The Shell Evolution of the Hydrocenidae of Malaysian Borneo

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This thesis is dedicated to my late father, Khalik;
my mother, Konia; my wife, Siti Khadijah;
my sons, Zaqwan and Zaeydan; and my daughter, Zulaika

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Chapter 1

A general perspective on animal diversification and the introduction to the model organism

A general perspective on diversification

Evolutionary biology is the field of study that aims to understand the evolutionary history of organisms through a certain period of time. One of its objectives is to understand how organisms respond genetically to environmental changes and the concomitant selection, and to determine which forces maintain, change, or deplete genetic variability (Houle, 1991). These responses may lead not only to anagenesis (evolution within a single lineage) but also to cladogenesis (speciation), which results in a tree-like multifurcation. Post-hoc, the relationship among two or more different species may be determined by reconstructing the phylogeny, based on molecular data and morphological characters.

Many morphological radiations in organisms occur due to new ecological opportunities in their surroundings, and this forces the organism to adapt, resulting in a continued persistence and branching out in the ecosystem (Mousseau and Roff, 1987). Accumulation of the adaptative results of selection could lead to multiple subsequent appearances or disappearances of genetic traits which, taken together, result in major phenotypic changes and the arising of new species (Chazot et al., 2016; Danowitz et al., 2015; Price et al., 2003) (**Figure 1.1**). In brief, this means that changes in the ecological system could induce changes in a species' morphological characters (Price et al., 2003). In many studies related to morphological character evolution and divergence, the researchers aim to link the morphological adaptation of their model organism with environmental change (e.g., Lovern et al., 2004; Hendenstrom and Moller, 1992; Freeman, 1981).

As niche characteristics may be geographically variable, species usually will display a spatially restricted range since it cannot establish itself beyond this range (Bridle and Vines, 2007). When such species do disperse beyond this range, they will suffer deleterious effects, leading to a negative population growth rate (Bridle and Vines, 2007). Only by evolving the ability to occupy a novel habitat and the accompanying niche, range expansion will be possible. However, this process generally requires long periods of time, from hundreds to millions of years (Pearman et al., 2008).

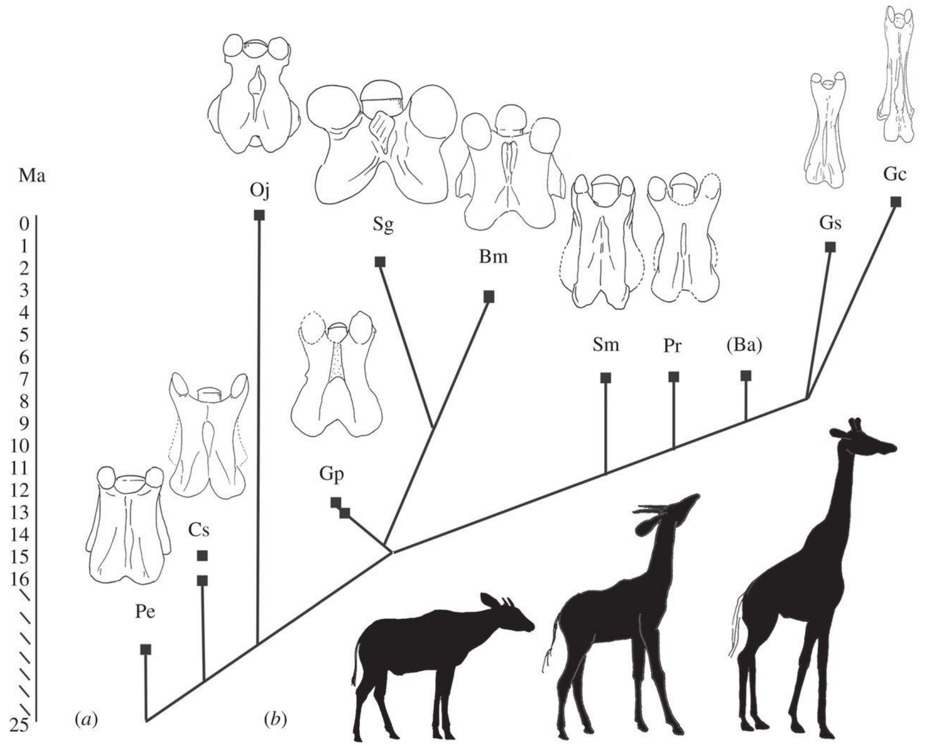


Figure 1.1 A cladogram with geological age of evaluated taxa and the dorsal view of their C3 vertebrae. Pe, *Prodremotherium elongatum*; Cs, *Canthumeryx sirtensis*; Oj, *Okapia johnstoni*; Gp, *Giraffokeryx punjabiensis*; Sg, *Sivatherium giganteum*; Bm, *Bramatherium megacephalum*; Sm, *Samotherium major*; Pr, *Palaeotragus rouenii*; Ba, *Bohlinia attica*; Gs, *Giraffa sivalensis*; Gc, *Giraffa camelopardalis*. The clades are terminated at the square points and each point indicates the age of the taxon in millions of years. The isometrically scaled V3 vertebrae are made to the equal length and they show shape/size changes through time (adapted from Danowitz et al., 2015).

In addition, we could observe that resource availability also plays an important role in the adaptive pattern of species in a complex niche area (Kohn, 1971). Competition for specific food resources in the same geographical region could induce selection to outcompete other individuals. Experimental physiology is one good approach to observe and determine the effects of different types of diets on organisms in the framework of adaptive radiation (e.g., Alföldi et al., 2011). This kind of study could explain how different types of diet (e.g., food item size) would affect the real adaptation process in the wild.

Determining the modes of adaptation and speciation based on a particular set of niche parameters is one key point in evolutionary biology. This assists the evolutionary biologist to infer the main force(s) that drive, channel, or impede the evolutionary trajectory of a particular species. The complete set of information, data, and various kinds of analyses could be used to reconstruct the evolutionary relationships among the selected species, finding the answers of the coexistence of species, and theoretically discuss the factors that drive the species to undergo phenotypic and genotypic variation.

Evolutionary radiations in gastropods

The class Gastropoda (snails and slugs) is the largest class within the phylum of the Mollusca and estimated to contribute approximately 80% of species to all molluscan taxa (Bieler, 1992). They are widely variable with distinct characters, which differentiate them from other classes within the Mollusca. Gastropods possess shells which are best described as coiled tubes made of calcium carbonate and proteins, an extra-cellularly deposited ‘organ’ common to all gastropods (except those who have secondarily lost it – i.e., true slugs). Recent studies of gastropod shell evolution focus on characters such as color, size, shape, growth, and presence or absence of an umbilicus (Barker, 2005; Fiorentino et al., 2010; Hausdorf, 2006; Kuris and Brody, 1976; Liew et al., 2014; Schilthuizen et al., 2005; Teshima et al., 2003).

Studies on the evolution of land snail shell shape and size (e.g., Fiorentino et al., 2010; Liew and Schilthuizen, 2016; Teshima et al., 2003) have shown that gastropods may display very distinct morphometric characters, even at the subgeneric level, and even among phylogenetically very closely related species. Major morphological evolution in gastropod shell shape may even occur within the same species, if different populations experience different selection pressures (Kemp and Bertness, 1984; Schilthuizen et al., 2005; Teshima et al., 2003). In addition to response to directional selection, shell evolution may also be steered by ‘constraints’, which may take the shape of genetic, selective, developmental, and functional constraints (**Figure 1.2**), that affect the evolutionary processes and history of a species (Arnold, 1992).

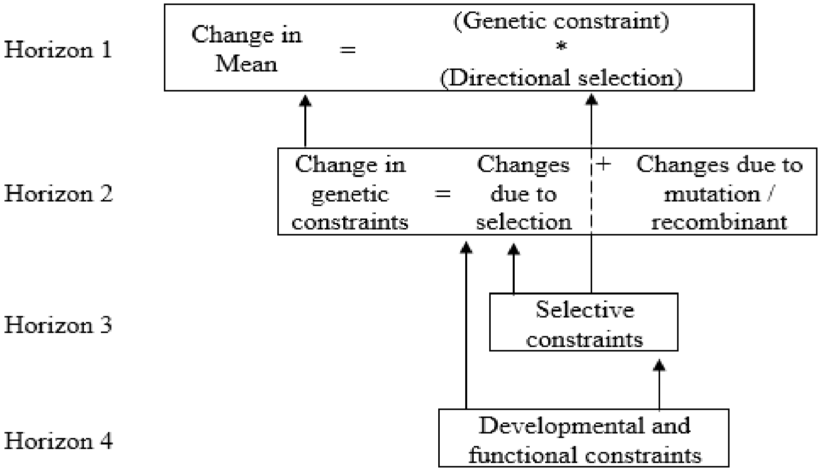


Figure 1.2 A framework for the main theoretical connection between genetic, selective, developmental, and functional constraint. Immediate consequences are shown by arrows. Adapted from Arnold (1992).

Interaction between shell and niche evolution of gastropods

The functional aspects of shell morphology are of great interest in the study of shell evolution, due to the fact that distinct characters or shell morphometrics could help unravel the evolutionary history of a population. (DeWitt et al., 2000; Hausdorf, 2006; Schilthuizen et al., 2006; Teshima et al., 2003). Teshima et al. (2003) explained adaptive shell morphology based on the patterns in a hybrid zone in *Ainohelix editha* in Japan. Using transects, they found that the angularity and globularity of the shells changed at two independent hybrid zones as the result of selective forces by the surrounding environment, with flat, keeled shells apparently adapted to either vertical or crevice-like substrates (Teshima et al., 2003). This is one example of how a new ecological niche will allow a species to change morphologically as well as to change or adjust its distribution range (Pearman et al., 2008).

In the perspective of predator-prey interaction, shelled organisms diverge in shell morphology in response to predation (Schilthuizen et al., 2006; Liew and Schilthuizen, 2014). As an example, *Plectostoma concinnum* evolved anti-predation traits (protruding radial ribs and distorted coiling of the shell), which appear to allow them to avoid predation by *Atopos* slugs. This study revealed how this defense mechanism works for this species, while the same characters do not seem to be the effect of adaptation to other (abiotic) ecological parameters.

As shown above, snails may evolutionarily adjust their shell morphology to suit biotic and abiotic ecological aspects of their environment, and may even experience speciation, either in sympatry or in allopatry, due to these processes. However, it is also possible that the evolution of the ecological niche is not reflected in the shell shape and that different cryptic species may be present, each with its own niche, which have solved the demands of these differing niches purely by behavioral and physiological, or soft-body morphological adaptation. Such cryptic species may only be detected and studied using genetic or anatomical means.

The model organism

Gastropoda from the clade Neritimorpha comprise four superfamilies: Helicenoidea, Hydrocenoidea, Neritoidea, and Neritopsoidea (Bouchet et al., 2005), which are thought to have diverged between the Paleozoic and early Mesozoic eras (Uribe et al., 2016). Hydrocenidae as the sole family in Hydrocenoidea consists of two widely accepted genera: *Hydrocena* and *Georissa* (Uribe et al, 2016). The genus *Georissa* is wide-spread in the Indo-West Pacific regions, and has approximately 70 described species. It is mainly characterised by dextral and calcareous shell whorls, with the presence of a rounded to ovate operculum, where a straight to curved peg emerges toward the inner part of the shell (**Chapters 2 and 3**; Thompson and Dance, 1983; Vermeulen et al., 2015).

As for the Bornean *Georissa*, Godwin-Austen (1889) conducted the first systematic study with the description of four conchologically distinct species: *G. hosei*, *G. niahensis*, *G. williamsi*, and *G. hungerfordi*. The work by Godwin-Austen (1889) was then followed by Smith (1893, 1895), Gredler (1902), and van Benthem-Jutting (1966), resulting in the description of an additional nine Bornean species. With the increasing amount of materials collected from Borneo, Thompson and Dance (1983) made the first overview on the systematics and biogeography of Bornean *Georissa*, and described three new species. Several additional Bornean *Georissa* have been described only recently by Haase and Schilthuizen (2007), Vermeulen and Junau (2007), and Vermeulen et al. (2015).

Habitat and distribution of the Bornean *Georissa*

Taking into account the work described in this thesis, there are now at least 29 known species of *Georissa* endemic to Borneo (see **Chapters 2 and 3**). They are particularly restricted to environments rich in calcium carbonate ($CaCO_3$) and can be found in variable abundances on wet and shaded limestone walls or rocks. *Georissa saulae* and *G. gomantongensis* on the other hand, are occasionally found on sandstone rocks and in vegetation, respectively, which are not associated with a rocky substrate (**Chapters 2 and 3**; Haase and Schilthuizen, 2007). Of all Bornean *Georissa*, at least three species are hypogean (cave species), while the rest are epigean.

The evolutionary dynamics of the Bornean *Georissa*

The overview presented by Thompson and Dance (1983) was, until now, the only revision for the Bornean *Georissa*. Furthermore, the evaluation of species validity has traditionally depended mostly on their morphological characters. Therefore, often conchologically similar species may have been misinterpreted as intraspecific variation of a species. For this reason, sympatric and/or cryptic diversity of the Bornean *Georissa* was not well understood.

Haase and Schilthuizen (2007) described the troglobitic *Georissa filiasaulae* from Sanaron cave in Sabah, Malaysian Borneo. *G. filiasaulae* has descended from the above-ground *G. saulae*, which lives on the surface of the limestone outcrop that holds Sanaron cave, and gene-flow between the two species is still occurring at cave entrances (Schilthuizen et al., 2012). *G. filiasaulae* has diverged strongly from *G. saulae* in shell sculpture, color, genitalia, and the digestive system, as well as in *16S* mtDNA sequences (Schilthuizen et al., 2005; Schilthuizen et al., 2012). These findings have led to a type of population and evolutionary studies of *Georissa* with systematic sampling design to understand the evolutionary pattern of this interesting minute terrestrial snail at a small geographical scale (see also Hendriks et al., 2019); this type of study has also been adopted in this thesis (see **Chapters 4 and 5**).

The aim of the study

In this PhD project, I am focusing on the evolutionary patterns of the *Georissa* species of Malaysian Borneo to reveal and interpret the divergence in two-character systems (a-b). To be able to correlate (a) shell shape changes in phylogeny with shifts in the environment, I will also undertake the study of (b) habitat niche evolution. Thus, the study aims to answer several questions related to physical and biological characters that occur within the genus *Georissa* which are:

- How do shell traits evolve in this genus?
- How do niche traits evolve in this genus?
- What correlations exist among the evolutionary pathways of these two sets of traits?

A first introduction to quantification and phylogenetic background of the shell characters in Bornean *Georissa*

Below, I present some preliminary attempts to quantify shell shape differences in Borneo *Georissa* and to generate divergence times for the species. I have decided to present these results here in the Introduction, rather than in any of the analytical chapters, because (a) the absence of regularly spaced landmarks on the outside of the shell, and the dissolution of the inner walls make it difficult to generate an ontogenetic axis, and (b) the absence of reliably dated calibration points renders the timetree quite imprecise. Nonetheless, I think these preliminary studies could provide a starting point for further work, which is why I have chosen to include them in my thesis, though not in any of the chapters that are intended for publication in peer-reviewed journals.

Micro-computed tomography for shell shape

The quantification of morphological divergence in gastropod shells has been discussed extensively (e.g., Chiba, 2009; Goodfriend, 1986; Haase and Misof, 2009; Liew and Schilthuizen, 2016; Schilthuizen et al., 2005). The diversity of shell morphologies may require different approaches of quantification. For example, Liew and Schilthuizen (2016) quantified the shell growth pattern of *Plectostoma* by using the ontogeny axis and the protruding apertural structures on the shell whorls. This was done by micro-computed tomography (μCT), to obtain the complete 3-dimensional (3D) structure of the shell. The quantification of the shell may provide an insight into how the shell shape of an individual species is generated. In *Georissa*, however, the dissolved inner wall and the absence of radial ribs make it hard to define the ontogeny axis. We did µCT-scanning of the shell of the *Georissa* to obtain their 3D structure in order to determine shell shape patterns, but we applied different ontogeny and measurement approaches than Liew and Schilthuizen (2016).

The measurements were carried out in Avizo 9.4 (FEI Visualisation Sciences Group, https://www.fei.com/software/avizo/materials-science/) on the 3D reconstructed images of the shell which were segmented using the same software. Each species was represented by an individual adult shell. We measured the distances from the tip of the protoconch (a reference point) to the ontogeny axis, which we assigned to the line of points on the whorls which had the largest distance to the vertical axis of the shell (**Figure 1.3**). The ontogeny axis was also determined by following the spiral ribbing on the shell whorls (if present). The distances from the reference point along the ontogeny axis were measured based on the applied Python script (Supplementary material 1, **File 1**; see also below) as implemented in Avizo 9.4. The measurement data of each shell were extracted and plotted with RStudio (RStudio Team, 2016).

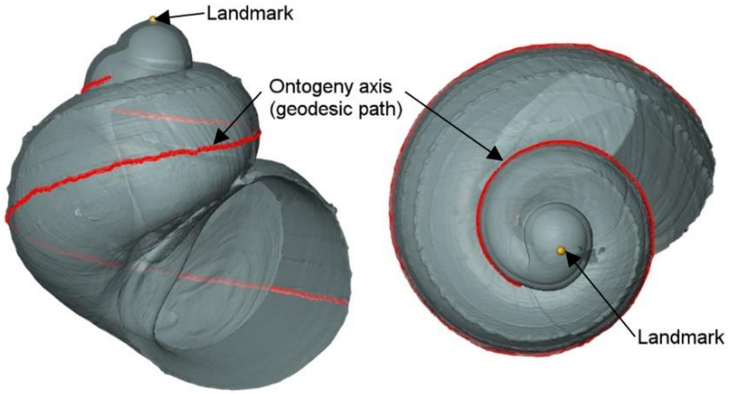
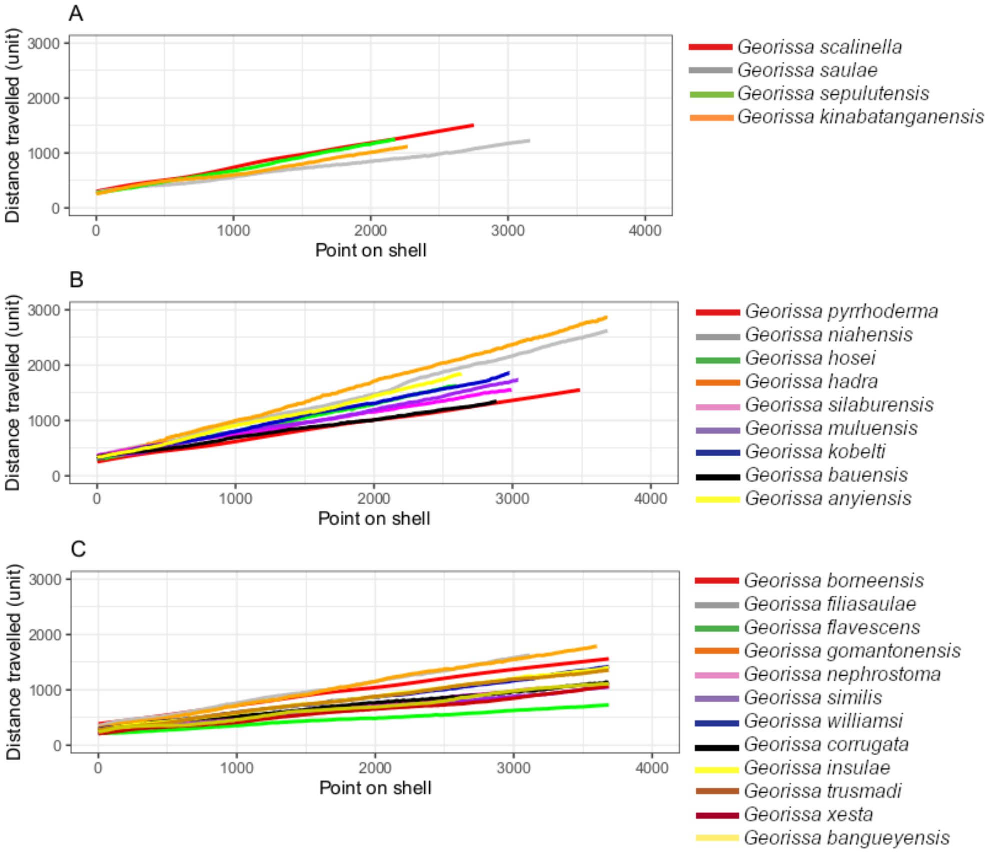


Figure 1.3 A landmark on the tip of the protoconch as a reference point for measurement purposes with an ontogeny axis, which we defined as the widest points on the shell whorl. The measurement is taken at each point on the shell whorl (pre-defined as in the Python script – one point at every one-pixel unit).

The measurement of the shape of each species of Bornean *Georissa* was conducted based on distance travelled from the tip of the protoconch along the ontogeny axis. The results of measurements on each shell (Supplementary material 2, **Table S1**) define the general shape of each species. In general, the Bornean species have a similar shell shape with linear shell growth as shown in **Figure 1.4**. The distance travelled from the landmark along the ontogeny axis serves as a reference to reflect shape of each species (i.e., greater slope indicates a more slender shell and vice versa), while the number of points indicate the length of the shell whorl. The number of points on the shell whorl were determined by the geodesic path on the shell as implemented in the Python script; one point at each 1-pixel unit or 1 µm.



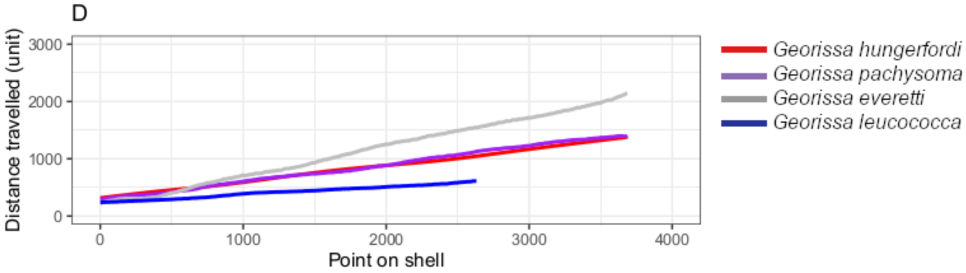


Figure 1.4 Plots of shell measurement based on the distance travelled from the tip of protoconch along the ontogeny axis (1000 units ~ 1 mm). **A** Measurement plot of the scaly *Georissa* of Sabah. **B** Measurement plot of the scaly *Georissa* of Sarawak. **C** Measurement plot of the non-scaly *Georissa* of Sabah. **D** Measurement plot of the non-scaly *Georissa* of Sarawak with two species (i.e., *G. everetti and G. leucococca*) ocurring in both Sabah and Sarawak.

All hydrocenid species have a simple coiled shell and show nearly linear shell tube expansion, resulting in an approximately regular cone (**Figure 1.4**). Slight deviations from a linear graph are discernible, which represent species-specific shell shape characteristics. Unlike the situation in *Plectostoma* (see Liew and Schilthuizen, 2016), our measurement plots provide no information regarding the shell growth rate, since there is no specific morphological feature(s) to determine increments with specific time intervals or different stages of shell growth. The plots form a visual summary of shell shape differences among species, independent of size, which are further elaborated upon in a qualitative manner in the taxonomic chapters (**Chapters 2 and 3**).

Estimation of divergence time of *Georissa*

We employed BEAST 2 (Bouckaert et al., 2014) to estimate the divergence times between the hydrocenid species and to obtain an ultrametric tree for the ancestral state reconstruction in Chapter 5. The parameters for the BEAST 2 analysis were set in BEAUTi 2 where the genes were unlinked for substitution model and linked for clock model as well as tree topology. We used bModelTest 1.1.2 (Bouckaert and Drummond, 2017) in the estimation of the Bayesian site models for each partition, selecting the transition + transversion split, since the rates of these substitutions are rarely equal. We applied an uncorrelated relaxed log-normal clock. The best fitted clock type was not tested. For the tree prior, we selected the Yule model of speciation. We ran the analysis with 50×10^6 generations and set the sampling frequency at every 1,000 generations. The tree was calibrated at the most recent ancestor of the genus *Nerita* assuming minimum and maximum ages of 49 and 56 my, respectively, following Frey and Vermeij (2008) and Uribe et al. (2016). The MCMC results were again inspected in Tracer 1.7.1 (Rambaut et al., 2018). The maximum clade credibility tree with mean node heights was generated after discarding a burn-in of 20% using TreeAnnotater 2.5.2 (a utility program distributed together with BEAST 2).

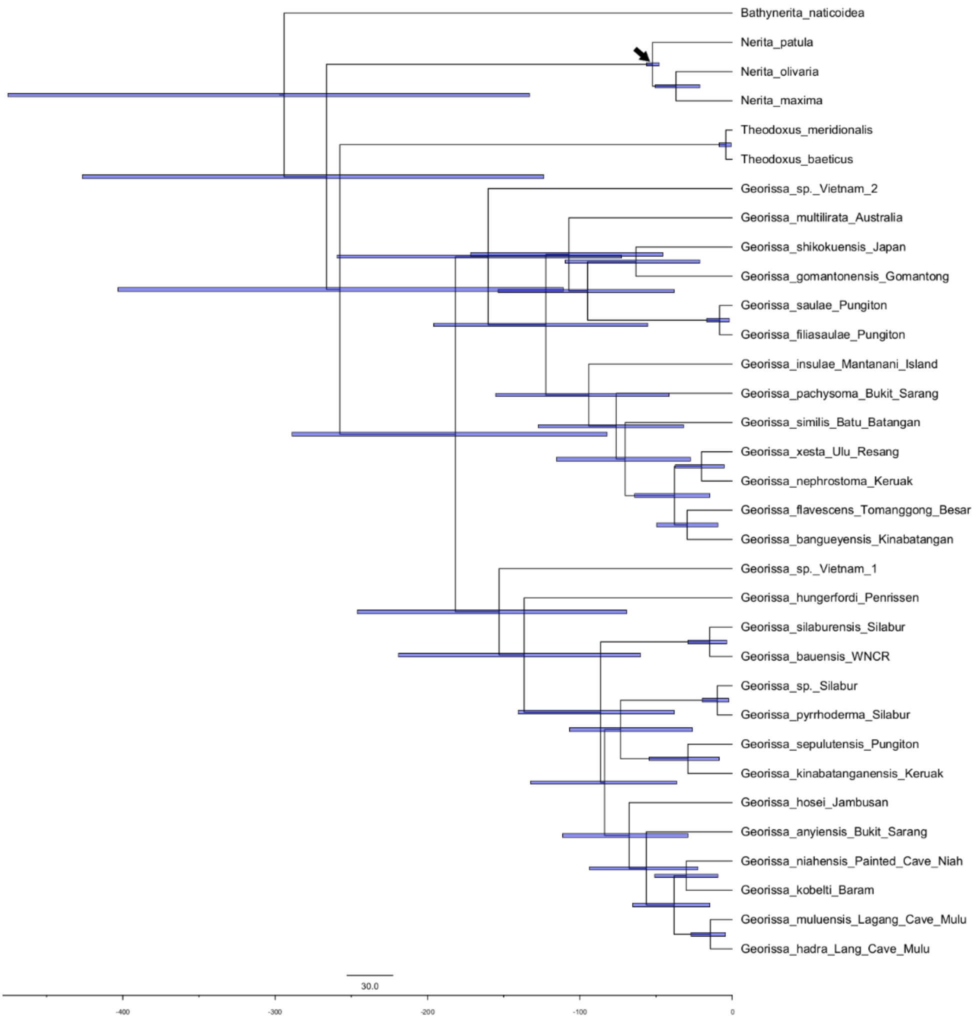


Figure 1.5 Chronogram of Hydrocenidae inferred by partial COI, 16S, 18S and 28S genes, calibrated at the node of genus *Nerita* (denoted by arrow). The horizontal bars represent the 95% highest posterior density intervals for time estimation in million years.

Our divergence time analysis based on BEAST 2 suggested that the divergence of the two major clades of Bornean *Georissa* took place between the Permian and Cretaceous (**Figure 1.5**). However, since any other calibrated time points are unavailable, these dates are to be considered tentative at best. The structure (though not the timing) of the ultrametric tree constructed in this analysis is used in our ancestral state reconstruction and phylogenetic signal analysis (**Chapter 5**).

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Supplementary material

1. File 1. Python script objects.
2. Table S1. Shell measurement.

Link to supplementary material
https://drive.google.com/drive/folders/1_iXsjYH1P2yfphCkNZN7hyJFUxgPyCo3

Chapter 2

A molecular and conchological dissection of the “scaly” *Georissa* (Gastropoda, Neritimorpha, Hydrocenidae) of Malaysian Borneo.

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Abstract

The Bornean hydrocenids have so far been understudied compared to other non-pulmonate snails in this region. In the present study, we review a first group of minute land snail species belonging to the genus *Georissa* (Gastropoda, Hydrocenidae) from Malaysian Borneo. This group is restricted to the species with conspicuous scale-like sculpture on the shell. Based on materials from recent fieldwork, museums, and personal collections, we find that Malaysian Borneo hydrocenids are more complex and diverse in shell characters than previously anticipated. Here, we present a molecular, conchological, and biogeographic study of this "scaly group". We recognise 13 species of which 6 are new to science, namely: *Georissa anyiensis* sp. n., *Georissa muluensis* sp. n., *Georissa bauensis* sp. n., *Georissa silaburensis* sp. n., *Georissa kinabatanganensis* sp. n., and *Georissa sepulutensis* sp. n.

Introduction

Over the past 25 years, the microsnail fauna of karst habitats in South East Asia has enjoyed an ongoing surge of attention. Detailed conchological and molecular studies in this region have revealed high allopatric and sympatric diversity (e.g., Liew et al., 2014; Rundell, 2008; Tongkerd et al., 2004), which has opened up this fauna for work in the fields of community ecology (Schilthuizen et al., 2005; Schilthuizen, 2011), speciation (Schilthuizen et al., 2006; Schilthuizen et al., 2012), and conservation biology (Clements et al., 2006; Clements et al., 2008; Schilthuizen et al., 2005). Although several families of non-pulmonate snails have featured prominently in these studies (in particular the Diplommatinidae and other cyclophoroids), the family Hydrocenidae (Neritimorpha) has so far been understudied. In this paper, we make a start with a first conchological and molecular characterisation of a surprisingly diverse group of species in the genus *Georissa* Blanford 1864.

The genus *Georissa* Blanford 1864 is characterized by a calcareous, rounded to ovate concentric, paucispiral operculum, with a calcareous peg emerging from the inner surface (Bandel, 2008; Thompson and Dance, 1983; Vermeulen et al., 2015). The shell is small, dextral, conical, and frequently presents conspicuous radial and spiral sculpture. The studies by Thompson and Dance (1983) and Vermeulen et al. (2015) showed that the Bornean *Georissa* are between 0.7 and 4.0 mm in adult shell height. The protoconch is usually distinctly hemi-spherically shaped, distinct in microsculpture and distinguishable from the post-embryonic whorls. The internal walls (some refer to these as septa) are resorbed, and the remaining wall ends more than one whorl before reaching the aperture; resorption also leads to excavation of the columella (Thompson and Dance, 1983; Bandel, 2008). The evolutionary causes for this internal shell restructuring remain to be studied. The snails are often found in moderate to high densities on rocks, especially limestone rocks, where they apparently forage moss, algae, and lichens (Berry, 1966). Cave-adapted species may forage on bacterial films (Schilthuizen et al., 2012).

Previous taxonomic treatments of Bornean *Georissa* (Godwin-Austen, 1889; Gredler, 1902; Haase and Schilthuizen, 2007; Smith, 1893, 1895; Thompson and Dance, 1983; van Benthem-Jutting, 1966, Vermeulen and Junau, 2007; Vermeulen et al., 2015) revealed that shell shape and size, as well as sculptural patterns on the whorls are important characters for species delimitation. Given the small size of these shells, great benefits can be had from the use of scanning electron microscopy and X-ray microtomography, which are able to show detailed microscopic sculpture patterns and the inner part of the shell.

Since the overview presented by Thompson and Dance (1983), no revisions have been made for the Bornean *Georissa*, although recently, several new Bornean *Georissa* have been described, i.e., *Georissa filiasaulae* Haase and Schilthuizen 2007, *Georissa pachysoma* Vermeulen and Junau 2007, *Georissa leucococca* and *Georissa nephrostoma* Vermeulen, Liew and Schilthuizen 2015. Our new studies of the *Georissa* of Malaysian Borneo reveal additional, previously unrecognized diversity, which warrants a series of revisions of the various species groups. In the present paper, we first address a group of species that we here call the "scaly group", chiefly consisting of species with conspicuous scale-like sculpture on the shell.

We present detailed species descriptions for a total of 13 Bornean *Georissa* from the “scaly group”, of which six species are new to science, namely: *Georissa anyiensis* sp. n., *Georissa muluensis* sp. n., *Georissa bauensis* sp. n., *Georissa silaburensis* sp. n., *Georissa kinabatanganensis* sp. n., and *Georissa sepulutensis* sp. n.

Materials and Methods

Materials and fieldwork

We examined collection materials from: (1) Naturalis Biodiversity Center, Leiden – RMNH and ZMA, (2) Natural History Museum, London – NHMUK, (3) Borneensis Collection, Universiti Malaysia Sabah – BORN, (4) Zoology Museum, Universiti Malaysia Sarawak – MZU, and (5) Jaap Vermeulen (personal collection) – JJV. In addition to these available data, we did fieldwork at limestone outcrops in Malaysian Borneo between September 2015 and May 2017. Manual searches were carried out to collect living and empty shells of *Georissa* on limestone walls and rocks, loose organic matter, and on/under living leaves. The living *Georissa* were directly stored in sample tubes containing ~96% ethanol. Ca. 5 litres of soil and leaf litter were sampled at each sampling location to collect empty shells by flotation (Vermeulen and Whitten, 1998). The holotypes, paratypes and all of the collected materials were deposited at the Zoology Museum (Universiti Malaysia Sarawak, Kota Samarahan, Malaysia), Borneensis Collection (Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia), and Naturalis Biodiversity Center (Leiden, The Netherlands).

Morphological analysis

Microscopy. Shells were observed with a Zeiss SteREO Microscope Discovery V20. The images of examined individuals were captured by AxioCamMRc5, Zeiss PlanApo S 1.0× FWD 60.0mm lenses. A complementary software package of the camera AxioVision Special Edition 64-bit version 4.9.1.0 was used for shell measurements, namely, shell height, shell width, aperture height, and aperture width, at ×30 -×60 magnification. The measurements of "scaly" *Georissa* were carried out following the shell measurement method of Vermeulen and Whitten (1998). **Scanning electron microscopy**. A representative adult shell for each species was cleaned using sodium hypochloride, dried, and sputter-coated with Pd/Pt coating agent before detailed examination with a JEOL JSM-6480LV scanning electron microscope (SEM). We obtained SEM images of the entire shell in top view and apertural view (including clear view of the sculpture), side and top views of the protoconch and the spire. **Micro-computed tomography**. The micro-computed tomography (μCT) scanning was carried out with an Xradia 520

Versa X-ray Microscope using accompanying software Zeiss Xradia Versa (11.1.6315). The X-ray images from the scanning (ca. 950 layers of images in TIFF format) were reconstructed into composite 3D images of the shells using software Scout-and-Scan^{TM} Control System Reconstructor (11.1.5707.17179). All shell materials were scanned in air medium at 80/7 voltage/power (kW/P) using objective lens unit 4 in 180° rotation. Detailed scanning parameters for each species are summarized in **Supplementary material 1**. We used reconstructed 3D images of representative adult shells of each species from µCT scanned data to examine the internal characters, including the operculum and its peg. We conducted 3D image reconstruction to preserve the original structure of the shells and avoid unintentional shell destruction. The 3D image analysis of the shells was carried out with Avizo ver. 9.2.0, FEI Company.

Molecular analysis

DNA extraction. Genomic DNA was extracted from 127 individuals of *Georissa* using the Qiagen DNeasy Blood and Tissue kit, following the manufacturer's protocol. Prior to the DNA extraction, the shells were removed and the entire soft tissue was used in the DNA extraction procedure. **DNA amplification**. We amplified two mtDNA regions, namely *16S* and *CO1*. DNA amplifications were conducted on a BIO-RAD C1000 Touch^{TM} Thermal Cycler. For the *16S* gene, a fragment of 422-464 bp was amplified using primer pair LR-J-12887 5'-CCGGTCTGAACTCAGATCACGT-3' (forward) and LR-N-13398 5'-CGCCTGTTTAACAAAAACAT-3' (reverse) (Schilthuizen et al., 2005) in 25.0 µL reaction volume, containing: 1.0 µL undiluted DNA template, 15.0 µL mQ (milli-Q, ultrapure water), 2.5 µL PCR chlorine buffer 10×, 2.5 µL $MgCl_2$ 25.0 mM, 0.25 µL BSA 100 mM, 1.0 µL forward primer 10 pmol/ µL, 1.0 µL reverse primer 10 pmol/ µL, 1.5 µL dNTPs 2.5 mM, and 0.25 µL Taq 5.0 U/ µL. The amplification was carried out with the following cycling protocol: initial denaturation at 95°C for 5 min, 36 cycles (of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min), and a final extension at 72°C for 5 min. A 546-603 bp fragment of *CO1* was amplified using primer pair LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' (forward) and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (reverse) (Folmer et al., 1994) in 25.0 µL reaction volume, containing: 1.0 µL DNA template, 16.8 µL mQ, 2.5 µL PCR chlorine buffer 10×, 1.0 µL $MgCl_2$ 25.0 mM, 1.0 µL BSA

100 mM, 1.0 µL forward primer 10 pmol/ µL, 1.0 µL reverse primer 10 pmol/ µL, 0.5 µL dNTPs 2.5 mM, and 0.25 µL Taq 5.0 U/ µL. The amplification was carried out with the following cycling protocol: initial denaturation at 94°C for 4 min, 40 cycles (of denaturation at 94°C for 15 s, annealing at 50°C for 30 s, extension at 72°C for 40 s), and a final extension at 72°C for 5 min. The unsuccessful amplication of *CO1* and *16S* genes were excluded in further phylogenetic analysis that used concatenated sequence alignment of both genes. **Sequencing**. The PCR products were Sanger sequenced in both directions at BaseClear B.V. (Leiden, The Netherlands) on the ABI3730XL sequencer from Life Technologies. All new *16S* mtDNA sequences used in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and *CO1* mtDNA sequences were deposited in GenBank via BOLD (http://boldsystems.org/), under accession numbers as listed in **Table 2.1**.

Sequence alignment and phylogenetic analyses

Sequence data and alignement. A total of 12 ingroup taxa of "scaly group" *Georissa* including an outgroup taxon, *Georissa gomantongensis* Smith 1893, were used for phylogenetic analyses (using a much larger hydrocenid taxon sampling, to be published elsewhere, we confirmed that *G. gomantongensis* indeed branches off basally to the scaly group). We added another six *16S* mtDNA sequences from GenBank, representing *Georissa saulae* (van Benthem-Jutting, 1966) (GenBank accession no. AY547380, AY547381, AY547384, and AY547385) and *Georissa sepulutensis* sp. n. (GenBank accession no. AY547387 and AY548388). We conducted our phylogenetic analyses based on 128 sequences for *16S* and 91 sequences for *CO1*. The forward and reverse nucleotide reads were assembled using *de novo* Geneious 10.0.7 assembler, manually checked and edited, and later aligned using default settings of MUSCLE alignment (Edgar, 2004). **Phylogenetic inference**. For *CO1* sequences, we selected the invertebrate mitochondrial genetic code at the second reading frame. Ambiguous nucleotide sequence ends were trimmed and removed prior to further analysis. ModelFinder (Kalyaanamoorthy et al., 2017) was used to select the most appropriate model, based on the corrected Akaike Information Creterion (AICc) for partial *16S* and *CO1* mtDNA genes. The best fitting models were TIM3+F+I+G4 for *16S* and TIM2+F+I+G4 for *CO1*. **Phylogenetic analysis**. Maximum likelihood analysis was performed using IQ-TREE 1.6.3 (Nguyen et al., 2015) on concatenated *16S* and *CO1* sequences of “scaly” *Georissa* using TIM3+F+I+G4 as the nucleotide substitution model with ultrafast bootstrapping (1000 replicates; Hoang et al., 2017). Bayesian Inference was performed using MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001) with the next closest nucleotide substitution model, GTR+I+G using the following MCMC settings: Chain length = 1,100,000 generations, heated chain = 4, subsampling frequency = one tree for each 200 generations, burn-in length = 100,000, and chain temperature = 0.2.

Table 2.1 List of specimens used in molecular analyses.

No.	Species	Voucher No.	Species name_sequence origin_location Town/District/Division, State. GPS coordinate	GenBank Accession No.	
				16S	*CO1*
1	*Georissa gomantongensis* Smith 1893	BOR/MOL 7389	G.gomantongensis_KPH01833.01_Kinabatangan Kinabatangan Valley, Sabah. 05°30.913’N, 118°16.889’E	MG982259	MH033876
2	*Georissa gomantongensis* Smith 1893	BOR/MOL 7389	G.gomantongensis_KPH01833.02_Kinabatangan Kinabatangan Valley, Sabah. 05°30.913’N, 118°16.889’E	MG982260	MH033875
3	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 2663-2667 (Schilthuizen et al., 2012)	G.saulae_AY547385_Sinobang Batu Sinobang, Sabah. 04°48.040’N, 116°37.035’E	AY547385	n.a.
4	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 2663-2667 (Schilthuizen et al., 2012)	G.saulae_hapA_AY547380_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	AY547380	n.a.
5	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 2663-2667 (Schilthuizen et al., 2012)	G.saulae_hapB_AY547381_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	AY547381	n.a.
6	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 2663-2667 (Schilthuizen et al., 2012)	G.saulae_hapC_AY547384_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	AY547384	n.a.
7	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 3493	G.saulae_ZI003_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	MG982261	n.a.
8	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 3493	G.saulae_KPH00181.02_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	MG982267	n.a.
9	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 12770	G.saulae_Sau-001_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410’N, 116°36.040’E	MG982262	n.a.

10	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 12770	G.saulae_Sau-002_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410’N, 116°36.040’E	MG982263	n.a.
11	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 12770	G.saulae_Sau-003_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410’N, 116°36.040’E	MG982264	n.a.
12	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 12770	G.saulae_Sau-004_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410’N, 116°36.040’E	MG982265	n.a.
13	*Georissa saulae* (van Benthem Jutting 1966)	BOR/MOL 12770	G.saulae_Sau-005_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410’N, 116°36.040’E	MG982266	n.a.
14	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.09	G.hosei_A001_Tongak Bukit Tongak, Bidi, Bau/Jambusan, Sarawak. 01°22.670’N, 110°08.325’E	MG982327	n.a.
15	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.09	G.hosei_A002_Tongak Bukit Tongak, Bidi, Bau/Jambusan, Sarawak. 01°22.670’N, 110°08.325’E	MG982331	MH033908
16	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.09	G.hosei_A003_Tongak Bukit Tongak, Bidi, Bau/Jambusan, Sarawak. 01°22.670’N, 110°08.325’E	MG982330	n.a.
17	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.09	G.hosei_A004_Tongak Bukit Tongak, Bidi, Bau/Jambusan, Sarawak. 01°22.670’N, 110°08.325’E	MG982329	MH033907
18	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.09	G.hosei_A005_Tongak Bukit Tongak, Bidi, Bau/Jambusan, Sarawak. 01°22.670’N, 110°08.325’E	MG982328	n.a.
19	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.09	G.hosei_A006_Tongak Bukit Tongak, Bidi, Bau/Jambusan, Sarawak. 01°22.670’N, 110°08.325’E	MG982326	n.a.

20	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C001_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982339	MH033904
21	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C002_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982338	MH033905
22	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C003_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982341	MH033902
23	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C004_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982340	MH033903
24	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C005_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982337	n.a.
25	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C006_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982336	MH033906
26	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C007_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982335	n.a.
27	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C008_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982334	n.a.

28	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C009_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. 01°24.050’N, 110°11.197’E	MG982333	n.a.
29	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.04	G.hosei_C0010_Liak Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak. N 01°24.050", E 110°11.197"	MG982332	n.a.
30	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.08	G.hosei_D001_Siboyuh Bukit Siboyuh, Kampung Skiat Baru, Jambusan, Sarawak. 01°22.909’N, 110°11.695’E	MG982346	MH033900
31	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.08	G.hosei_D002_Siboyuh Bukit Siboyuh, Kampung Skiat Baru, Jambusan, Sarawak. 01°22.909’N, 110°11.695’E	MG982342	MH033901
32	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.08	G.hosei_D003_Siboyuh Bukit Siboyuh, Kampung Skiat Baru, Jambusan, Sarawak. 01°22.909’N, 110°11.695’E	MG982345	MH033898
33	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.08	G.hosei_D004_Siboyuh Bukit Siboyuh, Kampung Skiat Baru, Jambusan, Sarawak. 01°22.909’N, 110°11.695’E	MG982344	MH033899
34	*Georissa hosei* Godwin-Austen 1889	MZU/MOL 16.08	G.hosei_D006_Siboyuh Bukit Siboyuh, Kampung Skiat Baru, Jambusan, Sarawak. 01°22.909’N, 110°11.695’E	MG982343	n.a.
35	*Georissa anyiensis* sp. n.	MZU/MOL 17.50	G.anyiensis_BSP2-01_Bukit Sarang Plot 2, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak.	MG982271	MH033929

			02°39.325’N, 113°02.432’E		
36	*Georissa anyiensis* sp. n.	MZU/MOL 17.50	G.anyiensis_BSP2-02_Bukit Sarang Plot 2, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak. 02°39.325’N, 113°02.432’E	MG982269	MH033930
37	*Georissa anyiensis* sp. n.	MZU/MOL 17.50	G.anyiensis_BSP2-03_Bukit Sarang Plot 2, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak. 02°39.325’N, 113°02.432’E	MG982268	MH033928
38	*Georissa anyiensis* sp. n.	MZU/MOL 17.50	G.anyiensis_BSP2-04_Bukit Sarang Plot 2, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak. 02°39.325’N, 113°02.432’E	MG982270	n.a.
39	*Georissa anyiensis* sp. n.	MZU/MOL 17.51	G.anyiensis_BSP11-01_Bukit Sarang Plot 11, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak. 02°39.325’N, 113°02.432’E	n.a.	MH033926
40	*Georissa anyiensis* sp. n.	MZU/MOL 17.51	G.anyiensis_BSP11-02_Bukit Sarang Plot 11, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak. 02°39.325’N, 113°02.432’E	MG982278	MH033927
41	*Georissa anyiensis* sp. n.	MZU/MOL 17.51	G.anyiensis_BSP11-03_Bukit Sarang Plot 11, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak. 02°39.325’N, 113°02.432’E	MG982280	MH033924
42	*Georissa anyiensis* sp. n.	MZU/MOL 17.51	G.anyiensis_BSP11-04_Bukit Sarang Plot 11, Bukit Lebik at Bukit Sarang, Bintulu, Sarawak. 02°39.325’N, 113°02.432’E	MG982279	MH033925
43	*Georissa anyiensis* sp. n.	MZU/MOL 17.60	G.anyiensis_BSP22-01_Bukit Sarang	MG982272	n.a.

			Plot 22, Bukit Anyi at Bukit Sarang, Bintulu, Sarawak. 02°39.252’N, 113°02.723’E		
44	*Georissa anyiensis* sp. n.	MZU/MOL 17.60	G.anyiensis_BSP22-02_Bukit Sarang Plot 22, Bukit Anyi at Bukit Sarang, Bintulu, Sarawak. 02°39.252’N, 113°02.723’E	MG982273	MH033931
45	*Georissa anyiensis* sp. n.	MZU/MOL 17.60	G.anyiensis_BSP22-03_Bukit Sarang Plot 22, Bukit Anyi at Bukit Sarang, Bintulu, Sarawak. 02°39.252’N, 113°02.723’E	MG982274	MH033933
46	*Georissa anyiensis* sp. n.	MZU/MOL 17.60	G.anyiensis_BSP22-04_Bukit Sarang Plot 22, Bukit Anyi at Bukit Sarang, Bintulu, Sarawak. 02°39.252’N, 113°02.723’E	MG982275	MH033934
47	*Georissa anyiensis* sp. n.	MZU/MOL 17.60	G.anyiensis_BSP22-05_Bukit Sarang Plot 22, Bukit Anyi at Bukit Sarang, Bintulu, Sarawak. 02°39.252’N, 113°02.723’E	MG982276	MH033935
48	*Georissa anyiensis* sp. n.	MZU/MOL 17.60	G.anyiensis_BSP22-06_Bukit Sarang Plot 22, Bukit Anyi at Bukit Sarang, Bintulu, Sarawak. 02°39.252’N, 113°02.723’E	MG982277	MH033932
49	*Georissa muluensis* sp. n.	MZU/MOL 17.31	G.muluensis_LGG-01_Mulu Plot 1, Lagang Cave, Mulu National Park, Mulu, Sarawak. 04°03.060’N, 114°49.372’E	MG982288	MH033893
50	*Georissa muluensis* sp. n.	MZU/MOL 17.31	G.muluensis_LGG-02_Mulu Plot 1, Lagang Cave, Mulu National Park, Mulu, Sarawak. 04°03.060’N, 114°49.372’E	MG982285	MH033891

51	*Georissa muluensis* sp. n.	MZU/MOL 17.31	G.muluensis_LGG-03_Mulu Plot 1, Lagang Cave, Mulu National Park, Mulu, Sarawak. 04°03.060'N, 114°49.372'E	MG982286	MH033892
52	*Georissa muluensis* sp. n.	MZU/MOL 17.31	G.muluensis_LGG-04_Mulu Plot 1, Lagang Cave, Mulu National Park, Mulu, Sarawak. 04°03.060'N, 114°49.372'E	MG982287	MH033890
53	*Georissa hadra* Thompson and Dance 1983	MZU/MOL 17.32	G.hadra_LC-01_Mulu Lang Cave, Mulu N.P., Mulu, Sarawak. 04°01.490'N, 114°49.482'E	MG982284	MH033896
54	*Georissa hadra* Thompson and Dance 1983	MZU/MOL 17.32	G.hadra_LC-02_Mulu Lang Cave, Mulu N.P., Mulu, Sarawak. 04°01.490'N, 114°49.482'E	MG982282	MH033897
55	*Georissa hadra* Thompson and Dance 1983	MZU/MOL 17.32	G.hadra_LC-03_Mulu Lang Cave, Mulu N.P., Mulu, Sarawak. 04°01.490'N, 114°49.482'E	MG982281	MH033894
56	*Georissa hadra* Thompson and Dance 1983	MZU/MOL 17.32	G.hadra_LC-04_Mulu Lang Cave, Mulu N.P., Mulu, Sarawak. 04°01.490'N, 114°49.482'E	MG982283	MH033895
57	*Georissa kobelti* Gredler 1902	MZU/MOL 17.36	G.kobelti_TC-01_Niah Trade Cave, Niah National Park, Niah, Sarawak. 03°49.137'N, 113°46.860'E	MG982296	MH033886
58	*Georissa kobelti* Gredler 1902	MZU/MOL 17.36	G.kobelti_TC-02_Niah Trade Cave, Niah National Park, Niah, Sarawak. 03°49.137'N, 113°46.860'E	MG982295	MH033889
59	*Georissa kobelti* Gredler 1902	MZU/MOL 17.36	G.kobelti_TC-03_Niah Trade Cave, Niah National Park, Niah, Sarawak. 03°49.137'N, 113°46.860'E	MG982293	MH033887
60	*Georissa kobelti* Gredler 1902	MZU/MOL 17.36	G.kobelti_TC-04_Niah Trade Cave, Niah National Park, Niah, Sarawak.	MG982294	MH033888

			03°49.137’N, 113°46.860’E		
61	*Georissa kobelti* Gredler 1902	MZU/MOL 17.38	G.kobelti_KJ1-01_Baram Plot 1, Bukit Kaijin, Baram, Sarawak. 03°41.753’N, 114°27.555’E	MG982290	MH033882
62	*Georissa kobelti* Gredler 1902	MZU/MOL 17.38	G.kobelti_KJ1-02_Baram Plot 1, Bukit Kaijin, Baram, Sarawak. 03°41.753’N, 114°27.555’E	MG982289	MH033883
63	*Georissa kobelti* Gredler 1902	MZU/MOL 17.38	G.kobelti_KJ1-03_Baram Plot 1, Bukit Kaijin, Baram, Sarawak. 03°41.753’N, 114°27.555’E	MG982292	MH033885
64	*Georissa kobelti* Gredler 1902	MZU/MOL 17.38	G.kobelti_KJ1-04_Baram Plot 1, Bukit Kaijin, Baram, Sarawak. 03°41.753’N, 114°27.555’E	MG982291	MH033884
65	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_PC-01_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982301	MH033965
66	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_PC-02_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982300	MH033878
67	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_PC-03_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982297	MH033877
68	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_PC-04_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982298	MH033954
69	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_GC-01_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982299	MH033879
70	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_GC-02_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982302	MH033880

71	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_GC-03_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982304	n.a.
72	*Georissa niahensis* Godwin-Austen 1889	MZU/MOL 17.25	G.niahensis_GC-04_Niah Painted Cave, Niah National Park, Niah, Sarawak. 03°48.688’N, 113°47.250’E	MG982303	MH033881
73	*Georissa silaburensis* sp. n.	MZU/MOL 17.05	G.silaburensis_SIG3-01_Silabur Plot 3, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982323	MH033949
74	*Georissa silaburensis* sp. n.	MZU/MOL 17.05	G.silaburensis_SIG3-03_Silabur Plot 3, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982324	MH033948
75	*Georissa silaburensis* sp. n.	MZU/MOL 17.05	G.silaburensis_SIG3-05_Silabur Plot 3, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982325	MH033944
76	*Georissa silaburensis* sp. n.	MZU/MOL 17.06	G.silaburensis_SIG4-01_Silabur Plot 4, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982320	MH033945
77	*Georissa silaburensis* sp. n.	MZU/MOL 17.06	G.silaburensis_SIG4-03_Silabur Plot 4, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982321	MH033952
78	*Georissa silaburensis* sp. n.	MZU/MOL 17.06	G.silaburensis_SIG4-06_Silabur Plot 4, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982322	MH033951
79	*Georissa silaburensis* sp. n.	MZU/MOL 17.07	G.silaburensis_SIG5-07_Silabur Plot 5, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982316	MH033946
80	*Georissa silaburensis* sp. n.	MZU/MOL 17.07	G.silaburensis_SIG5-08_Silabur Plot 5, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982317	MH033950
81	*Georissa silaburensis* sp. n.	MZU/MOL 17.07	G.silaburensis_SIG5-09_Silabur	MG982318	n.a.

			Plot 5, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E		
82	*Georissa silaburensis* sp. n.	MZU/MOL 17.07	G.silaburensis_SIG5-10_Silabur Plot 5, Gunong Silabur, Serian, Sarawak. 00°57.285’N, 110°30.228’E	MG982319	MH033947
83	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B002_WCave Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E	MG982306	MH033937
84	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B003_WCave Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E	n.a.	MH033938
85	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B004_WCave Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E	MG982309	MH033936
86	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B005_WCave Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E	MG982307	n.a.
87	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B007_WCave Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E	MG982308	n.a.
88	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B008_WCave Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E	MG982311	MH033939
89	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B009_WCave	MG982305	n.a.

			Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E		
90	*Georissa bauensis* sp. n.	MZU/MOL 16.01	G.bauensis_B010_WCave Wind Cave Passage 3, Wind Cave National Park, Bau, Sarawak. 01°24.810’N, 110°08.175’E	MG982310	n.a.
91	*Georissa bauensis* sp. n.	MZU/MOL 16.03	G.bauensis_Q001_Ayub Gunong Podam, near Sg. Ayup, Kampung Bogag, Bau, Sarawak. 01°21.158’N, 110°03.577’E	MG982313	MH033942
92	*Georissa bauensis* sp. n.	MZU/MOL 16.03	G.bauensis_Q002_Ayub Gunong Podam, near Sg. Ayup, Kampung Bogag, Bau, Sarawak. 01°21.158’N, 110°03.577’E	MG982312	n.a.
93	*Georissa bauensis* sp. n.	MZU/MOL 16.03	G.bauensis_Q003_Ayub Gunong Podam, near Sg. Ayup, Kampung Bogag, Bau, Sarawak. 01°21.158’N, 110°03.577’E	MG982314	n.a.
94	*Georissa bauensis* sp. n.	MZU/MOL 16.03	G.bauensis_Q004_Ayub Gunong Podam, near Sg. Ayup, Kampung Bogag, Bau, Sarawak. 01°21.158’N, 110°03.577’E	n.a.	MH033941
95	*Georissa bauensis* sp. n.	MZU/MOL 16.03	G.bauensis_Q005_Ayub Gunong Podam, near Sg. Ayup, Kampung Bogag, Bau, Sarawak. 01°21.158’N, 110°03.577’E	n.a.	MH033940
96	*Georissa bauensis* sp. n.	MZU/MOL 16.03	G.bauensis_Q006_Ayub Gunong Podam, near Sg. Ayup, Kampung Bogag, Bau, Sarawak. 01°21.158’N, 110°03.577’E	MG982315	MH033943

97	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.11	G.pyrrhoderma_SO3-01_Silabur Plot Outside 3-1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982366	MH033913
98	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.11	G.pyrrhoderma_SO3-02_Silabur Plot Outside 3-1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982364	MH033914
99	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.11	G.pyrrhoderma_SO3-03_Silabur Plot Outside 3-1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982367	MH033915
100	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.11	G.pyrrhoderma_SO3-04_Silabur Plot Outside 3-1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982365	MH033916
101	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.22	G.pyrrhoderma_SIO4-01_Silabur Plot SIO4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982376	MH033918
102	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.22	G.pyrrhoderma_SIO4-02_Silabur Plot SIO4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982377	MH033920
103	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.22	G.pyrrhoderma_SIO4-03_Silabur Plot SIO4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982378	MH033917
104	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.22	G.pyrrhoderma_SIO4-04_Silabur Plot SIO4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982379	MH033919
105	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.22	G.pyrrhoderma_SIO4-05_Silabur Plot SIO4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982380	n.a.

106	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.13	G.pyrrhoderma_SIE1-01_Silabur Plot SIE1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982372	n.a.
107	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.13	G.pyrrhoderma_SIE1-02_Silabur Plot SIE1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982373	MH033922
108	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.13	G.pyrrhoderma_SIE1-03_Silabur Plot SIE1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982374	MH033923
109	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.13	G.pyrrhoderma_SIE1-04_Silabur Plot SIE1, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982375	MH033921
110	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.16	G.pyrrhoderma_SIE4-01_Silabur Plot SIE4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982368	MH033910
111	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.16	G.pyrrhoderma_SIE4-02_Silabur Plot SIE4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982369	MH033909
112	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.16	G.pyrrhoderma_SIE4-03_Silabur Plot SIE4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982370	MH033911
113	*Georissa pyrrhoderma* Thompson and Dance 1983	MZU/MOL 17.16	G.pyrrhoderma_SIE4-04_Silabur Plot SIE4, Gunong Silabur, Serian, Sarawak. 00°57.451’N, 110°30.207’E	MG982371	MH033912
114	*Georissa kinabatanganensis* sp. n.	BOR/MOL 7289	G.kinabatanganensis_KPH01720.01_Pangi Kinabatangan Valley, Pangi, Sabah. 05°32.291’N, 118°18.376’E	MG982348	MH033963
115	*Georissa kinabatanganensis* sp. n.	BOR/MOL 7289	G.kinabatanganensis_KPH01720.02_Pangi Kinabatangan Valley, Pangi, Sabah. 05°32.291’N, 118°18.376’E	MG982347	MH033962

116	*Georissa kinabatanganensis* sp. n.	BOR/MOL 7289	G.kinabatanganensis_KPH01720.03_Pangi Kinabatangan Valley, Pangi, Sabah. 05°32.291’N, 118°18.376’E	n.a.	MH033961
117	*Georissa kinabatanganensis* sp. n.	MZU/MOL 17.26	G.kinabatanganensis_K001_Keruak Keruak, near Kinabatangan river, Sandakan, Sabah. 05°32.291’N, 118°18.376’E	MG982349	MH033959
118	*Georissa kinabatanganensis* sp. n.	MZU/MOL 17.26	G.kinabatanganensis_K002_Keruak Keruak, near Kinabatangan river, Sandakan, Sabah. 05°31.385’N, 118°17.113’E	MG982351	MH033958
119	*Georissa kinabatanganensis* sp. n.	MZU/MOL 17.26	G.kinabatanganensis_K005_Keruak Keruak, near Kinabatangan river, Sandakan, Sabah. 05°31.385’N, 118°17.113’E	MG982350	MH033960
120	*Georissa sepulutensis* sp. n.	BOR/MOL 39	G.sepulutensis_KPH00176.01_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	MG982357	MH033957
121	*Georissa sepulutensis* sp. n.	BOR/MOL 39	G.sepulutensis_KPH00176.02_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	MG982356	n.a.
122	*Georissa sepulutensis* sp. n.	BOR/MOL 39	G.sepulutensis_KPH00181.01_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052’N, 116°36.016’E	MG982359	MH033956
123	*Georissa sepulutensis* sp. n.	BOR/MOL 12278	G.sepulutensis_Sca-002_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410’N, 116°36.040’E	MG982361	MH033964
124	*Georissa sepulutensis* sp. n.	BOR/MOL 12278	G.sepulutensis_Sca-003_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410’N, 116°36.040’E	MG982360	MH033955
125	*Georissa sepulutensis* sp. n.	BOR/MOL 12278	G.sepulutensis_Sca-004_Pungiton	MG982362	MH033953

			Sepulut Valley, Gua Pungiton, Sabah. 04°42.410'N, 116°36.040'E		
126	*Georissa sepulutensis* sp. n.	BOR/MOL 12278	G.sepulutensis_Sca-005_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.410'N, 116°36.040'E	MG982363	n.a.
127	*Georissa sepulutensis* sp. n.	BOR/MOL 39	G.sepulutensis_ZA004_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052'N, 116°36.016'E	MG982354	n.a.
128	*Georissa sepulutensis* sp. n.	BOR/MOL 39	G.sepulutensis_ZB003_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052'N, 116°36.016'E	MG982355	n.a.
129	*Georissa sepulutensis* sp. n.	BOR/MOL 39	G.sepulutensis_ZC003_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052'N, 116°36.016'E	MG982358	n.a.
130	*Georissa sepulutensis* sp. n.	RMNH/MOL 333905	G.sepulutensis_ZE003_Simbaluyon Sepulut Valley, Bukit Simbaluyon, Sabah. 04°43.200'N, 116°34.140'E	MG982352	n.a.
131	*Georissa sepulutensis* sp. n.	RMNH/MOL 333905	G.sepulutensis_ZE004_Simbaluyon Sepulut Valley, Bukit Simbaluyon, Sabah. 04°43.200'N, 116°34.140'E	MG982353	n.a.
132	*Georissa sepulutensis* sp. n.	BOR/MOL 39 (Schilthuizen et al., 2005)	G.sepulutensis_hapA_AY547387_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052'N, 116°36.016'E	AY547387	n.a.
133	*Georissa sepulutensis* sp. n.	BOR/MOL 39 (Schilthuizen et al., 2005)	G.sepulutensis_hapB_AY547388_Sanaron Sepulut Valley, Batu Sanaron, Sabah. 04°42.052'N, 116°36.016'E	AY547388	n.a.

Species delimitation and description

For species delimitation, we combined the data of molecular phylogenetic analyses and the assessments of the morphology. We aimed for monophyly in species, allowing paraphyly under certain circumstances (Schilthuizen and Gittenberger, 1996), but disallowing polyphyly. Only when we found morphological characters that could distinguish DNA-based clades or paraphyletic groups, did we consider such groups as potential species. Although many forms in *Georissa* are allopatric, we did have a number of cases where two forms occurred sympatrically without forming intermediates, which also aided in determining species status by application of the biological species concept (Mayr, 1942). General shell characters were further divided into detailed sub-characters exclusively for the descriptions of the representatives of the "scaly group” of Bornean *Georissa*. The assessed morphological characters follow the descriptions made by Godwin-Austen (1889), Gredler (1902), Haase and Schilthuizen (2007), Smith (1893, 1895), Thompson and Dance (1983), van Benthem-Jutting (1966), Vermeulen and Junau (2007), and Vermeulen et al. (2015). Note that color indications always refer to living or freshly dead specimens, as the color in older specimens usually degrades, with an exception for *Georissa scalinella* (van Benthem-Jutting, 1966), where only old collection specimens were available.

CO1 genetic divergence

In addition to the molecular phylogenetic and morphological assessment in our species delimitation, we conducted divergence analysis of partial *CO1* genes to provide additional information to assist in the species delimitation of “scaly” *Georissa*. Several other studies on species delimitation in gastropods have also used *CO1* mtDNA successfully (see Liew et al., 2014; Puillandre et al., 2012a, 2012b). Pairwise genetic distances of *CO1* sequences from 89 individuals were computed based on Kimura 2-parameter with MEGA v. 7.0.26 (Kumar et al., 2016). These comprised 11 species, including the six new species.

Web interface species delimitation using 16S mtDNA

We carried out two more approaches of web interface species delimitation to provide more insight in our species delimitation, namely, Automatic Barcode

Gap Discovery (ABGD) (http://wwwabi.snv.jussieu.fr/public/abgd/-abgdweb.html) (Puillandre et al., 2012a), and Poisson Tree Processes (PTP) (http://species.h-its.org/ptp/) (Zhang et al., 2013). ABGD analysis was carried out using *16S* mtDNA sequences of the "scaly group" *Georissa* (excluding the outgroup). The parameters were set to default. For PTP analysis, we used the *16S* gene tree generated from IQ-TREE (Nguyen et al., 2015). The parameters were set to default. Both ABGD and PTP analyses were conducted using mtDNA *16S* sequences and gene tree based on the available data of all studied taxa. ABGD aims to partition the species based on the barcode gap (Puillandre et al., 2012a), while PTP focuses on the branching event of a rooted phylogenetic tree (Zhang et al., 2013).

Results and discussion

Morphology and phylogenetic analyses

Our morphological and phylogenetic studies lead us to conclude that there are at least 13 species of "scaly group" *Georissa* currently existing in Malaysian Borneo (For detailed morphological species descriptions, see the species treatments under the **Taxonomy** section). For one of these, *Georissa scalinella* van Benthem Jutting 1966, DNA data are yet unavailable. Detailed conchological assessments of the "scaly group" show that at least two species, *Georissa bauensis* sp. n. and *Georissa hosei* Godwin-Austen 1889, are highly variable (both intra- and inter-populationally) with regard to the "scaly" shell microsculpture characters (see **Figure 2.1**). Due to the high inter- and intraspecific variation of these species, identification based on morphological characters alone could be problematic without prior knowledge of the shell variation within these species. Furthermore, species similar in shell habitus and scale characters, like *Georissa pyrrhoderma* Thompson and Dance 1983 and *Georissa sepulutensis* sp. n., often have character combinations that overlap with either *G. bauensis* or *G. hosei*. Therefore, for identification of "scaly group" specimens, we found that thorough conchological examination of the shells aided by molecular data is most reliable.

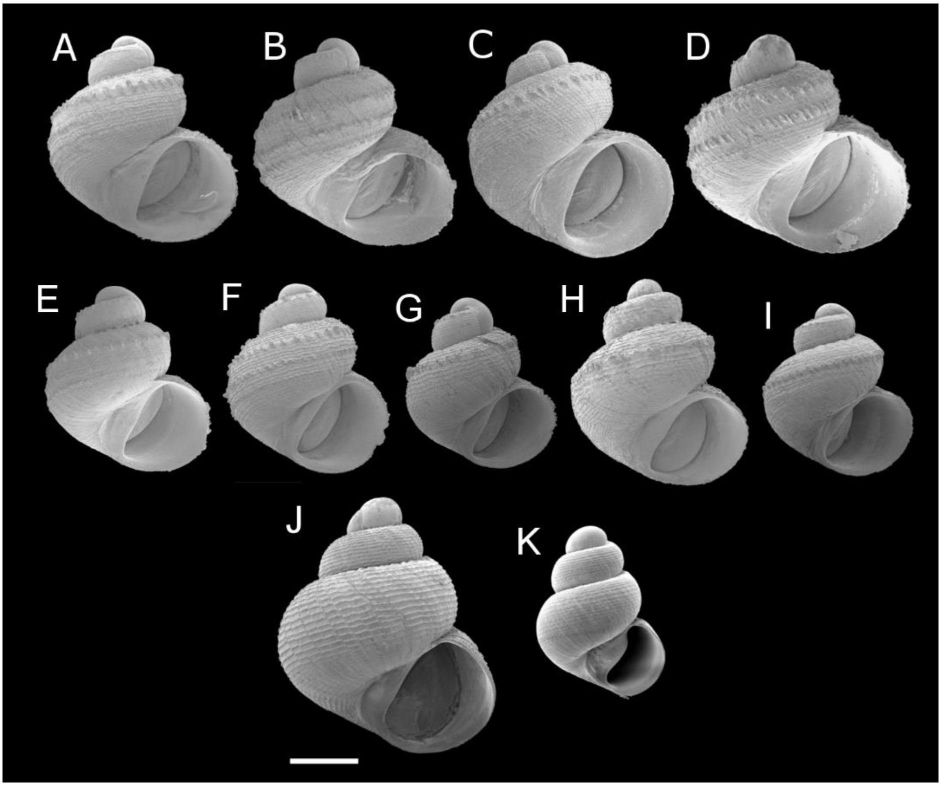

Figure 2.1 **A**-**D**. Intraspecific variation in shell shape and sculpture of *Georissa hosei* Godwin-Austen 1889. **E**-**G**. Intraspecific variation in shell shape and sculpture of *Georissa bauensis* sp. n. **H**. *Georissa pyrrhoderma* Thompson and Dance, 1983. **I**. *Georissa sepulutensis* sp. n. For comparison with the "scaly group", two additional species are shown that do not belong to the "scaly group", namely: **J**. *Georissa gomantongensis* Smith 1893 and **K**. *Georissa nephrostoma* Vermeulen et al 2015. Localities: **A** and **B**. Gunung Liak/Padang (Jambusan, Sarawak). **C**. Bukit Siboyuh (Jambusan, Sarawak). **D**. Bukit Tongak (Bau/Jambusan, Sarawak). **E** and **F**. Gunung Podam (Bau, Sarawak). **H**. Gunung Silabur (Serian, Sarawak). **I**. Batu Sanaron (Sanaron, Sabah). **J**. Gua Gomantong (Gomantong, Sabah). **K**. Keruak (Kinabatangan, Sabah) Scale: **A**-**K**. 500 µm.

Based on the molecular phylogenetic analyses of the “scaly group” *Georissa* we find multiple strongly supported monophyletic groups (bootstrap and posterior output values ranging from 89-100 and 97-100,) which correspond with subtly different conchologies (**Figure 2.2**). In contrast, *Georissa kobelti* Gredler 1902 is paraphyletic, and we treat this as a single species based on the conchological characters that support they are conspecific.

CO1 genetic divergence

Despite geographic proximity for some populations of morphologically highly similar forms, the *CO1* divergence analysis shows high genetic divergences (e.g., *G. bauensis* vs *G. hosei*, genetic divergence = 0.12). For some other species, the interspecific genetic divergence is lower, but such species may be surprisingly distinct in shell sculpture (e.g., *G. hadra* vs *G. muluensis*, genetic divergence = 0.07). As a consequence, we have sometimes given priority to genetic distinctness, sometimes to morphological distinctness in delimiting species, which means that intraspecific diversity may vary between species. For example, we found that *G. pyrrhoderma*, *G. hosei*, and *G. kobelti* are the three species to have the highest intraspecific divergence (0.06, 0.06 and 0.07, respectively) compared with the rest of the "scaly group", where all other species have an intraspecific divergence equal to or lower than 0.05 (see details in **Table 2.2**). Our study reveals that within group divergences of "scaly" *Georissa* do not exceed 0.07, while the divergences between all species pairs exceed 0.10, with the exception of *G. kinabatanganensis* vs *G. sepulutensis*, *G. bauensis* vs *G. silaburensis*, *G. hadra* vs *G. muluensis*, and *G. kobelti* vs *G. niahensis*.

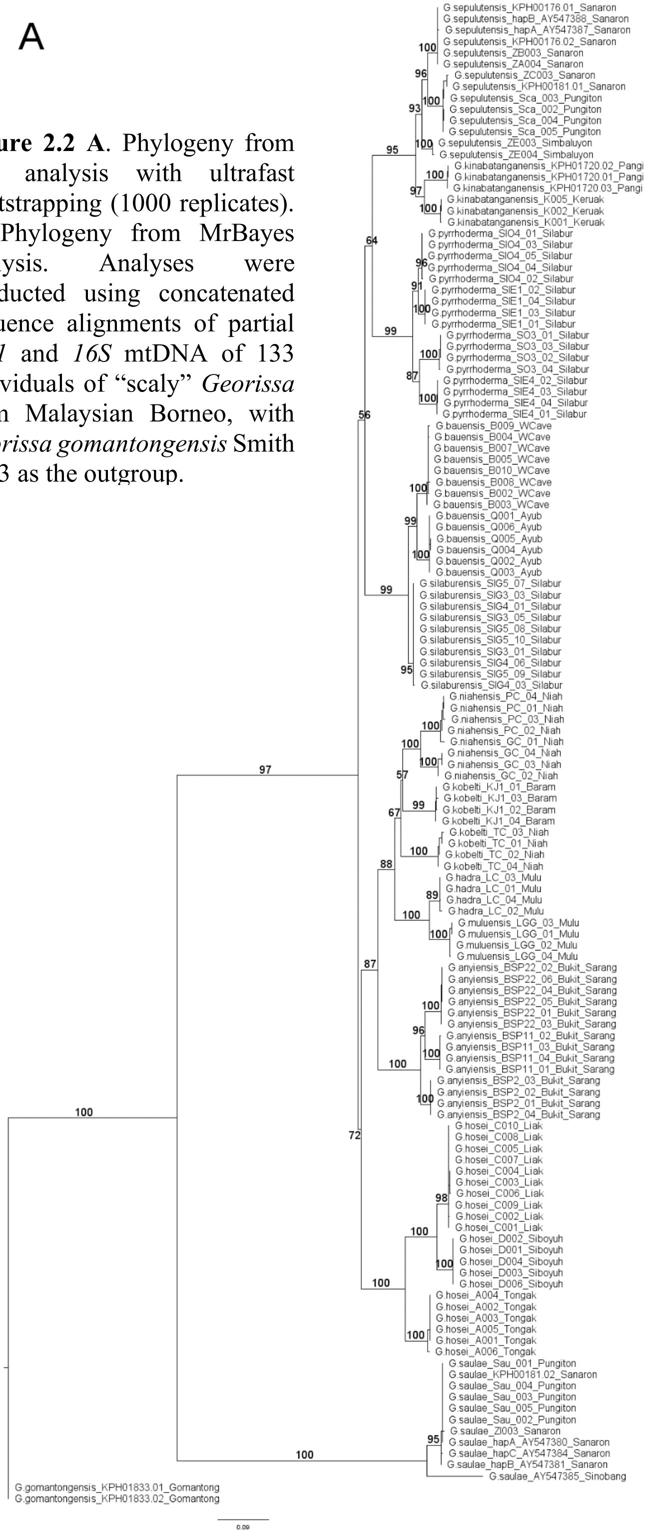

Figure 2.2 A. Phylogeny from ML analysis with ultrafast bootstrapping (1000 replicates). **B**. Phylogeny from MrBayes analysis. Analyses were conducted using concatenated sequence alignments of partial *CO1* and *16S* mtDNA of 133 individuals of “scaly” *Georissa* from Malaysian Borneo, with *Georissa gomantongensis* Smith 1893 as the outgroup.

Figure 2.2 *Cont…*

Web interface species delimitation using 16S mtDNA

To test to what extent automated procedures, based on genetic data alone, could reproduce our subjective species delimitation, we carried out ABGD and PTP analyses. ABGD recursive partition divided the “scaly group” *Georissa* into no more than six species at the lowest intraspecific divergence, while the highest divergence grouped all “scaly group” *Georissa* into a single species. The ABGD analysis further showed that partitioning into six species was due mostly to the separation of *G. saulae* into five different species while the rest of “scaly” *Georissa* were considered as a single species. This is possible due to the even higher intraspecific divergence of *16S* mtDNA of *G. saulae* compared to the rest of “scaly group” taxa (see **Supplementary material 2**).

While ABGD analysis underestimated the number of possible species in the “scaly group” of *Georissa*, PTP analysis based on maximum likelihood delimitation results devided the taxa in at least 15 possible species. The results from this species delimitation method therefore more closely match our preferred approach (in which we combined phylogenetic and morphometric assessment). The PTP analysis does, however, differ from our preferred delimitation at several crucial points. *G. saulae*, *G. kinabatanganensis*, *G. hosei*, *G. kobelti*, and *G. niahensis* are each split into two species, whereas the two sets of species composed of (i) *G. hadra* and *G. muluensis*, and (ii) *G. bauensis* and *G. silaburensis*, are each considered as a single species, which make another two species. Otherwise, PTP analysis resolves the same species as in our preferred resolution (see **Supplementary material 3**).

The results from *CO1* barcoding, ABGD, and PTP analyses reveals that objective species delimitation based solely on molecular data will not be successful for “scaly group” *Georissa*, at least if one wishes for the taxonomy to reflect morphology as well. Since most species are allopatric, and therefore the maintenance of species barriers can usually not be tested, we present our taxonomy as a compromise, which remains to be further tested by future workers.

Table 2.2 Intra- and inter-specific *CO1* sequence divergence of 11 species of scaly *Georissa*.

		Divergence within group	Number of specimens	*G. kinabatanganensis*	*G. sepulutensis*	*G. bauensis*	*G. silaburensis*	*G. anyiensis*	*G. pyrrhoderma*	*G. hosei*	*G. hadra*	*G. muluensis*	*G. kobelti*	*G. niahensis*
1	*G. kinabatanganensis*	0.05	6											
2	*G. sepulutensis*	0.02	5	0.06*										
3	*G. bauensis*	0.03	8	0.11	0.14									
4	*G. silaburensis*	<0.01	9	0.12	0.13	0.04*								
5	*G. anyiensis*	0.04	12	0.14	0.14	0.12	0.12							
6	*G. pyrrhoderma*	0.06	15	0.10	0.11	0.11	0.11	0.09						
7	*G. hosei*	0.06	11	0.14	0.13	0.12	0.12	0.10	0.12					
8	*G. hadra*	<0.01	4	0.18	0.18	0.16	0.15	0.12	0.15	0.14				
9	*G. muluensis*	<0.01	4	0.17	0.19	0.15	0.15	0.14	0.14	0.14	0.07*			
10	*G. kobelti*	0.07	8	0.11	0.13	0.12	0.12	0.09	0.10	0.09	0.10	0.09		
11	*G. niahensis*	0.04	7	0.13	0.15	0.13	0.14	0.10	0.12	0.10	0.11	0.13	0.05*	

*The average number of net base substitutions per site between species is equal or lower than 0.07, which is the highest number of base substitutions per site within a scaly species.

Systematics part

Class Gastropoda Cuvier, 1797
Family Hydrocenidae Troschel, 1856
Genus *Georissa* Blanford, 1864

“Scaly group”

We here define an informal group of 13 species of *Georissa* from Malaysian Borneo that are characterised by one or more spiral rows of scale-like sculpture. As far as they were known at the time, our “scaly group” corresponds to Thompson and Dance's (1983) “*hosei* group” + “*borneensis* group” p.p.

Conchological description of a generalised scaly group representative. *Protoconch*. Color (in living or freshly dead specimens): yellow, orange, red or brown. Sculpture: smooth, meshed, mixed or undefined. *Teleoconch*. Color (in living or freshly dead specimens): yellow, orange, red or brown. First whorl: convex, rounded to flat or angular. Subsequent whorl: convex, rounded, concave or tilted at the periphery, or flat, with well-impressed suture. Number of whorls: 2-3 ¼. Shell height (SH) (based on our conchological measurements of available studied materials stated in the methodology): 0.94-2.91 mm. Shell width (SW): 0.98-2.19 mm. Shell index (SI=SH/SW): 0.88-1.37. *Shell sculpture*. Radial sculpture: either absent or present. Growth lines: weak to strong. Spiral sculpture: absent or present; if present then weakly to strongly sculpted, continuous or discontinuous. Scales: between one and four spiral rows of vertical scales (any one of which may be more or less strongly pronounced than the others); scales can be minute to broad, low to acutely projecting. *Columella wall*. Smooth, translucent, and covering the umbilicus region. *Aperture*. Shape: oval to rounded, with straight to concave or convex parietal site, palatal edge either contiguous with or removed from the body whorl. Aperture height (AH): 0.50-1.33 mm. Aperture width (AW): 0.69-1.48 mm. Aperture index (AI=AH/AI): 0.65-1.02. *Peristome*. Simple, thickened inside, sharp toward the edge of the aperture. *Operculum*. Oval to rounded, with a peg facing inward, inner surface of the operculum has a small crater-like hole. Peg: straight or curved. The shell measurement of all measured "scaly group" *Georissa* are summarized in **Supplementary material 4**.

Anatomy. Haase and Schilthuizen (2007) described the anatomy of two closely related *Georissa*, viz. *G. saulae* and *G. filiasaulae*, and noted interspecific differences in radula, genital anatomy. Anatomical details of other scaly group representatives will be the focus of future studies and are not included in the present review.

Habitat and ecology. Members of the scaly group of *Georissa* live on limestone rocks, especially in wet and shaded environments. They are also found at lower density on dry limestone rocks, and occasionally on the limestone walls in cave systems (Haase and Schilthuizen, 2007).

Distribution. There are at least 9 species of this group distributed in Sarawak, and another 4 are distributed in Sabah (see **Figures 2.3** and 2.4). In the distribution maps, we combined the geographical coordinates of each species from the known previous fieldwork locations and the available data from the collection repositories. The distribution of "scaly group" *Georissa* was assigned based on the available locality data from the collection from NHMUK, RMNH, ZMA, BOR, ZMU, and JJV. Localities may contain Malay words, namely: Batu = rock; Bukit = hill; Gua = cave; Gunung = mountain. We provided two distribution maps (**Figure 2.3** and **2.4)** to avoid overlapping of species that co-occur at the same or nearby locations.

In the following systematic descriptions of "scaly" *Georissa*, the species are arranged based on the molecular phylogeny. *G. scalinella* (van Benthem-Jutting, 1966), for which no genetic data are available, is placed at the top of the list.

For the stacked images of the "scaly" *Georissa* (**Figure 2.5-2.17: A-C**), we decided not to remove the periostracum layers of the shells to retain the morphological characters of each species.

Since we needed fresh material to connect the morphology and molecular phylogenetics, we confined our study to Malaysian part of Borneo. We are aware that there might be species or populations in other parts of Borneo

(Kalimantan, Indonesian Borneo, and Brunei) which belong inside the "scaly group". However, we hope that our study will stimulate colleagues that study *Georissa* in Kalimantan or Brunei to compare their material with our analysis.

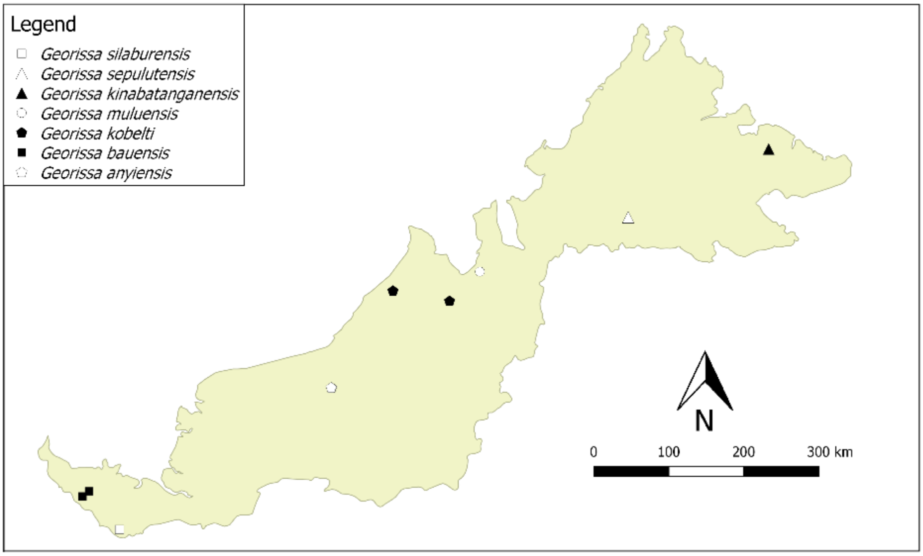

Figure 2.3 Distribution for 7 "scaly group" *Georissa* species in Malaysian Borneo (based on the materials examined from NHM, RMNH, ZMA, BORN, MZU, and JJV).

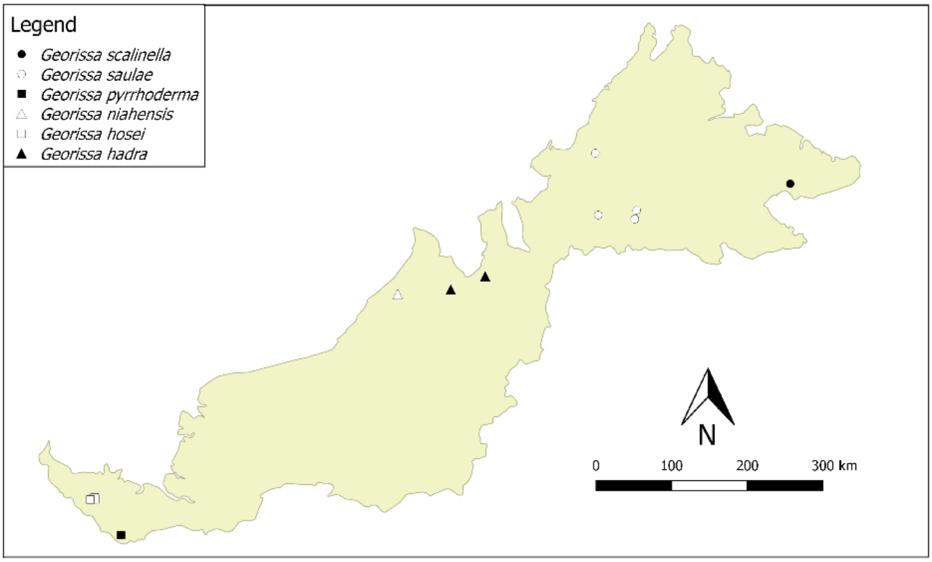

Figure 2.4 Distribution of 5 "scaly group" *Georissa* species in Malaysian Borneo (based on the materials examined from NHM, RMNH, ZMA, BORN, MZU, and JJV).

Georissa scalinella (van Benthem-Jutting 1966)

Hydrocena scalinella van Benthem-Jutting 1966: 39, fig. 1 (original description).

Hydrocena scalinella van Benthem-Jutting, Saul, 1967: 108.

Georissa scalinella (van Benthem-Jutting), Thompson and Dance, 1983: 119.

Georissa scalinella (van Benthem-Jutting), Phung et al., 2017: 68, fig. 8B.

Type locality. Lahad Datu Caves on Teck Guan Estate, Sabah.

Type material. *Holotype*. Lahad Datu Caves on Teck Guan Estate, Sabah: ZMA/MOLL 135736 (seen). *Paratypes*. Lahad Datu Caves on Teck Guan Estate, Sabah: ZMA/MOLL 135735 (seen), ZMA/MOLL 315596 (seen).

Description. *Protoconch*. Color: orange to red. Sculpture: smooth to meshed – semi oval mesh to undefined mesh pattern. Mesh width: 7-17 µm. *Teleoconch*. Color: orange. First whorl: flat at the shoulder. Subsequent whorls: flat above, slightly rounded below the periphery. Total number of whorls: 2 ¼-2 ½. SH: 1.56-1.80 mm, SW: 1.46-1.65 mm, SI: 1.03-1.15. *Shell sculpture*. Radial sculpture: absent, only weak to strong growth lines are visible. Spiral sculpture: present, and strongly sculpted, with continuous and discontinuous ribbing. Scales: a series of acute scales, low to highly projected, and regularly spaced. Intercept between growth lines and spiral ribbings form small pointed scale structures throughout the length of the body whorl. *Aperture*. Shape: oval., Basal side: rounded, angular at the columellar region. Parietal side: straight, palatal edge attached to the body whorl. AH: 0.78-0.94 mm, AW: 0.97-1.12 mm, AI: 0.75-0.89. *Holotype dimension*. SH: 1.88 mm, SW: 1.72 mm, AH: 0.84 mm, AW: 1.18 mm.

Cross diagnosis. *G. scalinella* has a series of scales at the shoulder. In habitus and scale characters, it resembles *G. pyrrhoderma* from Gunung Silabur, Sarawak. The angular shoulder and small scale-like nodular structure at the intersection of strong spiral ribbings and growth lines are diagnostic for *G. scalinella*.

Distribution. Known only from the type locality, Teck Guan Estate, Lahad Datu, Sabah, and also reported by Phung et al. (2017) at Pulau Tiga, Sandakan, Sabah. However, this may also refer to one of the other scaly group species from Sabah.

Discussion. *G. scalinella* was first described as *Hydrocena scalinella* van Benthem-Jutting 1966, before reclassified as *Georissa* by Thompson and Dance (1983). van Benthem-Jutting (1966) described *G. scalinella* as having strong spiral ribbing and multiple lines of scales.

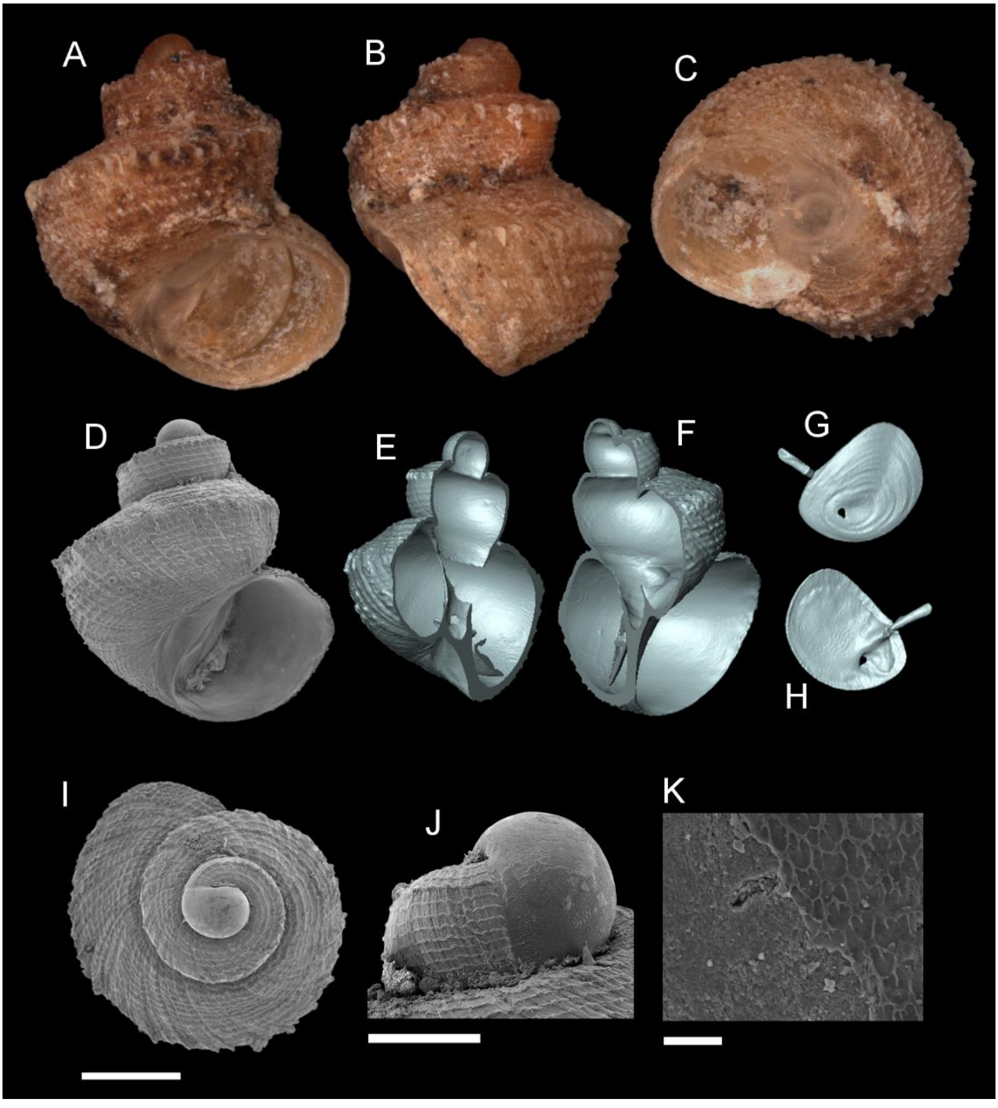

Figure 2.5 *Georissa scalinella* (van Benthem-Jutting 1966). **A**-**C**. Holotype: ZMA/MOL/ 135736. **D**-**K**. Paratypes: ZMA/MOLL 135735. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa saulae (van Benthem-Jutting 1966)

Hydrocena saulae van Benthem-Jutting 1966: 40, fig. 2 (original description).

Hydrocena saulae van Benthem-Jutting, Saul, 1967: 109.

Georissa saulae (van Benthem-Jutting), Thompson and Dance, 1983: 118, fig. 29, 53-54.

Georissa saulae (van Benthem-Jutting), Haase and Schilthuizen, 2007: 217, fig. 2.

Georissa saulae (van Benthem-Jutting), Clements et al., 2008: 2762.

Georissa saulae (van Benthem-Jutting), Schilthuizen et al., 2012: 278.

Georissa saulae (van Benthem-Jutting), Beron, 2015: 181.

Georissa saulae (van Benthem-Jutting), Phung et al., 2017: 68, fig. 8.

Georissa saulae (van Benthem-Jutting), Osikowski et al., 2017: 80.

Type locality. Malaysia, Borneo, Sabah, Laying cave, Keningau.

Type material. *Holotype*. Malaysia, Borneo, Sabah, Laying cave, Keningau: ZMA/MOLL 135731 (seen). *Paratypes*. Malaysia, Borneo, Sabah, Laying cave, Keningau: ZMA/MOLL 135598 (seen), ZMA/MOLL 135599 (seen).

Other material. Simbaluyon limestone hill, Sabah, Malaysia: RMNH/MOL 333913, RMNH/MOL 333919. Crocker Range National Park, Gua Laing, Keningau, Sabah (05°29.00’N, 116°08.00’E): RMNH/MOL 335180, ZMA/MOLL 315592, ZMA/MOLL 315593, JJV 1119. Sepulut Valley, Gua Pungiton, Sabah (04°42.41’N, 116°36.04’E): BOR/MOL 28, BOR/MOL 12770, JJV 7544. Sepulut valley, Gua Sanaron, Sabah (04°42.05’N, 116°36.01’E): BOR/MOL 29, BOR/MOL 32, BOR/MOL 3493, JJV 7660. Pinangah valley, Batu Urun (Bukit Sinobang), Sabah (04°48.40’N, 116°37.35’E): BOR/MOL 31, JJV 1144, JJV 5632, JJV 7993. Mahua, Sabah: BOR/MOL 33. Pun Batu, Sepulut, Sabah (04°45.00’N, 116°10.00’E): JJV 1268. Sepulut valley, Batu Punggul, Sabah: JJV 1904.

Description. *Protoconch*. Color: red to brown. Sculpture: meshed – ellipsoidal mesh pattern. Mesh width: 29-54 µm. *Teleoconch*. Color: brown to red. First whorl: convex to rounded. Subsequent whorls: convex to rounded. SH: 1.32-1.86 mm, SW: 1.14-1.48 mm, SI: 1.12-1.26. Total number of whorls: 2 ½-3 ¼. *Shell sculpture*. Radial sculpture: often present, when formed by vertical connections between corresponding scales on successive spiral ribs. These vertical connections, especially on the first whorls, form evenly spaced ribs that are raised when crossing a spiral rib. Spiral sculpture: present at the early teleoconch, subsequently becoming weaker, and later only short discontinuous lines are visible in between the radial sculptures. Scales: usually three or four discontinuous series of vertical, low to high-projecting scales, broad to pointed (only if the spiral series of scales are discontinuous). *Aperture*. Shape: rounded to slightly oval., Basal side: rounded, slightly angular before the columellar region. Parietal side: straight, connected to the palatal edge. AH: 0.58-0.83 mm, AW: 0.70-0.94 mm, AI: 0.76-0.92. *Holotype dimension*. SH: 1.60 mm, SW: 1.28 mm, AH: 0.66 mm, AW: 0.80 mm.

Cross diagnosis. *G. saulae* possesses clear diagnostic shell characters for distinction from other scaly *Georissa* species. *G. saulae* lacks a clear formation of spiral ribbing: although the spiral arrangement of the scales gives the impression of spiral sculpture, no underlying ribs are discernable. *G. scalinella, G. kinabatanganensis*, and *G. hosei*, on the other hand, have clear diagnostic spiral ribs. The shell whorls of *G. saulae* are broad but not as rapidly expanding as in *G. hosei, G. scalinella* or *G. kinabatanganensis*. It can also be distinguished from *G. scalinella* and *G. hosei* on the basis of a more elongate-conical shell shape and the aperture shape that is more rounded rather than oval.,

Distribution. The type locality of *G. saulae* is Laying cave, in the Crocker Range, Keningau, Sabah (a misspelling of Laing cave). Otherwise known from limestone outcrops in Sabah's interior, viz., Simbaluyon, Sinobang, Sanaron, and Pungiton, and also has been recorded from Mahua, Sabah, which is not a limestone area. Phung et al. (2017) also report it from Pulau Tiga, Sabah.

Molecular analysis. ML and Bayesian analyses show *G. saulae* (*16S*: n=11) as a monophyletic group with 100% BS and 100% PP. Schilthuizen et al. (2012) reported that *G. saulae* is a paraphyletic group from which emerges the cave-dwelling species *G. filiasaulae* (Haase and Schilthuizen, 2007), a fully unsculptured species that was not included in the present study. *G. saulae* + *G. filiasaulae* are sister to all other species in the scaly group (unpublished data).

Discussion. *G. saulae* was initially described as *Hydrocena saulae* (van Benthem-Jutting, 1966), then assigned to the genus *Georissa* by Thompson and Dance (1983). Thompson and Dance (1983) compared *G. saulae* with *G. scalinella*, and even suggested *G. saulae* might be a subspecies. In contrast, we find that *G. saulae* is a proper species with very distinct conchological characters, especially the presence of radial ribs on the shell, which makes it easy to identify. In some specimens from the entrance of the Batu Sanaron cave system, the vertical scales are spaced, and radial sculpture is weak. Such individuals presumably represent the hybrid zone with the cave-dwelling *G. filiasaulae* (Haase and Schilthuizen, 2007; Schilthuizen et al., 2012).

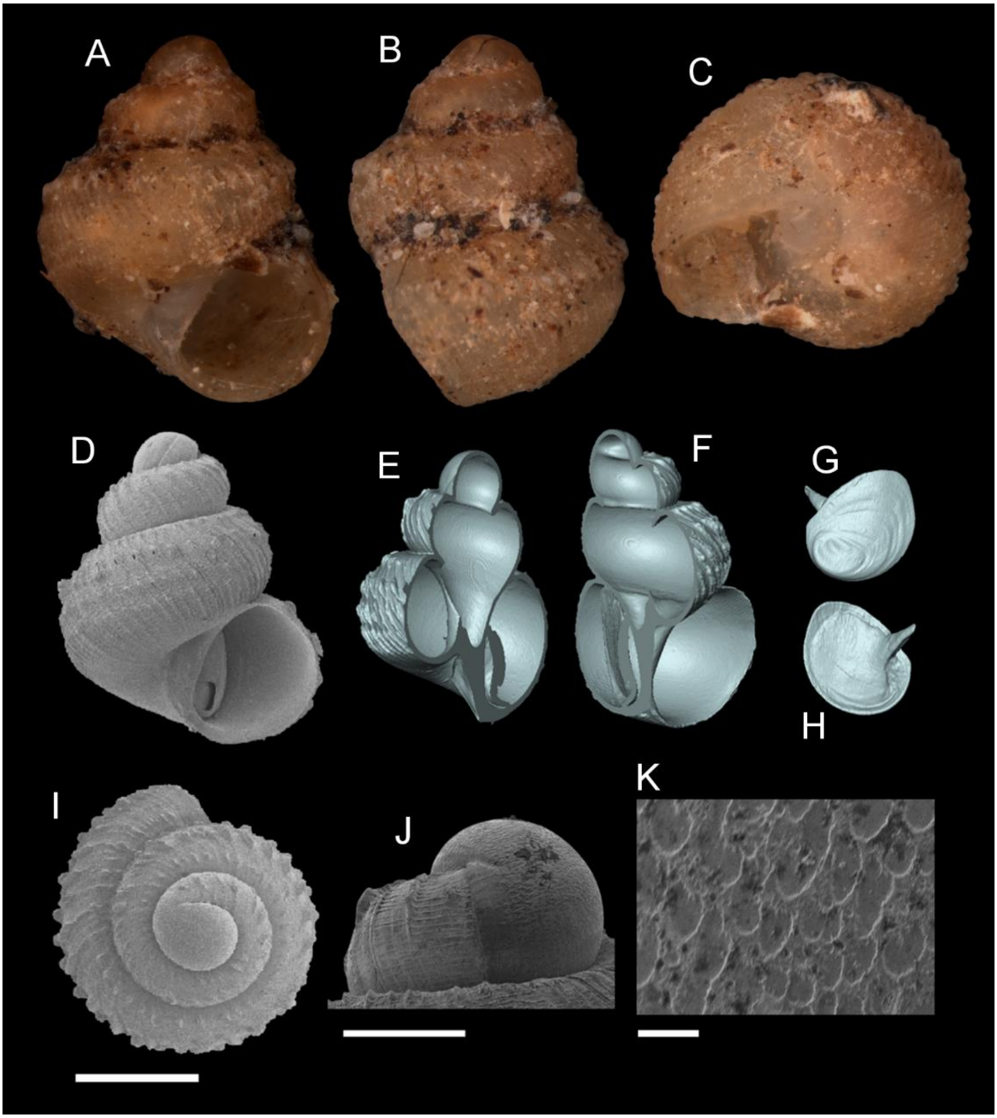

Figure 2.6 *Georissa saulae* (van Benthem-Jutting 1966), **A**-**C**. Holotype: ZMA/MOL 135599. **D**-**K**. BOR/MOL 3493. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa hosei Godwin-Austen 1889

Georissa hosei Godwin-Austen 1889: 353, fig. 11 plate XXXIX (original description).

Georissa hosei Godwin-Austen, Smith 1893: 351, fig. 27 plate XXV.

Georissa hosei Godwin-Austen, Thompson and Dance 1983: 116.

Type locality. Borneo (Unspecified).

Type material. *Lectotype* (Designated by Thompson and Dance, 1983). Borneo: NHMUK 1889.12.7.72 (glued on paper) (seen).

Other material. Jambusan, North Borneo: NHMUK 92.7.20.122, NHMUK 92.7.23.33-4. Gunung Liak/Padang, Kampung Skiat Baru, Jambusan, Sarawak (01°24.05’N, 110°11.19’E): MZU/MOL 16.04, MZU/MOL 16.05, MZU/MOL 16.06, MZU/MOL 16.07. Bukit Siboyuh, Kampung Skiat Baru, Jambusan, Sarawak (01°22.90’N, 110°11.69’E): MZU/MOL 16.08. Bukit Tongak, Bidi, Bau/Jambusan, Sarawak (01°22.67’N, 110°08.32’E): MZU/MOL 16.09.

Description. *Protoconch*. Color: red. Sculpture pattern: smooth. *Teleoconch*. Color: orange to red. First whorl: rounded or shouldered with flat surfaces above and below the shoulder. Subsequent whorls: convex to rounded; number of whorls: 2-2 ¼. SH: 1.06-1.55 mm, SW: 1.09-1.60 mm, SI: 0.94-1.12. *Shell sculpture*. Radial sculpture: absent, only weak growth lines. Spiral sculpture: present, weakly sculpted, continuous ribs, more prominent at the periphery. Scales: two to four series of low and broad vertical scales, regularly spaced, the upper scale series always the strongest, weaker series appear later at the spire, and the spaces between series are irregular. *Aperture*. Shape: oval., Basal side: rounded, angular at the columellar region. Parietal side: straight, palatal edge attached or removed at the body whorl. AH: 0.60-0.95 mm, AW: 0.80-1.16 mm, AI: 0.74-0.88.

Cross diagnosis. *G. hosei* has a diagnostic smooth protoconch. It possesses similar shell habitus and scale characters as *G. sepulutensis*, *G. pyrrhoderma*, and *G. kobelti*. However, the scales of *G. hosei* are rarely developed into large and acutely projected scales.

Distribution. Known from Gunung Liak/Padang and Bukit Siboyuh at Kampung Skiat Baru, Jambusan, and Bukit Tongak, in the area of Bau, which is close to Jambusan.

Molecular analysis. ML and Bayesian analyses shows that all *G. hosei* individuals (*16S*: n=21; *CO1*: n=11) group together in one clade with 100% BS and 100% PP, which is the sister group of all other scaly group species, except *G. saulae*.

Discussion. Godwin-Austen (1889), when he described the species, mentioned that the sides of the spire (whorls) are flat, which we find to be the case for the first whorl in our material (as well as in the lectotype). The exact type locality was not specified, but Smith (1893) reported that the specimens of *G. hosei* described by Godwin-Austen (1889) were from Jambusan, Sarawak. It has to be noted that *G. hosei* is highly variable in shell shape and sculpture, even within a local population. For example, material we collected at Gunung Liak/Padang have anything between two and four series of broad and low scales. Material from Bukit Tongak has three to four spiral threads with scales. Material from Bukit Siboyuh, finally, is brighter in color (orange), with only one or two spiral series of scales. These three limestone outcrops are all within the area of not more than 10 km radius. Thompson and Dance (1983) noted that *G. hosei* is widely distributed in Sarawak, and they give Baram, Marudi, Niah, Tatau, and Bukit Sarang as localities. However, as we elaborate in this paper, many of these populations are not conspecific with *G. hosei*. For example, the image of "*G. hosei*" provided by Thompson and Dance (1983) – UF 35919, from Batu Gading, Baram, appears conspecific to *G. kobelti*. Also, their "*G. hosei*" from Bukit Sarang we here describe as a *G. anyiensis* sp. n.

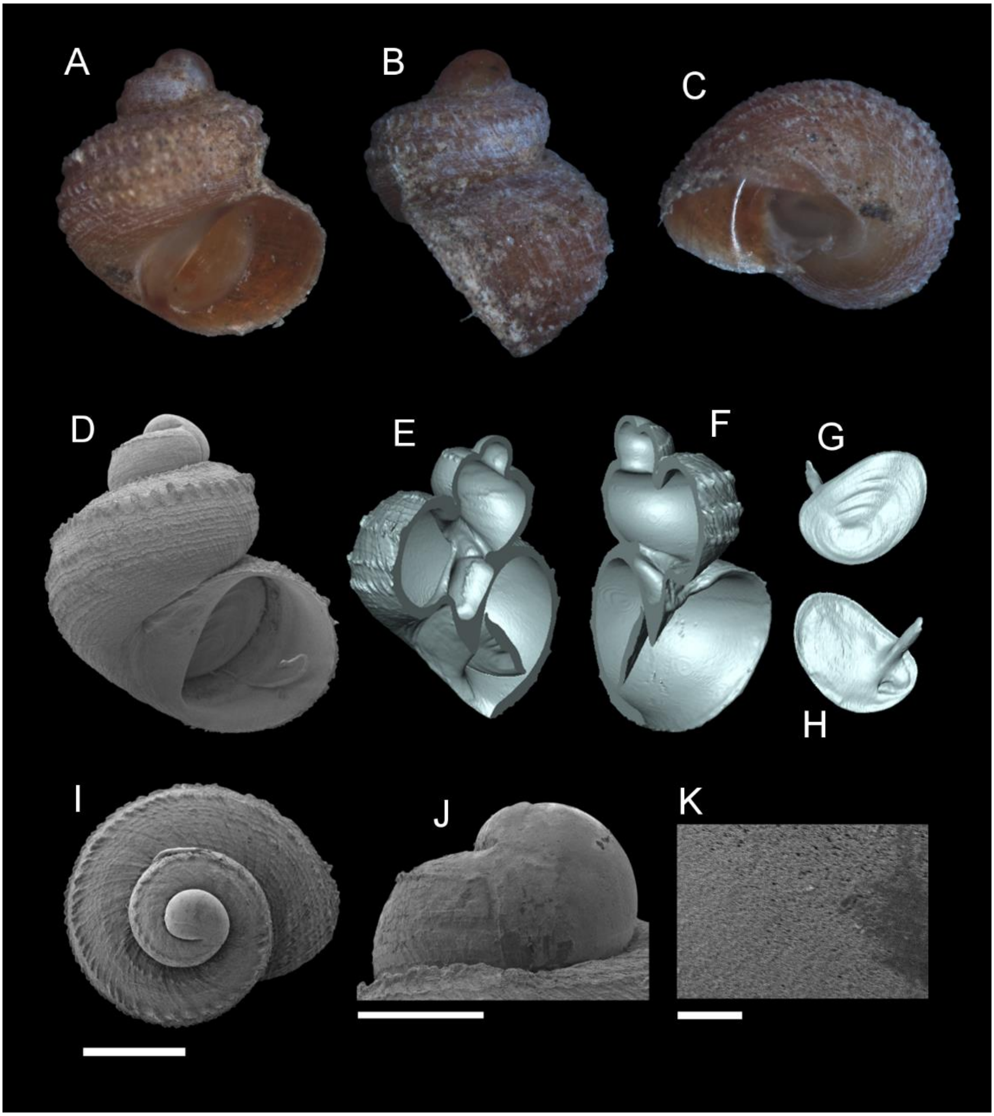

Figure 2.7 *Georissa hosei* Godwin-Austen 1889. **A**-**C**. MZU/MOL 16.05. **D**-**K**. MZU/MOL 16.04. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa anyiensis sp. n.

Georissa hosei Godwin-Austen, Thompson and Dance 1983: 117, materials from Tatau Valley, Bukit Sarang, Bintulu, Sarawak.

Type locality. Bukit Anyi at Bukit Sarang, Bintulu, Sarawak, Malaysia (02°39.25’N, 113°02.72’E).

Type material. *Holotype*. Bukit Anyi at Bukit Sarang, Bintulu, Sarawak, Malaysia (02°39.25’N, 113°02.72’E): MZU/MOL 17.90 (leg. MZ Khalik and SK Reduan). *Paratypes*. Bukit Anyi at Bukit Sarang, Bintulu, Sarawak (02°39.25’N, 113°02.72’E): MZU/MOL 17.53, MZU/MOL 17.54, MZU/MOL 17.55, MZU/MOL 17.56, MZU/MOL 17.57, MZU/MOL 17.58, MZU/MOL 17.59, MZU/MOL 17.60, MZU/MOL 17.61, JJV 12840 (40), JJV 12841 (1). Bukit Lebik at Bukit Sarang, Bintulu, Sarawak (02°39.32’N, 113°02.43’E): MZU/MOL 17.50, MZU/MOL 17.51, MZU/MOL 17.52, JJV 12842 (20), JJV 12843 (1). From Thompson and Dance 1983, Bukit Sarang, Tatau valley (20°45’ N, 113°02’ E): UF 35914, UF 35915, UF 35921 (not seen). Each lot of examined paratypes from MZU are more than 50 individuals.

Etymology. Named after the hill Bukit Anyi at Bukit Sarang, Bintulu, Sarawak, Malaysia, the type locality.

Description. *Protoconch*. Color: yellow to orange. Sculpture pattern: meshed – rounded to ellipsoidal or undefined mesh shape. Mesh width: 8-30 µm. *Teleoconch*. Color: yellow. First whorl: shouldered, cylindrical., Subsequent whorls: convex to rounded, with a deeply impressed suture. SH: 1.39-1.98 mm, SW: 1.32-1.72 mm, SI: 1.05-1.08. Total number of whorls: 2 ¼-2 ¾. *Shell sculpture*. Radial sculpture: absent, only weak to strong growth lines are visible. Spiral sculpture: present, strongly sculpted, continuous ribs, more prominent at the periphery. Scales: at the shoulder a continuous spiral row of highly projecting diagonal crown-like scales; subordinate to that, three to four series of tall, broad or acute diagonal scales, regularly spaced, the uppermost of these always stronger than the lower ones, inter-series pacing irregular. *Aperture*. Shape: oval to rounded. Basal side: rounded, angular at the columellar region. Parietal side: straight. AH: 0.67-0.91 mm, AW: 0.90-1.17 mm, AI: 0.74-0.93. *Holotype dimension*. SH: 1.91 mm, SW: 1.72 mm, AH: 0.90 mm, AW: 1.14 mm.

