Report on the microscopic identification of fruit in the faeces of flying-foxes

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Introduction

The black flying-fox (*Pteropus alecto*) has extended its range to the south by over 1000 km into areas that were previously solely occupied by the grey-headed flying-fox (*P. poliocephalus*) (Roberts *et al*. 2012). This has been happening since the start of the 20th Century. *Pteropus poliocephalus* is considered vulnerable under State and Commonwealth legislation and this change in distribution by *P. alecto* has been considered to be a threatening process for *P. poliocephalus* (Tidemann *et al*. 1999). The level of competition between these newly sympatric flying-foxes is, as yet uncertain. Nevertheless, competition for food between the two species has been suggested as a contributor to the population declines of *P. poliocephalus* (Tidemann *et al*. 1999).

Griffith *et al*. (2020) has identified some of the foods used by *P. poliocephalus* and *P. alecto* when they are occupying the same sites and preliminary work has shown that *P. alecto* eats a greater variety of native and cultivated fruits than does *P. poliocephalus*. However, while considerable work has been done in microscopically identifying the foods used by *P. poliocephalus* in the areas around Sydney (Parry-Jones and Augee 2001, Schmelitschek *et al.* 2009), the complete suite of foods that are available to both species of flying-foxes within the area of sympatry is not known. The identification of foods from faecal material is a method of determining not only which foods are used by flying-foxes but how the diet of the two species may differ. However, currently the microscopic identification of food items in faecal material is limited because many potential foods are not yet included in the reference collection.

The aim of this project is to expand the existing guides on the microscopic identification of foods from flying-fox faeces, to include a suite of possible flying-fox fruit obtained from sub-tropical areas of NSW and to use these to identify previously unidentified foods found during preliminary (Griffith *et al*. 2020) and current investigations. The photographs will form a permanent record of the identifiable structures within each fruit and will be used as a reference collection for subsequent projects. I have Ethics Permission (University of Sydney 2018/1466) and I have a NSW Research Licence (APP-0006026949) for projects deriving from this project.

The Project

Over 50 fruits were collected from mid and northern NSW. The fruits were kept cool during transport and prior to being processed.

To identify structures in the known fruit that can aid in identifying it in faecal samples, a small representative sample was teased apart and mixed with sufficient water to make a slurry. Two wet mounts were created from drops of the slurry then each slide was examined by light microscopy at low power (x100) and high power (x400) magnification. Continual scanning was carried out over both slides (Bumrunsri *et al*. 2013) and, using a digital microscope camera, photos were taken of structures that were characteristic of that fruit.

Flying-foxes eat fruit for its flesh. Most of the undigestible parts of the fruit are either spat out at source or pass through the animal into the faeces. So the identifiable structures within faecal material are generally pieces of fruit skin, fibres or sclereids. Sclereids are hard cells that are found scattered through the flesh of most fruits and among other things form the cores of apples and the shells of nuts. The gritty taste of pear and that of a lot of bush tucker is because of the large numbers of sclereids in their flesh. Microscopically they can be identified by their “solid” appearance: they are refractile and often have a double wall with a hole in the centre. They are a useful tool in the identification of fruit because their shape and size is often characteristic of the fruit and they appear unchanged in flying-fox droppings.

The images from the known fruits, were compared with fruits that were deemed “unknowns” in a previous project (Griffith *et al*. 2020) to see if a match could be made and the “unknown” identified.

Three categories of fruit were targeted during the collection: *Ficus* spp.; native fruit (known items of "Bush Tucker" in trees higher than 2 m) and tropical domestic or naturalised fruit.

Native Figs (*Ficus* spp.)

