Genetic characterisation of cercarial stages of *Choanocotyle* Jue Sue and Platt, 1998 (Digenea: Choanocotylidae) in a native Australian freshwater snail, *Isidorella hainesii* (Tryon)

Shokoofeh Shamsi\(^a,b,c\), Alara Nuhoglu\(^a\), Xiaocheng Zhu\(^a,b,c\), Diane P. Barton\(^a\)

\(^a\) School of Animal and Veterinary Sciences, Charles Sturt University, Wagga, Wagga, 2678, Australia
\(^b\) Graham Centre for Agricultural Innovation (Charles Sturt University and NSW Department of Primary Industries), Charles Sturt University, Wagga, Wagga, 2678, Australia
\(^c\) NSW Department of Primary Industries, Wagga Wagga, Australia

**ARTICLE INFO**

**Keywords:** Zoogeography, Molluscs, Life cycle

**ABSTRACT**

*Isidorella hainesii* (Tryon, 1866) is a native freshwater snail, belonging to the family Planorbiidae, commonly found on aquatic vegetation in south eastern parts of Australia. In the present study, we report natural infection with a species of *Choanocotyle* Jue Sue and Platt, 1998 (Digenea: Choanocotylidae) parasite in inland Australia for the first time, followed by characterisation of the parasite using both morphological and molecular approaches. Snails (n = 150) were collected from recently drained, natural ponds at a local fish farm located in the Riverina region, New South Wales, Australia. Parasites were subjected to preliminary morphological examination followed by DNA extraction to obtain their ITS-2, 18S and 28S sequences. Based on their sequence data and phylogenetic analyses they were identified as *Choanocotyle hobbsi* Platt and Tkach, 2003, which has only previously been described from *Chelodina oblonga* Gray, 1841 (snake-necked turtle) in Western Australia. Previous researchers suggested that in Australia, *C. oblonga* and its parasite fauna are separated from their eastern counterparts due to formation of impenetrable waterless desert in the country during the late Cretaceous. Our study extends the distribution of *Choanocotyle hobbsi* from Western Australia to the Murray Darling Basin in New South Wales, however, the definitive host remains unknown in New South Wales.

1. Introduction

Parasites belonging to the genus *Choanocotyle* Jue Sue and Platt, 1998 (Digenea: Choanocotylidae) are endemic species in Australia infecting freshwater turtles. Jue Sue and Platt (1998) established the family Choanocotylidae to accommodate two species belonging to the Order Plagiorchiida: *Choanocotyle elegans* Jue Sue and Platt (1998) from the small intestine of the freshwater turtles, *Chelodina expansa* Gray, 1857 and *Emydura macquarii* (Gray, 1830), and *Choanocotyle nematoides* Jue Sue and Platt (1998) from the large intestine of *E. macquarii*. The genus *Choanocotyle* currently comprises 5 species (Table 1).

Like many other aquatic Trematoda, *Choanocotyle* spp. have a three-host life cycle; however, our knowledge on the details of their natural life cycle is limited. Jue Sue and Platt (1998) established the life cycle experimentally and showed that *C. elegans* eggs are fully embryonated, containing a motile miracidium, which were hatched only after being ingested by suitable snail host, *Glyptophysa gibbosa* (Gould, 1846), where they developed to cercariae. In their study, *Glyptophysa gibbosa* was also successfully infected with the eggs of *C. nematoides*, which passed cercariae utilising snails and tadpoles as second intermediate hosts in the laboratory, without infecting fish. They found the metacercariae to naturally infect a glossiphonid leech from Grafton, northern New South Wales. Species of *Glossiphonia* Johnson, 1816 feed on snails and the leech probably became infected by ingesting cercariae from an infected snail. Although large numbers of metacercariae of various ages (recovered from experimentally infected snails and tadpoles) were fed to three laboratory-reared *Chelodina longicollis* (Shaw, 1794), infection did not occur. Jue Sue and Platt (1998) reported that heavy infection with sporocysts has led to death of snails in their experiments.

*Isidorella hainesii* (Tryon, 1866) is a native freshwater snail, belonging to the family Planorbiidae, which is commonly found on aquatic vegetation in ponds, billabongs, swamps and sluggish streams.
and rivers in south eastern part of Australia. In the present study, we report natural infection with a species of Choanoctyle parasite in *Isidorella hainesii* in inland Australia for the first time followed by its genetic characterisation using sequences of internal transcribed spacers (ITS), and small and large subunits ribosomal DNA (18S and 28S) regions.