Cross diagnosis. In general, *G. anyiensis* has a shell shape that is similar to *G. kobelti*, *G. scalinella*, and *G. muluensis*. However, *G. anyiensis* has an extremely prominent, crown-like spiral series of large scales on the shell periphery, which distinguishes it from other scaly *Georissa*.

Distribution. Known from Bukit Anyi and Bukit Lebik, two isolated hills at Bukit Sarang, Bintulu, Sarawak.

Molecular analysis. ML and Bayesian analyses show that the *G. anyiensis* individuals (*16S*: n=13; *CO1*: n=12) form a monophyletic group with 100% BS and 100% PP, sister group to the four species *G. niahensis* + *G. kobelti* + *G. hadra* + *G. muluensis*.

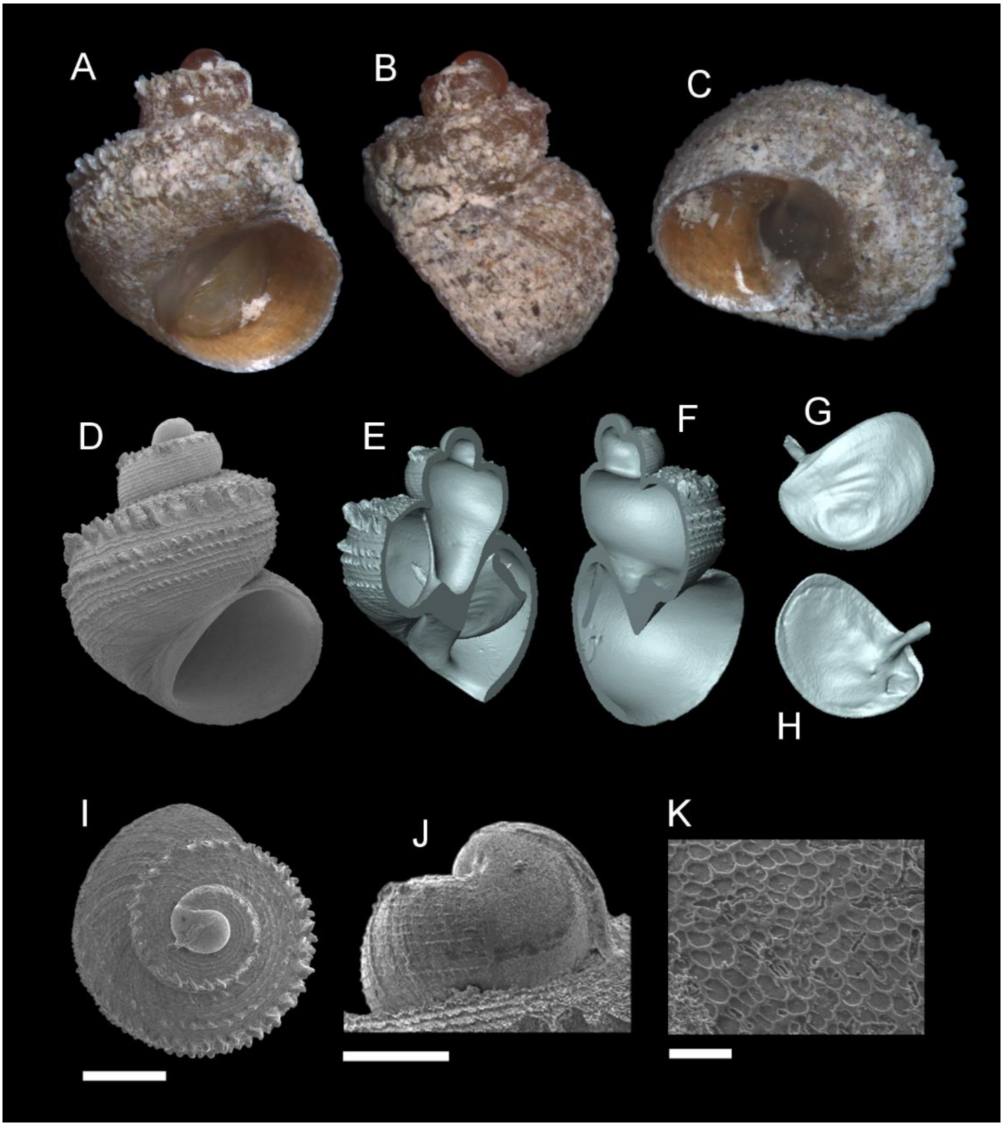

Figure 2.8 *Georissa anyiensis* sp. n. **A**-**C**. Holotype: MZU/MOL 17.90. **D**-**K**. Paratypes: MZU/MOL 17.55. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa muluensis sp. n.

Type locality. Lagang Cave, Mulu National Park, Mulu, Sarawak, Malaysia (04°03.06’N, 114°49.37’E).

Type material. *Holotype*. Lagang Cave, Mulu National Park, Mulu, Sarawak, Malaysia (04°03.06’N, 114°49.37’E): MZU/MOL 17.86 (leg. MZ Khalik and SK Reduan). *Paratypes*. Lagang Cave, Mulu National Park, Mulu, Sarawak (04°03.06’N, 114°49.37’E): MZU/MOL 17.30 (13), MZU/MOL 17.31 (9).

Other material. Deer Cave, Mulu National Park, Mulu, Sarawak: JJV 10533 (this sample, approximately 120 individuals, also contains specimens of *G. hadra*), JJV 10554 (this sample contains 5 individual of *G. muluensis*, 1 individual *G. hadra*), JJV 10533 (this sample, approximately 150 individuals, also contains specimens of *G. hadra* and *G. kobelti*). Mulu N.P., Mulu, Sarawak: JJV 10527.

Etymology. Named after Mulu National Park, Sarawak, Malaysia, the type locality.

Description. *Protoconch*. Color: yellow. Sculpture pattern: meshed – ellipsoidal mesh shape. Mesh width: 16-26 µm. *Teleoconch*. Color: yellow. First whorl: shouldered, above the shoulder flat, nearly horizontal; below the shoulder flat, cylindrical, but abruptly withdrawn into the deeply incised suture. Subsequent whorls: convex to rounded. SH: 1.67-2.05 mm, SW: 1.57-1.79 mm, SI: 1.08-1.18. Total number of whorls: 2 ¼-3. *Shell sculpture*. Radial sculpture: absent, only weak to strong growth lines are visible. Spiral sculpture: present, consisting of thin, but strongly sculpted and continuous ribs. Scales: two to three series of tall and diagonal scales, regularly spaced, the upper scale series always stronger than the lower ones, weaker series appear later at the spire or consist only of randomly spaced arrays of acute nodules, widely spaced between the first and second scale series, more densely spaced later. *Aperture*. Shape: rounded to slightly oval., Basal side: rounded, angular at the columellar region. Parietal side: straight to slightly curved. AH: 0.82-0.98 mm, AW: 1.03-1.18 mm, AI: 0.77-0.83. *Holotype dimension*. SH: 1.67 mm, SW: 1.53 mm, AH: 0.82 mm, AW: 1.07 mm.

Cross diagnosis. The wide spacing of the major spiral scale series of *G. muluensis* is similar to *G. kinabatanganensis*, but *G. muluensis* has a more elongated shell shape, rather than the more flattened habitus of *G.*

kinabatanganensis. In general shell shape and sculpture *G. muluensis* also resembles *G. hadra*, which, however, is larger and more elongated.

Distribution. Known only from the small area of Lagang Cave, Mulu National Park, Mulu, Sarawak, Malaysia.

Molecular analysis. ML and Bayesian analyses show that the individuals of *G. muluensis* (*16S*: n=4; *CO1*: n=4) form a monophyletic group with 100% BS and 100% PP, which is the sister group of *G. hadra*.

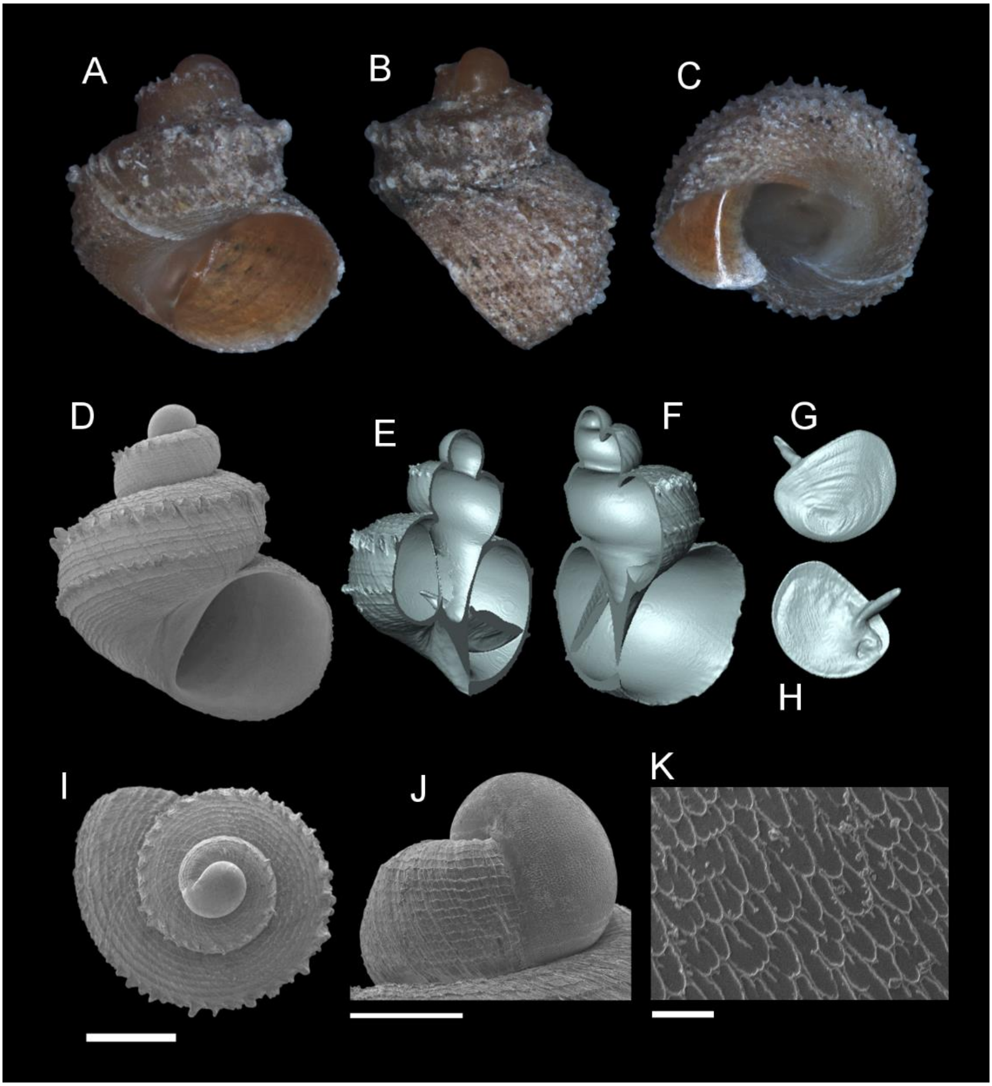

Figure 2.9 *Georissa muluensis* sp. n. **A**-**C**. Holotype: MZU/MOL 17.86. **D**-**K**. Paratypes: MZU/MOL 17.30. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa hadra Thompson and Dance 1983

Georissa hadra Thompson and Dance 1983: 115-116, fig. 32, 43-46 (original description).

Type locality. Butik Besungai, a small limestone hill 0.5 miles southwest of Batu Gading, and about 4 miles northeast of Long Lama, Baram Valley, Fourth Division, Sarawak. 03°52'N 114°25'E.

Type material. *Holotype*. Butik Besungai, a small limestone hill 0.5 miles southwest of Batu Gading, and about 4 miles northeast of Long Lama, Baram Valley, Fourth Division, Sarawak: UF36107 (not seen). *Paratypes*. Butik Besungai ½ mile SW. of Batu Gading, 4 miles NE. of Long Lama, Baram Valley, 4th Div., Sarawak, Malaysia: BMNH 1984004 (seen). Baram valley, Long Lama, Bt. Besungai 0.5 m SW of Batu Gading, Sarawak (03°52.00'N, 114°25.00'E): JJV 13421 (seen).

Other material. Lang Cave, Mulu N.P., Mulu, Sarawak (04°01.49'N, 114°49.48'E): MZU/MOL 17.32, MZU/MOL 17.33, MZU/MOL 17.34, MZU/MOL 17.35. Deer Cave, Mulu N.P., Mulu, Sarawak: JJV 10533 (this sample, approximately 120 individuals, also contains *G. muluensis*), JJV 10554 (5 individual of *G. muluensis*, 1 individual *G. hadra*), JJV 10533 (this sample, approximately 150 individuals, also contains *G. muluensis* and *G. kobelti*).

Butik = a misspelling of Bukit, a local name for hill.

Description. *Protoconch*. Color: orange. Sculpture pattern: meshed – rounded to ellipsoidal or undefined mesh shape. Mesh width: 12-24 µm. *Teleoconch*. Color: orange. First whorl: with a distinct shoulder (provided with a series of minuscule scales), above the shoulder flat and tapering towards the suture, below the shoulder flat and cylindrical., Subsequent whorls: distinctly scalariform, with three separate aspects separated by two or more main spiral series of scales: above the uppermost spiral series gently curved towards the suture; in between both spiral series flat and cylindrical; below the lowest spiral series abruptly narrowed towards the deeply impressed suture (on the final whorl these three aspects fuse, forming a uniformly rounded impression). SH: 2.61-2.91 mm, SW: 2.05-2.19 mm, SI: 1.21-1.37. Total number of whorls: 2 ¾-3 ¼. *Shell sculpture*. Radial sculpture: absent, but with strong and unevenly layered growth lines. Spiral sculpture: present, weakly sculpted, continuous to discontinuous. Scales: two to four irregularly spaced series of low to high, and minute to broad diagonal scales, densely spaced, the first scale series always the strongest, weaker series appearing later at the spire. *Aperture*. Shape: rounded, with a tilt below the palatal side. Basal side: rounded, angular at the columellar region. Parietal side: straight to curved. AH: 1.11-1.33 mm, AW: 1.32-1.48 mm, AI: 0.83-1.01.

Cross diagnosis. *G. hadra* has scales which are densely arranged, unlike *G. scalinella*, *G. hosei*, *G. muluensis*, *G. anyiensis*, and *G. kobelti*, which have more widely spaced scales. In shell shape, *G. hadra* is similar to the later three species but larger and more distinctly scalariform. *G. hadra* is similar in size to *G. niahensis*, but it has a more slender habitus and a more rounded periphery.

Distribution. The type locality for *G. hadra* is Bukit Besungai, Baram, Sarawak. We also obtained it at Mulu, Sarawak. Currently, therefore, the known distribution range is restricted to Mulu and Baram.

Molecular analysis. ML and Bayesian analyses retrieve *G. hadra* (*16S*: n=4; *CO1*: n=4) as a single clade with 89% BS and 100% PP, sister to *G. muluensis*.

Discussion. The paratypes of Thompson and Dance (1983) have a very pale orange color, compared to recently collected materials from Mulu; presumably the color has faded.

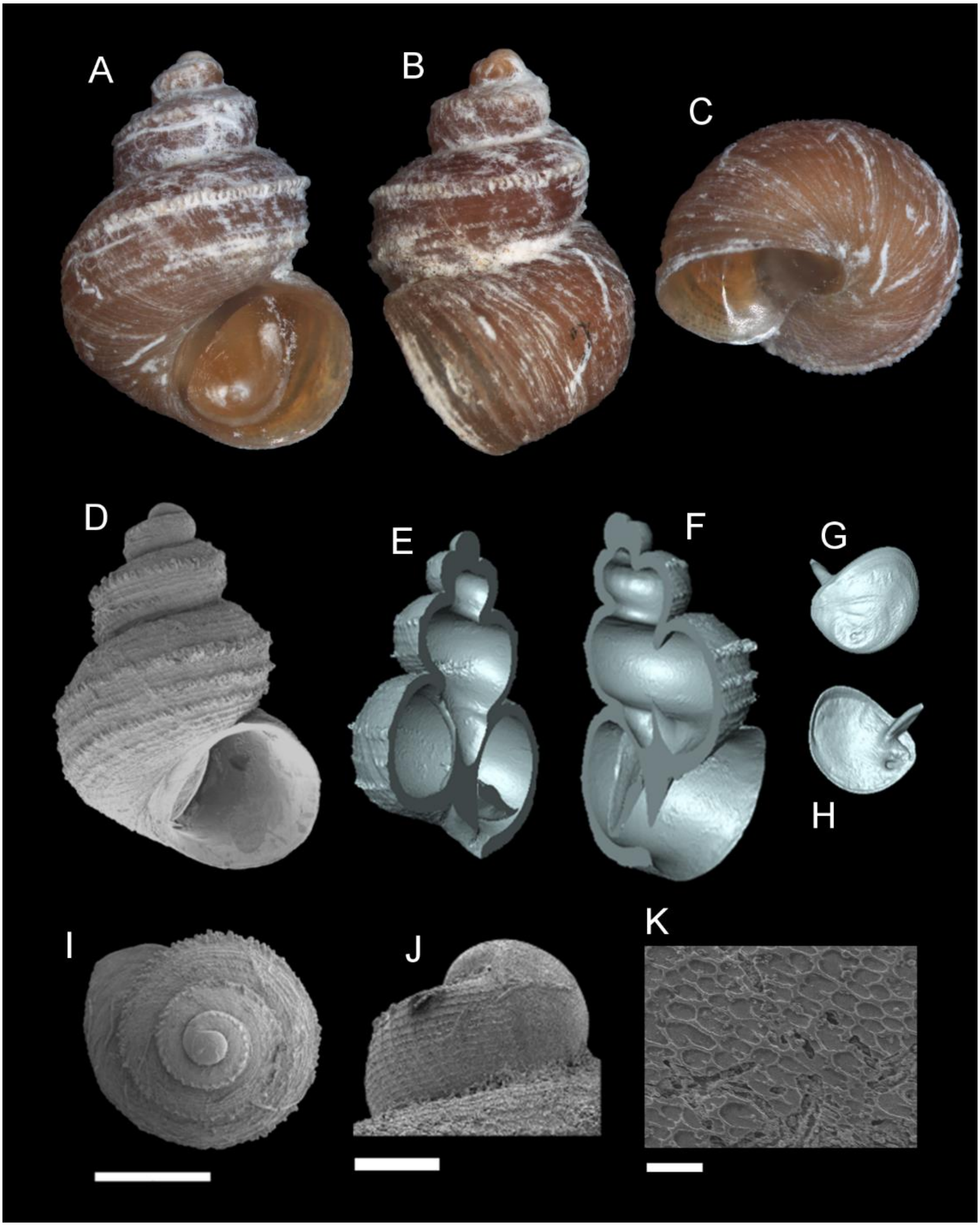

Figure 2.10 *Georissa hadra* Thompson and Dance 1983. **A**-**C**. MZU/MOL 17.33. **D**-**K**. ZMA/MOLL 17.32. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 1 mm. **J**: 200 µm. **K**: 10 µm.

Georissa kobelti Gredler 1902

Georissa kobelti Gredler 1902: 61 (original description).

Georissa kobelti Gredler, Zilch 1973: 265, fig. 11.

Georissa kobelti Gredler, Thompson and Dance 1983: 117, fig. 28, 50-52.

Georissa hosei Godwin-Austen, Thompson and Dance 1983: 117, fig. 47-49, material from Bukit Besungai at Baram Valley, Niah, Kejin trib. of Baram river.

Type locality. Niah, Baram (Sarawak, Borneo). Unspecified.

Type material. *Lectotype* (Designated by Zilch, 1973). Niah, Baram (Sarawak, Borneo): SMF 215893a (not seen).

Other material. Trade Cave, Niah National Park, Niah, Sarawak (03°49.13’N, 113°46.86’E): MZU/MOL 17.36. Great Cave, Niah National Park, Niah, Sarawak: MZU/MOL 17.37. Bukit Kaijin, Baram, Sarawak (03°41.75’N, 114°27.55’E): MZU/MOL 17.38, MZU/MOL 17.39, MZU/MOL 17.40, MZU/MOL 17.41, MZU/MOL 17.42, MZU/MOL 17.43, MZU/MOL 17.44, MZU/MOL 17.45, MZU/MOL 17.46, MZU/MOL 17.47, MZU/MOL 17.48, MZU/MOL 17.49, JJV 10217. Bukit Kasut, Niah N.P., Niah, Sarawak: JJV 10254. Niah N.P., Niah, Sarawak: JJV 1565, JJV 5466, JJV 10306, JJV 10392. Deer Cave, Mulu N.P., Mulu, Sarawak: JJV 10533 (the sample, approximately 150 individuals, also contains *G. muluensis* and *G. hadra*). Tatau river valley, Bukit Sarang, Bintulu, Sarawak: JJV 12551, JJV 12838. From Thompson and Dance 1983, Niah, Baram (Sarawak, Borneo): UF 35919, UF 36179 (not seen).

Description. *Protoconch*. Color: orange to red. Sculpture: meshed – semi-oval mesh shape. Mesh width: 11-22 µm. *Teleoconch*. Color: ranging from red to yellow. First whorl: convex to rounded. Subsequent whorls: convex to rounded. SH: 1.75-2.11 mm, SW: 1.48-1.75 mm, SI: 1.18-1.28. Total number of whorls: 2 ¾-3. *Shell sculpture*. Radial sculpture: absent, only weak growth lines. Spiral sculpture: present with thin but strongly continuous spiral ribs, forming small acute scales near the suture. Scales: three to four spiral rows of tilted, nearly vertical scales, the upper series stronger than the lower ones, scale prominence ranging from high to low and from small and acute to broadly sculpted and ear-like. Scales are regularly spaced, as are the scale series themselves. *Aperture*. Shape: rounded to oval., Basal side: rounded, angular before the columellar region. Parietal side: curved. AH: 0.82-1.04 mm, AW: 1.02-1.17 mm, AI: 0.71-0.90.

Cross diagnosis. The image of the *G. kobelti* lectotype by Zilch (1973) does not provide detailed information about the scale characters of *G. kobelti* as compared to the images of the individual from UF provided by Thompson and Dance (1983), which clearly shows the diagnostic characters of the ear-like scale pattern of this species. In shell habitus, *G. kobelti* is similar to some populations of *G. anyiensis*, *G. saulae*, and *G. hosei*, but these species differ from *G. kobelti* by the pattern of their diagonal scales.

Distribution. The lectotype in Senckenberg (SMF 215893a) was obtained from an unspecified location. As far as known, the species is restricted to the area of Niah to Baram, northern Sarawak. Thompson and Dance (1983) also stated that they examined this species from Beluru, which is located between Niah and Baram.

Molecular analysis. In the ML and Bayesian analyses of *G. kobelti* (*16S*: n=8; *CO1*: n=8), the Niah and Baram populations form highly supported clades (99% and 100% BS, respectively, and 100% PP for both clades), which are paraphyletic with respect to *G. niahensis*.

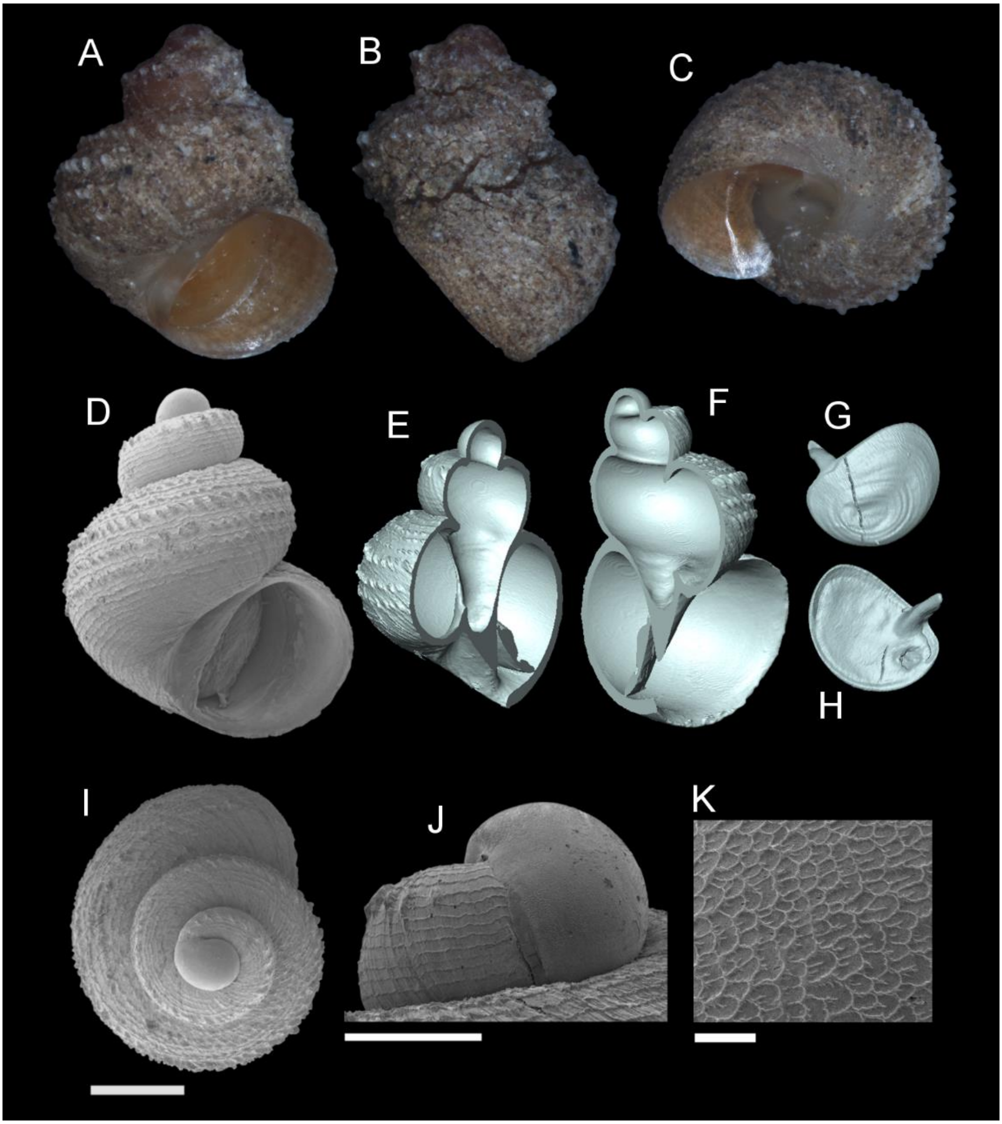

Figure 2.11 *Georissa kobelti* Gredler 1902. **A**-**C**. MZU/MOL 17.40. **D**-**K**. MZU/MOL 17.38. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa niahensis Godwin-Austen 1889

Georissa niahensis Godwin-Austen 1889: 353 (original description).

Georissa niahensis Godwin-Austen, Thompson and Dance 1983: 119.

Type locality. Niah Hills, Borneo. (Unspecified)

Type material. *Lectotype* (Designated by Thompson and Dance 1983). Niah Hills, Borneo: NHMUK 1889.12.7.69 (glued on paper) (seen). *Paralectotype*. Niah Hills, Borneo: NHMUK 1889.12.7.70 (glued on paper) (seen).

Other material. Painted Cave, Niah National Park, Niah, Sarawak (03°48.68'N, 113°47.25'E): MZU/MOL 17.25.

Description. *Protoconch*. Color: red. Sculpture pattern: smooth and meshed – ellipsoid to irregular mesh shape. Mesh width: 12-19 µm. *Teleoconch*. Color: orange to red. First whorl: curved above the shoulder, flat and cylindrical below the shoulder. Subsequent whorls: convex, angular at the periphery. SH: 1.81-2.53 mm, SW: 1.51-1.99 mm, SI: 1.10-1.29. Total number of whorls: 3-3 ¼. *Shell sculpture*. Radial sculpture: absent, only strong and unevenly layered growth lines. Spiral sculpture: present, strongly sculpted, continuous to discontinuous, well defined from the first whorl all the way to the peristome. Scales: a single spiral series of low and minute acute scales, regularly spaced at the first whorl, but weaker, grading to imperceptible on the body whorl. *Aperture*. Shape: rounded. Basal side: rounded, angular at the columellar region. Parietal side: straight to curved. AH: 0.85-1.24 mm, AW: 0.92-1.27 mm, AI: 0.83-1.02.

Cross diagnosis. *G. niahensis* has a distinctive single series of small scales on the whorl shoulder, close to the suture. *G. niahensis* is one of the largest Bornean *Georissa*, in shell size only matched by *G. hadra* (which, however, is more slender, angular at the shoulder and has a flat to slightly rounded whorls). In general shell shape, *G. niahensis* is closest to *G. kobelti*, but the latter species is more rounded, while *G. niahensis* has a distinctly convex periphery.

Distribution. Known to occur only at Niah, Sarawak.

Molecular analysis. ML and Bayesian analyses of *G. niahensis* (*16S*: n=8; *CO1*: n=7) showed that all *G. niahensis* specimens form one clade with 100%

BS and 100% PP. The sister group is the *G. kobelti* population from Baram (*G. kobelti* is paraphyletic).

Discussion. *G. niahensis* was described by Godwin-Austen (1889). Both Godwin-Austen (1889) and Thompson and Dance (1983) did not mention anything about the small scale-like nodules close to the suture of *G. niahensis*. Godwin-Austen (1889): “*Shell elongately conoid, solid, imperforate; sculpture a very in-distinct, ill-defined spiral liration, about 20 on the penultimate whorl, upon a rough surface crossed by transverse lines of growth; color ruddy ochre; spire high; apex pointed, finely papillated, minutely lirate; suture impressed; whorls 4 ½ convex; aperture oval, oblique; peristome simple, acute below; columellar margin straight*”. Thompson and Dance (1983): “*G. niahensis is similar in sculpture to G. williamsi but is much larger than G. niahensis also shows similarities to the hosei group in the depth of the suture and the relatively rapid expanding whorls, but it lacks the node-like sculpture found among species of that group*” The scales are relatively small which are not very conspicuous among the strong growth lines, and this is the reason why in the previous description of the species the scale characters were lacking. Thompson and Dance (1983) compared *G. niahensis* with what they call the *hosei* group, based on the size and the deeply impressed suture.

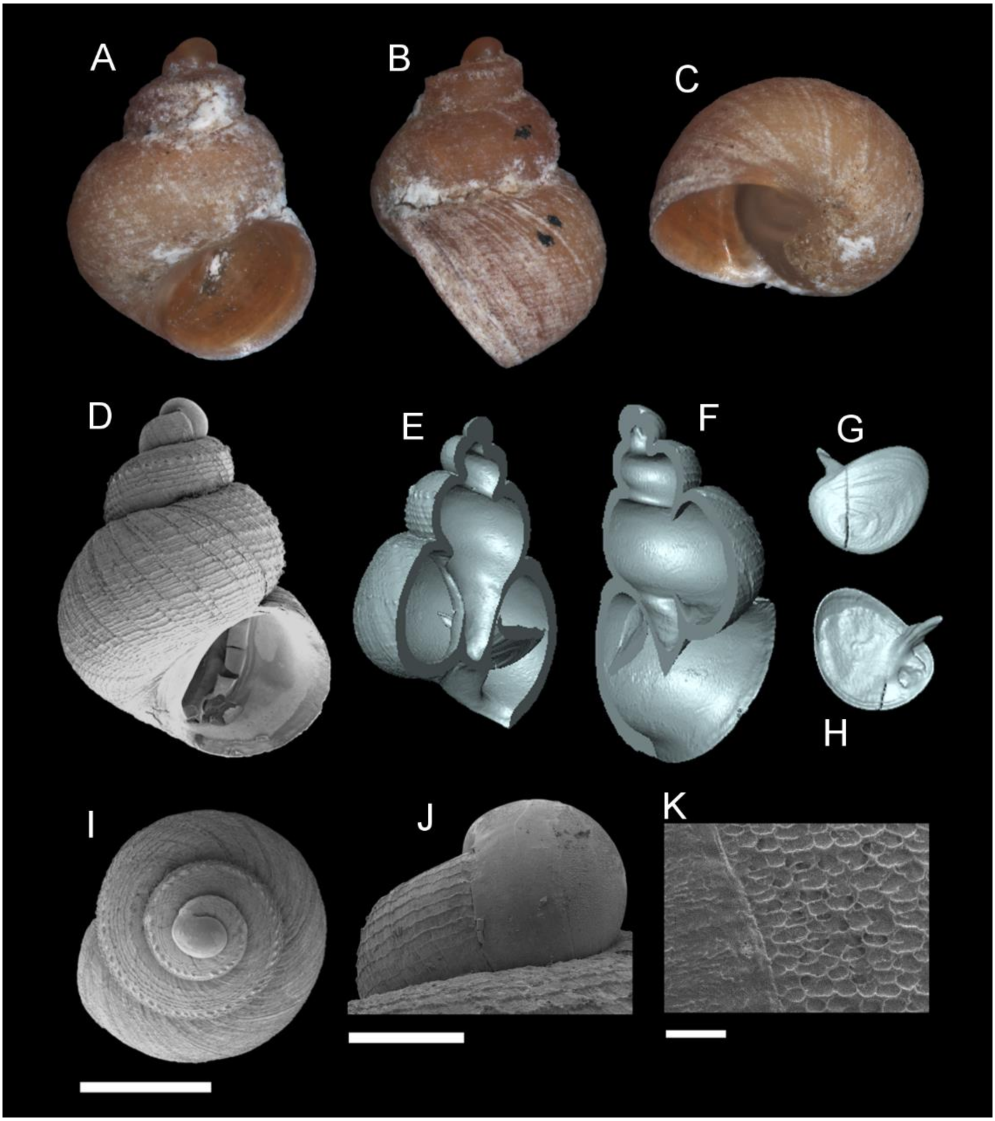

Figure 2.12 *Georissa niahensis* Godwin-Austen 1889. **A**-**K**. MZU/MOL 17.25. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 1 mm. **J**: 200 µm. **K**: 10 µm.

Georissa silaburensis **sp. n.**

Type locality. Gunong Silabur, Serian, Sarawak, Malaysia (00°57.28’N, 110°30.22’E).

Type material. *Holotype*. Gunong Silabur, Serian, Sarawak, Malaysia (00°57.28’N, 110°30.22’E): MZU/MOL 17.88 (leg. MZ Khalik and SK Reduan). *Paratypes*. Gunong Silabur, Serian, Sarawak, Malaysia (00°57.28’N, 110°30.22’E): MZU/MOL 17.01, MZU/MOL 17.02, MZU/MOL 17.03, MZU/MOL 17.04, MZU/MOL 17.05, MZU/MOL 17.06, MZU/MOL 17.07, MZU/MOL 17.08. Borneo, Sarawak, First Division, western side of Gunong Selabor, Semabang entrance to Lobang Batu Cave (00°55’N, 110°25’E): NHMUK 1984005 (seen). Each lot of examined paratypes from MZU are more than 50 individuals.

Etymology. Named after Gunung Silabur, Serian, Sarawak, Malaysia, the type locality.

Description. *Protoconch*. Color: red. Sculpture pattern: meshed – round to irregular mesh pattern. Mesh width: 8-18 µm. *Teleoconch*. Color: red. First whorl: rounded. Subsequent whorls: convex, number of whorls: 2-2 ¼. SH: 1.59-1.99 mm, SW: 1.50-1.76 mm, SI: 1.06-1.13. *Shell sculpture*. Radial sculpture: absent or weak to strong growth lines. Spiral sculpture: present, thin but strongly sculpted, continuous ribs, more prominent at the periphery. Scales: two to six or more randomly sculpted series of low and broad horizontal scales, or else acute horizontal nodules on the spiral sculpture, scale series irregularly spaced, which series is the most prominent is not consistent across individuals. *Aperture*. Shape: rounded. Basal side: rounded, angular at the columellar region. Parietal side: straight, palatal edge attached to slightly removed from the body whorl. AH: 0.95-1.09 mm, AW: 1.00-1.17 mm, AI: 0.92-0.99. *Holotype dimension*. SH: 1.68 mm, SW: 1.53 mm, AH: 0.95 mm, AW: 1.09 mm.

Cross diagnosis. The shell shape of *G. silaburensis* is distinct compared to other scaly group *Georissa*. It has rapid shell expansion like *G. hosei* and *G. scalinella*, but *G. silaburensis* has a different sculpture, consisting of horizontal, rather than vertical or diagonal scales. In addition, the whorls are rounded and convex, with the aperture almost circular, close to *G. saulae*.

Distribution. Known from the inside of the cave system of Gunung Silabur, Serian, Sarawak.

Molecular analysis. ML and Bayesian analyses show that the individuals of *G. silaburensis* (*16S*: n=10; *CO1*: n=9) form one clade with 95% BS and 98% PP, the sister group of *G. bauensis*.

Discussion. *G. silaburensis* was only found inside the cave entrance, with water flowing from the cave roof, and approximately less than 50% light penetration. In shell shape and reduced sculpture, it resembles another cave specialist, *G. filiasaulae*.

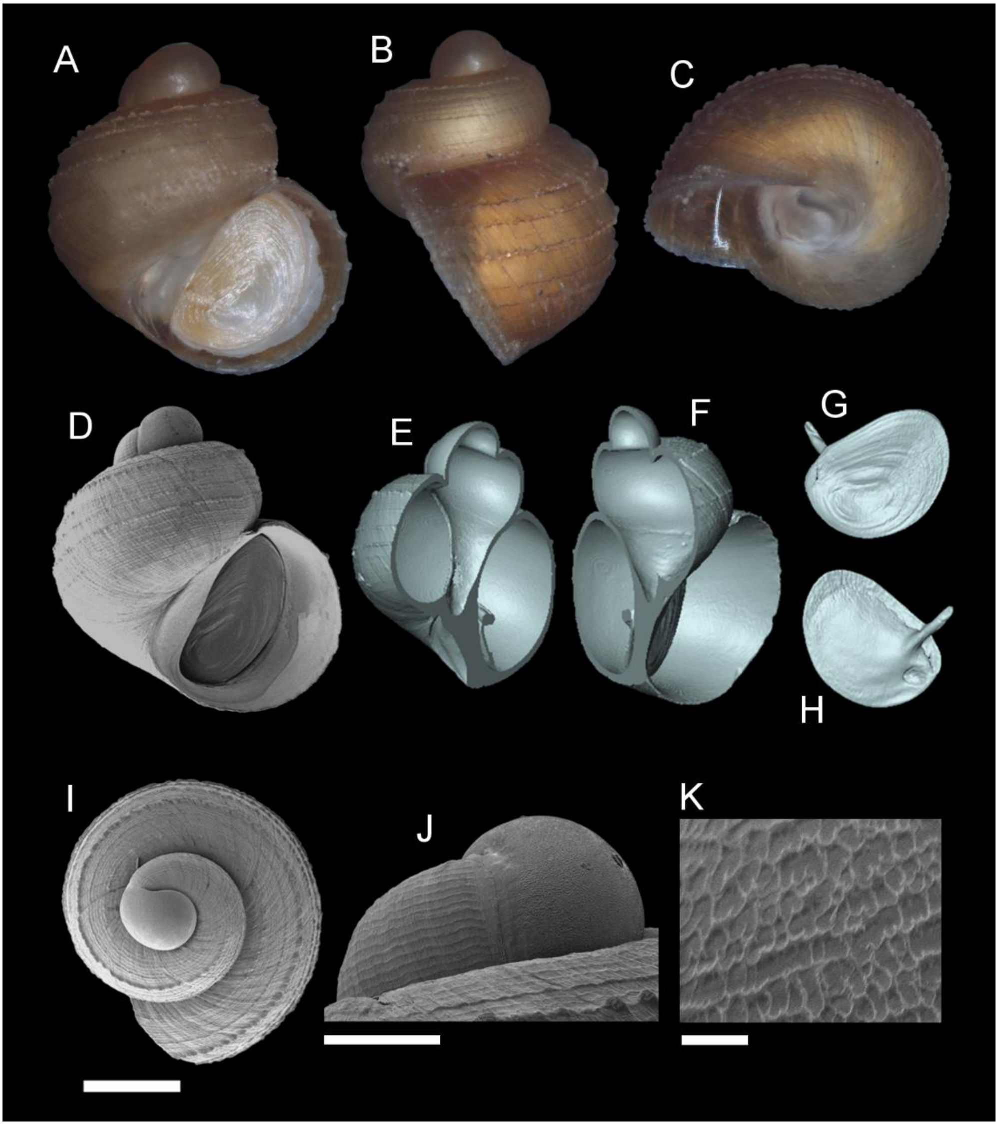

Figure 2.13 *Georissa silaburensis* sp. n. **A**-**C**. Holotype: MZU/MOL 17.88. **D**-**K**. Paratypes: MZU/MOL 17.04. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa bauensis sp. n.

Type locality. Wind Cave Passage 3, Wind Cave Nature Reserve, Bau, Sarawak, Malaysia (01°24.81’N, 110°08.17’E).

Type material. *Holotype*. Wind Cave Passage 3, Wind Cave Nature Reserve, Bau, Sarawak, Malaysia (01°24.81'N, 110°08.17'E): MZU/MOL 17.89 (leg. MZ Khalik). *Paratypes*. Wind Cave Passage 3, Wind Cave Nature Reserve, Bau, Sarawak, Malaysia (01°24.81'N, 110°08.17'E): MZU/MOL 16.01 (25), MZU/MOL 16.02 (>50). Gunung Podam, near Sungai Ayup, Kampung Bogag, Bau, Sarawak, Malaysia (01°21.15'N, 110°03.57'E): MZU/MOL 16.03 (5).

Etymology. Named after the district of Bau, Sarawak, Malaysia, where the type locality Wind Cave Nature Reserve is located.

Description. *Protoconch*. Color: red. Sculpture pattern: meshed – rounded or irregular mesh shape. Mesh width: 12-22 µm. *Teleoconch*. Color: orange to red. First whorl: shouldered, flat both above and below the shoulder. Subsequent whorls: convex shoulder and more rounded at the periphery. SH: 1.16-1.62 mm, SW: 1.06-1.30 mm, SI: 1.02-1.25. Total number of whorls: 2-2 ½. *Shell sculpture*. Radial sculpture: absent, only weak growth lines are visible. Spiral sculpture: present, weakly to strongly sculpted, continuous to discontinuous ribs, more prominent at the periphery. Scales: two to three major spiral series of low and small diagonal scales, regularly spaced, the upper series always stronger than the lower ones, scale series irregularly spaced. *Aperture*. Shape: rounded and tilted below. Basal side: rounded, angular at the columellar region. Parietal side: straight. AH: 0.57-0.78 mm, AW: 0.69-0.86 mm, AI: 0.74-0.96. *Holotype dimension*. SH: 1.16 mm, SW: 1.06 mm, AH: 0.58 mm, AW: 0.70 mm.

Cross diagnosis. *G. bauensis* is very similar to *G. kobelti* (although not closely related phylogenetically), in terms of general shell shape and spiral scale characters. However, *G. bauensis* is sufficiently variable to include specimens that are more similar to *G. hosei* and *G. scalinella*. Furthermore, *G. bauensis* has more strongly sculpted scales than *G. hosei*, and a more rounded and convex shell than *G. scalinella*.

Distribution. Known from Gunung Podam and Wind Cave Nature Reserve, Bau, Sarawak.

Molecular analysis. ML and Bayesian analyses resolve all individuals of *G. bauensis* (*16S*: n=13; *CO1*: n=8) as a monophyletic group with 99% BS and 100% PP, the sister group of *G. silaburensis*.

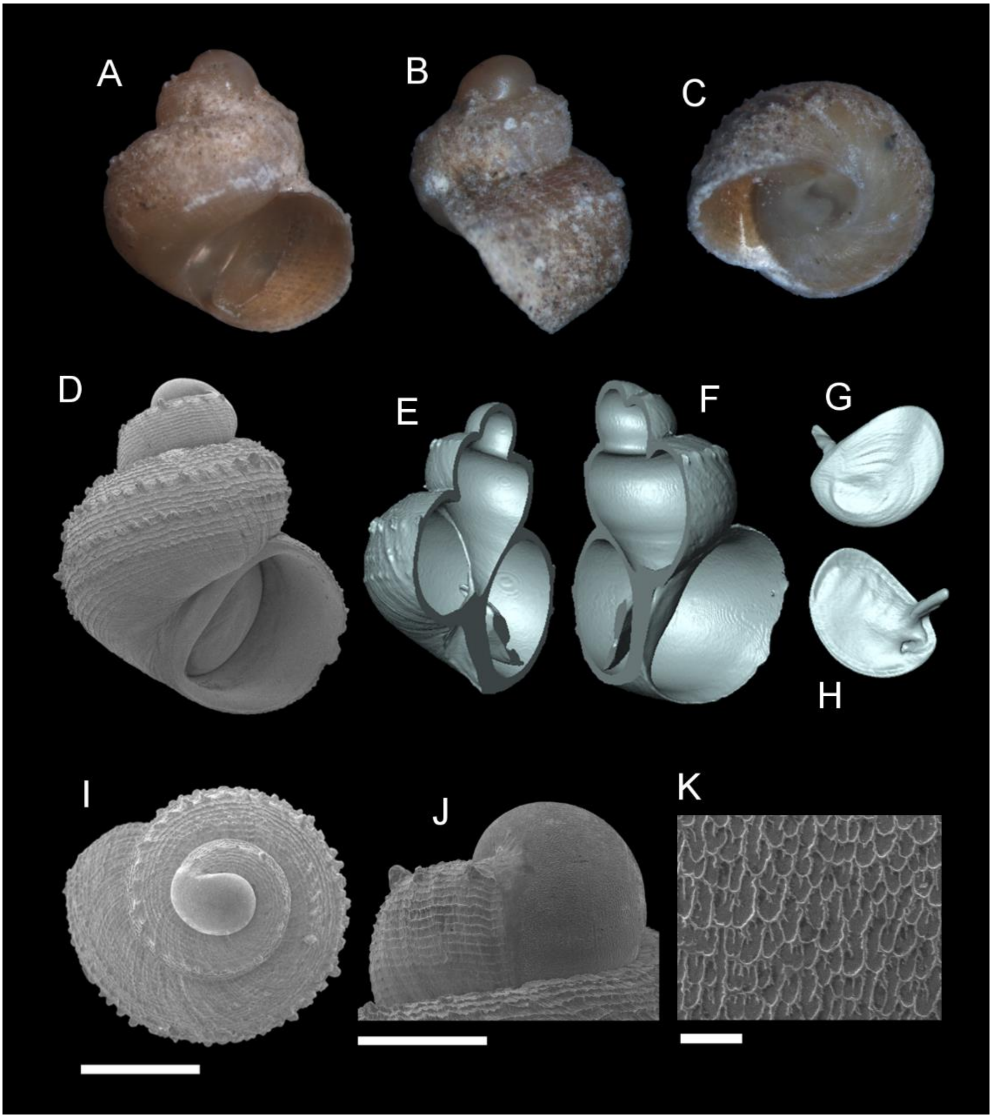

Figure 2.14 *Georissa bauensis* sp. n. **A**-**C**. Holotype: MZU/MOL 17.89. **D**-**K**. Paratypes: MZU/MOL 16.03. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa pyrrhoderma Thompson and Dance 1983

Georissa pyrrhoderma Thompson and Dance 1983: 123, fig. 64 (original description).

Georissa pyrrhoderma van Benthem-Jutting, in Beron 2015: 181.

Type locality. Borneo, Sarawak, First Division, western side of Gunong Selabor, Semabang entrance to Lobang Batu Cave (00°55’N, 110°25’E).

Type material. *Holotype*. Borneo, Sarawak, First Division, western side of Gunong Selabor, Semabang entrance to Lobang Batu Cave: UF36183 (not seen). *Paratypes*. Borneo, Sarawak, First Division, western side of Gunong Selabor, Semabang entrance to Lobang Batu Cave: UF 36184, UF 36185 (not seen).

Other materials. Gunong Silabur, Serian, Sarawak, Malaysia (00°57.45’N, 110°30.20’E): MZU/MOL 17.09, MZU/MOL 17.10, MZU/MOL 17.11, MZU/MOL 17.12, MZU/MOL 17.13, MZU/MOL 17.14, MZU/MOL 17.15, MZU/MOL 17.16, MZU/MOL 17.17, MZU/MOL 17.18, MZU/MOL 17.19, MZU/MOL 17.20, MZU/MOL 17.21, MZU/MOL 17.22, MZU/MOL 17.23, MZU/MOL 17.24.

Description. *Protoconch*. Color: red to brown. Sculpture pattern: smooth to meshed, with ellipsoid mesh shape. Mesh width: 11-26 µm. *Teleoconch*. Color: brown to red. First whorl: shouldered, slightly curved above the shoulder, flat, cylindrical below the shoulder. Subsequent whorls: initially shouldered, but soon grading into uniformly rounded and quickly expanding whorls, with a deeply impressed suture; number of whorls: 2 ¼-2 ½. SH: 1.16-1.31 mm, SW: 1.12-1.20 mm, SI: 1.03-1.09. *Shell sculpture*. Radial sculpture: absent, only weak to strong growth lines are visible. Spiral sculpture: present, strong spiral sculpture. Scales: a single series of low, small and acute, unevenly spaced scales above the periphery, occasionally, in the vicinity of the aperture, subordinate series of minute scales accompany the major series. *Aperture*. Shape: rounded, tilted below the palatal side. Basal side: rounded, strongly angular at the columellar region. Parietal side: straight, palatal edge attached to the body whorl. AH: 0.58-0.63 mm, AW: 0.75-0.85 mm, AI: 0.73-0.81.

Cross diagnosis. *G. pyrrhoderma* has a shell habitus that is similar to *G. kobelti*, *G. hosei*, and *G. sepulutensis*. The latter two species are high variable and are morphologically, especially in sculpture, closely related to *G. pyrrhoderma*. Therefore, *G. pyrrhoderma* is nearly indistinguishable from certain forms of these other species.

Distribution. Only known from the type locality, Gunung Silabur, Serian, Sarawak, Malaysia.

Molecular analysis. In the ML and Bayesian analyses, all *G. pyrrhoderma* (*16S*: n=28; *CO1*: n=26) individuals group together in one clade with 99% BS and 100% PP. Its sister clade is *G. scalinella* + *G. kinabatanganensis*.

Discussion. In the original description, Thompson and Dance (1983) did not compare *G. pyrrhoderma* with members of their *hosei*-group (which our molecular analyses show it belongs in). Instead, they considered it allied to *G. borneensis*. Possibly this misalignment was caused by the fact that the type specimens appear to lack the series of scales that is present on most of the specimens we collected. Nonetheless, given the restricted range of collection localities at Gunung Silabur and the degree of variability in our material, we consider our and Thompson and Dance's material as conspecific. The paratypes specimen NHMUK 1984005 (Semabang entrance to Lobang Batu Cave, W. side of Gunong Selabor, 1st. Div., Sarawak, Malaysia: seen) is *G. silaburensis*.

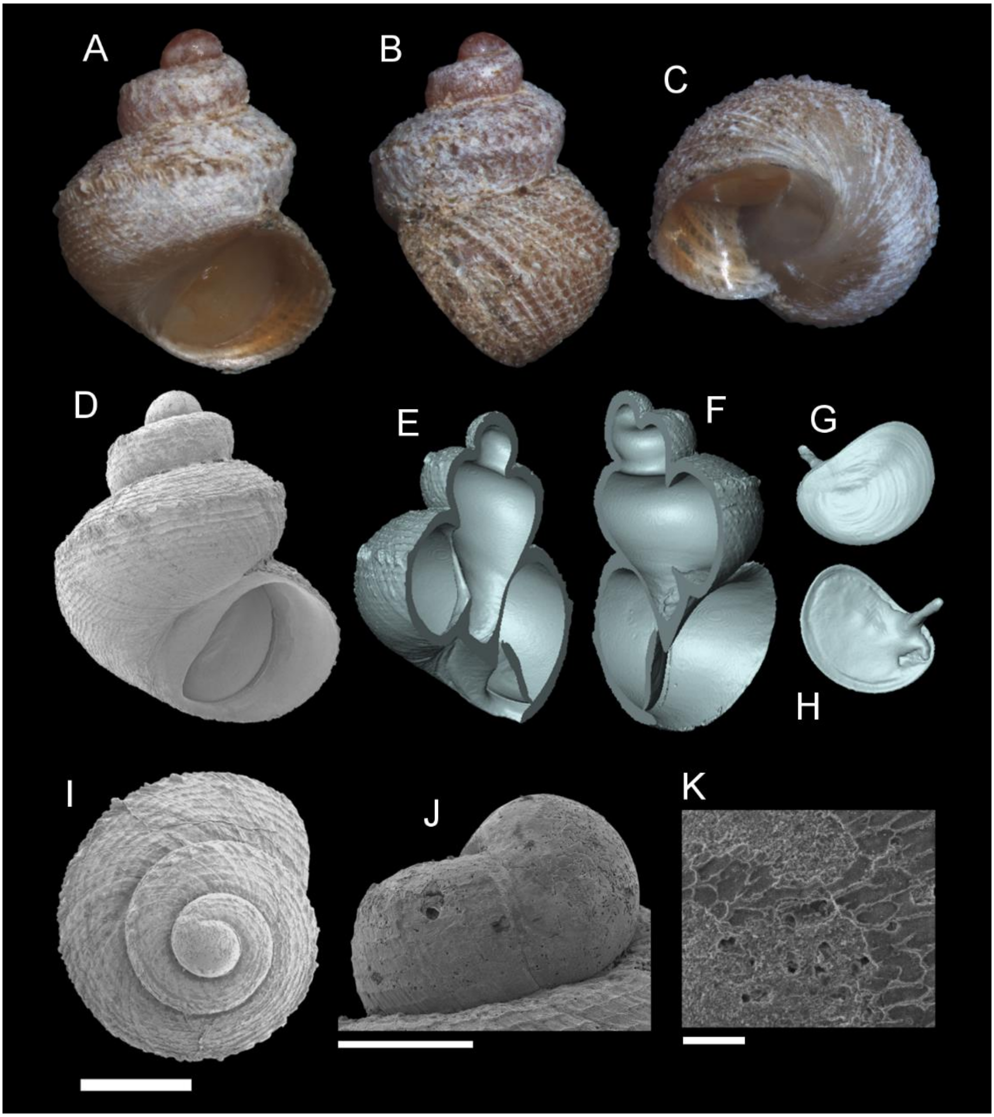

Figure 2.15 *Georissa pyrrhoderma* Thompson and Dance 1983. **A**-**C**. MZU/MOL 17.10. **D**-**K**. MZU/MOL 17.09. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

Georissa kinabatanganensis sp. n.

Type locality. Bukit Keruak, near Kinabatangan river, Sandakan, Sabah, Malaysia (05°31.385'N, 118°17.113'E).

Type material. *Holotype*. Bukit Keruak, near Kinabatangan river, Sandakan, Sabah, Malaysia (05°31.38’N, 118°17.11’E): BOR/MOL 13921 (leg. M Schilthuizen). *Paratypes*. Bukit Keruak, near Kinabatangan river, Sandakan, Sabah (05°31.38’N, 118°17.11’E): MZU/MOL 17.26 (>50). BOR/MOL 1458, BOR/MOL 11656, BOR/MOL 11665, BOR/MOL 11711, BOR/MOL 13871. Batu Pangi, Sabah: BOR/MOL 1455. Batu Tomanggong, Sabah: BOR/MOL 1456, BOR/MOL 1457, BOR/MOL 10530.

Etymology. Named after the district of Kinabatangan, Sabah, Malaysia, where the type locality Bukit Keruak is located.

