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Detection of Rabies Virus in a Yellow-throated Marten (*Martes flavigula chrysospila*) in Taiwan

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ABSTRACT: In June 2021, a yellow-throated marten (*Martes flavigula chrysospila*) submitted for postmortem examination was diagnosed as rabid through laboratory testing. The rabies virus detected was closest phylogenetically to viruses of ferret badgers (*Melogale moschata subaurantiaca*) in Taiwan, indicating spillover infection from the primary reservoir in this area, the ferret badger.

Rabies is an ancient and important zoonotic disease, threatening human and animal health, with tens of thousands of human deaths annually. All mammals are susceptible, with bats and carnivores as the primary reservoirs (Singh et al. 2017). In Taiwan, rabies was reported in wildlife during 2013. The ferret badger (*Melogale moschata subaurantiaca*) is considered the primary reservoir in Taiwan (Chiou et al. 2014; Tsai et al. 2016). By December 2022, rabies virus (RABV) had been detected in 889 ferret badgers, nine gem-faced civets (*Paguma larvata taivana*), one puppy (*Canis lupus familiaris*), and one house shrew (*Suncus murinus*); Bureau of Animal and Plant Health and Inspection Quarantine 2022). Most cases were found at altitudes ranging between 200 and to or - 600 m on Taiwan Island (Shih et al. 2018). Here we report a rabies case in a yellow-throated marten (*Martes flavigula chrysospila*), listed as a Level III conservation-deserving species (Ministry of Agriculture 2019), in Taiwan.

The yellow-throated marten, a subspecies of *Martes flavigula*, is a top predator and diurnal carnivore, mainly distributed in forests at altitudes of 300-3,900 m. It has a body weight of approximately 1–3 kg, body length of 45 cm, and tail length of 35 cm. A distinguishing characteristic is a large yellow spot on the throat. The yellow-throated marten usually hunts in groups of two or more. Prey includes small and medium mammals, birds, insects, and occasionally fruits (Taiwan Encyclopedia of Life 2013). Reports of rabies in this species are lacking.

On 10 June, 2021, an adult male yellow-throated marten was found in Taitung County (23°10′06.43867″ N, 121°08′33.04147″ E) by the Taitung Forest District Office, Forestry Bureau, Council of Agriculture, on a trail showing paralysis and lethargy; no aggressive behavior was observed. The animal was sent to the wildlife rescue center of the WildOne Wildlife Conservation Association for medical care. No traumatic injuries were observed. The combination of neurologic signs of ataxia, weakness, depression, and loss of concentration, with hyperphosphatemia detected, was diagnosed preliminarily as pesticide poisoning. The animal died on 11 June after intensive treatment. The carcass was frozen and submitted to the Animal Health Research Institute (AHRI) for postmortem examination and pesticide detection on 28 June, 2021.

The marten weighed 1.9 kg and showed no observable gross postmortem lesions. Because the AHRI is also responsible for nationwide rabies surveillance, the marten's brain tissue was tested for rabies virus (RABV) by the direct fluorescent antibody test and reverse transcription PCR (RT-PCR; Hsu et al. 2019). Both tests were positive (Supplementary Material Figs. S1, S2). Additionally, RABV RNA was detected in an oral swab and salivary glands. Viral loads were estimated by quantitative real-time RT-PCR (Hsu et al. 2018); the viral load in oral swab and salivary glands was 3.11×10^5 and 6.85×10^6 copy number/reaction, respectively.

For the whole genome amplification, 11 RT-PCR primer sets were designed based on the RABV sequences identified in ferret badgers

