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RESEARCH ARTICLE

Morphological, Molecular, Biochemical and Nutritional Characterization Of Three Major *Thais* Species From The Sindh Coast of Pakistan

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SUPPLEMENTARY FIGURES AND TABLES

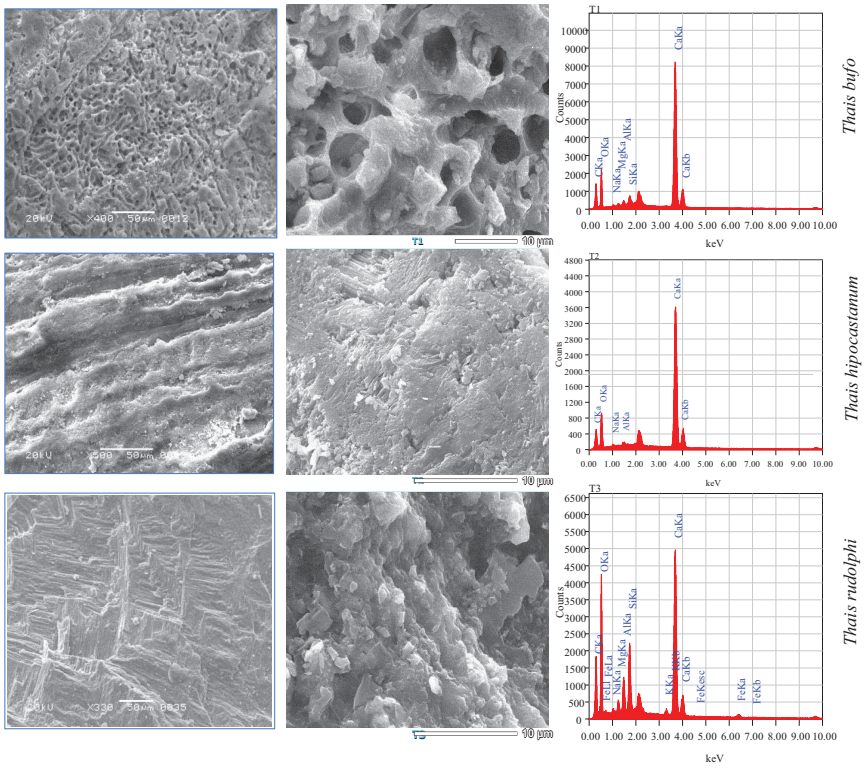

Fig. (S1). Scanning electron microscopy (SEM) energy dispersive X-ray spectrometry (EDX) spectra obtained from the shell of three *Thais sp.* showing calcium as the main inorganic component, while to a lesser concentration but differential Na, Mg, Al and Si peaks. See "Materials and Methods" for detail.

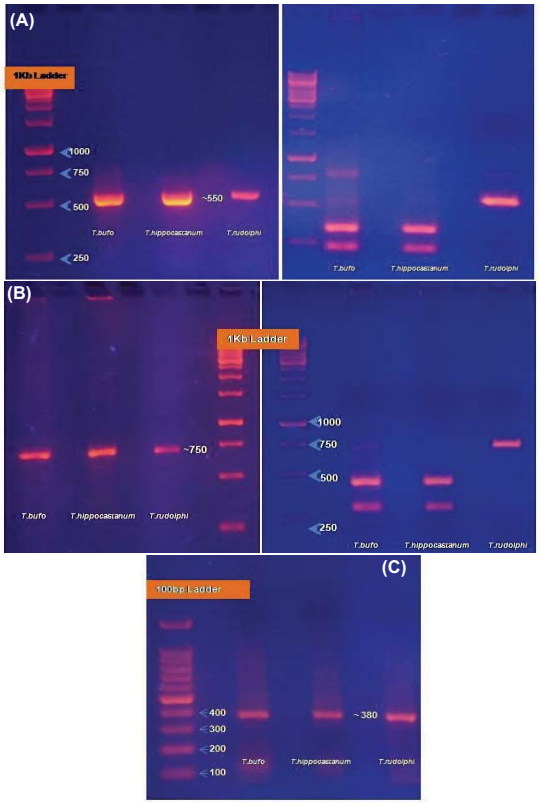

Fig. (S2). PCR based amplification of 16S rDNA (A), COI (B) and Histone H3 (C) genes from three major species of genus *Thais* collected from Buleji site Karachi, Pakistan. The RFLP analysis performed for the more variable 16S rDNA and COI genes are also shown in parallel.