2. Methodology

2.1. Sample collection

Snails (n = 150) were collected from a recently drained, natural pond at a local fish farm located in the Riverina region, New South Wales Australia. Combination of bore water and river water (Murrumbidgee River) was used for the pond which were mainly used to grow golden perch (*Macquaria ambiguа*, (Richardson, 1845)), a native Australian fish species. The ponds were with soil bottom and were frequented by cor- morants, duck, egrets, and pelicans, with turtles, yabbies, shrimp, small bivalves, water scorpions, and dipteran insects found at the bottom. Collection of snails took place late February–April 2019. The snails were collected in large specimen jars, approximately half-full of water, and were transported to the Parasitology Laboratory of Charles Sturt University. Snails were left in these jars with the lid loosely on (for air-flow), and with a lamp on over them for 12–48hrs. After this time, snails were examined for presence of parasites. Any parasite specimens found were preserved in 70 % ethanol. The best specimens were put on slides, some in lacto-phenol (25 % lactic acid, phenol, water and glycerine, each) and some in glycerine for morphology. The rest of the specimens were kept in 70 % ethanol for later molecular work.

2.2. Parasite study

Specimens were examined for distinguishing features of certain families of trematodes to estimate parasite identification. Where possible, total length (Total), body length (BL), body width (BW), tail length (TL), tail width (TW), tail width with fins (TWF), oral sucker diameter (OS), ventral sucker diameter (VS), and stylet length (SL) were measured. Illustrations were made using a microscope equipped with a drawing tube. All measurements are given in micrometres, unless otherwise stated. Mean measurements were specified, followed by the range in parenthesis. Photos were taken using a 9 MP Microscope Digital Camera (AmScope Model MU900). To prepare for DNA extraction, specimens had identical sequences with adult *S. Shamsi et al.*

| Parasite            | Host type | Stage      | Locality            | Reference |
|---------------------|-----------|------------|---------------------|-----------|
| *Auriculotrema lechneri* | Adult     | Queensland, Australia | Plant (2003) |
| *Eunuda kreffii*     | Adult     | Queensland, Australia | Plant (2003) |
| *E. elegans*        | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Bufo marinus*      | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Chelodina expansa* | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Chelodina rugosa*  | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Chelodina rugosa*  | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *C. nobilis*        | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *C. platii*         | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |

3. Results

Of the 150 *Isidorella hainesii* snails examined, 11 were infected by cercaria with the distinguishing characteristics of a stylet protruding from the oral sucker, a slightly larger oral sucker than ventral sucker, a short tail relative to body length and small terminal spines covering the body of the cercaria (Fig. 1). Measurements are based on 11 samples mounted in glycerine and are presented in Table 1 in comparison to cercaria reported by *Jue Sue and Platt* (1998) obtained from experimental infections. *Description*. Cercaria not examined alive, thus patterns of the excretory system and penetration glands could not be ascertained. Dark “patchy” area anterior to ventral sucker present, corresponding in location to penetration glands as described in *Jue Sue and Platt* (1998). Possible female genital rudiment immediately posterior to ventral sucker.

Sequences of the ITS, 18S and 28S regions for both cercariae and metacercaria were obtained and deposited in the GenBank (Supplementary Table 1 accession numbers: MW684083-9, MW686389-93 and MW682817-22). Alignment of our sequences with closely related species in the GenBank resulted in an alignment of 1318, 1215 and 1770bp for ITS, 28S RNA and 18S RNA regions, respectively. All three gene regions were identical for all our specimens. For the 18S region, our specimens had identical sequences with adult *C. hobbsi* reported from turtles, whereas for ITS and 28S regions, there was only 1 bp difference with adult *C. hobbsi*. Phylogenetic tree, using Bayesian inference, clustered our specimens into a single highly supported clade with *C. hobbsi* in all three gene regions, separate from other *Choanoctyle* species.

Table 1

| Parasite                              | Host type | Stage       | Locality            | Reference |
|---------------------------------------|-----------|-------------|---------------------|-----------|
| *Auriculotrema lechneri*              | Adult     | Queensland, Australia | Plant (2003) |
| *Eunuda kreffii*                      | Adult     | Queensland, Australia | Plant (2003) |
| *E. elegans*                          | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Bufo marinus*                        | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Chelodina expansa*                   | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Chelodina rugosa*                    | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *C. nobilis*                          | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *C. platii*                           | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |

3. Results

Of the 150 *Isidorella hainesii* snails examined, 11 were infected by cercaria with the distinguishing characteristics of a stylet protruding from the oral sucker, a slightly larger oral sucker than ventral sucker, a short tail relative to body length and small terminal spines covering the body of the cercaria (Fig. 1). Measurements are based on 11 samples mounted in glycerine and are presented in Table 1 in comparison to cercaria reported by *Jue Sue and Platt* (1998) obtained from experimental infections. *Description*. Cercaria not examined alive, thus patterns of the excretory system and penetration glands could not be ascertained. Dark “patchy” area anterior to ventral sucker present, corresponding in location to penetration glands as described in *Jue Sue and Platt* (1998). Possible female genital rudiment immediately posterior to ventral sucker.

