Short Communication

Morphological and scanning electron microscopy characterization of a spirurid in a wild Sunda pangolin (*Manis javanica*)

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Abstract

This study identifies and characterizes a spirurid in a wild Sunda pangolin (*Manis javanica*) using scanning electron microscopy (SEM). Native to Southeast Asia, the Sunda pangolin faces critical threats from poaching, illegal trafficking for its scales and meat, habitat loss, urban conflicts, and disease. Despite this, little is known about the biology of wild Sunda pangolins. In this research, spirurid parasites were recovered from a pangolin fatally injured in a vehicular collision. The parasites were preserved and analyzed with SEM, revealing key morphological features such as papillae, lip-like structures, and an anal opening in females. This study enhances our understanding of pangolin parasitology, stressing the value of detailed morphological methods for accurate parasite identification and assessing the clinical relevance of these findings. It underscores the need for continued pathogen monitoring in pangolins and stronger conservation measures to protect the species from parasitic infections and other threats.

Keywords: General morphology, scanning electron microscopy, spirurid, Sunda pangolin

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Introduction

The Sunda pangolin (*Manis javanica*) is one of eight species in the genus *Manis* and one of four species found in Asia. This critically endangered scaly anteater inhabits a wide range of landscapes across Southeast Asia, including Thailand, Laos, Vietnam, Cambodia, Peninsular Malaysia, Singapore, Sumatra, Java, and nearby islands, as well as Borneo (Challender *et al*., 2019). Unfortunately, Southeast Asia is a hub for illegal pangolin trafficking, driven by the high demand for their scales and meat, resulting in drastic population declines. As such, conservation initiatives are crucial to safeguarding this unique and vulnerable species (Zhang *et al*., 2023). These nocturnal animals are easily recognized by their sturdy bodies, covered in keratin scales that act as protective armor against predators (Liu *et al*., 2016). Pangolins primarily feed on ants, termites, and other small invertebrates (Wu *et al*., 2005). In Southeast Asia, the Sunda pangolin faces significant threats from illegal poaching and trafficking due to the demand for their scales and meat, particularly in East Asia, where they are mistakenly believed to have medicinal properties (Zhou *et al*., 2014). This exploitation has led to a sharp population decline, resulting in their classification as Endangered on the IUCN Red List (Cen *et al*., 2023). As pangolins forage by digging through soil and natural substrates, they may become more susceptible to gastrointestinal parasites. The foraging behavior of pangolins caused an infection of *Gendrespirura* in their stomachs (Gardiner and Werner, 1984). However, identifying and differentiating nematodes based on the external characteristics of eggs and adult specimens from the pangolin's digestive system remains difficult, leaving much to be discovered.

Scanning electron microscopy (SEM) is an invaluable tool for examining the external morphology of parasites, offering detailed insights at the microscale. SEM employs focused are not observable with light microscopy (Fischer *et al*., 2024). This method is especially useful for identifying specific morphological traits that distinguish closely related species (Boyde, 2012). When combined with molecular analysis, such as DNA or RNA sequencing, SEM provides a comprehensive approach to species identification. By comparing genetic sequences with known databases or reference sequences of related species, researchers can accurately identify the species of the nematode specimen (Li *et al*., 2024). This integrated approach complements traditional morphological techniques, offering a more complete understanding of nematode diversity and taxonomy. In this study, a spirurid parasite recovered from a deceased wild Sunda pangolin was identified using SEM.

Materials and Methods

Parasite recovery: A wild Sunda pangolin was found deceased near the southern boundary of the Central Catchment Reserve in Singapore and was sent to the Singapore Zoo, Mandai Wildlife Reserve, for post-mortem examination. The gross examination revealed that the specimen was an adult male (~7.35 kg) and had died from severe traumatic injuries, likely caused by a vehicular collision. A large number of adult nematodes were observed in the stomach lumen and gastric contents. Both live and dead nematode specimens were collected in saline and 10% buffered formalin saline (BFS) for further analysis. The specimens in saline were later transferred to chilled 2.5% glutaraldehyde for SEM analysis.

