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The first DNA barcodes for the Australian platypus tick *Ixodes ornithorhynchi* Lucas, 1846 (Acari: Ixodidae) to facilitate conservation efforts for a declining parasite and its host

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**ABSTRACT**

*Ixodes ornithorhynchi* is one of Australia’s most cryptic tick species and is found only on the platypus (*Ornithorhynchus anatinus*). The first cytochrome c oxidase subunit 1 (CO1) sequences for this species are presented to facilitate molecular identification and conservation of both this tick species and its host.

**Keywords** COI, parasite conservation, platypus inbreeding, bottleneck

**Zoobank** [http://zoobank.org/65AEFA11-8A3F-425B-B016-A7FBBA8BBCF9](http://zoobank.org/65AEFA11-8A3F-425B-B016-A7FBBA8BBCF9)

**Introduction**

*Ixodes ornithorhynchi* is a host-specific tick of the Australian platypus (*Ornithorhynchus anatinus*) (Roberts, 1970). In recent decades, many platypus populations have become fragmented and both the host and parasite are believed to have gone extinct on the mainland in at least one Australian state (Grant and Temple-Smith, 2003). The platypus has an extensive range spanning most of eastern Australia, but both it and *I. ornithorhynchi* are of conservation interest due to this pattern of decline (Menkhorst and Knight, 2011). Although tick population sizes can be monitored using morphological identification, assessing the conservation status of a tick population often requires molecular study to identify levels of genetic diversity and instances where genetic bottlenecks form. To better facilitate research on the conservation biology of *I. ornithorhynchi* and the platypus in Australia, the first DNA barcodes of *I. ornithorhynchi* are presented.

**Material and methods**

Twelve tick specimens (representing 9 nymphs and 3 adult females) collected from two platypuses (*O. anatinus*) in Jackson Creek, Sunbury, Victoria, Australia were identified using morphology-based keys provided by Roberts (1970) under a light microscope. Scanning electron micrographs were taken of specimens from which DNA barcodes were generated. These were then using a Hitachi TM3030 electron microscope and are presented in Figure 1.
Total genomic DNA was extracted from 12 individuals using the Nucleospin Tissue Kit (Machery-Nagel, Germany) following the manufacturer’s protocols. The mitochondrial COI gene was targeted with a primer combination adapted from Kwak et al. (2017). This comprised a forward primer, HCO2064 (5’-GGT GGG CTC ATA CAA TAA ATC C-3’) and a reverse primer, HCO1240 (5’-CCA CAA ATC ATA AAG ACA TTG G-3’) yielding a fragment of 846 bp in length. The COI gene segment was amplified from the purified genomic DNA using the Phusion HotStart Taq DNA polymerase (New England Biolabs, USA) with accompanying reagents according to the manufacturer’s protocols. The PCR was run on a BioRad model T100 thermocycler with a temperature cycle encompassing denaturation at 98 °C for 30 s, annealing at 48 °C for 30 s, and primer extension at 72 °C for 45 s. The cycle was repeated 30 times and bracketed by an initial denaturation step at 98 °C for 2 min and a terminal extension step at 72 °C for 5 min. PCR products were visualised by gel electrophoresis (1%), stained using SYBRSafe (Invitrogen, USA) and compared against size standards (Quick Load PCR Marker #N0475S, New England Biolabs, USA). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) following the manufacturer’s protocol and were submitted to the Australian Genome Research Facility (AGRF) for bidirectional Sanger sequencing using the same primers as those used for PCR.

A phylogenetic tree was constructed using the program MEGA6 (Tamura et al., 2013). Sequences were trimmed then aligned using the ClustalW function. The phylogeny was inferred using the neighbour-joining method with 10,000 bootstrap replications. Sequences of *I. ornithorhynchi* were compared against those of other *Ixodes* species present on GenBank and are presented in Table 1. Species used for comparison include those from the main Australian subgenera as well as the *Afrixodes* and *Partipalpiger* which exhibit morphological similarities with *I. ornithorhynchi*, as listed by Clifford et al. (1973). The southern reptile

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**Figure 1** Ventral view of *Ixodes ornithorhynchi* specimens from which DNA barcodes were generated. A – Adult female; B – Nymph.
### Table 1  *Ixodes* COI sequences used for the molecular analysis.

