

# Dietary partitioning in the sympatric *Paradoxurinae* civets in Borneo suggested by compound-specific nitrogen isotope analysis of amino acids

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## Research Article

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# Abstract

The sympatric four wild Paradoxurinae civet species in Borneo share similar ecological characteristics, but the coexistence mechanism of these species is unclear. We investigated their faunivory, reflected in their trophic positions (TP), by carbon and nitrogen stable isotope analysis of bulk hair and compound-specific nitrogen isotope analysis of amino acids. The bulk stable isotope analysis showed distinctly lower nitrogen isotope ratios in binturongs among the four subject species, suggesting the lowest degree of faunivory. The compound-specific stable isotope analysis confirmed little faunivory in binturong and showed a probably varying degree of faunivory in the other species. The estimated TP was the lowest in binturongs (2.0–2.1) and higher in small-toothed palm civets (2.4–2.5), masked palm civets (2.7), and common palm civets (2.9). Although the number of the measured sample for the compound-specific stable isotope analysis is small ( $n = 2$  for each species), our results suggest the varying degree of consumption of animal food sources, such as insects, is the key mechanism of niche partitioning in these four Paradoxurinae civet species in Borneo. Such subtle but important differences in closely related sympatric species would maintain high biodiversity in tropical regions.

## Introduction

The coexistence mechanism of closely related sympatric species is one of the major themes in ecology. In general, interspecific competition for resources among closely related sympatric species is intense because they have similar morphology, physiology, behavior, and ecology (Simberloff and Dayan 1991; Pianka 2000). As an evolutionary consequence of the competition, resource partitioning often occurs between/among closely related sympatric species (Pianka 2000).

Many ecologists have focused on the coexistence mechanisms of mammalian carnivores because they form community structures as apex or mesopredators (Ritchie and Johnson 2009). In mammalian carnivores, sympatric species usually exhibit differences in body size and behavior, which are often reflected in dietary, spatial, and temporal partitioning (Vanak et al. 2013; Lovari et al. 2014; Karanth et al. 2017; de Satgé et al. 2017; Hearn et al. 2018; Nakabayashi et al. 2021). Carnivore species richness is high in southeast Asia and central and southeast Africa (Loyola et al. 2009). Among them, rainforests in Asia possess a far larger number of sympatric carnivore species than those in the Neotropics and Africa (Corlett 2007). Civets (family Viverridae) are notable in their relatively greater number of sympatric species (Burgin et al. 2020). Up to eight species of civets coexist in Asian rainforests and share similar behaviors such as nocturnal and solitary behaviors.

Four civet species belonging to the subfamily Paradoxurinae inhabit in Borneo with the notable dietary, spatial, and temporal overlaps. These Paradoxurinae species include binturongs (*Arctictis binturong*), masked palm civets (*Paguma larvata*), common palm civets (*Paradoxurus philippinensis*), and small-toothed palm civets (*Arctogalidia trivirgata*), weighing 6–10 kg, 2.5–3 kg, 1.7–2.7 kg, and 1.5–2.6 kg, respectively (Yasuma and Andau 2000; Nakabayashi et al. 2017). Radio-tracking and camera-trapping studies of these species revealed that they occur sympatrically even in a small area (Brodie and Giordano

2011; Nakabayashi et al. 2017). The results of feeding observations showed that the temporal activity patterns of these civets are highly overlapped in Borneo (Nakabayashi et al. 2016). Observational studies revealed that *Ficus* fruits, hereafter called figs, dominate the diets of these civet species (Nakashima et al. 2010; Nakabayashi et al. 2016; Nakabayashi and Ahmad 2018; Nakabayashi 2020). *Ficus* comprises approximately 75.6% of the observed feeding patches for these civet species (Nakabayashi 2020). Although there are some species-specific differences in the use of plant parts (e.g. tree bark sap, nectar, oil palm pith, and unripe fruit), these civet species typically eat fruits of the same plant species (Nakabayashi 2020). Observational studies have suggested that faunivory (the generic term for feeding on animal flesh [carnivory] and insects [insectivory]) is rare in these four civet species, as no faunivory was observed in three binturong individuals during a total of 951 days of individual tracking (Nakabayashi and Ahmad 2018) and only ~ 5% ( $n = 3/55$ ) of fecal samples of common palm civets contained visible remains of arthropods (Nakabayashi 2020).

However, the observational evidence on the extensive dietary overlap and little faunivory in these four civet species is contrary to ecological and physiological expectations. Differentiating food resources is one of the most fundamental strategies in ecological communities for resource partitioning (Azevedo et al. 2006). Multiple sympatric species usually have difficulty in coexisting if their spatial and temporal activity patterns, as well as their food sources extensively overlap (Pianka 2000), and this is especially true in tropical forests of Borneo, where the availability of fruits is relatively scarce compared to the other Sundaic regions (Wich et al. 2011). It is also expected that a diet almost exclusively consisting of fruits cannot fulfill the nutritional and energetic demands of civets because of their typical morphology as mammalian carnivores, such as carnassial teeth (Anders 2005) and simple digestive tracts without fermentation of polysaccharides (Lambert et al. 2014), which predominantly limit types and amounts of ingestible fruit (Nakabayashi 2015). Therefore, we can expect unrevealed dietary partitioning in these civet species, though there are other examples of carnivores with extremely specialized herbivorous diets (e.g. pandas) or eating large amounts of fruit (e.g. some ursids, mustelids, canids, and procyonids (Draper et al. 2022)).

A complete picture of the diet of Bornean sympatric civet species remains unclear although there are some systematic observational studies on the diet of civets (masked palm civets in China: Zhou et al. 2008, common palm civets in Borneo: Nakashima et al. 2013, binturongs in Borneo: Nakabayashi and Ahmad 2018; Nakabayashi 2020). More specifically, it is not clear whether Bornean civets consume animal materials, and if so, how much of it they consume. Dietary estimation methods in field-based research, such as fecal analysis and direct observation of feeding animals, are usually a “snapshot” of reality, making it difficult to comprehensively determine animal diets and ecological resource uses (Moreno-Black 1978; Dickman and Huang 1988; Gales and Cheal 1992).