Native figs are a very important part of the flying-fox diet. There are at least 9 varieties of native fig that overlap the distribution of *P. poliocephalus* and *P. alecto*. While a lot of work has been done on the Moreton Bay fig (*Ficus macrophylla*), other species are likely to be just as important for flying-foxes but currently their importance may not be credited as they may not be able to be identified microscopically. As a result one of the aims of this project was to collect and photograph all the lesser studied native figs. Photos of two species of native fig, *Ficus watkinsiana* and *F. obliqua,* are given:

1) Strangler fig (*Ficus watkinsiana*) – Collected at Alstonville's Lumley Pk 30/07/2020. Under low power the fruit has large clear flesh cells, clear or coloured refractile sclereid cells and dark brown plant fibres (Figure 1). Under high power the structure and size of the fibres and both types of cells is clear and distinctive (Figure 2).
2) Small-leaved fig (*Ficus obliqua*) – Collected at Wingham Brush 08/09/2019. On low power it has characteristic small hairs and very small sclereids (Figure 3). On high power the sclereids are small and irregularly shaped but have the archetypal double wall with the hole in the centre (Figure 4).

"Bush Tucker"

Bush tucker or bush food is the name given to the foods eaten by Australia's indigenous peoples. Logically if these foods are good for humans to eat then they are likely to be of use to flying-foxes. Bush tucker fruits were targeted in this project, but as flying-foxes rarely forage below 2 m, only ones that grow higher than this were selected.

White aspen (or pidgeon berry) (*Acronychia oblongifolia*) – Collected at Bangalow 29/07/2020. *Acronychia oblongifolia* is a subtropical plant found in warm rainforests. It can grow as a shrub or a medium-sized tree. In spring it has clusters of white lobed fruit, each about 10 mm in diameter. It is known to be eaten by pied currawongs, blue-faced honeyeaters, green catbirds, wompoo fruit doves, satin bowerbirds and white-headed pigeons (tuckerbush.com.au), so was considered to potentially be a food that flying-foxes would eat. The fruit is fleshy and white and the skin looks unblemished but on high power the skin has spots (Figure 5). The flesh is unremarkable, but on high power the scleroids are characteristic, very long and irregular (Figure 6).

Domestic or Naturalised Fruit

Purple guava (*Psidium cattleianum*) – Collected at Port Macquarie 06/08/2020. Purple guava is native to Brazil and is considered a highly invasive species in many countries (e.g., New Zealand). It is sporadically naturalised in coastal northern NSW and Queensland. It grows as a tree up to 13 m and so potentially is a source of food for flying-foxes, especially as they eat other forms of guava. The sclereids in purple guava are irregularly amoeba-shaped but quite characteristic under low power (Figure 7). Another view of the sclereids on low power shows where the double wall and empty centre can be seen (Figure 8).

All four of these fruits were matched with fruits that have been labelled "unknowns" in a previous project that looked at flying-fox droppings (Griffith *et al*. 2020).

