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Leaf swallowing and parasitic infection of the Chinese lesser civet *Viverricula indica* in northeastern Taiwan

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Abstract

Background: Ingestion of plant parts purportedly for their non-nutritive and/or bioactive properties has been widely reported across the animal kingdom. Many of these examples are viewed as behavioral strategies to maintain health by controlling the level of parasite infections. One such behavior is leaf swallowing, the folding and swallowing of whole leaves without chewing. Void of any nutritional benefit, defecation of the whole leaves is associated with the physical expulsion of intestinal parasites. Fecal samples of the Chinese lesser civet *Viverricula indica* were collected along a fixed transect line monthly for 17 months in the Fushan Experimental Forest, northeastern Taiwan. We inspected samples for the occurrence of undigested leaves and parasite worms to test the possible antiparasitic function of the behavior in this species.

Results: Of the collected feces, 14.3% contained whole, folded, undigested leaves of grass. The co-occurrence of undigested grass and *Toxocara paradoxura* worms in the feces was statistically significant. Adult worms of *T. paradoxura* were trapped inside the fecal-grass mass or on the surface of leaves in these samples. Increases in the *T. paradoxura* prevalence and infection intensity were associated with a higher presence of whole leaves in the feces.

Conclusions: Reported for the first time in the context of self-medication for civet species, we propose that swallowing grass may facilitate expulsion of adult worms of *T. paradoxura*, which resembles behaviors widely reported in African great apes, bears, and geese.

Keywords: Intestinal parasite control; Undigested grass; Self-medication; *Toxocara paradoxura*

Background

Ingestion of plant parts purportedly for their non-nutritive and/or bioactive properties has been widely reported across the animal kingdom (Huffman 1997, 2011; Lozano 1998; Engel 2002). Many of these examples are viewed as behavioral strategies to maintain health by controlling the level of parasite infections. Studying the health maintenance of animals can help us better understand interactions across multiple trophic levels that shape the evolution of behavior, particularly self-medication (Hutchings et al. 2003; Forbey et al. 2009).

Leaf swallowing as a form of self-meditative behavior is widely documented in African great ape species (gorillas, *Gorilla gorilla*; chimpanzees, *Pan troglodytes*; and

bonobos, *Pan paniscus*) at multiple sites across Africa. Leaves of over 40 species are reported to be folded and swallowed by them without chewing (Huffman 1997, 2010). The leaves go through the digestive tract and appear in the feces folded and undigested along with either *Oesophagostomum stephanostomum* (Nematoda) adult worms or *Bertiella sturderi* (Cestoda) proglottids (Wrangham 1995; Huffman et al. 1996, 2009; Huffman and Caton 2001). The co-occurrence of folded, undigested leaves and these parasites was statistically significant at sites where the behavior has been studied in detail (e.g., *Oesophagostomum*: Huffman et al. (1996), McLennan and Huffman (2012) and *Bertiella*: Wrangham (1995)). Furthermore, ingesting leaves was positively correlated with the seasonal increase in the intensity of infection by these parasites in chimpanzees at these sites (Huffman et al. 1997).

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Species of leaves swallowed that expel these intestinal parasites all share a common physical feature: a rough, hairy surface caused by trichomes and/or sharp edges (Huffman 1997). The swallowing of whole leaves, already difficult to digest because of the abundance of stiff hairs made of silicate material, results in increased motility of the gastrointestinal tract that purges these parasites from the host within 6 h after ingestion of the leaves (Huffman and Caton 2001).

Leaf swallowing was also observed in other animal species such as wolves and dogs (Sueda et al. 2008) and was described in the North American brown bear and Canadian snow goose, as a habit for expelling other tapeworm species (see Huffman 1997). In the latter two species, leaves of a species of the Gramineae, typically eaten as food in spring when young and nutritious, were folded and swallowed in the fall when they had become too tough to chew and were void of nutritional value (B Gilbert and J Holmes, personal communication; cited in Huffman (1997)). In both species, this was associated with the mass expulsion of tapeworms before hibernation in bears and winter migration in snow geese (see Huffman 1997). Grass eating by wolves was similarly reported in the context of parasite expulsion (Murie 1944), where it was suggested to be a normal feeding behavior stemming from innate behavior in ancestral canids (Bjone et al. 2007; Sueda et al. 2008), and a response to a fiber-deficient diet (Kang et al. 2007) in domestic dogs. Ingestion of grass as an emetic was not supported by recent studies on domestic dogs (Bjone et al. 2007; Sueda et al. 2008).

