Morphological and Phylogenetic Analysis of *Bothridium pithonis* (Cestoda: Diphyllobothriidea) in Python Snake (*Malayopython reticulatus*) in Thailand

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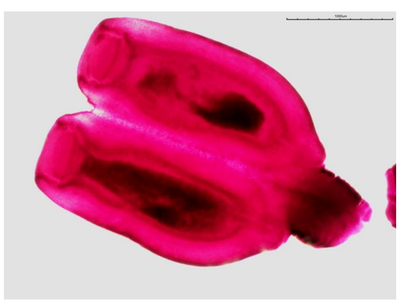
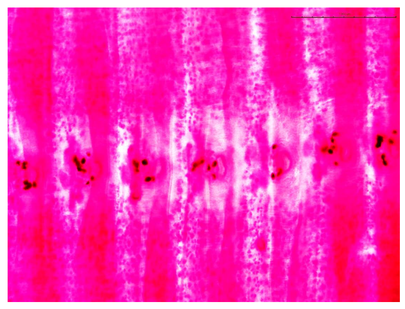
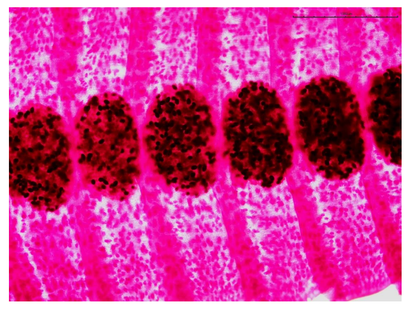
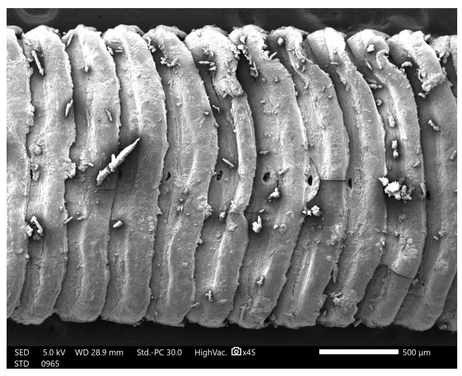
Permanent slide



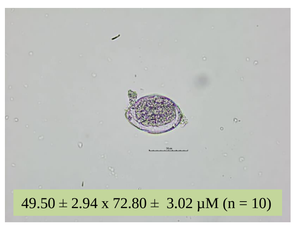




Scanning electron microscope



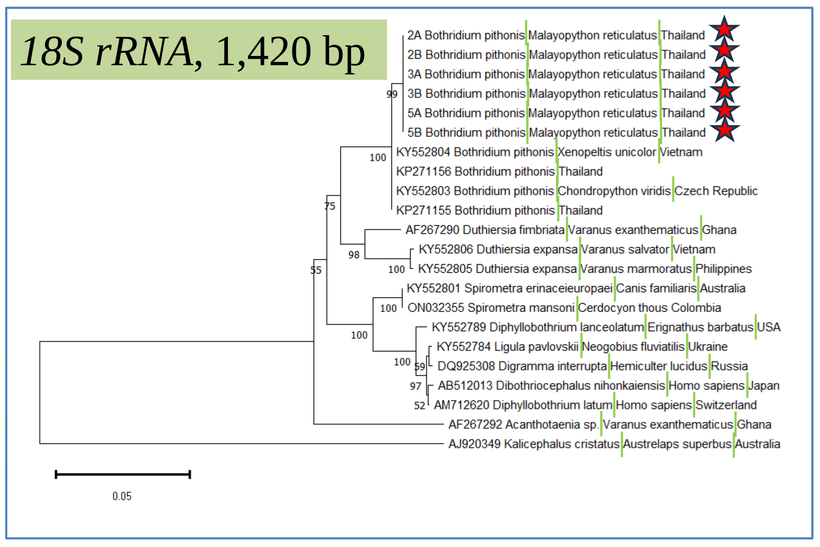
Egg morphology



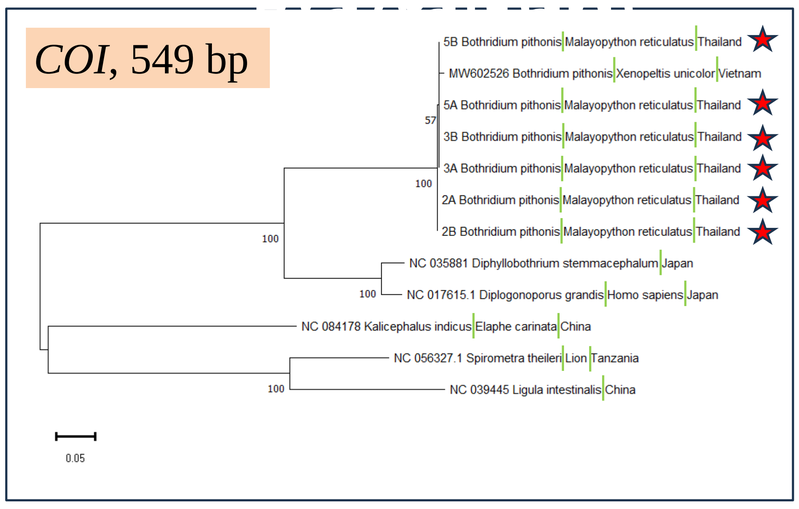
DNA extraction, PCR, sequencing, and phylogenetic analysis

