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Haematozoa of wild catfishes in northern Australia

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ABSTRACT

Very little is known about the diversity, prevalence, or pathogenicity of haematozoa in Australian freshwater fishes. Blood smears from 189 native catfishes, of six different species, from northern Australia were examined for haematozoa. Haematozoan infections were observed only in fishes from Queensland, at an overall prevalence of 0.191 (95% CI = 0.134–0.265). Intraerythrocytic haemogregarines were present in *Neoarius graeffei* from the Brisbane River at a prevalence of 0.35 (0.181–0.567). Trypanosomes were present in *Tandanus* species from four rivers, at prevalences ranging from 0.111 (0.020–0.330) to 1 (0.635–1), and in *N. graeffei* from one river in Queensland, at a prevalence of 0.063 (0.003–0.305). The haematozoans observed appeared to have little impact on their hosts. *Tandanus* spp. were significantly more likely to be infected with trypanosomes, suggesting a high parasite-host specificity. This is the first widespread survey of wild Australian freshwater catfishes for haematozoa, resulting in the first report of haemogregarines from Australian freshwater fish, and the first report of trypanosomes from *Neoarius graeffei* and *Tandanus tropicanus*.

1. Introduction

Parasitic haematozoa have been reported in a wide range of fish species worldwide. The most frequently reported haematozoa of fishes are kinetoplastids of the genera *Trypanosoma* and *Trypanoplasma* (*Cryptobia*), and apicomplexans belonging to genera of haemogregarines (Woo, 2006). Trypanosomes and haemogregarines are heteroxenous, and are believed to be transmitted to fish hosts by haematophagous vectors during feeding (Hamilton et al., 2005; Smit et al., 2006; Woo, 2006; Curtis et al., 2013), however a complete understanding of the life cycle of piscine haematozoa remains unknown.

Trypanosome species such as *Trypanosoma danilewskyi* and *T. murmanensis* develop into epimastigotes and metacyclic trypanosomes in the digestive system of their leech hosts (Qadri, 1962; Khan, 1976). Metacyclic trypanosomes accumulate in the proboscis of the leech, are presumably transmitted to their fish hosts during feeding (Woo, 2006), and once in a fish host, trypanosomes such as *T. danilewskyi* replicate as trypomastigotes in the blood (Woo, 1981). The life cycle of haemogregarines is also believed to be heteroxenous, whereby fishes are infected either through ingestion of an intermediate host, or via the introduction of sporozoites into the host through the bite of an infected vector (Davies, 1995).

Only two species of haematozoa have been recorded from Australian freshwater teleosts. Johnston and Cleland (1910) recorded *Trypanosoma bancrofti* in freshwater catfish *Tandanus tandanus* in Queensland, and *Trypanosoma anguillicola* in Australian marbled eel *Anguilla reinhardtii* from New South Wales and Queensland. Mackerras and Mackerras (1961) recorded *T. bancrofti* and *T. anguillicola* from the same host species. Although systematic parasite surveys of native fish species are increasing in Australia, most do not involve examination of blood samples, and therefore it is likely many haematozoan species have not been recorded (Adlard and O'Donoghue, 1998).

The effects of haematozoan infections on individual Australian freshwater fish or the health of fish populations are unknown. Parasites may influence host population dynamics by directly affecting host morbidity and mortality, modulating host growth and reproduction, and altering the likelihood of predation in the wild (Barber et al., 2000). Several studies in Australia, for example, suggest that haematozoa such as trypanosomes may be contributing to the decline of endangered terrestrial mammal species such as the woylie *Bettongia penicillata* (Botero et al., 2013; Thompson et al., 2014), and Gilbert's potoroo *Potorous gilbertii* (Austen et al., 2009) that are already threatened by wider ecosystem changes. Like many of Australia's mammals, native freshwater fishes in Australia are highly endemic, and are increasingly

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threatened by anthropogenic habitat alteration, including the introduction of invasive alien species, exotic disease emergence, and habitat destruction. As certain haemoparasite species are pathogenic to fishes (Ferguson and Roberts, 1975; Khan, 1985; MacLean and Davies, 1990; Woo, 2006), they represent a potential threat to wild populations already under pressure.

