The haematology of six species of native catfish from northern Australia

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Abstract

Haematology has the potential to be a valuable tool in determining the health status of wild fish populations and wider ecosystem health. However, limited haematological data are available for wild Australian fish species, and the interpretation and nomenclature of leukocytes is variable and inconsistent in fish. The morphology and cytochemical reactions of erythrocytes, thrombocytes and leukocytes of 189 wild eel-tailed catfish (Tandanus tandanus), Wet Tropics tandan (Tandanus tropicanus), Hyrtyl’s tandan (Neosilurus hyrtlii), black catfish (Neosilurus ater), lesser salmon catfish (Neoarius graeffei), and silver cobbler (Neoarius midgleyi) are described. Erythrocytes, thrombocytes, lymphocytes, monocytes and heterophils in all six species are morphologically similar to those reported in other Siluriformes. Basophils and eosinophils are rarely reported in fish; however basophils were observed in peripheral blood smears of T. tropicanus and N. ater, and eosinophils were observed in N. graeffei. Periodic acid Schiff positive granular leukocytes were observed in N. graeffei, N. midgleyi, N. ater and T. tandanus. This is the first report on the leukocyte morphology and cytochemistry of any native Australian catfish species, and provides useful baseline data for future assessments of fish health and ecosystem integrity in Australia.

Additional keywords: cytochemistry, leukocytes, PAS-GL.
Introduction

Haematology has the potential to be a valuable tool for health assessment in fish, as in conventional human and veterinary medicine (Blaxhall 1972; Bruno and Munro 1986; Rehulka and Minarik 2007); however, several factors currently encumber its general use. Nucleated erythrocytes and thrombocytes preclude the use of automated haematological analysers, necessitating manual techniques that rely on operator expertise. Manual identification of leukocytes in peripheral blood smears can be challenging, due to interspecies variation in the appearance of piscine leukocytes, and the presence of immature leukocytes in piscine peripheral blood smears (Campbell and Ellis 2007). Although cytochemical stains have greatly assisted in the identification of leukocytes in a variety of teleost species (Campbell and Ellis 2007), comparative studies are still required (Tavares-Dias 2006a).

Significant controversy surrounds the classification and nomenclature of teleost granulocytes. Leukocytes present in teleost blood smears are named on the basis of their similarity to mammalian leukocytes (Campbell and Ellis 2007). Neutrophils are a granulocyte commonly observed in mammalian blood smears, and are identified as cells with a segmented basophilic nucleus, and a clear cytoplasm containing unstained granules (Meinkoth et al. 2008). In comparison, granulocytes commonly classified as heterophils in avian and reptilian species have a segmented basophilic nucleus, and a clear cytoplasm containing eosinophilic-staining granules (Campbell and Ellis 2007). In both avian and reptilian species, studies indicate that heterophils are functionally similar to mammalian neutrophils (Campbell and Ellis 2007). Granulocytes resembling mammalian neutrophils have been reported in many teleost species, including common carp (Cyprinus carpio) (Tripathi et al. 2004), channel catfish (Ictalurus punctatus) (Williams and Warner 1976; Zinkl et al. 1991), brown trout (Salmo trutta) (Blaxhall and Daisley 1973) and big head carp (Aristichthys nobilis) (Tavares-Dias 2006a). Heterophils have been reported in many other teleost species (Zinkl et al. 1991; Canfield et al. 1994; Zhang et al. 2011), and in some species both neutrophils and heterophils have been reported (Tavares-Dias and Barcellos 2005). However, the differentiation between heterophils and neutrophils, and the nomenclature used to describe them, is inconsistent in fish. ‘Neutrophil’ is often used to describe the most common granulocyte in teleost peripheral smears, even
if its cytoplasmic granules do not stain neutral (Williams and Warner 1976; Campbell and Ellis 2007). In other cases, many authors use ‘heterophil’ to describe only those cells with eosinophilic and basophilic cytoplasmic granules, and still other authors use heterophil/neutrophil interchangeably (Zinkl et al. 1991). These inconsistencies further complicate the difficult process of leukocyte identification, and the establishment of haematological reference ranges.

