

## **Project Update: August 2021**

### **Summary**

*Rationale and objectives:* Otters in Malaysia are confined to inland and coastal wetland habitats that are vulnerable to a variety of anthropogenic disturbances. Three globally threatened species of otters, the smooth-coated otter, Asian small-clawed otter, and hairy-nosed otter occur along the North-central Selangor Coast (NCSC) including the Kuala Selangor Nature Park (KSNP) in Peninsular Malaysia. The aim of this project was to develop and test non-invasive methodologies to assess the spatial distribution and habitat relationships of these three species to identify critical habitats and patterns of spatial partitioning and overlap.

*Methods:* In 2021, we initiated a systematic survey of the NCSC. The study area was overlaid with 9-km<sup>2</sup> grid cells covering a range of different land use. Within 80 randomly selected grid cells, three to five 500 m transects were surveyed for otter sign (latrine sites, footprints, holts and direct sightings) or direct observations of individuals and groups. A sociological survey was conducted simultaneously to assess potential human-otter conflicts with fishermen and aquaculture communities. Spraint samples were collected for DNA extraction and amplification of the mitochondrial TanaD (d-loop region) and ND4 loci to identify species. In the following year, species location data will be overlaid with GIS layers of land use to map spatial distribution and assess habitat relationships. We will use occupancy models together with information theoretic methods to assess important covariates associated with occupancy and patterns of species co-occurrence.

*Results to date:* We surveyed 18 grid cells along the NCSC, of which 12 contained otter signs. We developed a successful PCR-RFLP method for otter species identification. We successfully amplified 32 and 17 samples using the TanaD-Mod and ND4 primers from a total of 33 field samples. Our current RFLP data successfully identified, (i) TanaD-Mod: seven Asian small-clawed otters and three smooth-coated otters, and (ii) ND4: eight Asian small-clawed otters, four smooth-coated otters and one hairy-nosed otter. In the 2<sup>nd</sup> year, we will survey the remainder of the NCSC. Thus far, four aquaculture farms were interviewed and collaboration with the Department of Fisheries Malaysia is underway to involve more aquaculture farms in the human-otter conflict documentation effectively.

*Future Plans and Implications:* In the subsequent months, we will continue the survey to cover the remainder of the NCSC and obtain sufficient sample sizes for occupancy analysis. The results of this study will provide natural resource agencies with suitable method to monitor the otter population, specific knowledge of otter distribution and habitat use, and ecological information crucial for the development of effective conservation policies for otter populations.

### **Introduction**

Southeast Asia contains natural habitats with high biodiversity, but the steep exponential growth of human populations in the region has caused a surge in conversion of natural habitats to urban or agricultural development (Sodhi *et al.*, 2010; Squires, 2013; Hughes, 2017). Otters are a prime example of species whose habitats have undergone significant alteration and degradation from anthropogenic activities. An important question is how contemporary otter populations have habituated to changes in land use and how interspecific

mechanisms of resource partitioning operate in such habitats to promote coexistence. An understanding of the mechanisms permitting coexistence in wildlife communities can aid conservation interventions and habitat management (Li *et al.*, 2021).

Landscape types, habitat selection and interspecific interactions all contribute to shaping spatial distribution patterns of species. Coexistence among different species is modulated by a combination of their own habitat preferences and the opposing effects of predation and competition (Shigesada *et al.*, 1979; Fedriani *et al.*, 1999; Pacala and Levin 1997). Compared to species of otter in other regions of the world, in Southeast Asia different species of otter have broadly overlapping geographic distributions (Foster-Turley, 1990).

There are four species of otter in Asia and all of them are found in Malaysia. The smooth-coated otter (*Lutrogale perspicillata*) and Asian small-clawed otter (*Aonyx cinereus*) are listed by the International Union for the Conservation of Nature (IUCN) as Vulnerable, the hairy-nosed otter (*Lutra sumatrana*) as Endangered and the Eurasian otter (*Lutra lutra*) as Near Threatened (Aadrean *et al.*, 2015; Wright *et al.*, 2015; de Silva *et al.*, 2015; Roos *et al.*, 2015).

In Peninsular Malaysia, records of the hairy-nosed otter are rare and those of the Eurasian otter even more scarce. Records of smooth-coated otters and Asian small-clawed otters are fairly abundant and from a wide range of habitats (Conroy, Melisch & Chanin, 1998; Ratnayeke *et al.*, 2018). There is strong evidence that the smooth-coated otter and Asian small-clawed otter are sympatric and occur in a variety of habitats including mixed dipterocarp forest, lowland forest, hill dipterocarp forest, peat swamp forest, mangrove forest and rice fields (Syakirah *et al.*, 2000; Norhayati *et al.*, 2009; Norma-Rashid & Teoh, 2012; Baker, 2013; Hedges *et al.*, 2013; Aihara *et al.*, 2016; Salahshour, 2016; Magintan *et al.*, 2017; Fernandez, 2018). Observations and remote camera data (Woo and Sawairnathan, 2019; Woo, 2021) suggest sympatry among smooth-coated otters, Asian small-clawed otter, and hairy-nosed otters at a site along the North-central Selangor Coast (NCSC). Abdul-Patah *et al.*, (2020) found that these same species co-occurred in seven of 138 sampling locations in the peninsula. Six of those seven sympatric locations were coastal habitats. A few studies have stressed the importance of coastal and sub coastal habitats including mangrove forest and rice fields for otters (Shariff, 1984, 1985; Burhanuddin & Norizan, 1990; Foster-Turley 1992). However, no systematic study has compared habitat use and resource partitioning for otters in the peninsula to date. Very little empirical data exists on the ecological requirements of most small carnivores in Malaysia; thus, there is urgent need for research on all Southeast Asian otters in terms of current distribution, habitat use, human-wildlife conflict, and site-specific threats to survival (Kruuk, 2011).