Carbon and nitrogen stable isotope analysis of animal tissues is an effective method to estimate the actual food sources consumed by individual animals. This is because the carbon and nitrogen stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) of animal tissues quantitatively reflect the nutritional contribution of ingested foods (Kelly 2000; Crawford et al. 2008; Crowley 2012; Ben-David and Flaherty 2012). In

terrestrial environments, the  $\delta^{13}\text{C}$  values of  $\text{C}_3$  plants are lower than those of  $\text{C}_4$  plants (Smith and Epstein 1971; O'Leary 1988), and such a difference is reflected in the consumers (Cerling et al. 1997). The  $\delta^{15}\text{N}$  values of bulk tissues ( $\delta^{15}\text{N}_{\text{bulk}}$  values) of organisms show a stepwise increase along with the increase in the trophic position (TP) in a food web (Minagawa and Wada 1984; Schoeninger and DeNiro 1984). While the precise estimation of the TP with the  $\delta^{15}\text{N}_{\text{bulk}}$  values is hampered in some cases by the fluctuations in baseline  $\delta^{15}\text{N}$  values of the ecosystem and physiological changes in diet–tissue offset values, compound-specific nitrogen isotope analysis of individual amino acids (hereafter, CSIA-AA) provides quantitative estimates of the TP of the individual animals (Chikaraishi et al. 2007, 2011; Steffan et al. 2013; Naito et al. 2016; Ohkouchi et al. 2017). Amino acids can be grouped into “source” and “trophic” amino acids, and the former fractionates  $^{15}\text{N}$  very little ( $< 0.5\text{‰}$ ) during trophic transfer but the latter is highly ( $\sim 6\text{--}8\text{‰}$ ) enriched in  $^{15}\text{N}$  in each trophic step (Popp et al. 2007; O'Connell 2017; Ohkouchi et al. 2017). Therefore, the  $\delta^{15}\text{N}$  values of each amino acid from an individual record information both on the baseline of the ecosystem and the individual's TP (McClelland and Montoya 2002; Chikaraishi et al. 2007). By applying these analyses for diet estimation, though the number of measurable samples in CSIA-AA is smaller due to its lower throughput, it is possible to investigate whether there is dietary partitioning among sympatric civet species.

In this study, we investigated the degree of faunivory of the four Paradoxurinae species in Borneo to investigate their dietary partitioning using bulk carbon and nitrogen stable isotope analysis and amino acid nitrogen isotope analysis. We hypothesized that the consumption of small animals, such as arthropods, has been underestimated in the diets of civets and that their contribution to dietary protein intake is important despite their low detectability. We tested this hypothesis with stable isotope analyses. In addition to bulk stable isotope analysis, we applied CSIA-AA to a limited set (i.e. two individuals per species) of representative samples to obtain a perspective on the species-specific difference in their degree of faunivory, reflected in their TPs. Since the nitrogen isotope ratios of consumer tissues mostly represent those of proteins in the food sources, the dietary protein contribution, rather than energy contribution, is investigated in this study. Also, this study only concerns dietary niche partitioning that appears in TP, which can be estimated from CSIA-AA. Niche partitioning in the same TP (e.g. dietary partitioning by eating different parts of the same plant) is not evaluated in this study.

## Materials and methods

### Study sites

We conducted this study in the Danum Valley Conservation Area (Danum) and Maliau Basin Conservation Area (Maliau) in Sabah, north-eastern Borneo, from May 2012 to May 2014, and from November 2015 to June 2018, respectively. Danum ( $4^{\circ}57' \text{N}$ ,  $117^{\circ}48' \text{E}$ ) is a  $438 \text{ km}^2$  protected area, and 90% of this area consists of mature lowland evergreen dipterocarp forest between 180 and 900 m a.s.l. (Marsh and Greer 1992; Newber et al. 1999). The study area was around the eastern boundary of the protected area. Maliau ( $4^{\circ}49' \text{N}$ ,  $116^{\circ}54' \text{E}$ ) is a  $588 \text{ km}^2$  protected area, including lowland dipterocarp

forests and at least 12 forest types between 300 and 1675 m a.s.l. (Hazebroek et al. 2004). The study area in Maliau was outside the basin and in a selectively logged dipterocarp forest.

### Sample collection

We conducted trapping of civet species using box traps (Supplementary Text 1.1) set on the ground or on the branches at heights ranging from 3–35 m. In total, we captured 6 individuals of two civet species from Danum and 21 individuals of five civet species from Maliau (Supplementary Table S1). Their hairs pulled out from the skin were used for stable isotope analyses. Trapping and handling of the animals conformed to the guidelines of the American Society of Mammalogists (Sikes et al. 2016).

We collected insects and fruits as potential food samples for the civet species. Two insect species (a dung beetle and a weevil) were found near the base camp and two fig species (*Ficus caulocarpa* and *F. annulata*) were obtained at the height of 3 m from the trees in a phenological survey plot. Based on the behavior observation of the civets, they strongly depend on figs as their diet (Nakabayashi 2020). Therefore, we select figs as representative plant food items.

Sample collection, transfer, and analyses were approved by Sabah Biodiversity Centre (Access Licence JKM/MBS.1000-2/2JLD.4(170), JKM/MBS.1000-2/2JLD.6(50), JKM/MBS.1000-2/2JLD.4(170), JKM/MBS.1000-2/2JLD.7(64) and Transfer Licence JKM/MBS.1000-2/3(66), JKM/MBS.1000-2/3JLD.3(100)).

### Stable isotope analyses

Samples were treated for stable isotope analyses based on the protocols described previously (Campbell et al. 2017). Briefly, hair and insect samples were defatted with chloroform and methanol. Strands of hairs and powders of insects/plants were used for both bulk stable isotope analysis and CSIA-AA. Carbon and nitrogen stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively) of bulk hair, insect, and plant samples (~0.6 mg) were measured by Shoko Science, Co., Ltd. with an elemental analyzer-isotope ratio mass spectrometer (EA/IRMS). Compound-specific isotope analysis of amino acid  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}_{\text{AA}}$ ) was performed for selected hair, insect, and plant samples. Samples for CSIA-AA were prepared based on the amino acid derivatization procedures described in Chikaraishi et al. (2015). The  $\delta^{15}\text{N}$  values of each amino acid were determined using a gas chromatograph coupled to an isotope ratio mass spectrometer (GC/C/IRMS) at the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) (Ishikawa et al. 2018, 2022). Detailed methods were described in Supplementary Text 1.2.