Siluriformes are well represented in Australia, and are important contributors to freshwater biodiversity (Allen et al. 2002) and ecosystem health. As anthropogenic pressures on aquatic ecosystems continue to increase, the ability to monitor ecosystem degradation and rapidly identify environmental contaminants will be invaluable to management and conservation efforts. Fish are being increasingly utilised as bioindicators of environmental contamination (Hodson 1976; Schmitt et al. 1993; Jenkins 2004; Costa et al. 2011), and studies of wild catfish species in other countries indicate that they are highly useful bioindicators (Azevedo et al. 2012; Pimpão et al. 2012; Galeb et al. 2013; Harabawy and Ibrahim 2014). As Australian Siluriformes exhibit a wide habitat and dietary range, and are widely distributed across Australia, they also have the potential to be valuable bioindicators. However, in order to utilise haematology to help assess fish population and ecosystem health in this way, reference baseline information on the normal blood cell morphology of individual native fish species is required.

In this study, the morphology and cytochemistry of erythrocytes, leukocytes and thrombocytes of six species of native catfish – eel-tailed catfish (*Tandanus tandanus*), Wet Tropics tandan (*Tandanus tropicanus*), Hyrtyl’s tandan (*Neosilurus hyrtlii*), black catfish (*Neosilurus ater*), lesser salmon catfish (*Neoarius graeffei*), and silver cobbler (*Neoarius midgleyi*) – sampled from 10 river systems in northern Australia were examined. Table 1 contains background information on the health of each sampled river. Most rivers sampled in this study have been impacted to varying degrees by urban development and agricultural practices. However, reports indicate that several of the rivers sampled, such as the Bloomfield River and Ross River in Queensland, are in good health (Wet Tropics Management Authority 2009; Gunn and Manning 2010). This is the first description of leukocyte
morphology of any Australian catfish species, which will be useful to assist in future assessments of wild fish health using haematology.

**Materials and methods**

As part of a larger health survey of wild catfish in Australia, 189 male and female catfish were sampled from 10 river systems in northern Australia. In total, 58 *N. graeffei* (28–2190 g and 15.2–59.5 cm long), 37 *T. tandanus* (18–1135 g and 13.1–47.5 cm long), 37 *T. tropicanus* (2–207.8 g and 4.2–30 cm long), 31 *N. hyrtlai* (1.9–205 g and 7.1–30.2 cm long), 13 *N. ater* (675–950 g and 44.5–48 cm long) and 13 *N. midgleyi* (120–535 g and 23.3–39.7 cm long) were collected by electrofishing, angling or in single-wing fyke nets, between May 2014 and February 2015. Fish were sampled from the Bloomfield River, the Barron River and its tributary Mazlin Creek, Goondaloo Creek (tributary of the Ross River), Bullyard Creek (tributary of the Tully River), Burnett River, Mary River, Brisbane River and Palm Tree Creek (tributary of Pioneer River) in Queensland, the Ord River in Western Australia, and Rapid Creek in the Northern Territory. Table 2 contains GPS locations of sampling, the catfish species caught at each sampling site, and specific holding conditions of fish before blood sampling.

Fish were euthanised in a prolonged bath of isoeugenol (AquIS) immediately before blood collection. Where possible, blood was collected by venepuncture of the caudal vertebral vein using a 21G or 23G needle and a 1- or 2-mL syringe. In smaller fish (<4–6 cm total length), blood was collected from the caudal vein using a heparinised microhaematocrit tube following excision of the caudal peduncle in the freshly killed fish. Where blood volume permitted, three fresh blood smears per fish were made on glass slides using the manual wedge technique. For each fish, one air-dried blood smear was fixed for 1 min in laboratory-grade methanol, and stained with Wright–Giemsa (Kinetik, Caboolture, Queensland). Blood smears were also stained with: (a) periodic acid Schiff (PAS) (Totty 2002); (b) 0.5% toluidine blue (the staining process utilised is similar to that outlined by Kiernan (1999); however, in this study the smears were stained with toluidine blue (CI 52040) for 40 s, differentiated
with 70% ethanol only, and then dehydrated using several changes of butanol); (c) 3, 3'-
diaminobenzidine (DAB) for peroxidase (Dako DAB and substrate chromogen system K3468 kit):
blood smears were immersed in DAB solution for 3 min, washed briefly with running water, stained
with haematoxylin (2 dips), washed briefly in water, immersed in Scott’s solution to blue nuclei,
dehydrated using ethanol, and cleared with xylene.

