PHYLOGEOGRAPHY OF *GEOTROCHUS* AND *TROCHOMORPHA* (GASTROPODA: TROCHOMORPHIDAE) IN SABAH

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ABSTRACT

Sabah is renowned for species richness and endemism, and this pattern is largely due to current ecological conditions and historical events. Phylogeography studies in Sabah have been focusing on plant taxa and several animal taxa with high mobility. There is a lack of the biogeographical knowledge of species with low mobility. Therefore, *Geotrochus* and *Trochomorpha* were selected to test the biogeography hypothesis in Sabah. The taxonomy status of the two genera has been based on morphology. As shell characters are strongly influenced by ecological factors, the evolutionary relationship of the two genera is currently not truly represented. Hence, this study presents the molecular phylogeny of six *Geotrochus* species and three *Trochomorpha* species based on mitochondrial genes (COI and 16S) and nuclear gene (ITS-1). Influences of temperature, elevation and precipitation on teleoconch sculpture pattern and four quantitative shell traits were statistically examined to elucidate the reliability of the diagnostic characters. Finally, biogeography patterns of *Geotrochus* and *Trochomorpha* in Sabah were predicted using MaxEnt. Determinants of the pattern were analysed using BEAST (Bayesian evolutionary analysis by sampling trees) and the MaxEnt built-in Jackknife test. Consensus trees of ML and BI revealed the polyphyly of *Trochomorpha* and indicated that the teleoconch sculpture is a homoplasy character. Shell width and aperture width are both negatively correlated with elevation (SW: r= -0.42; AW: r= -0.43) and precipitation (SW: r= -0.42; AW: r= -0.43) while being positively correlated with temperature (SW: r= 0.49; AW: r= 0.49). Shell height is only positively associated with temperature (r= 0.29) while aperture height is not correlated to any of the ecological variables. The biogeography patterns of the species appear to be highly influenced by the climate in Sabah, with the distribution of the highland species shaped by temperature, whereas lowland species appears affected by precipitation. *Geotrochus* and *Trochomorpha* diverged around Miocene to Pliocene, subsequent to the uplifting of the mountainous area and episode of sea lowering around Sabah. Furthermore, my analyses suggest that global warming will likely cause the extinction of mountaintop species such as *T. rhysa* and *T. haptoderma* while reducing the suitable habitat of *G. oedobasis* and *T. thelecoryphe* on Mt. Kinabalu. Based on my results, the taxonomy of *Geotrochus* and *Trochmorpha* still requires further revision and future studies should include more taxa and broader geographical scale.

Keywords:

Taxonomy, molecular phylogeny, biogeography, convergent evolution, species distribution modelling

ABSTRAK

PHYLOGEOGRAPHY GEOTROCHUS DAN TROCHOMORPHA (GASTROPODA: TROCHOMORPHIDAE) DI SABAH

Sabah terkenal dengan kekayaan spesies dan keendemisan. Corak ini banyak disumbangkan oleh keadaan ekologi semasa dan peristiwa sejarah. Kajian phylogeografi di Sabah memberi tumpuan kepada taksa tumbuhan dan beberapa taksa haiwan dengan mobiliti yang tinggi. Terdapat kekurangan dalam pengetahuan biogeografi spesies dengan mobiliti yang rendah. Oleh itu, Geotrochus dan Trochomorpha dipilih untuk menguji hipotesis biogeografi di Sabah. Status taksonomi kedua genera ini masih berasaskan kaedah morfologi. Oleh kerana cangkarang siput sangat dipengaruhi oleh faktor-faktor ekologi, hubungan evolusi kedua-dua genera masih tidak benar-benar ditunjukkan. Oleh itu, kajian ini membentangkan filogeni molekul enam spesies Geotrochus dan tiga spesies Trochomorpha berdasarkan gen mitokondria (COI dan 16S) dan gen nuklear (ITS-1). Pengaruh ekologi terhadap corak ukiran teleokonch serta 4 sifat kulit kuantitatif yang digunakan untuk mengklasifikasi spesies Geotrochus dan Trochomorpha telah diuji secara statistik untuk menjelaskan kebolehpercayaan ciri-ciri diagnostik. Akhir sekali, corak biogeografi Geotrochus dan Trochomorpha di Sabah telah diramalkan menggunakan MaxEnt dan penentu corak dianalisis menggunakan BEAST (analisis evolusi Bayesian oleh pokok sampingan) dan ujian Jackknife terbina dalam MaxEnt. Pokok konsensus ML dan BI mendedahkan polyphyly Trochomorpha menunjukkan bahawa corak teleokonch adalah watak homoplasy. Lebar cangkerang dan lebar apertur kedua-duanya secara statistik mempunyai kaitan negatif dengan ketinggian (SW: $r = -0.42$; AW: $r = -0.43$) dan pemendakan (SW: $r = -0.42$; AW: $r = -0.43$) manakala berkaitan secara positik dengan suhu (SW: $r = 0.49$; AW: $r = 0.49$). Ketinggian kulit hanya dikaitkan secara positif dengan suhu ($r = 0.29$) manakala ketinggian apertur tidak berkorelasi kepada mana-mana pembolehubah ekologi. Corak biogeografi spesies ini sangat dipengaruhi oleh iklim di Sabah dengan taburan spesies dataran tinggi yang dibentuk oleh suhu manakala spesies tanah rendah dipengaruhi oleh hujan. Penspesiesan Geotrochus dan Trochomorpha belaku dari Miocene ke Pliocene, berikutan peningkatan kawasan pergunungan dan episod penurunan aras laut di sekitar Sabah. Tambahan pula, analisis menunjukkan bahawa pemanasan global mungkin akan menyebabkan kepupusan spesies gunung seperti T. rhysa dan T. haptoderma sambil mengurangkan habitat G. oedobasis dan T. thelecoryphe yang sesuai di Mt. Kinabalu. Berdasarkan hasil dapatan di atas, taksonomi Geotrochus dan Trochomorpha memerlukan semakan lebih lanjut dan kajian masa depan perlu memasukkan lebih banyak taksonomi dan skala geografi yang lebih luas.

