

## A NEW *TRICHOMONAS* SP. IN AUSTRALIAN PIGEONS

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### INTRODUCTION

*Trichomonas gallinae* is a protozoan parasite of the avian upper gastrointestinal tract with a world-wide distribution and a broad range of known hosts. The Rock Dove, *Columba livia*, is considered its natural host, and by inference has often been considered to be either a natural parasite of various columbids (pigeons and doves) or an invasive pathogen occurring in columbid species which otherwise have no natural trichomonad. The discovery of a new *Trichomonas* sp. which has an Australian frugivorous pigeon species as its natural host, has the potential to dramatically change our understanding of the relationship between this protozoan genus and pigeons and doves and calls into question the origin of several trichomonads of significance to human and domestic animal health.

The Order Columbiformes, which includes the pigeons and doves, is a diverse lineage of at least 336 modern taxa, of which 100 are listed as being at risk and a further 15 are considered extinct (Birdlife International, 2008). Species of pigeons and doves are found on all continents except Antarctica and inhabiting almost all terrestrial habitat types including arid deserts and high mountain snowfields (Baptista et al., 1997). It has long been recognised that Australasia is home to a significant proportion of the world's columbid fauna and that, despite an unremarkable number of species for its land-size, mainland Australia has an extraordinary physical, behavioural and ecological diversity amongst its twenty-two species of native pigeons and doves (Frith, 1982; Goodwin, 1967). The discovery of plate tectonics in the 1960s and 1970s and the development of accessible genetic sequencing and phylogenetic analysis techniques have provided compelling evidence for a Gondwanan (Australian-Antarctic-South American) and Cretaceous origin for the pigeons and doves followed by radiation of this group in the Eocene (Hall, 2002; Pereira et al., 2007). Many of the pigeons and doves present in Australia have likely accompanied it on its lonely drift north after rifting with Antarctica 35-45 million years ago (Li and Powell, 2001).

Of the various lineages of pigeons and doves, the fruit-doves (*Ptilinopus* spp.) and imperial-pigeons (*Ducula* spp.) have exhibited amongst the most spectacular radiations (Gibb and Penny, 2010). Both *Ducula* and *Ptilinopus* are clearly of Australasian origin, yet despite having four species of fruit-doves mainland Australia has only one species of imperial-pigeon, the Pied Imperial-pigeon (*Ducula bicolor*) (Christidis, 2008). Most southern Australians are unfamiliar with this species, however it is very noticeable in the north due to its abundance, large size and enormous range from the Kimberley Division in Western Australia along the entire coastline and coastal hinterland of northern Australia (except the Joseph Bonaparte Gulf) to Broad Sound south of Mackay in Queensland (Figure 1) (Barrett et al., 2003). It is a prodigious frugivore and excellent seed-disperser for north Australian rainforests due to its wide ranging movements and gentle handling of seeds (Bhattacharyya, 1994; Brock, 2001; Cadow, 1933). Furthermore, and exceptionally, it is a recognised seasonal migrant with most of the

populations of the east and northern coasts departing their feeding grounds and enormous island breeding colonies between February and April to spend the dry season in Papua New Guinea and Indonesia (Higgins and Davies, 1996), to return again in later in the year (Figure 1).



**Figure 1.** Australia, New Guinea, the Bismarck archipelago and Wallacea with distribution of Pied Imperial-pigeons (*Ducula bicolor*) shaded in grey. Presumed (fine arrows) and known (thick arrows) paths of migration from Australia for this species are illustrated.

