).

Until this study, *B. australis* Nicholls, 1939 was the only known species for the genus in Australian waters, described from south Western Australia. *Biancolina australis* has uropods 1 and 2 rami subequal in length which readily distinguishes it from other *Biancolina*

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species. Ishimaru (1996) indicated this species should warrant its own genus, yet refrained from making a formal change in status, as type material of this species is lost. Recent museum collections from Western Australia have not turned up specimens of *Biancolina* (LEH pers. Obs; Peart 2004), yet this is to be expected for such small cryptic fauna. This paper is the first step to encouraging more work on small burrowing amphipods, reporting *B. japonica* from Botany Bay, New South Wales Australia and reviewing known algal hosts for all species in the genus.

Methods

Material is lodged in the Australian Museum, Sydney (AM). Specimens were dissected in 80 % ethanol. Illustrated were made from a temporary slide mount, except for mouthparts, where permanent slides were made using Aquatex™ mounting agent. Specimens were prepared for electron microscopy as follows: specimens were sonicated in a 10 % solution of the surfactant TWEEN 80 to remove detritus before being transferred back to 80 % ethanol; preserving solution was sequentially advanced in 5 % increments from 80 % to 100 % ethanol; critical point dried; mounted individually on pins and gold sputter coated. Images were captured using on a Zeiss EVO LS15 Scanning Electron Microscope with Robinson Backscatter Detector (SEM). Diagnoses are provided in bold text within the descriptions. Standard abbreviation on the plates are: A, antenna; C, coxa; Ep, epimeron; G, gnathopod; Md, mandible; Mx, maxilla; Mxp maxilliped; P, pereopod; T, telson; U, uropod; Ur, urosome; L, left and R, right.

Results

Systematics

Order AMPHIPODA Latreille, 1816

Family BIANCOLINIDAE J.L Barnard 1972

Genus *Biancolina* Della Valle, 1893

Biancolina japonica Ishimaru, 1996

(Figs. 1, 2, 3 and 4)

Biancolina japonica Ishimaru, 1996: 398–403, Figs. 1, 2, 3 and 4.

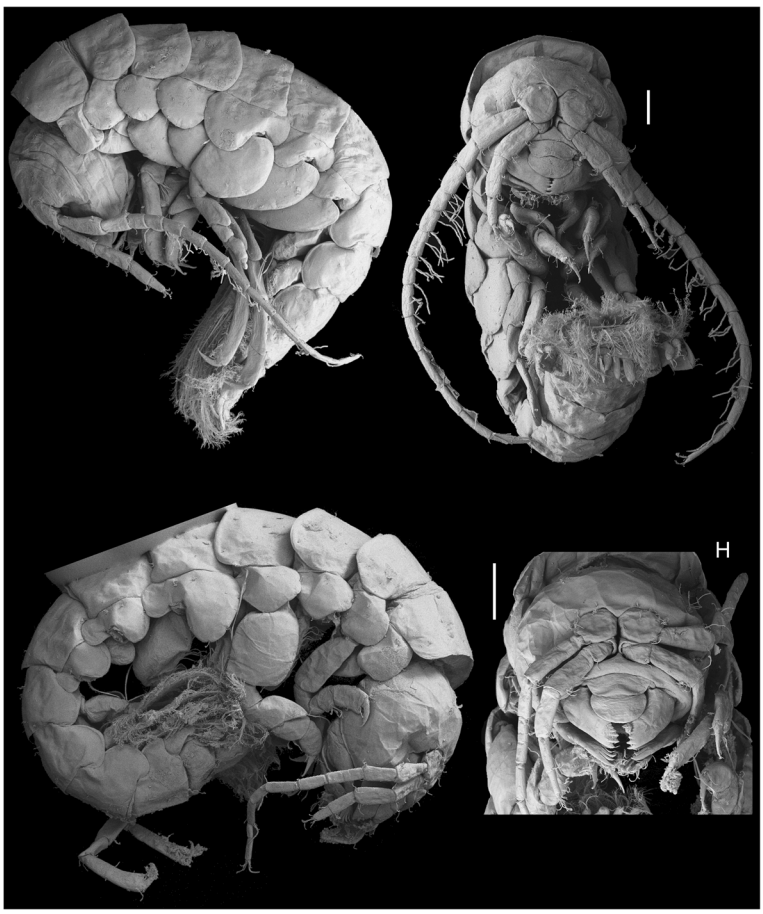

Fig. 1 *Biancolina japonica* Ishimaru, 1996, SEM image, upper specimen, male, 2.5 mm, AM P.98372, lower specimen, female, 2.2 mm, AM P.98370, Bare Island, Botany Bay, New South Wales, Australia. Scales 100 μm

