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Discovery of a host fish for glochidia of *Velesunio angasi* (Sowerby, 1867) (Bivalvia: Unionoida: Hyriidae) from the Fortescue River, Pilbara, Western Australia

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Abstract. Freshwater fishes are the most common hosts of the glochidia (parasitic larvae) of freshwater mussels (Bivalvia: Unionoida: Unionoidea). *Velesunio angasi* (Sowerby, 1867), is the only known hyriid species recorded from the Fortescue River in the Pilbara Region of Western Australia. Eleven species of fish (n = 516) were captured from pools of the Fortescue River in June 2010. Small, white, bladder-like cysts were observed on *Neosilurus hyrtlii* Steindachner, 1867, though not on any of the other 10 species examined. Light microscopy of sectioned cysts revealed that they contained glochidia that were of similar size and shape to those of *V. angasi*. Glochidia were found on 73.2% of 168 *N. hyrtlii* examined, with a mean intensity of 3.3 cysts per infested fish. Prevalence was significantly greater on smaller fish ($P < 0.01$), however, no relationship between size and intensity of infestation was observed. This represents the first record of glochidia infestation on fish from the Pilbara Region.

Keywords. Freshwater mussel, *Velesunio angasi*, *Neosilurus hyrtlii*, host fish, glochidia, Indian Ocean Drainage Division

Running Head. Host fish for *Velesunio angasi* from the Pilbara

Introduction

Freshwater mussels, of the order Unionoida, are an ancient group of bivalves found in freshwaters on all continents apart from Antarctica and include 854 species worldwide (Graf and Cummings 2009). Unionoids are dioecious and reproduce sexually; eggs are fertilised in specialised chambers in female gills known as 'marsupia', where embryos are brooded to become larvae (Bauer and Wächtler 2001; Strayer 2008). The two unionoid superfamilies are distinguished by larval forms (Bauer and Wächtler 2001). Larvae of Unionoidea (Hyriidae, Margaritiferidae and Unionidae) are known as 'glochidia' and are markedly different in morphology from the 'lasidia' or 'haustoria' of Etherioidea (Etheriidae, Iridinidae and Mycetopodidae). Eighteen species of unionoid mussels are found in Australia, all from the family Hyriidae (Walker *et al.* 2001; Graf and Cummings 2010).

Glochidia, lasidia and haustoria are all parasitic on fishes (Bauer and Wächtler 2001). Mature larvae are expelled in response to external stimuli and subsequently have a brief opportunity to attach to the body surfaces, fins, mouth or gills of a host (generally a fish) (Bauer and Wächtler 2001; Strayer 2008). Some glochidia are equipped with hooks, known as larval teeth, on the ventral edges of their shells, which may aid in attachment to their hosts (Bauer and Wächtler 2001; Strayer 2008). Australian Hyriidae, in particular, have interlocking, hooked larval teeth (Walker *et al*. 2001). Following attachment, larvae are then encapsulated with host epithelial tissue and remain as parasites for a period of weeks to months (Bauer and Wächtler 2001; Strayer 2008).

The fish hosts of freshwater mussels have been identified for a number of species in Europe and North America (see Bauer and Wächtler 2001; Strayer 2008). The number of known hosts varies widely among mussel species, with most being host generalists and only a few being specialized to a single host species (Strayer 2008). There have been only very limited studies of glochidia-host fish relationships for Australian hyriids. Host fish species have been identified for seven freshwater mussel species in Australia, but many of these reports are from limited geographic areas and unlikely to represent complete host lists (Hiscock 1951, Atkins 1979, Walker 1981, Humphrey 1984, Widarto 1993, DPIPWE 2009).

The Pilbara Region is located within the Indian Ocean Drainage Division of Western Australia and extends from the Greenough River in the south to the DeGrey River in the north (Morgan and Gill 2004). Distributions of fishes in the freshwaters of the region have only recently been recorded (Allen *et al.* 2002; Morgan and Gill 2004; Beesley 2006). Five of the 12 fish species found in the region are endemic (Morgan & Gill 2004). Within the Pilbara Region, there is apparently only one species of hyriid mussel, *Velesunio angasi* (Sowerby, 1867) (Graf and Cummings 2010). The species has a wide distribution ranging from the north-western Indian Ocean to the Timor Sea, Gulf of Carpentaria and North-East Coast Drainage Divisions, based on museum records; within the Pilbara Region, it had been found in the Ashburton River, Fortescue River, Kookhabinna Creek and Miaree Pool (Graf and Cummings 2010). In this study, we report, for the first time, glochidia-host relationships of *V. angasi* within the Pilbara Region.