| Subgenus       | Species                  | Genbank accession number |
|----------------|--------------------------|--------------------------|
| *Ceratixodes*  | *Ixodes uriae*           | KX360345.1               |
| *Coxixodes*    | *Ixodes ornithorhinchi*  | MH666077                 |
|                | *Ixodes ornithorhinchi*  | MH666078                 |
| *Endopalpiger* | *Ixodes fecialis*        | KX673872.1               |
|                | *Ixodes fecialis*        | KX673871.1               |
| *Exopalpiger*  | *Ixodes australiensis*   | KX673865.1               |
|                | *Ixodes australiensis*   | KX673864.1               |
|                | *Ixodes australiensis*   | KX673863.1               |
|                | *Ixodes tasmani*         | KY213772.1               |
|                | *Ixodes tasmani*         | KY2137869.1              |
|                | *Ixodes victoriensis*    | KY2137874.1              |
|                | *Ixodes victoriensis*    | KY2137873.1              |
|                | *Ixodes woyliei*         | KY2137881.1              |
|                | *Ixodes woyliei*         | KY2137880.1              |
|                | *Ixodes woyliei*         | KY2137879.1              |
| *Sternalixodes*| *Ixodes cornuatus*       | KY213793.1               |
|                | *Ixodes cornuatus*       | KY213792.1               |
|                | *Ixodes cornuatus*       | KY213791.1               |
|                | *Ixodes hirsti*          | KY213775.1               |
|                | *Ixodes hirsti*          | KY213774.1               |
|                | *Ixodes hirsti*          | KY213773.1               |
|                | *Ixodes holocyclus*      | KY213783.1               |
|                | *Ixodes holocyclus*      | KY213782.1               |
|                | *Ixodes holocyclus*      | KY213781.1               |
|                | *Ixodes trichosuri*      | KY213777.1               |
|                | *Ixodes trichosuri*      | KY213778.1               |

Tick (*Bothriocroton hydrosauri*) was used as an outgroup (GenBank reference: FJ584427.1). Bootstrap values <45 are not shown as the purpose of the phylogeny is to demonstrate the distinctiveness of the species from its relatives rather than to infer, and support, deeper evolutionary relationships.

## Results

The adult female specimens were identified as *I. ornithorhynchi* based on a combination of morphological features including unarmed coxae, the absence of cornua and auriculae, large porose areas, hypostomal dentition of 3/3, long palps in which all four articles are distinct and without spurs, and an anal groove which does not meet posteriorly. Nymphs were identified as *I. ornithorhynchi* based on a combination of morphological features including unarmed coxae, the absence of cornua and auriculae, hypostomal dentition of 2/2, long palps in which all four articles are distinct and without spurs, and an anal groove which does not meet posteriorly.

Unique sequences were obtained at the COI locus for two *I. ornithorhynchi* specimens representing one nymph and one adult female tick (Table 1). All other sequences were identical to that of the nymph.
Phylogenetic analysis (Figure 2) placed *I. ornithorhynchi* in a distinctly separate clade from all other tick species to which it was compared. Although several morphological similarities exist between *I. ornithorhynchi* and members of other subgenera, *I. ornithorhynchi* does not fall within any of these clades.

**Discussion**

Based on the phylogenetic tree presented in Figure 2, *I. ornithorhynchi* appears to be distinct from all other species to which it was compared. This is unsurprising, as the higher classification of *I. ornithorhynchi* has been far from settled historically due to the morphological distinctiveness of the species. Since Schulze (1941) erected the monotypic genus *Coxixodes* for *I. ornithorhynchi* in a short passage of German, few taxonomists have accepted and used it, even though it was reclassified as a subgenus. Roberts (1970) completely ignored the subgenus and Clifford *et al.* (1973) avoided the use of *Coxixodes*, citing limited available data to support its validity.

The evolutionary relationship between *I. ornithorhynchi* and the other *Ixodes* subgenera remains poorly understood. The genus *Ixodes* comprises over 240 known species, many of which are still without molecular data (Guglielmone *et al.*, 2010). This lack of data renders a total comparison of species at the COI locus impossible. As more DNA sequences of the COI locus become available for members of *Ixodes*, it may become possible to determine the evolutionary relationships between closely related *Ixodes* species. However, as demonstrated by Kwak *et al.* (2017), the COI marker only appears useful in distinguishing the evolutionary relationships between closely related members of the *Ixodes* genus. Therefore, the phylogeny presented in Figure 2 is only valuable to demonstrate the distinctiveness between *I. ornithorhynchi* and the other *Ixodes* to which it was compared. Analysis of more
highly conserved genetic markers will be required to make inferences about the evolutionary
relationships amongst species of this highly diverse genus.