The TP value was calculated based on the stable nitrogen isotope ratios of glutamic acid and phenylalanine ( $\delta^{15}\text{N}_{\text{Glu}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$ , respectively) as follows (Chikaraishi et al. 2011, 2014):

$$\text{TP} = (\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}} + 8.4)/7.6 + 1 \text{ [Equation 1]}$$

Glutamic acid and phenylalanine were used to calculate TP in this study, although recent meta-analyses and feeding experiments propose other combinations of amino acids for the calculation of TP (e.g. Ramirez et al. 2021; Whiteman et al. 2021). The metabolism of these amino acids has been studied well, and their positions in nitrogen metabolism represent one of the most obvious trophic and source amino acids, respectively (Ohkouchi et al. 2017, Ohkouchi 2023). Theoretically, TPs of primary producers (i.e. plants), primary consumers (i.e. obligate plant eaters, such as herbivorous animals), and secondary consumers (i.e. obligate eaters of primary consumers, such as some obligate insectivorous animals) are expected to be 1, 2, and 3, respectively. Propagated errors of TP are calculated based on Chikaraishi et al. (2009) and Ishikawa et al. (2022).

## Results

### Stable isotope ratios of bulk tissues

The results of stable isotope analyses are shown in Supplementary Table S1 and summarized in Table 1. First, the  $\delta^{15}\text{N}_{\text{bulk}}$  values of civets and plants were higher in Maliau than in Danum (Fig. 1). Mann–Whitney U tests showed that the  $\delta^{13}\text{C}_{\text{bulk}}$  ( $U = 36, p = 0.003$ ) and  $\delta^{15}\text{N}_{\text{bulk}}$  ( $U = 1, p = 0.009$ ) values of common palm civets from Maliau ( $n = 10$ ) were significantly higher (+ 0.4‰ and + 1.0‰, respectively) than those from Danum ( $n = 4$ ). Similarly, the  $\delta^{15}\text{N}_{\text{bulk}}$  values of small-toothed palm civets ( $n = 2$ ) and plant fruits ( $n = 5$ ) from Maliau were + 1.0‰ and + 3.5‰ higher than those from Danum ( $n = 2$  and 2), respectively, although no statistical test was applied due to their small sample sizes. Because of the more comprehensive coverage of civet taxa and the larger number of obtained samples, further CSIA-AA was performed only on the samples from Maliau, and the results from Maliau are described and discussed hereafter.

Table 1  
Summary of the stable isotope ratios of bulk samples from Danum and Maliau.

Site		$\delta^{13}\text{C}_{\text{bulk}}$		$\delta^{15}\text{N}_{\text{bulk}}$		n
		Mean	SD	Mean	SD	
Maliau	Binturong	-26.1	0.4	4.8	0.2	2
	Common palm civet	-24.0	0.7	8.6	0.3	10
	Malay civet	-22.6	1.0	7.7	0.3	4
	Masked palm civet	-24.7	0.3	8.1	0.6	3
	Small-toothed palm civet	-24.8	0.7	7.5	0.4	2
	Rodents	-25.6	0.7	6.1	1.6	3
	Fruits of <i>Ficus spp.</i>	-29.8	0.9	2.0	1.0	5
	Leaves of <i>Ficus spp.</i>	-31.6	1.2	2.5	0.7	6
	Insects	-27.2	7.8	6.7	3.4	2
	Danum	Common palm civet	-22.4	0.5	7.6	0.3
Small-toothed palm civet		-24.7	0.4	6.5	0.1	2
Fruits of <i>Ficus spp.</i>		-29.4	–	0.1	–	1
Fruits of <i>Microcos fibrocarpa</i>		-29.2	–	-0.4	–	1

The mean  $\delta^{13}\text{C}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{bulk}}$  values of civet species ranged from  $-26.1\text{‰}$  to  $-22.6\text{‰}$  and from  $4.8\text{‰}$  to  $8.6\text{‰}$ , respectively (Table 1; Fig. 2). Among the five civet species in Maliau, binturong showed the lowest  $\delta^{13}\text{C}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{bulk}}$  values, suggesting that the TP of binturongs is lower, although the feeding experiments were apparently inconsistent with this assumption (Supplementary Text 2). The  $\delta^{13}\text{C}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{bulk}}$  values of binturongs and other civets were similar to those of squirrel/porcupine and mouse, respectively (Fig. 2).

The mean  $\delta^{13}\text{C}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{bulk}}$  values of *Ficus* fruits were  $-29.8 \pm 0.9\text{‰}$  and  $2.0 \pm 1.0\text{‰}$ , respectively (Table 1). Compared with the  $\delta^{15}\text{N}_{\text{bulk}}$  values of fruits of *Ficus spp.*, those of binturong and other civets in Maliau were  $2.8\text{‰}$  and  $5.5\text{--}6.6\text{‰}$  higher, respectively (Table 1). The  $\delta^{13}\text{C}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{bulk}}$  values of insects varied widely (Fig. 2), which hinders the estimation of faunivory from bulk stable isotope ratios.

#### Stable isotope ratios of amino acids

The degree of faunivory was estimated quantitatively by applying CSIA-AA (Table 2; Supplementary Table S2; Fig. 3). The  $\delta^{15}\text{N}_{\text{Phe}}$  values, which mainly reflect baseline isotope ratios, were similar among the

analyzed samples in Maliau (8.6–13.5‰) except for the dung beetle (16.2‰), suggesting that *Ficus* fruits, snout beetle, and civets are part of the same food chain. The  $\delta^{15}\text{N}_{\text{Glu}}$  values, which typically increase with the increase in TP, were higher in civets (9.0–14.6‰) and snout beetle (11.9‰) than in *Ficus* fruits (3.8‰ and 4.6‰).

Table 2

Summary of the stable isotope ratios of amino acids of civet hair and food samples from Maliau.