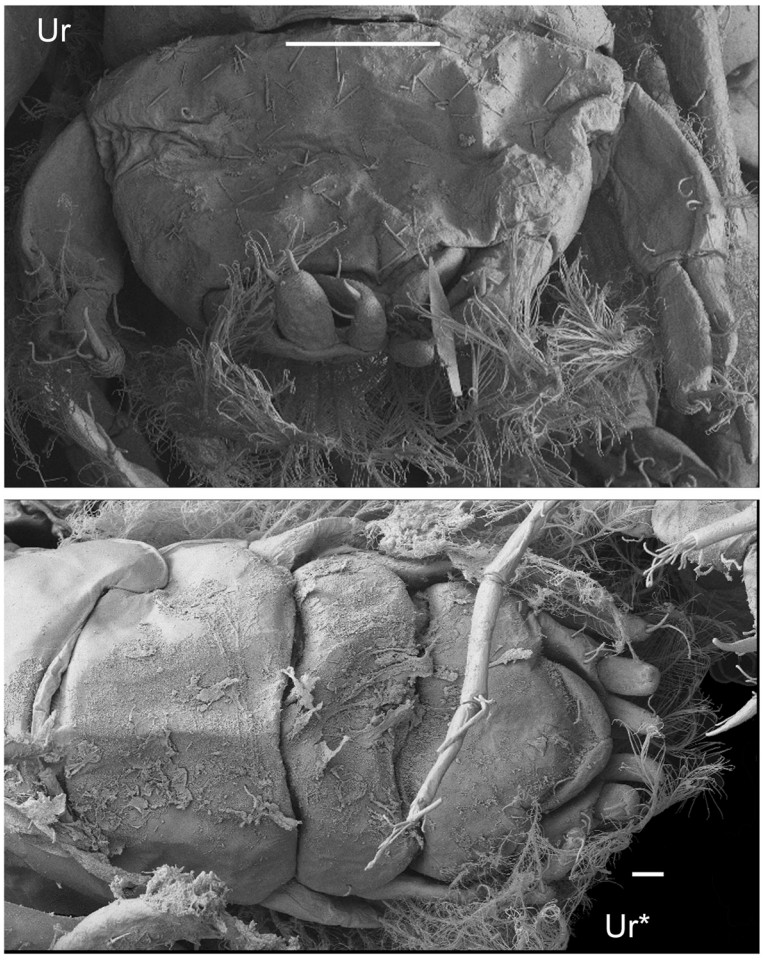

Fig. 2 *Biancolina japonica* Ishimaru, 1996, SEM image, female, 2.2 mm, AM P.98370, male, 2.5 mm, AM P.98372, Bare Island, Botany Bay, New South Wales, Australia. Scales 20 μm

Material examined

Female, 3.1 mm, dissected (urosome and carcass in microvial), 1 slide (mouthparts), AM P.98555; b female, 3.0 mm, dissected (carcass in microvial), 1 slide (head and mouthparts), AM P.98556; 1 male specimen, 2.5 mm, whole animal pin mount, AM P.98372; 1 'a' female specimen, 2.2 mm, whole animal pin mount, AM P.98370; 1 'b' female specimen, 2.4 mm, whole animal pin mount, AM P.98371; 25 specimens, AM P.42974, Bare Island, Botany Bay, New South Wales, Australia (34°00'S, 151°14'E), 2 m, brown alga *Sargassum linearifolium* (Turner) C. Agardh 1820, 4 May 1994, coll. A.G.B. Poore; 1 male, AM P.72591, west side of North Tollgate Island, New South Wales, Australia, (35°44'50"S, 150°15'28"E), 11 m, red calcareous *Corallina berteri* Montagne 1849, scattered low reefs, rocks and sand, 8 February 2003, coll. P.B. Berents, J. Eu, A.J. Millar and G.D.F. Wilson (Australian Museum, Marine Invertebrate station MI NSW 2019).