Although DNA barcodes of *I. ornithorhynchi* are useful for delimiting species boundaries,
they may also serve a purpose in future attempts to monitor and map the population dynamics
of platypus ticks and their hosts, both past and present. Nieberding and Olivieri (2007) suggested
that parasites could be used to map past population events in their hosts such as rapid range
expansions or contraction events causing genetic bottlenecks. Biek *et al.* (2006) successfully
demonstrated this when they showed how an examination of a directly transmitted parasite
could be used to map the recent demographic history of its host population. As *I. ornithorhynchi*
is a directly transmitted, host specific parasite, and the only tick species regularly found on
the platypus, it is a good candidate for such study. Haplotype diversity at the COI locus could
be used as a proxy to estimate historical levels of gene flow between platypus populations.
Although COI is a maternally inherited marker, it may still prove useful for providing a
raw estimation of population stability. Previously, COI has been used in New Zealand in
endangered *Amblyomma sphenodonti* populations to estimate gene flow and intrapopulation
genetic diversity (Miller *et al.*, 2007). Measuring haplotype diversity at the COI locus could
similarly be a valuable way to infer and monitor gene flow and relative genetic diversity in *I.
ornithorhynchi* populations to ensure the continued survival of one of Australia’s most cryptic
arthropods across its range.

References

Biek R., Drummond AJ. and Poss M. 2006. A virus reveals population structure and recent demographic
history of its carnivore host. Science, 311(5760): 538-541. doi:10.1126/science.1121360

Clifford CM., Soneshine DE., Keirans JE. and Kohls GM. 1973. Systematics of the subfamily Ixodinae
(Acarina: Ixodidae). 1. the subgenera of *Ixodes*. Annals of the Entomological Society of America
66(3): 489-500. doi:10.1093/aesa/66.3.489

Grant TR. and Temple-Smith PD. 2003. Conservation of the platypus, *Ornithorhynchus anatinus*: threats
and challenges. Aquatic Ecosystem Health & Management 6(1): 5-18. doi:10.1080/14634980301481

Guglielmone AA., Robbins RG., Apanaskevich DA., Petney TN., Estrada-Peña A., Shaw R. and Barker
SC. 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid
species names. Zootaxa 2528: 1-28.

Kwak ML., Beveridge I., Koehler AV., Malipatil M., Gasser RB. and Jabbar A. 2017. Phylogenetic
analysis of the Australasian paralysis ticks and their relatives (Ixodidae: *Ixodes*, *Sternalixodes*).
Parasites & Vectors 10(1): 122. doi:10.1186/s13071-017-2045-4

Menkhorst P. and Knight F. 2011. Field guide to the mammals of Australia (3rd Ed). Melbourne: Oxford
University Press.

Miller HC., Conrad AM., Barker SC. and Daugherty CH. 2007. Distribution and phylogenetic analyses
of an endangered tick, *Amblyomma sphenodonti*. New Zealand Journal of Zoology 34(2): 97-105.
doi:10.1080/03014220709510068

Nieberding CM. and Olivieri I. 2007. Parasites: proxies for host genealogy and ecology?. Trends in
Ecology & Evolution 22(3): 156-165. doi:10.1016/j.tree.2006.11.012

Roberts FHS. 1970. Australian ticks. Melbourne: CSIRO Publishing.

Schulze P. 1941. Das Geruchsorgan der Zecken. Untersuchungen über die Abwandlungen eines
Sinnesorgans und seine Stammesgeschichtliche Bedeutung. Zeitschrift für Morphologie und Ökologie
der Tiere 37: 491–564.

Tamura K., Stecher G., Peterson D., Filipski A. and Kumar S. 2013. MEGA6: molecular evolutionary
genetics analysis version 6.0.. Molecular biology and evolution 30(12): 2725-2729. doi:10.1093/molbev/ mst197