WILDLIFE BIOLOGY

*Short communication*

# Identification of scat samples from three terrestrial mustelids in Taiwan: a simple method

**Nian-Hong Jang-Liaw[1,2], Hao-Ming Huang[1], Yen-Jean Chen[3], Li-Min Yim[4], Nai-Cheng Yeh[5], Chia-Heng Chung[6] and Po-Jen Chiang[6]**

[1]Conservation Genetics Laboratory, Conservation and Research Center, Taipei Zoo, Taipei, Taiwan
[2]IUCN Species Survival Commission, Otter Specialist Group, Gland, Switzerland
[3]Department of Biology, National Museum of Natural Science, Taichung, Taiwan
[4]Yushan National Park Headquarters, Nantou, Taiwan
[5]Greenpeace East Asia, Taipei, Taiwan
[6]Formosan Wild Sound Conservation Science Center, Co., Ltd., Miaoli, Taiwan

**Correspondence: Nian-Hong Jang-Liaw (taco.tw@gmail.com)**

**Wildlife Biology**
**2025: e01364**
doi: 10.1002/wlb3.01364

Subject Editor:
S. J. Oyler-McCance
Editor-in-Chief: Ilse Storch
Accepted 2 December 2024

Among the five species of the Mustelidae found in Taiwan, four terrestrial species are potentially sympatric. These species include yellow-throated marten *Martes flavigula*, Siberian weasel *Mustela sibirica*, Taiwan least weasel *Mustela nivalis formosana*, and Chinese ferret-badger *Melogale moschata*. Scat from the first three mustelids are similar in morphology. Therefore, differentiating the scats of these three species is challenging, and without accurate species identification, scatology-related techniques such as dietary analysis are problematic. Here, we developed a novel set of primers capable of effectively identifying scat samples of these three species through a simple PCR amplification process. This allows for collection of more accurate field data on these species, enabling a better understanding of their ecological niche and basic biological information for future conservation strategies.

Keywords: agarose gel electrophoresis, scat identification, Siberian weasel *Mustela sibirica*, Taiwan least weasel *Mustela nivalis formosana*, yellow-throated marten *Martes flavigula*

## Introduction

Dietary analysis is crucial for understanding species' biology and their ecological interactions (Jang-Liaw 2021). Scatology, a method for studying wild animals through their scats, provides important information on aspects such as individual health, reproductive cycles, and feeding behavior with minimal disturbance (Seton 1925, Piggott and Taylor 2003). Since many studies on rare mammals rely on accurate identification from scat samples, the methods for scat identification must be more stringent,

NORDIC SOCIETY OIKOS

www.wildlifebiology.org

especially when the animal is endangered (Davison et al. 2002). However, species identification based on scat morphology can be challenging, especially for closely related small- to medium-sized carnivore species with overlapping ecological niches. In systems with multiple similar, sympatric species, field signs such as scats may be wrongly identified (Harrington et al. 2010). Incorrect species identification diminishes the academic and applied value of scat samples; hence, accurate species identification is essential for scatology research.

Taiwan, with its tropical to alpine climate zones and high biodiversity, hosts four terrestrial mustelid species: the yellow-throated marten *Martes flavigula*, Siberian weasel *Mustela sibirica*, Taiwan least weasel *Mustela nivalis formosana* and Chinese ferret-badger *Melogale moschata*. These species of mustelids are all widespread, and the first three show high similarities in the morphology of their scat. The yellow-throated marten (YTM) is an Asian species of martens, widely distributed from the Himalaya to eastern Russia, south to the Malay Peninsula, the Sunda Shelf, and Taiwan (Lim et al. 2015). The Siberian weasel (SW) is widely distributed across Palearctic Asia, with natural populations ranging from the western base of the Ural Mountains of Siberia to the Far East and south to Taiwan and the Himalaya (Law 2018). The least weasel *Mustela nivalis* is the smallest member of the genus *Mustela*, family Mustelidae and order Carnivora (Van Valkenburgh and Wayne 2010), and has a circumboreal range throughout the Holarctic (Sheffield and King 1994). The Taiwan least weasel (TLW) is a recently discovered new subspecies, previously thought to be juvenile weasels (Lin et al. 2010). Despite their wide distribution, the ecology and behavior of these mustelids have received little attention (Grassman et al. 2005, Zhou et al. 2011).