Species	ID	$\delta^{15}\text{N}_{\text{Glu}}$		$\delta^{15}\text{N}_{\text{Phe}}$		TP		n
		Mean	SD	Mean	SD	Mean	Propagated error	
Binturong	11	9.0	0.4	9.9	0.8	2.0	0.29	3
	12	9.0	0.3	9.4	0.2	2.1	0.27	3
Common palm civet	14	14.6	0.6	8.8	1.5	2.9	0.43	3
	15	14.4	0.1	8.8	0.6	2.9	0.38	3
Masked palm civet	31	14.4	0.4	9.9	0.8	2.7	0.37	3
	37	13.4	0.3	8.6	0.3	2.7	0.36	3
Small-toothed palm civet	16	11.6	0.1	8.9	0.9	2.5	0.34	3
	17	12.4	0.1	10.2	1.1	2.4	0.34	3
Fruits of <i>Ficus spp.</i>	24	3.8	0.7	11.0	0.6	1.2	0.23	3
	30	4.0	0.7	13.5	0.1	0.8	0.22	3
Insects	41	20.5	1.4	16.2	2.1	2.7	0.50	4
	43	12.2	1.3	8.5	1.3	2.6	0.41	3

The TPs calculated from the CSIA-AA of *Ficus* fruits were 0.8 and 1.2, and those of civets ranged from 2.0 to 2.9 (Table 2; Fig. 3). Among the four civet species analyzed, binturong had the lowest TP (2.0 and 2.1) compared with other species: common palm civets (2.9 and 2.9), masked palm civets (2.7 and 2.7), and small toothed civets (2.4 and 2.5). Their point measurements of TPs (n = 2 for each species) had species-specific clusters with no overlap (Fig. 4). The estimated TPs of insects were 2.6 and 2.7 for snout beetle and dung beetle, respectively (Table 2). This result suggests taxonomic differences in the degree of faunivory, although the small sample size prevents further statistical tests.

## Discussion

Trophic positions of Paradoxurinae civet species



Our findings show relatively high  $\delta^{15}\text{N}_{\text{bulk}}$  values and higher estimated TP among non-binturong civets, suggesting the possibility that the consumption of animal foods may have been previously underestimated. The results of  $\delta^{13}\text{C}_{\text{bulk}}$  and  $\delta^{15}\text{N}_{\text{bulk}}$  values of civets suggest that their diet is different among species, which is supported by TP values estimated from CSIA-AA. It is difficult to estimate the degree of faunivory from these results of varying  $\delta^{15}\text{N}_{\text{bulk}}$  values of possible food sources because the  $\delta^{15}\text{N}_{\text{bulk}}$  values reflect variations in both baseline isotope ratios and trophic discrimination. In other words, it is not clear whether the higher  $\delta^{15}\text{N}_{\text{bulk}}$  values of a given civet species are the result of higher trophic levels or elevated dietary  $\delta^{15}\text{N}_{\text{bulk}}$  values. It is not practical to collect and analyze dozens of dietary items from all civets (Nakabayashi 2020) to determine a stable isotopic baseline. As mentioned earlier, CSIA-AA provides information on both baseline isotope ratios and trophic positions, which enables the quantitative estimation of the TPs even from a limited set of representative samples.

The measured TP of plant-eating insects was higher ( $\geq 2.6$ ) than that expected for obligate plant eaters (i.e. 2), and it is possible that a small amount of insectivory inflates the TPs of civet species. The higher-than-expected insect TP may be due to the consumption of microorganisms with a higher TP than autotrophic organisms (Steffan et al. 2015, 2019). If dung beetles and weevils assimilated proteins from intestinal bacteria from feces or microbes grown on decaying wood, their TP would be higher than expected for a strict herbivorous insect. Even so, the TPs of masked palm civets (2.7) and common palm civets (2.9) were higher than those of insects, confirming faunivory in these civet species.

The point measurements of TP of the civet species ranged from 2.0 to 2.9, and each species showed clustered TPs that did not overlap with those of other species (Fig. 3). Although our critical assumption is that the TP estimates of two individuals per species represent their respective species, this result suggests possible species-specific differences in the degree of faunivory in Paradoxurinae civet species. CSIA-AA illustrated overall faunivory in common palm civets, masked palm civets, and small-toothed palm civets, and the tendency of faunivory seems to be stronger in this order if the analyzed individuals truly represent the diet of each species. Contrary to the other civet species, the TP of binturongs was almost one-level lower even within the same subfamily, suggesting that their diet consists almost entirely of plant-based materials such as fruits.

The diet of these civet species can be discussed more in detail with the existing knowledge from observational studies. First, we used the hairs of the two intensively radio-tracked female binturong individuals for the analysis (Nakabayashi et al. 2016; Nakabayashi and Ahmad 2018; Nakabayashi 2020). According to the results of individual tracking of these binturongs, spanning over 900 days in total, 79–86% of their diets consisted of fig fruits, and faunivory was not observed (Nakabayashi and Ahmad 2018). Therefore, the obviously low  $\delta^{15}\text{N}_{\text{bulk}}$  values and TP of binturongs, which are similar to those of plant-eating animals, compared to the other three Paradoxurinae species simply reflect their fruit-dominated diet (Figs. 2, 3, and 4). Furthermore, given that fig fruits generally contain pollinating fig wasps inside (Harrison et al. 2003), fig wasps seem to contribute little to protein intake in binturongs despite the large consumption of fig fruits (Nakabayashi et al. 2019). Popowics (2003) noted that binturong dentition

is relatively small compared to the body size and shows a decrease in shearing and crushing functions. Binturongs usually feed on mature fig fruits, and thus, large teeth to process large hard fruits may not be necessary for them. Our stable isotopic results, as well as the abovementioned observational studies, implying that faunivory seldom occur in wild binturongs, at least for females. This result is similar to the results of individual trackings (Nakabayashi and Ahmad 2018) and conclusions of more than 700 hours of observation of animals who visited fruiting fig trees in Borneo (Leighton and Leighton 1983; Shanahan 2000).