Ariid and plotosid catfishes represent a large component of total fish biomass in many northern Australian rivers (Bishop et al., 2001; Jardine et al., 2012), and silver cobbler *Neoarius midgleyorum* forms the basis of Western Australia's only freshwater finfish fishery. Eel-tailed catfish *Tandanus tandanus* is currently listed as Threatened in Victoria under the Flora and Fauna Guarantee Act 1988 (Department of Sustainability and Environment Victoria, 2005), and as Endangered in the Murray-Darling Basin in New South Wales (NSW) under the NSW Fisheries Management Act 1994 (Fisheries Scientific Committee, 2008). Freshwater catfishes are often host to a highly diverse range of tissue parasites (Lymbery et al., 2010), however, no widespread study on the hematozoa of catfishes has been undertaken in Australia. Here, we report on the haematozoa of catfishes sampled from freshwater systems in northern Australia, and investigate the effect of fish size and species on parasite prevalence.

2. Materials and methods

2.1. Sample collection and preparation

Native catfishes were sampled from 11 localities across Western Australia, Queensland and the Northern Territory, using a combination of fyke nets, handlines and electrofishing, between May 2014 and February 2015 (Table 1; Fig. 1). Fishes were euthanised using a prolonged anaesthetic bath of isoeugenol (AquiS, Lower Hutt, New Zealand), examined by eye for ectoparasites, and body weight and total body length were recorded. Blood was collected by caudal vertebral venepuncture, or following excision of the caudal peduncle in small fishes, as described by Kelly and Gibson-Kueh (2015), and major organs were collected and processed for histology as described by Kelly and Gibson-Kueh (2017). One air-dried blood smear per fish was fixed in methanol and stained with Wright-Giemsa (Kinetic, Caboolture, Queensland).

2.2. Microscopic evaluation

Blood smears were systematically scanned using 10x objective lens, followed by closer examination with 40x (high-dry) and 100x oil immersion objective lens (Stockham and Scott, 2008). A sample was considered uninfected if no haematozoa were observed after 15 min of scanning with the 100x oil immersion objective lens (Salkeld and Schwarzkopf, 2005). Histological sections of tissues from infected fishes were examined for the presence of tissue and blood borne parasite stages. Slides were examined on an Olympus BX41 laboratory microscope, and images taken on an Olympus BX51 system microscope, using an Olympus DP70 microscope digital camera and software (www.olympus.com).

2.3. Infection parameters

Prevalence was estimated separately for haemogregarine and trypanosome parasites (see Results), for each fish species in each locality, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa et al., 2000). Fisher exact tests were used to compare differences in prevalence between fish species or genera. Differences in length and weight between infected and uninfected fishes were tested using a non-parametric Wilcoxon test, with a normal approximation.

2.4. Morphometric analyses

Digital images were used to measure key morphological features of haemogregarines (Table 2), and trypanosomes (Table 3), as utilized by Smit et al. (2006) and Mackerras and Mackerras (1961) respectively, using Image J software (open source Java image processing program, available from http://imagej.net/Downloads; Schindelin et al., 2012). Trypanosomes were divided into two different morphological groups on the basis of one morphological trait (KN; see Results). Differences between these groups over all other morphological traits were tested using multivariate analysis of variance (MANOVA) and differences between groups for each trait were tested using one-way analyses of variance (ANOVA), with a Bonferroni correction to maintain an experiment-wide error rate of 0.05. All morphological data were log-transformed and the residuals from all analyses were normally distributed. Where the MANOVA showed a significant difference between groups, stepwise discriminant analysis was used to find the best combination of traits separating the groups. All statistical analyses were implemented in JMP®, Version 10.0 (SAS Institute Inc., Cary, NC).

3. Results and discussion

Blood smears from 189 catfishes, representing six species, were examined (Table 1). No haematozoa were observed in fishes from the

Table 1
Haematozoa present in catfishes by species and collection location.