Results

A summary of the leukocytes observed in each species is presented in Table 3. Nucleated
erythrocytes, thrombocytes, lymphocytes, monocytes and heterophils were identified in the peripheral
blood smears of all six catfish species examined. On Wright–Giemsa-stained blood films, mature
erthrocytes of all six catfish species appeared as oval cells with a central ovoid basophilic nucleus
surrounded by abundant acidophilic cytoplasm (Fig. 1a). Thrombocytes were predominantly elongate
or fusiform and occasionally round, with light blue cytoplasm often containing fine pink granules
(Fig. 1b). These granules stained PAS positive, but stained negatively for peroxidase using DAB.

In all six species, heterophils were large round cells with an eccentric nucleus, which varied in shape
from round to segmented (Fig. 1c). On Wright–Giemsa-stained blood smears, the cytoplasm of
heterophils stained light blue to grey, and contained both basophilic and eosinophilic granules of
variable shapes. Monocytes were large agranular cells with an eccentric nucleus, and dark blue
cytoplasm that was often vacuolated (Fig. 1d). Lymphocytes in all six catfish species were round,
variably sized cells, with a large round central nucleus and scant basophilic cytoplasm (Fig. 1e).

Basophils were observed only on Wright–Giemsa-stained blood smears of *T. tropicanus* and *N.
ater* (Fig. 1f). These cells were variable in size, with a large eccentric nucleus and deeply basophilic
granules of variable size. Eosinophils were identified only in the peripheral blood smear of *N.
graeffei*. On Wright–Giemsa-stained blood smears, eosinophils were spherical cells, with eccentric
nuclei and numerous fine eosinophilic cytoplasmic granules (Fig. 1g). PAS-positive granular
leukocytes (PAS-GL) were identified in *N. graeffei*, *N. midgleyi*, *T. tandanus* and *N. ater*. In Wright–
Giemsa-stained blood smears, they were medium to large granulocytes with a small spherical and eccentric nucleus, and abundant unstained cytoplasmic granules (Fig. 1h).

The PAS-GLs of N. ater and T. tandanus were strongly positive for PAS (Fig. 1i), and were only weakly positive for PAS in N. midgleyi and N. graeffei (Fig. 1j). Heterophils of all six catfish species also contained PAS-positive granules (Fig. 1k); however, the staining reaction to PAS was not as strong as observed in the PAS-GLs of N. ater or T. tandanus. Heterophils stained negative for peroxidase with DAB. In all six species, lymphocytes, eosinophils, basophils and monocytes stained negatively with both PAS and DAB (Fig. 1k), and no basophils with a metachromatic reaction to toluidine blue were observed.

**Discussion**

The thrombocyte and erythrocyte morphology of N. graeffei, N. midgleyi, N. ater, N. hyrtlii, T. tandanus and T. tropicanus were similar to those reported in other Siluriformes. PAS-staining indicated the presence of glycogen granules in the thrombocytes of all six catfish species studied. Glycogen granules have previously been reported in the thrombocytes of channel catfish (*Ictalurus punctatus*) (Tavares-Dias and de Moraes 2007), armoured catfish (*Hoplosternum littorale*) (Tavares-Dias and Barcellos 2005) and Tibetan catfish (*Glyptosternum maculatum*) (Zhang *et al.*. 2011), and are believed to help support the energy requirements of the thrombocytes’ phagocytic activity (Tavares-Dias 2006a; Nagasawa *et al.*. 2014).