*Trichomonas* are a genus of organisms historically considered to be much more limited in diversity and certainly not associated with Australasia in any particular way. The first discovered member of this genus is also the best studied, and it is little wonder considering that *Trichomonas vaginalis* was first identified more than 150 years ago (Thorburn, 1974) and according to the World Health Organisation is now the world's most common human sexually transmitted infection with effects such as decreased birth weights, increased risk of abortion, discomfort and arguably most significantly increased transmission of HIV. The entire genome of *T. vaginalis* has been sequenced (Carlton et al., 2007). Not long after *T. vaginalis* was discovered, a different species of *Trichomonas* was identified in the upper gastrointestinal tract of domestic pigeons (Stabler, 1954). This organism, named *T. gallinae*, is reported as causing disease (trichomonosis) characterised by caseous lesions leading to morbidity or death in the crop, oesophagus, oropharynx and sinuses as well as systemic infections in a range of avian hosts including birds of prey, owls, budgerigars, cuckoos, bustards and of course pigeons and doves (Jessup, 1980; Kocan and Sprunt, 1971; McKeon et al., 1997; Park, 2011; Silvanose et al., 1998; Stabler, 1951). References to disease consistent with trichomonosis (ie canker) in birds of prey exist in medieval treatises on falconry. Trichomonosis is recognised as a threatening process for several endangered columbids, especially the Mauritian Pink Pigeon (*Columba mayeri*) (Bunbury et al., 2007), and has likely caused mortality events and declines in populations of Mourning Doves (*Zenaida macroura*), Wood Pigeons (*Columba palumbus*) and in passerines in the UK (Hofle et al., 2004; Ostrand et al., 1995; Robinson et al., 2010).

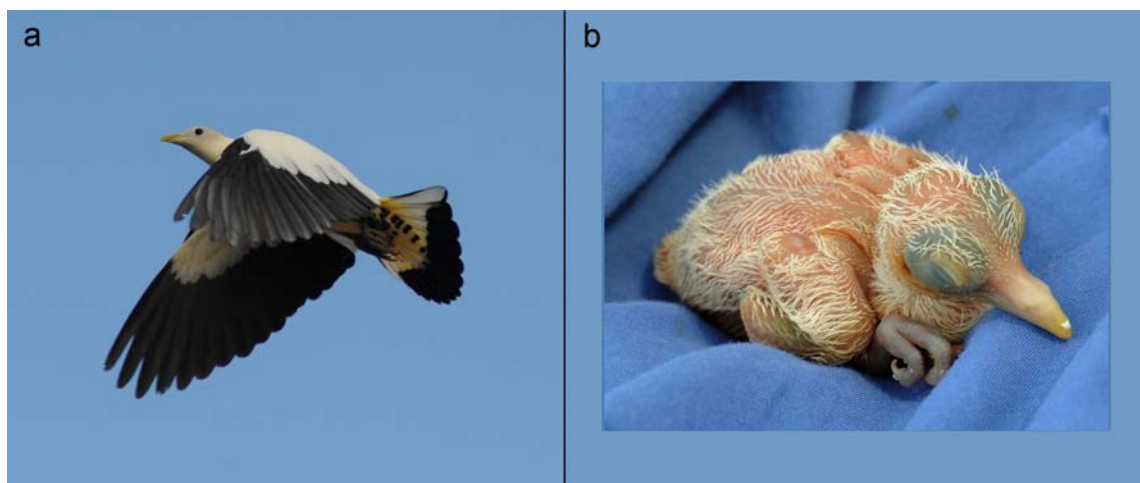
Several other species of *Trichomonas* are recognised including *Trichomonas tenax*, a presumed commensal of the human mouth, *Trichomonas canistomae*, a presumed commensal of the canine mouth, and an unknown *Trichomonas* sp. which was isolated from the crops of two Common Ground-doves (*Columbina passerine*) in the USA (Dobell, 1939; Gerhold et al., 2008; Hegner and Ratcliffe, 1927).

Here we describe a new species of *Trichomonas* that has the Pied Imperial-pigeon as its natural host.