Sampling location	Latitude (◦ S)	Longitude (◦ E)	Fish species collected (n)	Prevalence (95% CI)	
				Trypanosomes	Haemogregarines
Brisbane River	27·5447	152·7837	*Neoarius graeffei* (20)	0	0.350 (0.167–0.576)
Burnett River	25·2304	152·0116	*Neoarius graeffei* (16)	0.062 (0.003–0.305)	0
Barron River[a]	17·2611	145·5378	*Tandanus tandanus* (18)	0.111 (0.020–0.330)	0
Bloomfield River	15·9868	145·2882	*Tandanus tropicanus* (19)	0	0
Tully Catchment[a]	17·8818	145·8412	*Tandanus tropicanus* (18)	0.333 (0.156–0.586)	0
Palm Tree Creek (Pioneer River)	21·1540	148·7266	*T. tandanus* (3)	0	0
Mary River (site A)	26·0342	152·5106	*Neoarius graeffei* (10)	0	0
			Tandanus tandanus (8)	0.250 (0.046–0.635)	
Mary River (site B)	26·3319	152·7020	*Neosilurus hyrtlii* (1)	0	0
			Neoarius graeffei (1)	0	0
			Tandanus tandanus (8)	1 (0.635–1)	0
Goondaloo Creek (Ross River)	19·3232	146·7630	*Neosilurus hyrtlii* (1)	0	0
			Neosilurus ater (13)	0	
Ord River	15·7932	128·7177	*Neoarius graeffei* (11)	0	0
			Neoarius midgleyorum (13)	0	0
Rapid Creek	12·3955	130·8722	*Neosilurus hyrtlii* (29)	0	0

[a] At these sites, a number of locations were used to capture the required number of fishes and the coordinates refer to the modal locality.

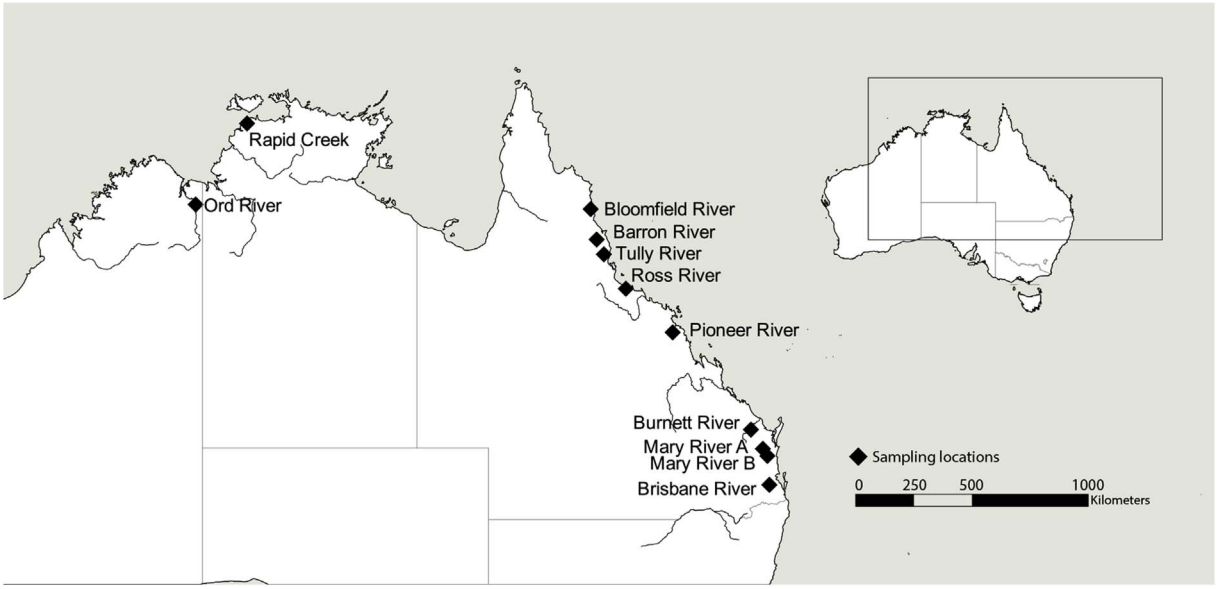

Fig. 1. Location of sampling sites. Map made using Natural Earth and Quantum Geographic Information System version 2.18.14 (Quantum GIS Development Team, 2017).