Methods

Five hundred and sixteen freshwater fishes of 11 different species were captured in June 2010, as part of the study by two of the authors (Morgan and Ebner) within groundwater-maintained pools of the Fortescue River between 21°19.040’ S, 116°09.374’ E and 21°18.875’ S, 116°09.313’ E,

using gill nets, double-winged fyke nets and seine nets. One empty mussel valve was collected and provisionally identified as *V. angasi* using taxonomic keys (McMichael and Hiscock 1958; Walker 2004).

All fishes were identified to species level and measured for total length (TL) (± 1mm). Whitish, bladder-like cysts on the surface of the fishes were provisionally identified as containing glochidia, and prevalence (percentage of fish infested) for each species was recorded from field examinations. A subsample (n = 87) of infested fish were anaesthetised in AQIS™, preserved in 100% ethanol and transported to the laboratory, where intensity (number of cysts per infested fish) was recorded. Portions of fins which contained cysts from two fish were examined under a dissecting microscope and dissected in preparation for histology. For histology, dissected cysts were dehydrated in graded ethanols, embedded in paraffin, serially sectioned (6 μm thick) and stained with Haematoxylin and Eosin.

Ninety five percent confidence intervals (CI) were calculated for prevalences, assuming a binomial distribution, and intensities, from 2,000 bootstrap replications, using the software Quantitative Parasitology 3.0 (Rozsa *et al.* 2000). The effect of TL on prevalence was tested using a t-test and the relationship between TL and intensity was tested by regression analysis.

Results

Of the 11 species of fish examined, cysts were found only on Hyrtl's tandan, *Neosilurus hyrtlii* Steindachner, 1867 (Table 1; Fig. 1). Histological examination confirmed the presence of glochidia in fish cysts (Fig. 2). Cysts were found only on the fins and body surfaces of *N. hyrtlii*.

Glochidial cysts were found on 73.2% (95% CI = 65.8 – 79.8%) of the 168 *N. hyrtlii* that were collected. Infested fish had a significantly lower TL (109.9 mm ± 2.1 SE) than uninfested fish (121.8 mm ± 4.2 SE) ($t = 2.8$, $P < 0.01$, n=168). Of infested fish, the intensity of infestation

varied from 0 – 12, with a mean of 3.3 (95% CI = 2.7 – 4.0, n=87). Regression analysis found no relationship between TL and intensity.

Discussion

The glochidia in our study were of similar size and shape to the glochidia of *V. angasi* described previously by Humphrey (1984). Using morphology to identify encysted glochidia to species level is difficult because the larval teeth are usually not visible; nevertheless, larval tooth morphology has been suggested as a taxonomic tool (Atkins 1979; Humphrey 1984; Jones *et al.* 1986; Jupiter and Byrne 1997; Walker 1981; Walker *et al.* 2001). Humphrey (1984) described the structure of larval teeth on *V. angasi* as well-developed, dual-pronged interlocking hooks. The shape and size of the periostracum outline of glochidia and distinct larval teeth of glochidia in the Fortescue River (Fig. 1) appear morphologically similar to those described by Humphrey (1984). On this basis, together with the fact that *V. angasi* is the sole species of Hyriidae recorded from the Fortescue River (Graf and Cummings 2010), encysted glochidia found on *N. hyrtlii* were interpreted as *V. angasi.* Pekkarinen and Englund (1995) reported that glochidia with well-developed larval teeth are often attached to fins and skin, rather than the gills of fish, which may explain why no glochidia were observed on the gills of *N. hyrtlii* in this study.