In Taiwan, these three mustelids, whose scats are visually similar, overlap in distribution, and rodents constitute their primary prey (Moors 1975, Zhou et al. 2011, Law 2018). Visually distinguishing their scats for species identification is difficult. Establishing an effective and rapid method for species identification from scat samples is essential for practical scatology operations in areas where these three species coexist. The scats of other native carnivorous animals in Taiwan of similar body size can generally be distinguished morphologically from those of the three mustelid species discussed in this study. However, the morphology of scats is not entirely consistent within species due to factors such as age, health condition, and dietary composition. Even experienced field researchers may misidentify scat species based on morphology alone. This study provides a simple method employing novel primer sets designed for PCR amplification and agarose gel electrophoresis to identify scat samples of these three terrestrial mustelid species in Taiwan. Our objectives were to 1) develop and test a method that could differentiate mustelid species from scat DNA, and 2) evaluate whether the method would rule out other carnivores in Taiwan.

## Material and methods

### Primers design

Three sets of mitochondrial gene primers were designed based on full-length mitochondrial DNA reference sequences of the YTM and SW obtained from GenBank (accession no. AP017414-417 for SW; no. KM347744 and no. FJ719367 for YTM; Jang and Hwang 2016, Shalabi et al. 2016). These primer sets shared the same forward primer (JLSFF) located at positions 7739–7758 on the $tRNA^{Lys}$ gene (the gene locations refer to the published Siberian weasel complete mtDNA sequence, GenBank no. AP017414). The first reverse primer (JLMFR) was designed for YTM, located approximately at positions 8217–8194 (Table 1). The second and third reverse primers (JLMSR1 and JLMSR2) were designed for the SW, located at positions 8703–8680 and 8896–8879, respectively, both within the COIII gene (Table 1). Successful PCR amplification using these primer sets yields DNA fragments of lengths approximately 479, 965, and 1158 bp.

### Experiment 1-primers and PCR conditions test

To test the efficacy of these primers, muscle tissue samples from the mammal Cryobank collection of the National Museum of Natural Science, Taichung, Taiwan (NMNS) were obtained. The samples included muscle tissue samples from a YTM (NMNS7745), an SW (NMNS17962), and a TLW (NMNS18021). Additionally, two scat samples collected from the field were selected to test the newly designed primers. Before testing these scat samples, we first sequenced them to confirm that they were indeed the species we believed them to be. To do this, we followed the PCR protocol of Jang-Liaw et al. (2023) consisting of an initial denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 50 s, annealing at 50°C for 50 s, extension at 72°C for 1 min, and a final extension step at 72°C for 5 min, using primers 12SV5F and 12SV5R targeting the 12S rRNA gene region of vertebrates (Riaz et al. 2011). PCR products were purified using ExoSAP-It (USB Corp., Cleveland, OH, USA) and Sanger-sequenced on an Applied Biosystems 3730XL DNA Analyser bidirectionally using the primers for PCR and the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit ver. 3.1 (Applied Biosystems). Unfortunately, due to the rarity of TLW populations in the wild, no scat samples of this species were available to test. The scat samples of SW are also very limited, with only two SW scat samples available for testing. We selected the mud-like material in the scat samples for DNA extraction and excluded other materials such as hair, muscle remains, or bone fragments that may come from prey. DNA extraction from the samples followed Liu et al. (2024), using the Qiagen DNeasy tissue kit for muscle tissues and Jang-Liaw (2021) for scats. These DNA samples were stored in the Cryobank at the Taipei Zoo.

PCR testing was divided into two batches. The first batch included a mixture of the three primers (JLSFF, JLMFR, and

Table 1. PCR primers designed and tested in this study.

| Set | Primer name | Direction | sequence 5′–3′ | Position | Approx. sequence length (bp) | Cover genes | Target species |
|---|---|---|---|---|---|---|---|
| I/II/III | JLSFF | Forward | TAAGTTAAAGATTGAGAGCA | 7739–7758 | – | – | Siberian weasel, yellow-throated marten |
| I | JLMFR | Reverse | GGTAAATGAATGTGGTAAGAGTCC | 8217–8194 | 479 | $tRNA^{Lys}$, ATP8, ATP6 | Yellow-throated marten |
| II | JLMSR1 | Reverse | ATGTTGTAAGCAGGGCGCGAAAGAG | 8703–8680 | 965 | $tRNA^{Lys}$, ATP8, ATP6 | Siberian weasel |
| III | JLMSR2 | Reverse | AAAAAGACTTCGGATGTG | 8896–8879 | 1158 | $tRNA^{Lys}$, ATP8, ATP6, COIII | Siberian weasel |