Table 2
Dimensions and standard errors (S.E.) of morphological features of haemogregarines observed in *N. graeffei* sampled from the Brisbane River.

Feature[a]	No. of organisms measured	Observed range (μm)	Mean ± S.E. (μm)
TL	43	10.418–14.191	12.19333 ± 0.13157
W	43	1.682–3.213	2.2915 ± 0.04851
NL	35	2.915–5.052	4.03306 ± 0.105037
NW	34	0.997–2.03	1.6225 ± 0.046642

[a] TL = total length (measured along midline), W = width (measured across nucleus), NL = length of nucleus, NW = width of nucleus.

Northern Territory (n = 29) or Western Australia (n = 24). Haematozoa were observed in 19.1% (n = 26, 95% CI = 13.39–26.54%) of fishes sampled in Queensland, and infected fishes were present in 62.5% (n = 5, 95% CI = 30.6–86.3%) of rivers sampled in Queensland. Specifically, haematozoa were detected in the blood of 32.4% (n = 12, 95% CI = 19.6–48.5%) of *T. tandanus,* 17% (n = 8, 95% CI = 8.9–30.1%) of *N. graeffei* and 16.2% (n = 6, 95% CI = 7.7–31.1%) of *T. tropicanus* sampled in Queensland, and were not observed in any sampled *N. ater* (n = 13) or *N. hyrtlii* (n = 2). All catfishes appeared healthy on external examination, and no evidence of ectoparasites were observed.

Monomorphic intraerythrocytic inclusions consistent with haemogregarine parasites (Davies, 1995) were observed in 35% (n = 7, 95% CI = 18.1–56.7%) of *Neoarius graeffei* sampled from the Brisbane River (Fig. 2). Haemogregarines were slender in shape with slightly tapering ends (Table 2), light blue cytoplasm and a central nucleus containing 9–13 coarse chromatin clumps on Wright-Giemsa stained smears.

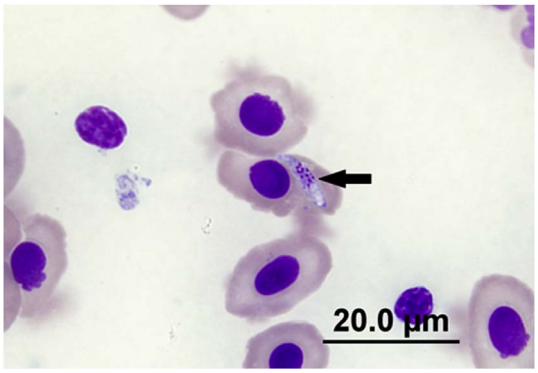

Fig. 2. Intra-erythrocytic haemogregarine in *N. graeffei* from the Brisbane River (Giemsa stain, 100x oil immersion).

Rarely, free parasites were observed attached to an erythrocyte remnant. No haemogregarine species has previously been recorded from *Neoarius graeffei.* The majority of haemogregarine species are reported from marine fishes (Davies, 1995), and many reports of haemogregarines from freshwater fishes are from euryhaline species that may have spent time in the marine environment (Davies, 1995). Several haemogregarines from marine fishes exhibit a wide host-range (Davies, 1995) and, as some populations of *N. graeffei* are euryhaline, there exists the possibility of transmission from inshore marine fishes. Superficial morphological similarities exist between the haemogregarines observed in this study and intraerythrocytic *Haemogregarina balistapi n. sp.* described in orange-lined triggerfish *Balistapus undulatus* from Lizard Island, QLD (Smit et al., 2006), however further studies are required for

Table 3
Means and standard errors (S.E.) of morphological features of the broad and slender trypanosomes observed in this study.

Trypanosome forms	Morphological feature[a]					
	n	TL	W	PK	KN	NA
Broad	29	56.6 ± 1.40	5.47 ± 0.287	1.69 ± 0.230	25.2 ± 0.613	25.16 ± 0.716
Slender	32	22.4 ± 0.396	1.96 ± 0.0926	0.724 ± 0.0868	9.86 ± 0.341	9.11 ± 0.264

[a] TL = total length (measured along midline), W = width (measured across nucleus, includes undulating membrane), PK = posterior to kinetoplast (distance between the kinetoplast, and the posterior end of the trypomastigote body), KN = kinetoplast to nucleus (distance between the kinetoplast, and the posterior edge of the nucleus), NA = nucleus to anterior (distance between anterior edge of the nucleus, to the anterior end of the trypomastigote body).

molecular identification.