Scanning electron microscopy: Adult nematode specimens preserved in glutaraldehyde were submitted to the Scientific and Technological Research Equipment Centre of Chulalongkorn University (STREC) for SEM analysis and morphological examination. The specimens were fixed in glutaraldehyde, incubated for several hours at room temperature or overnight at 4°C, then rinsed with a buffer solution to eliminate excess fixative. Dehydration followed, using a series of ethanol solutions with concentrations progressively increasing from 30% to 100%, each stage lasting 10-15 minutes. To avoid surface tension artifacts during drying, Critical Point Drying (CPD) was applied. Ethanol was substituted with liquid CO_2, which was subsequently converted to gas without transitioning through a liquid phase. The dehydrated samples were mounted on stubs using conductive adhesive or carbon tape for optimal viewing alignment. To prevent charging under the electron beam and to enhance imaging quality, a thin conductive layer, such as gold or platinum, was sputter-coated onto the specimens. The prepared samples were placed into the SEM chamber, where parameters such as accelerating voltage, magnification, and resolution were adjusted before imaging. The electron beam scanned the samples, capturing high-resolution images of the nematodes at various magnifications to examine their surface structures, morphology, and specific features of interest.

Results and Discussion

The microscopic examination of the parasite revealed that the head of the female specimen has a mouth with a chitinized vestibule. In the tail region, an anus was observed. The body length of the female worm measures 1.7 cm. Additionally, the parasite's eggs were visible, with some in immature stages and others fully developed (Fig. 1). Scanning Electron Microscopy (SEM) analysis revealed that the nematode has a stout, medium-sized body with a faint transverse striped pattern running along its entire length (Fig. 2a, b). The small cephalic region is distinguished by four distinct papillae (Fig. 2c). The mouth area features upper and lower lip-like structures, with larger paired teeth inside that are more prominent than those along the sides (Fig. 2c). In the tail region, an anal opening is present, a characteristic feature of the female nematode (Fig. 2d).

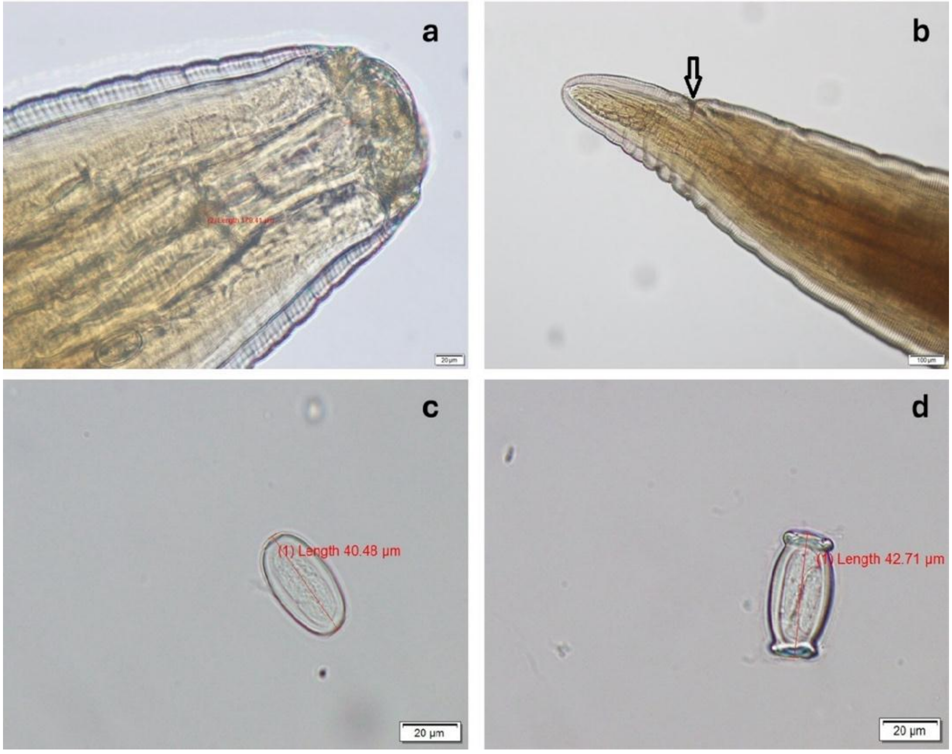

Figure 1 Physical characteristics of female specimens. Anterior end displaying a chitinized vestibule (a). The posterior end showing the anus (arrow) and a striated cuticle (b). Immature egg extracted from the uterus (c). Mature egg with a characteristic barrel shape, also extracted from the uterus (d).