## METHODS

### Sample collection

Adult Pied Imperial-pigeons (Figure 2a) were caught over two weeks in November 2009 using mist-nets suspended from adjustable poles up to 8 m high at Low Isles (Figure 1) in Queensland. This breeding colony supports up to 14,000 breeding pairs of Pied Imperial-pigeons (Low Isles Preservation Society, personal communication) and is situated on a mangrove-covered coral cay lying 15 km off the coast of Port Douglas. Birds were caught as they returned to feed their chicks after foraging during the day in coastal lowland rainforest and suburban fruiting trees. Chicks aged from newly hatched (Figure 2b) to near fledging were taken out of nests by hand and sampled. While young birds were abandoned by their attending parent when approached they appeared to do well after being returned to the nest and all chicks were observed to continue to grow and be cared for on subsequent days.



**Figure 2.** a) An adult Pied Imperial-pigeon (*Ducula bicolor*) in flight as it approaches its breeding colony at Low Isles.  
b) A newly hatched Pied Imperial-pigeon chick removed from its nest for sampling at Low Isles.

The crop of each bird was swabbed with two sterile cotton-tipped applicators, one of which was immediately used to inoculate a commercially available media pouch (InPouch TF, Biomed Diagnostics). The tip of the remaining applicator was placed in a 1.5mL microcentrifuge tube and stored at -20°C. Inoculated pouches were incubated at 37°C and were inspected every 24h for three days using a light microscope under 10× objective for the presence of flagellated protozoans. After the final 72h inspection the pouches were frozen and stored at -20°C.

## Laboratory procedures

DNA was extracted and purified from the applicator tips that were not used to inoculate the pouches and from ten of the pouches that had cultured flagellated protozoans using commercially available kits (DNeasy Blood & Tissue, Qiagen).

1 $\mu$ L of extracted DNA was used as a template for PCR amplification of the ITS1/5.8S/ITS2 region of the small subunit ribosomal gene using trichomonad-specific primers TFR1 and TFR2 and reaction parameters as previously described (Felleisen, 1997). Stained (SyberSafe, Invitrogen) agar gel electrophoresis was used to determine likely positive samples, which exhibited bands at approximately 400bp. PCR products were purified (DNeasy PCR Purification Kit, Qiagen) and submitted to the Australian Genome Research Facility Ltd., Sydney, for bidirectional sequencing using an automated AB 3730xl DNA sequencer (Applied Biosystems). PCR primers TFR1 and TFR2 were used for sequencing.

## Alignment and phylogenetic analysis

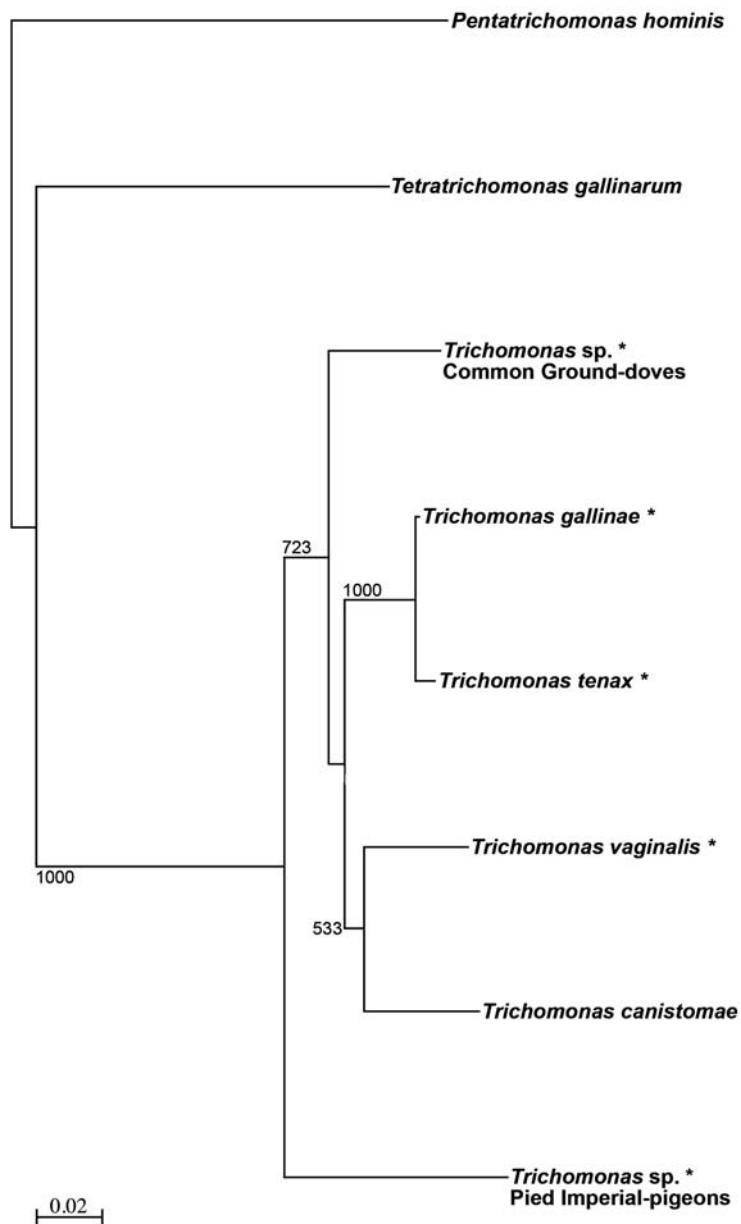
Clean bidirectional sequences were aligned and the resulting contig examined for ambiguities using Sequencher (Gene Codes Corporation). Primers were excised leaving a 326bp sequence that was homologous between Pied Imperial-pigeons. An index sequence was chosen to represent this isolate and was compared to those in existing databases using the nucleotide BLAST service provided by GenBank.