JLMSR1) in the PCR, while the second batch included a mixture of the same three primers but with JLMSR2 instead of JLMSR1. The PCR thermal cycling conditions were the same as mentioned above. Two additional annealing temperatures (47 and 54°C) were tested as well, resulting in a total of three different annealing temperatures (47, 50, and 54°C) for each batch of samples. The PCR reaction mixture (20 µl) contained 1 µl of DNA template (or $ddH_2O$ for negative control, NC) and 0.4 µM of each primer. PCR products (5 µl) were loaded onto a 1.5% agarose gel containing HealthView Nucleic Acid Stain (0.02 µl/ml; Cat. GN-NAS-100; GENOMICS Ltd, TAIWAN) in 1× TAE buffer and electrophoresed at 100 V for 15 min; 2 µl of 50 bp DNA size marker (Cat. DM012-R500, GeneDireX Inc., Taiwan) was loaded and was run at the same time. The DNA band was detected under ultraviolet light and photographed by DigiGel DGIS-12s (TOPBIO Co., Taiwan).

### Experiment 2-testing scat under optimal PCR conditions

After confirming the optimized primer composition and PCR conditions, we used 11 additional YTM and 1 SW scat samples to validate this method. In addition, due to the absence of scat sample of TLW, two museum tissue samples (NMNS18022, 11345) of TLW were added to this stage to further confirm the effectiveness of this method in species identification.

### Experiment 3-testing on other similar carnivores native to Taiwan

To determine whether the method used in this study could produce similar bands in other native carnivorous animals of similar size – potentially leading to species misidentification – we tested the DNA of six carnivorous species: the ferret-badger *Melogale moschata* (NMNS19488), Eurasian otter *Lutra lutra* (NMNS19887), small Indian civet *Viverricula indica taivana* (NMNS19239), Formosan gem-faced civet *Paguma larvata taivana* (NMNS17479), crab-eating mongoose *Herpestes urva formosanus* (NMNS6760), and leopard cat *Prionailurus bengalensis chinensis* (NMNS19492). These samples were tested in two batches using primer pairs with a reaction temperature of 50°C, and the band patterns of the PCR products were observed on electrophoresis gels. An SW tissue sample (NMNS17962) was run through PCR alongside the samples mentioned above as a positive control (PC).

### Scat samples used in this study

Scat freshness affects the proportion of detectable prey DNA (McInnes et al. 2017). All mustelid scat samples used in this study were collected in the mountainous areas of northern and central Taiwan and collected by researchers walking on paths (Table 2). Only 'fresh' (when the animal was observed defecating) and 'recent' (when the scat was still wet but the animal was not observed defecating) scats were collected following the sample freshness catalog of McInnes et al. (2017).

Table 2. Detailed information regarding the scat and tissue samples employed in this study. NMNS: catalogue numbers of mammal collection of the National Museum of Natural Science, Taichung, Taiwan; ZF: scat DNA collection of Conservation Genetics Lab of Taipei Zoo.