Description. *Protoconch*. Color: orange. Sculpture pattern: smooth to meshed – rounded to undefined mesh pattern. Mesh width: 14-21 µm. *Teleoconch*. Color: orange. First whorl: flat and angular at the shoulder. Subsequent whorls: angular, slightly rounded at the periphery, with number of whorls: 2-2 ¼. SH: 1.00-1.32 mm, SW: 1.13-1.37 mm, SI: 0.85-0.99. *Shell sculpture*. Radial sculpture: absent, only weak to strong growth lines are visible. Spiral sculpture: present, and strongly sculpted, with continuous to discontinuous ribbings. Scales: two series of diagonal vertical scales, widely spaced in between, both series are strongly sculpted, broad, and the scales are regularly placed. *Aperture*. Shape: oval., Basal side: rounded, angular at the columellar region. Parietal side: straight, palatal edge attached to the body whorl. AH: 0.54-0.66 mm, AW: 0.75-0.86 mm, AI: 0.65-0.80. *Holotype dimension*. SH: 1.00 mm, SW: 1.18 mm, AH: 0.54 mm, AW: 0.78 mm.

Cross diagnosis. *G. kinabatanganensis* has less variation in shell sculpture compared with *G. hosei* and *G. scalinella*. *G. kinabatanganensis* has two series of acutely projected scales on the whorls. In some cases, the second scale series is weaker than the first, and creates a series of nodular structures at the periphery. Often the shell is wider than high, which gives it a flattened appearance. In addition, *G. kinabatanganensis* has widely spaced between the scale series, similar to *G. muluensis*.

Distribution. Known from Bukit Keruak, Batu Tomanggong, and Pangi, in the region of Kinabatangan, Sabah.

Molecular analysis. RAxML and Bayesian analyses show *G. kinabatanganensis* (*16S*: n=6; *CO1*: n=6) forming a clade with 97% BS and 97% PP and as a sister clade to *G. sepulutensis*.

Discussion. *G. kinabatanganensis* is the only species in "scaly group" *Georissa* to have a flat shell habitus, all examined individuals have a shell that is broader than high.

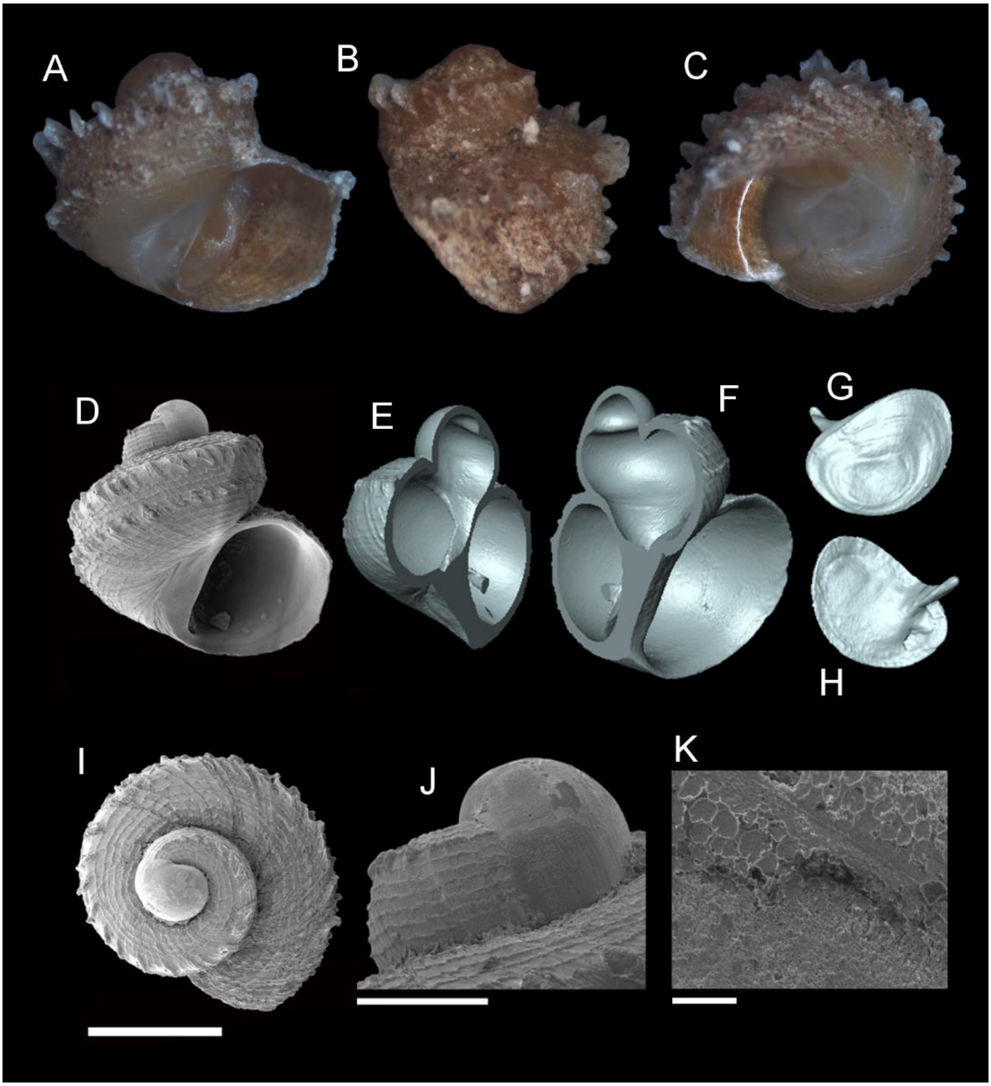

Figure 2.16 *Georissa kinabatanganensis* sp. n. **A**-**C**. Holotype: BOR/MOL 13921. **D**-**K**. Paratypes: MZU/MOL 17.26. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**.

Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

***Georissa sepulutensis* sp. n.**

Georissa scalinella van Benthem-Jutting, Schilthuizen et al., 2005: 134-135.

Type locality. Sepulut valley, Gua Pungiton near Kg. Labang, Sabah, Malaysia (04°42.41'N, 116°36.04'E).

Type material. *Holotype*. Sepulut valley, Gua Pungiton near Kg. Labang, Sabah, Malaysia (04°42.41'N, 116°36.04'E); BOR/MOL 13922 (leg. M Schilthuizen). *Paratypes*. Simbaluyon limestone hill, Sabah: RMNH/MOL 333905 (18), RMNH/MOL 333982 (23), RMNH/MOL 334006 (7). Tinahas, Sabah: RMNH/MOL 333984 (>50), RMNH/MOL 334013 (>50). Sepulut valley, Gua Sanaron, Sabah (04°42.05'N, 116°36.01'E): BOR/MOL 36, BOR/MOL 39, BOR/MOL 13870 (1). Sepulut Valley, Gua Pungiton, Sabah (04°42.41'N, 116°36.04'E): BOR/MOL 12278. Sepulut valley, Batu Punggul, Sabah: RMNH/MOL 187650, BOR/MOL.40. Baturong, Sabah: BOR/MOL 37.

Etymology. Named after the town of Sepulut, Sabah, Malaysia, near which where the type locality Gua Pungiton, as well as the other known localities, is located.

Description. *Protoconch*. Color: red to brown. Sculpture: smooth to meshed – semi-oval mesh to undefined mesh pattern. Mesh width: 7-17 µm. *Teleoconch*. Color: red. First whorl: flat to rounded at the shoulder. Body whorl: rounded, with number of whorls: 2-2 ¾. SH: 1.11-1.52 mm, SW: 1.11-1.37 mm, SI: 0.94-1.07. *Shell sculpture*. Radial sculpture: absent, only weak growth lines are visible. Spiral sculpture: present, and strongly sculpted. Scales: a series of pointed vertical scales, acute and highly projected, and regularly spaced. *Aperture*. Shape: oval and tilted below. Basal side: rounded, angular at the columellar region. Parietal side: straight, palatal edge attached to the body whorl. AH: 0.62-0.81 mm, AW: 0.76-0.96 mm, AI: 0.72-0.87.

Holotype dimension. SH: 1.34 mm, SW: 1.23 mm, AH: 0.65 mm, AW: 0.82 mm.

Cross diagnosis. Unlike *G. kinabatanganensis*, *G. sepulutensis* has a series of scales only at the shoulder, which makes it resemble in habitus and scale characters *G. pyrrhoderma* from Gunung Silabur, Sarawak.

Distribution. Distributed in the Sepulut Valley, Sabah; known from the following limestone localities: Simbaluyon, Sanaron, Tinahas, and Pungiton.

Molecular analysis. ML and Bayesian analyses show *G. sepulutensis* (*16S*: n=10; *CO1*: n=2) as two clades with 93% BS and 97% PP, and as the sister species to *G. kinabatanganensis* sp. n.

Discussion. *G. sepulutensis* and *G. kinabatanganensis* were previously included in *G. scalinella* (van Benthem-Jutting 1966). Based on the genetic and morphological distinctness, we here consider them as separate species.

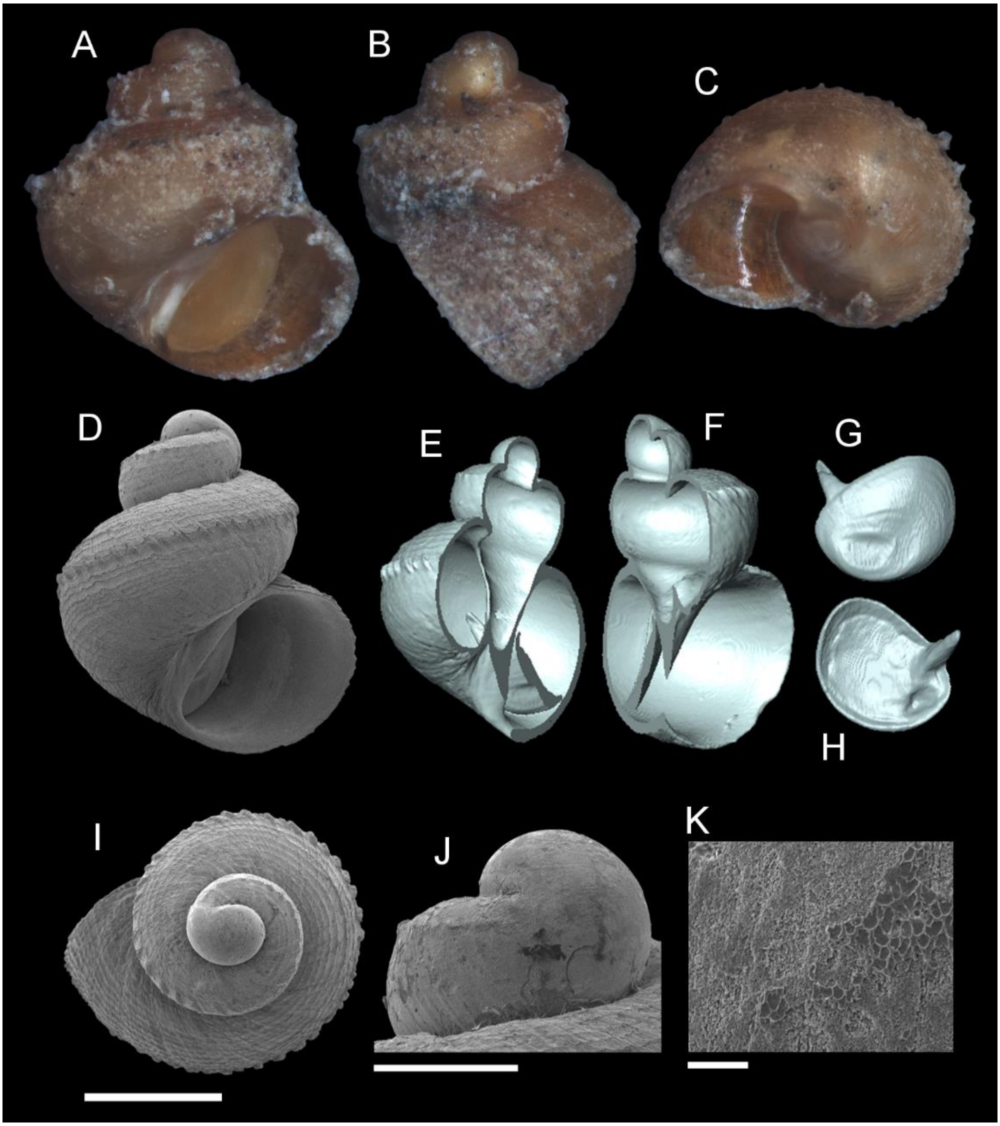

Figure 2.17 *Georissa sepulutensis* sp. n. **A**-**C**. Holotype: BOR/MOL 13922. **D**-**K**. Paratypes: BOR/MOL 12278. **A** and **D**. Shell apertural view. **B**. Shell side view. **C**. Shell rear view. **E**-**F**. Shell cross-section from 3D model. **G**-**H**. Operculum frontal and ventral view. **I**. Shell top view. **J**. Protoconch side view. **K**. Close up of protoconch from top at 1000× magnification. **A**-**I**: 500 µm. **J**: 200 µm. **K**: 10 µm.

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Supplementary material

1. An overview of scanning parameters of each examined “scaly” *Georissa*.
   https://doi.org/10.3897/zookeys.773.24878.suppl1
2. “Scaly” *Georissa* partitioning based on ABGD species delimitation.
   https://doi.org/10.3897/zookeys.773.24878.suppl2
3. PTP species delimitation of “scaly” Georissa.
   https://doi.org/10.3897/zookeys.773.24878.suppl3
4. Shell measurement of “scaly” *Georissa*.
   https://doi.org/10.3897/zookeys.773.24878.suppl4
5. Synoptic view of 13 species of “scaly” *Georissa*, and their 3D model.
   https://doi.org/10.3897/zookeys.773.24878.suppl5

Chapter 3

Conchological and molecular analysis of the "non-scaly" Bornean *Georissa* with descriptions of three new species (Gastropoda, Neritimorpha, Hydrocenidae).

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Abstract

The Bornean representatives of the genus *Georissa* (Hydrocenidae) have small, dextral, conical, calcareous shells consisting of about three teleoconch whorls. Our recent study on the *Georissa* of Malaysian Borneo have revealed high intra- and inter-specific variation in the "scaly group" (a group of species with striking scale-like surface sculpture). The present study on the "non-scaly" *Georissa* is the continuation of the species revision for the genus. We find that the "non-scaly" species are also diverse in shell sculpture. This informal group comprises *Georissa* with subtle spiral and/or radial sculpture. The combination of detailed conchological assessment and molecular analyses provides clear distinctions for each of the species. We present conchological, molecular and biogeographic details for a total of 16 species of "non-scaly" *Georissa*. Three of these are new to science, namely *Georissa corrugata* sp. n., *Georissa insulae* sp. n., and *Georissa trusmadi* sp. n.

Introduction

The genus *Georissa* Blanford 1864 (Hydrocenidae) comprises minute terrestrial snails, generally characterized by a small, dextral, conical, calcareous shell with about three teleoconch whorls (Bandel, 2008; Thompson and Dance, 1983; Vermeulen et al., 2015). *Georissa* is primarily restricted to environments rich in calcium carbonate ($CaCO_3$). They are found in variable abundances on wet and shaded limestone walls or rocks, but occasionally on sandstone rocks and in vegetation that is not associated with a rocky substrate (Chapter 2; Haase and Schilthuizen, 2007). They have a calcareous operculum, constructed in a concentric paucispiral manner and a peg attached at the inner surface. The hemi-spherically shaped protoconch has a distinct microsculpture, which often shows species-specific distinctness (Chapter 2).

Until recently, simple conchological analyses have been the main approach to describe and study the species of Bornean *Georissa*. In our recent systematic study of the "scaly" *Georissa* (see Chapter 2), however, we combined molecular, detailed conchological examination and biogeographic data of each species to assist in the process of species delimitation. We have revealed that this group of minute land snails has high intra- and inter-specific variation, especially in shell shape, size, aperture, and sculptural characters, as well as high allopatric diversity. Here, we provide a complete list of known “scaly” *Georissa* of Borneo: *G. scalinella* (van Benthem-Jutting, 1966); *G. saulae* (van Benthem-Jutting, 1966); *G. hosei* Godwin-Austen, 1889; *G. anyiensis* Khalik et al., 2018; *G. muluensis* Khalik et al., 2018; *G. hadra* Thompson and Dance, 1983; *G. kobelti* Gredler, 1902; *G. niahensis* Godwin-Austen, 1889; *G. silaburensis* Khalik et al., 2018; *G. bauensis* Khalik et al., 2018; *G. pyrrhoderma* Thompson and Dance, 1983; *G. kinabatanganensis* Khalik et al., 2018; *G. sepulutensis* Khalik et al., 2018. Striking allopatric patterns are well-known from several other microsnail taxa of Southeast Asia (see Liew et al., 2014, Hoekstra and Schilthuizen, 2011; Rundell 2008, Tongkerd et al. 2004). These studies have led to the realisation that the geographic variation of different populations needs to be well understood and used as an important guideline for species delimitation. Previous phylogenetic studies on the Bornean *Georissa* based on *16S* and *CO1* mtDNA allowed species to be recognised as monophyletic clades. There were at least two exceptions to this pattern, *G. kobelti* and *G. saulae*, which are paraphyletic with respect to the locally endemic, conchologically distinct *G. niahensis* and *G. filiasaulae*, respectively (Chapter 2; Schilthuizen et al., 2005). Such paraphyletic patterns are not unexpected when microgeographic speciation yields recently evolved, locally endemic species branched off from more widespread ancestors (Schilthuizen and Gittenberger, 1996).

In this paper, the second part of our work on the Bornean *Georissa*, we apply the same approach of combining information from multiple datasets to 16 species of Bornean *Georissa* that belong to the informal “non-scaly group”, characterised mainly by weak to strong spiral and/or radial sculptures without conspicuous scale-like sculpture on the shell. We also present the phylogenetic relationships among all Bornean *Georissa* and their distribution. We describe three species new to science, namely *Georissa corrugata* sp. n., *Georissa insulae* sp. n., and *Georissa trusmadi* sp. n.

Materials and methods

Fieldwork and collection material

We examined collection material from:

RMNH	Naturalis Biodiversity Center (previously collection from Rijksmuseum van Natuurlijke Historie), Leiden,

ZMA	Naturalis Biodiversity Center (previously collection from Zoological Museum of Amsterdam), Leiden,
NHMUK	Natural History Museum, London,
BORN	Borneensis Collection, Universiti Malaysia Sabah,
MZU	Zoology Museum, Universiti Malaysia Sarawak,
MFN	Museum für Naturkunde, Berlin, and,
JJV	Jaap Vermeulen (personal collection).

We conducted series of fieldwork at limestone outcrops in Malaysian Borneo between September 2015 and October 2018. We searched for living *Georissa* on the limestone walls and rocks, loose organic matter, and living leaves. The collected specimens were directly sorted and stored in sample tubes with ~96% ethanol. Ca. 5 liters of soil and leaf litter were sampled at every sampling location, which were later sieved and soaked in water to collect the empty shells by flotation (Vermeulen and Whitten, 1998). The floating organic matter was extracted and dried. The shells of *Georissa* were manually picked from the dried organic matter using the stereomicroscope and sorted. The holotypes, paratypes and other materials were deposited at MZU, BORN, and RMNH.

Morphological analysis

Microscopy. The shells were observed under a stereomicroscope for species identification and detailed examination at 40–100× magnification. The 2-dimensional images of the individual shell of each *Georissa* species were captured in three views, namely apertural (aperture as the frontal view), side (the right side of the shell as the frontal view), and rear (umbilical region as the frontal view) views, using AxioCamMRc5, Zeiss PlanApo S 1.0×FWD 60.0mm lenses. The apertural view images of each individual shell were then measured to obtain shell height (SH), shell width (SW), aperture height (AH), and aperture width (AW). **Scanning electron microscopy (SEM).** We selected a representative adult shell of each species for detailed character examination by using scanning electron microscopy. The shells were first cleaned in sodium hypochloride (household bleach, 10× dilution, for 3-5 minutes) dried and then sputter-coated with Pd/Pt coating agent. We used a JEOL JSM-6480LV machine for SEM imaging to obtain detailed shell characters of the teleoconch and protoconch. **Micro-computed tomography**

(µ-CT). The µ-CT scanning was carried out using an Xradia 520 Versa X-ray Microscope (see **Supplementary material 1** for µ-CT scanning parameters). We obtained ca. 995 layers of X-ray images of an individual shell per scanning, which we then used to reconstruct a composite 3-dimensional image of the shell. These images were then segmented in Avizo ver. 9.4.0 (FEI Company), to examine the operculum, peg, and inner part of the shell.

Molecular analysis

DNA extraction. The sample preparation prior to DNA extraction procedure followed the method from **Chapter 2**. We extracted the genomic DNA from 52 individuals using the Qiagen DNeasy Blood and Tissue kit, and applied the protocol provided by the manufacturer. **DNA amplification.** We used the primer pairs LR-J-12887 5’-CCGGTCTGAACTCAGATCACGT-3’ (forward) and LR-N-13398 5’-CGCCTGTTTAACAAAAACAT-3’ (reverse) (Schilthuizen et al., 2005) to amplify a fragment of 458-466 bp of *16S* gene, and LCO1490 5’-GGTCAACAAATCATAAAGATATTGG-3’ (forward) and HCO2198 5’-TAAACTTCAGGGTGACCAAAAAATCA-3’ (reverse) (Folmer et al., 1994) to amplify a fragment of 585-603 bp *CO1* gene. We amplified both these mtDNA regions on a BIO-RAD C1000 Touch^{TM} Thermal Cycler. The PCR master mix and amplification procedures followed as in **Chapter 2**. **DNA Sequencing.** PCR products were sent to BaseClear B.V. (Leiden, The Netherlands) and Sanger sequenced in forward and reverse directions using the ABI3730XL sequencer, Life Technologies.

Sequence alignment and phylogenetic analyses

Sequence data. From GenBank, we downloaded *16S* and *CO1* mtDNA sequences of representatives of the “scaly group” species, *G. gomantonensis* (Chapter 2), a full mitochondrial genome of *G. similis* (Uribe et al., 2016), and, as an outgroup, *Bathynerita naticoidea* (Arellano et al., 2016). We extracted the *16S* and *CO1* regions from the *G. similis* full mitochondrial genome to be included among the sequences in our phylogenetic analysis. The newly sequenced data were assembled using *de novo* Geneious 10.2.3 assembler, manually edited, and trimmed for ambiguities. This resulted in a total of 68 and 53 sequences of *16S* and *CO1* mtDNA, respectively. Sequences were deposited in GenBank via BankIt (https://www.ncbi.nlm.nih.gov/-

WebSub/) and BOLD (http://boldsystems.org/). **Sequence alignment.** The *16S* and *CO1* mtDNA sequences were aligned to their respective genes using default parameters of MUSCLE (Edgar, 2004). The alignments were manually checked and edited. **Phylogenetic inference.** The alignment of *CO1* mtDNA was set to invertebrate mitochondrial genetic code at the third reading frame. The best fit nucleotide substitution models of the concatenated *16S* and *CO1* sequence alignment was determined using ModelFinder (Kalyaanamoorthy et al., 2017) based on corrected Akaike Information Criterion (AICc). The best fit nucleotide model for the concatenated sequence alignment is GTR+F+R4. **Phylogenetic analysis.** We performed a maximum likelihood analysis using the concatenated alignment using GTR+F+R4 nucleotide substitution model with ultrafast bootstrapping (5000 replicates) (Hoang et al., 2017) in IQ-TREE 1.6.3 (Nguyen et al., 2015). We used MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001) for Bayesian Inference using the following settings: GTR+I+G nucleotide substitution model; 1,100,000 number of generations; tree subsampling for every 200 generation; 100,000 burn-in length; 4 heated chains with heated chain temperature at 0.2. Details of the newly sequenced data and their accession number are listed in **Table 3.1**.

Species delimitation and description

Species delimitation of the “non-scaly group” Bornean *Georissa* was carried out based on detailed examination of the shell characters which are exclusive to the group, combined with the molecular analyses. While morphological analysis is widely accepted for species identification in gastropods, this conventional way of species delimitation could become very challenging when applied to the genus *Georissa* which show high morphological variation within and between populations. For this reason, we applied a similar species delimitation approach as done in the “scaly group” *Georissa* (Chapter 2). In view of the considerations given in Chapter 2, we refrained from web-based species delimitation in this case.

CO1 genetic divergence

CO1 genetic divergence was performed to determine the genetic distances between species of the “non-scaly group” *Georissa*. We conducted genetic distance analysis within and between species groups. We computed pairwise genetic distances of *CO1* sequence alignment based on the nucleotide substitution model Kimura 2-parameter in MEGA v. 7.0.26 (Kumar et al., 2016) which includes the transition + transversion, gamma distribution, and 1000 bootstraps for variance estimate. We conducted the analysis based on *CO1* sequence data of 40 individuals comprised of nine species, including three newly described species.

Table 3.1 List of specimens used in molecular analyses.

No.	Species	Voucher No.	Species name_sequence origin_location Town/District/Division, State. GPS coordinate	GenBank Accession No.	
				16S	*CO1*
1	*Georissa saulae* (van Benthem Jutting, 1966)	BOR/MOL 2663-2667	G.saulae_AY547385_Sinobang Batu Sinobang, Sabah. 04°48.04'N, 116°37.03'E	AY547385 (Schilthuizen et al. 2012)	n/a
2	*Georissa saulae* (van Benthem Jutting, 1966)	BOR/MOL 12770	G.saulae_Sau-001_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.41'N, 116°36.04'E	MG982262 (Khalik et al. 2018)	n/a
3	*Georissa saulae* (van Benthem Jutting, 1966)	BOR/MOL 12770	G.saulae_Sau-002_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.41'N, 116°36.04'E	MG982263 (Khalik et al. 2018)	n/a
4	*Georissa filiasaulae* Haase and Schilthuizen, 2007	BOR/MOL 12768	G.filiasaulae_002_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.41'N, 116°36.04'E	MK411785	MK505425
5	*Georissa filiasaulae* Haase and Schilthuizen, 2007	BOR/MOL 12768	G.filiasaulae_003_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.41'N, 116°36.04'E	MK411786	MK505426
6	*Georissa filiasaulae* Haase and Schilthuizen, 2007	BOR/MOL 12768	G.filiasaulae_005_Pungiton Sepulut Valley, Gua Pungiton, Sabah. 04°42.41'N, 116°36.04'E	MK411787	MK505427
7	*Georissa pachysoma* Vermeulen and Junau, 2007	MZU/MOL 17.63	G.pachysoma_BSM2-01_Bukit Sarang Bukit Sarang, Bintulu, Sarawak. 02°39.31’N, 113°02.47’E	MK411789	MK505443
8	*Georissa pachysoma* Vermeulen and Junau, 2007	MZU/MOL 17.63	G.pachysoma_BSM2-02_Bukit Sarang Bukit Sarang, Bintulu, Sarawak. 02°39.31’N, 113°02.47’E	MK411788	MK505442
9	*Georissa pachysoma* Vermeulen and Junau, 2007	MZU/MOL 17.63	G.pachysoma_BSM2-03_Bukit Sarang Bukit Sarang, Bintulu, Sarawak. 02°39.31’N, 113°02.47’E	MK411791	MK505441

10	*Georissa pachysoma* Vermeulen and Junau, 2007	MZU/MOL 17.63	G.pachysoma_BSM2-04_Bukit Sarang Bukit Sarang, Bintulu, Sarawak. 02°39.31’N, 113°02.47’E	MK411790	MK505440
11	*Georissa similis* Smith, 1893	MZU/MOL 16.14	G.similis_E001_Batu Batangan Batu Batangan, Sabah. 05°27.61’N, 118°06.17’E	MK411792	MK505446
12	*Georissa similis* Smith, 1893	MZU/MOL 16.14	G.similis_E002_Batu Batangan Batu Batangan, Sabah. 05°27.61’N, 118°06.17’E	MK411795	MK505444
13	*Georissa similis* Smith, 1893	MZU/MOL 16.14	G.similis_E003_Batu Batangan Batu Batangan, Sabah. 05°27.61’N, 118°06.17’E	MK411793	n/a
14	*Georissa similis* Smith, 1893	MZU/MOL 16.14	G.similis_E004_Batu Batangan Batu Batangan, Sabah. 05°27.61’N, 118°06.17’E	MK411794	MK505445
15	*Georissa bangueyensis* Smith, 1895	RMNH/MOL 5005090	G.bangueyensis_KPH01627.01_NewLocation1 New Location 1, Kinabatangan River, Sabah. 05°27.40’N, 118°08.76’E	MK403002	MH254770
16	*Georissa bangueyensis* Smith, 1895	RMNH/MOL 5005090	G.bangueyensis_KPH01627.02_NewLocation1 New Location 1, Kinabatangan River, Sabah. 05°27.40’N, 118°08.76’E	MK402999	MH254645
17	*Georissa bangueyensis* Smith, 1895	RMNH/MOL 5005052	G.bangueyensis_KPH01589.01_NewLocation1 New Location 1, Kinabatangan River, Sabah. 05°27.40’N, 118°08.76’E	MK402996	n/a
18	*Georissa bangueyensis* Smith, 1895	RMNH/MOL 5005052	G.bangueyensis_KPH01589.02_NewLocation1 New Location 1, Kinabatangan River, Sabah. 05°27.40’N, 118°08.76’E	MK402993	MH254230
19	*Georissa bangueyensis* Smith, 1895	RMNH/MOL 5005052	G.bangueyensis_KPH01589.05_NewLocation1 New Location 1, Kinabatangan River, Sabah. 05°27.40’N, 118°08.76’E	MK402998	MH254559

20	*Georissa bangueyensis* Smith, 1895	RMNH/MOL 5005057	G.bangueyensis_KPH01594.01_NewLocation1 New Location 1, Kinabatangan River, Sabah. 05°27.40’N, 118°08.76’E	MK402997	MH254416
21	*Georissa flavescens* Smith, 1895	BOR/MOL 7660	G.flavescens_KPH02157.12_Pangi Batu Pangi, Kinabatangan valley, Sabah. 05°32.01’N, 118°18.24’E	MK402995	MH254340
22	*Georissa flavescens* Smith, 1895	BOR/MOL 7638	G.flavescens_KPH02135.11_Pangi Batu Pangi, Kinabatangan valley, Sabah. 05°31.89’N, 118°18.37’E	MK402989	MH254024
23	*Georissa flavescens* Smith, 1895	BOR/MOL 7626	G.flavescens_KPH02123.07_Tomanggong Besar Batu Tomanggong Besar, Kinabatangan valley, Sabah. 05°31.83’N, 118°18.26’E	MK403001	MH254706
24	*Georissa flavescens* Smith, 1895	BOR/MOL 7293	G.flavescens_KPH01725.08_Tomanggong Besar Batu Tomanggong Besar, Kinabatangan valley, Sabah. 05°31.52’N, 118°18.41’E	MK402992	MH254160
25	*Georissa flavescens* Smith, 1895	BOR/MOL 7638	G.flavescens_KPH02135.08_Pangi Batu Pangi, Kinabatangan valley, Sabah. 05°31.89’N, 118°18.37’E	MK402990	MH254028
26	*Georissa flavescens* Smith, 1895	BOR/MOL 7416	G.flavescens_KPH01860.09_Tomanggong Besar Batu Tomanggong Besar, Kinabatangan valley, Sabah. 05°31.38’N, 118°17.89’E	MK403003	MH254769
27	*Georissa flavescens* Smith, 1895	BOR/MOL 7299	G.flavescens_KPH01733.03_Tomanggong Besar Batu Tomanggong Besar, Kinabatangan valley, Sabah. 05°31.33’N, 118°18.06’E	MK402994	MH254313
28	*Georissa flavescens* Smith, 1895	BOR/MOL 7294	G.flavescens_KPH01727.13_Tomanggong Besar Batu Tomanggong Besar, Kinabatangan valley, Sabah.	n/a	MH254614

			05°31.46’N, 118°18.14’E		
29	*Georissa nephrostoma* Vermeulen et al., 2015	MZU/MOL 17.29	G.nephrostoma_K001_Keruak Batu Keruak, Kinabatangan valley, Sabah. 05°32.291'N, 118°18.376'E	MK411797	MK505439
30	*Georissa nephrostoma* Vermeulen et al., 2015	MZU/MOL 17.29	G.nephrostoma_K002_Keruak Batu Keruak, Kinabatangan valley, Sabah. 05°32.291'N, 118°18.376'E	MK411798	n/a
31	*Georissa nephrostoma* Vermeulen et al., 2015	MZU/MOL 17.29	G.nephrostoma_K003_Keruak Batu Keruak, Kinabatangan valley, Sabah. 05°32.291'N, 118°18.376'E	MK411800	n/a
32	*Georissa nephrostoma* Vermeulen et al., 2015	MZU/MOL 17.29	G.nephrostoma_K004_Keruak Batu Keruak, Kinabatangan valley, Sabah. 05°32.291'N, 118°18.376'E	MK411796	n/a
33	*Georissa nephrostoma* Vermeulen et al., 2015	MZU/MOL 17.29	G.nephrostoma_K005_Keruak Batu Keruak, Kinabatangan valley, Sabah. 05°32.291'N, 118°18.376'E	MK411799	n/a
34	*Georissa xesta* Thompson and Dance, 1983	BOR/MOL 7258	G.xesta_KPH02048.12_Materis Materis, Kinabatangan valley, Sabah. 05°31.39’N, 118°10’E	MK403000	MH254698
35	*Georissa xesta* Thompson and Dance, 1983	BOR/MOL 7303	G.xesta_KPH01738.05_Ulu Resang Ulu Sungai Resang, Kinabatangan valley, Sabah. 05°30.67’N, 118°20.39’E	MK402991	MH254122
36	*Georissa xesta* Thompson and Dance, 1983	BOR/MOL 7311	G.xesta_KPH01746.06_Ulu Resang Ulu Sungai Resang, Kinabatangan valley, Sabah. 05°31.16’N, 118°19.78’E	n/a	MH254082
37	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.11	G.hungerfordi_G001_Mawah Gunong Mawah, Padawan/Penrissen, Sarawak. 01°16.15’N, 110°15.46’E	MK411771	n/a
38	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.11	G.hungerfordi_G002_Mawah Gunong Mawah, Padawan/Penrissen, Sarawak. 01°16.15’N, 110°15.46’E	MK411773	n/a

39	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.11	G.hungerfordi_G003_Mawah Gunong Mawah, Padawan/Penrissen, Sarawak. 01°16.15’N, 110°15.46’E	MK411770	MK505432
40	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.11	G.hungerfordi_G004_Mawah Gunong Mawah, Padawan/Penrissen, Sarawak. 01°16.15’N, 110°15.46’E	MK411772	n/a
41	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.10	G.hungerfordi_I001_Regu Regu, Padawan/Penrissen, Sarawak. 01°12.82’N, 110°16.82’E	MK411775	MK505428
42	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.10	G.hungerfordi_I002_Regu Regu, Padawan/Penrissen, Sarawak. 01°12.82’N, 110°16.82’E	MK411774	MK505438
43	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.10	G.hungerfordi_I003_Regu Regu, Padawan/Penrissen, Sarawak. 01°12.82’N, 110°16.82’E	MK411777	MK505437
44	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.10	G.hungerfordi_I004_Regu Regu, Padawan/Penrissen, Sarawak. 01°12.82’N, 110°16.82’E	MK411776	MK505436
45	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.13	G.hungerfordi_H001_Sirat Gunong Sirat, Padawan/Penrissen, Sarawak. 01°12.42’N, 110°16.52’E	MK411784	MK505431
46	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.13	G.hungerfordi_H002_Sirat Gunong Sirat, Padawan/Penrissen, Sarawak. 01°12.42’N, 110°16.52’E	MK411783	MK505430
47	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.13	G.hungerfordi_H003_Sirat Gunong Sirat, Padawan/Penrissen, Sarawak. 01°12.42’N, 110°16.52’E	MK411778	MK505429
48	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.13	G.hungerfordi_H004_Sirat Gunong Sirat, Padawan/Penrissen, Sarawak. 01°12.42’N, 110°16.52’E	MK411782	n/a

49	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.12	G.hungerfordi_F001_Duai Gunong Seduai, Padawan/Penrissen, Sarawak. 01°12.25’N, 110°17.00’E	MK411780	MK505435
50	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.12	G.hungerfordi_F002_Duai Gunong Seduai, Padawan/Penrissen, Sarawak. 01°12.25’N, 110°17.00’E	MK411781	MK505434
51	*Georissa hungerfordi* Godwin-Austen, 1889	MZU/MOL 16.12	G.hungerfordi_F004_Duai Gunong Seduai, Padawan/Penrissen, Sarawak. 01°12.25’N, 110°17.00’E	MK411779	MK505433
52	*Georissa insulae* sp. n.	MZU/MOL 18.02	G.insulae_Man_001 Pulau Mantanani Besar, Sabah. 06°43.06’N, 116°20.50’E	MK411801	n/a
53	*Georissa insulae* sp. n.	MZU/MOL 18.02	G.insulae_Man_002 Pulau Mantanani Besar, Sabah. 06°43.06’N, 116°20.50’E	MK411803	n/a
54	*Georissa insulae* sp. n.	MZU/MOL 18.02	G.insulae_Man_003 Pulau Mantanani Besar, Sabah. 06°43.06’N, 116°20.50’E	MK411804	n/a
55	*Georissa insulae* sp. n.	MZU/MOL 18.02	G.insulae_Man_004 Pulau Mantanani Besar, Sabah. 06°43.06’N, 116°20.50’E	MK411802	n/a

Results and discussion

Morphological and phylogenetic analyses

The “non-scaly” *Georissa* from Borneo are characterised by the simple spiral and/or radial sculpture on the shell, unlike the distinct scale-like structures of the “scaly group”. These two informal groups of *Georissa* could be used as an initial framework for future species identification. Previously, Thompson and Dance (1983) divided the Bornean *Georissa* into four groups, namely the “*hosei*”, “*borneensis*”, “*everetti*”, and “*williamsi*” groups. The “*hosei*” group and a species of the “*borneensis*” group (i.e., *G. pyrrhoderma*) are species with scaly sculpture. Thompson and Dance (1983) included *G. monterosatiana* from Peninsular Malaysia in the “*hosei*” group, which does not have obvious scales on the shell. The rest of the groups of Thompson and Dance (1983) consist of the “non-scaly” species, which were further distinguished based on their color and ribbing. Although shell color may help in species-level taxonomy, we suggest not to use color as a character for species grouping, given the wide range of shell color variation in most Bornean *Georissa*.

Our previous work on the “scaly group” Bornean *Georissa* (Chapter 2) together with this present study on the “non-scaly group” have resulted in a complete revision of the Bornean *Georissa*. To date, we recognise 29 species of Bornean *Georissa*, of which 13 are in the “scaly group” and 16 are in the “non-scaly group”. Since we have studied and examined all shell materials from BORN, MZU, ZMA, RMNH, MFN, NHMUK and JJV, we find that it is useful to highlight some issues related to the “non-scaly group” that could be beneficial for future understanding. Firstly, the name *G. williamsi* was mentioned in several publications to refer to a species with distinct spiral ribs (Thompson and Dance, 1983; Clements et al., 2008; Nurinsiyah et al., 2016; Maassen, 2003; O’Loughlin and Green, 2016; Vermeulen and Whitten, 1998). After examination of the holotype of *G. williamsi* in the NHMUK, we find that this species name has often been misapplied. The images provided by Thompson and Dance (1983, figs. 66-68), Phung et al. (2017, fig. 8C), and Vermeulen and Whitten (1998, fig. 15) show entirely different spiral sculpture than the ‘true’ *G. williamsi*. Based on the taxonomy presented in this paper, the specimens illustrated in Thompson and Dance (1983) are *G. bangueyensis* Smith, 1895; those in Phung et al. (2017) are *G. insulae* sp. n.; and that in Vermeulen and Whitten (1998) is *G. javana* Möllendorff, 1897.

There is a similar confusion with *G. borneensis*, a name widely applied to both *G. similis* and *G. corrugata* sp. n. in the collection materials. Schilthuizen et al. (2003) mentioned *G. similis*, but it is presently not sure if this refers to the true *G. similis* or otherwise, because the collection numbers of the specimens used in their studies (materials deposited in BORN/RMNH) was not mentioned. *G. similis* and *G. corrugata* are conchologically distinct from *G. borneensis* (see detailed description in **Systematic part**).

Clements et al. (2008) and Schilthuizen et al. (2003, 2011) refer to several species of “non-scaly” *Georissa*, namely, *G. borneensis*, *G. bangueyensis*, *G. similis*, and *G. williamsi*. Again, we cannot be sure whether the specimens were correctly assigned since we could not examine the materials studied by these authors, due to similar case as above.

On the one hand, we find that “non-scaly” *Georissa* have strongly supported monophyletic groups with bootstrap and posterior output values in our phylogenetic analyses, ranging from 96–100 and 100, respectively. This corresponds to conchological characters of the respective taxa. On the other hand, we find *G. xesta* is paraphyletic. Discussions for each species treatment are in the **Systematics part**.

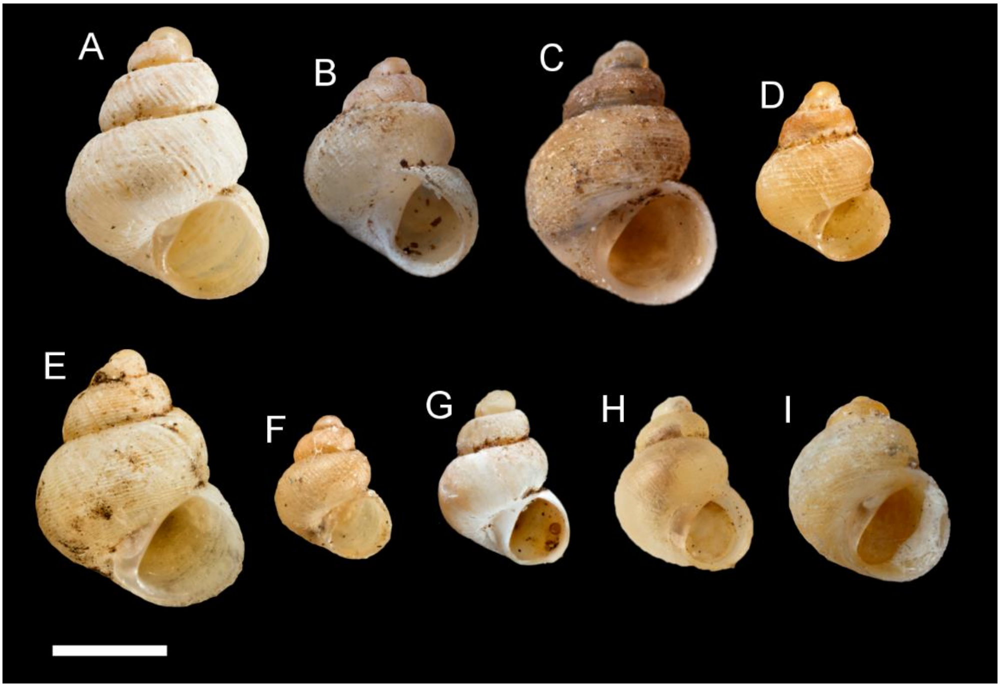


Figure 3.1 The types specimens of the “non-scaly” *Georissa* of Borneo from NHMUK. **A** *Georissa borneensis* Smith, 1895 **B** *Georissa everetti* Smith, 1895 **C** *Georissa williamsi* Godwin-Austen, 1889 **D** *Georissa hungerfordi* Godwin-Austen, 1889 **E** *Georissa gomantonensis* Smith, 1893 **F** *Georissa similis* Smith, 1893 **G** *Georissa xesta* Thompson and Dance, 1983 **H** *Georissa bangueyensis* Smith, 1895 **I** *Georissa flavescens* Smith, 1895. Scale bar = 1 mm. Photos by NHMUK.

A

Figure 3.2 A Maximum likelihood phylogenetic reconstruction with ultrafast bootstrapping (5000 replicates) **B** Bayesian phylogenetic reconstruction with posterior probabilities, constructed using MrBayes analysis. Phylogenetic analyses were conducted using concatenated sequence alignments of partial *16S* and *CO1* mtDNA. The analyses consist of 69 ingroup taxa (11 taxa representing the “scaly group” and 58 taxa representing the “non-scaly” group), and *Bathynerita naticoidea* as an outgroup. “Scaly” taxa in the phylogenies are with the red branches.

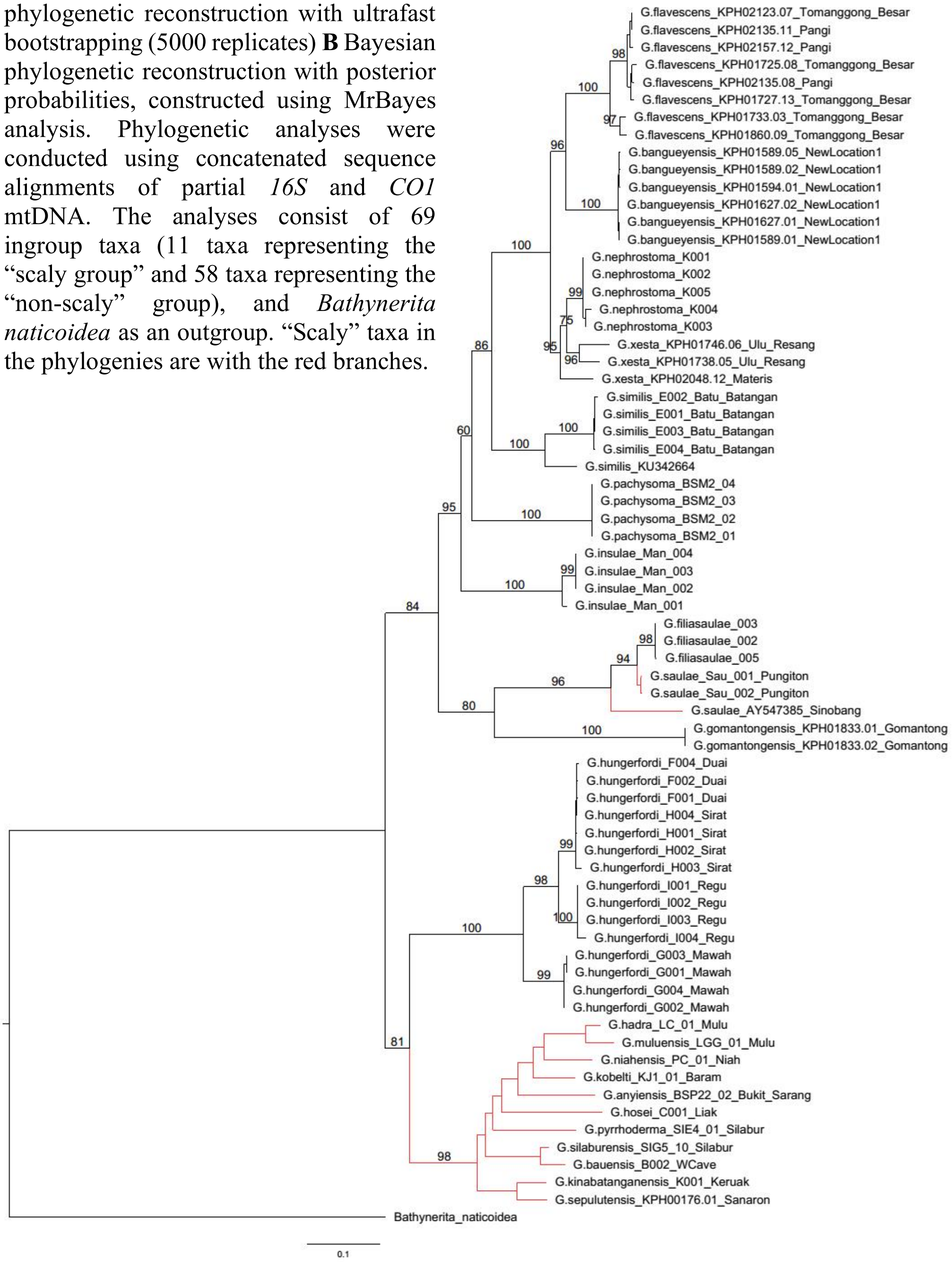
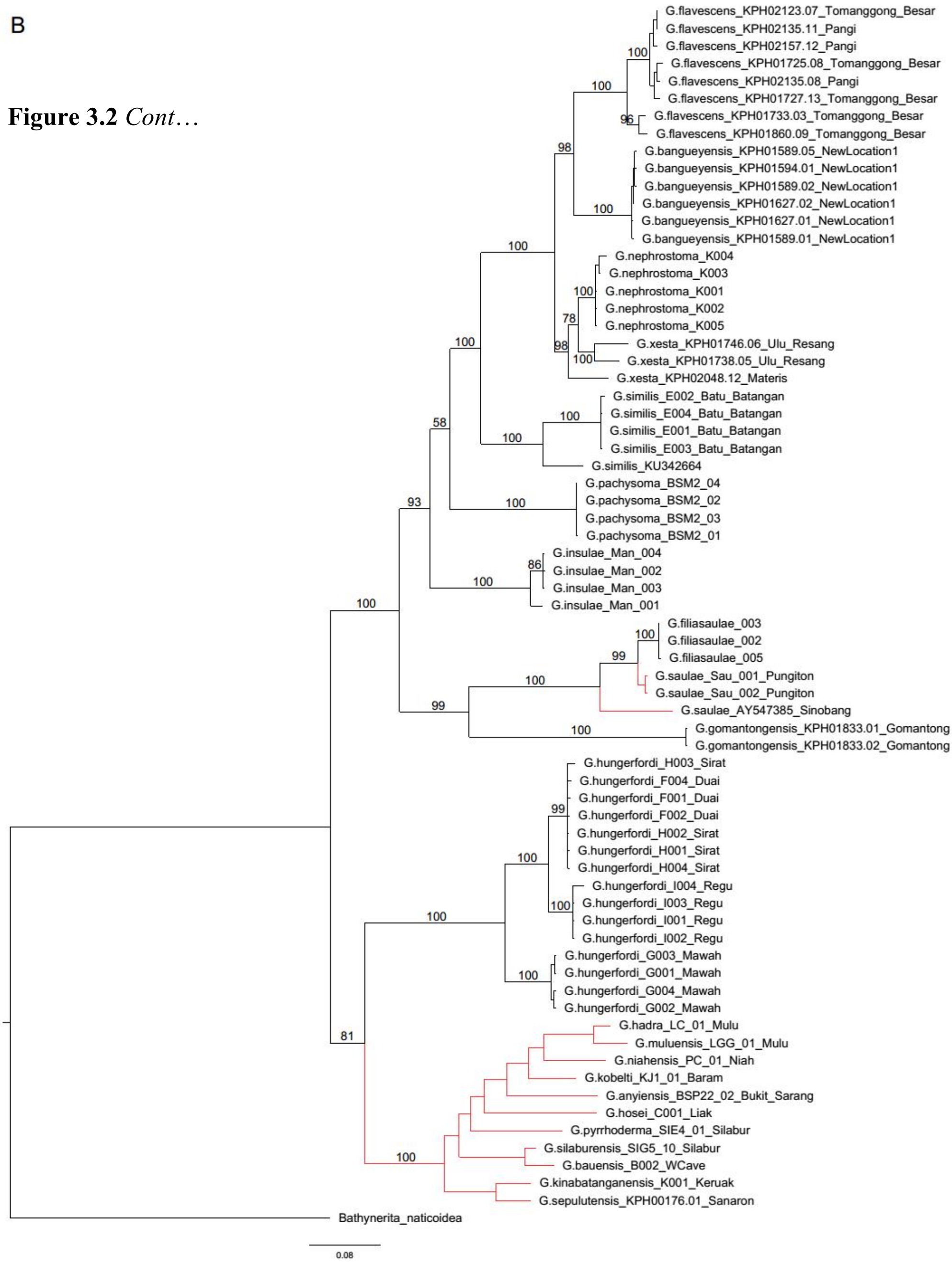


Figure 3.2 *Cont…*



CO1 genetic divergence

Species delimitation based solely on morphological analysis of a group of closely related minute gastropods such as *Georissa* could be challenging, especially when the studied taxa have high intra-specific variation (see Chapter 2; Liew et al., 2014). The analysis of molecular data provides a large benefit in the process of species delimitation. Previous systematic studies of gastropods have reported to successfully delimit the studied taxa to a species level by using *CO1* divergence (see Chapter 2; Boeters and Knebelsberger, 2012; Liew et al., 2014; Puillandre et al., 2012), but provide no specific genetic barriers for each studied taxon.

The *CO1* genetic divergence (**Table 3.2**) shows the Kimura 2-parameter distances within a group of species and net average distances between groups of *Georissa* sequences. This reveals that between-species genetic divergence of the "non-scaly" species exceeded 0.10, with the exception of *G. xesta* vs *G. flavescens*, *G. xesta* vs *G. bangueyensis*, and *G. xesta* vs *G. nephrostoma*. Although the divergences of these three species pairs are considerably low, they comprise groups of species with distinct morphological characters. This is similar to what was found with the "scaly" *Georissa*, for example, *G. silaburensis* vs *G. bauensis* is a conchologically distinct species pair that has a *CO1* divergence as low as 0.04 (Chapter 2). We also find that the intraspecific divergence within each "non-scaly" species is equal or does not exceed 0.05, with the exception of *G. xesta* (0.11).

Hoekstra and Schilthuizen (2011) suggested that intraspecific divergence for a limestone-dwelling microsnail (*Gyliotrachela hungerfordiana* Möllendorff, 1886) of Peninsular Malaysia would not exceed 0.10, which we find in the Bornean *Georissa* as well, with the exception of *G. xesta*. We find that the genetic divergence analysis of the Bornean *Georissa* provides useful information for species delimitation. There is, however, no specific genetic divergence limit that separates intraspecific from interspecific distances, since the divergence within a species and divergence between species often overlap (the highest value for intraspecific divergence = 0.11, while the lowest value for intraspecific divergence = 0.03).

Table 3.2 Intra- and inter-specific divergence of partial *CO1* sequences of nine species of the "non-scaly" *Georissa*.

		Divergence within group	Number of specimens	*G. gomantonensis*	*G. filiasaulae*	*G. hungerfordi*	*G. pachysoma*	*G. similis*	*G. flavescens*	*G. bangueyensis*	*G. nephrostoma*	*G. xesta*
1	*G. gomantonensis*	0.00	2									
2	*G. filiasaulae*	0.00	3	0.24								
3	*G. hungerfordi*	0.05	11	0.21	0.22							
4	*G. pachysoma*	<0.01	4	0.22	0.25	0.20						
5	*G. similis*	<0.01	3	0.22	0.22	0.20	0.17					
6	*G. flavescens*	0.03	8	0.19	0.22	0.16	0.20	0.19				
7	*G. bangueyensis*	<0.01	5	0.22	0.23	0.17	0.23	0.20	0.11*			
8	*G. nephrostoma*	-	1	0.20	0.23	0.16	0.17	0.17	0.13	0.12		
9	*G. xesta*	0.11	3	0.17	0.18	0.13	0.12	0.13	0.09*	0.07*	0.03*	

*The average number of net base substitutions per site between species is equal or lower than 0.11, which is lower or equal to the highest number of base substitutions per site within a "non-scaly" species.

Systematics part

Class Gastropoda Cuvier, 1797
Family Hydrocenidae Troschel, 1856
Genus *Georissa* Blanford, 1864

“Non-scaly group”

We previously described the first informal group of Bornean *Georissa*, the “scaly group” which consists of 13 species (**Chapter 2**). In the current paper, we describe the remaining group of Bornean *Georissa*, consisting of 16 species which do not have conspicuous scale sculpture and are characterised mainly based on species-specific patterns of more subtle radial and/or spiral sculpture. Our “non-scaly group” corresponds to Thompson and Dance’s (1983) “*williamsi*”, “*everetti*” and “*borneensis*” (p.p.) groups. A species of the “*borneensis* group”, *Georissa pyrrhoderma* Thompson and Dance, 1983 has been previously included by us in the “scaly group” (**Chapter 2**).