ITS1/5.8S/ITS2 sequences were obtained from GenBank for *Trichomonas vaginalis* (FJ492751), *T. gallinae* (AY349182), *T. tenax* (U86615), *T. canistomae* (EU215359), *Trichomonas* sp. isolated from a Common Ground-dove in the USA (AY244652) with *Pentatrachomonas hominis* (U86616) and *Tetratrachomonas gallinarum* (AY244649) as outgroups. These were aligned with the *Trichomonas* sp. from Pied Imperial-pigeons using ClustalX 2 (Larkin et al., 2007) which was also used to create a bootstrapped neighbour-joining tree of the aligned sequences (Felsenstein, 1981; Saitou and Nei, 1987). 1000 bootstrap trials were run, excluding gaps, and *P. hominis* was used to root the tree. Clades were considered supported if present in more than 500 of the bootstrap trials (Berry and Gascuel, 1996). The tree produced was visualised using Njplot and text was edited using Photoshop CS3 (Adobe).

## RESULTS

### Culture

Swabs of the crop were collected and culture pouches inoculated from 131 Pied Imperial-pigeons (of which 35 were chicks). 52 birds (40%) cultured positive for trichomonads. Positive cultures were observed in 17 of 35 chicks (49%) and 35 of 96 adults (36%).



**Figure 3.** Neighbour-joining phylogram of *Trichomonas* spp. rooted with *Pentatrachomonas hominis* and *Tetratrachomonas gallinarum*. Bootstrap values (/1000) are provided for supported clades. Species marked with an asterisk have been isolated from the crops of pigeons or doves.

### Phylogenetic analysis

67% of crop swabs from the Pied Imperial-pigeons produced PCR products using trichomonad-specific primers of 326bp. 10 of these products were sequenced and found to be homologous between birds. BLAST analysis of the edited ITS1/5.8S/ITS2 sequences confirmed the presence in Pied Imperial-

pigeons of an undescribed species of *Trichomonas* that was at least 10% divergent in this relatively conserved gene from the nearest known trichomonad. Neighbour-joining phylogenetic tree construction (Figure 3) confirmed the placement of this organism within the genus *Trichomonas* however the new species did not group closely with any other species of *Trichomonas* and indeed appeared to be a basal lineage within the genus.

## DISCUSSION

A new species of *Trichomonas* has been found in a frugivorous, migratory species of pigeon in northern Australia.