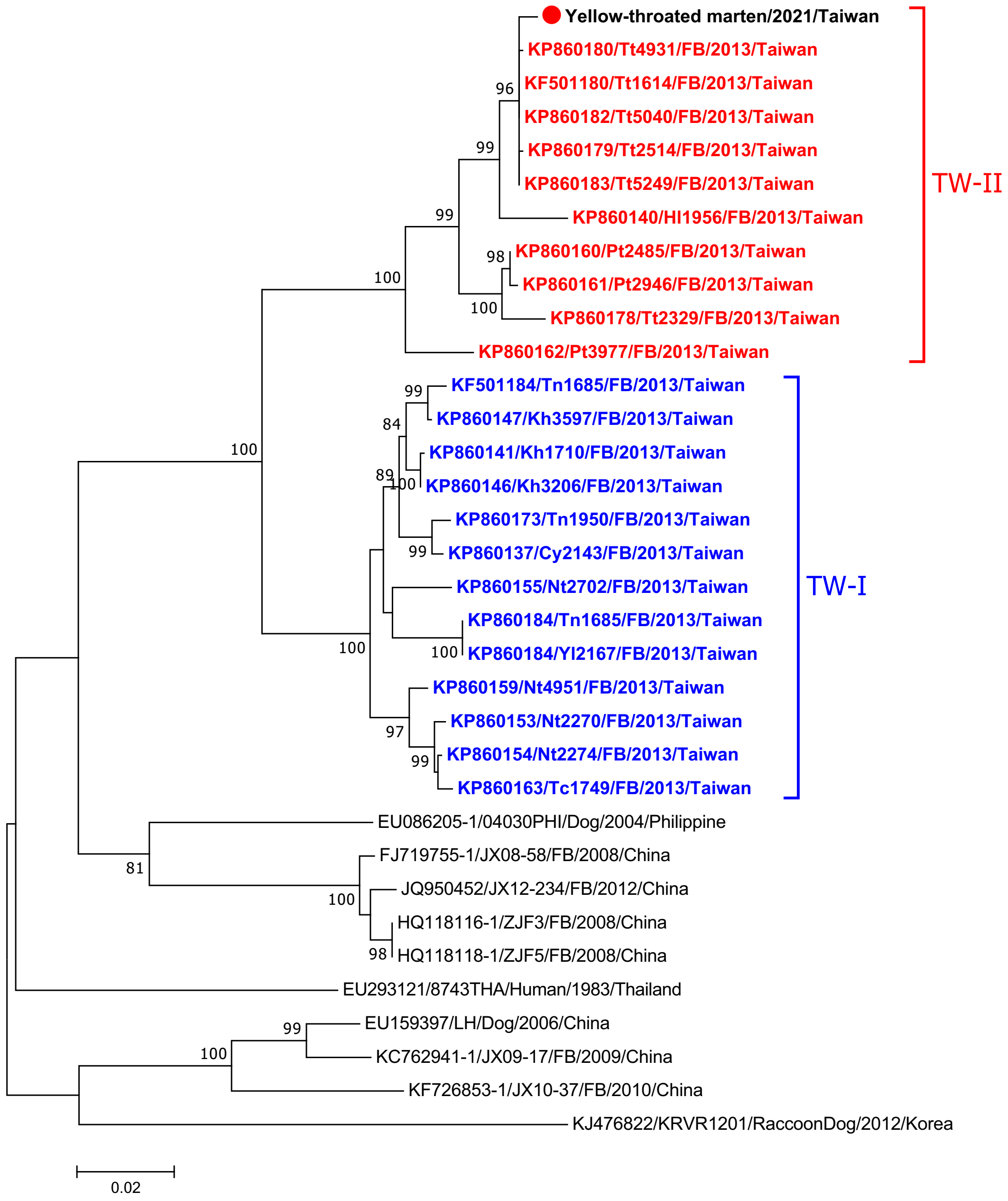

FIGURE 1. Phylogenetic analysis of rabies viruses in Taiwan and neighboring countries. The evolutionary tree was inferred on the basis of the nucleoprotein gene by the maximum likelihood method. The percentage of replicate trees in which the associated viruses clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. The rabies viruses from ferret badgers (*Melogale moschata subaurantiaca*) in Taiwan, presented as red and blue characters, were divided into two groups: the TW-I and TW-II lineages. The tree shows that the virus from the yellow-throated marten (*Martes flavigula chrysospila*) detected in Taiwan in 2021 presented as a closed circle clustered with the viruses of the TW-II lineage.