The aim of this project is to assess spatial distribution of three species of otter, namely the Asian small-clawed otter, smooth-coated otter and hairy-nosed otter, along the North-central Selangor coast, Selangor of Malaysia. Our specific objective are to:

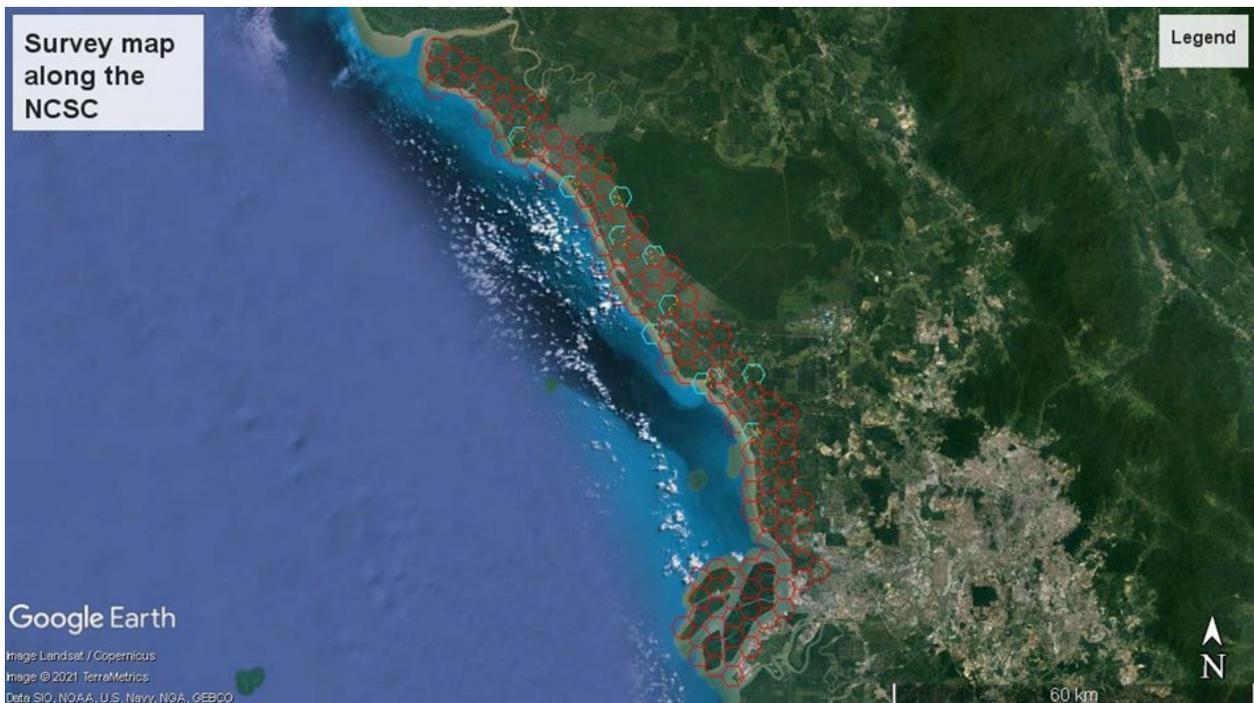
- To develop a suitable method for identifying the three species of otter from signs.
- To assess patterns of species co-occurrence and relationships to habitat type.

- To document incidents of human-otter conflict among fishermen and aquaculture farm communities along the North-central Selangor coast, Selangor.

### Methodology

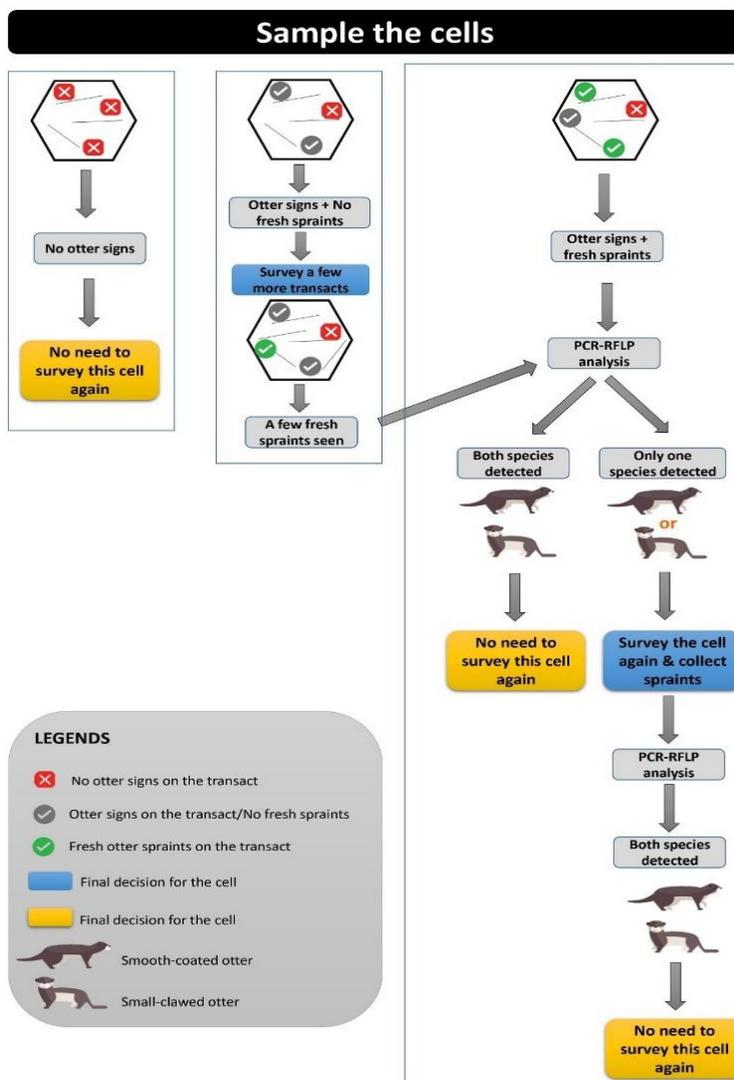
We established 136 grid cells, sized 9 km<sup>2</sup> each, along the North Central Selangor Coast (NCSC) and subcoastal area as sample units for occupancy surveys (Figure 1) (MacKenzie *et al.*, 2006). From the total grid cells (N = 136) spanning the study area, 80 grid cells were targeted between January and June 2021 for the survey and selected based on the presence of waterways and with the aim of sampling a gradient of land use covariates.

We used spatial replication, from which detection probabilities will be calculated. In each grid cell, three to five predetermined 500 m-long transects with random starting points were sampled during a single visit to build encounter histories. Transects were at least 1 km apart. Otter presence was documented based on footprints, spraints, holt sites and direct observation. A fibreglass fishing boat or inflatable kayak was used for surveying large rivers. Grid cells that overlapped mangrove forest or larger rivers were surveyed between periods of high and low tide to facilitate accessibility. Otter signs and sighting locations were marked with a GPS device and the approximate age, size, shape and composition of spraints were recorded. A transect was assigned '1' if otter sign was observed and '0' for no detection. We used augmented sampling to address sites where only old spraints were detected by repeating the survey at these transects within a few days (Figure 2) (Specht *et al.*, 2017).



**Figure 1.** The survey map of the North Central Selangor Coast and subcoastal areas which made up of a total 136 9 km<sup>2</sup> grid cells (in red colour) and the selected grid cells in blue colour contains three to five 500 m transects for the first survey.