Type locality

Sado Strait, Japan Sea (37°56'N 138°36'E).

Description

Based on female specimen, 3.1 mm, AM P.98555.

Body cylindrical, head subglobular. *Antennae 1*peduncle article 1 length 1.1 x width; article 2 length 3 x width, 1.7 x article 1 length; article 3 length twice width, subequal to article 1 length; flagellum 8-articlulate (male AM P. 98372, 13–15 articulate). *Antennae 2* less than half length of antennae 1, peduncule article 4 length 1.5 x width, slightly longer than article 5; flagellum 3-articlulate. Upper lip broader than long, ventral margin rounded. *Mandible* incisors with 6 teeth. *Maxilla 1* inner plate reduced, apically rounded with 1 lateral slender seta; outer plate with 8 setal teeth, palp absent. *Maxilla 2* inner plate slender with 2 lateral and 4 apical slender setae; outer plate broad, longer than inner plate,

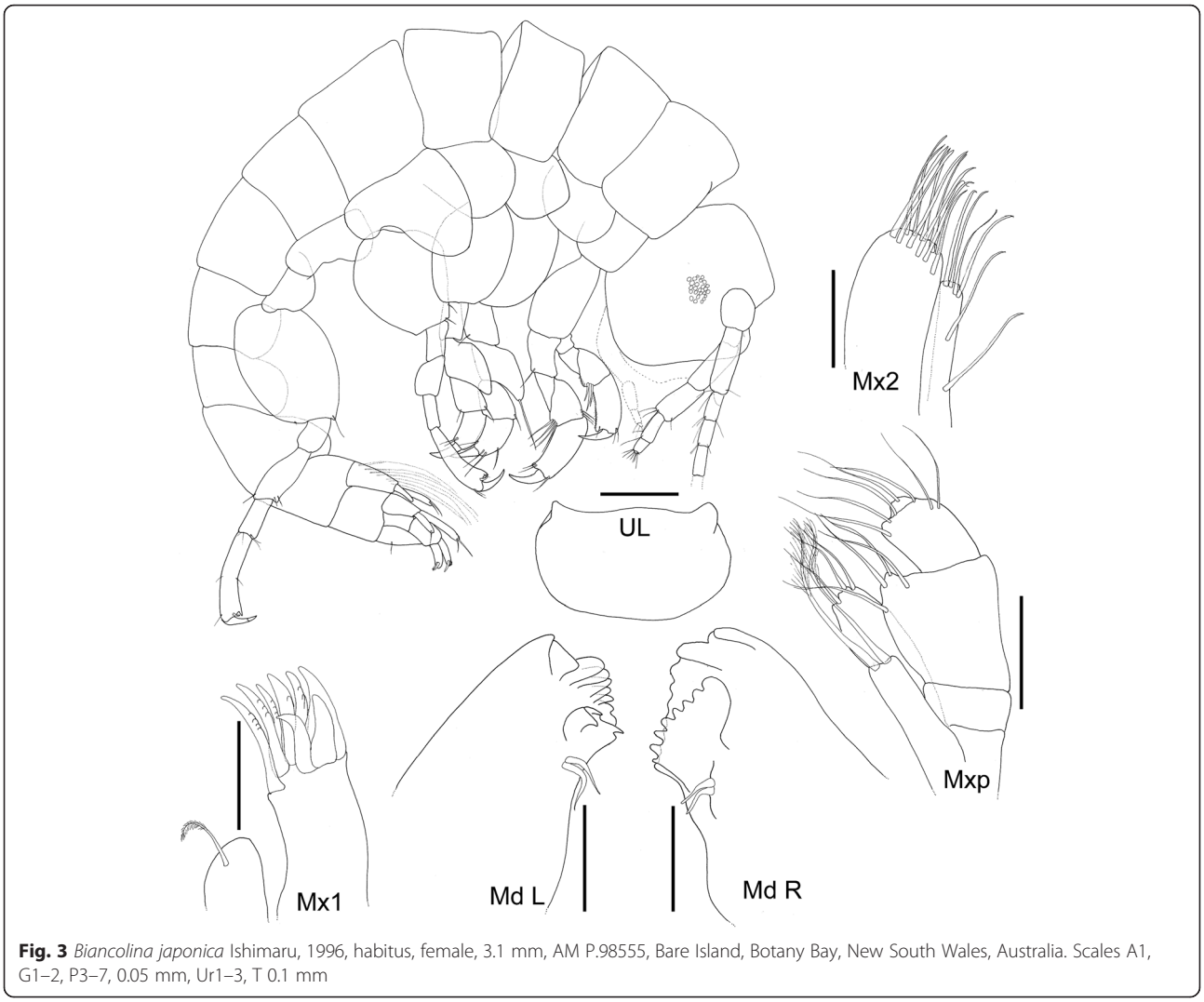

Fig. 3 *Biancolina japonica* Ishimaru, 1996, habitus, female, 3.1 mm, AM P.98555, Bare Island, Botany Bay, New South Wales, Australia. Scales A1, G1–2, P3–7, 0.05 mm, Ur1–3, T 0.1 mm

apically rounded with 10 apical slender setae. *Maxilliped* small; inner plate narrow, not reaching extent of outer plate, with 2 long apical setae; outer plate with slender apical setae; palp exceeding outer plate, article 2 with medial lobe, article 4 broad.

Coxae 1–4 similar in size. *Gnathopod 1* parachelate, coxa 1 subquadrate, ventral margin straight; carpus subtriangular, length 1.2 x width; propodus length 2.6 x width, palm transverse, entire, straight; dactylus overreaching palm (twice length of palm), posterior margin with