***Kata kunci**: Taksonomi, filogeni molekul, biogeografi, evolusi konvergen, pemodelan pengedaran spesis*

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LIST OF ABBREVIATIONS

AUC	-	Area under the receiver-operator curve
Beast	-	Bayesian evolutionary analysis by sampling trees
COI	-	Cytochrome *c* oxidase 1
DNA	-	Deoxyribonucleic acid
GPS	-	Global Positioning System
ITS	-	Internal Transcribed Spacer
Km^2	-	Square kilometer
LGM	-	Last Glacial Maximum
LSU	-	Large subunit
M	-	Meter
MaxEnt	-	Maximum Entropy Modeling
mm	-	Millimeter
Mya	-	Million years ago
PCR	-	Polymerase Chain Reaction
Sp.	-	Species
SSU	-	Small subunit

LIST OF SYMBOLS

°	-	Degree
%	-	Percent
μ	-	Micro
Δ	-	Difference
×	-	Magnification
*	-	Signification

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CHAPTER 1

INTRODUCTION

1.1 Study Background

Southeast Asia is one of the well-known biodiversity hotspots in the world (Mittermeier *et al.*, 2005; Woodruff, 2010). Establishment of effective conservation strategies in the biodiversity hotspot has never been more imperative nowadays in the face of escalating environmental deterioration in the region pressured by the expanding human population (Sodhi & Brook 2006; Sodhi *et al.*, 2010; Woodruff, 2010). However, the four biogeographic hotspots identified by Myer (2000) within Southeast Asia are spatially too coarse to ensure comprehensive conservation management. Hence, identification of a finer scale hotspot by understanding the biogeographic pattern and the factors structuring the pattern within the mega- biodiversity hotspots are needed to guide effective regional conservation planning (Noroozi *et al.*, 2018).

Within the Sundaic hotspot lies the Malaysian state of Sabah which is located at the northernmost of Borneo. It occupies approximately 10% of the total area of Borneo. This state is especially renowned for its species and endemism richness (Raes *et al.*, 2009; Sheldon, 2016), which is mainly concentrated in the central Crocker Range, the area west of the Crocker Range as well as eastern Sabah (MacKinnon, 1996; Wong, 1998; Raes *et al.*, 2009). The exceptional richness and endemicity of Sabah is believed to be not only attributed to the current climatic condition in the area (Raes *et al.*, 2009) but also due to the complex Tertiary geological and the Quaternary climatological histories in the area that not only promoted species diversification but also served as a barrier for species range expansion (Gawin *et al.*, 2014; Sheldon, 2016; Camacho-Sanchez *et al.*, 2018). During the Tertiary period, an extensive and topographically complex area emerged in Sabah (Hall, 2013). This created different environmental gradients of temperature and precipitation that could lead to allopatric speciation (Roberts *et al.*, 2011). In the Pleistocene, changes in sea level and climate linked to the glacial cycle also profoundly influenced the geography and vegetation in the area. (Heaney, 1991; Voris, 2000; Gathorne-Hardy *et al.*, 2002; Bird *et al.* 2005; Cannon *et al.*, 2009). Particularly, lowered temperatures temporary expanded the distribution of montane rainforest in Sabah (Gathorne-Hardy *et al.*, 2002; Cannon *et al.*, 2009) and hence promoted the richness of the accommodated rainforest species (Camacho-Sanchez *et al.*, 2018). The lowering of sea levels, on the other hand, intermittently connected several surrounding islands to Sabah mainland (Voris, 2000). It provided potential routes that facilitated the range expansion of species across the landmass (Woodruff *et al.*, 2010).