PAS-staining also revealed numerous glycogen granules within the heterophils of N. graeffei, N. midgleyi, N. ater, N. hyrtlii, T. tandanus and T. tropicanus, a finding common for both heterophils and neutrophils in fish (Blaxhall and Daisley 1973; Zinkl *et al.*. 1991; Tavares-Dias and Barcellos 2005). In this study, heterophils were the predominant granulocyte observed in the peripheral smears of all catfish species studied. These cells were named as heterophils based on the presence of eosinophilic and basophilic cytoplasmic granules on Wright–Giemsa-stained blood smears, and a negative result to peroxidase (DAB) stain. Differentiation between heterophils and neutrophils in fish is often not
undertaken. Some authors suggest that the term ‘neutrophil’ be confined only to mammals, and ‘heterophil’ to non-mammals (Canfield 1998), and in other cases the terms are often used interchangeably (Zinkl et al. 1991; Ainsworth 1992). However, this may be erroneous, as studies in fish support the presence of heterophils and neutrophils as distinct entities. Although neutrophils and heterophils of fish share many similar cytochemical properties including positivity to PAS and a negative reaction to non-specific esterase (Zinkl et al. 1991; Tavares-Dias and Barcellos 2005), only neutrophils are positive for peroxidase in fish (Zinkl et al. 1991; Tavares-Dias and Barcellos 2005; Campbell and Ellis 2007; Campbell 2012). Given the differences in morphological appearance and cytochemistry, it may be prudent to differentiate between heterophils and neutrophils until further research can shed light on their role in immune function. A standardised method of differentiation will also help avoid confusion and inconsistencies in identification and nomenclature of these granulocytes in fish. On this basis, we suggest that the term ‘neutrophil’ be used for cells containing neutral-staining cytoplasmic granules that contain peroxidase, similar to mammalian neutrophils, and ‘heterophil’ for cells containing coloured granules that are peroxidase negative, similar to avian heterophils (Campbell and Ellis 2007).

The morphological and cytochemical features of *T. tandanus* and *N. ater* PAS-GLs were similar to those described in duckbill catfish (*Sorubim lima*) (Bianchi et al. 2014), bighead carp (*Aristichthys nobilis*) (Tavares-Dias 2006a), and 30 other marine and freshwater fish species by Barber and Westermann (1978). Similar cells with a weak PAS reaction, as seen in *N. graeffei* and *N. midgleyi* in this study, have been reported by Zinkl et al. (1991) in granular leukocytes of goldfish (*Carassius auratus*). In white suckers (*Catostomus commersoni*), injection of compound 48/80, which promotes histamine release, altered the morphology and number of PAS-GLs (Barber and Westermann 1978b). Since many of these histamine-induced morphological changes were similar to those observed in mammalian basophils and mast cells during histamine release, and basophils were absent in the peripheral blood of *C. commersoni*, it was suggested that PAS-GLs may be functionally equivalent to, or evolutionary precursors of, basophils or mast cells (Barber and Westermann 1978b; Tavares-Dias 2006a). However, both PAS-GLs and basophils were present in the peripheral blood of *N. ater* in this
study, and the concurrent presence of metachromatic basophils and PAS-GLs has been reported in other teleost species, such as *S. lima* (Bianchi et al. 2014). This evidence suggests that PAS-GLs may not be the evolutionary precursor of basophils, as suggested by Barber and Westermann (1978b); however, it is possible that PAS-GLs are a cell lineage precursor to basophils.

Basophils and eosinophils are rarely reported in the peripheral blood of teleosts (Tavares-Dias 2006b); however, this study revealed cells typical of basophils in Wright–Giemsa-stained blood smears of *T. tropicanus* and *N. ater*. The morphological features of the basophils observed in *T. tropicanus* and *N. ater* were similar to those described in other Siluriformes such as *I. punctatus* (Williams and Warner 1976; Tavares-Dias and de Moraes 2007) and moustache catfish (*Synodontis membranacea*) (Owolabi 2011). Metachromatic staining of mucopoysaccharide granules in toluidine blue occurs in mammalian basophils (Cooper and Cruickshank 1966), and the use of 0.5% alkaline (pH 9) toluidine blue, as used in this study, has revealed metachromatic granules within basophils in several fish species (Tavares-Dias 2006b). Although basophils containing metachromatic granules were not observed in 0.5% toluidine blue–stained blood smears of *T. tropicanus* and *N. ater*, a study by Tavares-Dias (2006b) suggests that basophil staining with toluidine blue is variable, and several methods, including methanol fixation, as used in this study, can negate the metachromasia reaction. Thus, although no basophils exhibiting metachromasia to toluidine blue were observed in this study, this may be due to a failure in basophil granulation preservation, rather than a true absence of basophils in the peripheral blood of *T. tropicanus* and *N. ater*.