Undigested leaves were found in the feces of the African civet *Civettictis civetta* and were thought to help with digestion and possibly to be of medicinal value (Bekele et al. 2008). Folded and undigested grass was also reported in the feces of the Chinese lesser civet *Viverricula indica* at various locations across Taiwan (Chuang and Lee 1997; H Su, unpublished data); however, this behavior and its possible function have not yet been described. Ecological studies of the Chinese lesser civet are limited. Its diet was studied and compared to two other sympatric carnivore species, the Formosan ferret-badger *Melogale moschata* and crab-eating mongoose *Herpestes urva* (Chuang and Lee 1997). The Chinese lesser civet was found to consume both animal and plant matter. Insects are the most important food item in its diet. It was also pointed out that the average daily travel distance of the civet is about 500 m (Lee and Chou 2004). Information on activity patterns and social organization is currently not available.

Based on those previous reports of leaf and grass swallowing in various wild animal species, and specifically in civet spp., we hypothesized that one of the functions of ingesting grass without chewing it by civets is to facilitate control of the parasite load by expelling intestinal parasites. We examined patterns of leaf swallowing in the Chinese

lesser civet by investigating the occurrence of undigested leaves and parasite worms in its feces and looked for possible patterns of infection by parasite species temporally and directly associated with its leaf swallowing behavior.

Methods

Study subjects and location

Civet fecal samples were systematically collected in the Fushan Experimental Forest (24°46'N, 121°34'E) in north-eastern Taiwan. This is one of the six ecological research sites in Taiwan designated for long-term ecosystem monitoring and research (Smithsonian Tropical Research Institute 2010). The main geographical characteristic of the region is its steep mountainous terrain, with elevations ranging 500 ~ 1,400 m. This area is predominantly covered by subtropical moist hardwood forest that mainly consists of species belonging to the Lauraceae, Fagaceae, and Symplocaceae. The monthly average daily maximum temperature of 29.5°C occurred in July, and the minimum temperature of 9.4°C occurred in January. The humidity is high (near or over 90%) year round. The average annual rainfall is 4,096.4 mm (Lu et al. 2009).

Sample collection, preparation, and analysis

Fecal samples were routinely collected along a 5-km route, located at elevations of 500 ~ 700 m. This survey route combined a path using roads, established hiking trails, and dry riverbeds and was systematically surveyed three consecutive days every month to collect all civet feces encountered in July 2005, in March 2006, and from August 2006 through October 2007. All feces not found on the survey route the previous day was collected. Samples found on the first day of a monthly collection were examined for freshness (≤ 24 h) by shape, the presence of mucus, smell, and the surface moisture level. All fresh feces was fixed in a sodium acetate-acetic acid-formalin (SAF; 1.5 g sodium acetate (1.5%), 2.0 ml glacial acetic acid (2.0%), 4.0 ml 40% formaldehyde commercial solution (1.6%), and 92.5 ml H₂O (94.9%)) solution. Fixed samples were then taken back to the laboratory for examination. Information on the location of the collected fecal samples was recorded with a Global Positioning System.

SAF-fixed fecal samples were analyzed for contents, including the presence of folded undigested grass, food remains, and adult parasite worms. Parasite worms were examined with a scanning electron microscope and identified by morphological and anatomical characteristics. Fecal samples for an entire year were analyzed for helminth eggs to investigate the intestinal parasite load and its variation over the course of a year. To count eggs per gram of feces (EPG), the McMaster method was used for 1 g of sediments obtained from a fixed sample (Mbora and Munene 2006). Identification of helminth eggs was based on the morphology of the egg.