Sprints were assigned to species using a Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) approach that we developed specifically for otters in Southeast Asia (Sunway University unpublished data). Samples of fresh and medium-fresh spraint collected during sign surveys were stored in separate 5 ml cryotubes containing 97% alcohol. Total genomic DNA was extracted using the GF-1 Soil Sample DNA extraction kit (Vivantis Technologies, Malaysia) using around 250mg - 500mg starting material. Amplification was conducted in a final volume of 25  $\mu$ L which consisted of 12.5  $\mu$ L DreamTaq™ PCR Master Mix (2X) (Thermo Fisher Scientific, USA), 1.5  $\mu$ L of 10 pmol/ $\mu$ L forward and reverse primers, 2  $\mu$ L extracted DNA sample and 7.5  $\mu$ L distilled water. We designed two sets of primers targeting portions of the mitochondrial D-loop region (Table 1). Amplifications for the TanaD-modified primers were performed according to thermocycling parameters described in Masuki et al. (2008). Amplification for the ND4 marker was as follows: initial denaturation at 94°C for 10 min followed by 45 cycles of amplification (denaturing 94°C for 30s, annealing at 62°C for 30s, extension at 72°C for 1 min) and a final extension at 72°C for 7 min.



**Figure 2.** Three different occasions that determine the use of the augmented sampling approach for the survey in this project.

**Table 1.** Primer sequences designed for species identification of Southeast Asian otters. The primers target partial fragments of the mitochondrial D-loop region.

Name of the primers	No. of fragments	Forward primer	Reverse primer
TanaD-Modified, designed in this study from an amplified sequence by Masuki et al. (2008)	194 bp	5'- CACCATGC CTCGA GAAACCA - 3'	5'- GGGCTGATTAGTC ATTAGTCCATC -3'
NADH dehydrogenase subunit 4 (ND4)	304 bp	5'- GGCAACC AAACA GAACGCC - 3'	5'- GTTAAGGAGTAC GGCGGCAA -3'

Restriction digests were conducted on amplified PCR products. After conducting predictions *in silico*, we conducted the following PCR-RFLP protocols for the TanaD-Modified amplicons and selected the one producing the best results.

- A single digest protocol using the Hin1II/CviAll restriction enzyme.
- A double digest protocol using the NdeI and FspI restriction enzymes. The digestion protocol with the clearer and accurate PCR-RFLP species-specific profiles was selected for analysis of all samples.

We used the BsuRI restriction enzyme for single digests of the ND4 amplicons, to obtain species-specific RFLP profiles. We conducted restriction digests in 30 µL reactions containing 10 µL PCR products, 1 µL of each enzyme, 2 µL buffer (Thermo Fisher Scientific, USA) and distilled water for the remaining volume. Restriction digests were incubated for two hours at 37 °C (NdeI and BsuRI). RFLP profiles were visualised in 2.5% agarose gel for better separation and species identity was assessed based on fragment band patterns. This approach was designed to achieve a cost- and time-effective alternative to sequencing.

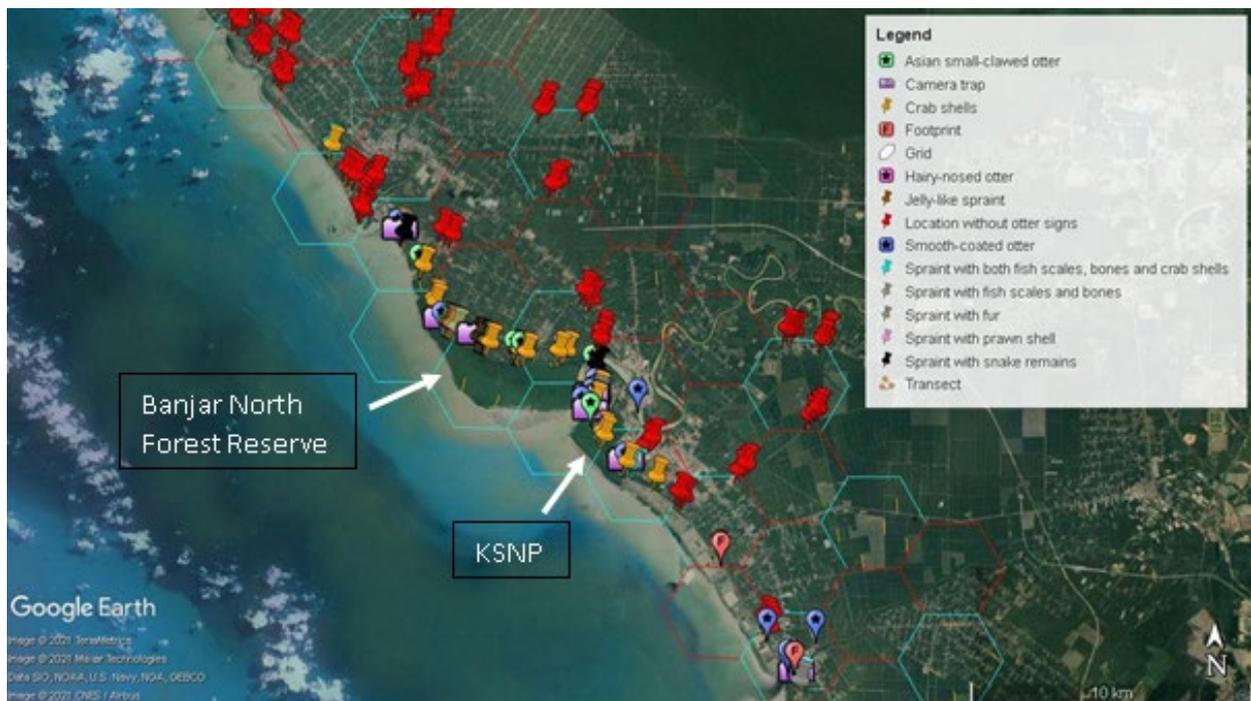
We designed a questionnaire for surveys of human-otter interactions and conflicts. Questionnaires were designed for semi-structured interviews. Sampling was purposive, targeting interviewees who were most likely to have first-hand knowledge or experience with otter conflicts or otter activity. After the completion of the questionnaire survey (currently ongoing), data will be coded and summarized to yield relative frequency statistics.

### Progress to date

Table 2 showed the up-to-date grid survey results. Currently our survey has been concentrated in the coastal and the subcoastal areas of the Kuala Selangor region. The ongoing COVID-19 pandemic, which peaked in May 2021 in Malaysia, resulted in a total lockdown in June 2021. Laboratory research is permitted, but fieldwork has paused since June. We are awaiting the lifting of restrictions. The Malaysian Government has continued to lift restrictions as the vaccination programme has gained momentum.

**Table 2.** The current results from the survey of otter signs around Kuala Selangor districts and adjacent wetlands in Sabak Bernam district.