Effects of the historical events on Sabah' biota is mirrored in the current distribution of the extant biota, especially those organisms with limited dispersal ability. Land snails are a good candidate for phylogeographic studies and have been employed around the world to test biogeographical hypotheses (Cameron *et al.*, 2006; Uit de Weerd *et al.*, 2016; Fiorentino *et al.*, 2016). Due to their low vagility, the genetic variation patterns that developed during colonisation tend to be conserved (Cruzan & Templeton, 2000). Sabah is especially rich in land snail diversity, with currently approximately 340 species recorded to date (Phung *et al.*, 2017). Among these, they are two genera of land snails with a low conical shell: *Geotrochus* and *Trochomorpha*. Species within these two forest-dependent genera can be widely distributed across Sabah or locally endemic (Vermeulen *et al.*, 2015). Hence, *Geotrochus* and *Trochomorpha* are suitable model taxa to investigate biogeographical patterns in Sabah.

To better understand the biogeography of any species, its taxonomy should accurately reflect its evolutionary relationships. However, the classification of *Geotrochus* and *Trochomorpha* has been traditionally based on morphology; the latter species is consistently differentiated from the former based on its coarser nodular teleoconch sculpture. Species of these two genera have only been described and classified based on the subjective conchological characters that are often the result of convergent evolution. Convergent evolution is common among taxa such as land snails that have low mobility and become locally adapted to their environment (Hirano *et al.*, 2014;

Köhler & Criscione, 2015; Holznagel *et al.*, 2010; Hyman & Ponder, 2010; Dowle *et al.*, 2015). Thus, the classification of *Geotrochus* and *Trochomorpha* remains uncertain, and a molecular phylogenetic approach is needed to elucidate the taxonomy of these genera.

In this study, I investigate the phylogeography of *Geotrochus* and *Trochomorpha* in Sabah. First, the phylogenetic relationship of *Geotrochus* and *Trochomorpha* were constructed using two mitochondrial genes (16S and COI) and one nuclear gene (ITS-1), which are useful in resolving the evolutionary relationship of land snails at the species level (Phung *et al.*, 2017; Elejalde *et al.*, 2009). Second, the reliability of the teleoconch sculpture pattern and four quantitative shell traits as diagnostic characters between *Geotrochus* and *Trochomorpha* species were tested. Finally, the contemporary distribution of *Geotrochus* and *Trochomorpha* were predicted using Maximum Entropy (MaxEnt) modelling and their biogeographic patterns were explained using our results obtained from the built-in jackknife test of MaxEnt and molecular clock analyses.

1.2 Justification

I have chosen to investigate the phylogeography of two land snail genera, *Geotrochus* and *Trochomorpha* mainly because related studies in Sabah have only focused on more mobile animals (Gawin *et al.*, 2014; Hawkin *et al.*, 2016; Robert *et al.*, 2011). As previous quantitative analyses of biogeographical patterns in Sabah have focused in mountainous areas (Md, 2001; Liew *et al.*, 2010; Grytnes *et al.*, 2006), my study will also involve land snail species with distributions across a broader spatial scale in Sabah. To date, broad-scale biogeographical studies in Sabah have mainly examined on plant data (Raes *et al.*, 2009; Slik *et al.*, 2003). Considering the rapid loss of rainforest in Sabah (Raes *et al.*, 2009), my study can help provide additional biogeographical knowledge on species with low vagility to support regional conservation planning in Sabah.

With the extraordinary land snail diversity in Sabah, numerous land snail species can be used in the phylogeographic analysis. However, I chose *Geotrochus* and *Trochomorpha* because species from these genera range from widespread to restricted distributions; some are even highly endemic to mountainous areas in Sabah are therefore are of high conservation concern. As the taxonomy of these two genera have been morphologically based, and nine new species has just been added into the genus during a recent taxonomic revision (Vermeulen *et al.*, 2015), a phylogenetic study is timely in order to resolve species limits in these two genera.

CHAPTER 2

LITERATURE REVIEW

2.1 Diversity of *Geotrochus* and *Trochomorpha* in Sabah

Geotrochus in Sabah consists of both lowland and highland species and is more diverse than *Trochomorpha,* which is mainly made up of highland species. At the same time, *Geotrochus* also has a widespread distribution in Sabah, in contrast to *Trochomorph*a, which is only found in the central mountainous area of Sabah.