Undigested and folded leaves were examined in detail on a subset of 15 fecal samples which contained whole leaves to assess the average number of leaves swallowed at one time, the length of each fold, and the number of folds per leaf. Species of folded leaves found in these fecal samples were also recorded.

The monthly sample prevalence of each parasite species was calculated as the percentage of fecal samples containing parasite eggs or larvae in a given month. The monthly mean intensity of a given parasite species was calculated by dividing the total EPG of the species by the number of fecal samples examined containing that species in a given month.

Results

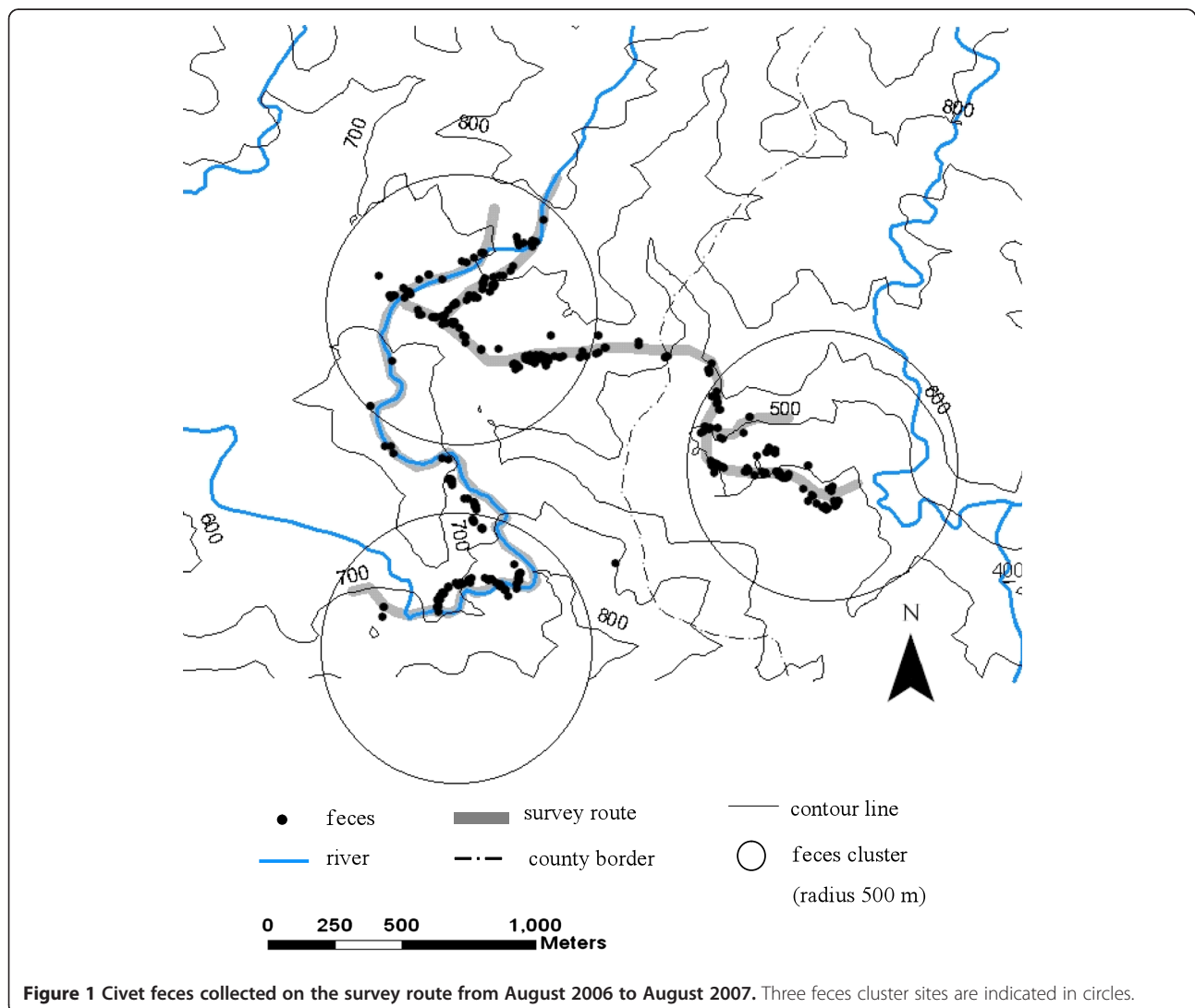
Distribution of samples collected along the survey route