Our research represents the first time *N. hyrtlii* has been identified as a host for *V. angasi*, although Widarto (1993) reported *N. hyrtlii* as a host for the closely related *Velesunio ambiguus* (Philippi, 1847) from the Ross River, north-eastern Queensland. Considering that *N. hyrtlii* is one of Australia's most widely distributed freshwater fishes (Allen *et al.* 2002), the limited reports of the species as a host of hyriid glochidia is surprising.

The greater prevalence of glochidia on small fish, found here, may indicate that larger (and presumably older) *N. hyrtlii* become more resistant to infestation with *V. angasi*, as has

been found previously in mussel/fish host relationships (e.g. Bauer 1987; Hastie and Young 2001). Not all fish species appear to develop acquired immunity to glochidia however, as some studies have shown glochidial cysts appear to be more prevalent on large-bodied hosts, presumably because larger fish provide more surface area for colonisation and because large bodied individuals are typically longer lived and have greater opportunity to gain infection (Martel and Lauzon-Guay 2005; Blažek and Gelnar 2006).

Globally, glochidia of most freshwater mussel species, particularly those with hooked teeth, have been found to be host generalists, with a number of fish species involved in the life-cycle (Haag and Warren 1998; Wächtler *et al.* 2001; Martel and Lauzon-Guay 2005; Blažek and Gelnar 2006). In the few studies that have examined fish species for glochidia of Australian hyriids, a number of host species have also been identified (Hiscock 1951; Atkins 1979; Walker 1981; Humphrey 1984; Widarto 1993; DPIPWE 2009). It is thus surprising that glochidia were only found on one fish species in the current study, considering that Humphrey (1984) identified 17 fish species as hosts for *V. angasi* in the Alligator Rivers Region, Northern Territory, and that five of these fish species were examined in the current study. Future studies of glochidia-host fish relationships in the Pilbara Region of Western Australia are warranted to determine the specificity of mussel/host relationships in this area and to compare this with relationships in other regions.

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Permits were issued by the Department of Fisheries, as required by the Fisheries Management Act (1994).

References

Allen, G. R., Midgley, S. H. and Allen, M. (2002). 'Field Guide to the Freshwater Fishes of Australia.' (Western Australian Museum: Perth.)

Atkins, L. (1979). Observations on the glochidial stage of the freshwater mussel *Hyridella* (*Hyridella*) *drapeta* (Iredale) (Mollusca : Pelecypoda). *Australian Journal of Marine and Freshwater Research* **30**, 411–416.

Bauer, G. (1987). The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) II. Susceptibility of brown trout. *Archiv für Hydrobiologie* **Supplementband 76**, 403-412.

Bauer, G. and Wächtler, K. (2001). 'Ecology and Evolution of the Freshwater Mussels Unionoida.' (Springer-Verlag: New York).

Beesley, L. (2006). Environmental stability: its role in structuring fish communities and life history strategies in the Fortescue River, Western Australia. Ph.D. Thesis, University of Western Australia, Perth.

Blažek, R. and Gelnar, M. (2006). Temporal and spatial distribution of glochidial larval stages of European unionid mussels (Mollusca: Unionidae) on host fishes. *Folia Parasitologica* **53**, 98-106.

DPIPWE. (2009). Status of fish communities and observations on South Esk freshwater mussel (*Velesunio moretonicus*) populations in the Macquarie River catchment upstream of Lake River. Water Assessment Aquatic Ecology Report Series, Report No. WA 09/02. Water

and Marine Resources Dision. Department of Primary Industries, Parks, Water and Environment, Hobart, Tasmania.

Graf, D. and Cummings, K. S. (2010). The Mussel Project (Online) at: http://www.mussel-project.net

Haag, W. R. and Warren, M. L. (1998). Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 297-306.

Hastie, L. C. and Young, M. R. (2001). Freshwater pearl mussel (*Margaritifera margaritifera*) glochidiosis in wild and farmed salmonid stocks in Scotland. *Hydrobiologia* **445**, 109-119.

Hiscock, I. D. (1951). A note on the life history of the Australian freshwater mussel, *Hyridella australis* Lam. *Transactions of the Royal Society of South Australia* **74**, 146-148.