The presence of eosinophils in teleost peripheral blood smears is reportedly equally rare (Campbell and Ellis 2007). Eosinophils have been reported in several catfish species including *H. littorale* (Tavares-Dias and Barcellos 2005), *S. membranacea* (Owolabi 2011), and Mekong giant catfish (*Pangasionodon gigas*) (Phoonsamran et al. 2008). Williams and Warner (1976) also reported eosinophils in *I. punctatus* on Romanowsky-stained smears; however, Tavares-Dias and de Moraes (2007) and Zinkl et al. (1991) found no eosinophils using cytochemical and Romanowsky stains in the same species. Although eosinophils were observed only in *N. graeffei*, and basophils only in *T. tropicanus* and *N. ater*, these cells were observed in only very low numbers in the peripheral blood.
smears, and their absence from smears of the other species in this study does not preclude their existence in these species.

In conclusion, the leukocyte morphology of *N. ater*, *N. graeffei*, *N. midgleyi*, *N. hyrtlii*, *T. tandanus* and *T. tropicanus* in Wright–Giemsa-stained peripheral blood smears was similar to those reported in other teleost species. The morphology and cytochemistry of lymphocytes and monocytes for all six catfish species were similar to those recorded in other siluriform species. The PAS-GLs of *N. graeffei*, *N. midgleyi*, *N. ater* and *T. tandanus* may be precursors of basophils or mast cells, although further studies are required to determine the true identity and role of these granulocytes in immune processes. Data establishing the normal leukocyte morphology of wild fish species, as presented in this study, forms an important basis for future haematological studies, and the future application of haematology as a tool to assess wild fish health and investigate disease.

**Acknowledgements**

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References


Table 1. Health of sampled rivers

<table>
<thead>
<tr>
<th>River</th>
<th>River health</th>
<th>Method of assessment</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brisbane River</td>
<td>Poor</td>
<td>Healthy Waterways’ Monitoring Program – abiotic and biotic factors (Healthy Waterways 2014a)</td>
<td>Highly modified urban stream, large volumes of runoff, riparian vegetation has been cleared, exotic fish species present (i.e. tilapia).</td>
<td>Healthy Waterways (2014b)</td>
</tr>
<tr>
<td>Bloomfield River</td>
<td>Good</td>
<td>Modified pressure-state-response approach developed by the Organisation for Economic Cooperation and Development (OECD 1993).</td>
<td>90–100% native vegetation cover, only minor change in hydrological conditions, few threatened species. No exotic fish species recorded.</td>
<td>Wet Tropics Management Authority (2009); Burrows (2009)</td>
</tr>
<tr>
<td>Barron</td>
<td>Impacted</td>
<td>Water quality and exotic fish introductions.</td>
<td>Levels of diazinon and chlorpyrifos exceed established freshwater 99% species protection guidelines. Three exotic fish species, and 36 translocated native species recorded.</td>
<td>O’Brien et al. (2014); Burrows (2009)</td>
</tr>
<tr>
<td>Rapid Creek</td>
<td>Severely impaired</td>
<td>Australian River Assessment System (AUSRIVAS) – macroinvertebrate populations (Lloyd and Cook 2002).</td>
<td>Urban creek, significant nutrient and metal runoff.</td>
<td>Fortune (2015)</td>
</tr>
<tr>
<td>Lake Kununurra (Ord River)</td>
<td>Modified</td>
<td>Abiotic and biotic factors.</td>
<td>Residual levels of DDT and other organochlorides recorded in fish, high volume of weeds significantly impacting native vegetation. No exotic fish species reported.</td>
<td>Morgan et al. (2011); Fredericks and Palmer (2008)</td>
</tr>
<tr>
<td>River (state)</td>
<td>GPS coordinates</td>
<td>Species sampled</td>
<td>Collection method</td>
<td>Time between collection and blood sample</td>
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<td>----------------------------------------</td>
</tr>
<tr>
<td>Goondaloo Creek (Qld)</td>
<td>−19.3232, 146.7630</td>
<td>1 <em>N. hyrtii</em>, 13 <em>N. ater</em></td>
<td>Electrofishing</td>
<td>&lt;1 day (fish held overnight in outdoor tanks)</td>
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<tr>
<td>Brisbane River (Qld)</td>
<td>−27.5447, 152.7837</td>
<td>20 <em>N. graeffei</em></td>
<td>Electrofishing, hand line</td>
<td>3–4 days (fish held in outdoor tanks)</td>
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<td>Palm Tree Creek (Qld)</td>
<td>−21.1540, 148.7266</td>
<td>3 <em>T. tandanus</em></td>
<td>Electrofishing, single-wing fyke nets</td>
<td>Sample taken in the field, immediately after caught</td>
</tr>
<tr>
<td>Bloomfield River (Qld)</td>
<td>−15.9868, 145.2882</td>
<td>19 <em>T. tropicanus</em></td>
<td>Electrofishing, single-wing fyke nets</td>
<td>1–3 days, kept in holding nets in the river for 1 day, then held in indoor tanks</td>
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<td>Barron River and Mazlin Creek (Qld)</td>
<td>−17.2611, 145.5378 and −17.2533, 145.4769</td>
<td>18 <em>T. tandanus</em></td>
<td>Electrofishing, single-wing fyke nets</td>
<td>2 weeks, held in outdoor tanks</td>
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<td>Bullyard Creek (Qld)</td>
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<td>Electrofishing, single-wing fyke nets</td>
<td>2 weeks, held in outdoor tanks</td>
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<td>Burnett River (Qld)</td>
<td>−25.2304, 152.0116 and −25.1336, 151.9835</td>
<td>16 <em>N. graeffei</em></td>
<td>Electrofishing, single-wing fyke nets</td>
<td>Sample taken in the field, immediately after caught</td>
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<td>Mary River (Qld)</td>
<td>−26.0342, 152.5106</td>
<td>8 <em>T. tandanus</em>, 10 <em>N. graeffei</em></td>
<td>Electrofishing, single-wing fyke nets</td>
<td>Sample taken in the field, immediately after caught</td>
</tr>
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<td>Mary River (Qld)</td>
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<td>8 <em>T. tandanus</em>, 1 <em>N. hyrtii</em>, 1 <em>N. graeffei</em></td>
<td>Electrofishing, single-wing fyke nets</td>
<td>Sample taken in the field, immediately after caught</td>
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<td>29 <em>N. hyrtii</em></td>
<td>Electrofishing</td>
<td>1 week, held indoor in tanks</td>
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<td>Ord River (WA)</td>
<td>−15.7932, 128.7177</td>
<td>11 <em>N. graeffei</em>, 13 <em>N. midgleyi</em></td>
<td>Single-wing fyke nets</td>
<td>1–2 weeks, <em>N. graeffei</em> held in indoor tanks, <em>N. midgleyi</em> held in outdoor tanks</td>
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Table 2. River, species and collection method of fish sampled
Table 3. Leukocytes observed in sampled catfish species