In total, 444 civet fecal samples collected along the survey route were examined macroscopically for the presence of

folded leaves and adult parasite worms. Of the samples we obtained, 92.6% were clustered within one of three distinct cluster sites, with the closest peripheral sample of a cluster separated by ≥ 500 m (the average daily travel distance of a civet (Lee and Chou 2004)) from the next closest peripheral sample in the other two adjacent cluster sites (Figure 1). By clustering the feces in these sites, we were able to determine that it was highly unlikely that the feces we analyzed belonged to the same individuals or just a few of the animals inhabiting the study site.

Seasonality of occurrence of whole grass in the feces

A subset of 307 fecal samples collected from August 2006 to August 2007 was microscopically analyzed for parasite eggs and larvae. We found folded clumps of leaves in 14.3% of this subset of samples (44/307, with monthly occurrences ranging 4.3% ~ 18.5%). These leaves were not chewed but instead were folded into a bundle and



swallowed whole. From the EPG data of this subset of samples, we were able to compare infection by *Toxocara paradoxura* with the occurrence of folded grasses during this period. While there was no strict seasonal pattern (Figure 2a), a tendency was observed for peaks in the mean monthly prevalence of *T. paradoxura*, followed in the next few months by peaks in the number of fecal samples with whole leaves (Figure 2b,c).

Four different grass species were identified in 15 fecal samples containing leaf bundles. *Setaria palmifolia* was

predominant and was recovered from eight samples. Seven fecal samples contained undigested leaves of two different species. All leaves (*S. palmifolia*, *Miscanthus floridulus*, *Axonopus* sp., and one unidentified species) belonged to the Gramineae and were similarly sharp edged and covered with trichomes. These leaf bundles consisted of 4 ~ 17 folded leaf blades which were stacked in layers of leaf segments ranging from 5 to 75 layers. The leaf blades were either bent or bitten off, and leaf segments ranged 1.3 ~ 7.1 cm long. Such a bolus of indigestible material would be expected to present a significant challenge to the GI tract as it passes through the civet.