slender seta, unguis present. *Gnathopod 2* similar to gnathopod 1, parachelate, coxa 2 subquadrate, basis stout, length twice width; ischium long, subequal to merus length; carpus subtriangular, length 1.2 x width; propodus length 2.6 x width, longer than gnathopod 1 propodus, palm transverse, distally convex, dactylus overreaching palm, unguis present. *Pereopods 3–4* similar, coxa ventral margin convex; basis subovate, length 1.2 x width; ischium, merus, carpus and propodus subequal in length; merus anterior margin expanded with single long anterodistal seta. *Pereopods 5–7* similar, coxa anterior lobe well-developed (decreasing in size from coxa 5 to 7); basis subovate length 1.1 x width; ischium, merus and carpus subequal in length; propodus weakly prehensile, distally expanded, length 4 x width, palm with 1 large robust setae; dactylus with unguis present. *Epimera 1–3* shallow, posterior and ventral margins convex. *Urosome* dorsoventrally flatterned, urosomite 1 largest; urosomite 3 fused with telson. *Uropod 1* peduncle length 1.5 x width, lateral margins lined with long plumose setae; outer ramus length 3 x width, half length of inner ramus with single apical slender seta; inner ramus length 3.5 x width, with 2 apical slender setae. *Uropod 2* peduncle long, length 3.3 x width, lateral margin with setae; outer ramus length 2.5 x width, less than half length of inner ramus with single apical slender seta; inner ramus length 4.2 x width, with 1 distolateral and 2 apical slender setae. *Uropod 3* peduncle broad, length 1.1 x width, lateral margin without setae, outer ramus length twice width,