General conchological description of a “non-scaly group” representative. *Protoconch*. Color (in living or freshly dead specimens): white, yellowish green, orange, red, or brown. Sculpture pattern: smooth (no sculpture on the protoconch), straight lines (the sculpture is raised in a pattern of straight lines), rounded to ellipsoidal (the sculpture is rounded and/or ellipsoidal), mixed (a combination of more than one sculpture patterns), or irregular (the present sculpture comprises of no uniform shape or pattern). *Teleoconch*. Color (in living or freshly dead specimens): white, yellowish green, orange, red, or brown. First and subsequent whorls: convex (the whorls are partially circular in shape), rounded (the whorls are semi-circular in shape), and/or flat. Suture: deeply-impressed. Shoulder: narrow or extended. Number of whorls: 2 ¼–3 ½. Shell height (SH): 0.62–2.23 mm. Shell width (SW): 0.60–1.82 mm. Shell index (SI=SH/SW): 0.97–1.51. *Shell sculpture*. Radial sculpture: absent or present; if present then either raised in wavy and/or regular form, with narrow or wide interval. Growth lines: weak or strong, for species without clear formation of radial sculpture. Species with radial sculpture normally do not have clear growth lines since these are covered by the radial sculpture; such species generally have a row of nodules at the shoulder close to and parallel to the suture or away from the suture on the whorls. Spiral sculpture: absent, weak or strong, continuous or discontinuous, frequently the orientation is distorted by the radial sculpture (if present). *Columella*. Smooth and translucent. Umbilicus: open or closed. *Aperture*. Shape: semi-elliptic, ovoid or rounded, with straight, concave or convex parietal side, palatal edge either contiguous with the body whorl or with the parietal side. Aperture height (AH): 0.31–1.07 mm. Aperture width (AW): 0.33–1.09 mm. Aperture index (AI=AH/AW): 0.81–1.02. *Peristome*. Simple, thickened inside, sharp toward the edge of the aperture. *Operculum*. Shape: ovoid to rounded, the inner surface of the operculum has a small crater-like structure next to the peg. Peg: straight or curved. The shell dimensions of the "non-scaly" *Georissa* are summarised in **Supplementary material 2**.

All species of Bornean *Georissa* have a broadly developed callus that fully covers the umbilicus, except *G. leucococca*, which has this callus incompletely developed. Hence, the umbilical region of this species is partially open. *G. nephrostoma* is the only known Bornean *Georissa* with a 'bulb'-like callus covering the umbilical region. This is an inflation of the columella along the parietal wall. As a result, the aperture of *G. nephrostoma* is partly obstructed, unlike any other aperture of the Bornean *Georissa*. Of all the "non-scaly" *Georissa*, the operculum is available, except for *G. corrugata*, *G. williamsi*, and *G. leucococca*.

Habitat and ecology. Like the "scaly group", the members of the "non-scaly group" *Georissa* are usually restricted to limestone areas. They can be found on the limestone walls, rocks located in wet and shaded environments, and occasionally at a low density on dry limestone walls and rocks, in the vegetation away from the limestone (e.g., *G. gomantonensis*), on other, non-limestone rocky substrates (e.g., *G. saulae*), and on limestone walls inside cave systems with partial or no exposure to the sunlight (e.g., *G. silaburensis* and *G. filiasaulae*).

Distribution. We provide distribution maps of the "non-scaly" *Georissa* of Malaysian Borneo in **Figures 3.3** and **3.4**. The species are divided into two distribution maps to avoid overlapping. There are at least twelve species of the “non-scaly group” in Sabah, two species in Sarawak, and another two species in both Sabah and Sarawak.

Remark. For the type material that was not examined during this study, we make a note in each of the species treatment that the type specimen was not seen.

In the following systematic descriptions of the “non-scaly” *Georissa*, the species treatment is arranged partly based on the molecular phylogeny (**Figures 3.2 A-B**). We start with the description of six species for which no DNA-data are available, namely (i) *Georissa borneensis* Smith, 1893, (ii) *Georissa corrugata* sp. n., (iii) *Georissa everetti* Smith, 1895, (iv) *Georissa williamsi* Godwin-Austen, 1889, (v) *Georissa trusmadi* sp. n., and (vi) *Georissa leucococca* Vermeulen, Liew and Schilthuizen, 2015, followed by the remaining ten species, treated in the order in which they appear in the phylogenetic tree. The numbers of individuals of the newly described species are stated in brackets (if available) right after the collection number. The locality data may contain the following Malay words: Batu = rock; Bukit = hill; Gua = cave; Sungai/Sungei/Sg. = river; Gunung/Gunong = mountain; Pulau = island; Kampung = village.

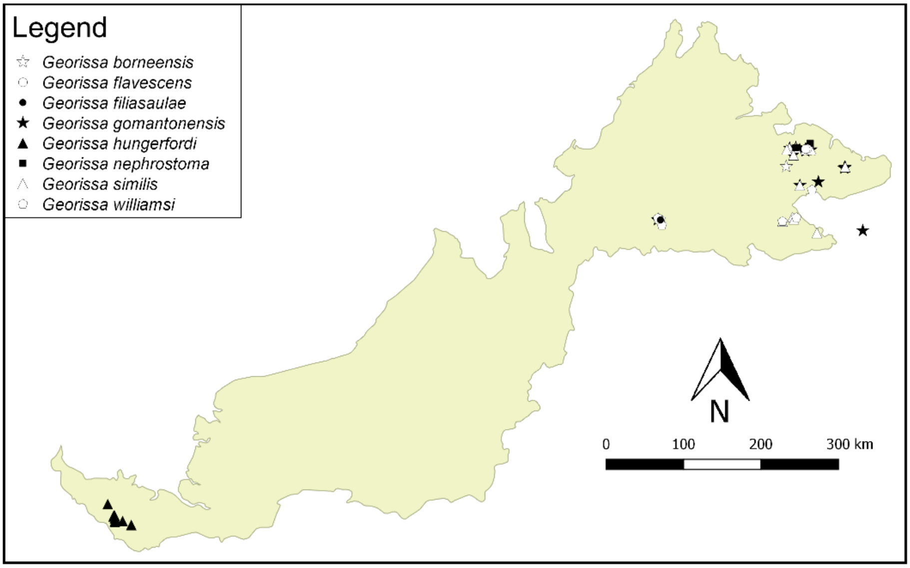


Figure 3.3 The distribution of eight species of the “non-scaly” *Georissa* of Malaysian Borneo, based on studied materials.

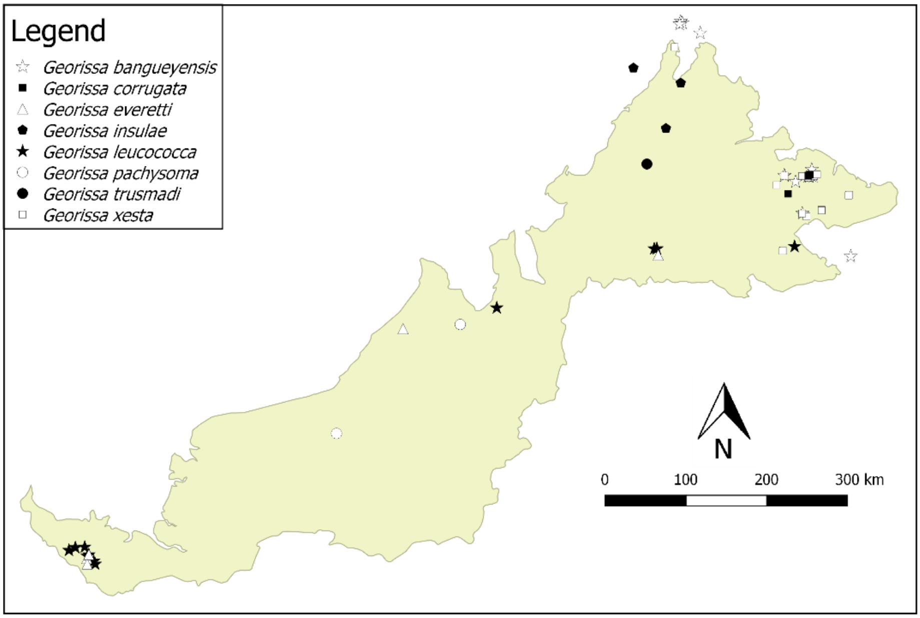


Figure 3.4 The distribution of another eight species of the "non-scaly" *Georissa* of Malaysian Borneo, based on studied materials.

Georissa borneensis Smith, 1895

Georissa borneensis Smith, 1895: 126, fig. 18 plate IV; Thompson and Dance, 1983: 122, figs. 18, 61–62.

Type locality. Gomanton, N.E. Borneo.

Type material. *Lectotype* (Designation by Thompson and Dance, 1983) (fig. 3.1A). Gomanton, N. Borneo: NHMUK 1894.7.20.61 (glued on paper). *Paralectotypes*. Gomanton, N. Borneo: NHMUK 1894.7.20.62, NHMUK 1894.21.54-57 (glued on paper).

Other material. N. Borneo: RMNH/MOL 152748, ZMA/MOLL 315546 (fig. 3.5). Gomanton, N. Borneo: MFN 47552, MFN 47942. Kinabatangan valley, Gomantong Hill 30 km South of Sandakan, Sandakan Province, Sabah (05°19.20'N, 118°3.60'E): JJV 1613.

Description. *Protoconch*. Color: white to pale orange, darker than the rest of the shell. Sculpture pattern: irregular sculpture pattern, from base to apex end with no specific sculptural shape. Mesh width: 2.5–8.0 µm. *Teleoconch*. Color: white to pale orange. First whorl: flat, convex close to the suture. Subsequent whorls: flat, convex and angular at the periphery. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ¾–3 ½. SH: 1.91–2.23 mm. SW: 1.65–1.82 mm. SI: 1.12–1.28. *Shell sculpture*. Radial sculpture: present, weak or flattened, densely sculpted on the whorls, about two to three ribs per 0.1 mm. Spiral sculpture: present, but thin and weak, only visible under high magnification (> ×100 magnification), strongest at the first whorl, weaker at subsequent whorls. *Aperture*. Shape: semi-elliptic, straight parietal side, palatal edge contiguous with the body whorl, palatal side tilted and angular, basal side convex. AH: 0.82–1.07 mm. AW: 1.00–1.09 mm. AI: 0.75–1.02.

Cross diagnosis. The flat whorls that are strongly convex at the periphery, giving the shell an angular shape, are diagnostic. The sculpture of *G. borneensis* resembles that of *G. similis* and *G. corrugata*, but is weaker and more flattened than in those species. The spiral sculpture of *G. corrugata* is also more irregular. The adult shell *G. borneensis* is larger than in adult *G. similis* and *G. corrugata*. Also, the base to apex end sculpture of the protoconch of *G. borneensis* is distinct compared to these species (*G. similis* has a rounded protoconch sculpture and *G. corrugata* has straight line protoconch sculpture).

Distribution. The species is known only from Gomantong hill in the Kinabatangan region of Sabah.

Discussion. The identification of *G. borneensis* can be confusing when we refer to the sketches by Thompson and Dance (1983: figs. 61 and 62). These appear to reflect the radial sculpture of *G. corrugata*, which is wavy/irregular and strongly sculpted. Smith (1895) and Thompson and Dance (1983) described *G. borneensis* referring to the absence of spiral sculpture, which, however, is present but only visible under high magnification. The bright red color of the peristome as described by Smith (1895) could not be observed by us, probably due to the faded condition of the shells. We also find there is no association based on coloration of *G. borneensis* with *G. pyrrhoderma* and the “*williamsi*”. Thompson and Dance (1983) grouped the ‘*borneensis*’ based on their reddish shell color, while ‘*williamsi*’ with their light brown color, of which we find these colors are often a variation within these groups of species.

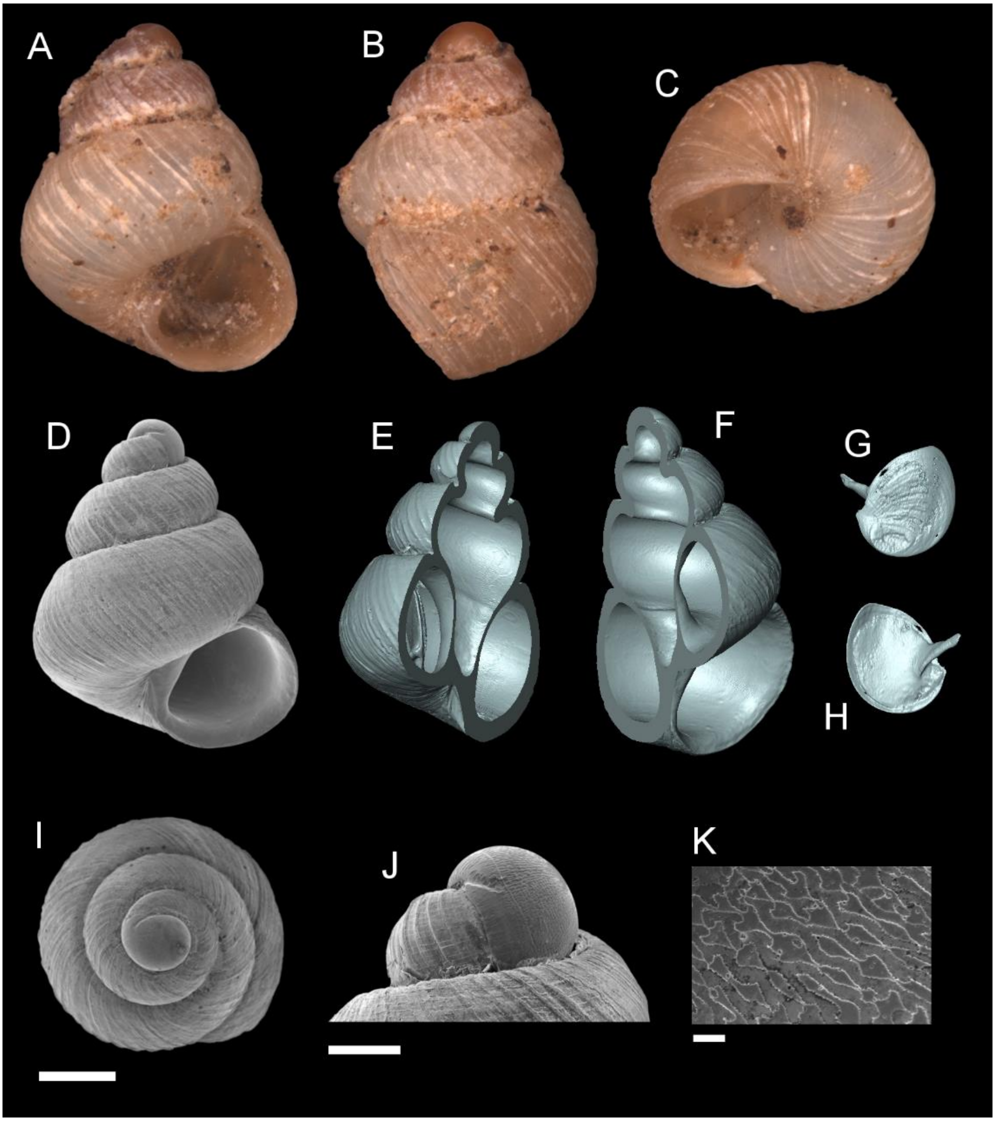


Figure 3.5 *Georissa borneensis* Smith, 1895. **A–K** ZMA/MOLL 315546 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa corrugata sp. n.

Type locality. Batu Tomanggong, Kinabatangan valley, Sandakan, Sabah, Malaysia (05° 31.86’N, 118°18.24’E).

Type material. *Holotype*. Batu Tomanggong, Kinabatangan valley, Sandakan, Sabah, Malaysia (05° 31.86’N, 118°18.24’E): MZU/MOL 16.15 (fig. 3.6 A-C) *Paratypes*. Batu Tomanggong, Kinabatangan valley, Sandakan, Sabah, Malaysia (05° 31.86’N, 118°18.24’E): MZU/MOL 16.16 (fig. 3.6 D-I). Batu Punggul, Sepulut valley, Sabah: JJV 1903 (1). North Borneo: RMNH/MOL 152848. Batu Keruak, Kinabatangan valley, Sabah (05°31.38’N, 118°17.10’E): BOR/MOL 1467, BOR/MOL 1844, BOR/MOL 11661 (1). Unnamed hill, Kinabatangan valley, Sabah (05°31.11’N, 118°17.23’E): BOR/MOL 2218 (1, juvenile).

Etymology. The name is derived from the Latin word *corrugatus*, meaning “wrinkled”, referring to the coarse and irregular radial sculpture.

Description. *Protoconch*. Color: white. Sculpture pattern: parallel lines of varying width, widening before splitting in two. Mesh width: 3.1–6.2 µm, with the distance between each of the sculptural units as wide as the mesh width. *Teleoconch*. Color: white. First whorl: rounded. Subsequent whorls: rounded. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ½–3. SH: 1.14–1.43 mm. SW: 1.01–1.11 mm. SI: 1.10–1.29. *Shell sculpture*. Radial sculpture: present, more prominent after the first whorl, wavy, irregular and widely spaced, often distinctly higher and strongly projected on and above the periphery. Spiral sculpture: present, regularly spaced, thin and discontinuous due the interruption by radial sculpture. *Aperture*. Shape: rounded to slightly ovoid, parietal side straight, palatal edge contiguous with the parietal side, palatal and basal sides convex. AH: 0.82–1.07 mm. AW: 1.00–1.09 mm. AI: 0.75–1.02. *Holotype dimensions*. SH: 1.71 mm, SW: 0.99 mm, AH: 0.52 mm, AW: 0.58 mm.

Cross diagnosis. The wavy and irregular, widely spaced and strong radial sculpture, with thin regularly arranged spiral sculpture in between is diagnostic for *G. corrugata*. *G. similis* and *G. borneensis* have a somewhat similar arrangement of radial and spiral sculpture, but do not carry the protoconch sculpture consisting of parallel lines. The shell shape and size of *G. corrugata* are similar to *G. similis*. Besides the difference in the protoconch sculpture, the latter species also has more densely arranged radial sculpture on the teleoconch.

Distribution. *G. corrugata* is distributed on the limestone hills of the lower Kinabatangan valley, known to occur from Gomantong to Batu Tomanggong, but always in low densities compared to other *Georissa* species. The species has also been found in the Sepulut valley, about a hundred km further to the southwest.

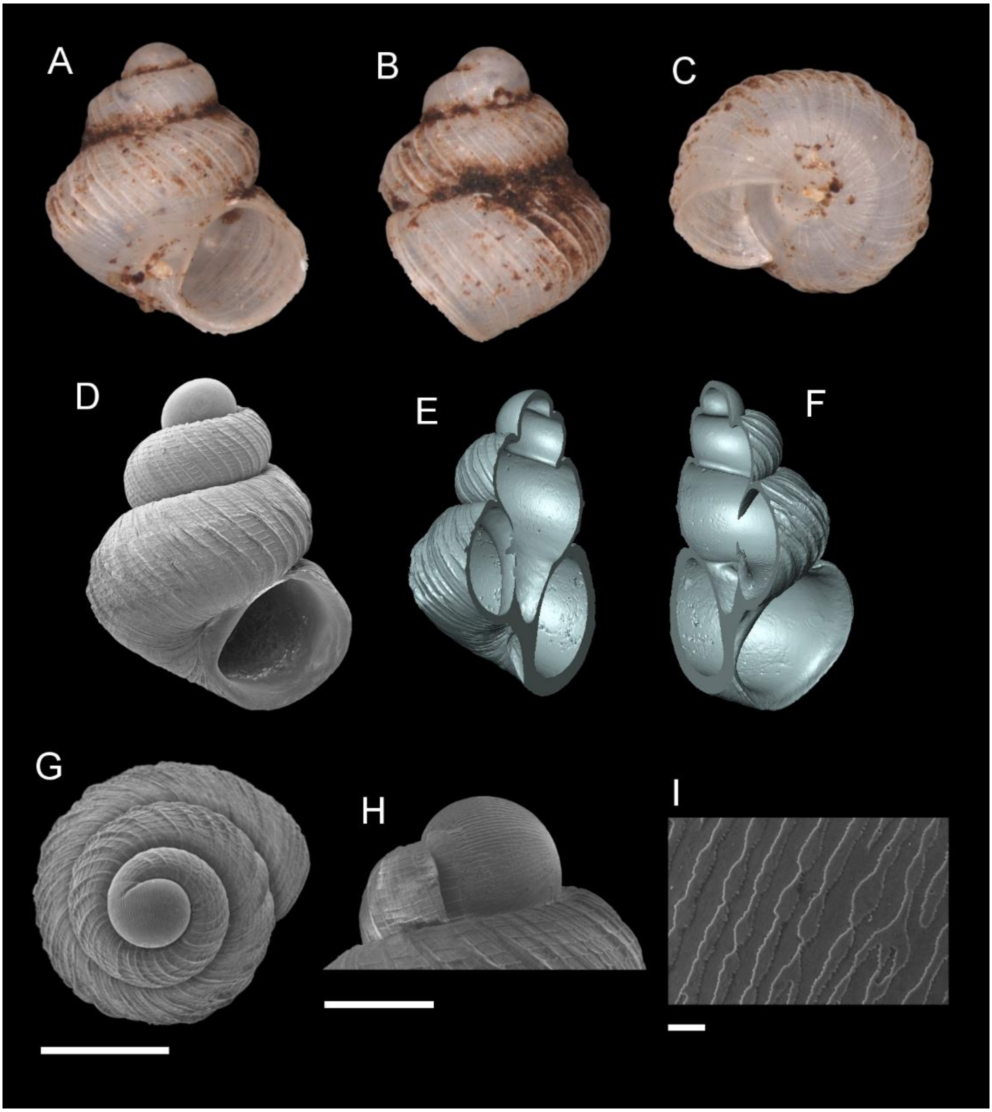


Figure 3.6 *Georissa corrugata* sp. n. **A–C** Holotype: MZU/MOL 16.15 **D–I** Paratype: MZU/MOL 16.16 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G** shell top view **H** Protoconch side view **I** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–G**); 200 µm (**H**); 10 µm (**I**).

Georissa everetti Smith, 1895

Georissa everetti Smith, 1895: 125, Plate IV fig. 15; Thompson and Dance, 1983: 120, figs. 55-57.

Type locality. Rumbang, W. Sarawak.

Type material. *Holotype* (Holotype by original monotypy). Rumbang, Sarawak: NHMUK 1893.6.7.69 (glued on paper) (fig. 3.1B) (Thompson and Dance, 1983).

Other material. Kampung Giam, Lower Penrissen valley, Sarawak: JJV 12546. Kampung Benuk, Lower Penrissen valley, Sarawak (01°18.47'N, 110°17.29'E): JJV 12548. Kampung Temurang, Upper Penrissen valley, Sarawak (01°12.15'N, 110°16.18'E): JJV 12547. Great Cave, Niah National Park, Sarawak: RMNH/MOL 336264, JJV 10185 (fig. 3.7), JJV 13119. Batu Punggul, Sepulut valley, Interior Province, Sabah: JJV 1906. Bukit Tinahas, Sepulut valley, Interior Province, Sabah (04°38.28'N, 116°37.05'E): JJV 7622.

Description. *Protoconch*. Color: orange to red. Sculpture: rounded to ellipsoidal mesh pattern, mixed with irregular sculptural shapes (whenever two or more rounded or ellipsoidal meshes are connected or combined). Mesh width: 4–30 µm. *Teleoconch*. Color: orange to red. First whorl: convex. Subsequent whorls: convex, with relatively wide penultimate and final whorls. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ¾–3 ¼. SH: 1.82–2.23 mm. SW: 1.52–1.75 mm. SI: 1.16–1.30. *Shell sculpture*. Radial sculpture: present, thin, forming small nodules when intersecting with spiral sculpture; these nodules are also present on the shoulder close to the suture. Spiral sculpture: present, thin, regularly spaced, oblique, appearing immediately after the protoconch, distorted/discontinuous by radial ribs. *Aperture*. Shape: rounded to ovoid, straight to concave parietal side, palatal edge contiguous with the body whorl, basal side convex. AH: 1.05–0.92 mm. AW: 1.09–0.96 mm. AI: 0.89–0.99.

Cross diagnosis. The strong and thin oblique spiral sculpture on its shell is diagnostic for *G. everetti*. *G. similis* has a somewhat similar knitted sculpture pattern resulting from the intersection of radial and spiral ribbing, but the shell shape is entirely distinct, with broad penultimate and final whorls. Based on the shell shape and habitus, *G. everetti* resembles *G. gomantonensis* and *G. williamsi*, which, however, have clear, regular, spiral shell ribs.

Distribution. *G. everetti* is widely distributed in Sabah and Sarawak, but is found in low abundances. The species known to occur from Penrissen/Padawan, Sarawak in the South (where Rumbang, the type locality is located), to further north, Niah, Sarawak, and Sepulut valley, Sabah.

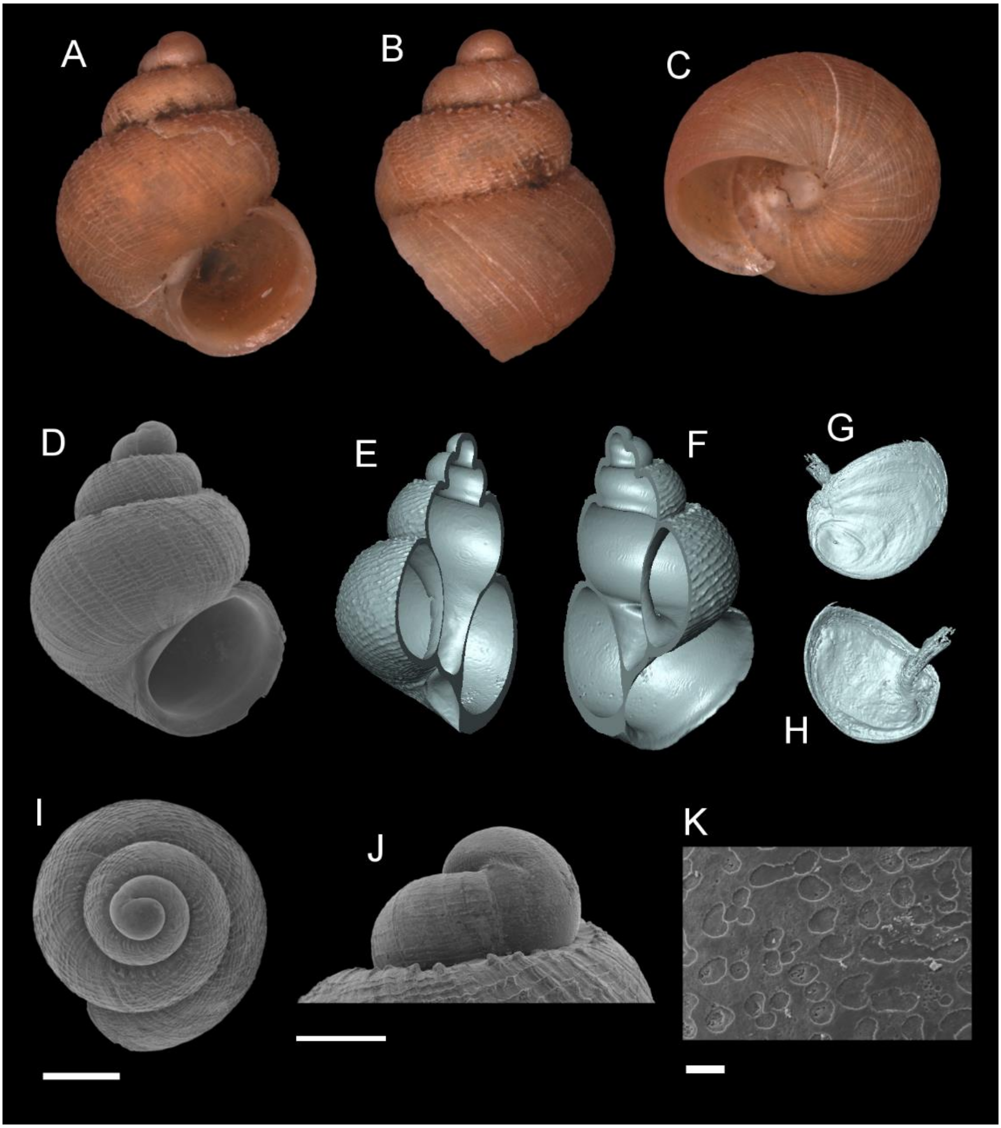


Figure 3.7 *Georissa everetti* Smith, 1895. **A–K** JJV 10185 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa williamsi Godwin-Austen, 1889

Georissa williamsi Godwin-Austen, 1889: 353, Plate XXXIX fig. 10; Thompson and Dance, 1983: 124 (**non** *G. hungerfordi* Godwin-Austen, 1889; *G. javana* Möllendorff, 1897; *G. javana intermedia* Möllendorff, 1897).

Hydrocena williamsi (Godwin-Austen, 1889): Saul, 1967: 109.

Georissa sp.1 (Godwin-Austen, 1889): Clements et al., 2008: Appendix D.

Type locality. Borneo.

Type material. *Holotype* (Holotype by original monotypy). Borneo: NHMUK 1889.12.7.71 (glued on paper) (fig. 3.1C) (Thompson and Dance, 1983).

Other material. Batu Punggul, Sepulut valley, Interior province, Sabah (04°39.00’N, 116°37.00’E): RMNH/MOL 187642, BOR/MOL 57, JJV 1907. Gua Pungiton, Sepulut valley, Interior province, Sabah (04°42.41’N, 116°36.04’E): BOR/MOL 55, JJV 7543. Bukit Tinahas, Sepulut valley, East end of Batu Punggul limestone, Interior province, Sabah (04°38.28’N, 116°37.05’E): JJV 7623. Tinahas limestone hill, Interior Province, Sabah (04°38.46’N, 116°37.08’E): RMNH/MOL 333928, RMNH/MOL 334016, BOR/MOL 56, BOR/MOL 59. Batu Temurung, Sepulut valley, Interior province, Sabah (04°42.45’N, 116°34.40’E): BOR/MOL 58, BOR/MOL 60, JJV 8037. Simbaluyon limestone hill, Interior Province, Sabah, (04°43.25’N, 116°34.22’E): RMNH/MOL 333922, RMNH/MOL 333946 (fig. 3.8), RMNH/MOL 334007. Batu Baturong c. 50 km W.S.W. of Lahad Datu, Tawau province, Sabah (04°41.00’N, 118°1.00’E): JJV 1830. Madai limestone hill, Tawau Province, Sabah (04°43.66’N, 118°10.71’E): RMNH/MOL 337817, RMNH/MOL 337827, RMNH/MOL 337834. Cave on Teck Guan estate, Lahad Datu, Sabah: ZMA/MOLL 315607, ZMA/MOLL 315608, ZMA/MOLL 315609, ZMA/MOLL 315622.

Description. *Protoconch*. Color: orange to red. Sculpture: a mix of rounded, ellipsoidal to irregular sculptural shape. Mesh width: 2–6 µm. *Teleoconch*. Color: orange to red. First whorl: convex. Subsequent whorls: convex. Suture: well-impressed. Shoulder: narrow. Number of whorls: 3–3 ¼. SH: 1.58–1.91

mm. SW: 1.17–1.42 mm. SI: 1.30–1.38. *Shell sculpture*. Radial sculpture: absent, only weak to strong growth lines are visible at irregular intervals. Spiral sculpture: present, thin, regularly spaced at the first whorl, appearing immediately after the protoconch, on later whorls the spiral sculpture weakens and becomes distorted by the growth lines; more than 20 spiral ribs on the body whorl of the adult individual. *Aperture*. Shape: semi-elliptic, straight to concave parietal side, palatal side rounded, palatal edge contiguous with the body whorl, basal side slightly convex. AH: 0.63– 0.81 mm. AW: 0.71–0.87 mm. AI: 0.89–0.95.

Cross diagnosis. *G. williamsi* has a broad final whorl, in which it is similar to *G. gomantonensis* and *G. everetti*. However, these three species are all distinctly sculptured, where *G. gomantonensis* has raised spiral sculpture, *G. everetti* has oblique spiral sculpture, but *G. williamsi* has thin, hardly raised, and densely arranged spiral sculpture (4-6 ribs in every 0.1 mm), despite the similar shell habitus.

Distribution. *G. williamsi* occurs over a large part of Sabah from the Sepulut valley in the west-central to Tawau and Lahad Datu in the east.

Discussion. The type locality of *G. williamsi* is ‘Borneo’, with no specific location stated by Godwin-Austen (1889). Saul (1966) in her note on “Shell collecting in the limestone cave of Borneo” mentioned that during her trip to Lahad Datu, Sabah, they collected *G. williamsi* (syn. *Hydrocena williamsi*). Based on the characters of *G. williamsi* described by Godwin-Austen (1889) and the type material we have examined, the species does not have very prominent spiral sculpture. *G. williamsi* was previously misinterpreted as having highly raised spiral sculpture, and the name was therefore misapplied to forms like *G. hungerfordi*, *G. insulae*, and *G. javana* (Thompson and Dance, 1983; Phung et al., 2017; Vermeulen and Whitten, 1998).

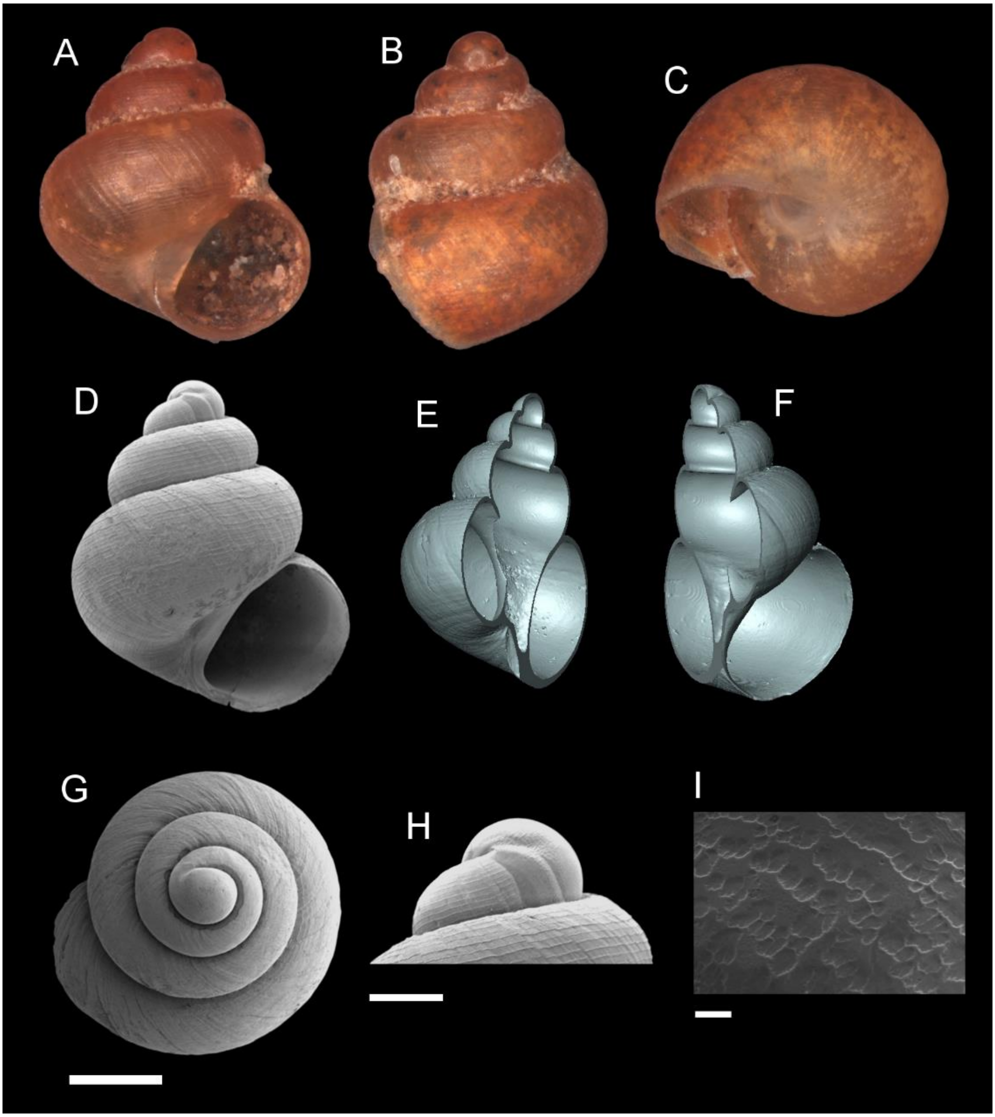


Figure 3.8 *Georissa williamsi* Godwin-Austen, 1889. **A–I** RMNH/MOL 333946 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G** shell top view **H** Protoconch side view **I** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–G**); 200 µm (**H**); 10 µm (**I**).

Georissa trusmadi sp. n.

Type locality. Loloposon Cave, Gunung Trus Madi, Sabah, Malaysia (5°39.00’N, 116°29.51’E).

Type material. *Holotype*. Loloposon Cave, Gunung Trus Madi, Sabah, Malaysia (5°39.00’N, 116°29.51’E): MZU/MOL 16.17 (fig. 3.9 A-C). *Paratypes*. Loloposon Cave, Gunung Trus Madi, Sabah, Malaysia (5°39.00’N, 116°29.51’E): MZU/MOL 16.18 (8) (fig. 3.9 D-K). Gunung Trus Madi slopes, Gua Loloposon, Interior province, Sabah (5°39.00’N, 116°29.51’E) (20): JJV 13231.

Etymology. The species is named after the type locality, Gunung Trus Madi, Sabah.

Description. *Protoconch*. Color: orange. Sculpture pattern: rounded to irregular sculptural shape. Mesh width: 3–30 µm. *Teleoconch*. Color: orange. First whorl: convex. Subsequent whorls: convex. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ¾–3 ½. SH: 1.40–1.89 mm. SW: 1.12–1.37 mm. SI: 1.22–1.38. *Shell sculpture*. Radial sculpture: absent, weak growth lines present throughout the shell surface. Spiral sculpture: present, strong spiral ribs, broadly spaced above the whorls, ca. 5–7 strongly raised spiral ribs on the body whorl of the adult individual, appearing immediately after the protoconch, thin spiral ribs in between the stronger ones, more densely spaced and weaker at the basal part of the body whorl. *Aperture*. Shape: rounded to slightly ovoid, straight to convex parietal side, palatal edge contiguous with the parietal side, basal side convex. AH: 0.59– 0.72 mm. AW: 0.66–0.79 mm. AI: 0.85–0.91. *Holotype dimensions*. SH: 1.67 mm, SW: 1.28 mm, AH: 0.68 mm, AW: 0.75 mm.

Cross diagnosis. *G. trusmadi* is characterised by the highly raised spiral sculpture. The number of strong spiral ribs on the first whorl is lower (3–5) than on the later whorls (5–7). The spiral sculpture is similar to *G. insulae* and *G. hungerfordi*, but always shows fewer ribs. Based on the shell habitus, it is similar to *G. hungerfordi* from Sarawak. The latter species, however, has the spiral ribs on the body whorl less strongly raised.

Distribution. *G. trusmadi* is only known from Gunung Trus Madi, Sabah.

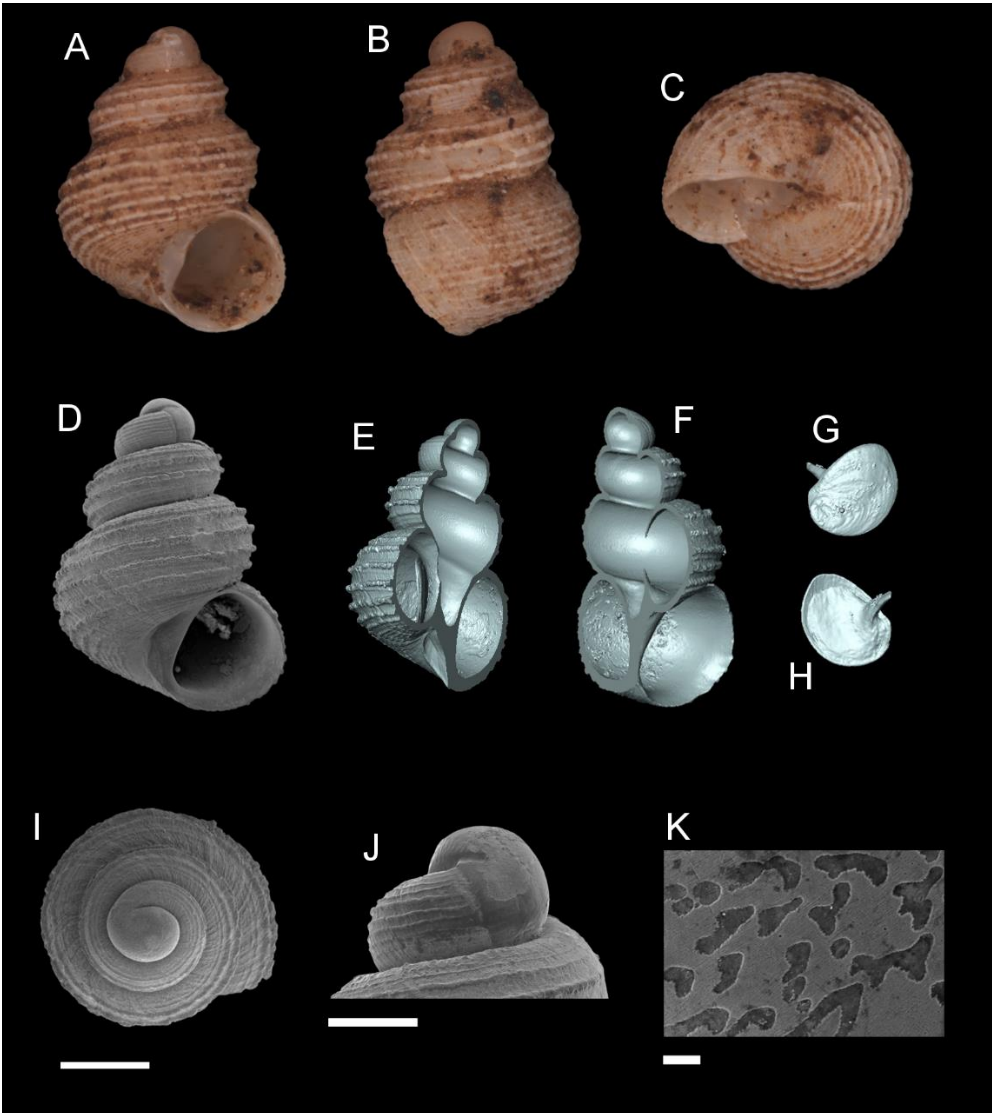


Figure 3.9 *Georissa trusmadi* sp. n. **A–C** Holotype: MZU/MOL 16.17 **D–K** Paratype: MZU/MOL 16.18 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa leucococca Vermeulen, Liew, and Schilthuizen, 2015

Georissa leucococca Vermeulen et al., 2015: 33, fig. 19 A-B; Marzuki and Foon, 2016: 317; Khalik et al., 2018: 2.

Type locality. Malaysia, Sabah, Interior Province, Sepulut valley, Gua Pungiton (04°42.41’N, 116°36.04’E).

Type material. *Holotype* (Holotype by original designation). Malaysia, Sabah, Interior Province, Sepulut valley, Gua Pungiton (04°42.41’N, 116°36.04’E): RMNH/MOL 5003956 (not seen, we were unable to locate the material in RMNH collection). *Paratypes*. Malaysia, Sabah, Interior Province, Sepulut valley, Gua Pungiton (04°42.41’N, 116°36.04’E): NHMUK 20150572, JJV 8081.

Other material. Gua Sanaron, Sepulut valley, Sabah (04°42.52’N, 116°36.16’E): JJV 8068. Gua Pungiton, Sepulut valley, Sabah: BOR/MOL 61. Gua Madai, Tawau province, Sabah (04°44.00’N, 118°8.00’E): JJV 1736. Batu Temurung, Sepulut valley, Sabah (04°42.45’N, 116°34.40’E): JJV 12681. Clearwater Cave, Mulu National Park, Sarawak: JJV 13098. Bukit Sarang group, Lower Tatau River valley, Sarawak: JJV 12571, JJV 12848, JJV 12849. Gunung Segu near Kampung Benuk, Penrissen valley, Sarawak (01°18.47’N, 110°17.29’E): JJV 12569. Bt. Krian, Upper Penrissen valley, Sarawak (01°12.20’N, 110°21.54’E): JJV 14217. Kampung Semedang, Lower Penrissen valley, Sarawak (01°17.49’N, 110°16.24’E): JJV 14221. Gunung Aup, Bau, Sarawak (01°21.36’N, 110°4.04’E): JJV 12570. Gunung Rapih, Bau, Sarawak (01°23.15’N, 110°8.29’E): JJV 12572 (fig. 3.10). Gunung Chupak, Sungei Bukar headwaters, Sarawak (01°14.05’N, 110° 20.50’E): JJV 14218, JJV 14219. Batu Staat, Sungei Sarawak Kiri valley, Sarawak (01°23.55’N, 110°14.55’E): JJV 14220.

Description. *Protoconch*. Color: white. Sculpture pattern: minutely formed, a mix of rounded, semi-elliptic to ellipsoidal. Mesh width: 1–2 µm. *Teleoconch*. Color: white. First whorl: convex. Subsequent whorls: convex. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ¼. SH: 0.62–0.72 mm. SW: 0.60–0.70 mm. SI: 0.97–1.06. *Shell sculpture*. Radial sculpture: absent, only weak growth lines at irregular intervals are visible. Spiral sculpture: present, thin, regularly spaced, appearing immediately after the protoconch, distorted by the growth lines, more prominent at the upper whorls, becoming weaker when closer to the columellar region. *Aperture*. Shape: semi-elliptic, straight to concave parietal side, palatal side rounded, palatal edge contiguous with the body whorl, basal side convex. Umbilicus: open, with a narrow space underneath the reflected columellar peristome. AH: 0.31– 0.37 mm. AW: 0.33–0.38 mm. AI: 0.87–0.97.

Cross diagnosis. *G. leucococca* has spiral sculpture that is more prominent at the upper part of the whorls, similar to *G. bangueyensis*. *G. leucococca* is so far the only known Bornean *Georissa* with an open umbilicus and with an adult shell size of hardly over 1 mm. It has an angular shell shape, similar to *G. borneensis*.

Distribution. *G. leucococca* is widely distributed in Malaysian Borneo. The species is known to occur from west Sarawak to east Sabah.

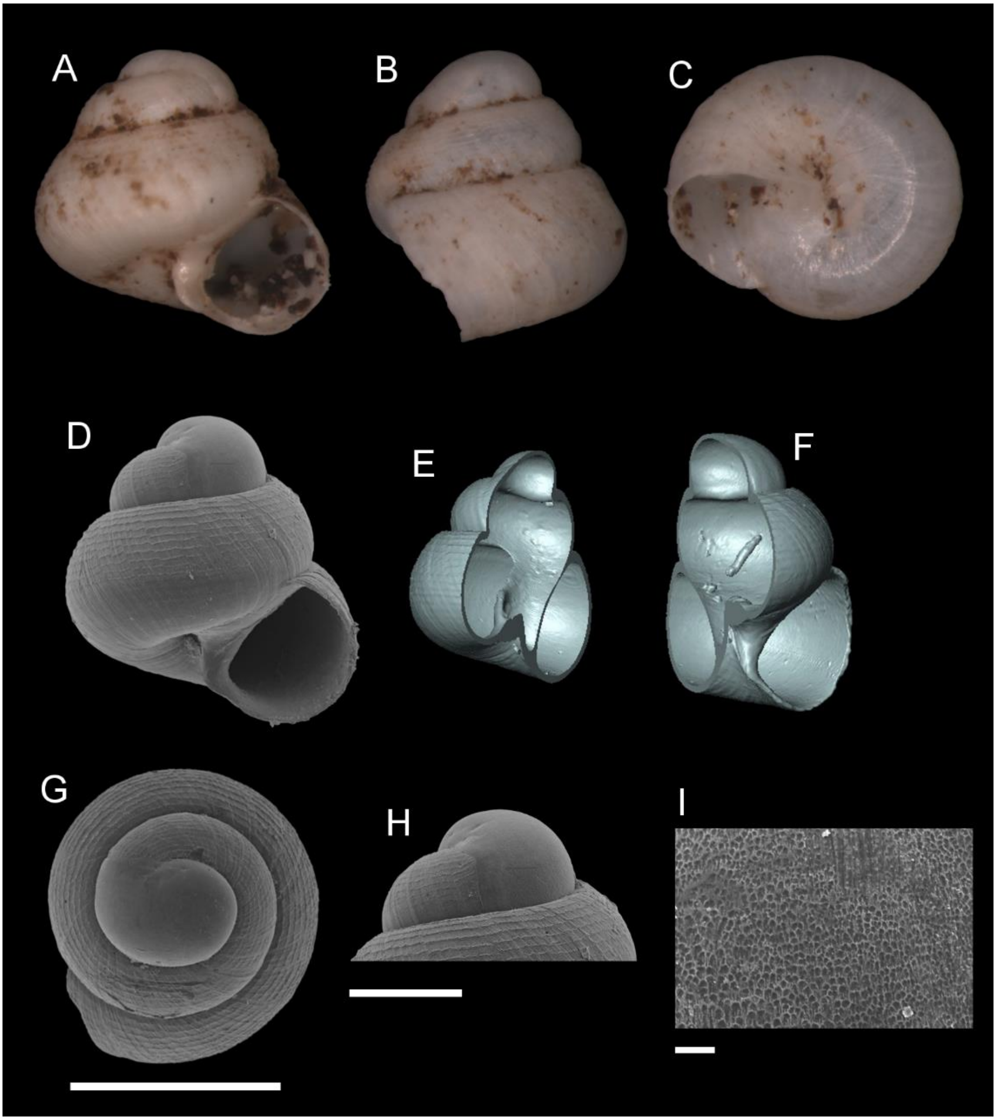


Figure 3.10 *Georissa leucococca* Vermeulen et al., 2015. **A–I** JJV 12572 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G** shell top view **H** Protoconch side view **I** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–G**); 200 µm (**H**); 10 µm (**I**).

Georissa hungerfordi Godwin-Austen, 1889

Georissa hungerfordi Godwin-Austen, 1889: 354, fig. 9 Plate XXXIX.

Georissa lowi Smith, 1893: 351.

Georissa williamsi Godwin-Austen: Thompson and Dance, 1983: 124 (**non** *G. williamsi* Godwin-Austen, 1889; *G. javana* Möllendorff, 1897; *G. javana intermedia* Möllendorff, 1897).

Type locality. Borneo.

Type material. *Lectotype* (Designation by Thompson and Dance, 1983). Borneo: NHMUK 1891.3.17.864 (glued on paper) (fig. 3.1D).

Other material. Rumbang, Sarawak: NHMUK 1893.6.7.71, NHMUK 1893.6.7.108-110, NHMUK 94.7.21.58 (glued on paper), NHMUK 94.7.20.63-4 (glued on paper). Regu, Kampung Timurang, Padawan/Penrissen, Kuching, Sarawak (01°12.82'N, 110°16.82'E): MZU/MOL 16.10. Gunong Mawah, Kampung Bengoh, Padawan/Penrissan, Kuching, Sarawak (01°16.15'N, 110°15.46'E): MZU/MOL 16.11 (fig. 3.11). Gunong Seduai/Duai, Kampung Timurang, Padawan/Penrissen, Kuching, Sarawak (01°12.25'N, 110°17.00'E): MZU/MOL 16.12. Gunong Sirat, Kampung Timurang, Padawan/Penrissen, Kuching, Sarawak (01°12.42'N, 110°16.52'E): MZU/MOL 16.13. Gunung Bra'ang, upper Penrissen valley, Kuching, Sarawak (01°14.12'N, 110°16.21'E): JJV 12451. Gunung Babu, upper Penrissen valley, Kuching, Sarawak (01°12.15'N, 110°16.18'E): JJV 12542. 12 km NNE of Padawan village, upper Penrissen valley, Kuching, Sarawak: JJV 13067, JJV 13070. Upper Penrissen valley, Bt. Krian, Kuching, Sarawak (01°12.20'N, 110°21.54'E): JJV 14222. Gunung Manok, upper Penrissen valley, Kuching, Sarawak (01°11.56'N, 110°16.16'E): JJV 14224. Gunung Kayan, upper Penrissen valley, Kuching, Sarawak (01°15.45'N, 110°15.30'E): JJV 14225. Sungei Bukar headwaters, G. Buros S of Gunung Nambi, Kuching, Sarawak (01°09.55'N, 110°27.59'E): JJV 14223. Gunung Pangga, Bau, Sarawak: JJV 2165. Gunung Jambusan, Bau, Sarawak: JJV 2213. Gunung Kapor, Bau, Sarawak: JJV 2274.

Description. *Protoconch*. Color: orange. Sculpture pattern: smooth. *Teleoconch*. Color: orange. First whorl: convex. Subsequent whorls: convex, shell shape slender to broad. Suture: well-impressed suture, straight to slightly concave, and narrow shoulder. Number of whorls: 2 ½–3 ¼. SH: 1.35–1.85 mm. SW: 1.38–1.20 mm. SI: 1.16–1.36. *Shell sculpture*. Radial sculpture:

absent, only weak to strong growth lines present throughout the shell surface. Spiral sculpture: present, strong spiral ribs, regularly spaced, with ca. 7–10 spiral ribs on the body whorl of the adult individual, appearing immediately after the protoconch, sometimes distorted/discontinuous by growth lines, more densely spaced spiral cords at the basal part of the body whorl. *Aperture*. Shape: rounded to slightly ovoid, straight to concave parietal side, palatal edge contiguous with the body whorl, basal side convex. AH: 0.63– 0.79 mm. AW: 0.67–0.83 mm. AI: 0.89–1.06.

Cross diagnosis. *G. hungerfordi* is characterised by the strong spiral sculpture with ca. 7–10 spiral ribs on the body whorl. The shell shape approaches the shape of *G. trusmadi* and *G. pachysoma*. *G. hungerfordi* has stronger spiral sculpture compared to *G. pachysoma* but weaker compared with *G. trusmadi*. The spiral ribbings resemble *G. insulae*, which has, however, a more elongated and slender shell shape.

Distribution. *G. hungerfordi* is distributed from Bau to Padawan/Penrissen, Kuching, Sarawak.

Discussion. Thompson and Dance (1983) considered *G. hungerfordi* a junior synonym of *G. williamsi*. We are, however, of the opinion that *G. hungerfordi* is a valid species based on the distinctly raised spiral sculpture of the shell compared to *G. williamsi*. *G. lowi* (Smith, 1893) is a junior synonym of *G. hungerfordi*. See also discussion in *G. williamsi* Godwin-Austen, 1889.

Molecular analysis. ML and Bayesian analyses show that the samples of *G. hungerfordi* (*16S*: n = 14; *CO1*: n = 11) form a monophyletic group with 100% BS and 100% PP, sister group to the "scaly group", except *G. saulae*.

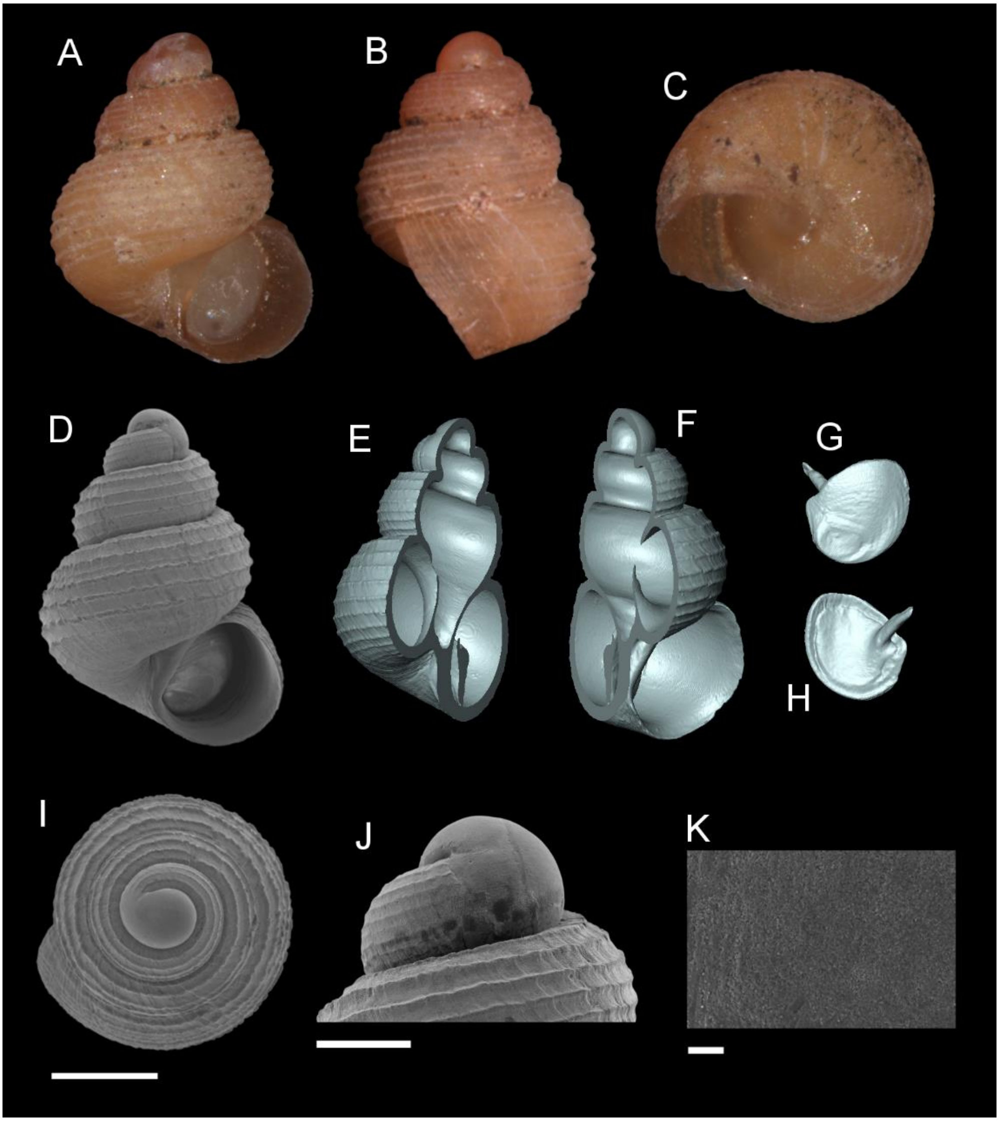


Figure 3.11 *Georissa hungerfordi* Godwin-Austen, 1889. **A–K** MZU/MOL 16.11 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa gomantonensis Smith, 1893

Georissa gomantonensis Smith, 1893: 351, Plate XXV fig. 25; Thompson and Dance, 1983: 121, figs. 36, 58-60; Schilthuizen et al., 2003: 41.