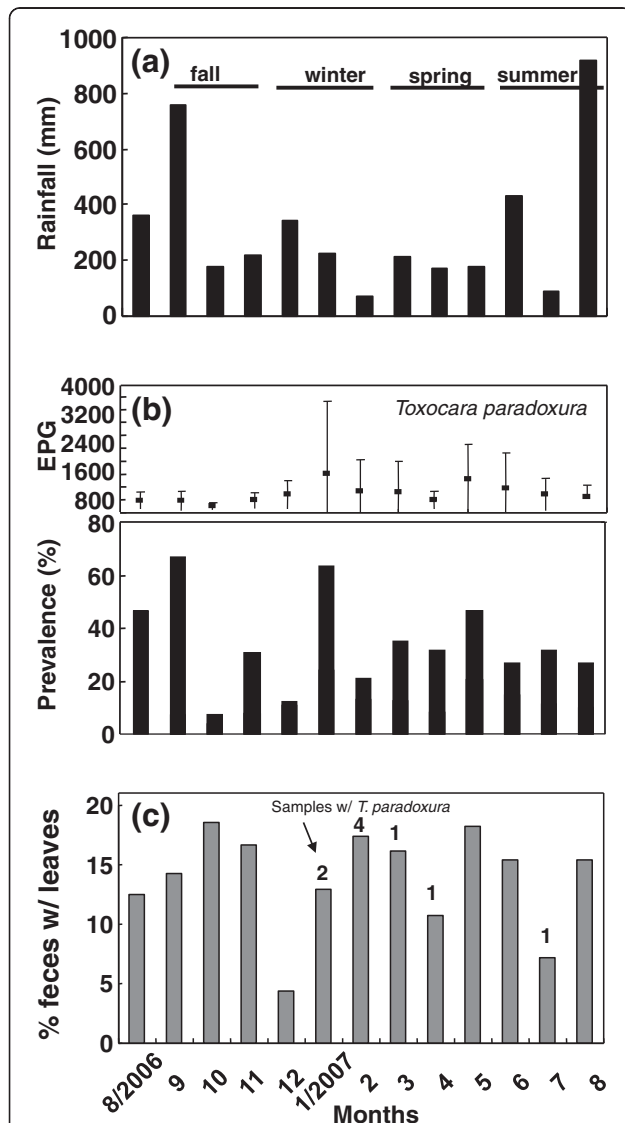


Figure 2 Rainfall patterns, distribution of *T. paradoxura* infection, and the number of fecal samples with whole leaves. The mean monthly variation in rainfall patterns (a), distribution of *T. paradoxura* infection (prevalence and EPG; mean \pm SD) (b), and the occurrence of undigested, folded leaves of grass and worms in the feces of the Chinese lesser civet (c).

Parasites recovered from the feces: seasonality of *T. paradoxura* infection

Twelve parasite species that are infectious to civets were recovered (Table 1). The five most prevalent species were *Capillaria* sp. (63.9%), *Ancylostoma* sp. (39.8%), *T. paradoxura* (36.6%), *Strongyloides* sp. (36.0%), and *Trichuris* sp. (14.1%). The mean intensities of *Capillaria* sp. and *Trichuris* sp. infections were highest among the intestinal parasite species harbored (2,411 and 2,161 eggs/g of feces, respectively). Among these prevalent parasite species, the only adult worms detected in civet fecal samples were of *T. paradoxura*.

Eggs of *T. paradoxura* were detected each month, and the monthly prevalence of *T. paradoxura* varied, with the highest prevalence (63.3%) and mean intensity (1,210 eggs/g of feces) occurring in January (Figure 2b). Monthly mean intensities of *T. paradoxura* infections tended to be higher in winter and spring than in summer and fall (Mann–Whitney *U* test: $Z = 1.81$; $p = 0.07$; $n_{\text{winter-spring}} = 6$; mean \pm standard deviation (SD), $7,53.7 \pm 310.0$; $n_{\text{summer-fall}} = 6$; mean \pm SD, 388.5 ± 122.1).

Table 1 Intestinal parasites recovered from the feces of Chinese lesser civets

Species	Stage	Prevalence (%)
<i>Capillaria</i> sp.	Egg	63.9
<i>Toxocara paradoxura</i>	Egg	36.6
<i>Ancylostoma</i> sp.	Egg	39.8
<i>Strongyloides</i> sp.	Larva	36.0
<i>Spirocerca</i> sp.	Egg	8.1
<i>Paragonimus</i> sp.	Egg	1.3
<i>Trichuris</i> sp.	Egg	14.1
<i>Mesocostoides litteratus</i>	Egg	1.3
<i>Isoospora</i> spp. (<i>felis</i> and <i>reiota</i>)	Oocyst	2.9
<i>Cryptosporidium</i> sp.	Oocyst	N/A
<i>Cyclospora</i> sp.	Oocyst	N/A

N/A, not applicable.

Association between undigested grass and *T. paradoxura* worms in the feces

The presence of *T. paradoxura* worms was detected in 8 months in 12 fecal samples located in three different clusters along the survey transect (any two clusters were at least 500 m apart). Four of the fecal samples containing *T. paradoxura* were collected in February 2007 from two different cluster sites on the same day (Figure 3), and the other eight were collected in seven different months. Worms of both sexes were recorded, and a maximum of 25 worms was recovered from a single fecal sample (range 1 ~ 25, $n = 12$). In 61% (8/12) of these fecal samples, only one adult *T. paradoxura*

worm was detected. Proglottids of the cestode *Mesocestoides litteratus* were recovered in one sample.