| No. | Sample type | Sample ID | Species | Collection Date | Locality | Altitude (m a.s.l.) | Used in |
|---|---|---|---|---|---|---|---|
| 1 | Tissue | NMNS7745 | YTM | 12/15/2003 | Aowanda, Nantou | ca 1230 | Exp. 1 |
| 2 | Tissue | NMNS17962 | SW | 2006 | Mt Daxue, Taichung | ca 2120 | Exp.1, 3 |
| 3 | Tissue | NMNS18021 | TLW | 7/1/2010 | Cui Pond, Tai'an, Maoli | ca 3500 | Exp. 1 |
| 4 | Scat | ZF-YS011 | YTM | 1/21/2024 | Shinyi, Nantou | 2494 | Exp. 1 |
| 5 | Scat | ZF-YS002 | SW | 11/7/2023 | Shinyi, Nantou | 2623 | Exp. 1 |
| 6 | Tissue | NMNS19488 | Ferret-badger | 12/17/2013 | Taiping, Taichung | 150 | Exp. 3 |
| 7 | Tissue | NMNS19887 | Eurasian otter | 12/29/2017 | Jinhu, Kinmen | 10 | Exp. 3 |
| 8 | Tissue | NMNS19239 | Small Indian civet | 3/12/2016 | Yuanshan, Yilan | ca 580 | Exp. 3 |
| 9 | Tissue | NMNS17479 | Formosan gem-faced cive | 11/27/2010 | Yangmingshan, Beito, Taipei | ca 510 | Exp. 3 |
| 10 | Tissue | NMNS6760 | Crab-eating mongoose | 11/9/1997 | Fushan, Yuanshan, Yilan | 550 | Exp. 3 |
| 11 | Tissue | NMNS19492 | Leopard cat | 2/2/2016 | Zhuolan, Miaoli | ca 330 | Exp. 3 |
| 12 | Scat | ZF-Mf2110-1 | YTM | 10/7/2021 | Shinyi, Nantou | 2650 | Exp. 2 |
| 13 | Scat | ZF-Mf2110-2 | YTM | 10/17/2021 | Shinyi, Nantou | 2540 | Exp. 2 |
| 14 | Scat | ZF-Mf220111-1 | YTM | 1/11/2022 | Shinyi, Nantou | 2670 | Exp. 2 |
| 15 | Scat | ZF-Mf220111-2 | YTM | 1/11/2022 | Shinyi, Nantou | 2630 | Exp. 2 |
| 16 | Scat | ZF-Mf22011901 | YTM | 1/19/2022 | Shinyi, Nantou | 2615 | Exp. 2 |
| 17 | Scat | ZF-Mf220726 | YTM | 7/26/2022 | Shinyi, Nantou | 2630 | Exp. 2 |
| 18 | Scat | ZF-YS015 | YTM | 3/15/2024 | Shinyi, Nantou | 2630 | Exp. 2 |
| 19 | Scat | ZF-Mf2107-1 | YTM | 7/31/2021 | Shinyi, Nantou | 2630 | Exp. 2 |
| 20 | Scat | ZF-MfGP-8 | YTM | 10/6/2021 | Shinyi, Nantou | 2710 | Exp. 2 |
| 21 | Scat | ZF-Mf220112 | YTM | 1/12/2022 | Shinyi, Nantou | 2670 | Exp. 2 |
| 22 | Scat | ZF-TP230220-7 | YTM | 2/20/2023 | Cuifeng Lake, Nan'ao, Yiland | 1868 | Exp. 2 |
| 23 | Scat | ZF-Mf230823-1 | SW | 8/23/2023 | Mt. Hehuan, Ren-ai, Nantou | 2986 | Exp. 2 |
| 24 | Tissue | NMNS18022 | TLW | 6/15/2009 | Mt. Hehuan, Xiulin, Hualien | ca 3000 | Exp. 2 |
| 25 | Tissue | NMNS11345 | TLW | 7/27/2006 | Meifeng, Ren-ai, Nantou | ca 2100 | Exp. 2 |

Each sample was preserved individually in 99% alcohol and stored at −80°C until examination. Species identification was conducted through DNA comparison with the Genbank database. As in Experiment 1, PCR and sequencing were performed using primers 12SV5F and 12SV5R, which target the vertebrate-specific 12S rRNA gene region. All scat samples were confirmed to species level.

## Results and discussion

The first batch of tests, which included a mixture of JLSFF, JLMFR, and JLMSR1 primers, demonstrated good resolution and identification capabilities for all three target species at the three annealing temperatures tested (Fig. 1). The YTM DNA sample exhibited a clear single band at approximately 480 bp in length, while the SW DNA sample showed two bands at approximately 480 and 970 bp, respectively. The TLW DNA sample displayed a single band slightly shorter than 1000 bp, obviously different to the YTM. At the lower annealing temperature (47°C), both the SW and TLW samples exhibited multiple nonspecific bands of approximately 100 bp in size (Fig. 1), which decreased in intensity at higher annealing temperatures without interfering with species identification due to distinct fragment sizes. This batch of tests also showed good resolution for the other two scat DNA samples from YTM and SW. Notably, with a higher annealing temperature of 54°C, the DNA sample of TLW showed a faint band of approximately 480 bp (Fig. 1).

In the second batch of PCR tests using primers JLSFF, JLMFR, and JLMSR2, it was possible to distinguish between two species, the YTM and the SW, but not easily between the YTM and the TLW (Fig. 1). Unlike the results from the first batch of tests, the PCR product of the TLW in the second batch only showed a single band at the same position as the YTM (approximately 480 bp). Although the TLW displayed weak multiple nonspecific bands in the gel electrophoresis test under low to moderate Tm conditions, the YTM did not, but this is insufficient to clearly differentiate these two species. When identifying DNA samples from the scat of YTMs and SWs, the primer combination in the second batch still provides sufficient resolution and may have the potential to identify different mustelids in other regions.