Sequences of the ITS, 18S and 28S regions for both cercariae and metacercaria were obtained and deposited in the GenBank (Supplementary Table 1 accession numbers: MW684083-9, MW686389-93 and MW682817-22). Alignment of our sequences with closely related species in the GenBank resulted in an alignment of 1318, 1215 and 1770bp for ITS, 28S RNA and 18S RNA regions, respectively. All three gene regions were identical for all our specimens. For the 18S region, our specimens had identical sequences with adult *C. hobbsi* reported from turtles, whereas for ITS and 28S regions, there was only 1 bp difference with adult *C. hobbsi*. Phylogenetic tree, using Bayesian inference, clustered our specimens into a single highly supported clade with *C. hobbsi* in all three gene regions, separate from other *Choanoctyle* species.

Table 1

| Parasite                              | Host type | Stage       | Locality            | Reference |
|---------------------------------------|-----------|-------------|---------------------|-----------|
| *Auriculotrema lechneri*              | Adult     | Queensland, Australia | Plant (2003) |
| *Eunuda kreffii*                      | Adult     | Queensland, Australia | Plant (2003) |
| *E. elegans*                          | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Bufo marinus*                        | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Chelodina expansa*                   | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *Chelodina rugosa*                    | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *C. nobilis*                          | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
| *C. platii*                           | Adult     | Queensland, Australia | Jue Sue and Platt (1998) |
Fig. 1. Cercaria of Choanoctyle hobbsii. A. Ventral view of whole mount. Scale bar 100 μm. B. Stylet. Scale bar 10 μm.
study were not examined alive so the patterns of the excretory system and penetration glands could not be ascertained. Additionally, cercaria were dissected from the snail, rather than after release, which may have influenced the range of measurements recorded. Future work should include study of live free-swimming cercaria to enable a more accurate description.

In experimental infections conducted by Jue Sue and Platt (1998), _C. elegans_ infected _Glyptophasa gibbosa_, but not _Austropeplea lessoni_ or _Glyptophasa sp._; metacercaria were found in naturally infected _Glyptophasa_ spp., including _G. gibbosa_. Similarly, _C. nematoides_ infected _G. gibbosa_ but not _A. lessoni_ nor _Glyptophasa sp._ Choanoctyle _elegans_ were found to apparently naturally infect _Isidorella newcombii_ (mentioned in abstract by Jue Sue and Platt, 1998; but no details provided in the text of the paper).

In this study, Choanoctyle cercariae were found in _Isidorella hainesii_ (Planorbidae) in aquaculture ponds. Although Jue Sue and Platt (1998) stated that _I. newcombii_ was infected with _C. elegans_, the distribution of this species is more inland, in arid to semi-arid areas; _I. hainesii_, on the other hand, is found along the coastal edge of southern Queensland and NSW (Ponder et al. 2016) and is more likely to be the snail host studied by Jue Sue and Platt (1998). If this is the case, then this study confirms the presence of Choanoctyle cercaria in naturally infected _Isidorella hainesii_ specimens.

### Funding

This project has been funded by Charles Sturt University (A512-828-xxx-66770 awarded to SS).

### Ethics approval

Not applicable.

### Consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

Data supporting the conclusions of this article are included in the article and its additional files. Raw data are available upon request to the first author.

### Declaration of competing interest

The authors declare that they have no competing interests.

### Acknowledgements

We would like to acknowledge an anonymous commercial fishery in the Riverina region for allowing us to collect snail samples. Authors are grateful to Charles Sturt University for financial support toward Open Access and costs of the research through Senior Research Fellowship Program awarded to SS.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2021.08.001.