Georissa gomantongensis Smith: Vermeulen and Junau, 2007: 217; Clements et al., 2008: 2762; Khalik et al., 2018: 19, fig. 1J.

Type locality. Gomanton, N. Borneo.

Type material. *Holotype* (Holotype by original monotypy). Gomanton, N. Borneo: NHMUK 1892.7.20.39 (glued on paper) (fig. 3.1E) (Thompson and Dance 1983).

Other material. Gua Gomantong, Kinabatangan, Sabah (05°32.00’N, 118°06.00’E): BOR/MOL 7632, BOR/MOL 7389 (fig. 3.12), JJV 1612. Batu Tai (not Bod Tai) near Gomantong, Kinabatangan valley, Sabah (05°32.35’N, 118°10.32’E): JJV 9590. Batu Pangi, Kinabatangan valley, Sandakan province, Sabah (05°31.59’N, 118°18.43’E): BOR/MOL 10829, JJV 9648. Batu Keruak 2 near Sukau, Kinabatangan valley, Sabah (05°32.00’N, 118°18.00’E): JJV 9801. Kampung, Kinabatangan, Sabah (05°30.90’N, 118°16.86’E): BOR/MOL 10866, BOR/MOL 12545. Batu Tomanggong Besar 1, Kinabatangan, Sabah (05°31.26’N, 118°18.06’E): BOR/MOL 10561, BOR/MOL 11296. Batu Tomanggong Besar, lower Kinabatangan valley, Sandakan, Sabah (05°31.02’N, 118°18.21’E): BOR/MOL 2253, BOR/MOL 2282. Tomanggong 2, lower Kinabatangan valley, Sandakan, Sabah (05°31.00’N, 118°18.00’E): BOR/MOL 1462. Batu Keruak, Kinabatangan, Sabah (05°31.32’N, 118°17.10’E): BOR/MOL 1460, BOR/MOL 1883, BOR/MOL 11697. Bod Tai, Kinabatangan, Sabah (05°31.00’N, 118°13.00’E): BOR/MOL 1465, BOR/MOL 11256. Bukit Mawas, lower Kinabatangan valley, Sabah (05°27.00’N, 118°08.00’E): BOR/MOL 1463, BOR/MOL 1990. Unnamed hill 1, lower Kinabatangan valley, Sabah (05°31.11’N, 118°17.23’E): BOR/MOL 2152, BOR/MOL 2185. Unnamed hill 2, lower Kinabatangan valley, Sabah (05°30.00’N, 118°17.00’E): BOR/MOL 1461. Sabahmas Cave, Segama valley, Tawau, Sabah (05°08.52’N, 118°26.01’E): JJV 7452. Segama River, Segama valley, near bridge of road Sandakan to Lahad Datu, Tawau, Sabah (05°06.10’N, 118°13.12’E): JJV 7496. Tabin River, Segama valley, Sandakan, Sabah (05°18.49’N, 118°44.39’E): JJV 7753. Batu Temurung, Sepulut valley, Sabah (04°42.45’N, 116°34.40’E): JJV 8035. Pulau Mataking, Easternmost island of the Semporna-Sulu Chain, Sandakan: JJV 11523. Tabin Wildlife Reserve, Lahad Datu, Sabah (05°18.81’N, 118°44.65’E): BOR/MOL 19, BOR/MOL 20. Ulu Sungai Resang, lower Kinabatangan limestone hill, Sabah (05°31.00’N, 118°21.00’E): BOR/MOL 1464. N end of limestone ridge on E bank of Tabin River Sandakan Province, Sabah (05°18.04’N, 118°44.03’E): BOR/MOL 18. Batu Temurung, Sepulut Valley, Interior province, Sabah (04°42.04’N, 116°34.04’E): BOR/MOL 17.

Description. *Protoconch*. Color: greenish yellow. Sculpture: ellipsoidal mesh to irregular sculptural shape. Mesh width: 4–16 µm. *Teleoconch*. Color: greenish yellow. First whorl: convex. Subsequent whorls: convex. Suture: well-impressed. Shoulder: slightly extended, with a row of regularly spaced granules. Number of whorls: 3–3 ¼. SH: 1.95–2.17 mm. SW: 1.67–1.68 mm. SI: 1.17–1.29. *Shell sculpture*. Radial sculpture: absent, only weak growth lines present throughout the shell surface. Spiral sculpture: present, strongly sculpted spiral ribs, at regular intervals, appearing immediately after the protoconch, sometimes distorted/discontinuous by growth lines, reduced in strength when reaching the columellar region, ca. 14–18 spiral ribs visible on the body whorl in the adult individual. *Aperture*. Shape: rounded to slightly ovoid, straight to concave parietal side, palatal edge contiguous with the body whorl, basal side convex. AH: 0.94– 0.97 mm. AW: 1.04–1.08 mm. AI: 0.87–0.92.

Cross diagnosis. *G. gomantonensis* is characterised by its bright greenish yellow color, broad final whorl and strong spiral sculpture. The spiral sculpture pattern is similar to *G. insulae*, but the shell habitus of *G. gomantonensis* is much broader and inflated. *G. everetti* and *G. williamsi* have a similar shell habitus as *G. gomantonensis*, but differ because lacking of the oblique (*G. everetti*) and densely arranged (*G. williamsi*) spiral sculpture.

Distribution. *G. gomantonensis* is widely distributed throughout Sabah. More commonly found in the vegetation of the limestone forest, rather than on the limestone rocks themselves.

Molecular analysis. ML and Bayesian analyses show that the individuals of *G. gomantonensis* (*16S*: n = 2; *CO1*: n = 2) form a monophyletic group with 100% BS and 100% PP, sister group to the paraphyletic *G. saulae* + *G. filiasaulae*.

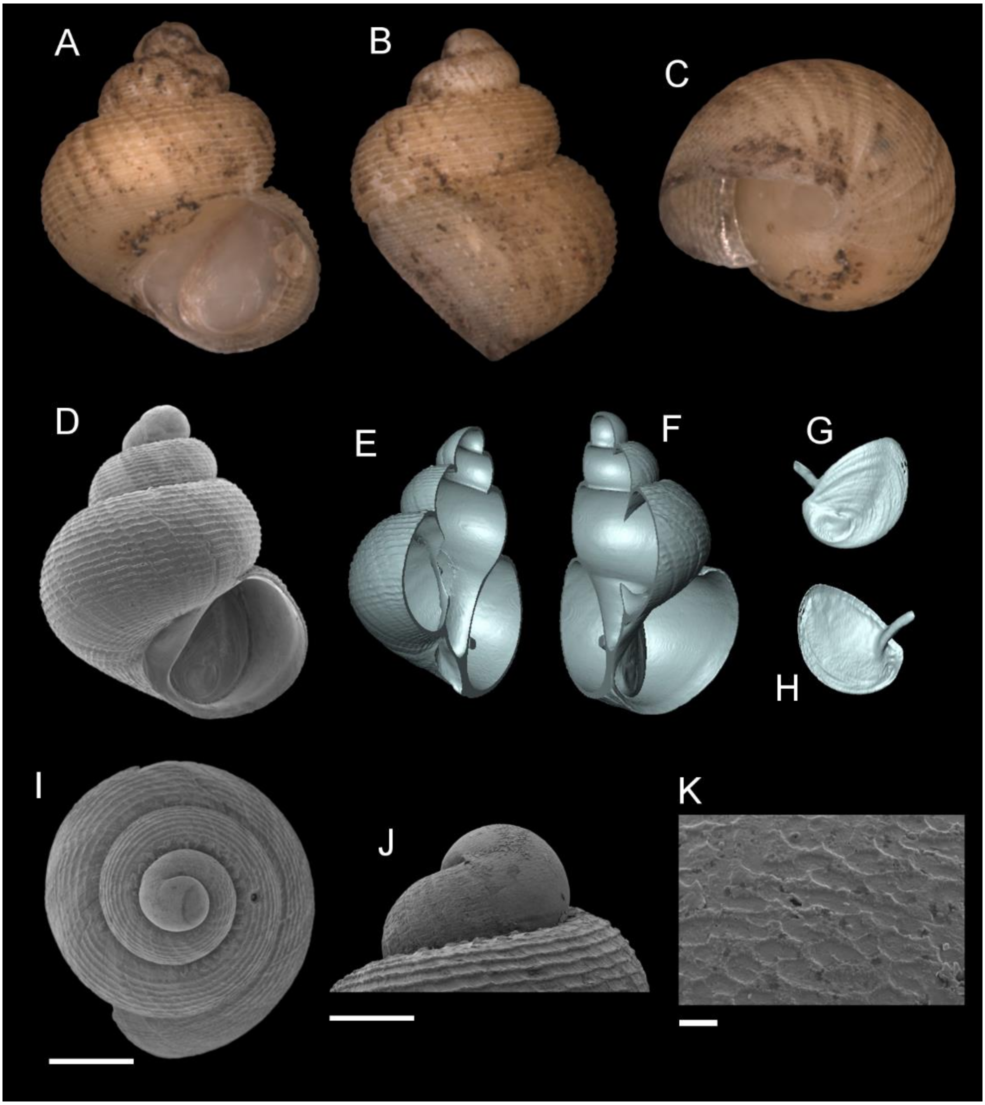


Figure 3.12 *Georissa gomantonensis* Smith, 1893. **A–K** BOR/MOL 7389 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**). Shell view image (fig. 13D) is the same image used in Khalik et al. (2018, fig. 1J).

Georissa filiasaulae Haase and Schilthuizen, 2007

Georissa filiasaulae Haase and Schilthuizen, 2007: 216, figs. 2A-B and 2E; Clements et al., 2006: 736; Clements et al., 2008: Appendix D; Schilthuizen et al., 2012; Khalik et al., 2018.

Type locality. Malaysia, Sabah, Sepulut valley, Interior province, Batu Sanaron (04°42.05'N, 116°36.01'E).

Type material. *Holotype* (Holotype by original designation). Malaysia, Sabah, Sepulut valley, Interior province, Batu Sanaron (04°42.05'N, 116°36.01'E): BOR/MOL 3795. *Paratypes*. Malaysia, Sabah, Sepulut valley, Interior province, Batu Sanaron (04°42.05'N, 116°36.01'E): BOR/MOL 3491 (7); ZMB 107143-107149 (7) (not seen).

Other material. Batu Sanaron, Interior province, Sepulut valley, Sabah: BOR/MOL 532, BOR/MOL 3405. Batu Pungiton, Interior province, Sepulut valley, Sabah Batu Pungiton, Sabah (04°42.41'N, 116°36.04'E): BOR/MOL 12768 (fig. 3.13).

Description. *Protoconch*. Color: white. Sculpture: a mix of rounded, ellipsoidal to irregular sculptural shape. Mesh width: 2.5–20 µm. *Teleoconch*. Color: white. First whorl: convex. Subsequent whorls: convex, shell shape slender to broad. Suture: well-impressed. Shoulder: slightly extended, regularly spaced nodules. Number of whorls: 2 ½–3. SH: 1.21–1.68 mm. SW: 1.67–1.68 mm. SI: 1.08–1.27. *Shell sculpture*. Radial sculpture: absent, weak to strong growth lines. Spiral sculpture: present, rather weak and thin, densely spaced on the first whorl, the ribbing appears immediately after the protoconch, sometimes distorted/discontinuous by the growth lines, superficially smooth on the later whorls. *Aperture*. Shape: rounded to semi-elliptic, straight to slightly convex parietal side, palatal side rounded, palatal edge partially contiguous with the body whorl and parietal side, basal side convex. AH: 0.67– 0.79 mm. AW: 0.69–0.83 mm. AI: 0.93–0.97.

Cross diagnosis. *G. filiasaulae* has weak, thin and densely arranged spiral sculpture with nodular structure on the shoulder. The shell color and thickness are most similar to *G. corrugata*, which has white and partially transparent shell.

Distribution. *G. filiasaulae* is a cave specialist*,* known from the cave system of Batu Sanaron and Batu Tinahas in the Sepulut valley. Schilthuizen et al. (2012) studied the population genetics of *G. filiasaulae* and its sister species, *G. saulae*. They found narrow hybrid zones between the two species in cave entrances.

Molecular analysis. ML and Bayesian analyses of *G. filiasaulae* (*16S*: n = 3; *CO1*: n = 3) show that *G. filiasaulae* form one clade with 98% BS and 100% PP. The sister group is the *G. saulae* population from Pungiton (*G. saulae* is paraphyletic).

Discussion. *G. filiasaulae* is one of the two known Bornean *Georissa* that is troglobitic. Khalik et al. (2018) described *G. silaburensis*, another species of Bornean *Georissa* from the “scaly group” as a possible troglobite from Gunung Silabur, Serian, Sarawak. *G. filiasaulae* differs from *G. saulae* by the absence of any scale-like sculpture, reduced shell pigmentation, and relatively larger shell size and broader shell shape. Population genetic studies suggest that the hybrid zone between the two is restricted to a narrow region at the cave entrances, rendering the two species as independent evolutionary units. Therefore, considering them as separate species is warranted (Schilthuizen, 2000).

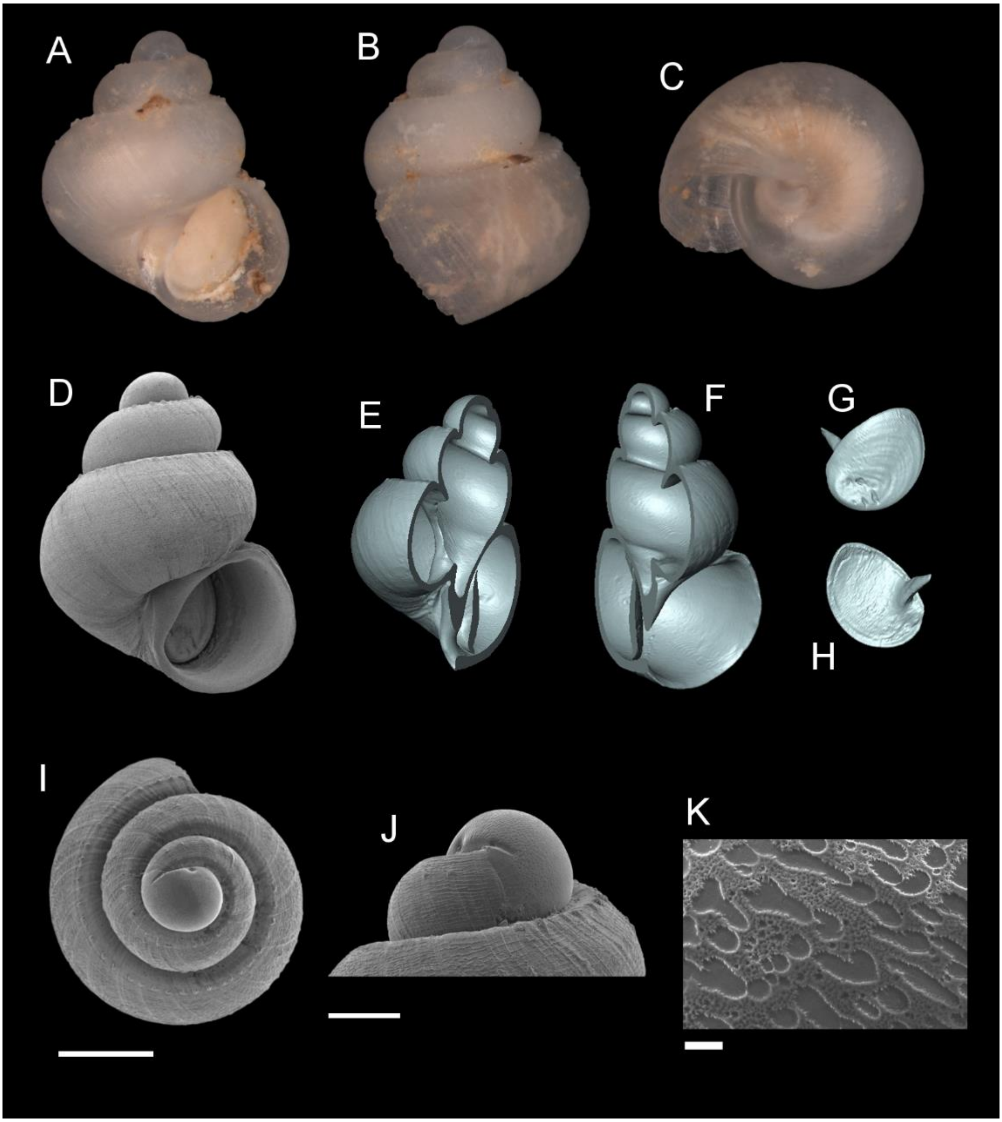


Figure 3.13 *Georissa filiasaulae* Haase and Schilthuizen, 2007. **A–K** BOR/MOL 12768 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa insulae sp. n.

Georissa williamsi Godwin-Austen: Clements et al., 2008: Appendix D; Phung et al. (2017), fig. 8C.

Type locality. Pulau Mantanani Besar, Sabah, Malaysia (06°43.06’N, 116°20.50’E).

Type material. *Holotype*. Pulau Mantanani Besar, Sabah, Malaysia (06°43.06’N, 116°20.50’E): MZU/MOL 18.01 (fig. 3.14 A-C). *Paratypes*: Pulau Mantanani Besar, Sabah, Malaysia (06°43.06’N, 116°20.50’E): MZU/MOL 18.02 (fig. 3.14 D-K). Pulau Mantanani Besar, West Coast Province, Sabah: JJV 9845 (8), JJV 9860 (>50), BOR/MOL 3718, BOR/MOL 7161 (1), BOR/MOL 7174 (9). Pulau Lungisan, Sabah: BOR/MOL 3744. Kinabalu N.P., Poring Hot Springs, along path to waterfall, West Coast Province, Sabah: JJV 13003 (1). Gua Mundau, Pitas, Sabah (06°33.02’N, 116°52.07’E): BOR/MOL 4373 (1, broken shell).

Etymology. The name is a genitive singular of the Latin word *insula*, meaning ‘island’, which refers to the Mantanani islands, the main collecting locality.

Description. *Protoconch*. Color: orange. Sculpture pattern: a mix of rounded and ellipsoidal sculpture. Mesh width: 1.5–12.0 µm. *Teleoconch*. Color: orange. First whorl: convex. Subsequent whorls: convex. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ¾–3 ¼. SH: 1.78–2.11 mm. SW: 1.21–1.40 mm. SI: 1.42–1.51. *Shell sculpture*. Radial sculpture: absent, weak growth lines present throughout the shell surface. Spiral sculpture: present, strong, regularly spaced, ca. 10–12 spiral ribs on the body whorl of the adult individual, developed immediately after the protoconch; more densely spaced spiral ribs at the basal part of the body whorl, becoming weaker closer to the columellar region. *Aperture*. Shape: rounded to slightly ovoid, straight to concave parietal side, palatal edge contiguous with the body whorl, basal side convex. AH: 0.66– 0.79 mm. AW: 0.74–0.84 mm. AI: 0.89–0.94. *Holotype dimensions*. SH: 2.11 mm, SW: 1.40 mm, AH: 0.79 mm, AW: 0.84 mm.

Cross diagnosis. *G. insulae* is characterised by the strong and regularly spaced spiral ribs throughout the entire shell. This shell sculpture is similar to that of *G. hungerfordi* and *G. trusmadi*, but less raised than in these two species. *G. insulae* has a greater number of spiral ribs, about 10-12 ribs on its shell compared to these two species. The shell habitus is distinctly elongated compared to other spirally ribbed Bornean *Georissa*.

Distribution. Known from islands of Mantanani Kecil, Mantanani Besar, Lungisan, and on the mainland from Pitas to Kinabalu National Park, Sabah.

Molecular analysis. ML and Bayesian analyses of *G. insulae* (*16S*: n = 4) show that *G. filiasaulae* form one clade with 100% BS and 100% PP. Sister to the rest of "non-scaly" Georissa, except for *G. hungerfordi* + *G. gomantonensis* + *G. filiasaulae*.

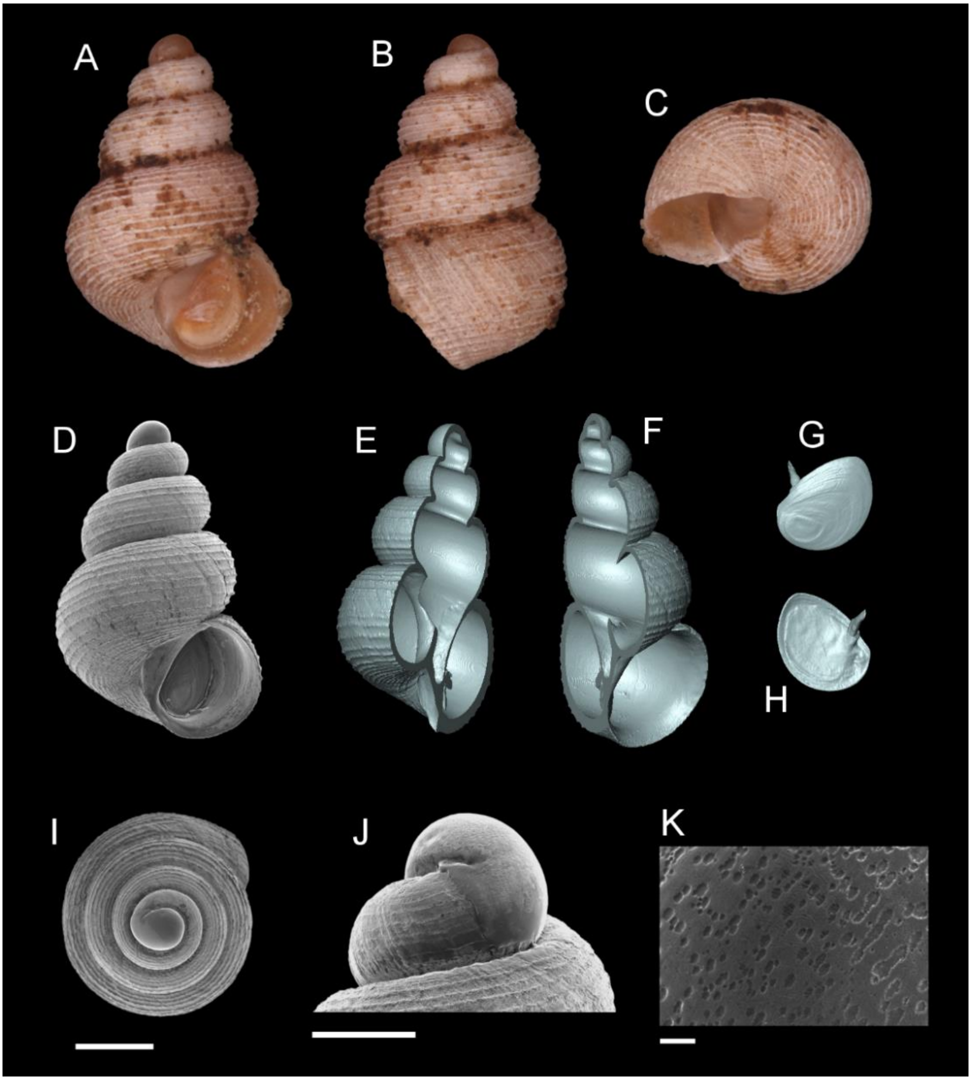


Figure 3.14 *Georissa insulae* sp. n. **A–C** Holotype: MZU/MOL 18.01 **D–K** Paratype: MZU/MOL 18.02 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa pachysoma Vermeulen and Junau, 2007

Georissa pachysoma Vermeulen and Junau, 2007: 216, fig. 7.

Type locality. Malaysia, Sarawak, 2nd div.: Lower Tatau River valley, Bukit Sarang group, Bukit Lebik.

Type material. *Holotype* (Holotype by original designation). Malaysia, Sarawak, 2nd div.: Lower Tatau River valley, Bukit Sarang group, Bukit Lebik: RMNH/MOL 109084. *Paratypes*. Malaysia, Sarawak, 2nd div.: Lower Tatau River valley, Bukit Sarang group, Bukit Lebik: JJV 12628 (10), JJV 12837 (2).

Other material. Bukit Sarang group, Lower Tatau River valley: JJV 12626, JJV 12844, JJV 12845, JJV 12846. Upper Tatau River valley, upper Kakus River limestone scarps: JJV 12847. Bt. Besungai 0.5 m SW of Batu Gading, Long Lama, Baram valley (03°52.00’N, 114°25.00’E): JJV 4940. Slopes and cliffs along path to Great Cave, Niah National Park: JJV 10216. N side of limestone area, Painted Cave, Niah National Park: JJV 10391. Bukit Lebik and Bukit Anyi, Bukit Sarang, Bintulu, Sarawak (02°39.31’N, 113°02.47’E): MZU/MOL 17.62–MZU/MOL 17.84.

Description. *Protoconch*. Color: red to brown. Sculpture: a mix of triangular, rounded and irregular sculptural shapes. Mesh width: 2–12 µm. *Teleoconch*. Color: red to brown. First whorl: convex. Subsequent whorls: convex, broad at the final whorl. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ¾–3 ¼. SH: 1.20–1.65 mm. SW: 0.95–1.23 mm. SI: 1.19–1.34. *Shell sculpture*. Radial sculpture: absent, weak growth lines at irregular intervals only. Spiral sculpture: present, rather thin, widely spaced in the center of the whorls, densely spaced close to the suture and the periphery, ribs appear immediately after the protoconch, ca. 12–15 medium raised spiral ribs, distorted/discontinuous where they are crossed by the growth lines. *Aperture*. Shape: rounded to semi-elliptic, straight to concave parietal side, palatal side convex and tilted below, palatal edge contiguous with the body whorl, basal side convex. AH: 0.54– 0.70 mm. AW: 0.57–0.72 mm. AI: 0.90–1.00.

Cross diagnosis. *G. pachysoma* is characterised by a dark red to brown color of the shell, similar to *G. nephrostoma*, but the latter species has wavy spiral ribs while *G. pachysoma* does not. In shell habitus, *G. pachysoma* closely resembles *G. hungerfordi*, but the color and spiral sculpture of *G. hungerfordi* (orange in color in living or freshly dead specimens, with highly raised spiral sculpture) is entirely different from *G. pachysoma*.

Distribution. *G. pachysoma* is widely distributed from Bukit Sarang, Bintulu to further north in Baram and Niah, Sarawak.

Molecular analysis. ML and Bayesian analyses of *G. pachysoma* (*16S*: n = 4; *CO1*: n = 4) show that *G. pachysoma* forms one clade with 100% BS and 100% PP, sister to the rest of the "non-scaly group" species, except for *G. hungerfordi* + *G. gomantonensis* + *G. filiasaulae* + *G. insulae*.

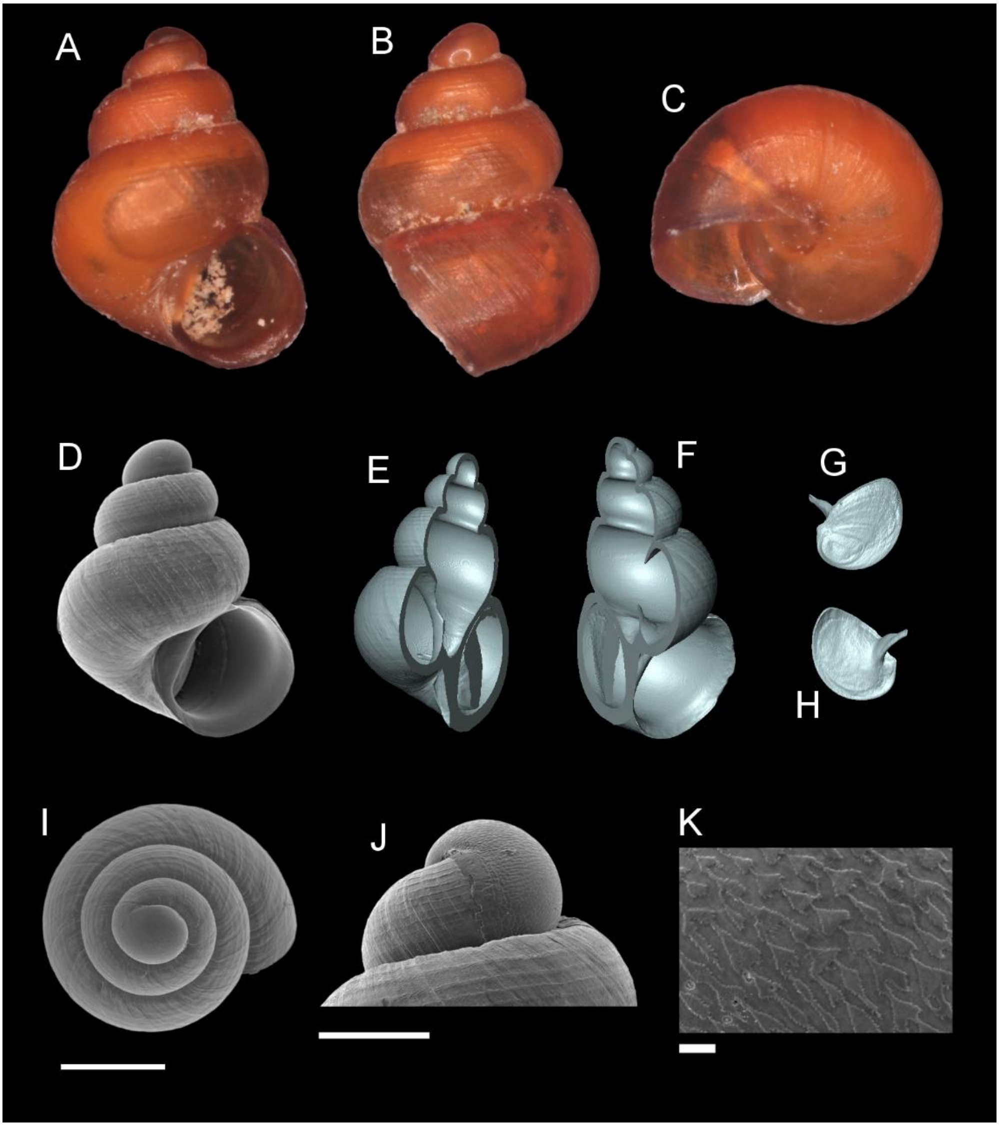


Figure 3.15 *Georissa pachysoma* Vermeulen and Junau, 2007. **A–K** MZU/MOL 17.64 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa similis Smith, 1893

Georissa similis Smith, 1893: 351, Plate XXV fig. 26; Thompson and Dance, 1983: 126, figs. 37, 42, 73-75.

Georissa sp.3 (Smith, 1893): Clements et al., 2008: Appendix D.

Type locality. Gomanton Hill, N. Borneo.

Type material. *Lectotype* (Designation by Thompson and Dance, 1983). Gomanton Hill, N. Borneo: NHMUK 1892.7.23.51 (glued on paper) (fig. 3.1F). *Paralectotype*. Gomanton Hill, N. Borneo: NHMUK 1892.7.23.52 (1) (glued on paper).

Other material. Gomanton, N. Borneo: NHMUK 94.7.20.59-60 (glued on paper), NHMUK 94.7.21.50-3 (glued on paper). Gomantong hill, Kinabatangan valley, Sandakan, Sabah (05°32.00'N, 118°06.00'E): JJV 1614. Gua Gomantong, Sabah (05°31.03'N, 118°04.01'E): BOR/MOL 52, BOR/MOL 3644. Bukit Mawas, lower Kinabatangan valley, Sabah (05°27.20'N, 118°08.67'E): BOR/MOL 1989. Batu Pangi, Kinabatangan valley, Sandakan, Sabah (05°31.59'N, 118°18.43'E): JJV 9831. Batu Tai (not Bod Tai) near Gomantong, Kinabatangan valley, Sandakan, Sabah (05°32.35'N, 118°10.32'E): JJV 9830, BOR/MOL 2686. Batu Keruak, lower Kinabatangan valley, Sabah (05°31.00'N, 118°17.00'E): BOR/MOL 1466. Ulu Sungai Resang, lower Kinabatangan, Sabah (05°31.00'N, 118°21.00'E): BOR/MOL 1447. Batu Batangan, Sabah (05°27.54'N, 118°06.18'E): MZU/MOL 16.14 (fig. 3.16). Gua Madai, Tawau, Sabah (04°44.00'N, 118°08.00'E): BOR/MOL 53, JJV 1738, JJV 7693. Segarong Hills, Bukit Pababola, Semporna, Tawau, Sabah (04°33.00'N, 118°25.00'E): JJV 1772, JJV 1817. Batu Baturong, Tawau, Sabah (04°41.00'N, 118°01.00'E): JJV 1829, BOR/MOL 1446. Limestone hill on North bank Segama river, Tawau, Sabah (05°06.10'N, 118°13.12'E): JJV 7823. Tabin Wildlife reserve, Lahad Datu, Sabah (05°18.81'N, 118°44.65'E): BOR/MOL 54.

Description. *Protoconch*. Color: red. Sculpture: rounded to ellipsoidal mesh pattern. Mesh width: 2.8–7.0 µm. *Teleoconch*. Color: red. First whorl: convex to flat at the upper part of the whorl. Subsequent whorls: convex. Suture: well-impressed. Shoulder: narrow to slightly extended. Number of whorls: 2 ½–3 ¼. SH: 0.96–1.44 mm. SW: 0.85–1.06 mm. SI: 1.13–1.36. *Shell sculpture*. Radial sculpture: present, dense and regularly spaced, always stronger than the spiral sculpture. Spiral sculpture: present, raised but thin, appearing immediately after the protoconch, spiral sculpture often interrupted due to highly developed radial ribs; the overlapping radial and spiral sculptures form knitted structures on the shell. *Aperture*. Shape: semi-elliptic to rounded, straight to slightly concave parietal side, palatal edge contiguous with the parietal side, basal side convex. AH: 0.49– 0.64 mm. AW: 0.50–0.67 mm. AI: 0.85–0.98.

Cross diagnosis. *G. similis* is characterised by the dense radial sculpture. The radial ribs intersect with the thin spiral ribs and form knitted structures throughout the shell surface. The sculpture pattern is similar to *G. everetti* but not oblique, and the radial sculpture is more raised in *G. similis*. The shell shape is similar to *G. corrugata* and *G. xesta*, but these species differ entirely in their shell and protoconch sculpture (*G. corrugata* has irregular radial shell sculpture and straight line protoconch sculpture; *G. xesta* does not have radial sculpture and the protoconch sculpture is a mix of irregular shapes).

Distribution. *G. similis* is widely distributed in the east of Sabah, from Sandakan in the north to Tawau in the south and Lahad Datu in the east.

Molecular analysis. ML and Bayesian analyses of *G. similis* (*16S*: n = 5; *CO1*: n = 5) show that *G. similis* form one clade with 100% BS and 100% PP, sister to the group of *G. xesta* + *G. nephrostoma* + *G. bangueyensis* + *G. flavescens*.

Discussion. Uribe et al. (2016) have published the mitochondrial genome of *G. similis* (GenBank acc. no. KU342664) which was previously identified as *G. bangueyensis* (see phylogenetic trees, figs. 2A and B). Phylogenetic analyses have shown that it is possible to identify the identity of a *Georissa* even when shell data are not available.

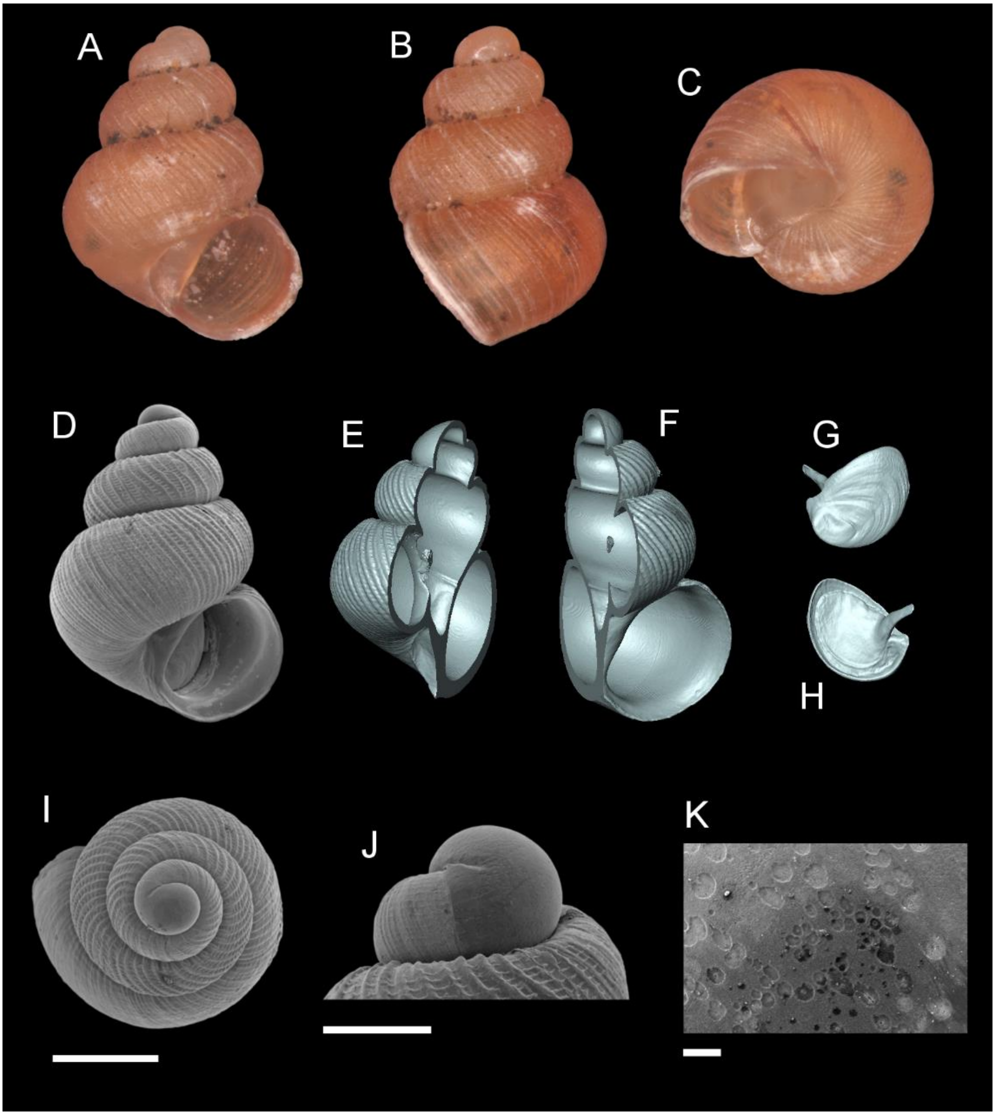


Figure 3.16 *Georissa similis* Smith, 1893. **A–K** MZU/MOL 16.14 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa xesta Thompson and Dance, 1983

Georissa xesta Thompson and Dance, 1983: 125, figs. 69-70.

Type locality. A small limestone ridge quarried for rock 5 mi W Kudat, Sabah, Borneo (06°57.00’N, 116°48.00’E).

Type material. *Holotype* (Holotype by original designation). A small limestone ridge quarried for rock 5 mi W Kudat, Sabah, Borneo (06°57.00’N, 116°48.00’E): UF 35968 (not seen). *Paratypes*. A small limestone ridge quarried for rock 5 mi W Kudat, Sabah, Borneo (06°57.00’N, 116°48.00’E): UF 35969 (not seen), UF35970 (not seen), SMF 255740/6 (not seen), NHMUK 1984.006 (5) (fig. 3.1G), JJV 13424.

Other material. Kinabatangan valley, Batu Tulug (Batu Putih) along road Lahad Datu-Sandakan, North of bridge over Kinabatangan River, Sandakan province, Sabah (05°25.00’N, 117°56.00’E): JJV 1481. Kinabatangan valley, Batu Keruak 2 near Sukau, Sandakan province, Sabah (05°32.00’N, 118°18.00’E): JJV 9786. Kinabatangan valley, Batu Tomanggong Kecil, Sandakan province, Sabah (05°30.12’N, 118°18.10’E): JJV 9828. Batu Tomanggong Besar, Kinabatangan valley, Sandakan, Sabah (05°31.02’N, 118°18.21’E): BOR/MOL 1437, BOR/MOL 2252, BOR/MOL 2281. Batu Tomanggong Besar 2, Kinabatangan valley, Sandakan, Sabah (05°31.16’N, 118°18.33’E): BOR/MOL 1440. Batu Tomanggong Kecil, Kinabatangan valley, Sandakan, Sabah (05°30.21’N, 118°18.18’E): BOR/MOL 2025, BOR/MOL 2053. Batu Keruak, Kinabatangan valley, Sandakan, Sabah (05°32.00’N, 118°18.00’E): BOR/MOL 2687. Lower Kinabatangan valley, Sabah; Unnamed limestone hill 1 (05°31.11’N, 118°17.23’E): BOR/MOL 2151, BOR/MOL 2184, BOR/MOL 2217; Unnamed limestone hill 2 (05°30.00’N, 118°17.00’E): BOR/MOL 1441. Batu Materis, Kinabatangan valley, Sabah (05°31.21’N, 118°01.31’E): BOR/MOL 2113, BOR/MOL 2083. Bod Tai, Kinabatangan valley, Sabah (05°31.00’N, 118°13.00’E): BOR/MOL 1443. Bukit Mawas, lower Kinabatangan valley, Sabah: BOR/MOL 1444. Pangi, Kinabatangan valley, Sandakan province, Sabah (05°31.59’N, 118°18.43’E): BOR/MOL 1442. Ulu Sungai Resang, lower Kinabatangan valley, Sabah (05°31.00’N, 118°21.00’E): BOR/MOL 1438, BOR/MOL 7303 (fig. 3.17), BOR/MOL 7311. Segama valley, ‘Kirk’s Cave’ 8 km North of Lahad Datu, Tawau province, Sabah (05°04.00’N, 118°16.00’E): JJV 1236. Segama valley, hill N.W. of crossing road Sandakan-Lahad Datu with the Segama River, Tawau province, Sabah (05°06.00’N, 118°13.00’E): JJV 1687. Segama valley, Sabahmas Cave, Tawau province, Sabah (05°08.52’N, 118 26.01’E): JJV 7453. Batu Baturong, North slope, Tawau province, Sabah (04°41.46’N, 118°0.45’E): JJV 7583. Segama valley, North end of limestone ridge on East bank Tabin River, Sandakan province, Sabah (05°18.49’N, 118°44.39’E): JJV 7755. Tabin Wildlife Reserve, Lahad Datu, Sabah: BOR/MOL 12, BOR/MOL 13. Limestone hill on N bank Segama River, Tawau province, Sabah (05°06.01’N, 118°13.01’E): BOR/MOL 9. Sabahmas Cave, Tawau Province, Sabah (05°08.05’N, 118°26.00’E): BOR/MOL 8. Batu Baturong, Tawau Province, Sabah (04°41.04’N, 118°00.04’E): BOR/MOL 10. N end of limestone ridge on E bank Tabin River, Sandakan Province, Sabah (05°18.04’N, 118°44.03’E): BOR/MOL 11. Tomanggong Sukau, Sandakan, Sabah (05°32.01’N, 118°23.00’E): BOR/MOL 14. Sabah, Malaysia: RMNH/MOL 335369. Sabah, N. Borneo: ZMA/MOLL 315545. Materis, Kinabatangan, Sabah (05°31.38’N, 118°01.02’E): BOR/MOL 7258.

Description. *Protoconch*. Color: pale orange to orange. Sculpture: irregular sculptural shape resulted from a combination of rounded to ellipsoidal sculpture patterns. Mesh width: 1–10. *Teleoconch*. Color: orange, the color of the teleoconch always darker than the protoconch. First whorl: convex. Subsequent whorls: convex. Suture: well-impressed. Shoulder: narrow to slightly extended. Number of whorls: 2 ¾–3 ½. SH: 1.05–1.68 mm. SW: 0.84–1.18 mm. SI: 1.22–1.45. *Shell sculpture*. Radial sculpture: absent, only weak growth lines present. Spiral sculpture: present, ca. 20–25 thin and weak spiral ribs, superficially smooth, densely arranged, appearing immediately after the protoconch, distorted by growth lines. *Aperture*. Shape: rounded to slightly ovoid, straight to concave parietal side, palatal edge partially contiguous with the body whorl and the parietal side, basal side convex. AH: 0.48– 0.66 mm. AW: 0.50–0.74 mm. AI: 0.76–0.98.

Cross diagnosis. *G. xesta* has densely arranged spiral sculpture (8–10 ribs in every 0.1 mm), unlike *G. bangueyensis* (4–5 ribs in every 0.1 mm), which has more space in between the spiral ribs. The shell of *G. xesta* looks superficially smooth under a stereomicroscope at low contrast with less than ×20 magnification. The dense spiral sculpture is similar to the spiral ribbing pattern of *G. williamsi*, but the shell habitus of these two species is entirely different, where *G. williamsi* has a broad ultimate whorl but *G. xesta* does not. Based on the shell shape, *G. xesta* is similar to *G. similis* and *G. corrugata*, but both of these species have strongly raised radial sculpture.

Distribution. *G. xesta* is widely distributed in Sabah, especially in the coastal areas around Kudat, Sandakan, Lahad Datu, and Tawau.

Molecular analysis. In the ML and Bayesian analyses of *G. xesta* (*16S*: n = 2; *CO1*: n = 3), the Materis and Ulu Resang populations form highly supported clades 96% BS and 100% PP, which are paraphyletic with respect to *G. nephrostoma*.

Discussion. The type series of *G. xesta* from NHMUK seems to be partially eroded. However, the densely arranged thin spiral sculpture which is the diagnostic character of the species is still visible in the type series.

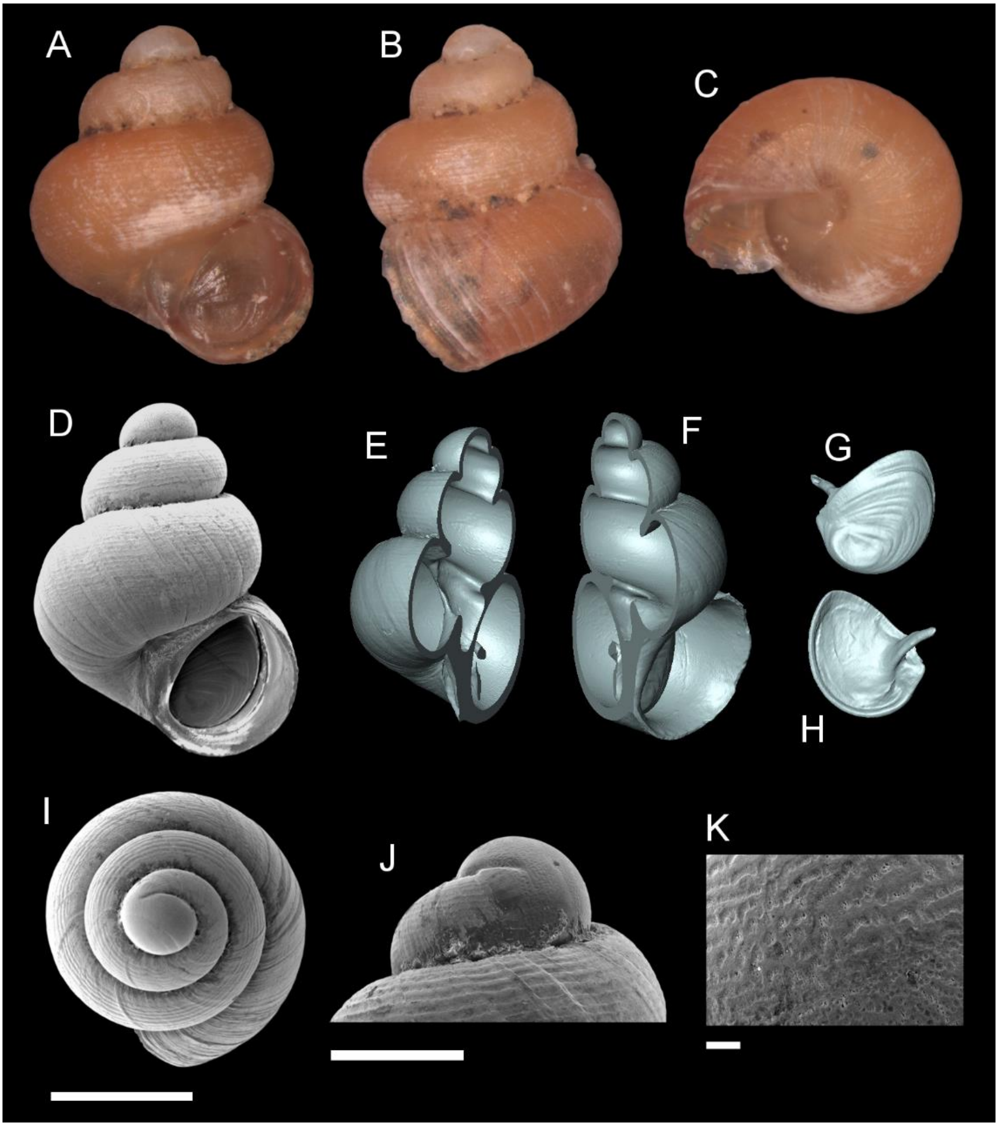


Figure 3.17 *Georissa xesta* Thompson and Dance, 1983. **A–K** BOR/MOL 7303 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa nephrostoma Vermeulen, Liew, and Schilthuizen, 2015

Georissa nephrostoma Vermeulen et al., 2015: 34, fig. 20.

Type locality. Malaysia, Sabah, Sandakan Province, Kinabatangan valley, Batu Keruak 2 near Sukau.

Type material. *Holotype* (Holotype by original designation). Malaysia, Sabah, Sandakan Province, Kinabatangan valley, Batu Keruak 2 near Sukau (05°32.00’N, 118°18.00’E): RMNH/MOL 5003955 (not seen, we were unable to locate the material in RMNH collection). *Paratypes*. Malaysia, Sabah, Sandakan Province. Kinabatangan valley, Batu Keruak 2 near Sukau (05°32.00’N, 118°18.00’E): NHMUK 20150573, BOR/MOL 1449, BOR/MOL 1450, BOR/MOL 1845, JJV 9795; Batu Pangi (05°31.59’N, 118°18.43’E): BOR/MOL 1452, JJV 9833; Batu Tai near Gomantong (05°32.35’N, 118°10.32’E): JJV 9832; Tandu Batu (05°35.47’N, 118°20.34’E): JJV 9834; Limestone hills near Sukau Police Station: BOR/MOL 2186, BOR/MOL 2153, BOR/MOL 1451.

Other material. Batu Keruak, Sandakan Province, Sabah (05°32.00’N, 118°18.00’E): BOR/MOL 1454, MZU/MOL 17.29 (fig. 3.18). Tandu Batu, Sandakan Province, Sabah (05°35.47’N, 118°20.34’E): BOR/MOL 2685.

Description. *Protoconch*. Color: red to brown. Sculpture: rounded, ellipsoidal to irregular sculptural shape. Mesh width: 2–10 µm. *Teleoconch*. Color: red to brown. First whorl: rounded to convex. Subsequent whorls: rounded to convex. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ½–2 ¾. SH: 0.87–1.24 mm. SW: 0.69–0.92 mm. SI: 1.26–1.43. *Shell sculpture*. Radial sculpture: absent, densely spaced weak to strong growth lines, no formation of true radial ribs. Spiral sculpture: present, appearing immediately after the protoconch; the ribs are low but narrow to broadly sculpted, regularly spaced, wavy, ca. 12–14 spiral ribs at the upper part of the body whorl; near the aperture, the spiral sculpture is weakened and flattened approaching the columellar region. *Aperture*. Shape: semi-elliptic, highly convex and bulky parietal side, palatal side rounded, palatal edge contiguous with the parietal side, basal side convex. AH: 0.40–0.55 mm. AW: 0.43–0.60 mm. AI: 0.92–0.95.

Cross diagnosis. *G. nephrostoma* is characterised by the wavy formation of the spiral sculpture and the inflated parietal side of the aperture. The wavy sculpture pattern of *G. nephrostoma* is similar to *G. flavescens* but the two species differ entirely based on the shell habitus, where *G. flavescens* has a more compressed shell habitus. In shell shape, *G. nephrostoma* resembles *G. similis*, *G. xesta* and *G. bangueyensis*, but none of these species have the uniquely inflated parietal side of the aperture.

Distribution. *G. nephrostoma* is distributed from Sukau to Gomantong, Kinabatangan region, Sabah.

Molecular analysis. ML and Bayesian analyses of *G. nephrostoma* (*16S*: n = 5; *CO1*: n = 1) showed that all *G. nephrostoma* specimens form one clade with 99% BS and 100% PP. The sister group is the *G. xesta* population from Materis and Ulu Resang (*G. xesta* is paraphyletic).

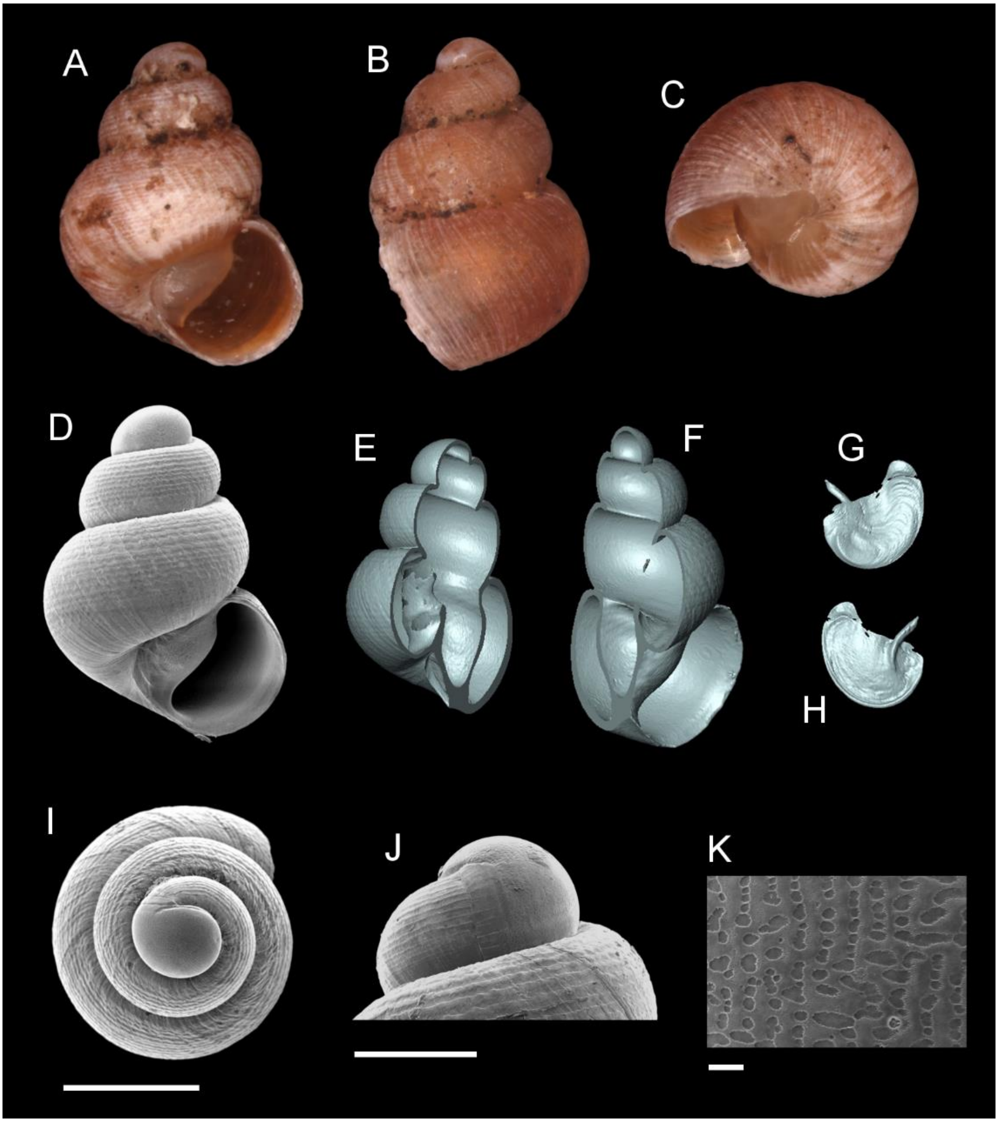


Figure 3.18 *Georissa nephrostoma* Vermeulen et al., 2015. **A–K** MZU/MOL 17.29 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**). Shell image of the apertural view (fig. 18D) is the same as shown in Khalik et al. (2018, fig. 1K).