Combining the above tests, it is evident that the primer combination JLSFF, JLMFR, and JLMSR1 from the first batch can be applied to identify the DNA samples of these three species, including DNA samples extracted from scats (Fig. 2). An optimal PCR result can be obtained at an annealing temperature of 50°C. Most scat samples (Fig. 2) displayed clear bands similar to those in Fig. 1, yet some samples

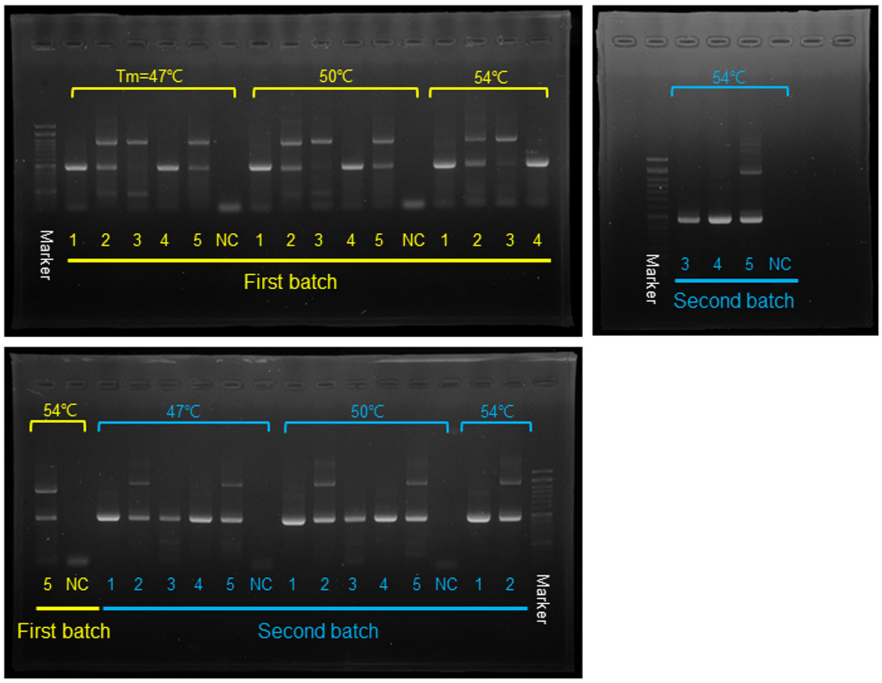


Figure 1. Results of 1.5% agarose gel electrophoresis of the primer combinations and PCR reaction temperature tests. Tm: annealing temperature of PCR. Samples of both batches: 1, YTM tissue DNA; 2, SW tissue DNA; 3, TLW tissue DNA; 4, YTM scat DNA; 5, SW scat DNA; NC, negative control. Detailed sample information can be found in Table 2. The first batch of tests included a mixture of JLSFF, JLMFR, and JLMSR1 primers; the second batch included a mixture of JLSFF, JLMFR, and JLMSR2 primers.

showed faint or unexpected bands. Regardless, they still do contain recognizable bands at the expected locations, providing support for the identification results. It must be stated that due to the lack of TLW scat samples for testing, although we believe this method is effective for TLW tissue samples and should also be effective for TLW scat samples, it has not yet been tested on TLW scat samples (Fig. 3).

Apart from these three species, there are no other mustelids with similar scat morphology in Taiwan (Chuang and Lee 1997). Two native mustelid species, the Eurasian otter and Chinese ferret-badger, exist in Taiwan. The former is semi-aquatic yet has not been recorded in Taiwan recently (Chang et al. 2019, Jang-Liaw et al. 2023, Li et al. 2024). Further, it feeds mainly on fish, so its scats (spraints) usually contain fish remains (Jang-Liaw 2021). The latter mainly consumes invertebrates, amphibians, and plants (Chuang and Lee 1997, Wu 1999), and its diet is very different from that of other mustelids. Both the appearances of scats of Eurasian otter and Chinese ferret-badger are quite different from that of other mustelids, and their scats can be easily differentiated

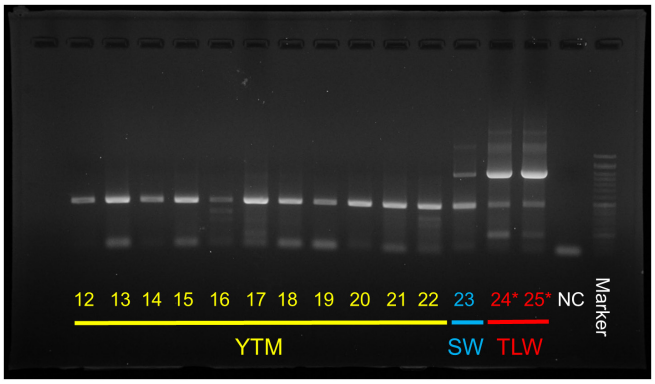


Figure 2. Results of 1.5% agarose gel electrophoresis showing additional PCR results from scat samples (11 YTM and 1 SW) and 2 TLW tissue samples in Experiment 2. Detailed sample information can be found in Table 2.