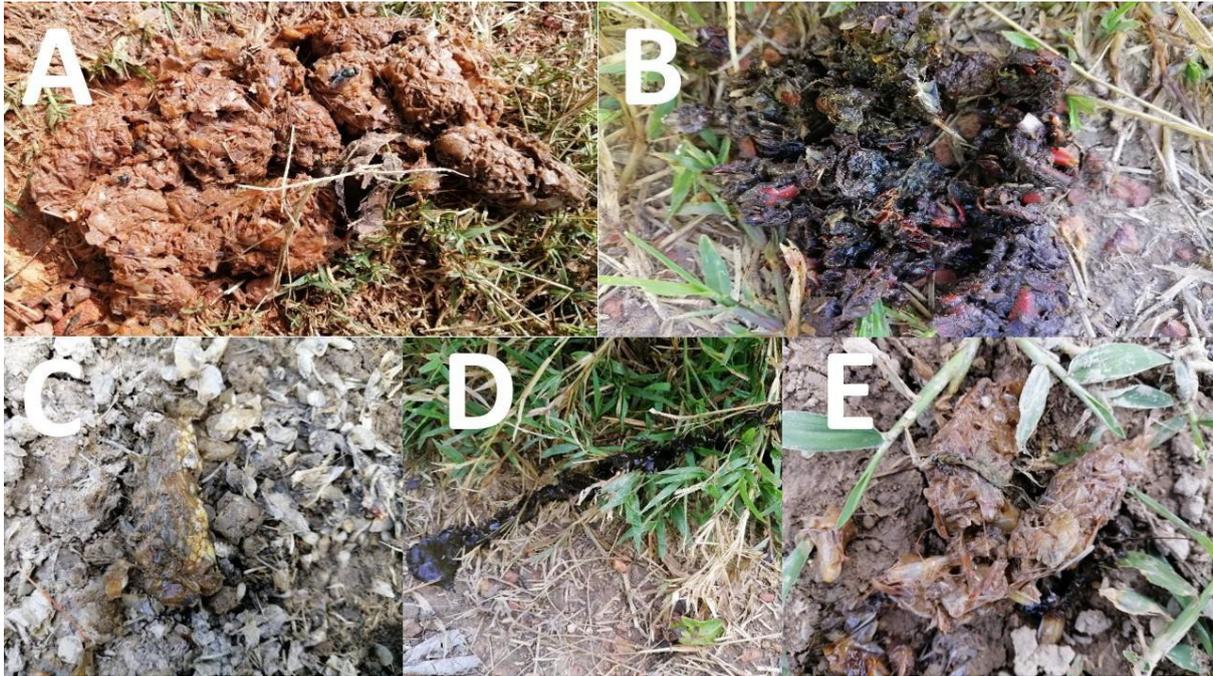
Number of grid cells surveyed so far	18
Number of grid cells with otter sign	12
Number of transects with sign	21
Number of transects without sign	47
Number of spraint samples collected	46
Number of direct observations of otters	9



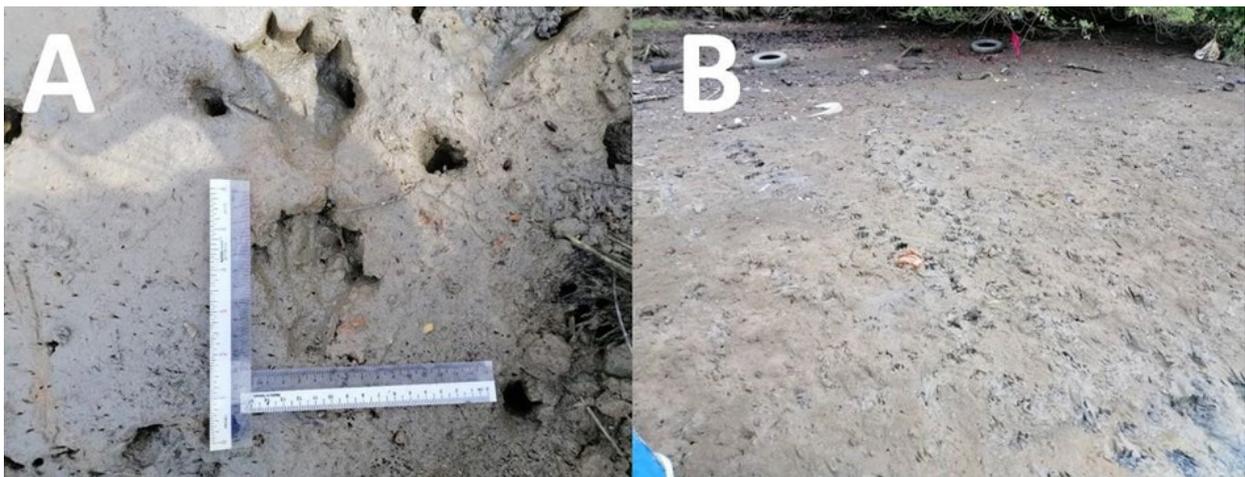
**Figure 3.** The map of otter sign's locations which comprised of latrine sites, footprints and direct observations in Kuala Selangor Nature Park and adjacent wetlands around Kuala Selangor district.

Thus far, we have encountered six types of spraint contents (Figure 4): fish scales and fish bones, crab shells, prawn shells, snake remains, unknown hair and jelly-like secretions or a mixture of the two.

The other signs that were used to document the presence of otter is their footprints. But it is difficult to distinguish the species of otter based on footprint and only able to deduce the presence of otter at that survey site. So far, we had only spotted three footprint sites. Two of the footprints were found at the boundary of aquaculture farms and another site was found during boat survey on the mud (Figure 5). The reason behind of the low detections of footprints when compared to spraint might be due to the tide that wash away the footprint on the mud periodically along the coastline.



**Figure 4.** Otter spraint containing (A) fish scales and bones; (B) crab shells; (C) snake remains; (D) jelly-like substance; (E) prawn shells. © Woo Chee Yoong.



**Figure 5.** (A) Footprint clearly showing fully webbed feet and confirming the presence of otter at the boundary of aquaculture farm in Sg Buloh. (B) Multiple footprints of otters showing evidence of a group present in Sungai Buloh Sasaran mudflat. © Woo Chee Yoong.

Direct observation is another method that we can confirm the species of the otter in the fastest manner during the survey. We had a total of nine direct observations, and all had been confirmed as the smooth-coated otter. Out of the nine encounters, five of the observations were made during boat survey (Figure 6), one in oil palm plantation (Figure 7), two in between mangrove and oil palm plantation (Figure 8), one in between mangrove and secondary forest and one in between mangrove forest and artificial tidal lagoon of KSNP. The numbers of individuals in the group ranged from one to 20.