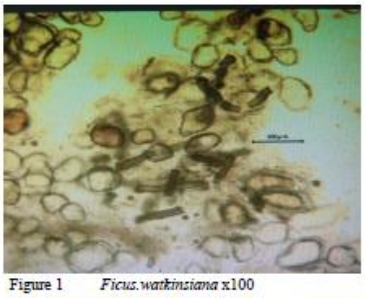

Figure 1 *Ficus.watkinziana* x100

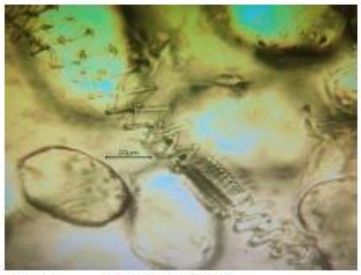

Figure 2 *F.watkinziana* x400

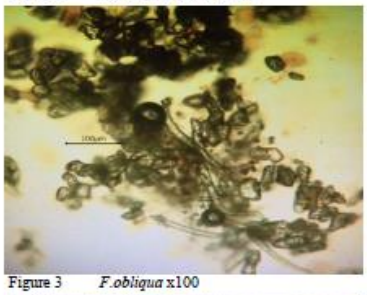

Figure 3 *F.obliqua* x100

Figure 4 *F.obliqua* x400

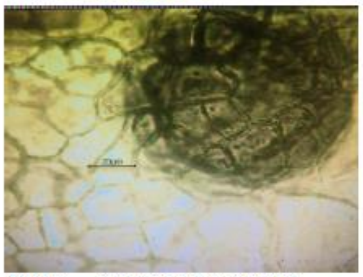

Figure 5 *Acronychia oblongifolia* x400

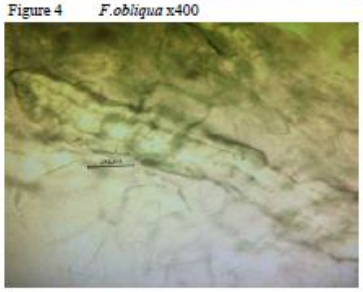

Figure 6 *A. oblongifolia* x400

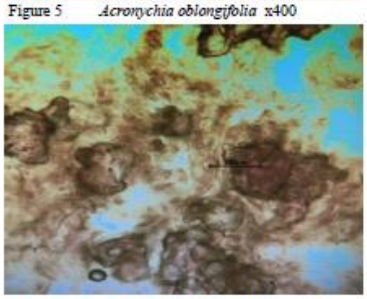

Figure 7 *Psidium cattleianum* x100

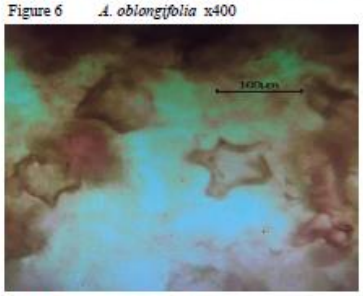

Figure 8 *P. cattleianum* x100

Figures 1-8 showing low (x100) and high (x400) magnification of characteristic structures in the fruit flesh of flying-fox food species

Discussion

As Robert Burns said “The best laid schemes o’ Mice an’ Men Gang aft agley”. It should have been an easy project: primarily, it involved seven trips, nicely spaced over a year to obtain fruit as it was fruiting around the Border region of NSW. In addition, as the behaviour of the two species, in particular their foraging behaviour is a function of their morphology, the plan was to collect flying-fox carcasses kept in freezers along the eastern seaboard from vets and rehabilitators for a combined project with the Australian Museum.

It should have been easy but it wasn’t. The first trip was in early September 2019 but it was only moderately successful as the worst drought in decades was playing havoc with fruiting patterns. It was also playing havoc with the flying-fox breeding season, as a continuing food shortage that had started in February was playing itself out, with starving animals making mistakes and ending up on barbed wires, in netting and on electric wires. While some dead flying-foxes were collected, the rehabilitators were really busy trying to cover the huge number of rescues and incoming animals.

The season only got worse with the dry conditions enhancing horrendous bush fires along the East Coast and so the November trip was cancelled for safety reasons. Four billion animals died in those fires and the toll among flying-foxes will never be known. The cancellation of the northern trips also resulted in finding it difficult to collect flying-fox bodies from the rehabilitators who were suffering overflowing freezers. A lot of animals had to be buried because, over the summer, no courier service would collect them – they did not want frozen flying-foxes (even ones safely placed in polystyrene boxes) anywhere near their rigs.

Fire conditions were no better later in the 2019-2020 summer. Field trips were cancelled throughout that period, in the understanding that many food species would fruit in autumn and the climatic chaos should have calmed down as the weather got cooler in March.

But then COVID-19 descended. Two field trips have been made since travelling restrictions have been partially lifted but there are at least another four trips planned so that samples of fruit produced over summer and autumn can be added to the reference collection. A number of flying-fox carcasses were collected and that project has commenced. Being half way through a project that by now should have been finished is frustrating however considering the problems that 2019/2020 has caused other people, it is a minor set-back. A number of fruits have been collected and these have enabled the identification of several “unknowns” from other projects. As more fruits are collected and processed, it can be assumed that more of the gaps in the identification of fruits in flying-fox faeces will be filled.

Acknowledgements

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