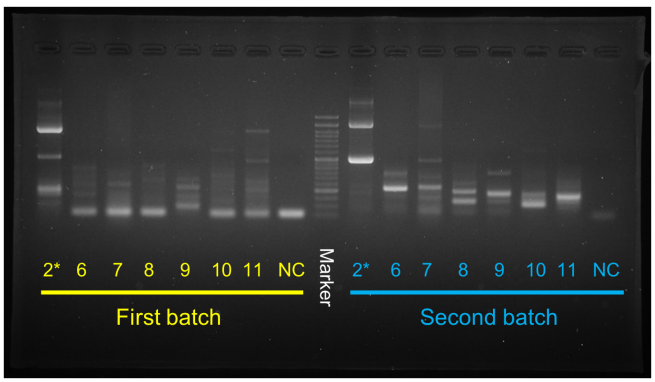


Figure 3. Results of 1.5% agarose gel electrophoresis of PCR for six carnivorous species in Experiment 3: 6, ferret-badger; 7, Eurasian otter; 8, small Indian civet; 9, Formosan gem-faced civet; 10, crab-eating mongoose; and 11, leopard cat. Detailed sample information can be found in Table 2. An asterisk ('*') indicates the results for SW as a positive control (PC).

from those of other three mustelid species (Fig. 4). In addition to mustelids, there are other native carnivorous species on the island that are similar in size, yet their scats are very different from the mustelids in this study. The primer panel used in this study will not amplify these carnivore species with the same band patterns as those of the three mustelid species in the gel electrophoresis test (Fig. 3).

For mustelid-like scat collected from Taiwan's mountainous areas, these newly designed primers can be used to quickly identify species, facilitating subsequent scatology-related research for various ecological investigations. When applying this species identification method in other regions, it is necessary to consider whether there are other sympatric mustelid species, and evaluation of the primers' discrimination ability for different species would be required. Xiong et al. (2017) and Zhao et al. (2022) encountered similar issues where the scat of certain species selected for dietary studies appeared quite similar morphologically, making species identification difficult. They accomplished species identification of scat samples by sequencing a fragment of the mtDNA 16S rRNA gene and comparing it to the GenBank database. However, DNA sequencing is more expensive and time consuming than traditional PCR. Earlier studies used PCR-RFLP technology to identify species of sympatric mustelids in scat samples (Gómez-Moliner et al. 2004, Colli et al. 2005, Ruiz-González et al. 2008); however, this method relied on only a few informative DNA sequence positions, necessitating the use of multiple restriction enzymes to achieve accurate identification. In such cases, the use of different enzymes resulted in highly complex RFLP patterns that were challenging to interpret. Additionally, this method is not suitable for automation and standardization because it requires a substantial amount of high-quality DNA (Pereira et al. 2008) and DNA extracted from scat is generally of low quality.

Our method skips the sequencing process, directly determining species identification results through PCR followed by electrophoresis, accelerating the differentiation process for these three species. However, these sets of primers are only applicable to the yellow-throated marten, Siberian weasel, and Taiwan least weasel, and not to other native carnivorous animals in Taiwan, such as the small Indian civet and crab-eating mongoose (there are no PCR products similar to those of three mustelid species; see Experiment 3, Fig. 3). If scat samples from other species are accidentally collected, species identification cannot be performed using this method.

The first batch of primers JLSFF, JLMFR, and JLMSR1, introduced in this study has already been applied in research on the dietary analysis of mustelids in Taiwan's mountain areas, assisting in the preliminary identification of scat samples from one of the three mustelid species. However, if no PCR product is obtained after amplification, the scat sample may not belong to a mustelid species (for example, misidentified scat samples from the small Indian civet or crab-eating mongoose, provided by volunteers without experience in distinguishing between the morphological traits of scats of civets, mongooses, and mustelids). Alternatively, the scat may have been exposed to air for an extended period, leading to DNA degradation. To address these challenges, the use of PCR amplification with mixed primers (JLSFF, JLMFR, and JLMSR1) combined with gel electrophoresis can serve as a quality control (QC) procedure for scat samples.