The occurrence of adult *T. paradoxura* was significantly associated with the presence of undigested, folded leaves (chi-squared with Yates's correction, $\chi^2 = 4.31$, $d.f. = 1$, $p = 0.038$). Live worms were found within the folded grass and/or in the mucus on the surface of the leaves (Figure 4). The four fecal samples containing both folded leaves and *T. paradoxura* worms were collected at two different cluster sites on the survey transect. We judged them to be from different individuals within their respective territories.

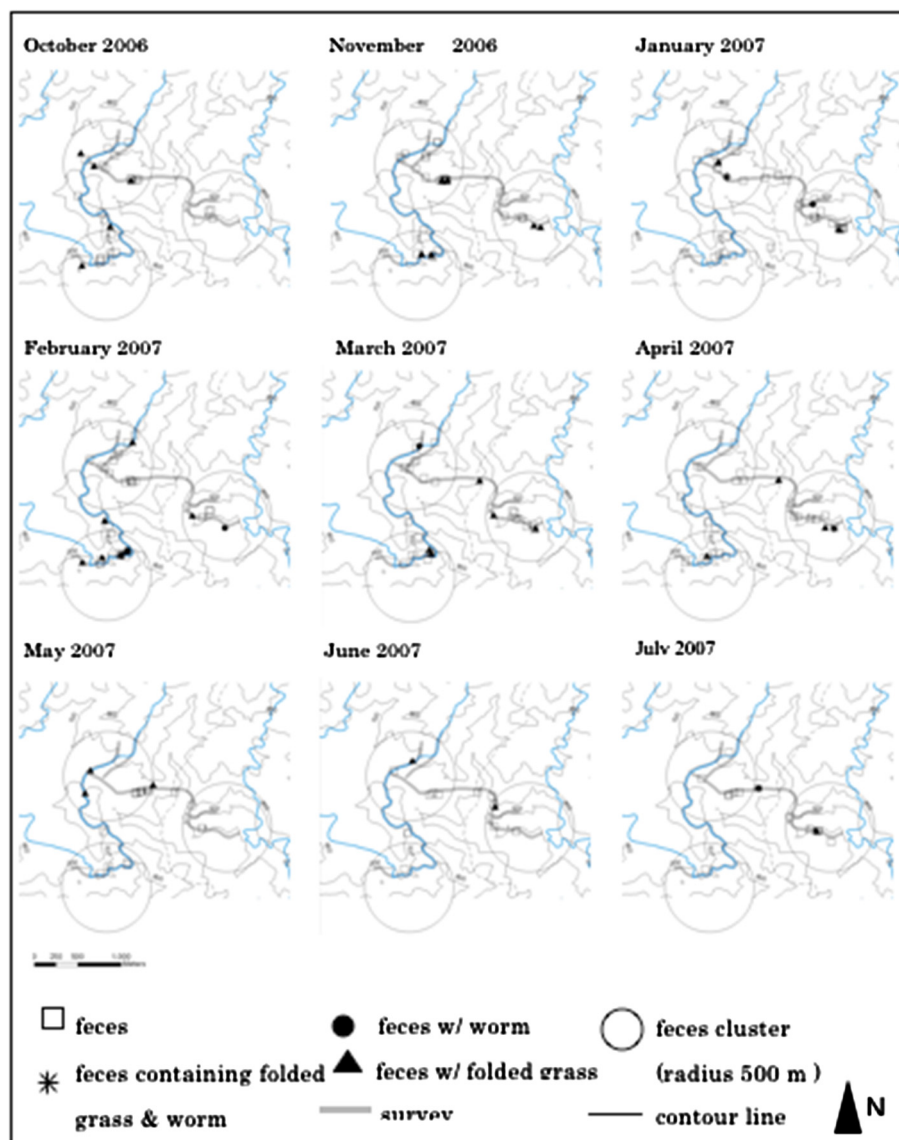


Figure 3 Civet feces collected in February 2007. Four samples containing *T. paradoxura* worms were distributed in two feces cluster sites.