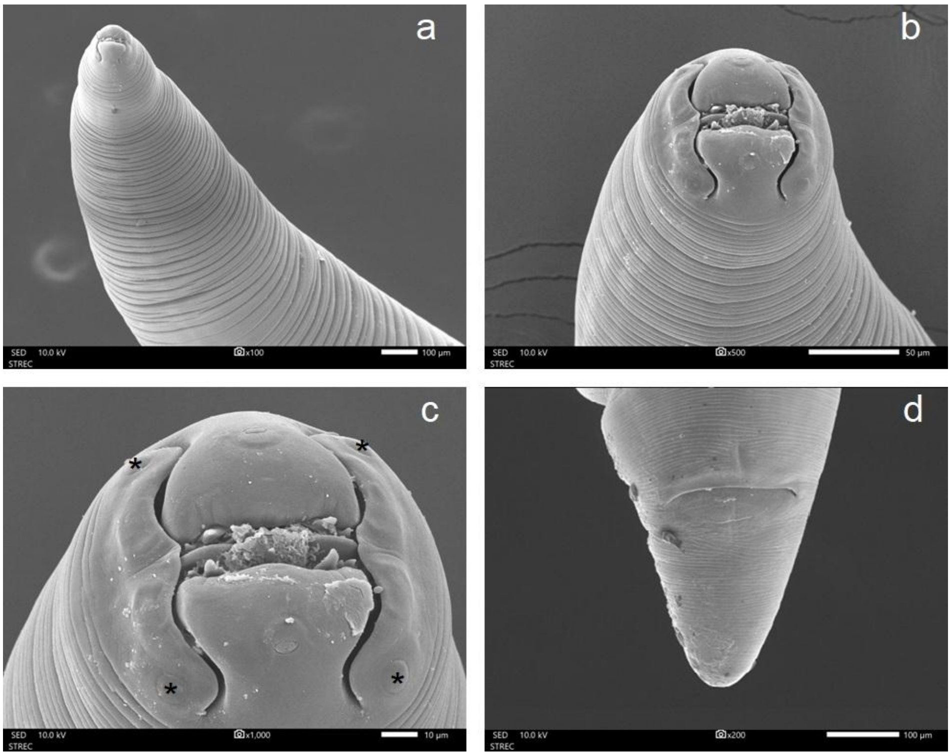

Figure 2 Scanning electron microscopic images of a Spirurid retrieved from a dead wild Sunda pangolin (*Manis javanica*). Body cuticle showing annulations and having a small head (a). Cephalic extremities have upper and lower lips (b). On the head, 4 round distinct papillae (*) are located (c). Inside the mouth, teeth of the parasite are found (c) and the inner paired teeth are larger than the outer pairs. Posterior extremities of the female ending showing the presence of an anal opening (d).

This is the first report and identification of a Spirurid in a wild Sunda pangolin (*Manis javanica*) by SEM. The detailed morphological and molecular description provides significant insights into the parasitic fauna of Sunda pangolins. *Spiruromorpha* was recently identified in the Chinese pangolin (*Manis pentadactyla*) by molecular methods, but the clinical significance of this detection is unknown (Li *et al*., 2024). Other nematodes previously reported in the Sunda pangolin include *Brugia malayi*, *B. pahangi*, *Necator americanus,* and *Gendrespirura* sp. (Mohapatra *et al*., 2016; Wicker *et al*., 2020; Barton *et al*., 2022) but their clinical and pathological significance are unclear.

Measurements from SEM showed that the parasite's head was 95.87 µm vertically and 99.78 µm horizontally. The upper and lower lip width measured 77.52 µm, while the round node ranged between 10.11 and 11.52 µm. The widest part of the body measured 635 µm, with an overall body length of 17,000 µm. Additionally, the anal opening was 179.4 µm wide and located 300.05 µm from the tip of the tail.