2.1.1 Taxonomy

Trochomorpha (Albert, 1850) and *Geotrochus* (Van Hasselt, 1823) are two genera under the Trochomorphidae (Möllendorff, 1890), a family with a complicated taxonomic history (Bouchet & Rocroi, 2005; Bouchet & Rocroi, 2017). *Trochomorpha* was first classified under the subfamily Trochomorphinae as it was assumed to be closely related to the family Zonitidae (Solem, 1959). During the 19th century, *Trochomorpha rhysa* was the only *Trochomorpha* species recognised in Sabah and was placed in the genus *Trochomorpha* due to the simplicity of its penial structure and by having unicuspid central and lateral radular teeth (Tillier & Boucher, 1988). Three species were then added to the genus as they shared similar coarse sculpture patterns with *T. rhysa* (Vermeulen *et al.*, 2015). To date, there are currently four *Trochomorpha* species recognised in Sabah. The conchological differences among these species are slight and have been mainly classified based on their sculpture pattern and shell shape (Figure 2.1; Table 2.1).

Although *Geotrochus* shares several common characteristics with *Trochomorpha* such as the shell shape and the umbilicus pattern, it can be readily distinguished from *Trochomorpha* by the smooth surface of its shell, as opposed to the *Trochomorpha* which has a coarse shell (Figure 2.2; Vermeulen *et al.*, 2015). In Sabah, 11 species of *Geotrochus* are currently recognised. Six species were newly described by Vermeulen et al. (2015), while the remaining five species were described in the 19th century and had been assigned in the genus *Trochonanina* (Smith, 1895). Sabah *Geotrochus* are highly similar in their appearance, which was separated into two informal groups by Vermeulen et al. (2015): (1) species with a suture coinciding with the shell periphery (Table 2.2 and Table 2.3), and (2) species with the suture slightly below the periphery (Table 2.4). The latter informal group was further subdivided into two groups based on the shell width of the adult specimen: shell size 10mm- 12.5mm and shell size 13.5mm-24.5mm.

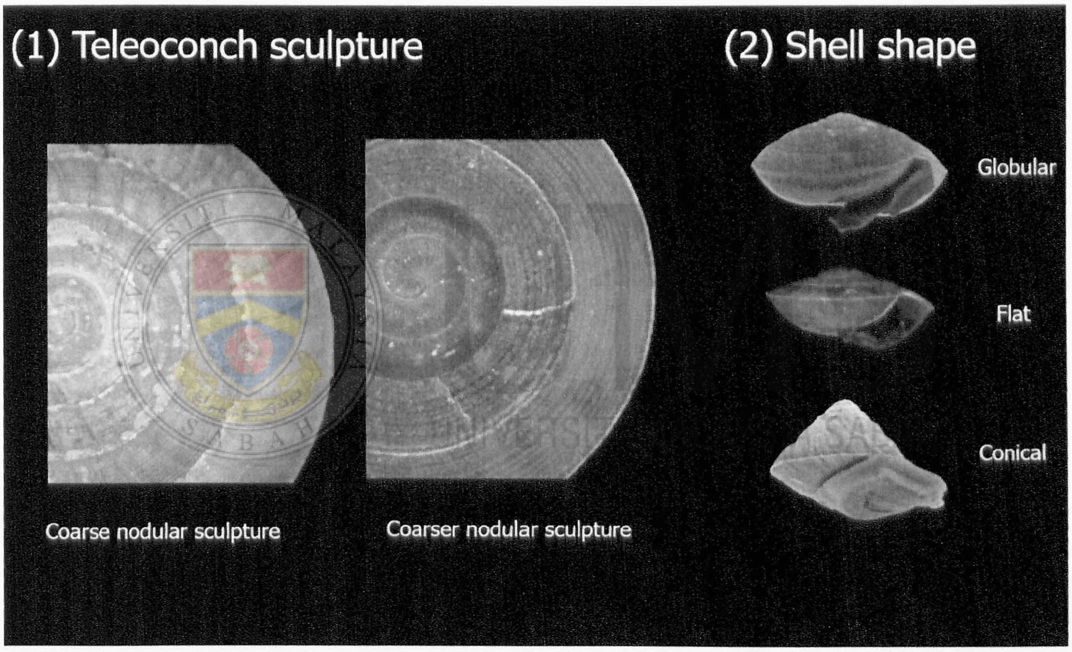

Figure 2.1 : Illustrated diagnostic shell characters used to differentiate *Trochomorpha* species