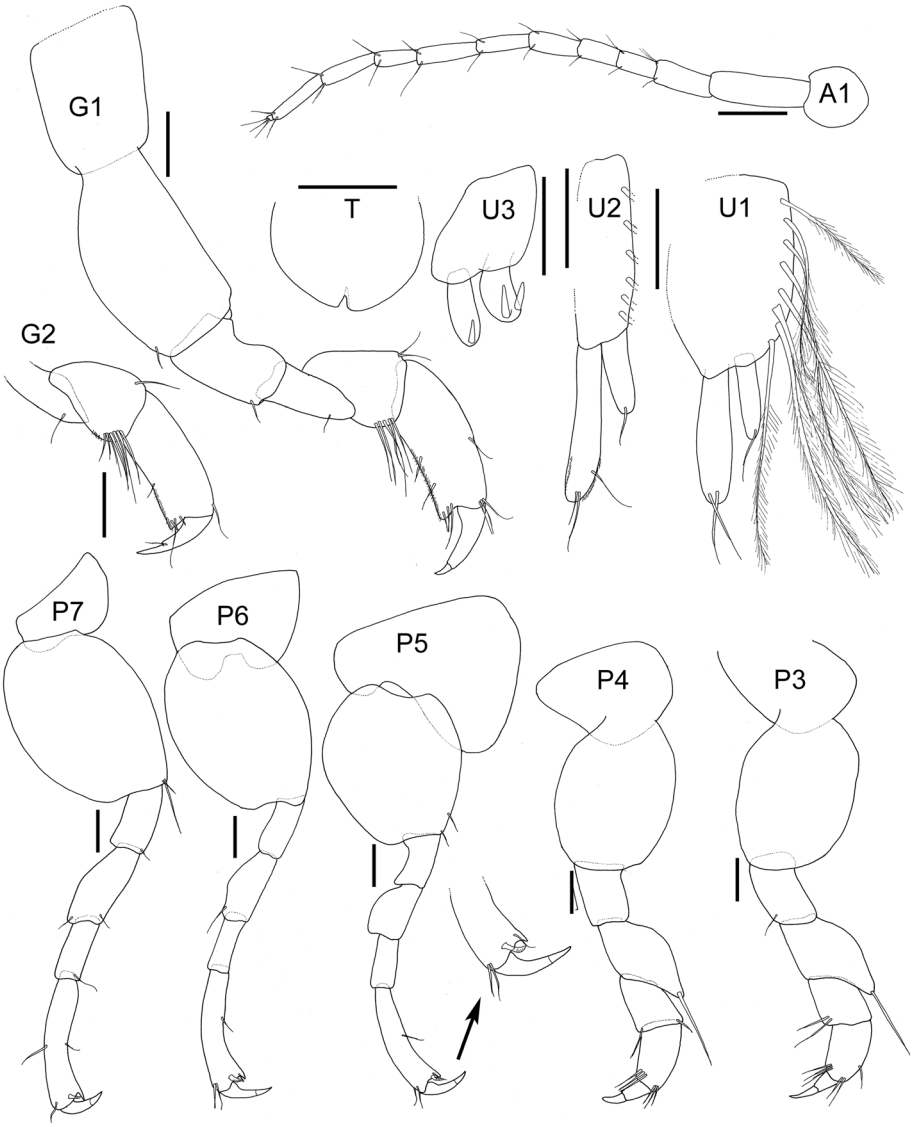

Fig. 4 *Biancolina japonica* Ishimaru, 1996, female, 3.1 mm, AM P.98555, Bare Island, Botany Bay, New South Wales, Australia. Scale 0.05 mm

subequal to inner ramus, with 2 recurved robust setae; inner ramus with 1 recurved robust setae. *Telson* subovate, tissue fleshy, apically notched/incised (note: notch not observable in SEM images).

Remarks

Biancolina japonica Ishimaru, 1996 may be separated from *B. australis* Nicholls, 1939, which also occurs in Australian waters, by the uropod 1 and 2 which have a shorter outer ramus, while the rami are subequal in *B. australis*. The coxae 1 to 2 ventral margin is reported as convex in the original description of *B. japonica*. SEM prepared specimens from Port Jackson include individuals with straight and convex coxal margins. As the genus is not heavily calcified and the tegument supple, this feature is now considered an artefact of tissue movement and not a species level character.

Distribution

Japan (Ishimaru, 1996). Australia: Botany Bay and Tollgate Island, New South Wales (current study).

Discussion

Specimens attributed to *B. japonica* are now reported from Japan and Australia. This small cryptic living species may represent a widely distributed taxon, a species complex or potentially an invasive species of unknown geographic origin. There is potential for widespread dispersal of these algal associated invertebrates on rafting material of their *Sargassum* hosts. Another species in

Table 1 Distribution and known host algae of all species of *Biancolina*. References are publications that include information on the macroalgal species inhabited by each species of *Biancolina*