Compared with binturongs, the difference in TP among other civet species is less clear. To further investigate their dietary niche partitioning in TP, more samples need to be analyzed by CSIA-AA. Even though, these civet species have occasional species-specific feeding behaviors, especially in small-toothed palm civets and binturongs. Common palm civets showed similar TP (2.9) to masked palm civets (2.7), which is equivalent to omnivorous diet. Scat analyses of common palm civets revealed that they sometimes consume rodents in Borneo (Nakashima et al. 2010, 2013; Colon and Sugau 2012), and their occasional faunivory was supported by our CSIA-AA. These two species are genetically close compared to the other two Paradoxurinae species (Patou et al. 2008; Zhou et al. 2017) and show overlap in several food items (Nakabayashi 2020). However, competition could be mitigated by their habitats being different in altitude. While the occurrence records of common palm civets are concentrated in the lowlands, those of masked palm civets are concentrated in the highlands at an altitude of over 700 m in Borneo (Mathai et al. 2010; Brodie and Giordano 2011; Nagano et al. 2019; Nakabayashi et al. 2021). Geographical differences may be a more critical niche partitioning factor than diet for these two species. The measured TP for small-toothed palm civets (2.4 and 2.5) is between those of common palm civets and binturongs, suggesting their unique dietary niche among Paradoxurinae civets as omnivores consuming both plant and animal materials. This is partly supported by their unique dietary habits of feeding on tree bark sap, nectar, oil palm pith, and unripe fruits which the other Paradoxurinae civets do not consume in Borneo (Nakabayashi 2020). Contribution from these unique food sources would increase the overall dietary protein contribution from plants and lower the TP of small-toothed palm civets compared to common palm civets and masked palm civets.

### Ecological significance

Our stable isotopic results suggested faunivory in several Paradoxurinae civet species in Borneo, which has never been confidently shown in previous observational studies (e.g., Harrison 1961). Paradoxurinae civet species are thought to be largely dependent on plant foods (Nakabayashi 2020) although their dental morphology (Anders 2005) and digestive tract anatomy (Gahkod 1878; Liu et al. 1997; McGrosky et al. 2016) present clear characteristics of Carnivora. Such an adaptation is not unique to civets because some other mammals in Carnivora largely depend on plant foods, such as extant giant pandas and extinct cave bears (Naito et al. 2020). However, this study suggested that the postulated assumption of a plant-dominated diet is only applicable for binturongs among the Paradoxurinae civet species in Borneo. Common palm civets, masked palm civets, and small-toothed palm civets showed TP values greater than 2.4, suggesting their omnivorous diet (Table 2, Fig. 4). Such an unexpected omnivorous diet was also

suggested in South American extinct ground sloth *Mylodon* by CSIA-AA (Tejada et al. 2020). Considering that small-toothed palm civets evolutionarily diverged from the group containing these four species first, followed by binturongs and masked palm civets (Patou et al. 2008), the possible differences in TP are not explained by the evolutionary relationship among the subject species. In Viverridae civets, larger species feed on fibrous low-quality food (Gittleman 1985), such as figs. Binturongs have the largest body size and they cannot digest fruits efficiently (Crapo et al. 2002; Lambert et al. 2014), indicating that they need large amounts of food to extract sufficient energy for survival and reproduction compared to the other three species (Kleiber 1961). Because *Ficus* reproduces fruits year-round due to its obligate mutualism with pollinating fig wasps and has a relatively large crop size (Harrison et al. 2003), it is one of the most stable food resources in the Bornean rainforest where fruit production is unstable and low (Wich et al. 2011). Therefore, binturongs may strongly depend on figs for their diet, at least in Borneo. Considering that Asian rainforests possess a much larger number of sympatric carnivore species than the other tropical regions (Corlett 2007), strong dependence on plant foods in binturongs may reduce competition for faunivorous diets among sympatric carnivores.

Direct observation of food items in wild civet species is usually difficult, and previous field-based observational studies, such as focal individual observations and fecal content analysis (Nakabayashi 2020), failed to detect dietary partitioning in Paradoxurinae civet species. Most civets' behaviors are not observable due to their nocturnal, solitary, and semiarboreal habits above 10–60 m canopies (Nakabayashi et al. 2017; M Nakabayashi personal observation). Observation of opportunistic consumption of small insects is quite difficult, and their faunivory had been underestimated. Furthermore, civets' feces are difficult to obtain for fecal content analysis because several civet species, such as small-toothed palm civets and binturongs, defecate higher in the canopy (Nakabayashi et al. 2019; M Nakabayashi personal observation), making the detectability of these feces on the ground extremely low. Even if their fecal samples are obtained, some soft-bodied insects such as larvae and annelids are easily digested and thus are morphologically undetectable through the identification of macro remains in feces. This study shows that stable isotope analysis, as well as direct observation, is useful to reveal the entire diet of the subject species. Additionally, metagenomic and metaproteomic analyses of feces can be utilized further to reveal the detailed food items of mammalian species (e.g. Mallot et al. 2017; Tsutaya et al. 2021). The application of isotopic and biomolecular analyses, such as that conducted in this study, to animal species that are difficult to observe or have been studied little can illuminate unrevealed mechanisms of coexistence and facilitate efficient conservation approaches especially for non-charismatic medium- and small-sized mammals (Trimble and Van Aarde 2010; Troudet et al. 2017).

As with this study, stable isotope analyses have revealed nuanced dietary partitioning in various sympatric terrestrial mammalian taxa, such as Malagasy lemurs (Dammhahn and Kappeler 2014), great apes (Oelze et al. 2014), bats (Campbell et al. 2017; Oelbaum et al. 2019), rodents, and Bovidae (Djagoun et al. 2020). In general, dietary overlap in sympatric carnivores is extensive, especially for confamilial species (Arbogast et al. 2017; Webster et al. 2021), because of the morphological and physiological limitations to digestible diets (Stevens and Hume 2004). In this regard, the inclusion of and dependence on plant foods, in addition to faunal foods, enabled the Paradoxurinae civet species to compensate for

their spatial, temporal, and taxonomic overlaps and similarities (Nakabayashi et al. 2016, 2017) and to occur sympatrically. Such subtle but important differences in closely related sympatric species would maintain high biodiversity in tropical regions (Whitmore 1984). It is possible that anthropogenic disturbances would destroy such a subtle difference, such that by exacerbating competition for fruits (Meijaard et al. 2005), and thus the exquisite coexistence mechanism and biodiversity are impaired.