Primer name	Target (gene)	Primer sequence (5'→3')	Product size (bp)	Polymorphic sites	Evolution divergence (%)
Bothri_18S-F Bothri_18S-R	*18S rRNA*	ACGGGTCCTTCAAATGTCTG GTACAAAGGGCAGGGACGTA	1,690	0	0.00
Bothri_COI-F Bothri_COI-R	*COI*	GTTCTGTAACTATGATTATAGGCGTTC GACTATGGTAAGGCAAGGGAAG	600	10	0.00-0.01
Bothri_16S-F Bothri_16S-R	*16S rRNA*	GAGGAAGTTGTGTGACGGGT ACATCGAGGTGGCAAACAGT	585	10	0.00-0.02
Bothri_12S-F Bothri_12S-R	*12S rRNA*	TGAAAGGGGATAAGGCACAG ACGCCAACACCCTGAATAAG	465	1	0.00-0.01

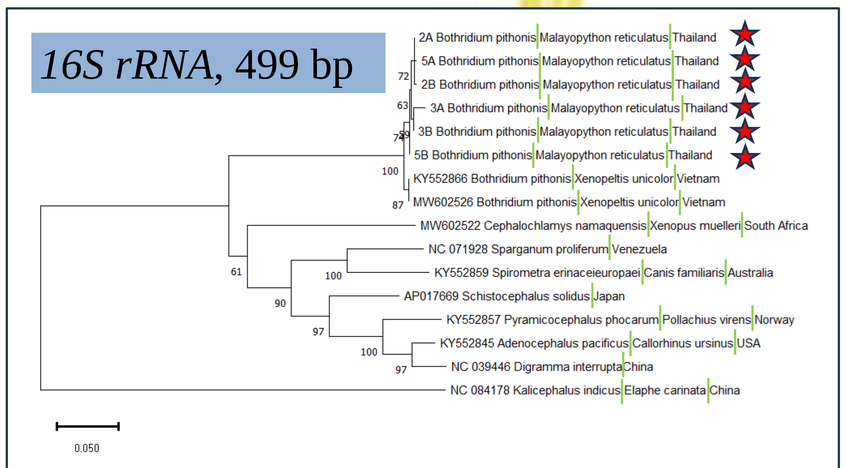
18S rRNA, 1,420 bp



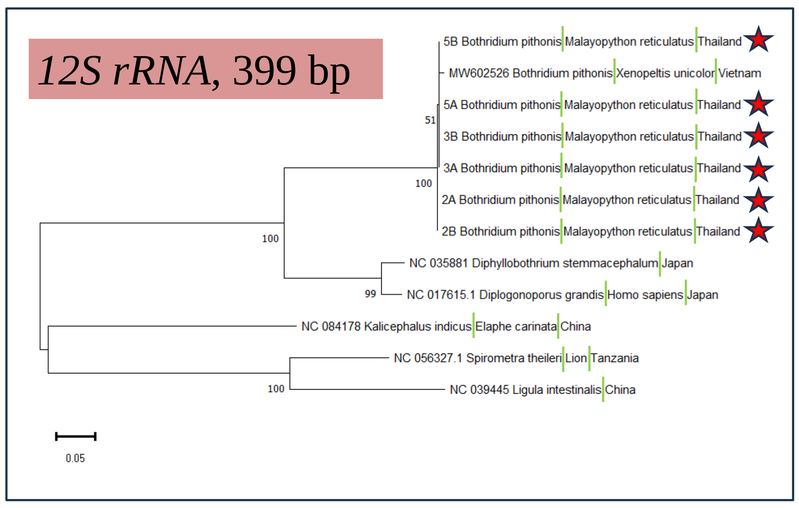
COI, 549 bp



16S rRNA, 499 bp



12S rRNA, 399 bp



The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown below the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site.

Conclusion

This study supports the use of the *18S rRNA*, *COI*, *16S rRNA*, and *12S rRNA* genes as a genetic marker for studying the molecular characteristics of *Bothridium pithonis* in python, to understand intra- and inter-species genetic distances. Moreover, both mitochondrial genes (*COI* and *12S rRNA*) are suitable for future genetic and biodiversity studies of this tapeworm.

Acknowledgements

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