<table>
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<tr>
<th></th>
<th>Lymphocyte</th>
<th>Heterophil</th>
<th>Monocyte</th>
<th>Eosinophil</th>
<th>Basophil</th>
<th>PAS-GL</th>
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<td><em>T. tandaicus</em></td>
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<td>Present</td>
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<td>Not observed</td>
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<td>Present</td>
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<td>Present</td>
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<tr>
<td><em>N. hyrtlisi</em></td>
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<td>Present</td>
<td>Present</td>
<td>Not observed</td>
<td>Not observed</td>
<td>Not observed</td>
</tr>
<tr>
<td><em>N. groovei</em></td>
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<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Not observed</td>
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<tr>
<td><em>N. midgleyi</em></td>
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<td>Present</td>
<td>Not observed</td>
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Fig. 1. (a) Mature erythrocytes and one immature erythrocyte of *T. tropicanus* (Wright–Giemsa). (b) Elongate thrombocytes of *T. tropicanus*, containing fine pink granules (Wright–Giemsa). (c) Heterophil of *N. ater*, with a segmented nucleus (Wright–Giemsa). (d) Monocyte of *T. tropicanus* (Wright–Giemsa). (e) Lymphocyte of *T. tropicanus* (Wright–Giemsa). (f) Basophil of *T. tropicanus* (Wright–Giemsa). (g) Eosinophil of *N. graeffei* (Wright–Giemsa). (h) Large PAS-GL of *N. ater* (Wright–Giemsa). (i) Small PAS-GL of *N. ater* (PAS). (j) Large PAS-GL of *N. midgleyi* (PAS). (k) A monocyte (left) and heterophil of *T. tandanus* (PAS). All images 100× magnification; scale bar = 10.0 μm.