## Conclusions

The diet and TP of four Paradoxurinae civet species in Maliau, Borneo were investigated by applying bulk stable isotope analysis of hairs and CSIA-AA. Although the TP of binturongs (~ 2.0) suggested that their diet almost entirely consists of plant foods, the TPs of small-toothed palm civets, masked palm civets, and common palm civets (2.4–2.9) suggested their omnivorous diets. These results support our hypotheses that faunivory is more common than previously assumed in Paradoxurinae civet species in Borneo and imply that its degree systematically differs among the sympatric species. Such a subtle dietary difference would enable the coexistence of closely related civet species and ensure high biodiversity in tropical regions.

## Declarations

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### Conflicts of interest

The authors declare that they have no conflict of interest.

### Ethics approval

Sample collection, transfer, and analyses were approved by Sabah Biodiversity Centre (Access Licence JKM/MBS.1000-2/2JLD.4(170), JKM/MBS.1000-2/2JLD.6(50), JKM/MBS.1000-2/2JLD.4(170),

JKM/MBS.1000-2/2JLD.7(64) and Transfer Licence JKM/MBS.1000-2/3(66), JKM/MBS.1000-2/3 JLD.3(100)).

### **Consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Availability of data and material**

All data are included as Supplementary Information files

### **Code availability**

All codes used in this study are included as Supplementary Information files.

### **Authors' contributions**

MN and TT conceptualized the initial idea. MN obtained the field data. AHA arranged the sampling in the field. TT, YS, NOO, NFI, and NO performed and interpreted the isotope analyses. MN and TT wrote the first draft of the manuscript. All authors contributed to the final version of the manuscript.

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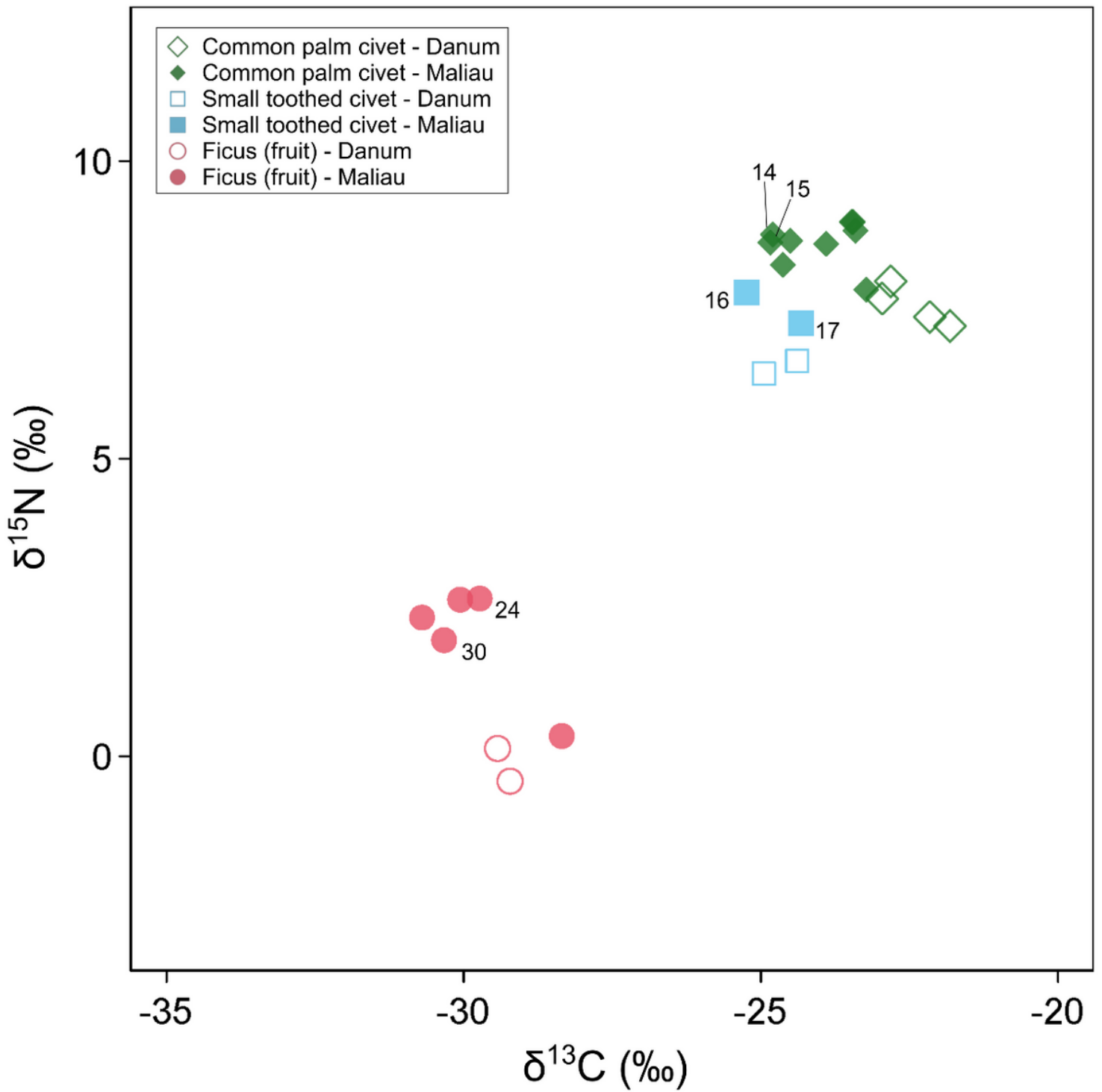
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## Supplementary Informations