The application of this study significantly reduces the time and cost required for species identification in scat samples, enables the early elimination of poor-quality scat DNA samples, and lowers the risk of underestimating prey species content due to DNA degradation. These primers have already been used for species identification in wildlife scat samples from mountainous areas of Taiwan, helping to delineate the distribution patterns of these three mustelids and advancing wildlife ecology research in areas such as dietary behavior and parasitology. The success of this study also encourages the future development of specific primers tailored for species


Figure 4. Photos of scats from various small- to medium-sized carnivore species in Taiwan: (a) YTM; (b) ferret-badger; (c) and (d) Eurasian otter; (e) small Indian civet; (f) Formosan gem-faced civet; (g) crab-eating mongoose; and (h) leopard cat.

identification in different regions and species, supporting the ongoing progress of wildlife management and research.

*Acknowledgements* – We would like to express our appreciation to the anonymous reviewers for their careful reading of our manuscript and their insightful comments and suggestions.
*Funding* – This study was supported by the Animal Adoption Plan of Taipei Zoo (11206).
*Permits* – All tissue samples were deposited in the Wildlife Cryobank of Taipei Zoo, and were used here with permission by the Forestry Bureau, the Council of Agriculture, the Executive Yuan, Taiwan, per the following authorization: 1096011590 (2020–22).

### Author contributions

**Nian-Hong Jang-Liaw**: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Methodology (lead); Resources (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Hao-Ming Huang**: Formal analysis (supporting); Writing – review and editing (supporting). **Yen-Jean Chen**: Data curation (supporting); Resources (supporting); Writing – review and editing (supporting). **Li-Min Yim**: Investigation (supporting); Resources (supporting); Writing – review and editing (supporting). **Nai-Cheng Yeh**: Investigation (supporting); Resources (supporting); Writing – review and editing (supporting). **Chia-Heng Chung**: Investigation (supporting); Resources (supporting); Writing – review and editing (supporting). **Po-Jen Chiang**: Resources (supporting); Writing – review and editing (supporting).

### Transparent peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/wlb3.01364.

### Data availability statement

All data has been disclosed in this paper.

## References

Chang, S.-W., Chang, H.-L. and Hsu, S.-L. 2019. History of Eurasian otters on the island of Taiwan. – Nat. Conserv. Q. 106: 12–23.

Chuang, S.-A. and Lee, L.-L. 1997. Food habits of three carnivore species (*Viverricula indica*, *Herpestes urva*, and *Melogale moschata*) in Fushan Forest, northern Taiwan. – J. Zool. 243: 71–79.

Colli, L., Cannas, R., Deiana, A. M., Gandolfi, G. and Tagliavini, J. 2005. Identification of mustelids (Carnivora: Mustelidae) by mitochondrial DNA markers. – Mamm. Biol. 70: 384–389.

Davison, A., Birks, J. D. S., Brookes, R. C., Braithwaite, T. C. and Messenger, J. E. 2002. On the origin of faeces: morphological versus molecular methods for surveying rare carnivores from their scats. – J. Zool. 257: 141–143.

Gómez-Moliner, B. J., Cabria, M. T., Rubines, J., Garin, I., Madeira, M. J., Elejalde, A., Aihartza, J., Fournier, P. and Palazón, S. 2004. PCR-RFLP identification of mustelid species: European mink (*Mustela lutreola*), American mink (*M. vison*) and polecat (*M. putorius*) by analysis of excremental DNA. – J. Zool. 262: 311–316.

Grassman Jr, L. I., Tewes, M. E. and Silvy, N. J. 2005. Ranging, habitat use and activity patterns of binturong *Arctictis binturong* and yellow-throated marten *Martes flavigula* in north-central Thailand. – Wildl. Biol. 11: 49–57.

Harrington, L. A., Harrington, A. L., Hughes, J., Stirling, D. and Macdonald, D. W. 2010. The accuracy of scat identification in distribution surveys: American mink, *Neovison vison*, in the northern highlands of Scotland. – Eur. J. Wildl. Res. 56: 377–384.

Jang, K. H. and Hwang, U. W. 2016. Complete mitochondrial genome of Korean yellow-throated marten, *Martes flavigula* (Carnivora, Mustelidae). – Mitochondrial DNA A 27: 1785–1786.

Jang-Liaw, N.-H. 2021. A barcoding-based scat-analysis assessment of Eurasian otter *Lutra lutra* diet on Kinmen Island. – Ecol. Evol. 11: 8795–8813.

Jang-Liaw, N.-H., Tan, Y.-C., Chang, C.-J., Juan, C.-H., Hou, H.-Y., Chung, L.-W., Cao, H.-S., Waku, D., Chang, S.-W. and Lee, L.-L. 2023. Genetic diversity and structure of Eurasian otters on Kinmen Island. – Conserv. Genet. 24: 589–606.