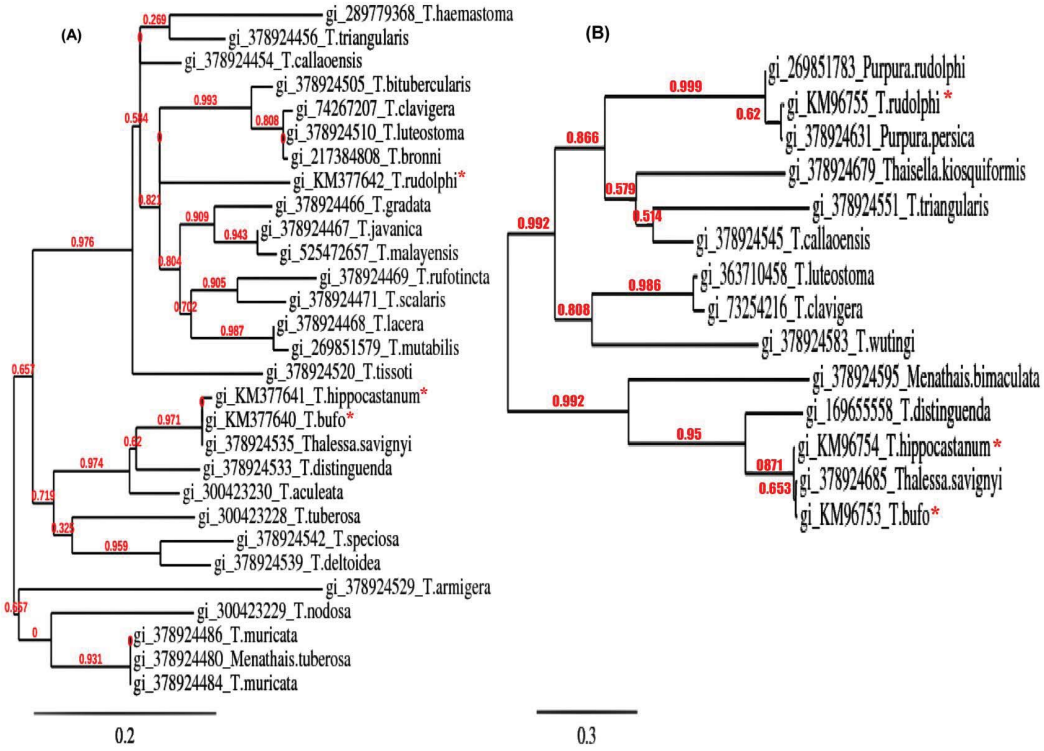

Fig. (S3). Phylogeny of out groups based on analysis of 16S rDNA (A) and COI (B). * Indicates the three *Thais* sp. subjected for molecular identification in the present study. See Table S1 and "Materials and Methods" for detail.

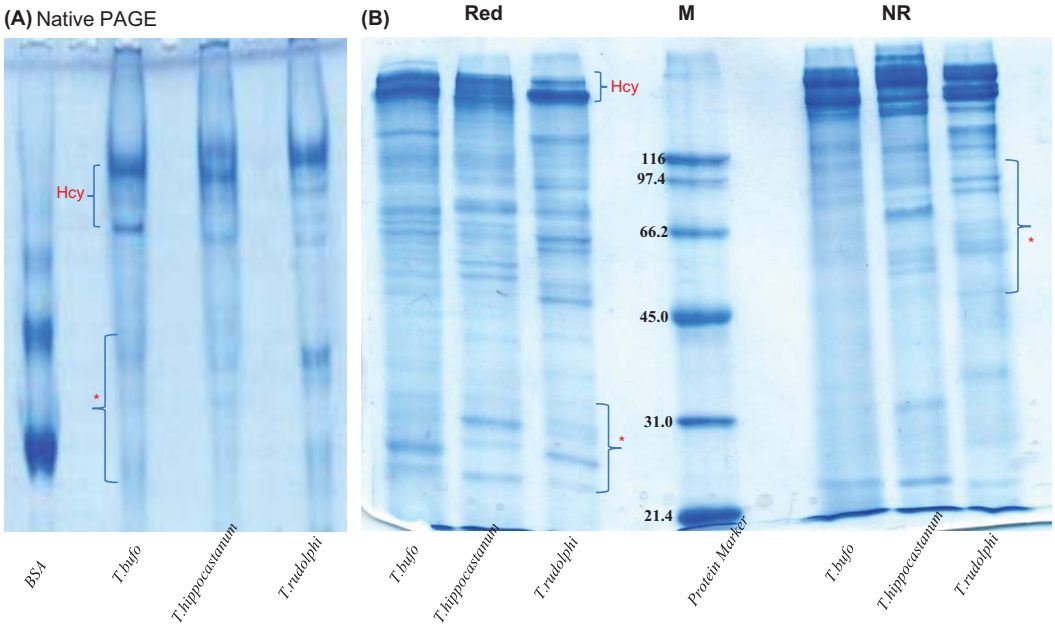

Fig. (S4). Biochemical protein finger printing of three *Thais sp.* using 10% polyacrylamide gel electrophoresis under Native (A), and dissociating (NR, non-reduced) and dissociating/denaturing (Red, reduced) conditions (B). Lane BSA and M, are the known molecular weight marker proteins. Asterisk marks the differential bands and Hcy, as predominant blue cupper-containing oxygen carrier protein (i.e. hemocyanins).