Trypomastigotes of *Trypanosoma* spp. were observed in 32.4% (n = 12, 95% CI = 19.6–48.5%) of *Tandanus tandanus,* 16.2% (n = 6, 95% CI = 7.65–31.1%) of *Tandanus tropicanus,* and 2.1% (n = 1, 95% CI = 0.380–11.1%) of *Neoarius graeffei* sampled in Queensland (Table 1). Over all rivers in which trypanosome infections were found, *Tandanus* spp. were more likely to be infected than *Neoarius* spp. or *Neosilurus* spp. (Fisher exact test, p = .001). However, fish species is confounded with site in these analyses. At both sites where *Tandanus* spp. were sampled with co-existing *Neoarius* spp. or *Neosilurus* spp. (Mary River, sites A and B), trypanosomes were present only within *Tandanus* spp., at prevalence rates of 0.250 (n = 2) at the first site and 1 (n = 8) at the second site. Mary River site A was the only site in which sufficient numbers of different species were captured for analysis; although *Tandanus tandanus* was more heavily infected than *Neoarius graeffei*, the difference in prevalence was not significant (Fisher exact test, p = .18). The absence of trypanosomes in *Neosilurus* and *Neoarius* species co-existing with infected *Tandanus* species suggests, however, that the trypanosomes may be host specialists.

The absence of haematozoa from fishes collected in the Northern Territory and Western Australia may be influenced by the fact that larger numbers of catfishes were sampled from Queensland (n = 136) than the Northern Territory (n = 29) and Western Australia (n = 24), and that *Tandanus* species, clearly a species susceptible to trypanosome infection, are not present in these localities. However, it may also reflect the absence of suitable vectors or transmission dynamics (i.e. host/vector interaction) for haematozoa in these locations. Aquatic leeches and isopod crustaceans may serve as vectors for trypanosomes and haemogregarines in aquatic species (Hamilton et al., 2005; Smit et al., 2006; Woo, 2006; Curtis et al., 2013). Within Australia, *Trypanosoma binneyi,* which falls into a clade of trypanosomes from aquatic hosts, is believed to be transmitted to platypuses *Ornithorhynchus anatinus* by aquatic leeches (Paparini et al., 2014), however similar studies have not been conducted for wild freshwater fishes in Australia, and no ectoparasites were observed in this study to suggest a potential vector.

The absence of haematozoa from *N. ater* and *N. hyrtli* sampled in Queensland may be influenced by the fact that few *Neosilurus* species were sampled (n = 15), compared to *Tandanus* (n = 74) or *Neoarius* species (n = 47). However, it may also be due to differences in habitat preference or behaviour, which may influence exposure to suitable vectors. *Neosilurus ater* and *Neosilurus hyrtlii* are nocturnal, shoaling species (Lintermans, 2007; Burrows and Perna, 2006), frequently recorded in slow or still waters with a sandy or muddy substratum (Pusey et al., 2004a). *N. graeffei* occur in a wide range of habitats (Kailola and Pierce, 1988; Pusey et al., 2004b) across a range of salinities, and may undertake lateral movement into floodplains (Pusey et al., 2004b). *T. tropicanus* is a newly described species, however it is closely related to *T. tandanus,* which is a largely nocturnal species with a limited home range and high site fidelity (Koster et al., 2015), that is most frequently reported in lentic habitats (Allen et al., 2002; Lintermans, 2007) where macrophytes and wood provide cover (Koster et al., 2015).