(Chiou et al. 2014). The RT-PCR was performed as described (Hu et al. 2018). The complete genome sequence of the RABV identified in the marten was uploaded to the GenBank database (accession no. OQ473645). The nucleotide sequence of the nucleoprotein gene was compared with representative RABVs from Taiwan and neighboring countries. Nucleotide identities between the virus from the yellow-throated marten and all represented RABVs were calculated by BioEdit software (version 7.0.5.3; Hall 1999). Phylogenetic analyses of the nucleoprotein genes were conducted using the maximum likelihood method with the General Time Reversible+I+γ_4 model in the software Molecular Evolutionary Genetics Analysis (version 7; Kumar et al. 2016). A previous study had indicated that RABVs in Taiwan were divided into two groups, the TW-I and TW-II lineages, probably because of segregation imposed by geographic barriers (Tsai et al. 2016). Our nucleoprotein gene–based phylogenetic analysis showed that the virus detected in the yellow-throated marten clustered with those viruses of the TW-II lineage (Fig. 1). The nucleotide identities of the viral nucleoprotein gene between the RABV of the yellow-throated marten and those of ferret badgers in the same county (Taitung County) ranged from 99.4% to 99.6% (Fig. 2).

On the basis of these results, we believe that, rather than forming an independent branch, this case was a spill-over of rabies. The most likely source was a rabid ferret badger. The clinical signs of lethargy, weakness, depression, and paralysis, without aggressive behavior, observed in this case are common in the terminal stage of rabies (Hanlon 2013). The detection of viral RNA in the oral swab and salivary glands indicated the virus might be shed by the marten. People who engage in wildlife research or rescue should be cautious and wear appropriate personal protective equipment when handling animals; rabies pre-exposure vaccination is also recommended for high-risk groups. Members of the general public should follow the government's instructions in Taiwan to vaccinate pets, not abandon their pets, and not to contact wild animals to minimize the exposure risk in human and animals.

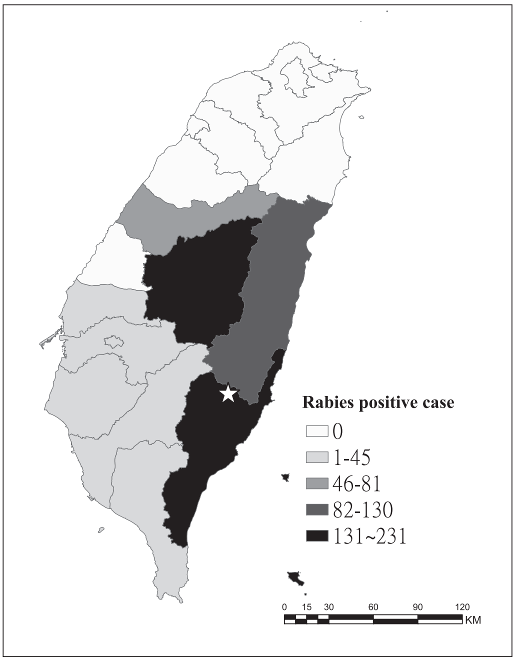

FIGURE 2. Distribution of rabies virus–affected counties and cities in Taiwan. Shaded areas include the counties and cities where rabid ferret badgers (*Melogale moschata subaurantiaca*) were detected during 2013–2020. The location of the rabid yellow-throated marten (*Martes flavigula chrysospila*) detected in 2021 is indicated with a white star.

The AHRI had not previously received yellow-throated marten samples for rabies surveillance. The sampling bias might be due to the small population size of yellow-throated martens in Taiwan, difficulty in detecting unwell or dead animals, and difficulties in shipping samples from mountainous areas. Incidental cases of RABV spillover infection in non–host species have been detected elsewhere, such as in a sand cat (*Felis margarita*) in Saudi Arabia (Fagbo et al. 2021). The discovery of such incidental host highlights the incompleteness of a passive surveillance system and limitations for detection of virus spread in Taiwan. Enhancement of rabies surveys in mountainous areas and education of the general public are urgently needed.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/JWD-D-23-00039.

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