**Figure 6.** A group of smooth-coated otters photographed on Sungai Buloh Sasaran mudflat during a boat survey in Selangor on 19<sup>th</sup> May 2021. © Woo Chee Yoong.

Thus far, 11 camera trap stations were established. Both the smooth-coated otter (Figure 9) and Asian small-clawed otter (Figure 10) were documented in these camera trap locations.

Tissue samples of smooth-coated otter and Asian small-clawed otter from the Department of Wildlife and National Parks (DWNP) Peninsular Malaysia, and spraint samples of Asian small-clawed otter (Sunway Zoo) were used as positive controls during laboratory protocol optimisation procedures. We extracted and amplified DNA from 33 field samples and PCR products were visualised in a 2% agarose gel. The amplification success for TanaD-Modified was 97% (N=33) and ND4 was 55% (N=33) (Table 3). Thus, smaller amplicons had greater amplification success.

**Table 3.** Amplification success and RFLP species ID of field samples (N=33).

	Primers	
	TanaD-Mod	ND4
<b>Total field samples</b>	33	33
<b>Amplification success</b>	32	17
<b>RFLP Species ID</b>		
1. Asian small-clawed otter	7	8
2. Smooth-coated otter	3	4

3. Hairy-nosed otter	0	1
4. Unidentified	-	4
5. In-progress	22	-



**Figure 7.** A solitary smooth-coated otter that we observed in a canal running through an oil palm plantation in Sungai Buloh on the 26<sup>th</sup> of March 2021. © Woo Chee Yoong.



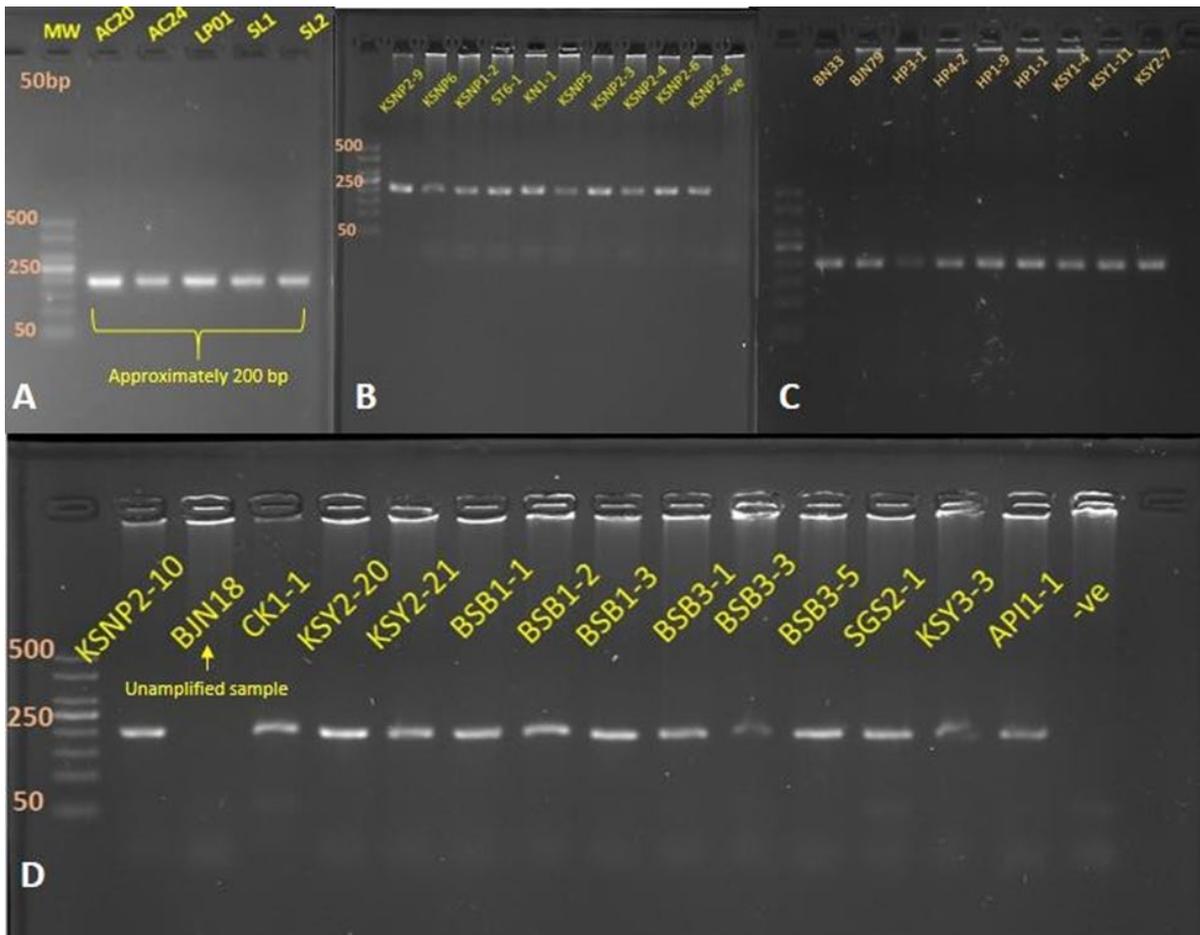
**Figure 8.** (A) A smooth-coated otter with its catch (fish) was captured via remote camera in Kuala Selangor Nature Park. (B) A pair of smooth-coated otters were captured through the camera trap in Banjar North Forest Reserve. © Malaysian Nature Society.