Humphrey, C. L. (1984). Biology and ecology of the freshwater mussel *Velesunio angasi* (Bivalvia: Hyriidae) in the Magela Creek, Alligator Rivers region, Northern Territory. Ph.D. Thesis, University of New England, Armidale.

Jones, H. A., Simpson, R. D. and Humphrey, C. L. (1986). The reproductive cycles and glochidia of freshwater mussels (Bivalvia: Hyriidae) of the Macleay River, northern New South Wales, Australia. *Malacologia* **27**, 185-202.

Jupiter, S. D. and Byrne, M. (1997). Light and scanning electron microscopy of the embryos and glochidia larvae of the Australian freshwater bivalve Hyridella depressa (Hyriidae). *Invertebrate Reproduction and Development* **32**, 177-186.

Martel, A. L. and Lauzon-Guay, J. S. (2005). Distribution and density of glochidia of the freshwater mussel *Anodonta kennerlyi* on fish hosts in lakes of the temperate rain forest of Vancouver Island. *Canadian Journal of Zoology* **83**, 419-431.

McMichael, D. F. and Hiscock, I. D. (1958). A monograph of the freshwater mussels (Mollusca: Pelecypoda) of the Australian Region. *Australian Journal of Marine and Freshwater Research* **9**, 372-507.

Morgan, D. L. and Gill, H. S. (2004). Fish fauna in inland waters of the Pilbara (Indian Ocean Drainage Division) of Western Australia – evidence for three subprovinces. *Zootaxa* **636**, 1-43.

Pekkarinen, M. and Englund, V. P. M. (1996). Anatomy of the glochidia of the freshwater pearl mussel, *Margaritifera margaritifera* (L.). *Archiv für Hydrobiologie* **137**, 411-423.

Rozsa, L., Reiczigel, J. and Majoros, G. (2000). Quantifying parasites in samples of hosts. *Journal of Parasitology* **86**, 228-232.

Strayer, D. L. (2008). 'Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance.' (University of California Press: Berkley.)

Wächtler, K., Dreher-Mansur, M. C. and Richter, T. (2001). Larval types and early postlarval biology in naiads (Unionoida). In 'Ecology and Evolution of the Freshwater Mussels Unionoida'. (Eds G. Bauer and K. Wächtler.) pp. 93-125. (Springer-Verlag: New York).

Walker, K. F. (1981). Ecology of freshwater mussels in the River Murray. Australian Water Resources Council Technical Paper No. 63. Department of national Development and Energy Research Project No. 78/101. (Australian Government Publishing Service: Canberra.)

Walker, K. F., Byrne, M., Hickey, C. W. and Roper, D. S. (2001). Freshwater mussels (Hyriidae) of Australia. In 'Ecology and Evolution of the Freshwater Mussels Unionoida'. (Eds G. Bauer and K. Wächtler.) pp. 5-31. (Springer-Verlag: New York.)

Walker, K. F. (2004). 'A guide to the provisional identification of the freshwater mussels

(Unionoida) of Australasia.' CRC Freshwater Ecology Identification Guide 51. (Murray Darling Freshwater Research Centre: Albury.)

Widarto, T. H. (1993). Aspects of the biology of *Velesunio ambiguus* Philippi from a tropical freshwater environment, Ross River, Townsville, Australia. M.Sc. Thesis, James Cook University, Townsville.