Georissa bangueyensis Smith, 1895

Georissa bangueyensis Smith, 1895: 125, Plate IV fig. 16; Thompson and Dance, 1983: 126.

Type locality. Banguey Island, N. Borneo.

Type material. *Lectotype* (Designation by Thompson and Dance, 1983). Banguey Island, N. Borneo: NHMUK 1893.6.7.9 (fig. 3.1H).

Other material. Banggi Island, South end, Kudat province, Sabah (07°06.32’N, 117°5.07’E): RMNH/MOL 152746, JJV 1423, JJV 1451, JJV 9467, JJV 9497. Pulau Banggi, Kudat Dist., Sabah: BOR/MOL 15. Bod Gaya Island, Tun Sakaran Marine Park, Semporna, Sabah: BOR/MOL 4729. Gomantong limestone hill, Sabah: BOR/MOL 3320. Balambangan Island, Sabah (07°14.00’N, 116°52.00’E): BOR/MOL 3684. Kok simpul, Pulau Balambangan, Kudat Province, Sabah (07°13.03’N, 116°53.14’E): BOR/MOL 1445. S end Batu Sireh, Pulau Balambangan, Kudat Province, Sabah (07°12.29’N, 116°51.30’E): BOR/MOL 1439. Segama valley, limestone hill on North bank Segama River, near bridge of road Sandakan to Lahad Datu, Tawau province, Sabah (05°06.10’N, 118°13.12’E): JJV 7495. Materis, Kinabatangan, Sabah (05°31.38’N, 118°01.02’E): BOR/MOL 11820, BOR/MOL 11851, BOR/MOL 11910, BOR/MOL 11945. Bukit Mawas, lower Kinabatangan valley, Sabah (05°27.20’N, 118°08.67’E): BOR/MOL 1954, RMNH/MOL 5004968 (fig. 3.19). Kampung, Kinabatangan, Sabah (05°30.72’N, 118°16.92’E): BOR/MOL 10901, BOR/MOL 10922. Ulu Resang, Kinabatangan, Sabah (05°30.66’N, 118°20.40’E): BOR/MOL 9284, BOR/MOL 9311, BOR/MOL 9345, BOR/MOL 9595, BOR/MOL 9610, BOR/MOL 9617. Batu Payung, Kinabatangan, Sabah (05°35.34’N, 118°19.44’E): BOR/MOL 8952, BOR/MOL 8967, BOR/MOL 8976, BOR/MOL 9003. Tomanggong Kecil, Kinabatangan, Sabah (05°30.54’N, 118°17.94’E): RMNH/MOL 152858, RMNH/MOL 152859, BOR/MOL 7473, BOR/MOL 9619, BOR/MOL 9685, BOR/MOL 9903, BOR/MOL 9943, BOR/MOL 9952, BOR/MOL 9983. Tomanggong Besar 1, Kinabatangan, Sabah (05°31.86’N, 118°18.24’E): BOR/MOL 10560, BOR/MOL 10806, BOR/MOL 11318, BOR/MOL 11361. Tomanggong Besar 2, Kinabatangan, Sabah (05°31.32’N, 118°17.88’E): BOR/MOL 10385, BOR/MOL 10411, BOR/MOL 10531, BOR/MOL 11352. “NewLocation1”, Kinabatangan, Sabah (05°27.40’N, 118°08.76’E): RMNH/MOL 5004826.

Description. *Protoconch*. Color: red. Sculpture: irregular sculptural shape to smooth. Mesh width: 1–20 µm. *Teleoconch*. Color: red. First whorl: convex. Subsequent whorls: convex. Suture: well-impressed. Shoulder: narrow. Number of whorls: 2 ¾–3. SH: 1.00–1.33 mm. SW: 0.77–0.96 mm. SI: 1.22–1.42. *Shell sculpture*. Radial sculpture: absent, only weak growth lines are here and there visible. Spiral sculpture: present, appearing immediately after the protoconch, regularly arranged; ca. 8–10 spiral ribs on the first whorl, on the later whorls the sculpture is more prominent at the upper part of the whorl, weaker and flattened closer to the columellar region. *Aperture*. Shape: semi-elliptic to rounded, straight to slightly concave parietal side, palatal edge contiguous with the parietal side, basal side convex. AH: 0.40– 0.53 mm. AW: 0.45–0.60 mm. AI: 0.81–1.00.

Cross diagnosis. *G. bangueyensis* is characterised by its clear spiral ribs at the upper part of the body whorl, similar to *G. flavescens* and *G. nephrostoma*, but the two latter species have wavy spiral ribs. Spiral sculpture on the lower whorl is weaker and less obvious closer to the columellar region. In shell sculpture, it is most similar to *G. xesta*, but the latter species has more densely arranged spiral sculpture (see discussion in *G. xesta*).

Distribution. *G. bangueyensis* is widely distributed in the coastal regions of northern and eastern Sabah.

Molecular analysis. ML and Bayesian analyses of *G. bangueyensis* (*16S*: n = 6; *CO1*: n = 6) show that *G. bangueyensis* forms a monophyletic clade with 100% BS and 100% PP, and is sister to *G. flavescens*.

Discussion. Thompson and Dance (1983) questioned the validity *G. bangueyensis* as a proper species based on a limited number of specimens. We propose that *G. bangueyensis* is a proper species with distinct characteristics, as compared to *G. xesta*.

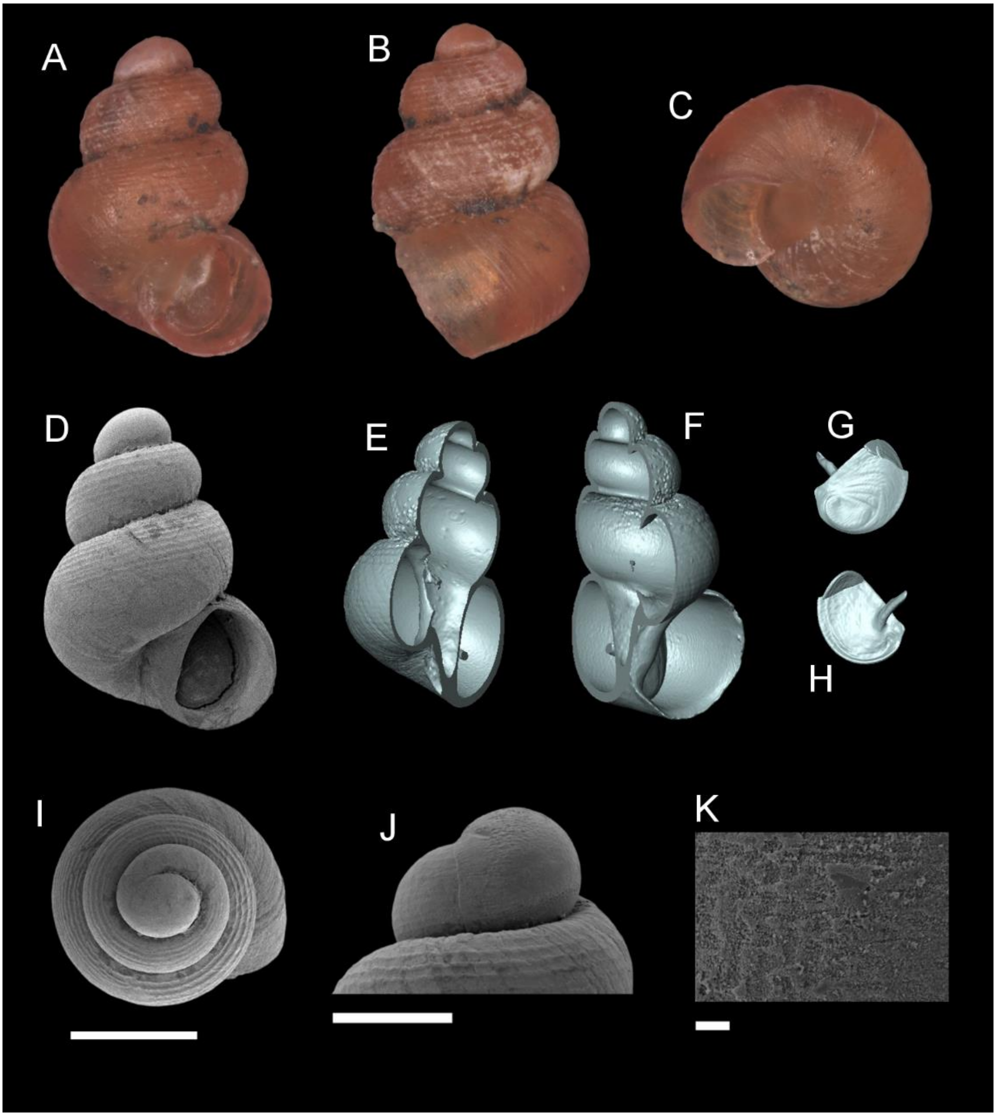


Figure 3.19 *Georissa bangueyensis* Smith, 1895. **A–K** RMNH/MOL 5004968 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

Georissa flavescens Smith, 1895

Georissa flavescens Smith, 1895: 126, Plate IV fig. 17; Thompson and Dance, 1983: 121.

Type locality. Gomanton, N.E. Borneo.

Type material. *Lectotype* (Designation by Thompson and Dance 1983). Gomanton, N.E. Borneo: NHMUK 1893.6.8.11 (fig. 3.1I). *Paralectotypes*. Gomanton, N.E. Borneo: NHMUK 1893.6.8.12-13.

Other material. Tomanggong Besar 1, Kinabatangan, Sabah: BOR/MOL 7299. Tomanggong Besar 2, Kinabatangan, Sabah: BOR/MOL 7626. Batu Pangi, Kinabatangan valley, Sandakan province, Sabah (05°31.59’N, 118°18.43’E): JJV 9827, BOR/MOL 7288 (fig. 3.20), BOR/MOL 9261, BOR/MOL 9325 (*G. flavescens* mixed with *G. xesta*), BOR/MOL 10816, BOR/MOL 10830, BOR/MOL 10835. Batu Keruak, Kinabatangan, Sabah (05°31.38’N, 118°17.16’E): BOR/MOL 11621.

Description. *Protoconch*. Color: orange. Sculpture: rounded, ellipsoidal to irregular sculptural shape. Mesh width: 2–10 µm. *Teleoconch*. Color: orange. First whorl: convex. Subsequent whorls: convex, and slightly angular at the penultimate whorl. Suture: well-impressed. Shoulder: narrow to slightly extended. Number of whorls: 2 ½–2 ¾. SH: 0.87–1.20 mm. SW: 0.73–0.95 mm. SI: 1.15–1.26. *Shell sculpture*. Radial sculpture: absent, weak growth lines. Spiral sculpture: present, appearing immediately after the protoconch, wavy, thin, and regularly arranged ribs at the first whorl, more raised at the later whorls. *Aperture*. Shape: semi-elliptic, straight to concave parietal side, palatal side rounded, palatal edge contiguous with the palatal side, basal side convex. AH: 0.43– 0.55 mm. AW: 0.47–0.58 mm. AI: 0.82–0.95.

Cross diagnosis. *G. flavescens* is characterised by the wavy spiral sculpture, which it only shares with *G. nephrostoma*, but the latter species, with its narrow spire and inflated columella, is entirely distinct in shell habitus. The shell shape of *G. flavescens* is similar to *G. gomantonensis*, *G. williamsi*, and *G. everetti*, but its size is reduced compared to these three species.

Distribution. *G. flavescens* is restricted to four limestone hills, Batu Pangi, Batu Keruak, Batu Gomantong, and Batu Tomanggong, in the Lower Kinabatangan valley of Sabah.

Molecular analysis. ML and Bayesian analyses of *G. flavescens* (*16S*: n = 7; *CO1*: n = 8) show that *G. flavescens* forms a monophyletic clade with 100% BS and 100% PP, a sister species of *G. bangueyensis*.

Discussion. Thompson and Dance (1983) synonymised *G. flavescens* to *G. gomantonensis*, without stating any reason of the species conspecificity. We otherwise find that *G. flavescens* is a proper species based on detailed conchology and molecular analysis.

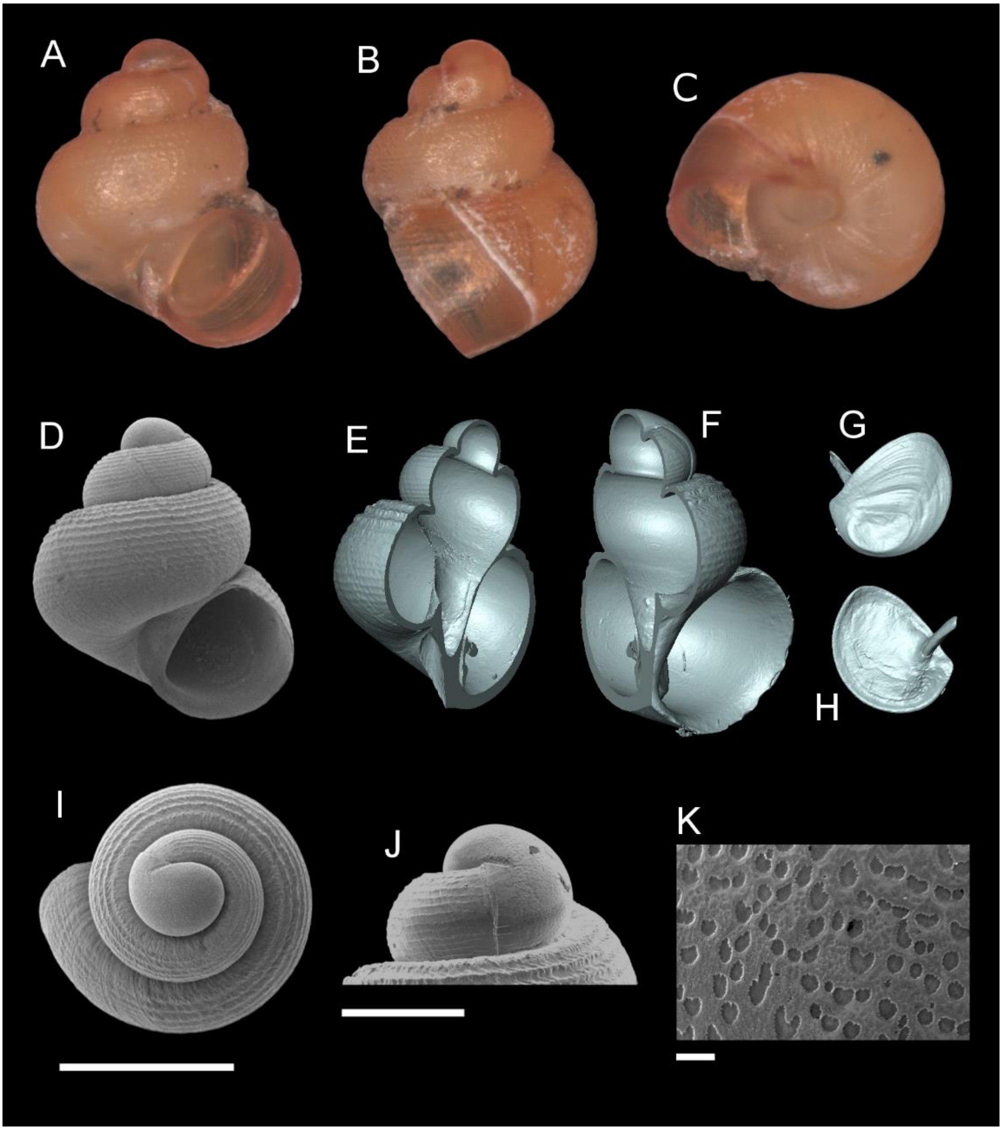


Figure 3.20 *Georissa flavescens* Smith, 1895. **A–K** BOR/MOL 7288 **A, D** Shell apertural view **B** Shell side view **C** Shell rear view **E–F** Shell cross-section from 3D model **G–H** Operculum frontal and ventral view from 3D model **I** shell top view **J** Protoconch side view **K** Close up of protoconch from top at 1000× magnification. Scale bars: 500 µm (**A–I**); 200 µm (**J**); 10 µm (**K**).

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Supplementary material

1. An overview of scanning parameters of each "non-scaly" *Georissa*. https://doi.org/10.3897/zookeys.840.33326.suppl1
2. Shell measurement of the "non-scaly" *Georissa*. https://doi.org/10.3897/zookeys.840.33326.suppl2

Chapter 4

Morphological parallelism of sympatric cave-dwelling microsnails of the genus *Georissa* at Mount Silabur, Borneo (Gastropoda, Neritimorpha, Hydrocenidae).

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Published chapter

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Abstract

Parallel evolution in phenotype may result when closely related taxa are adapting in the face of similar ecological pressures. Here, we discuss possible parallelism in shell morphology in the context of the microgeographic phylogeography of two conchologically distinct sympatric hydrocenid snails inhabiting a limestone outcrop and its cave system, *Georissa pyrrhoderma* and *Georissa silaburensis*, respectively, at Mount Silabur in Sarawak, Malaysian Borneo. Our results show a certain degree of morphological parallelism between *G. silaburensis* and a third, possibly new, cryptic *Georissa* species within the same cave that diverged from its above-ground sister species, *G. pyrrhoderma*. We found that both sympatric cave species have shifted from a more sculptured, conical shell towards a broader, less sculptured form.

Introduction

Convergent evolution and parallelism have been a subject of studies related to genotype and phenotype evolution in a wide range of taxa, including mammals (Madsen et al., 2001), reptiles (Revell et al., 2007; Stayton, 2006), birds (Fain and Houde, 2004; Grenier and Greenberg, 2005), and fishes (Rüber and Adams, 2001; Hulsey et al., 2008; Qi et al., 2012). Convergence and parallelism are the outcomes of evolutionary processes in which different species independently converge towards similar, adaptive phenotypes. Usually, the term convergence is reserved for superficially similar but non-homologous traits that evolve in distantly related taxa, whereas parallelism refers to similar morphologies involving homologous structures in more closely related taxa (Alejandrino et al., 2011). To address and understand the processes involved, researchers often investigate the evolutionary patterns in organisms that respond to similar environmental changes and concomitant selection (Houle, 1991; Rundle and Nosil, 2005). Using molecular-phylogenetic reconstruction, detailed phenotype changes in multiple species make further analysis and interpretation of selected taxa possible, allowing an understanding of their evolutionary diversification.

Studies on convergent evolution and morphological parallelism have demonstrated that similar phenotypic traits often result when taxa are facing similar ecological pressures (in, e.g., microhabitat, climate, diet, predator pressure, etc.). For example, Kaeuffer et al. (2010) studied the intraspecific phenotypic diversity of threespine stickleback fish from different aquatic environments and concluded that morphological characters such as body depth and gill raker numbers show strong morphological convergence. In a different study, Lindgren et al. (2012) investigated the interspecific morphological divergence and convergence of Cephalopoda; they found convergent evolution in cephalopods of similar habitat types, even in distantly related taxa. For example, taxa with an autogenic photophore were always associated with a pelagic habitat, while the benthic habitat harboured taxa characterised by the presence of corneas and accessory nidamental glands. Both these studies employed detailed study of morphological characters and molecular data to reconstruct the evolutionary relationship among different taxa, and interpreted these in the context of the ecological factors that drive the evolution of similar morphological traits.

Rock-dwelling land snails of Southeast Asia often show high allopatric and sympatric diversity with many species endemic to small geographic regions (Liew et al., 2014; Rundell, 2008; Tongkerd et al., 2004), especially species inhabiting karstic environments. This can sometimes make it difficult to decide on the taxonomic status of similar-shelled forms. Usually, these are considered as conspecific based on shared shell characters (**Chapters 2 and 3**; Foon and Liew, 2017; Liew et al. 2014; Thompson and Dance, 1983; Vermeulen et al., 2015). Furthermore, phylogenetic analyses often reveal that the detailed conchology follows the patterns of phylogenetic relatedness (**Chapters 2 and 3**; Schilthuizen et al., 2005; Schilthuizen et al., 2012). Nonetheless, possible cases of morphological parallelism due to ecological similarity may be overlooked.

Shell variation in a single land snail species has often been shown to be correlated with variation in habitat (Baur, 1988; Cameron and Cook, 1989; Chiba, 2004; Chiu et al., 2002; Goodfriend, 1986; Haase and Misof, 2009). This means that different ecological systems provide different selective pressures and induce adaptive changes in a population's morphological characters. Eventually, this may lead to speciation (Chazot et al., 2016; Danowitz et al., 2015; Price et al., 2003). Hirano et al. (2014) recently showed that a molecular phylogenetic and morphological analysis revealed parallelism in multiple lineages of camaenid land snails. They found that an angularity of the shell periphery appeared at least seven times in the phylogeny. Although their study focused on the discordance between shell morphology and molecular phylogenetics which could impinge on taxonomic classification, their study also provides an important example of parallelism.

Here, we investigate microgeographic patterns of molecular phylogeny and conchometric characters of sympatric species of Hydrocenidae Troschel, 1856 (microsnails of the genus *Georissa* Blanford, 1864) inhabiting the above and below-ground areas of the limestone hill Mount Silabur in Sarawak, Malaysian Borneo. Due to the common occurrence and high abundance of hydrocenids in the region, we were able to conduct a systematic sampling design to understand the evolutionary patterns, specifically shell character changes inferred by molecular phylogeny. The above-ground limestone outcrop and the below-ground cave systems are two microhabitats with extremely different environmental conditions. In general, the below-ground conditions are darker, with smaller temperature and humidity fluctuations, a less complex foodweb, and lower energy availability (Barr, 1967; Poulson and White, 1969).

Thus, we attempt to reconstruct the phylogenetic relationships among three sympatric hydrocenid forms of the genus *Georissa*, originating from 19 different populations sampled from the limestone outcrop and cave. Two sympatric *Georissa* species were previously known to occur at the Mount Silabur limestone hill, namely *G. pyrrhoderma* Thompson and Dance, 1983, inhabiting the limestone outcrops surrounding the hill, and *G. silaburensis* Khalik, Hendriks, Vermeulen, and Schilthuizen, 2018, inhabiting the cave interior (**Chapter 2**). Similarly to what was found for the troglobitic species *G. filiasaulae* Haase and Schilthuizen, 2007 from Sepulut, Sabah), the cave-inhabiting *G. silaburensis* has a relatively large, broad shell, reduced shell sculpture, and is less pigmented compared to the sympatric epigean species inhabiting the outcrops, typical (and variant) *G. pyrrhoderma* (**Figure 4.3A**). Based on shell morphology, we found a third form, inhabiting the cave environment, *Georissa* “sp. Silabur” (**Figure 4.3C**), of which the shell shape is similar to the typical *G. silaburensis* whereas the scale characters are similar to the typical *G. pyrrhoderma*.

In this study we therefore subject this complex of three shell forms to a morphological and molecular analysis to reveal patterns of phylogenetic relatedness among them, and offer a scenario for their evolution.

Material and Methods

Study site

The sampling was conducted at an isolated limestone hill, Mount Silabur, Serian, Sarawak, Malaysian Borneo (00°57.407'N, 110°30.276'E). Mount Silabur is approximately 350 metres high and measures roughly 500 by 300 metres. The cave entrance is about 150 metres from the foot of the hill. The cave itself consists of a main chamber connecting the opposite sides of the hill. There are multiple high chambers (Wilford, 1964) that we excluded from our sampling for safety reasons. Our first fieldwork was on the 3rd of March 2017, when random sampling was carried out at two locations at the limestone outcrops and one location in the cave. The second sampling took place on the 16th of April 2017, when we conducted systematic sampling and sketched a map of the cave system in which we also indicated the location of the plots (**Figure 4.1**). Populations of hydrocenids were collected at 19 different plots surrounding the outcrops and inside of the cave.

Sampling method

The sampling locations consist of 13 plots (plots SIE1-SIE6, SIO1-SIO6, and SO3) outside the cave and six plots (plots SIG1-SIG6) inside the cave of Mount Silabur. Apart from these plots, we also encountered 10 sites where no hydrocenids were found (**Figure 4.1**). The plots surrounding the hill were located approximately 5-10 metres away from the established trails. We generally selected humid, shaded limestone rocks covered with vegetation. The plots inside of the cave were mainly the vertical limestone walls of the main chamber. The distance between two plots was set to be at least 20 metres. We spent about 20 minutes at each (approximately circular) plot with two collectors searching on the limestone rocks and walls, vegetation and the surrounding areas, within an approximately five metres diameter. The collected living materials were directly stored in ~96% ethanol.

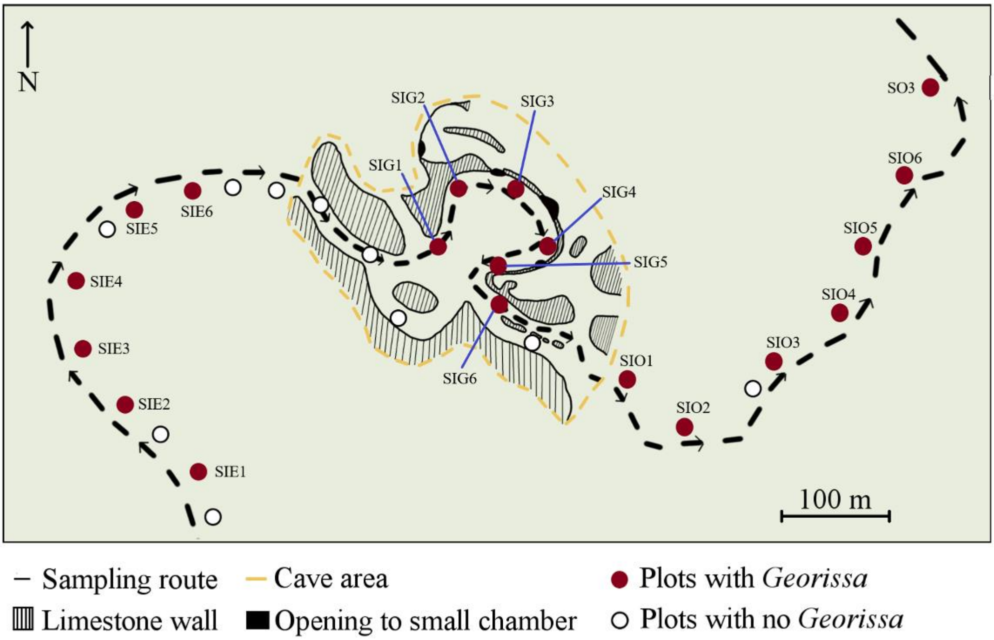

Figure 4.1 Sampling transect and plots at Mount Silabur outcrops and cave environments. Abbreviation used for each sampling plot during the fieldwork; S/SI = Silabur; E = before cave; G = inside cave; O = after cave.

Shell measurement and scanning electron microscope (SEM)

A composite 2D image of each individual shell was obtained from the focus-stacked images captured with a Zeiss SteREO Discovery.V20 stereo-microscope. The images were taken at 40× magnification and 100 µm layer thickness at constant light intensity. We used the apertural view of the individual shell to measure the shell height (SH), shell width (SW), aperture height (AH), and aperture width (AW). Details on the shell measurements are in Supporting Information (Table S1).

For each shell form we determined the characters based on the shell shape and sculpture patterns. We performed scanning electron microscopy (SEM) using a JEOL JSM-6480LV to obtain detailed views of the sculptural patterns. All hydrocenid shells were grouped into shell form categories based on their detailed shell shape and surface characters.

A total of 274 adult shells of hydrocenids from all sampling plots were used in the morphometric analysis. We also included specimens of the closest relative of *G. silaburensis*, viz. *G. bauensis* Khalik, Hendriks, Vermeulen, and Schilthuizen, 2018 (17 adult shells) from Wind Cave Nature Reserve (WNCR), Bau, Sarawak (approximately 50 km from Mount Silabur). We made scatterplots of measured characters in RStudio (RStudio Team, 2016) to visualise the morphological variation of the measured adult shell forms (Supporting Information Table S1).

DNA extraction and amplification

Genomic DNA of the snails was extracted with Qiagen DNeasy Blood and Tissue kit using the manufacturer's protocol. Prior to the extraction, the shells were crushed and removed (partially). The entire soft tissue was used in the extraction. Three partial DNA regions were amplified, *16S* ribosomal RNA ("*16S*") and *cytochrome c oxidase subunit I* ("*COI"*) for mitochondrial markers, and *28S* ribosomal RNA ("*28S*") for a nuclear marker. A fragment of *16S* region was amplified using primer pair LR-J-12887 5'-CCGGTCTGAACTCAGATCACGT-3' (forward) and LR-N-13398 5'-CGCCTGTTTAACAAAAACAT-3' (reverse) (Schilthuizen et al., 2005). For the *COI* region, we used primer pair LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' (forward) and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (reverse) (Folmer et al., 1994) in the amplification process. We also amplified a partial *28S* DNA nuclear region using primer set D23F 5'-GAGAGTTCAAGAGTACGTG-3' (forward) and D6R 5'-CCAGCTATCCTGAGGGAAACTTCG-3' (reverse) (Park and Ó Foighil, 2000).

PCR were performed in 25.0 µL reaction volume containing 1.0 µL undiluted DNA template, which consist of: 12.5-17.0 µL ultrapure water milli-Q, 5.0 µL Qiagen Q-solution (only for *28S* amplification), 2.5 µL Qiagen PCR chlorine buffer 10×, 1.0-2.5 µL Qiagen $MgCl_2$ 25.0 mM, 0.25-1.0 µL Promega BSA 100 mM (only for *16S* and *COI* amplification), 1.0 µL forward primer 10 pmol/ µL, 1.0 µL reverse primer 10 pmol/ µL, 0.5 µL dNTPs 2.5 mM, and 0.25 µL Qiagen Taq 5.0 U/ µL. The PCR program started with initial denaturation at 95°C for 5 min, followed by 36-40 cycles of denaturation at 95°C for 15-45 s, annealing at 50-55°C for 30-40 s, extension at 72°C for 1-2 min, and a final extension at 72°C for 5-10 min. DNA amplifications were performed on a BIO-RAD C1000 Touch^{TM} Thermal Cycler. The PCR products were then Sanger sequenced in both directions at BaseClear B.V. (Leiden, The Netherlands). The amplification length of each *16S*, *COI*, and *28S* genes are 530-532, 708-710, and 830-834 bp, respectively, including primers of both directions. The newly obtained sequences were assembled using the *de novo* Geneious 10.2.3 assembler, manually checked and edited, and primer sequences at both ends were trimmed. The resulted sequence lengths of each *16S*, *COI*, and *28S* genes are 481-486, 602, and 700-788 bp, respectively. The new sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/WebSub/) and BOLD (http://boldsystems.org/). Details of the specimens used in this study are listed in Supporting Information (Table S2). The accession number for *16S*, *CO1* and *28S* genes are MK775735-MK775820, MK811455-MK811541 and MK775829-MK775944, respectively.

Phylogenetic analyses

We used *16S* and *COI* mtDNA sequences of other Bornean *Georissa* species, namely *G. gomantonensis*, *G. hosei*, *G. sepulutensis*, *G. kinabatanganensis*, and *G. bauensis*, and the outgroups *Bathynerita naticoidea*, *Nerita maxima* and *Nerita patula*. These sequences were obtained from our previous studies, Aktipis and Giribet (2010), and Frey and Vermeij (2008), respectively. Our previous study (see **Chapters 2 and 3**) have shown that *G. bauensis* is sister to *G. silaburensis*, while *G. sepulutensis* and *G. kinabatanganensis* are sister to *G. pyrrhoderma*. For these particular reasons, we included these three taxa in our current study. The sequences were aligned using default parameters of MUSCLE (Edgar, 2004). The alignment of *COI* mtDNA was set to invertebrate mitochondrial genetic code at the second reading frame.

We performed a maximum likelihood (ML) analysis using the concatenated *16S* and *COI* mitochondrial and nuclear *28S* genes, with 5000 rapid bootstrap replicates (Hoang et al., 2017) using IQ-TREE 1.6.3 (Nguyen et al., 2015). The best fit nucleotide substitution model for each gene was determined using ModelFinder (Kalyaanamoorthy et al., 2017) based on the corrected Akaike Information Criterion (AICc). The best fit nucleotide substitution models for *28S*, *16S*, and *COI* were TIM3+F+G4, HKY+F+G4, and TIM+F+G4, respectively. In addition, we also conducted separate ML analyses of mitochondrial and nuclear genes. Bayesian inference (BI) was performed with MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001) using the following MCMC settings: GTR+G+I nucleotide substitution model; 1,100,000 generations; tree subsampling for every 200 generation; a burn-in of 100,000; 4 heated chains with heated chain temperature at 0.2.

Haplotype network and genetic divergence

A total of 112 *COI* sequences of hydrocenids including those of *G. silaburensis* and *G. pyrrhoderma* from **Chapter 2** were used for the haplotype study. The sequences were aligned using MUSCLE (Edgar, 2004), and both ends were trimmed to give a set of 603 bp sequences, with no gaps. The haplotype groups were determined with DNAsp ver. 6.12.01 (Rozas et al., 2017) and the median joining network was calculated and constructed in Network ver. 5.0.1.1 (Fluxus Technology Ltd, Kiel, Germany).

To determine the genetic divergence among the sympatric conchologically distinguishable forms, we conducted genetic distance analysis in MEGA v. 7.0.26 (Kumar et al., 2016) based on the same *COI* sequence alignment as used in the phylogenetic analysis above. We computed pairwise genetic distances between each pair of individuals and between different shell forms based on the Kimura 2-parameter nucleotide substitution model, selecting the transition + transversion, uniform rates among sites, and 1000 bootstraps for variance estimation.

Results

Microhabitats and shell characteristics

Our fieldwork resulted in a thorough population sampling of *Georissa* at Silabur. Initially, we made 29 plots comprising 10 plots in the cave and 19 above-surface plots at the limestone outcrops. We were able to find and collect *Georissa* at six plots in the cave and 13 plots at the outcrops. Sampling started at the foot of the hill, plot SIE1 (00°57.407'N, 110°30.276'E) at approximately 50 metres a.s.l., which was the lowest sampling point. The highest sampling plot was SIO2 (00°57.388'N, 110°30.161'E) at approximately 168 metres a.s.l. Our random survey of the higher levels of the hill failed to yield any living hydrocenids. This may have been due to the fact that the upper part of hill is directly exposed to sunlight and consists of dry limestone rocks, which might not be suitable for these minute land snails. Examples of different microhabitats where we did encounter *Georissa* are shown in **Figure 4.2**.

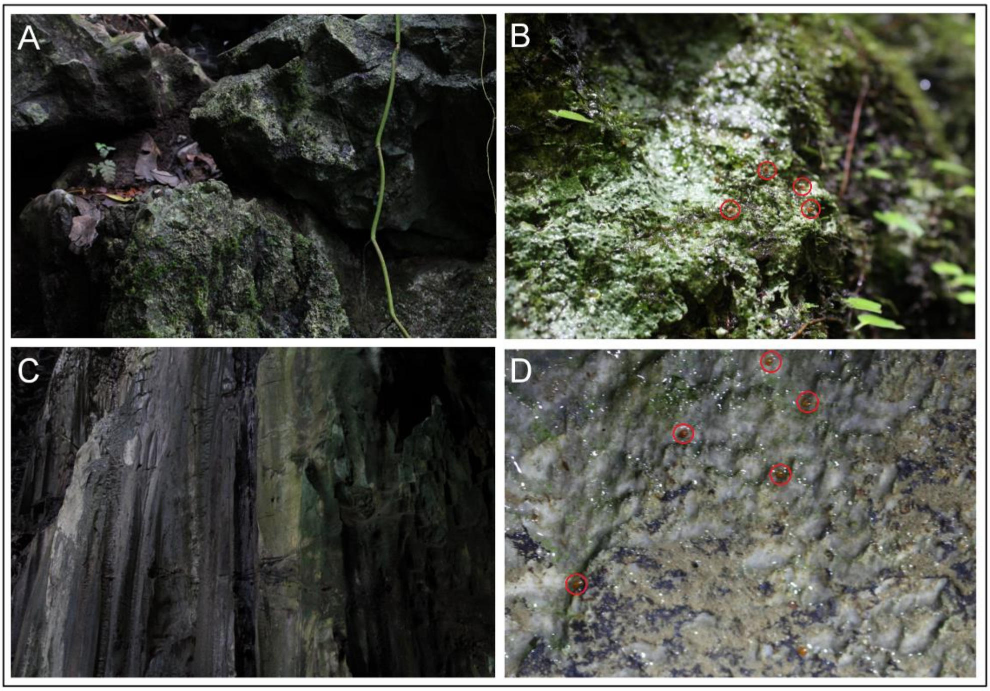

Figure 4.2 An overview of the different microhabitats of the studied hydrocenids. **A** Wet and shaded limestone rocks covered with lichen, a microhabitat of *Georissa* at the outcrops. **B** Close up image of the substrate showing *Georissa* foraging on the rock. **C** Vertical limestone cave wall with water slowly flowing from the cave ceiling. **D** Close up image of the cave wall showing *Georissa* foraging on the calcareous substrate. *Georissa* can be observed in **B** and **D**, which are minute, orange/red shells (circled in red).

During our morphological assessment of the hydrocenid shell, we were able to determine the morphological variation in each shell form. Qualitatively different sets of shell forms and their variation are shown in **Figure 4.3** and explained in **Table 4.1**.

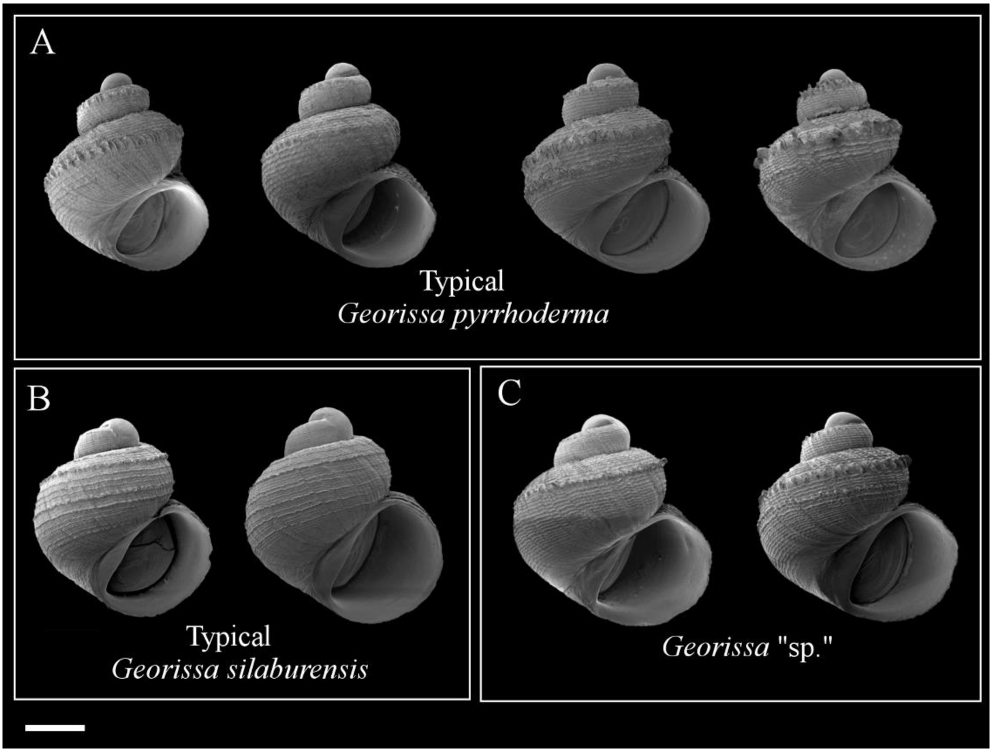

Figure 4.3 Scanning electron microscopy (SEM) images of detailed sculptural patterns of different forms of hydrocenids and their sculptural variation from Mount Silabur. **A** Typical *G. pyrrhoderma*. **B** Typical *G. silaburensis*. **C**. *Georissa* “sp. Silabur”. See **Table 4.1** for explanations. Scale bar = 500 µm.

Table 4.1 Main characters and microhabitat of different forms of *Georissa*.

Shell forms	Microhabitat	Shell shape	Scales	Scale series
Typical *G. pyrrhoderma*	Outside of the cave	More conical	Wide and/or minute; reduced and/or raised	One or two
Typical *G. silaburensis*	Cave	More globular	Minute and reduced	Multiple
Georissa “sp. Silabur”	Cave	More globular	Minute and/or wide; reduced and/or raised	One or two

We made two shell measurement plots of hydrocenids from Mount Silabur, including *G. bauensis* from WNCR (**Figure 4.4**). The plots show that, in the cave, shell width is always greater with reference to shell height compared to the above-ground sister species of relatively similar shell height. As a result, a more globular shell shape is observed in the cave-dwelling species. Similar shape changes in general shell form and aperture appear when each surface-dwelling form is compared with the respective cave-dwelling sister form, although the differences are stronger for the pair *G. bauensis* vs. *G. silaburensis* than for *G. pyrrhoderma* vs. *Georissa* "sp. Silabur"

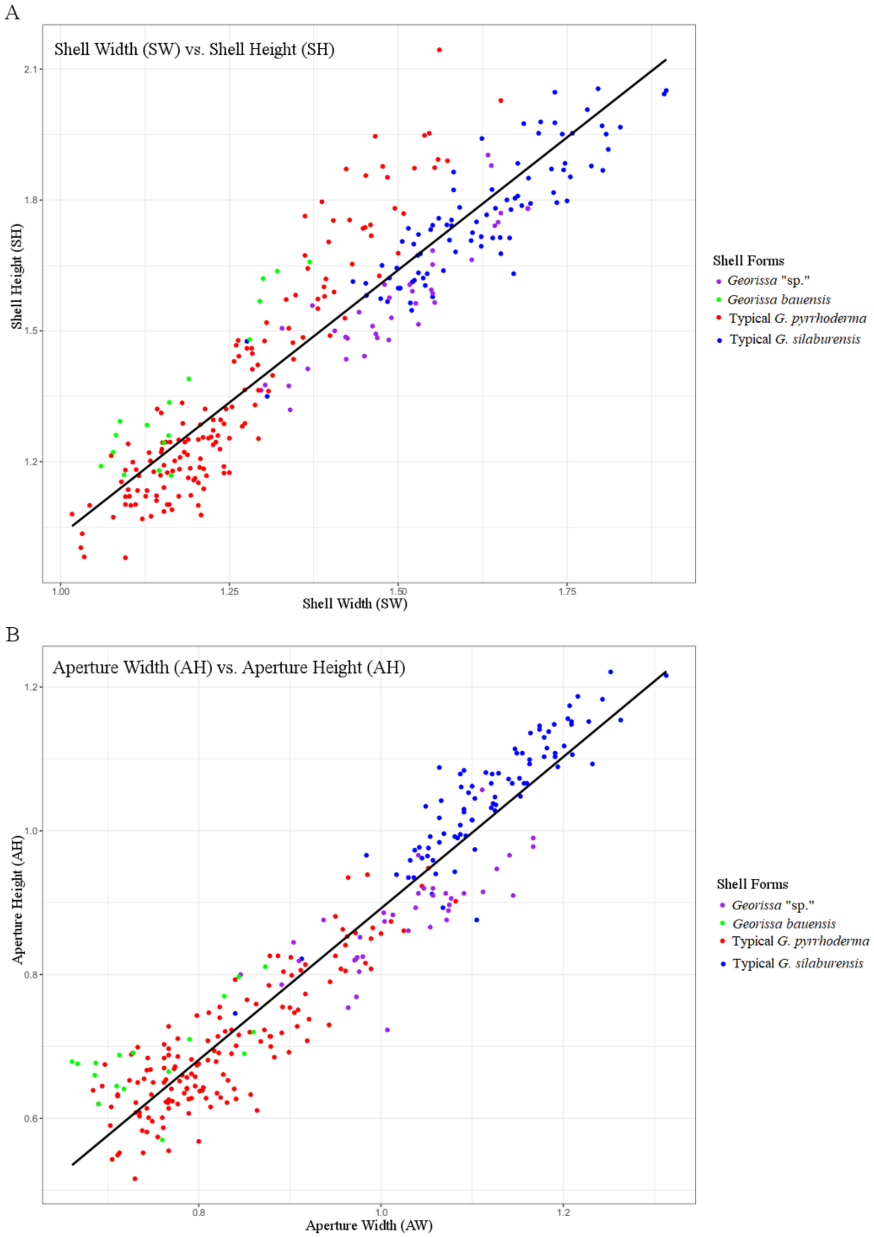

Figure 4.4 Shell measurement plots of a total 274 adult shells of different hydrocenid shell forms from Mount Silabur, including 17 adult shells of *G. bauensis* from WCNR. **A** Shell width vs. shell height. **B** Aperture width vs. aperture height.

Phylogenetic analyses

The maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses result in slightly different tree topologies (**Figure 4.5 and 4.6**). Nonetheless, the results suggest that typical *G. silaburensis* and cryptic *Georissa* “sp. Silabur”, which inhabit the cave environment are unrelated, despite their very similar (but not identical, see **Figures 4.3, 4.4a and 4.4b**) shell morphology and quantification. Typical *G. pyrrhoderma*, which inhabits the external part of the limestone outcrops is the sister species of *G. sepulutensis* and *G. kinabatanganensis* from Sabah, and paraphyletic with respect to *Georissa* “sp. Silabur”.

Overall, we find the sympatric hydrocenids are divided into two major lineages, with typical *G. silaburensis* closely related to *G. bauensis* from WNCR, which is approximately 50 km away, and, typical *G. pyrrhoderma* and *Georissa* “sp. Silabur” phylogenetically close to *G. sepulutensis* and *G. kinabatanganensis* from Sabah, despite their geographical distance.

The ML cladogram of mitochondrial genes (Supporting Information Figure S2) shows a similar topology to the phylogenetic analyses (**Figures 4.5 and 4.6**). Additionally, we find the ML cladogram of the *28S* gene (Supporting Information Figure S3) is also in concordance with the ML and BI trees, which might indicate hybridisation between below-ground *Georissa* “sp. Silabur” and above-ground *G. pyrrhoderma*. Neither in the mitochondrial nor in the nuclear tree do we see any indication of introgression between the sympatric cave species (*G. silaburensis* and *Georissa* “sp. Silabur”), although they were found next to each other in at least three different plots, namely, SIG1, SIG3, and SIG5.

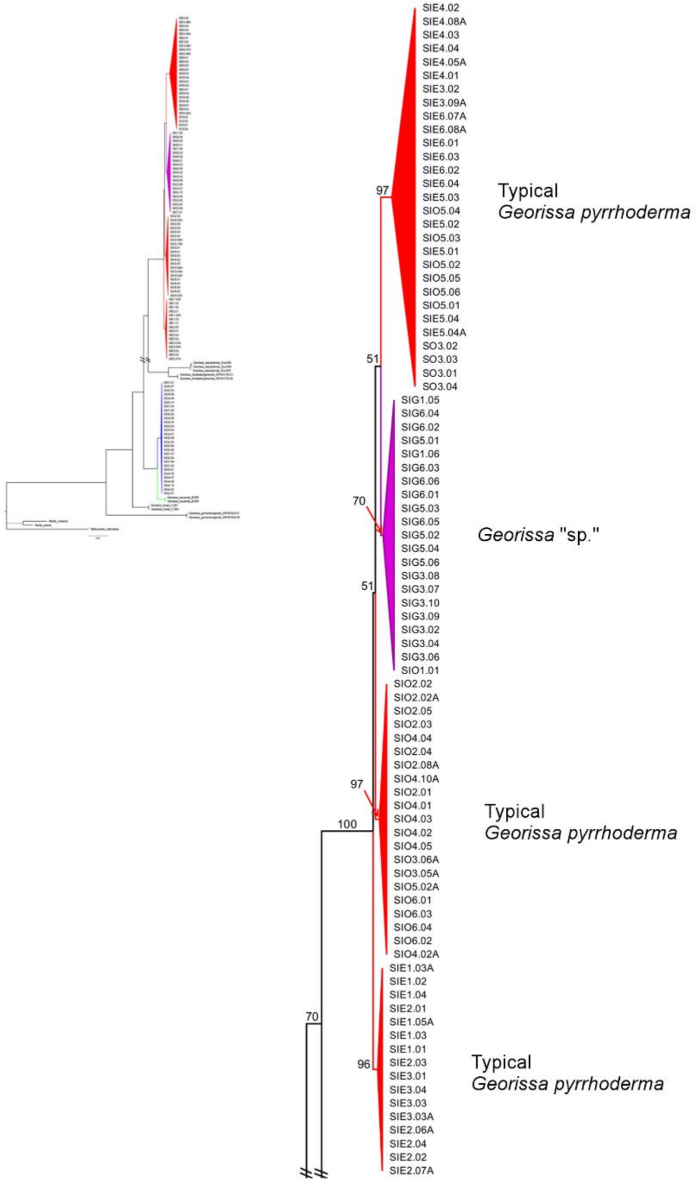

Figure 4.5 Maximum likelihood tree inferred by partitioned data of partial *16S* and *COI* mtDNA, and *28S* nDNA. The analysis was conducted with ultrafast bootstrapping (5000 replicates) with the respective nucleotide substitution models for each gene enforced in IQ-TREE 1.6.3. The tree consists of 127 individuals of Bornean hydrocenids, including *G. gomantonensis*, *G. hosei*, *G. bauensis*, *G. kinabatanganensis* and *G. sepulutensis* from Malaysian Borneo, with three outgroups taxa, namely, *Bathynerita naticoidea*, *Nerita patula* and *Nerita maxima*. Different colors of the clades indicate different form of hydrocenids (blue = typical *G. silaburensis*; red = typical *G. pyrrhoderma*; purple = *Georissa* “sp. Silabur”; green = *G. bauensis*).

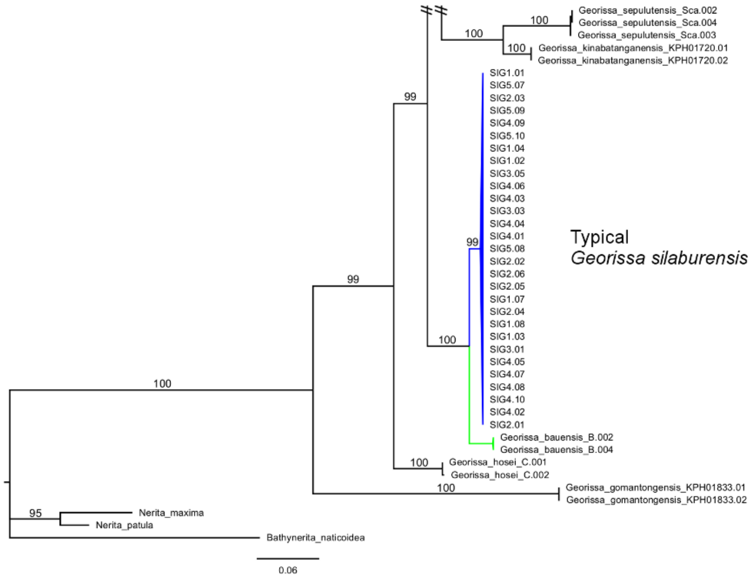

Figure 4.5 *Cont…*

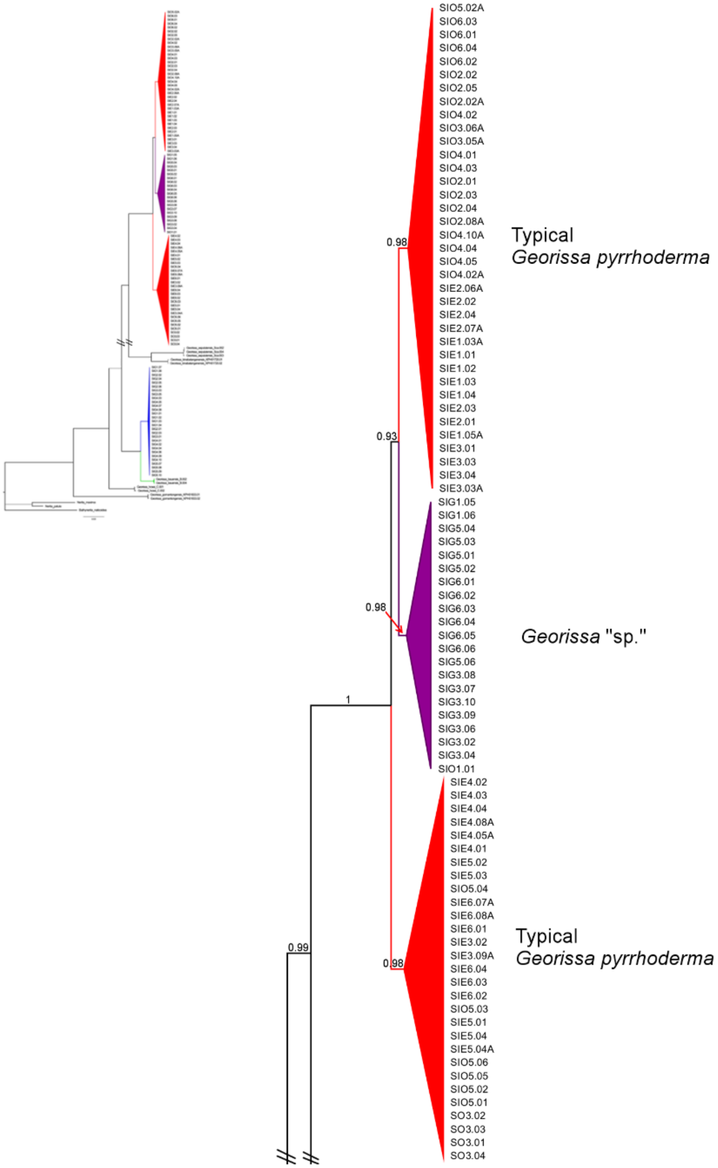

Figure 4.6 Bayesian inference tree inferred by concatenated sequence alignments of partial *16S* and *COI* mtDNA, and partial *28S* nDNA. The analysis consists of 127 individuals of Bornean hydrocenids, including *G. gomantonensis*, *G. hosei*, *G. bauensis*, *G. kinabatanganensis* and *G. sepulutensis* from Malaysian Borneo, with three outgroups taxa, namely, *Bathynerita naticoidea*, *Nerita patula* and *Nerita maxima*. Different colors of the clades indicate different form of hydrocenids (blue = typical *G. silaburensis*; red = typical *G. pyrrhoderma*; purple = *Georissa* "sp. Silabur"; green = *G. bauensis*).