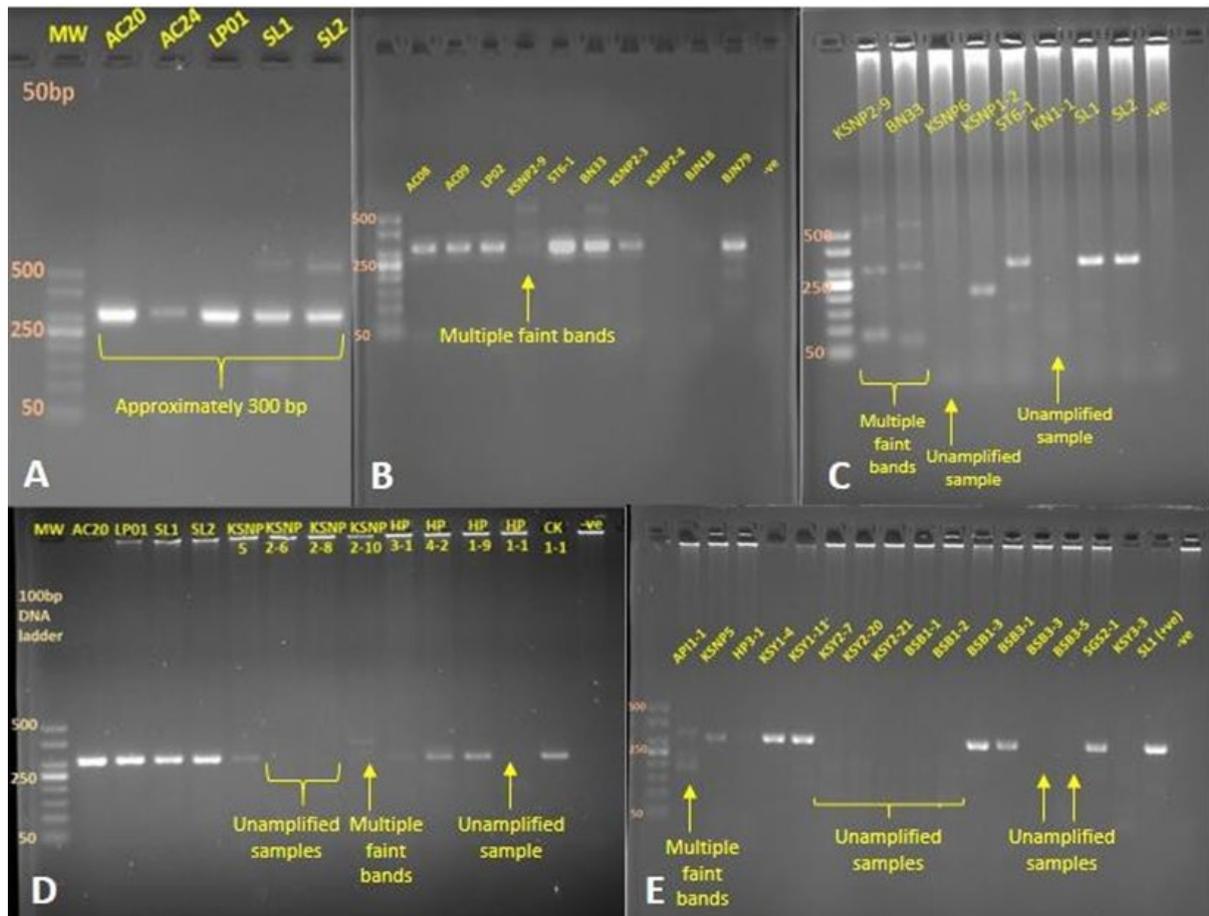
**Figure 9.** Two juvenile smooth-coated otters with fishes brought by their mother photographed in a canal running between mangrove and oil palm plantation in Bagan Sungai Burong on the 29<sup>th</sup> April 2021. © Woo Chee Yoong.



**Figure 10.** (A) A group of Asian small-clawed otter with pups were captured via remote camera in Kuala Selangor Nature Park. (B) A group of Asian small-clawed otters were captured through camera trap in Banjar North Forest Reserve. © Malaysian Nature Society.



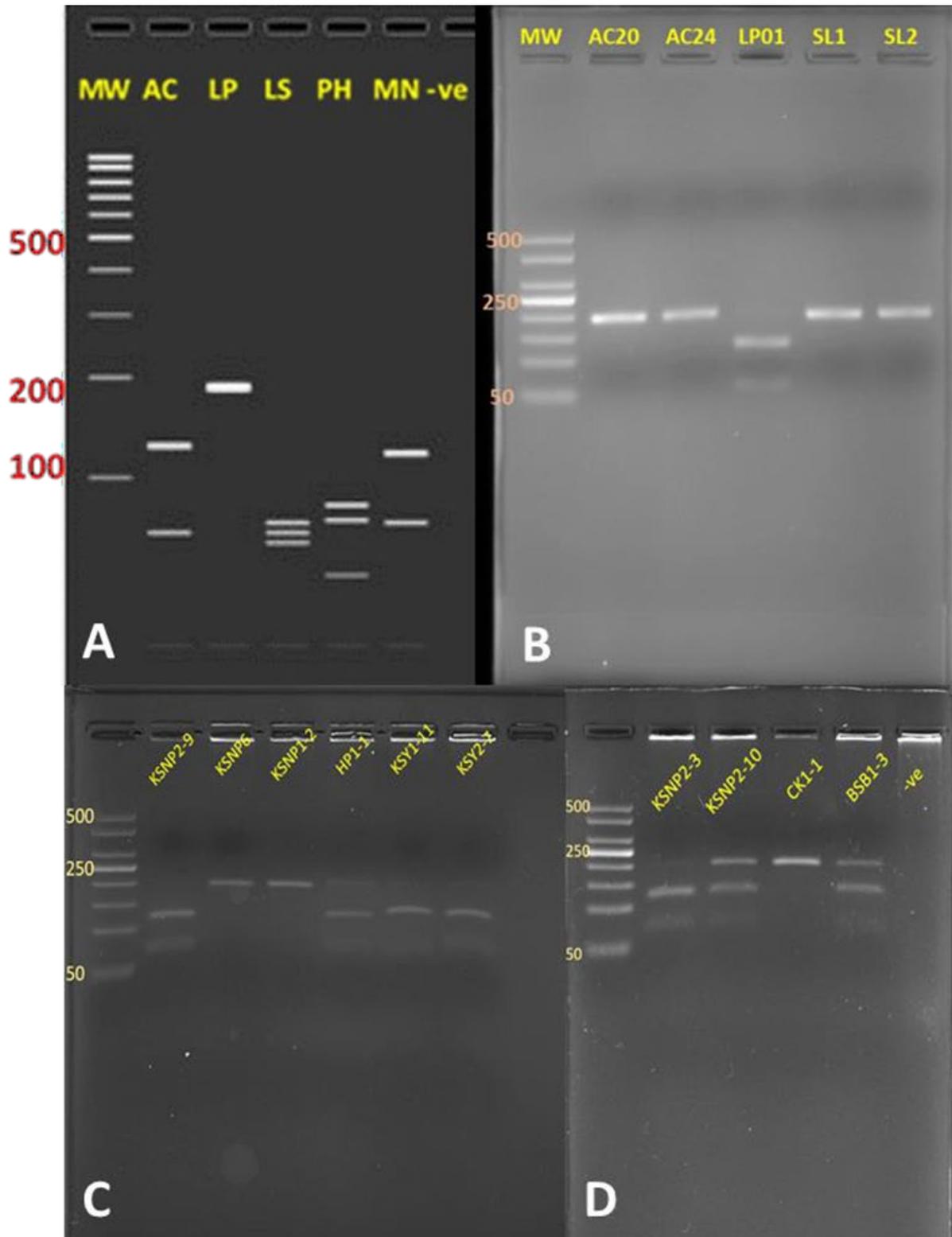
**Figure 11.** Amplification success for TanaD-Modified primer which targets a 194 bp region of the D-Loop region was 32/33 or 97% (N=33). Tissue samples (AC20, AC24 and LP01) from Department of Wildlife and National Parks Peninsular Malaysia and spraint spraints samples of Asian small-clawed otter from Sunway Zoo (SL1 and SL2) are shown in (A). Field samples are shown in (B), (C) and (D).