Law, C. J. 2018. *Mustela sibirica* (Carnivora: Mustelidae). – Mamm. Species 50: 109–118.

Li, S.-H., Yeh, C.-F., Jang-Liaw, N.-H., Chang, S.-W., Lin, Y.-H., Tsai, C.-E., Chiu, C.-C., Chen, C.-W., Ke, H.-R., Wang, Q., Lu, Y., Zheng, K., Fan, P., Zhang, L. and Liu, Y. 2024. Low but highly geographically structured genomic diversity of East Asian Eurasian otters and its conservation implications. – Evol. Appl. 17: e13630.

Lim, S.-J., Min, J.-H. and Park, Y.-C. 2015. Analysis of habitat characteristics of the yellow-throated marten *Martes flavigula* (Carnivora : Mustelidae) using geographic information system (GIS). – J. Forest Environ. Sci. 31: 261–266.

Lin, L.-K., Motokawa, M. and Harada, M. 2010. A new subspecies of the least weasel *Mustela nivalis* (Mammalia, Carnivora) from Taiwan. – Mamm. Study 35: 191–200, 110.

Liu, C.-W., Hou, H.-Y., Hsieh, H.-I. and Jang-Liaw, N.-H. 2024. Sex identification of birds in Taipei Zoo. – Zoo Biol. 43: 268–275.

McInnes, J. C., Alderman, R., Deagle, B. E., Lea, M.-A., Raymond, B. and Jarman, S. N. 2017. Optimised scat collection protocols for dietary DNA metabarcoding in vertebrates. – Methods Ecol. Evol. 8: 192–202.

Moors, P. J. 1975. The food of weasels (*Mustela nivalis*) on farmland in north-east Scotland. – J. Zool. 177: 455–461.

Pereira, F., Carneiro, J. and Amorim, A. 2008. Identification of species with DNA-based technology: current progress and challenges. – Recent Pat. DNA Gene Seq. 2: 187–199.

Piggott, M. P. and Taylor, A. C. 2003. Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. – Wildl. Res. 30: 1–13.

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P. and Coissac, E. 2011. EcoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. – Nucl. Acids Res. 39: e145.

Ruiz-González, A., Rubines, J., Berdión, O. and Gómez-Moliner, B. J. 2008. A non-invasive genetic method to identify the sym-

patric mustelids pine marten (*Martes martes*) and stone marten (*Martes foina*): preliminary distribution survey on the northern Iberian Peninsula. – Eur. J. Wildl. Res. 54: 253–261.

Seton, E. T. 1925. On the study of scatology. – J. Mammal. 6: 47–49.

Shalabi, M. A., Abramov, A. V., Kosintsev, P. A., Lin, L.-K., Han, S.-H., Watanabe, S., Yamazaki, K., Kaneko, Y. and Masuda, R. 2016. Comparative phylogeography of the endemic Japanese weasel (*Mustela itatsi*) and the continental Siberian weasel (*Mustela sibirica*) revealed by complete mitochondrial genome sequences. – Biol. J. Linn. Soc. 120: 333–348.

Sheffield, S. R. and King, C. M. 1994. *Mustela nivalis*. – Mamm. Species 454: 1–10.

Van Valkenburgh, B. and Wayne, R. K. 2010. Carnivores. – Curr. Biol. 20: R915–R919.

Wu, H.-Y. 1999. Is there current competition between sympatric Siberian weasels (*Mustela sibirica*) and ferret badgers (*Melogale moschata*) in a subtropical forest ecosystem of Taiwan? – Zool. Stud. 38: 443–451

Xiong, M., Wang, D., Bu, H., Shao, X., Zhang, D., Li, S., Wang, R. and Yao, M. 2017. Molecular dietary analysis of two sympatric felids in the mountains of southwest China biodiversity hotspot and conservation implications. – Sci. Rep. 7: 41909.

Zhao, D., Liu, Z., Tan, M., Wang, Y., Dai, W., Ge, J. and Feng, L. 2022. Molecular dietary analysis of three sympatric Mustelidae in northeast China. – Animals 12: 3290

Zhou, Y.-B., Newman, C., Buesching, C. D., Zalewski, A., Kaneko, Y., Macdonald, D. W. and Xie, Z.-Q. 2011. Diet of an opportunistically frugivorous carnivore, *Martes flavigula*, in subtropical forest. – J. Mammal. 92: 611–619.