### References

- Cann, J., 1998. Australian freshwater turtles. Beaumont Pub.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9, 772-772.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95-98.
- Hillis, D.M., Dixon, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q Rev Biol 66, 411-453.
- Iwaki, T., Sata, N., Hasegawa, H., Matsuo, K., Na, T., 2020. _Ochetosoma kanaeae_ (Plagiochiida: Ochetosomatidae) from native snake species in Japan. Jpn. J. Zoo Wild. Med. 25, 129-134.
- Jue Sue, L., Platt, T.R., 1998. Description and life-cycle of two new species of _Choanoctyle_ n. g. (Trematoda : Plagiochiidae), parasites of Australian freshwater turtles, and the erection of the family _Choanoctyidae_. Syst. Parasitol. 41, 47-61.
- Karl, E.L., Font, T.J., Font, W.F., Cristione, C.D., 2014. _Alloglossidium floridense_ n. sp. (Digenea: Macroderoididae) from a spring run in North Central Florida. J. Parasitol. 100, 121-126.
- Karl, E.L., Font, W.F., Cristione, C.D., 2018. Resolving evolutionary changes in parasite life cycle complexity: molecular phylogeny of the trematode genus _Alloglossidium_ indicates more than one origin of precociousness. Mol. Phylogenet. Evol. 126, 371-381.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547-1549.
- Littlewood, D.T.J., Olston, P.D., 2001. Small subunit rDNA and the Platyhelmintes: signal, noise, conflict and compromise. Interrelationships of the Platyhelmintes, 262-262.
- Manning, B., 1996. Evolution and zoogeography of Australian freshwater turtles. Memoirs of the Queensland Museum 39, 328-331.
- Olsón, P., Cribb, T., Tkach, V., Bray, R., Littlewood, D., 2003. Phylogeny and classification of the _Digenes_ (Platyhelmintes: Trematoda). Int. J. Parasitol. 33, 733-755.
- Platt, T.R., 2003. Description of _Auculotremena lechmeri_ n. gen., n. sp. ( _Digenes_ : _Choanoctyinae_), a parasite of freshwater turtles ( _Testudines : Pleurodira : Chelidae_ ) from Queensland, Australia. J. Parasitol 889, 141-144.
- Platt, T.R., Tkach, V.V., 2003. Two new species of Choanoctyle Jue Sue and Platt, 1998 ( _Digenes_ : _Choanoctyidae_ ) from an Australian freshwater turtle ( _Testudines : Pleurodira : Chelidae_ ). J. Parasitol. 89, 145-150.
- Pulis, E.E., Tkach, V.V., Newman, R.A., 2011. _Helinthm parasites of the wood frog, Lithobates sylvaticus_, in prairie pothole wetlands of the Northern Great Plains. Wetlands 31, 675-685.
- Rambaut, A., 2014. FigTree v1.4.2, A Graphical Viewer of Phylogenetic Trees, Shamsi, S., Briand, M.J., Justine, J.-L., 2017. Occurrence of _Anisakis_ (Nematoda: _Anisakidae_ ) larvae in unusual hosts in southern hemisphere. Parasitol. Int. 66, 837-840.
- Svitin, A.O., Bashinskyi, I., Liviinshuk, S., Neymark, L., Ivanov, A.Y., Ermakov, O., Vedenirkov, A., Dubois, A., 2019. A mollusk _Planorbarius corneus_ is an intermediate host of the infectious agent of rostand’s anomaly _p’_ in green frogs. Russ. J. Herpetol. 26.
- Tkach, V., Mills, A., 2011. _Alloglossidium fonti_ sp. nov. ( _Digenes_, _Macroderoididae_ ) from black bullheads in Minnesota with molecular differentiation from congers and resurrection of _Alloglossidium kent_. Acta Parasitol. 56, 154-162.
- Tkach, V., Pawlowski, J., Mariaux, J., 2000. Phylogenetic analysis of the suborder _Plagiochiata_ (Platyhelmintes, _Digenes_) based on partial _rDNA_ sequences. Int. J. Parasitol. 30, 83-93.
- Tkach, V.V., Snyder, S.D., 2007. _Choanoctyle platii_ sp. nov from the northern long-necked turtle, _Chelodina nigra_ ( _Pleurodira : Chelidae_ ) in Australia. Acta Parasitol. 52, 318-324.
- Tkach, V.V., Snyder, S.D., 2007a. _Aptorchis megacetabulus_ n. sp. ( _Platyhelmintes : Digenes_ ) from the northern long-necked turtle, _Chelodina nigra_ ( _Pleurodira : Chelidae_ ), in Australia. J. Parasitol 93, 404-408.
- Tkach, V.V., Snyder, S.D., Swiderski, Z., 2001. On the phylogenetic relationships of some members of _Macroderoididae_ and _Ochetosomatidae_ ( _Digenes_, _Plagiochiidae_ ). Acta Parasitol. 46, 267-275.
- Ponder, W.F., Hallan, A., Shea, M., Clark, S.A., 2016. Australian freshwater mollusks (Accessed 12 August 2021).