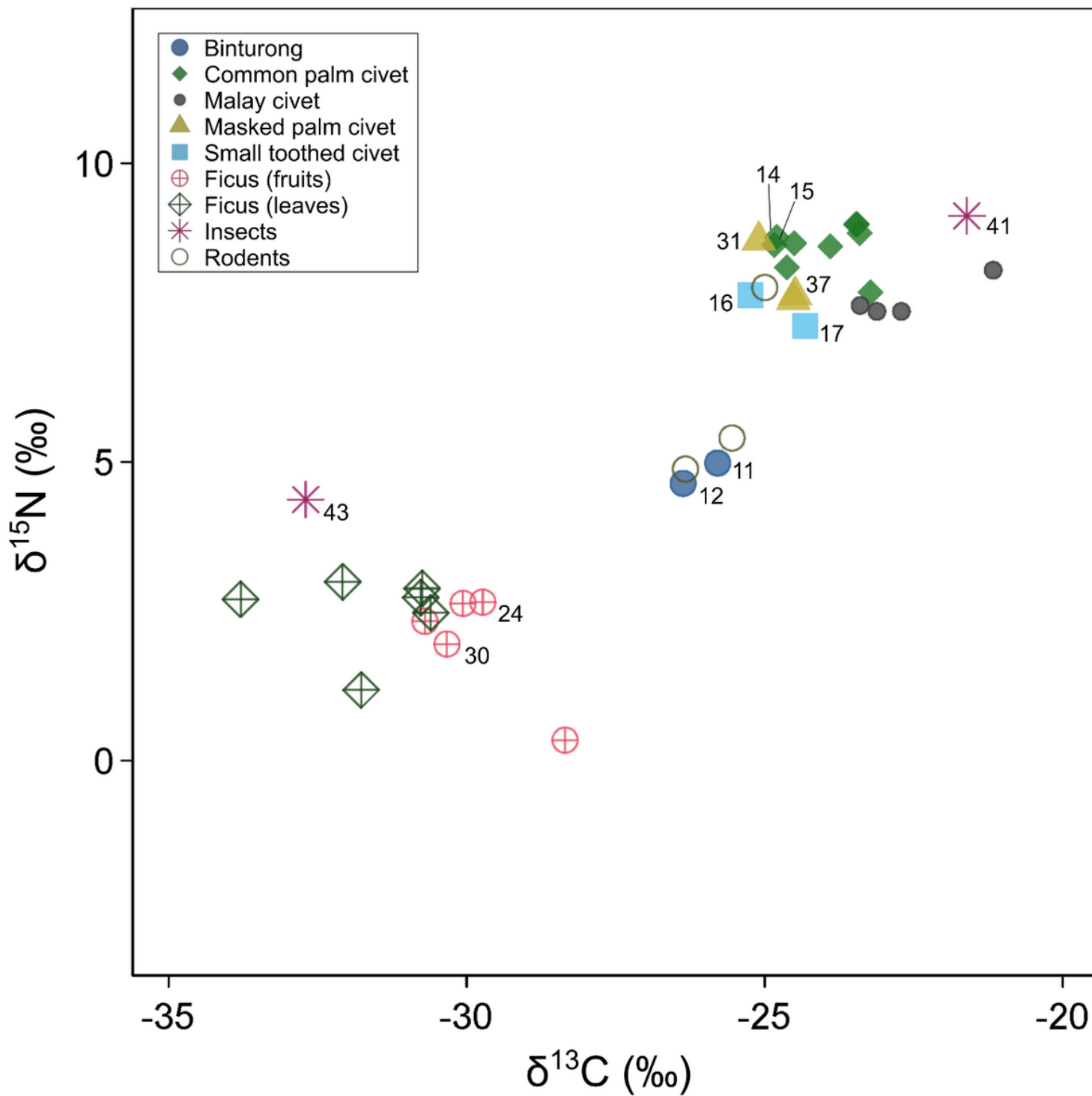
Supplementary Text and Supplementary Tables are not available with this version.