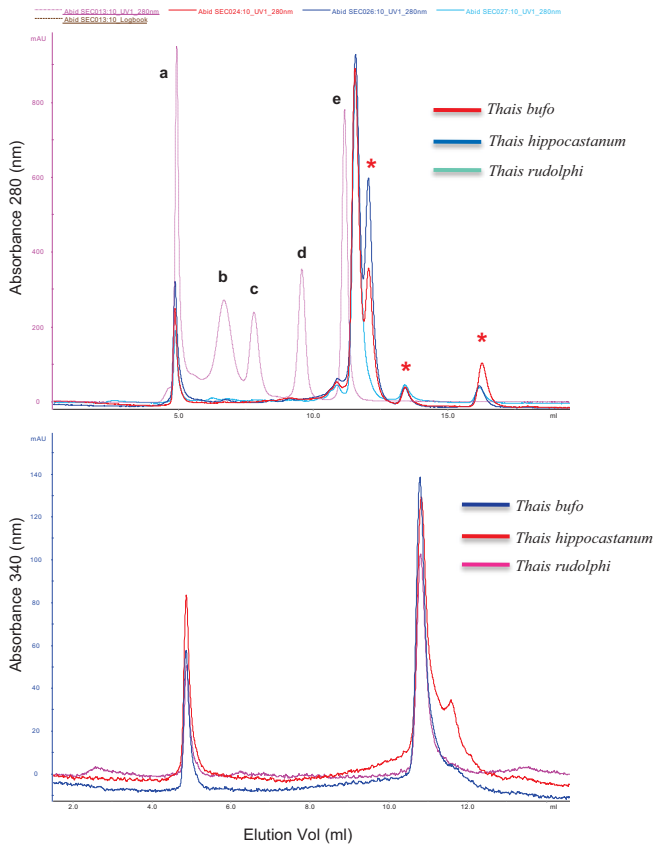

Fig. (S5). Size-exclusion fast protein liquid chromatography (SEC FPLC) of the three *Thais sp*. Top chromatograms monitored at 280 nm for proteins and bottom 340 nm specific for hemocyanins. Asterisk marks the differential peaks in the chromatograms. Column was calibrated with known molecular weight marker proteins (profile with broken line) are labeled as a to e (i.e. 670, 158, 41, 17, and 1.3 kDa, respectively).

Table S1. List of the PCR primers and conditions used for the amplification of marker genes for molecular identification of *Thais* sp.

Gene region	Species	GenBank Acc. No	Amplicone Size (bp)	Primers Name	Sequences 5' → 3'	PCR program	References
COI	*Thais bufo*	KM96753	685	COI-1490F	GGTCAACAAATCATAAAG ATATTGG	94°C 3 min, (94 °C 45 s, 50°C 90 s, 72 °C 120 s) x30 72 °C 5 min	Nakano et al.., (2009)
	Thais hippocastanum	KM96754	680				
	Thais ruolphi	KM96755	684	COI-2198R	TAAACTTCAGGGTGACCA AAAAATCA		
16S rDNA	*Thais bufo*	KM377640	522	16S-H	CGCCTGTTTATCAAAAACAT	94 °C 5 min, (94 °C 40 s, 52 °C 1 min, 72 °C 5 min) x30 72 °C,10 min	Dayrat et al., (2011)
	Thais hippocastanum	KM377641	527				
	Thais ruolphi	KM377642	520	16S-R	CCGGTCTGAACTCAGATCACGT		
Histone H3	*Thais bufo*	NS	390	H3AF	ATGGCTCGTACCAAGCAGACVGC	94 °C 3 min, (94 °C 45 s, 54 °C 90 s, 72 °C 120 s) x30 72 °C 5 min	Nakano et al., (2009)
	Thais hippocastanum	NS	390				
	Thais ruolphi	NS	380	H3AR	ATATCCTTRGGCATRATRGTGAC		

NS = Not Submitted

Table S2. Elemental composition of shell material determined using energy-dispersive X-ray spectroscopy (EDX) and the associated analytical program EDX Analysis Station.

Element	*Thais bufo*	*Thai hippocastanum* (% mass)	*Thais rudolphi*
C	12.4	8.3	17.58
O	47.84	49.95	53.24
Na	0.51	0.96	0.48
Mg	0.56	-	1.11
Al	0.9	0.64	2.76
Si	1.08	-	4.94
Ca	36.71	40.14	18.49
K	-	-	0.57
Fe	-	-	0.84