For both species of *Tandanus*, infected fishes were larger than uninfected fishes, possibly due to the greater potential for parasite exposure over time, and a larger surface area of larger fishes for the attachment of vectors. For *T. tandanus*, the mean length of infected fish was 39.46 cm (SE 2.52 cm) and the mean weight was 631.25 (97.25) g compared to 31.91 (1.85) cm and 426.59 (71.82) g for uninfected fish (z = 2.16, P = .03 for length; z = 1.80, P = .07 for weight). For *T. tropicanus*, the mean length of infected fish was 19.13 (2.28) cm and the mean weight was 81.17 (16.74) g compared to 12.74 (1.61) cm and 19.42 (11.84) g for uninfected fish (z = 1.92, P = .05 for length and z = 1.88, P = .06 for weight).

Two morphological forms of trypanosomes were observed in blood smears, and identified as “broad” and “slender” trypomastigotes. Both forms were elongate, with tapering posterior and anterior ends, a basophilic cytoplasm on Wright-Giemsa-stained slides, a terminal kinetoplast, and no apparent free flagellum. Broad trypomastigotes contained a prominent, lightly eosinophilic nucleus on Giemsa-stained slides, and a well-developed undulating membrane (Fig. 3), however these structures were less prominent in slender trypomastigotes (Fig. 4). Broad and slender trypomastigotes could be differentiated on the basis of non-overlapping measurements in the distance between the kinetoplast and nucleus (KN < 15 μm for slender forms and KN > 20 μm for broad forms). From the MANOVA, there was a significant difference between groups over all other morphological traits (Wilks λ = 0.05; $F_{4,56}$ = 246.22, P < .0001) and univariate tests also found significant differences between groups for total length (TL; $F_{1,59}$ = 978.38), width (W; $F_{1,59}$ = 210.88), distance between posterior end and kinetoplast (PK; $F_{1,59}$ = 12.32) and distance between nucleus and anterior end (NA; $F_{1,59}$ = 580.92) (P < .05 for all tests, with the Bonferroni) (Table 3). Discriminant analysis correctly classified 100% of cases along one canonical discriminant function, loading most heavily for TL (0.93), followed by PK (−0.22), W (0.15) and NA (0.07).

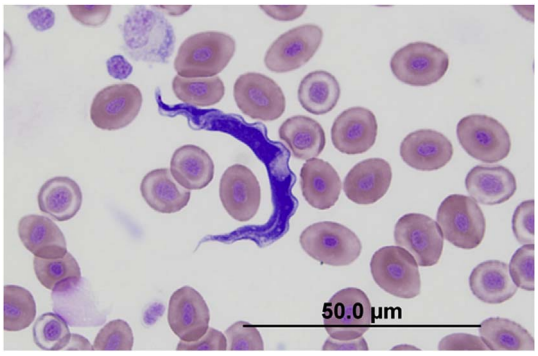

Fig. 3. Broad trypanosome in *T. tropicanus* from the Tully River (Giemsa stain, 100x oil immersion).

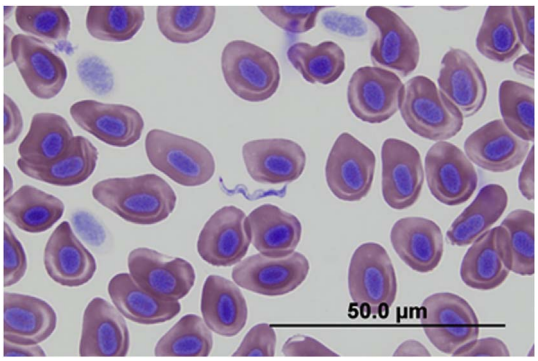

Fig. 4. Slender trypanosome in *N. graeffei* from the Burnett River (Giemsa stain, 100x oil immersion).

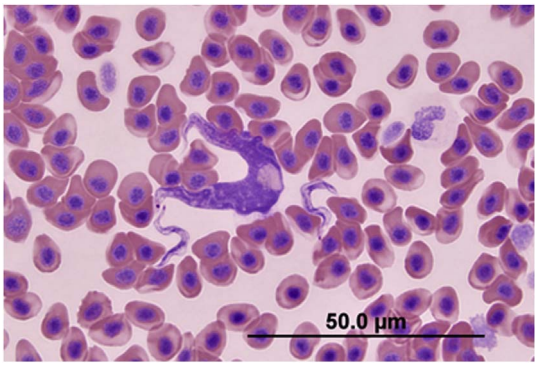

Fig. 5. Broad and slender trypanosomes in *T. tandanus* from the Mary River (Giemsa stain, 100x oil immersion).