The existence of this species of *Trichomonas* at such a high prevalence in Pied Imperial-pigeons (40% by culture, 67% by direct PCR of swabs) in the absence of clinical signs such as oropharyngeal lesions or poor body condition is strongly supportive of the Pied Imperial-pigeon being a natural host for the organism. Furthermore this *Trichomonas* appears to be widespread in the host and the author (Peters) has now isolated the same species of *Trichomonas* over two years from four different populations of Pied Imperial-pigeons extending from Cairns to Darwin. It is clear that birds are infected at a very young age, with the youngest sampled bird (Figure 2b) being positive on culture and with the finding of a similar prevalence between chicks and adults. This suggests strongly that adults infect chicks almost immediately upon hatching, possibly through the first feed. Such a high prevalence of this *Trichomonas* sp. in the population and likely high abundance in individuals (considering the sensitivity of culture) it is probable that if indeed entirely parasitic the metabolic cost of this organism on its host is small beyond impacting fitness. Alternatively, and more significantly, it challenges the notion of where this *Trichomonas* lies on the spectrum between mutualism and parasitism and forces consideration of a possible trade-off for any metabolic costs to the host. Considering that this is the first *Trichomonas* sp. to be demonstrably within its natural host this has implications for the way in which all members of this genus are considered, especially regarding the existence of pathogenicity.

While this is not the first time *Trichomonas* has been found in a fruit-eating columbid (Kocan and Sprunt, 1971), this is to the author's knowledge the first report of the protozoan in a pigeon species with such marked migratory behaviour. The implications for this on the interregional movement of this endoparasite seem intuitive however there is a need for the organism to be characterised in Pied Imperial-pigeons across their east-west and north-south clines of migratory range to understand the role of geography, rather than just host species, in the distribution of avian *Trichomonas*.

The existence of the new, possibly basal lineage of *Trichomonas* described here may guide the development of a theory on the origin of this genus. *T. gallinae* has for long been considered a natural parasite of the crop and oesophagus of the pigeon (specifically the Rock Dove) with a high prevalence and worldwide distribution in this species and disease only occurring with particular, virulent strains (Stabler, 1948a; Stabler, 1948b; Stabler, 1954). It seems highly relevant that variants of *T. vaginalis* have been cultured from wild doves and that a new species of *Trichomonas* was found in two Common Ground-doves in the USA (Gerhold et al., 2008), although the significance of these findings was not discussed in that study. Furthermore the author (Peters) has cultured variants of *T. tenax* from a number of native species of Australian pigeons and doves, several species of which exhibit 100% prevalence for this organism. By contrast, almost all molecular studies confirming the place of the common human oral protozoan *T. tenax* within the genus *Trichomonas* have used a single commercially available isolate which was cultured (without record) from the mouth of a human female in north America once. It remains to be proven that the organism deposited in this culture

collection is indeed the common human oral commensal, with the single molecular study examining more than one flagellate isolated from the human oral cavity being unable to find a *Trichomonas* species and finding *Tetratrichomonas* instead (Kutisova et al., 2005).

With five of the six known *Trichomonas* species occurring in wild columbids (Figure 3), and the last, *T. canistomae*, being represented by only three ribosomal gene sequences (two of which come from unpublished sources and two of which differ by 25% despite supposedly being the same gene from the same species) it seems parsimonious that the ancestral *Trichomonas* probably also had the upper gastrointestinal tract of ancestral columbids as its natural environment. The implications for *T. vaginalis* are significant and much work remains to be done to determine if, when and how this important human pathogen changed its natural environment from the dove to the human reproductive tract. The concept of co-evolution of *Trichomonas* and the pigeons and doves has implications too for possible co-radiation in host and parasite and also predator-host-parasite tritrophism, particularly considering the recognised impact the parasite can have on some birds of prey while others which feed heavily on pigeons, such as the Peregrine Falcon (*Falco peregrines*), are seldom affected.

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