## Figures



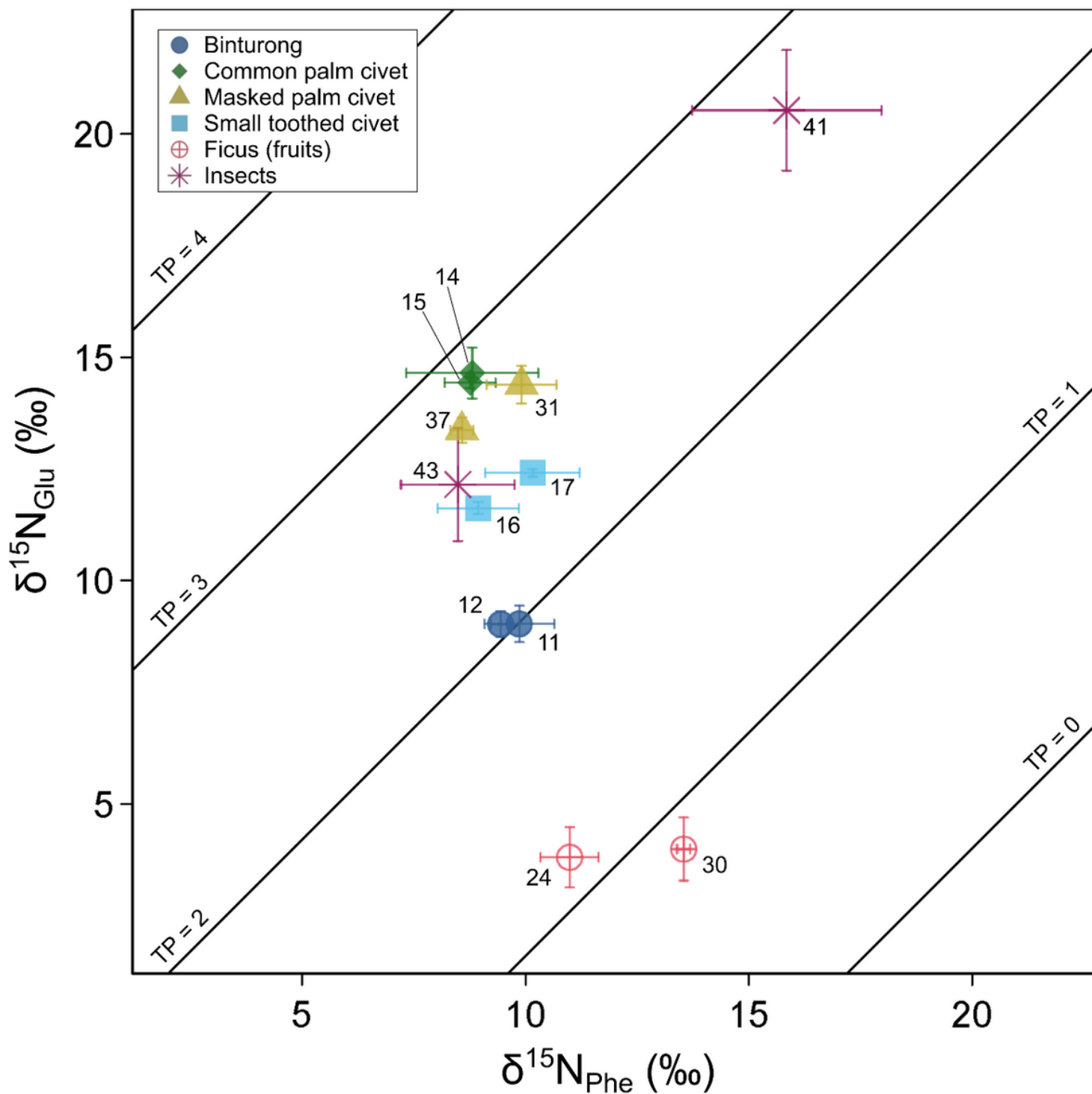
**Figure 1**

Comparison of bulk stable isotope ratios of subject species from Danum and Maliau. Samples analyzed in CSIA-AA are shown with their ID.



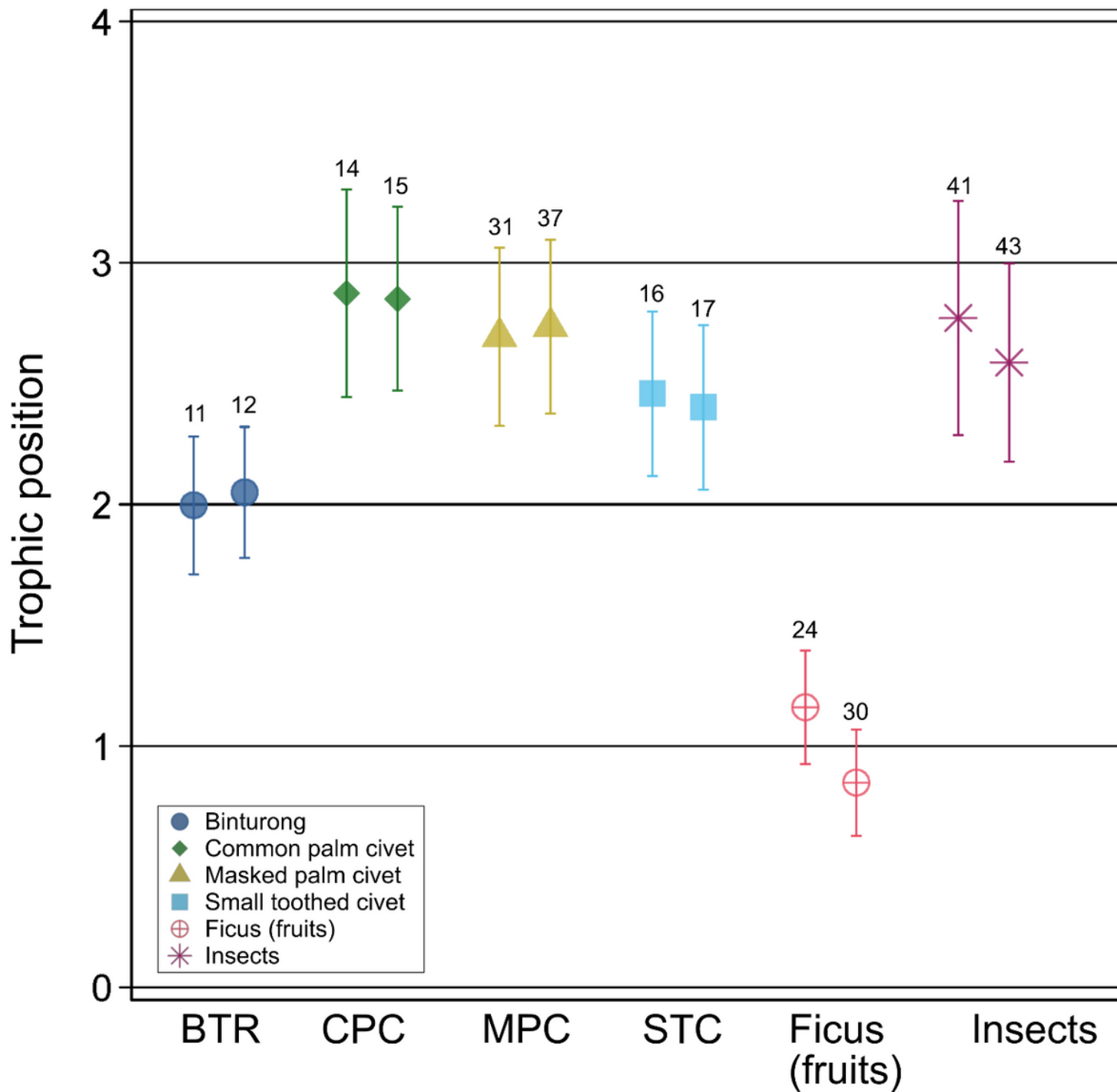
**Figure 2**

Carbon and nitrogen stable isotope ratios of bulk samples from Maliau. Samples analyzed in CSIA-AA are shown with their ID.



**Figure 3**

The  $\delta^{15}\text{N}_{\text{Glu}}$  and  $\delta^{15}\text{N}_{\text{Phe}}$  of Paradoxurinae civet species and their potential food sources from Maliau. Sample ID and estimated TPs calculated based on Equation 1 are also shown.



**Figure 4**

Calculated TP of Paradoxurinae civet species. The error bars represent 1 SD range of propagated uncertainties. Sample ID was also shown.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [aa.txt](#)
- [bulk.txt](#)
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