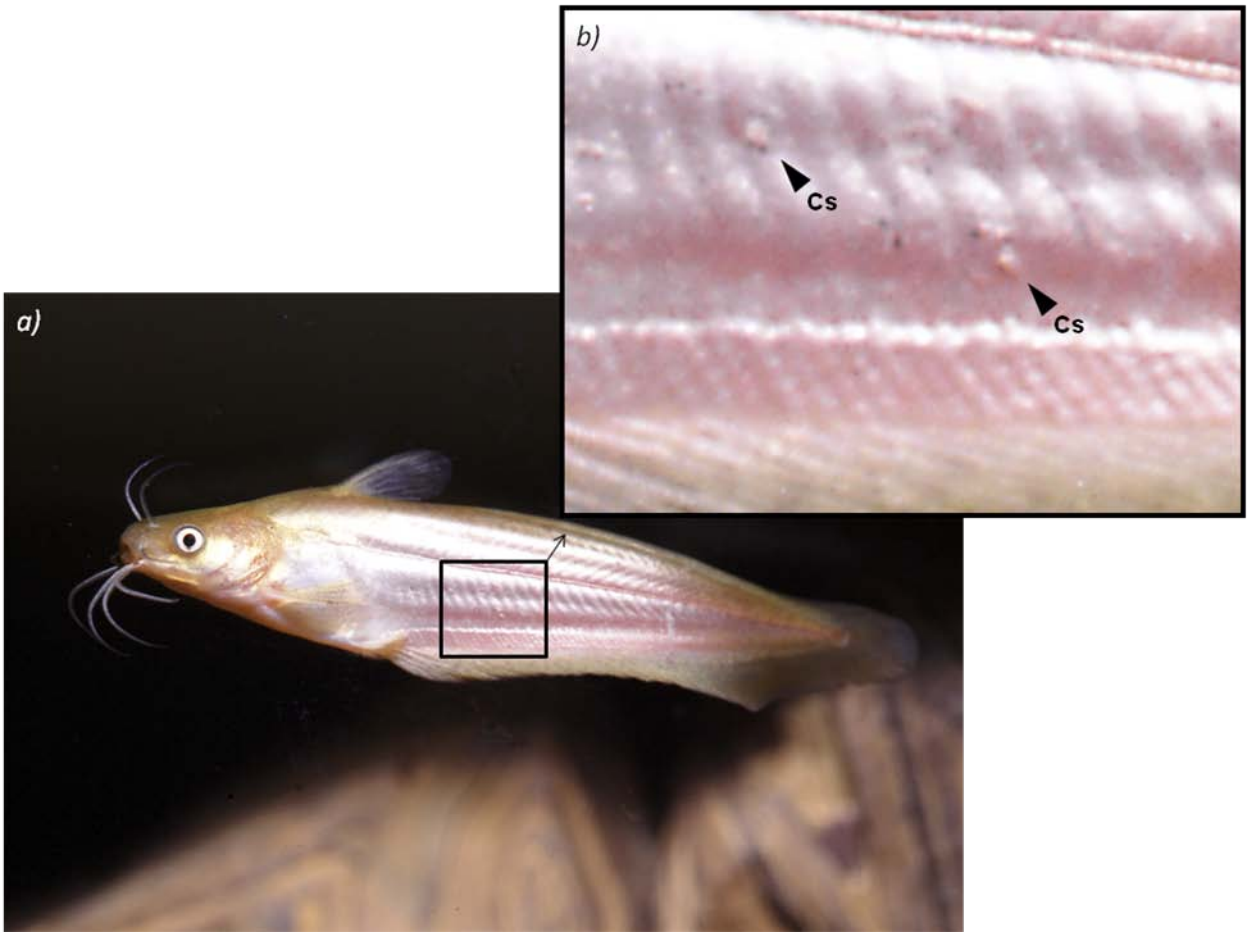

Fig. 1

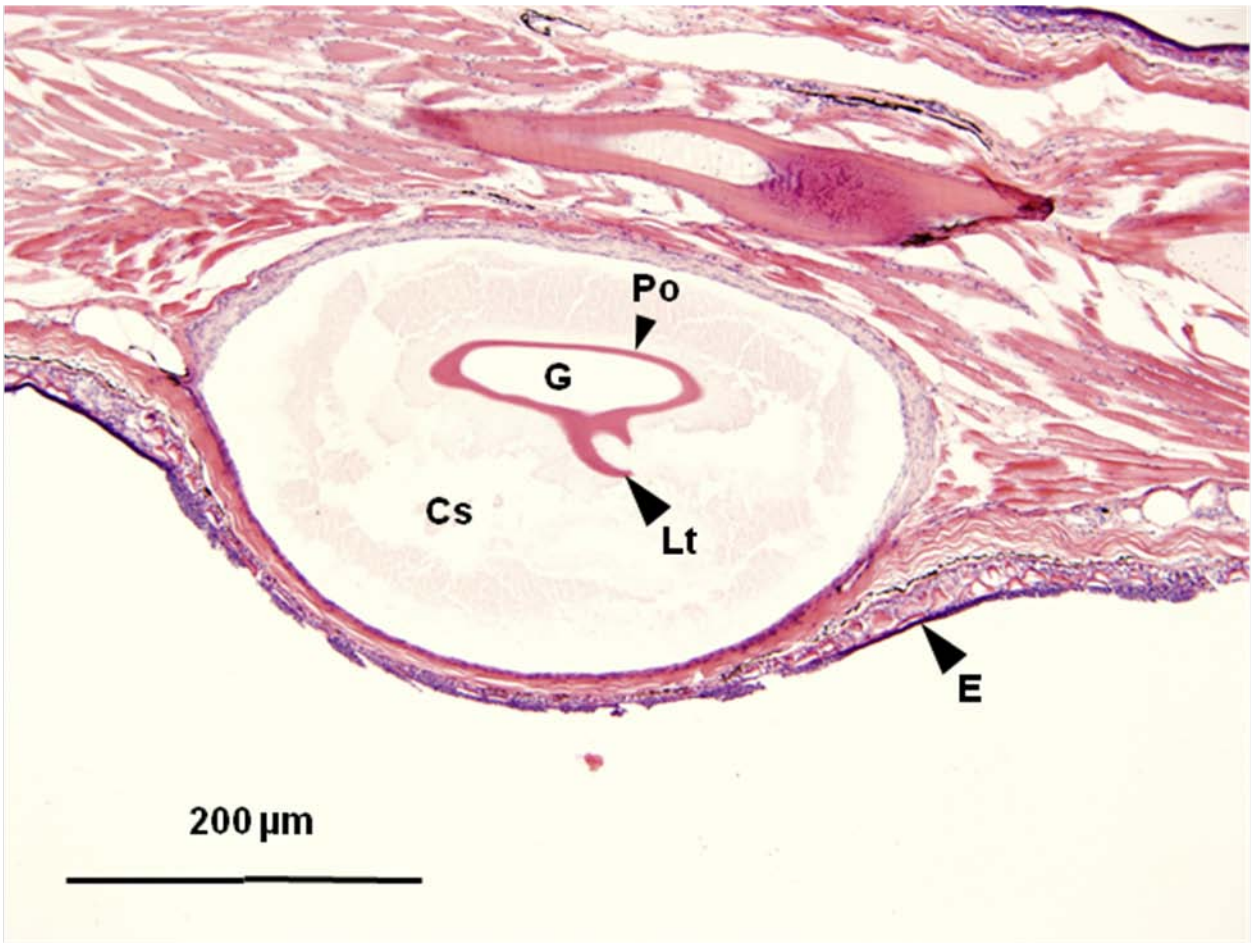

Fig. 2

Table 1. The number of fishes collected and examined for parasites from the Fortescue River, Pilbara, Western Australia, and the proportion of each species infested by glochidia.

Fish Family	Fish Species	Number of individuals examined	Prevalence of glochidia (%)
Ariidae	**Lesser salmon catfish** *Neoarius graeffei* (Kner & Steindachner, 1867)	16	0
Chanidae	**Milkfish** *Chanos chanos* Forsskål, 1775	3	0
Clupeidae	**Bony bream** *Nematalosa erebi* (Günther, 1868)	46	0
Gobiidae	**Flathead goby** *Glossogobius giurus* (Hamilton, 1822)	21	0
Megalopidae	**Oxeye herring** *Megalops cyprinoides* (Broussonet, 1782)	3	0
Melanotaeniidae	**Western rainbowfish** *Melanotaenia australis* (Castelnau, 1875)	100	0
Mugilidae	**Green mullet** *Liza subviridus* (Valenciennes, 1836)	5	0
Plotosidae	**Hyrtl's tandan** *Neosilurus hyrtlii* Steindachner, 1867	168	73.2
Terapontidae	**Barred grunter** *Amniataba percoides* (Günther, 1864)	36	0
	Fortescue grunter *Leiopotherapon aheneus* (Mees, 1963)	100	0
	Spangled perch *Leiopotherapon unicolor* (Günther, 1859)	18	0
	TOTAL	**516**	