**Figure 12.** Amplification success with primer ND4 was 17/33 or 55% (N=33). Tissue samples (AC20, AC24 and LP01) from Department of Wildlife and National Parks Peninsular Malaysia and spraints samples of Asian small-clawed otter from Sunway Zoo (SL1 and SL2) are shown in (A). Other tissue samples (AC08, AC09 and LP02) from Department of Wildlife and National Parks Peninsular Malaysia and field samples are shown in (B), (C) and (D) and (E).

After PCR amplification, we proceeded with the restriction digest for the amplified PCR products of TanaD-Modified and ND4. Currently, the ND4 primer with the BsuRI restriction enzyme has been tested for all amplified samples (N=17) whereas the TanaD-Mod primer with the Hin1II/CviAII restriction enzyme has been tested for 10 of the 32 amplified samples.

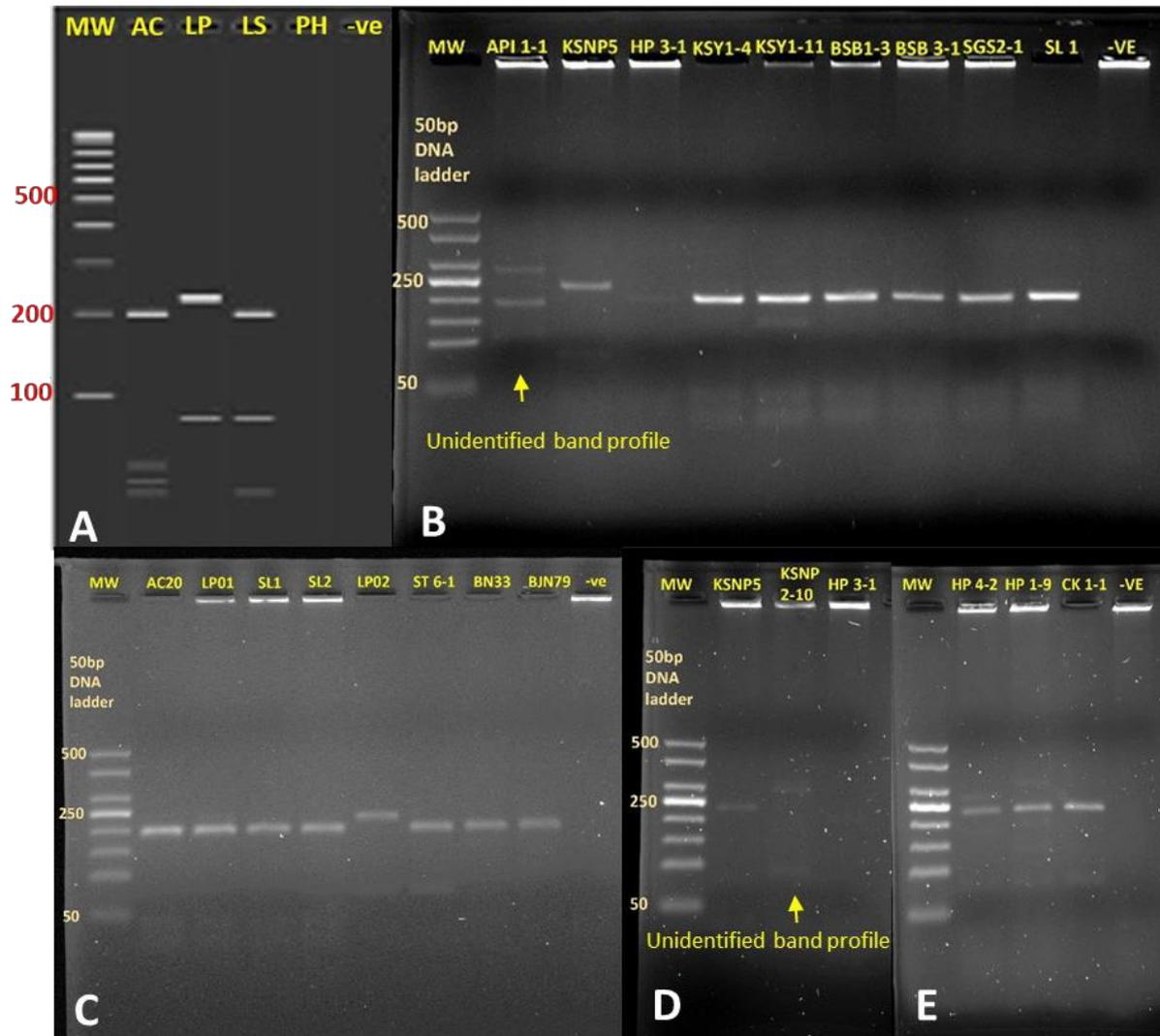
Based on our preliminary findings, both TanaD-Mod and ND4 primers together with the Hin1II/CviAII and BsuRI restriction enzymes reliably assigned species identity to all three species of otter in the study area. Figure 13 and 14 display band patterns that aligned with the expected fragments produced in silico, thus establishing the presence of the three species of otter in the study site. Combined with data on direct observations, three species of otter were identified in 9 out of 12 grids containing sign of otter.