Table 4
Comparison of the morphological features of broad and slender form trypomastigotes observed in this study, with *Trypanosoma bancrofti*.

Trypanosome	Morphological features[a]					
	TL	W	PK	KN	NA	FF
Broad	41.3–71.0	3.43–9.01	0–4.55	20.5–30.8	15.7–33.7	0
Slender	19.1–30.3	1.04–3.07	0–1.89	7.18–14.8	5.86–12.9	0
T. bancrofti ("narrow form")[b]	27–31	< 2-3	1	8–12.5	9	4–11
T. bancrofti ("broad form")[b]	50–53.5	4.45–7	1.78	12.5	11	21.5
T. bancrofti ("intermediate form")[b]	34	3.5				
T. bancrofti[c]	21.5–23	2°2.5	0	10.5–11.5	7.5–10	9–11.5

[a] TL = total length (measured along midline), W = width (measured across nucleus, includes undulating membrane), PK = posterior to kinetoplast (distance between the kinetoplast, and the posterior end of the trypomastigote body), KN = kinetoplast to nucleus (distance between the kinetoplast, and the posterior edge of the nucleus), NA = nucleus to anterior (distance between anterior edge of the nucleus, to the anterior end of the trypomastigote body), FF = free flagellum.
[b] Johnston and Cleland (1910).
[c] Mackerras and Mackerras (1960).

Broad trypomastigotes were found in *T. tropicanus* from the Tully River (Fig. 3), while slender trypomastigotes were present in *T. tandanus* from the Barron and Mary River Site A, and *N. graeffei* from the Burnett River (Fig. 4). Both broad and slender trypomastigotes were observed concomitantly within individual *T. tandanus* from the Mary River Site B (Fig. 5). The ranges of all morphological features for slender form trypomastigotes observed in this study overlap with *T. bancrofti* reported by Mackerras and Mackerras (1961), and the "narrow form" *T. bancrofti* reported by Johnston and Cleland (1910) (Table 4). The length, width and PK ranges for broad form trypomastigotes in this study, and "broad form" *T. bancrofti* (Johnston and Cleland, 1910) also overlap (Table 4). *T. bancrofti* is reported to be highly polymorphic (Johnston and Cleland, 1910; Mackerras and Mackerras, 1961), however this has not been confirmed with molecular methods. It is possible that the two forms of trypomastigotes in this study are morphological variants of one polymorphic species, as has been previously suggested for *T. bancrofti* (Johnston and Cleland, 1910; Mackerras and Mackerras, 1961), however it is also possible that they may be different species, particularly considering their concomitant occurrence in individual fish hosts. Further molecular studies are required to test these hypotheses.

No tissue stages or pathology associated with haematozoan infection was observed on examination of histological sections, and the trypanosomes and haemogregarines appear to have had little effect on their hosts. In general, both haemogregarines and trypanosomes are believed to be of low pathogenicity in fishes (Davies, 1995; Woo, 2006), however the pathogenicity of parasites may vary due to host factors such as immunocompetence, and environmental factors such as pollution or temperature, and thus present a potential threat to wild fish populations.

4. Conclusion

Despite its sampling limitations, this study has recorded haematozoa in two new host species of catfish, and has provided the first report of haemogregarines from freshwater fish in Australia, indicating that additional studies to investigate haematozoan diversity in Australian freshwater fishes are likely to be rewarding. Further studies are required for the molecular identification of the parasites observed in this study, and to determine the diversity and life cycles of haematozoa infecting other ecologically important species of fish in Australia. Although trypanosomes and haemogregarines are rarely associated with significant pathology or mortality in wild fishes, knowledge of the normal parasite fauna of wild fish species is important, as host and environmental changes can influence parasite abundance and virulence (Møller et al., 2013), and parasites themselves are important contributors to biodiversity. Australian freshwater ecosystems are increasingly threatened by anthropogenic practices, and identification and knowledge of the potential threats to wild fish health, such as haematozoa, is important for the conservation of Australia's unique freshwater biodiversity.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2017.12.002.

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