Figure 4 Worms of *T. paradoxura* and undigested, folded grass expelled with the feces of a Chinese lesser civet. Arrows indicate *T. paradoxura* worms.

Discussion

Relationship of leaf swallowing with the expulsion of worms

From fecal samples collected over our 17-month study, we found that the occurrence of grass was significantly associated with that of *T. paradoxura* worms together in the feces. A daily travel distance of ≤ 500 m was reported for civets of Fushan (Lee and Chou 2004). Given the feces that was collected from three different cluster sites (i.e., separated by >500 m), it is likely that the samples collected from these different clusters on the same day were from different individuals of the study site's population. That samples with leaves were found on the same days at different locations and across the study period is consistent with our proposal that this habit is widely established among individuals in the Fushan population of civets and is not an isolated anomaly of just one

individual or a product of sampling bias. This being an anomaly is further refuted by reports of leaf swallowing behavior in the species elsewhere in Taiwan (H Su, unpublished data) and captive individuals in southern Taiwan (C Chen, personal communication).

Three captive civets in the Pingtung Rescue Center for Endangered Wild Animals in southern Taiwan are occasionally provided grass (*M. floridulus*). Undigested, folded grass was subsequently found in their feces (H Su, unpublished data). Since these animals are subjected to regular intestinal parasite control by the center's veterinary staff, they are not likely to be suffering from parasitic infections. This was also observed in captive chimpanzees housed in enclosures experimentally given rough leaves (Huffman and Hirata 2004; Huffman et al. 2010), suggesting that this behavior can be spontaneously induced.

The leaves were evacuated intact. It is clear that the civets obtained very little, if any, nutritional or energetic benefit from this type of ingestive behavior. Ingesting grass leaves by the Chinese lesser civet may function to remove *Toxocara* worms, which live in the intestinal lumen and share several life history traits in common with *Oesophagostomum*, another nematode species (Anderson 2000; Holland and Smith 2006) closely associated with leaf swallowing in chimpanzee and bonobo populations across Africa (Huffman 1997, 2010; Dupain et al. 2002; Fowler et al. 2007; McLennan and Huffman 2012). At the very least, these data suggest that infections by this parasite stimulate the swallowing of grass by the civet and point toward a relationship between leaf swallowing and the expulsion of parasites, as noted for other wild carnivore species (Murie 1944; Huffman 1997).

Leaf characteristics and the mechanism of parasite expulsion

Leaves of Gramineae species recovered from civet feces in this study were sharp edged and trichome-covered. They belonged to common grass species available year round in its habitat. We recovered up to 17 neatly folded blades of grass from a single fecal sample. Folded segments of whole grass were recovered from the feces intact and undigested. How a civet folds and swallows these long blades of grass was not observed, but fecal examination suggests that they collect several leaves at a time, folding and swallowing them whole without chewing. The mechanism by which folded, undigested grass expels *T. paradoxura* remains unclear, but we assert that the basic mechanism should be similar to that previously reported for apes and other species (Huffman and Caton 2001) since the basic digestive system of both hosts and the habits of the parasite species involved are similar. In domestic dogs, *Canis familiaris*, a higher undigested fiber content in the diet was suggested to result in shorter gut transit times and higher water contents in the feces (Burrows et al. 1982). The civet may react to leaf swallowing by increased gut motility in a similar way, as was found for wild chimpanzees (Huffman and Caton 2001).

Conclusions

Reported for the first time in the context of self-medication for civet species, we propose that swallowing grass may facilitate expulsion of adult worms of *T. paradoxura*. The association between the presence of folded grass and *T. paradoxura* worms in the civet feces was significant. The ingestion of grass tended to coincide well with the increased intensity of *T. paradoxura* infection. This proposed self-medication of leaf swallowing in civets resembles behaviors widely reported in African great apes, bears, and geese.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HS and YS carried out the sample collection and parasite analyses. HS and MAH drafted the manuscript and conceived of the study and its design and coordination. All authors read and approved the final manuscript.

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