Species	Type locality	Distribution	Host algae	References
B. algicola Della Valle 1893	Bay of Naples, Italy	Mediterranean Sea, Black Sea, Portugal	*Cystoseira amantacea*, C. *barbata, C. foeniculacea, C. sauvageauana, C. spinosa, C. tamariscifolia, Phyllophora* sp.	Ruffo & Wieser 1951; Krapp-Shickel 1993; Pereira *et al.* 2006; Tsytsugina 2011; Uzunova 2011
B. australis Nicholls 1939	Rottnest Island, Western Australia	Western Australia		
B. brassicacephala Lowry 1974	Sargasso Sea	Gulf stream off south east USA, Sargasso Sea, Gulf of Mexico	*Sargassum fluitans, S. natans*	Fine 1970; Steele & Collard 1981; Stoner & Greening 1984; LeCroy 2002; Huffard *et al.* 2014
B. japonica Ishimaru 1996	Sado Strait, Japan Sea	Japan, Australia	*Sargassum horneri*, *S. ilicifolium*, *S. linearifolium, S. vestitum.*	Ishimaru 1996; Poore *et al.* 2000; Taylor & Steinberg 2005
B. lowryi Ortiz & Lalana 1996	Cayo Mendoza, Cuba	Cuba	*Sargassum* sp.	Ortiz & Lalana 1996
B. mauihina Barnard 1970	Kawela Bay, Hawaii	Hawaii, Fiji, Moluccas	*Sargassum* sp., *Turbinaria* sp., *Ulva* sp.	Barnard 1970; Ledoyer 1979; Myers 1985
B. obstusata Tzvetkova 1976	Possjet Bay	Sea of Japan	*Cystoseira hakodatensis*	Tzvetkova & Kudrjashov 1985
Not identified to species		North Carolina, USA	*Sargassum filipendula*	Duffy 1990
		Japan	*Sargassum confusum*	Kito 1975
		Florida, USA	*Laurencia poiteaui*	Lewis 1987

the genus, *B. brassicacephala*, occurs largely on floating *Sargassum* in the Sargasso Sea and Gulf of Mexico (Table 1). Broader sampling and genetic study is required to investigate relationships among these morphologically similar populations.

Biancolina japonica is found within burrows that run lengthwise in the fronds of *Sargassum*. The burrows can extend for over 10 mm and have a diameter that is close to the thickness of the frond. Once complete, the sides of these burrows can become eroded leaving an empty slot that runs parallel to the main axis of the frond. Any individuals that have become disassociated from their algal host following collection have been observed to rapidly initiate a new burrow on contact with an algal frond. The burrowing starts in a central position on the frond and within minutes the individual amphipod has burrowed within the algal tissues.

In the shallow subtidal algal beds of the Sydney region, *Biancolina japonica* is found exclusively on two species of *Sargassum* (Poore *et al.* 2000). Such feeding specificity is rare among herbivorous amphipods (Poore et al. 2008). A review of host use of all known species of *Biancolina*, however, indicates that the genus as a whole displays a very restricted range of algal hosts (Table 1). Almost all records of *Biancolina* derive from collections of brown algae from just two genera, *Sargassum* and *Cystoseira*, both from the Sargassaceae in the order Fucales (Table 1). These hosts differ from those used by other burrowing amphipods, with *Amphitholina cuniculus* (Ampithoidae) recorded from *Fucus* and *Bifurcaria* (Fucales) (Gestoso et al. 2014), *Peramphithoe stypotrupetes* and *P. lessionophila* (Ampithoidae) from kelps (Laminariales) (Conlan and Chess 1992), *Najna* and *Carinonajna* (Najnidae) recorded from a wide variety of seagrasses and kelps (Bousfield and Marcoux 2004) and species from the Eophliantidae from kelps and *Carpophyllum* (Fucales) (Lorz et al., 2009).

Conclusions

Biancolina japonica, previously known only from Japan, is reported for the first time in Australian waters. Like other members of the genus, *B. japonica* burrows into the blades of brown algae and feeds from within those burrows. In contrast to most herbivorous amphipods, which are generalist consumers and found on many genera of host algae, this species is found almost exclusively on algae from one genus (*Sargassum*). A review of the known algal hosts of all eight species of *Biancolina* indicates high levels of host specialisation, with nearly all records derived from few genera of brown algae in the order Fucales.

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Availability of data and material

The specimens of *B. japonica* examined in this study are held in the Australian Museum (voucher numbers AM P.98556, AM P.98372, AM P.98370, AM P.98371, AM P.42974, and AM P.72591).

Authors' contributions

LEH and AGBP conceived of the study. LEH prepared the figures and species description. AGBP reviewed the ecological literature. LEH and AGB wrote the manuscript. Both authors read and approved the final manuscript.

Authors' information
LEH is a Research Fellow at the Australian Museum with research interests in crustacean systematics. AGBP is an Associate Professor at the University of New South Wales, Australia, with research interests in the ecology and evolution of marine invertebrates, seagrasses and macroalgae.

Competing interests
The authors declare that they have no competing interests.

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