The use of SEM in this study provided a detailed morphological description of the Spirurid, including the presence of distinct papillae on the cephalic region, paired teeth inside the mouth, and an anal opening characteristic of the female parasite. These morphological features are consistent with the descriptions of spirurid nematodes found in previous studies, such as the work by Li *et al*. (2024), who reported similar features in *Spiruromorpha sp*. identified in the Chinese Pangolin. However, SEM analysis is limited by the requirement for expensive equipment and technical expertise. This research represents a significant advancement in the understanding of pangolin parasitology and increases the knowledge of parasitic diseases in wild Sunda pangolins and their effects on health. Future studies should continue to employ a multi-disciplinary approach to explore the genetic diversity and phylogenetic relationships of nematodes in pangolins and other wildlife species.

In conclusion, the SEM in this study has provided a detailed and accurate identification of a spirurid parasite in a wild Sunda pangolin. Where possible, this approach should be adopted in future parasitological studies to enhance the understanding of parasite biodiversity and inform conservation strategies for endangered species like the Sunda pangolin.

References

Barton DP, Martelli P, Worthington BM, Lam TT-Y, Zhu X and Shamsi S 2022. Nematode and acanthocephalan parasites of confiscated Sunda pangolins, *Manis javanica Desmarest*, 1822 (Mammalia: Pholidota: Manidae) with an updated list of the parasites of Pangolins. Diversity. 14: 1039.

Boyde A 2012. Scanning electron microscopy. Physics Procedia. 9: 307-312.

Cen X, Zhou Z and Lin Y 2023. Conservation status of pangolins in Southeast Asia. Conserv Biol. 37: 567-575.

Challender D, Willcox DHA, Panjang E, Lim N, Nash H, Heinrich S and Chong J 2019. *Manis javanica*. The IUCN Red List of Threatened Species. e.T12763A123584856. Available from: https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T12763A123584856.en.

Fischer ER, Hansen BT, Nair V, Hoyt FH, and Dorward DW 2024. Scanning electron microscopy. Curr Protoc Microbiol. 4, e1034. doi: 10.1002/cpz1.1034.

Gardiner CH and Werner RM 1984. Polypoid gastritis in a scaly anteater caused by larvae of Gendrespirura sp. J Wildl Dis 20(1):68–70. doi:10.7589/0090-3558-20.1.68.

Li H, Chen W, Qi W, Ren Z, Pan X, Shen F, Lu J, Zhai J, Wu Y, Zou J, Xiao L, Feng Y and Yuan D 2024. Molecular characterization of a novel *Spiruromorpha* species in wild Chinese pangolin by mitogenome sequence analysis. Parasitol Res. 123: 137.

Liu ZQ, Jiao D, Weng ZY and Zhang ZF 2016. Structure and mechanical behaviors of armored pangolin scales and the effects of hydration and orientation. J Mech Behav Biomed Mater. 56: 165-174.

Mohapatra RK, Panda S, Nair MV and Acharjyo LN 2016. Check list of parasites and bacteria recorded from pangolins (*Manis sp.)*. J Parasit Dis. 40(4):1109-1115.

Wicker LV, Lourens K and Hai LK. 2020. Veterinary health of pangolins. In: Challender DWS, Nash HC, Waterman C, editors. Biodiversity of World: Conservation from Genes to Landscapes, Pangolins. Academic Press; p. 461-493.

Wu S, Liu N, Li Y and Sun R 2005. Observation on food habits and foraging behavior of Chinese pangolin (Manis pentadactyla). Chin J Appl Environ Biol. 11(3): 337-341.

Yodsheewan R, Sukmak M, Sangkharak B, Kaolim N, Ploypan R and Phongphaew W 2021. First report on detection of Babesia spp. in confiscated Sunda pangolins (Manis javanica) in Thailand. Vet World. 14(9): 2380-2385.

Zhang F, Xi F, Tang X, Cen P and Wu S 2023. The illegal trade network of pangolin meat in Chinese mainland and its implications for the implementation of key interventions. Biodivers Sci. 31(10): 23079.

Zhou Z, Lin Y and Kawashima T 2014. Illegal pangolin trade: a brief overview. Biodivers Conserv. 23: 2877-2891.