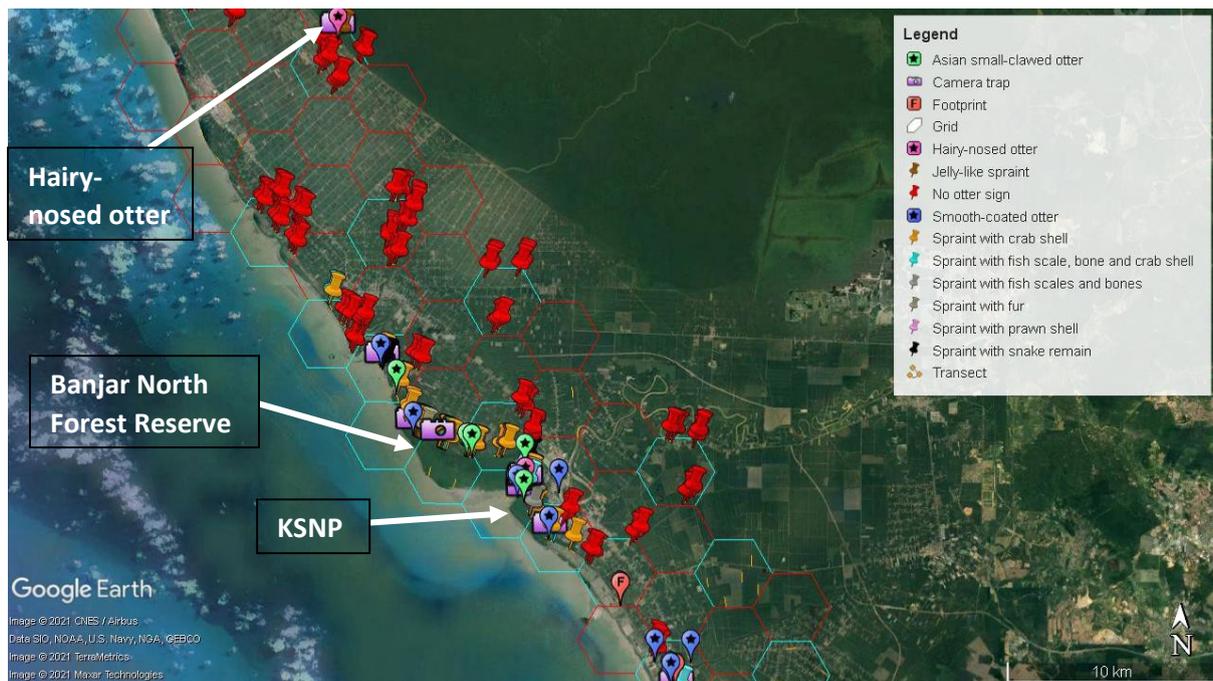
**Figure 13.** The expected fragment lengths for species identification of the three species of otter using Hin1II (or CviAll isoschizomer) produced through (A) in silico. The representatives for each acronym are stated as following: AC: *Aonyx cinereus*; LP: *Lutrogale perspicillata*; LS: *Lutra sumatrana*; PH: *Paradoxurus hermaphroditus* (common palm civet); MN: *Mustela nudipes* (Malay weasel). TanaD-Mod-amplified tissue (AC20, AC24 and LP01) and spraints samples of Asian small-clawed otter from Sunway Zoo (SL 1 and SL2) were digested with Hin1II (B) whereas field samples

(KSNP2-9, KSNP6, KSNP1-2, HP1-1, KSY1-11, KSY2-7, KSNP2-3, KSNP2-3, KSNP2-10, CK1-1 and BSB1-3) were

digested with the isoschizomer CviAll (C and D). RFLP band profiles of both Asian small-clawed otter and smooth-coated otter field samples are shown. KSNP6, KSNP1-2 and CK1-1 showed the desire bands to indicate smooth-coated otter. The remaining samples are of Asian small-clawed otter the RFLP fragments are partially digested.



**Figure 14.** The expected fragment lengths for species identification of the three species of otter amplified with the ND4 primer and digested with BsuRI are produced through in silico (A). The representatives for each acronym are stated as following: AC: *Aonyx cinereus*; LP: *Lutrogale perspicillata*; LS: *Lutra sumatrana*; PH: *Paradoxurus hermaphroditus* (common palm civet). Field samples, API 1-1, KSNP 5, HP 3-1, KSY 1-4, KSY 1-11, BSB 1-3, BSB 3-1 and SGS 2-1) are shown (B); ST6-1, BN33 and BJJN79 are shown in (C); KSNP5, KSNP2-10 and HP3-1 are shown in (D); and HP4-2, HP1-9 and CK1-1 are shown (E). Tissue (AC20 and LP01) and spraints samples of Asian small-clawed otter from Sunway Zoo (SL 1 and SL2) are shown in (B) and (C). The DNA fragment sizes and ID of KSNP5 LP02, HP 4-2, HP 1-9 and CK 1-1 are of smooth-coated otter, ST 6-1 is of hairy-nosed otter and the remaining samples are of Asian small-clawed otter except API 1-1 and KSNP 2-10, which are unidentifiable.



**Figure 15.** Study area displaying the locations of surveyed grid cells (blue outlines), and otter sign. Sign of hairy-nosed otter (star icon in pink colour) located at the boundary of the North Selangor Peat Swamp Forest complex was confirmed through our PCR-RFLP method. Starred icons in blue and green represent positive IDs of smooth-coated otter and Asian small-clawed otter, respectively, from spraint analysis and direct observation.

## Conclusion

We have developed an effective PCR-RFLP method to successfully identify three sympatric species of otter using spraints collected in the field and mapped otter occurrence and potential hotspots along the coastline and subcoastal areas of Kuala Selangor district. Preliminary data on human-otter conflict has been collected. We will continue to survey the grids in Klang and Sabak Bernam districts, collect samples for species identification through PCR-RFLP and determine the spatial distribution and habitat relationships of these three species to identify critical habitats and patterns of spatial partitioning and overlap. Human-otter conflict in the landscape will be documented simultaneously for future conservation management.

## Publication

We have submitted an article to the Malaysian Naturalist (MN), a local popular magazine that Malaysian Nature Society (MNS) produced quarterly to showcase MNS's efforts and conservation issues in and around Malaysia. The article will be posted in the September 2021 issue.



Wed 11/8/2021 4:54 PM

Hui-Min Khor <khor.hm@gmail.com>

Re: MN Sep issue - otter article

To Woo Chee Yoong

Cc Sonny Wong

 You replied to this message on 11/8/2021 5:14 PM.

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Dear Woo,

Thank you for sending in the otter article. I've downloaded the photos from WeTransfer.

I will include it in the next issue of the Naturalist.

Thank you again.

Best regards,

Hui Min

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**Figure 16.** Proof of acceptance on the submission of an article to MN sent by the editor through email.

At the same time, we are currently preparing a manuscript and aiming to publish in the Global Ecology and Conservation (GECCO).

### Public engagement

During the granted period, I had delivered two public talks on the project.

On the 16<sup>th</sup> October 2020, I was invited by the Have Hope to deliver a talk with the title of "Otters – Malaysia's Unsung Mammal". The link to the talk's recording is attached as the link below: <https://fb.watch/6VaYiaJ5tr/>

On the 26<sup>th</sup> May 2021, I was invited by the International Otter Survival Fund to deliver a talk with the title of "Research on the Kuala Selangor Otters (including the rare Hairy-nosed otter) and the work of the Malaysian Otter Network" in conjunction of World Otter Day 2021. The link to the talk's recording is attached as the link below: [https://www.youtube.com/watch?v=Y6wWGgZz\\_Tc&t=177s](https://www.youtube.com/watch?v=Y6wWGgZz_Tc&t=177s)

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