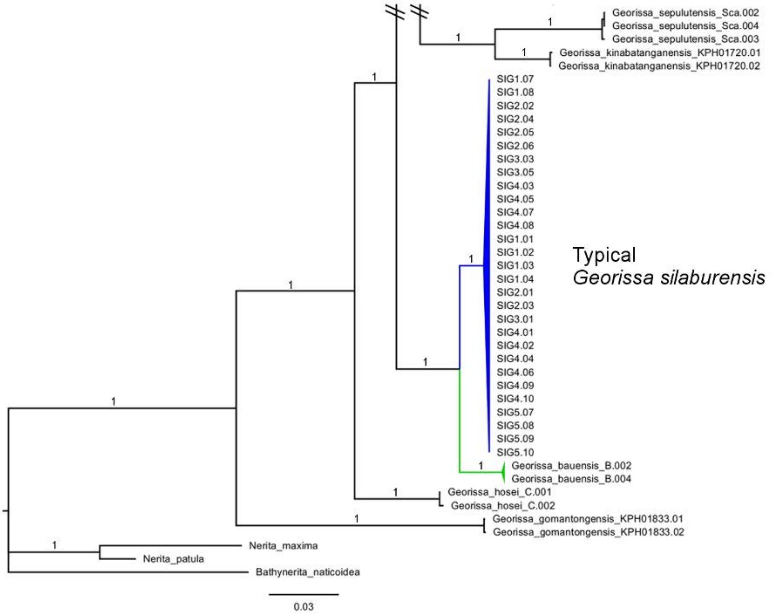

Figure 4.6 *Cont…*

Haplotype and genetic divergence

Sequence analysis of the partial *COI* gene resulted in a total of 32 unique haplotypes, with no insertions or deletions, differing only in base substitutions. From the median joining haplotype network (**Figure 4.7**), we are able to group the haplotypes into four major lineages, namely, H1 to H4, H17 to H28, H29 to H32, and H5 to H16, as the first, second, third and fourth lineages, respectively.

The haplotype network is consistent with our phylogenetic analyses, where organisms of different shell shape and size are grouped together in one clade. The shell form of typical *G. pyrrhoderma*, which is paraphyletic with respect to *Georissa* “sp. Silabur” in the phylogenetic trees, is divided into two major lineages in the haplotype study.

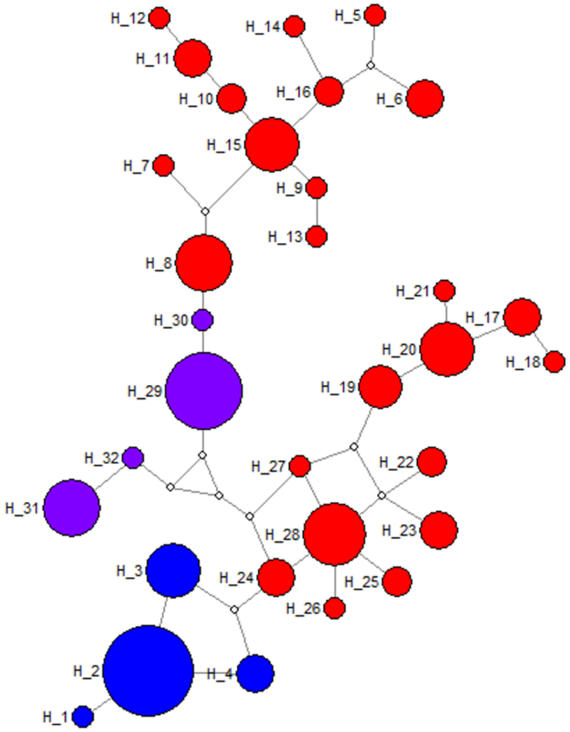

Figure 4.7 Median-joining haplotype network of 112 individuals of three major shell forms of *Georissa* of Mount Silabur based on *COI* mtDNA. Each haplotype is represented by a circle which is proportionate to the number of individuals bearing this haplotype. The colors represent different shell forms, namely, typical *G. silaburensis* (four haplotypes, blue), typical *G. pyrrhoderma* (24 haplotypes, red), and *Georissa* "sp. Silabur" (four haplotypes, purple). Details of the haplotype group of each individual are included in Supporting Information (Table S3).

COI genetic divergence

Our phylogenetic reconstructions and haplotype network have shown significant molecular divergences among all hydrocenid forms at Mount Silabur. Despite the similarity in morphological forms, the *COI* genetic divergences between sympatric cave species are high (distance of typical *G. silaburensis* to *Georissa* "sp. Silabur" = 13%), as shown in **Table 4.2**. The divergences between typical *G. silaburensis* (from the cave) to all other forms are 13% or more, whereas between typical *G. pyrrhoderma* (above-ground species) and *Georissa* "sp. Silabur" is lower (distance of *G. pyrrhoderma* to *Georissa* "sp. Silabur" = 5%).

Table 4.2 Genetic divergence of *COI* mtDNA between sympatric hydrocenids of Mount Silabur.

	Within group divergence	Number of specimens	Typical *G. silaburensis*	*Georissa* "sp. Silabur"
Typical *G. silaburensis*	1.13×10^{-3}	29		
Georissa "sp. Silabur"	1.40×10^{-2}	21	0.13	
Typical *G. pyrrhoderma*	4.71×10^{-2}	66	0.14	0.05

Discussion

Our results on phylogenetic analyses inferred by mitochondrial and nuclear genes show clear separation between each sympatric taxon and between each shell form (**Figures 4.5 and 4.6**). A combination of conchometric and phylogenetic analysis has provided a clear distinction among sympatric taxa, typical *G. silaburensis*, *G.* "sp. Silabur", and *G. pyrrhoderma*. Moreover, the phylogenetic analyses (**Figures 4.5 and 4.6**) indicate reproductive isolation between two morphologically very similar hydrocenid forms (typical *G. silaburensis* and *Georissa* "sp. Silabur") inhabiting the cave habitat. Additionally, *G. silaburensis* and *G.* "sp. Silabur" populations inside the cave habitat are completely mix. Based on our phylogenetic analysis, it shows population structuring between these two hydrocenid forms. The inferred phylogenies therefore provide evidence of parallelism in the shell size and shape of taxa of different lineages that independently have invaded the same cave habitat. In this case, we find a broader and more globular shell form with reduced sculpture in the troglobitic taxa, having evolved from the presumed ancestral state, a more conical, strongly sculptured shell form in the epigean taxa. Although we could not specifically address the selective pressures underlying this morphological parallelism, the fact that a similar kind of morphological divergence was found in the species pair *G. saulae* and *G. filiasaulae* in a different cave in northern Borneo (Haase and Schilthuizen, 2007; Schilthuizen et al., 2012), makes it very likely that a general broadening of the shell, relatively bigger shell size and a reduction in sculpture are a predictable response, at least in hydrocenids, to one or more ecological factors characteristic of the tropical cave environment. As in other environments, these factors may include the abiotic environment (Baur, 1988; Cameron and Cook, 1989; Chiba, 2004; Chiu et al., 2002; Goodfriend, 1986; Haase and Misof, 2009), predation pressure (Moreno-Rueda, 2009; Schilthuizen et al., 2006) and physical restrictions (Okajima and Chiba, 2009).

The maintenance of a distinct morphology in a radically different habitat despite (presumably) gene-flow near the cave entrance, is the hallmark of incipient parapatric species. Under this scenario, the split of the daughter species from only a selection of lineages of the parental species renders the latter paraphyletic (Schilthuizen and Gittenberger, 1996). In a previous paper (Schilthuizen et al., 2005), we followed this reasoning to assign species status to *G. filiasaulae*, and we prefer to do the same here. Therefore, *Georissa* “sp. Silabur” should, in the future, be described as a separate species. (This discovery was made after finalising the taxonomic treatments in Ch. 2 and 3, which is why *Georissa* “sp. Silabur” was not formally described and named yet.)

The non-monophyletic relationship inferred by mitochondrial and nuclear DNA between hydrocenids of Mount Silabur indicates the multiple origins of the hydrocenid fauna here. Both ML and BI topologies (**Figures 4.5 and 4.6**) suggest that typical *G. pyrrhoderma* is sister to *G. sepulutensis* and *G. kinabatanganensis*, *Georissa* “sp. Silabur” is monophyletic, branching off from within the above-ground species *G. pyrrhoderma* (which is thereby rendered paraphyletic), while typical *G. silaburensis* derives from an entirely different ancestral lineage, and is sister to *G. bauensis*. Our ML analyses on separate mitochondrial and nuclear genes show some indications of occasional hybridisation between troglobitic *Georissa* “sp. Silabur” with epigean *G. pyrrhoderma* (see Supporting Information Figures S2 and S3). This is similar to the case of a zone of hybridization between *G. saulae* and *G. filiasaulae* in a cave near Sepulut, Sabah (Schilthuizen et al., 2012). During our sampling, we found only one individual of *Georissa* near the cave entrance, which belongs to the *Georissa* “sp. Silabur” haplotype. So, we suggest that hybridisation between *Georissa* “sp. Silabur” and *G. pyrrhoderma* at Mount Silabur is probably best explained by the rainwater flowing from the upper chambers of the cave to the deep cave habitat.

The haplotype network suggests the hydrocenids of Mount Silabur are clustered based on their shell morphologies, which is in concordance with our phylogenetic and conchometric analyses. We could not find the shell forms of *G. silaburensis* and *Georissa* “sp. Silabur” in the populations of *G. pyrrhoderma*, or the other way around, despite the variation in shell shape and sculptures of the sympatric species. The similar morphologies in spite of their genetic distinctness between the cave species (*G. silaburensis* and *Georissa* “sp. Silabur”) provide evidence for parallelism and cryptic diversity of cave hydrocenids. The cave hydrocenids were collected on the cave walls with continuous slow-flowing water from the cave ceiling which indicates a constant level of moisture, whereas the availability of water or moisture in the external outcrop environment probably fluctuate more strongly. Also, the different food availability of the cave and external outcrop habitats suggest different diet compositions of epigean and hypogean hydrocenids, with green plants available outside, but lacking inside the cave environment. These might be two of many abiotic and biotic factors that lead toward evolving a larger and broader shell morphology in hydrocenids of Mount Silabur.

While it is widely known that rock-dwelling microsnails of Southeast Asia region possess great allopatric divergence in shell shape (for example in the genera *Diplommatina*, *Georissa*, *Gyliotrachela*, *Hungerfordiana*, *Opisthostoma*, and *Plectostoma*; Liew et al., 2014; Rundell, 2008; Schilthuizen et al., 2006; Schilthuizen et al., 2012; Tongkerd et al., 2004; **Chapters 2 and 3**; Yamazaki, Yamazaki, and Ueshima, 2013), our findings provide evidence that morphological convergence may also occur.

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Supplementary materials

Link to supplementary materials1-9
https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjzs.12352andfile=jzs12352-sup-0001-Supinfo.pdf

1. **Table S1** Shell measurement of all hydrocenids used in Principal Component Analysis (PCA) and their shell form.
2. **Table S2** List of specimens used in molecular analyses and their GenBank accession number.
3. **Table S3** Haplotype group of each individuals based on partial *COI* mtDNA.
4. **Figure S1a** Plots of AW vs. SH of different hydrocenid shell forms at Mount Silabur.
5. **Figure S1b** Plots of AW vs. SH of different shell forms at Mount Silabur.
6. **Figure S1c** Plots of AH vs. SW of different shell forms at Mount Silabur.
7. **Figure S1d** Plots of AW vs. SW of different shell forms at Mount Silabur.
8. **Figure S2** Maximum likelihood tree inferred by concatenated partial *16S* and *CO1* mtDNA.
9. **Figure S3** Maximum likelihood tree inferred by *28S* nDNA.
10. **Sequence alignment A1** *16S* sequence alignment. https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjzs.12352andfile=jzs12352-sup-0002-AlignmentA1.fasta
11. **Sequence alignment A2** *28S* sequence alignment. https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjzs.12352andfile=jzs12352-sup-0003-AlignmentA2.fasta
12. **Sequence alignment A3** *COI* sequence alignment. https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fjzs.12352andfile=jzs12352-sup-0004-AlignmentA3.fasta

Chapter 5

Shell evolution of the Bornean *Georissa* in a phylogenetic context

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Abstract

Gastropod shells have experienced divergent, convergent, and parallel evolution at various taxonomical levels. In this paper, we attempt to elucidate the patterns of conchological character evolution in the minute *Georissa* land snails from Borneo. We performed phylogenetic analyses, incorporating taxa from Malaysian Borneo and other regions, inferred by mitochondrial (*COI* and *16S*) and nuclear (*18S* and *28S*) DNA sequences. Within this phylogenetic context we then investigated the evolutionary patterns of selected morphological features using a maximum likelihood ancestral state reconstruction and phylogenetic signal analysis. We found that the Bornean *Georissa* show significant parallelism in shell characters (i.e., shell scales, shell size, and general shape) as well as habitat. In addition, the non-monophyly of Bornean *Georissa* might indicate multiple invasions of ancestral species to Borneo and a history of long-distance dispersal.

Introduction

Gastropods are primarily single-shelled organisms which (except those who have secondarily lost their shell - i.e., true slugs) display great morphological diversity in shell shape. Generally, qualitative and quantitative approaches are applied in evaluating the evolution of gastropod shells. Although many taxa possess high intraspecific morphological variation, which may be due to either different selection pressures between different populations (Kemp and Bertness, 1984; Schilthuizen et al., 2005; Teshima et al., 2003) or phenotypic plasticity (Clewing et al., 2015; Verhaegen et al., 2018), the presence of unique morphological traits among closely related species could provide taxonomically informative characters for species identification and these characters could be used to explore evolutionary diversification of a particular taxon.

However, although detailed assessment of shell characters can provide substantial information for gastropod classification and identification, due to their long evolutionary history and diversification, gastropods also often possess similar phenotypic traits at various taxonomical levels as a result of morphological convergence (e.g., Dowle et al., 2015; Emberton, 1995; Goodacre and Wade, 2001; Köhler and Criscione, 2015; Pfenninger and

Magnin, 2001; Vermeij, 2017; Wagner and Erwin, 2006; West and Cohen, 1996) and parallelism (e.g. Butlin et al., 2014; Gittenberger and Schilthuizen, 1996; van Moorsel et al., 2000).

Recent taxonomic treatments of the Bornean *Georissa* have revealed that this genus of minute land snails is more diverse than previously anticipated. Our recent study of the Bornean species (**Chapters 2 and 3**) revealed a total of 29 species, many of which are narrowly endemic, and which vary in a number of trait systems, most conspicuously in the type and degree of expression of the sculpture of their shells. These varying conchological features make this group of minute snails a suitable and interesting model to study character evolution which may provide a basis to understand the adaptive function of these shell characters.

Study system

Georissa is the only genus from the family Hydrocenidae that is widespread in the limestone areas of Borneo. In general, the shell of *Georissa* consists of a moderate number of whorls with distinct morphological surface features. The internal shell walls are dissolved (**Chapters 2 and 3**; Haase and Schilthuizen, 2007; Thompson and Dance, 1983; Vermeulen and Junau, 2007; Vermeulen et al., 2015). Species diversity is particularly displayed in the presence or absence of protruding sculpture on the shell whorls and varying morphological features of the protoconch and teleoconch (see **Chapters 2 and 3**); for example, the number of whorls, ribbing patterns, shell shape, and shell size. The projected scale features on the whorls are superficially similar to those seen in the minute land snails of the genera *Opisthostoma*, *Plectostoma*, and *Diplommatina* (Diplommatinidae) of the same region (Webster, van Dooren and Schilthuizen, 2012). Studies on morphological evolution of those features suggest a function in anti-predation defense (see Liew and Schilthuizen, 2014; Schilthuizen et al., 2006), and this might be one of many evolutionary responses displayed by minute terrestrial snails in this region.

In Bornean *Georissa*, phylogenetically closely related species often share unique conchological characters relating to shell shape, size, and color (**Chapters 2 and 3**). Their adult height can range between 0.7 and 4.0 mm (see **Chapters 2 and 3**; Thompson and Dance, 1983; Vermeulen et al., 2015), their shape ranges from taller than wide to wider than tall, and they vary in shell color from unpigmented (white) over moderately pigmented (yellow to orange) to highly pigmented (red to brown).

The Bornean *Georissa* are usually found foraging at limestone outcrops and nearby vegetation, while some species (e.g., *G. filiasaulae*, *G. silaburensis*, *Georissa* "sp.") have diverged from the aboveground environment by occupying a cave environment. The first recorded hypogean species, *G. filiasaulae* from the interior province of Sepulut, Sabah (see Schilthuizen et al., 2005; Haase and Schilthuizen, 2007) showed a marked niche shift accompanied by morphological changes. Similarly, a microgeographic study of *Georissa* from Mount Silabur, Sarawak (see **Chapter 4**), revealed morphological parallelism between two sympatric, but reproductively isolated, species (*G. silaburensis* and *Georissa* "sp. Silabur"), both occupying a hypogean environment.

Here, we explore the evolution of shell shape in the Bornean *Georissa*. We conducted ancestral character state reconstruction by employing the ultrametric tree presented in **Chapter 1**. Additionally, we performed phylogenetic signal analysis to determine whether genetically closely related species are likely to share similar morphological traits. We aim to obtain answers to the question to what extent are character state changes conserved within the phylogeny of *Georissa*?

Materials and Methods

Taxon sampling for molecular phylogenetic analysis

We sampled 23 species of Bornean *Georissa*, including the paraphyletic species *G. kobelti*, *G. saulae*, *G. xesta*, and *G. pyrrhoderma* (**Chapters 2 and 3**; Schilthuizen et al., 2012), each represented by one individual. Six species could not be investigated, because we failed to obtain genetic sequences (namely, *G. borneensis*, *G. leucoccoca*, *G. trusmadi*, *G. williamsi*, *G. corrugata*, and *G. scalinella*). As the Bornean species are not likely to form a monophyletic group, we also incorporated two species from Vietnam (*Georissa* sp. Vietnam 1 and *Georissa* sp. Vietnam 2), one from Australia (*Georissa multilirata*) and one from Japan (*Georissa shikokuensis*). For the outgroup, we obtained the DNA sequences of *Bathynerita naticoidea*, *Theodoxus baeticus*, *T. meridionalis*, *Nerita patula*, *N. maxima*, and *N. olivaria* from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Details of specimens used in this study and their accession numbers are listed in **Table 5.1**.

Shell characters and morphological assessment

The morphological characters were compiled for the species used in the molecular phylogeny based on shells examined from newly collected specimens, museum material, as well as literature data. The resulting character matrix contained one continuous and nine discrete shell characters as listed in **Table 5.2**. We categorized the continuous shell character into three discrete traits, which we defined in **Table 5.2**. This set of characters is considered as taxonomically informative for the Bornean *Georissa* (**Chapters 2 and 3**; Haase and Schilthuizen, 2007; Thompson and Dance, 1983; Vermeulen and Junau, 2007; Vermeulen et al., 2015). We used the previously taken shell measurements from **Chapters 2, 3 and 4** for shell height, shell width, aperture height, aperture width, and angles. The measurements were taken as shown in **Figure 5.1**. We measured 4 to 20 adult shells of each species (based on the available material). For shell color, we referred only to freshly collected specimens. Details of all scored characters are presented with reference to representative shells in **Figures 5.1 and 5.2**.

Table 5.1 List of specimens used in molecular analyses with their voucher, locality and GenBank accession numbers. Sources for the sequences are encoded as follows: [a]Aktipis and Giribet (2010); [b]Bunje and Lindberg (2007); [c]Frey and Vermeij (2008); [d]Khalik et al. (2018); [e]Khalik et al. (2019a); [f]Khalik et al. (2019b); [g]Current study.

Species	Voucher	Locality	GenBank accession numbers			
			COI	16S	28S	18S
Bathynerita naticoidea	MCZ DNA 102209	-	FJ977768[a]	FJ977721[a]	-	-
Theodoxus baeticus	-	Quart, Spain	AY771277[b]	AY771234[b]	-	-
Theodoxus meridionalis	-	Sicily, Italy	AY771291[b]	AY771252[b]	-	-
Nerita patula	-	Philippines	EU732286[c]	EU732123[c]	-	-
Nerita maxima	-	Papua New Guinea	EU732274[c]	EU73211 [c]	-	-
Nerita olivaria	-	Indonesia	EU732281[c]	EU732118[c]	-	-
Georissa hungerfordi	MZU/MOL 16.12	Sarawak, Borneo	MK505435[e]	MK411780 [e]	Current study	Current study
Georissa sepulutensis	BOR/MOL 12278	Sabah, Borneo	MH033955[d]	MG982360[d]	MK775833[f]	Current study
Georissa kinabatanganensis	MZU/MOL 17.26	Sabah, Borneo	MH033959[d]	MG982349[d]	Current study	Current study
Georissa silaburensis	MZU/MOL 17.07	Sarawak, Borneo	MH033946[d]	MG982316[d]	MK775941[f]	Current study
Georissa bauensis	MZU/MOL 16.01	Sarawak, Borneo	MH033936[d]	MG982309[d]	Current study	Current study

Georissa pyrrhoderma	MZU/MOL 17.21	Sarawak, Borneo	MK811486[d]	MK775808[d]	MK775903[f]	Current study
Georissa “sp. Silabur”	MZU/MOL 17.05	Sarawak, Borneo	MK811455[f]	MK775775[f]	MK775892[f]	Current study
Georissa hosei	MZU/MOL 16.04	Sarawak, Borneo	MH033905[d]	MG982338[d]	MK775914[f]	Current study
Georissa anyiensis	MZU/MOL 17.51	Sarawak, Borneo	MH033927[d]	MG982278[d]	Current study	Current study
Georissa muluensis	MZU/MOL 17.31	Sarawak, Borneo	MH033893[d]	MG982288[d]	Current study	Current study
Georissa hadra	MZU/MOL 17.32	Sarawak, Borneo	MH033897[d]	MG982282[d]	Current study	Current study
Georissa kobelti	MZU/MOL 17.38	Sarawak, Borneo	MH033882[d]	MG982290[d]	Current study	Current study
Georissa niahensis	MZU/MOL 17.25	Sarawak, Borneo	MH033954[d]	MG982298[d]	Current study	Current study
Georissa gomantonensis	BOR/MOL 7389	Sabah, Borneo	MH033876[d]	MG982259[d]	MK775829[f]	Current study
Georissa saulae	BOR/MOL 12770	Sabah, Borneo	Current study	MG982266[d]	Current study	Current study
Georissa filiasaulae	BOR/MOL 12768	Sabah, Borneo	MK505425[e]	MK411785[e]	Current study	Current study
Georissa insulae	MZU/MOL 18.02	Sabah, Borneo	Current study	MK411801[e]	Current study	Current study
Georissa pachysoma	MZU/MOL 17.63	Sarawak, Borneo	MK505443[e]	MK411789[e]	Current study	Current study
Georissa similis	MZU/MOL 16.14	Sabah, Borneo	MK505446[e]	MK411792[e]	Current study	Current study
Georissa flavescens	BOR/MOL 7416	Sabah, Borneo	MH254769[e]	MK403003[e]	Current study	Current study
Georissa bangueyensis	RMNH/MOL 5005057	Sabah, Borneo	MH254416[e]	MK402997[e]	Current study	Current study

Georissa xesta	BOR/MOL 7258	Sabah, Borneo	MH254698[e]	MK403000[e]	Current study	Current study
Georissa nephrostoma	MZU/MOL 17.29	Sabah, Borneo	Current study	MK411798[e]	Current study	Current study
Georissa "sp. 1 Vietnam"	-	Vietnam	Current study	Current study	Current study	Current study
Georissa "sp. 2 Vietnam"	-	Vietnam	Current study	Current study	Current study	Current study
Georissa multilirata	-	Australia	Current study	Current study	Current study	Current study
Georissa shikokuensis	-	Japan	Current study	Current study	Current study	Current study

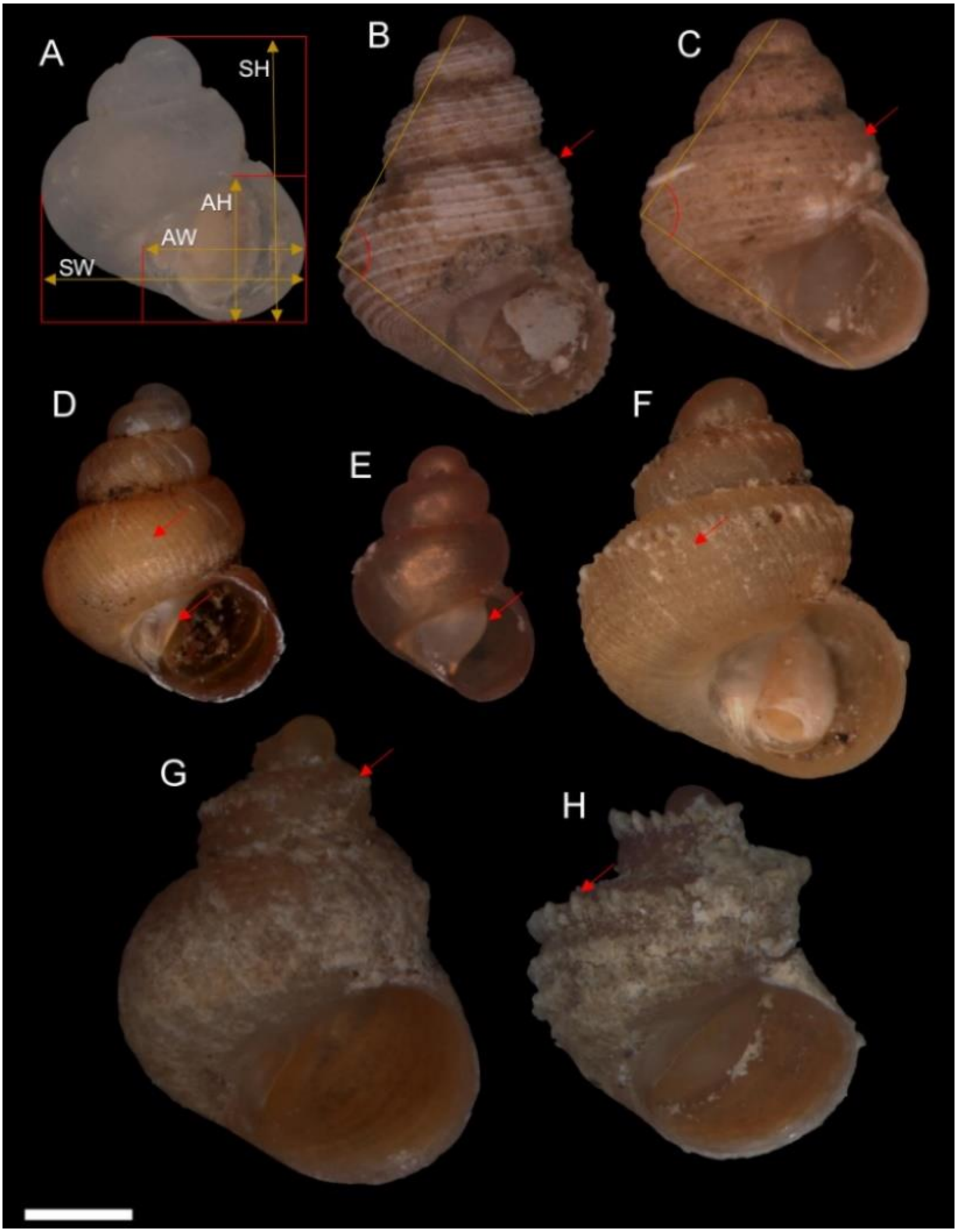

Figure 5.1 Morphological characters of the Bornean *Georissa* shown by representative species. The examined characters include shell height (SH), shell width (SW), aperture height (AH), aperture width (AW), spiral and radial ribbings, columella, shell shape, color and size (see Table 5.2). Measurements were taken as shown in **A** (*G. filiasaulae*). Other characters are indicated by red arrows on representative species (detailed in Table 5.2). **B** and **C**: spiral ribs and shell shape-angle (< 90° = broad, > 90° = slender) (*G. insulae* and *G. hungerfordi*, respectively), **D**: radial ribs and columellar wall (*G. similis*), **E**: columellar wall (*G. nephrostoma*), **F**: radial ribs (*G. saulae*), **G** and **H**: scales (*G. niahensis* and *G. anyiensis*, respectively). Scale bar equals 500 µm.

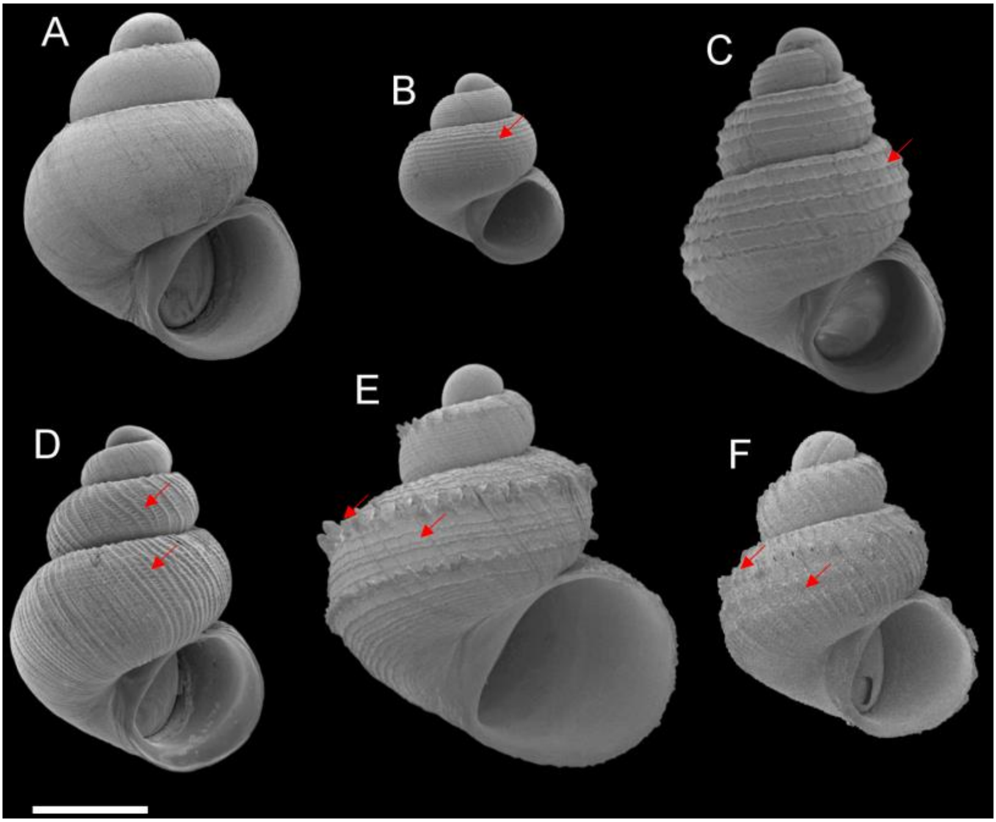

Figure 5.2 Morphological details shown by SEM. Red arrows indicate characters on representative shells (detailed in Table 5.2). **A**: smooth shell (*G. filiasaulae*), **B** and **C**: spiral ribs (*G. flavescens* and *G. hungerfordi*, respectively), **D**: radial ribs (*G. similis*), **E**: scales and spiral ribs (*G. muluensis*), **F**: scales and radial ribs (*G. saulae*). Scale bar equals 500 µm.

Table 5.2 List of characters and scoring of seven morphological traits, with references to examples illustrated in Figs 5.1 and 5.2. Selected models for ancestral state reconstruction (ASR) were determined as explained further in the methodology section.

Characters	States	ASR model	Examples
Shell surface	(0) smooth; (1) ribbed; (2) scaly	ER	Smooth: Figs 5.1A, and 5.2A Ribbed: Figs 5.1B-D and F, 5.2B-F Scaly: Figs 5.1F-H, 2E-F
Primary scale pattern	(0) none; (1) low; (2) acute	SYM	Low: Figs 5.1F-G, and 2F Acute: Figs 5.1H, 2 E
Spiral rib pattern	(0) none; (1) straight; (2) wavy/distorted	SYM	None: Figs 5.1A, 5.2A Straight: Figs 5.1B-C, 5.2D-E Wavy: Fig 5.2B Distorted: Fig 5.2D
Radial rib pattern	(0) none; (1) straight; (2) distorted	SYM	Straight: Fig 5.2D Distorted: Fig 5.2F
Shell shape	(0) slender; (1) broad	ARD	Slender: Fig 5.1B Broad: Fig 5.1C The apical angle for a slender shell > 90°, for a broad shell < 90°.
Shell size	(0) small; (1) moderate; (2) large	ER	Small: shell height < 1.5 mm Moderate: 1.5 mm < shell height < 2.5 mm Large: shell height > 2.5 mm
Shell color	(0) unpigmented; (1) moderately pigmented; (2) highly pigmented	SYM	Unpigmented: Fig 5.1A (white) Moderately pigmented: Figs 5.1C, 5.1F (from yellow to orange) Highly pigmented: Figs 5.2D-E (from red to brown)

DNA extraction, amplification, and sequencing

We extracted genomic DNA of the specimens with the Qiagen DNeasy Blood and Tissue kit using the manufacturer's protocol. The whole organism was used in the extraction procedure, with the shell (partially) included. We amplified three partial ribosomal genes, namely, *16S* (mitochondrial), *18S* (nuclear) and *28S* (nuclear), and also the partial *COI* (mitochondrial) protein coding gene. Polymerase chain reactions (PCR) were performed in 25 µL reaction volumes containing 1.0 µL undiluted DNA template following the master-mix and temperature profiling as in **Chapters 2 and 3** for *16S* and *COI* genes, Park and O'Foighil (2000) for *28S*, and Webster et al. (2012) for *18S*. Details on each primer set and temperature profiling are listed in Supplementary material 1 (**Table S1**).

The amplified fragments were Sanger-sequenced at BaseClear B.V. (Leiden, The Netherlands). The genes for which we failed to obtain sequences were treated as missing data. Accession numbers of newly obtained sequences and sequences retrieved from GenBank are given in **Table 5.1**.

Sequence alignment and phylogenetic analyses

The sequenced products were assembled in Geneious 10.2.3 assembler, manually checked and edited, and primer sequences were trimmed. The sequences were aligned using default parameters of MUSCLE (Edgar, 2004). The alignments were checked and edited including removal of the ambiguous ends. For *COI* sequences, the codon positions were identified in Geneious 10.2.3. We inferred the best fitting substitution models for each gene (partial *16S*, *18S*, and *28S* genes) and all three codon positions of *COI* in MrModelTest 2.4 (Nylander, 2004) based on the Akaike Information Criterion (AIC). The sequence data were concatenated and partitioned for further analysis. We performed phylogenetic analyses with Bayesian inference (BI) and maximum likelihood (ML) methods.

Bayesian inference was performed with MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2012) with the following MCMC parameters: number of generation (ngen) = 20 × 10^6; number of runs (nruns) = 2; number of chains (nchains) = 4; temperature profile (temp) = 0.2, tree sampling frequency (samplefreq) = 1,000. The log data of each independent run and both combined were inspected in Tracer 1.7.1 (Rambaut et al., 2018) to check for convergence of parameter estimates with effective sample sizes > 200. We discarded the initial 20% of the generated trees as burn-in and used the remaining trees to generate the consensus tree. We performed a maximum likelihood (ML) analysis using the same dataset with 10,000 ultrafast bootstrap replicates (Hoang et al., 2017) using IQ-TREE 1.6.3 (Nguyen et al., 2015) using a command line *-s -spp -m MF -bb 10000 -AIC* (Kalyaanamoorthy et al., 2017). The best suited nucleotide substitution models were determined using ModelFinder based on Akaike Information Criterion (AIC). We then evaluated the posterior probability (PP) for Bayesian inference and bootstrap support (BS) for maximum likelihood analyses.

Ancestral state reconstruction

The ancestral state reconstructions of the selected characters listed in **Table 5.2** were conducted in a maximum likelihood framework (Bouckaert et al., 2014) using the *ace* function in the package *phytools* (Revell, 2012), implemented in RStudio based on the maximum clade credibility tree from the BEAST 2 analysis. The best-suiting models were determined by comparing the *p*-values of log likelihoods between equal rates (ER), symmetrical (SYM), and all rates different (ARD). For each character, we did stochastic mapping on the tree with 1,000 replicates using the function *make.simmap* and *describe.simmap* (Bollback, 2006; Revell, 2012) to calculate the transition and proportion of time spent of each character state on the branches. For the ASR, we used the ultrametric tree we presented in **Chapter 1**.

Phylogenetic signal

The phylogenetic signal of each trait was estimated again in a ML framework based on the same ultrametric tree. We used two different models, *Blomberg's K* (Blomberg et al., 2003) and *Lambda* (Pagel, 1999) applying the *phylosig* function in the *phytool* package implemented in RStudio. In addition, we applied 1,000 simulation tests on all character state estimations for both models for an estimate of robustness.

Results

Sequence alignment and phylogenetic analyses

Our alignment of concatenated sequences of *COI*, *16S*, *28S*, and *18S* gene fragments had a total length of 3,009 base pairs, including gaps. The concatenated sequences were partitioned based on their codon positions and gene fragments. The model selection analysis showed that the best fitting nucleotide substitution model was GTR+I+G for all data partitions except for *18S*, where the K80+I substitution model gave a better fit. We applied these substitution models in our BI analysis in MrBayes.

Both ML and BI produced nearly identical topologies, except for Clade B, where *G. bauensis* + *G. silaburensis* are sister to all the scaly species in the BI analysis, while in the ML analysis they are not (see Supplementary material 2, **Figures S1**). *Georissa* splits into two likewise well supported clades A and B. Both clades consist of species of Sabah and Sarawak (Malaysian Borneo), and other regions (i.e., Australia, Japan, and Vietnam). Thus, the Bornean species are not monophyletic (**Figure 5.5**). Our ML and BI analyses show multiple well-supported nodes, with PP >0.95 and BS >75% values.

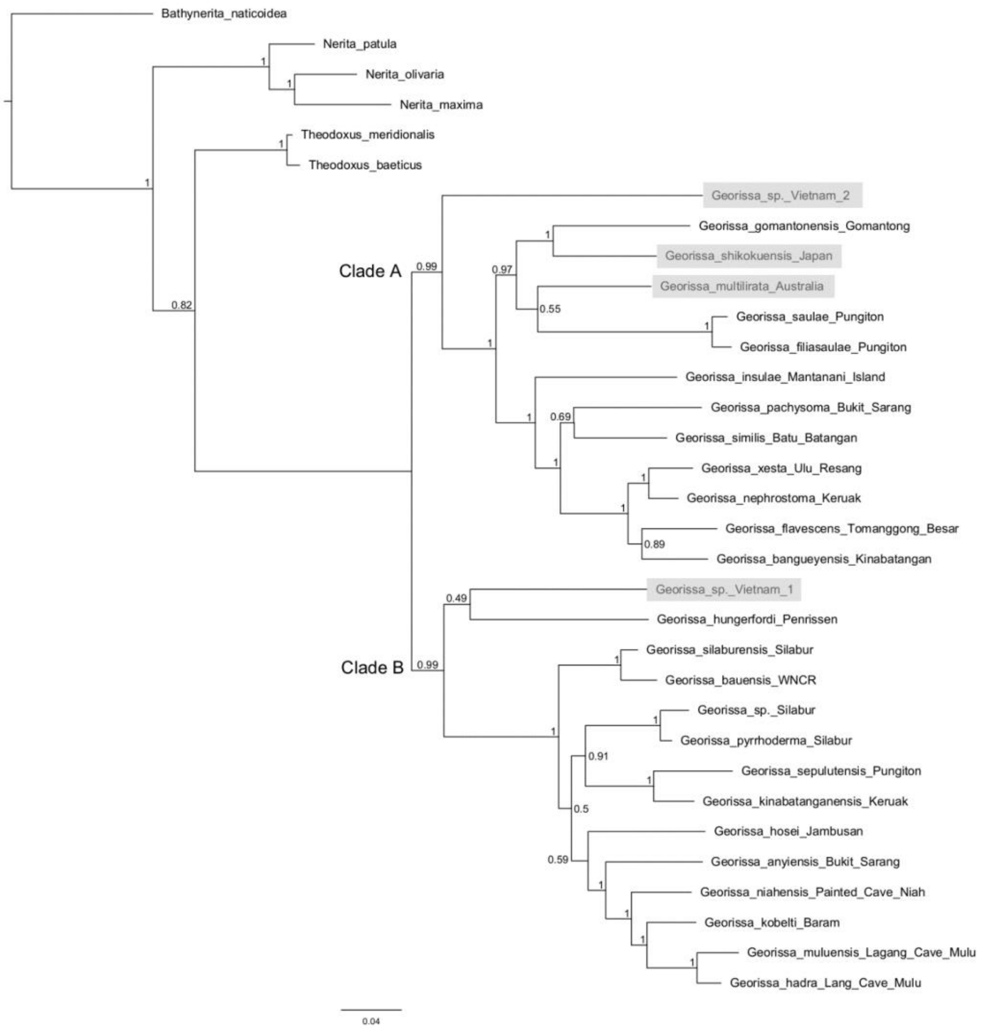

Figure 5.5 Phylogenetic tree from Bayesian inference. Non-Bornean species shaded in grey. Node support given as posterior probabilities. Scale in substitutions per site.

Ancestral state reconstruction

The results for the ancestral state reconstructions are shown in **Figures 5.6-5.9**. The absence of scale-like projections on the shell whorls of *Georissa* is likely to be ancestral. Hence, it is inferred that the character state change in the evolution of scales occurred at least twice in the phylogeny (**Figure 5.6**). For the “scaliness” of the primary scale (the first scale series on the whorls), we found that the species of clade B tend to possess a more acute scale projection (i.e., *G. anyiensis*, *G. hosei*, *G. kinabatanganensis*, and *G. muluensis*). With respect to other sculptural patterns (i.e., the radial and spiral ribbings on the shell whorls), we found that among the Bornean species, only *G. filiasaulae* has non-conspicuous sculpture and a smooth shell, and two species evolved conspicuous spiral and radial sculptures, *G. saulae* and *G. similis* (see **Figure 5.7**). For ribbing patterns, our ASR shows absence of radial ribbing as the ancestral character state of *Georissa*, while for spiral ribbing the ancestral state cannot be reconstructed unambiguously as both states "straight" and "distorted" are similarly common and scattered across the taxa suggesting multiple events of convergence.

Regarding shell shape (apical angle) and color (**Figures 5.8 and 5.9**) we found similar character state distributions in the two lineages of the Bornean *Georissa*. Broad-shelled species are generally poorly pigmented or unpigmented, with the exception of the more strongly pigmented *G. bauensis*, *G. saulae*, and *G. sepulutensis*. For shell size, medium size (between 1.5 to 2.5 mm) was reconstructed as ancestral in the Bornean species. Large-shelled species from Borneo evolved only in northern Sarawak (e.g., *G. hadra* and *G. niahensis*). As for the habitat, at least three Bornean species (e.g., *Georissa* "sp. Silabur", *G. silaburensis*, and *G. filiasaulae*) are known to occupy a hypogean habitat, while all others occupy epigean environments. However, only *G. filiasaulae* has lost the epidermal pigment in the cave environment.

Figure 5.6 Ancestral state reconstructions for shell surface (ER) and primary scale patterns (SYM). Characters mapped on the ultrametric tree resulting from BEAST 2 analysis.

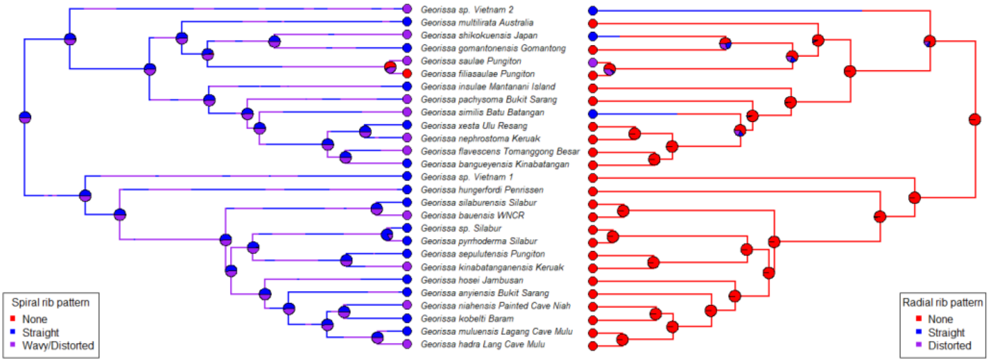

Figure 5.7 Ancestral state reconstructions for spiral rib (SYM) and radial rib (ARD) patterns. Characters mapped on the ultrametric tree resulting from BEAST 2 analysis.

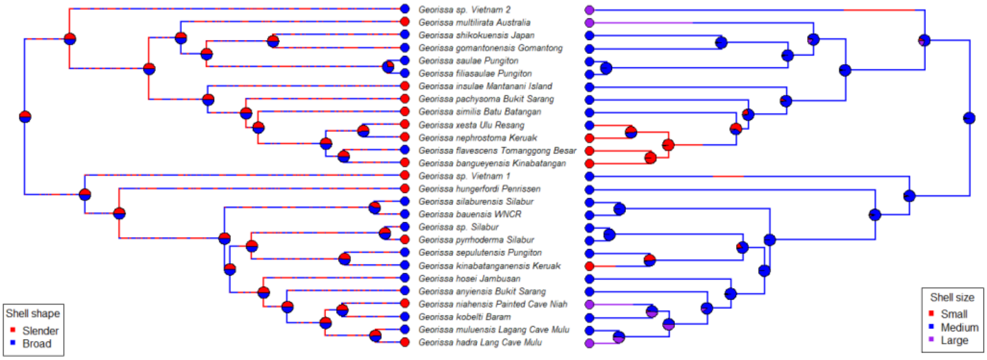

Figure 5.8 Ancestral state reconstructions for shell shape (ARD) and shell size (ER). Characters mapped on the ultrametric tree resulting from BEAST 2 analysis.

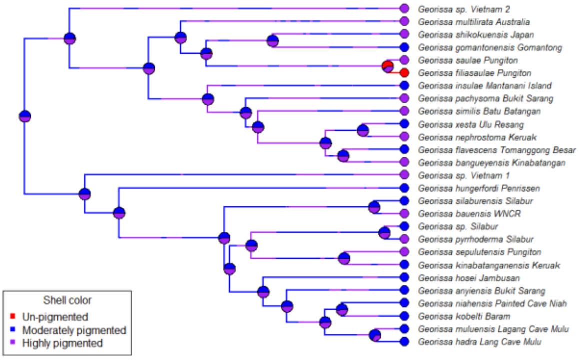

Figure 5.9 Ancestral state reconstruction for shell color (SYM). Character mapped on the ultrametric tree resulted from BEAST 2 analysis.

Phylogenetic signal

For morphological characters, our phylogenetic signal analysis based on both *lambda* (λ) and *Blomberg's K* show significant ($p < 0.05$) signal only for primary scale pattern. For shell surface, λ is significant ($p = < 0.001$) but *Blomberg's K* is not ($p = 0.533$). In addition, both methods are concordant for the remaining morphological characters, which appeared to evolve uncorrelated with the phylogeny, namely, spiral rib pattern, radial ribbing pattern, shell shape, size, and color.

Table 5.3 Phylogenetic signal and maximum likelihood model for each character in the ancestral state reconstruction analysis.

	Blomberg *K* method		Lambda method			
	K	*p*	Lambda	logL	logL0	*p*
Shell surface	0.261	0.533	0.463	-15.699	14.542	< 0.001
Primary scale pattern	0.723	0.005	0.672	-20.837	17.781	< 0.001
Spiral rib pattern	0.132	0.998	< 0.001	-22.382	-0.001	1
Radial rib pattern	0.154	0.893	0.144	-17.886	0.572	0.450
Shell shape	0.301	0.299	0.253	-19.111	0.636	0.425
Shell size	0.447	0.073	0.477	-21.287	1.205	0.272
Shell color	0.144	0.981	< 0.001	-22.738	< -0.001	1

Discussion

Phylogenetic relationships of the Bornean Georissa

Recent taxonomic treatment of the Bornean species (**Chapters 2 and 3**) delimited the species based on the combination of their geographical distribution, detailed conchology, and molecular phylogenetics. These studies have shown that the Bornean species have high allopatric and sympatric diversity. At least four species are known to be paraphyletic (*G. kobelti*, *G. saulae*, *G. xesta*, and *G. pyrrhoderma*). Our phylogenetic analyses inferred by both mitochondrial and nuclear genes, in which we incorporated the paraphyletic taxa, recovered similar relationships (see Supplementary material 2, **Figure S2**). Our results for this study are concordant with **Chapters 2 and**

3, where we also found that the “scaly” species grouped together in Clade B, with the exception of *G. saulae*.

Similarly, phylogenetic analyses showed that the Bornean *Georissa* are composed of two distantly related ancestral lineages, each of which also includes non-Bornean species, resulting in non-monophyly for the Bornean *Georissa*. Presumably, multiple invasions of several ancestral species took place in the region, similar to the situation with other minute land snails (e.g., *Diplommatina* and *Opisthostoma*; see Webster et al., 2012; Rundell, 2008).

Evolution of characters related to shell sculpture

The protruding microsculpture (i.e., ‘scales’) on the shell whorls of *Georissa* are one of the phenotypically informative traits for species identification in the genus. Our results (**Figure 5.6**) suggest a “non-scaly” origin of the Bornean *Georissa*. Based on the phylogeny and our ancestral state reconstruction, scales were obtained at least twice by the Bornean species. Although the precise function of the scales remains unclear, environmental selection in subterranean conditions could possibly cause the loss (e.g., *G. filiasaulae*) or reduction (e.g., *G. silaburensis* and *Georissa* “sp. Silabur”) of the scales on the shell whorls (see **Chapter 4;** Schilthuizen et al., 2005; Haase and Schilthuizen, 2007). As in other minute land snails from Borneo (i.e., Diplommatinidae), a stronger expression of protruding sculpture may relate to the evolution of mechanisms that defend against predators (Liew and Schilthuizen, 2014; Schilthuizen et al., 2006). *Georissa* could possibly experience similar ecological forces that lead toward similar morphological features.

Our phylogenetic analysis of the Bornean *Georissa* shows that most of the “scaly” species are grouped together in Clade B. Only the scaly *G. saulae* is grouped with Clade A, otherwise entirely consisting of “non-scaly” species.

Regarding acuteness of the scales, we found that only medium to small shelled *Georissa* possess acute projections of the scales. Our ancestral state reconstruction shows that scale acuteness diverged at least four times, and reversed at least twice in the phylogeny (Clade B). The development of acute scales could be a structural modification in response to predation. Large shelled species (e.g., *G. hadra* and *G. niahensis*) tend to possess low and minute scales on the whorls, which may indicate that the size and robustness of their shell is sufficient to resist predation.

Other phenotypically informative features of the Bornean *Georissa* are the spiral and radial ribs on the shell whorls. Despite species specificity, we found that these traits contain little phylogenetic signal (only one significant signal for Blomberg *K* and *lambda*, shell surface), and therefore probably evolve independently and repeatedly. Furthermore, there are only two not closely related species of the Bornean *Georissa* (e.g., *G. similis* and *G. saulae*) with conspicuous radial ribbing, one species with a smooth shell (e.g., *G. filiasaulae*) and one species with inflated columellar aperture wall (e.g., *G. nephrostoma*). These species are strongly diverged from their sister species, by independently having evolved novel morphological traits.

Shell size

Given the strong correlation, genus-wide, of shell height and width, we may use shell height to represent the shell size of each species. Our results based on the ancestral state reconstruction and phylogenetic signal analyses show that shell height of *Georissa* is phylogenetically conserved. We found that the Bornean *Georissa* possess a medium shell size, despite some recently diverged species having attained a larger shell (i.e., *G. niahensis* and *G. hadra*).

In addition, *Georissa* from the lower Kinabatangan valley, Sabah, possess a small shell compared to species from other geographical areas, while *Georissa* from Northern Miri, Sarawak, tend to possess relatively large shells. Thus, phylogenetically closely related species tend to evolve independently and show concerted shifts in shell size. In more specific cases (see **Chapter 4** and Haase and Schilthuizen, 2007), below-ground (cave) species often appear to have a relatively large and broad shell compared to closely related above-ground species. In general, strong shell size divergence among closely related land snail species is well known (e.g., see Chiba, 2004; Fiorentino et al., 2008; Hoso and Hori, 2008; Teshima et al., 2003), similar to what we found for *Georissa*.

Shell color

The analysis of shell color was somewhat restricted by the type of materials available for examination. Old dry specimens show decolorisation of the shell. We found no correlation between shell color and sculpture based on our ancestral state reconstruction. The hypogean species (e.g., *G. filiasaulae*, *G. silaburensis* and *Georissa* "sp. Silabur") show less pigmentation than closely related above-ground species, which indicates that shell color divergence may be driven by different habitat types (e.g., Baur, 1988; Cameron and Cook, 1989; Chiba, 2004; Chiu et al., 2002; Goodfriend, 1986).

Conclusion

Our current study revealed that the Bornean species of *Georissa* are divided into two major lineages (clades A and B). Clade A comprises species with no protruding sculptures on the shell surface, with the exception of *G. saulae*, and clade B comprises subclades of the "non-scaly" (*G.* "sp. Vietnam 1" + *G. hungerfordi*) and the "scaly" groups (all "scaly" species, except *G. saulae*). Our ancestral character state reconstructions show that many character states have evolved repeatedly and retain little phylogenetic value, with the exception of those relating to surface sculpture. Future studies which focus on morphological evolutionary patterns in *Georissa* might need to assess the ecological pressures, behavior, and diets of the individual species that possibly play important roles to the morphological divergence of the genus *Georissa*.

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Supplementary material

1. Supplementary material 1. Table S1. Primers and their respective sequences used in DNA extraction.
2. Supplementary material 2. Maximum likelihood phylogenies.

Link to supplementary material:
https://drive.google.com/drive/folders/1YBeLAomLIv8n88xGOUC9ALnwYOA23jjc

Chapter 6

Discussion and future prospects

Discussion

Tropical land snails are known to possess high allopatric and sympatric diversity, especially those inhabiting limestone karsts (as discussed in **Chapters 2 and 3**). The spatial isolation of limestone hills greatly contributes to this diversity and ultimately leads to the high incidence of local endemism (Clements et al., 2008). A few studies have shown that this isolation does not only induce endemism, but also leads to cryptic diversity in land snails (e.g., Köhler and Burghardt, 2016; Rundell, 2008; von Oheimb et al., 2019). This study was conducted to understand the evolutionary history of the genus *Georissa* of Borneo, which is particularly distributed in karst areas.

Land snails of the genus *Georissa* Blandford 1864 were collected at various localities in Malaysian Borneo (Sabah and Sarawak). I then applied molecular and morphological approaches to understand (i) the systematics, taxonomy, and biogeography, (ii) morphological variation, and, (iii) shell character evolution. In this chapter, I will summarize the most important findings of this study. In addition, I will briefly discuss the future prospects and potential further studies of *Georissa*.

Systematics, taxonomy, and biogeography

In general, species identification in *Georissa* and other minute land snails is done by applying detailed morphological assessment and biogeographic data. By doing so, a geographically and morphologically coherent species can often be defined. For the Bornean *Georissa*, our study shows that these two approaches could be improved by adding molecular data in species delimitation, because I found:

a. cryptic molecular diversity of two or more morphologically similar species, and
b. high degrees of morphological variation within a single, genetically uniform population, suggesting morphological plasticity.

Therefore, I did a comprehensive systematic evaluation of the Bornean *Georissa* by combining these three approaches in species delimitation. Initially, I grouped the species into two informal groups, namely, (i) the “scaly group” (**Chapter 2**) and (ii) the “non-scaly group” (**Chapter 3**). As a result, I identified a total of 13 scaly species, of which six were new to science (i.e., *Georissa anyiensis* sp. n., *Georissa muluensis* sp. n., *Georissa bauensis* sp. n., *Georissa silaburensis* sp. n., *Georissa kinabatanganensis* sp. n., and *Georissa sepulutensis* sp. n.) and 16 non-scaly species, of which three were new to science (i.e., *Georissa corrugata* sp. n., *Georissa insulae* sp. n., and *Georissa trusmadi* sp. n.).

Overall, the defined species show strong bootstrap support (**Chapters 2 and 3**). On the one hand, high *COI* genetic divergences were observed between species with similar features (e.g., *Georissa bauensis* and *Georissa hosei*). On the other hand, I found species with very distinct morphological features that might be genetically closely related (e.g., *Georissa hadra* and *Georissa muluensis*). This already suggests that morphological evolution is not neutral, and may include both stasis and rapid divergence (see further below).

In **Chapter 2**, I carried out web-based delimitation by using Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012) and Poisson Tree Processes (PTP) (Zhang et al., 2013) on the “scaly” group *Georissa*. I found that ABGD divided the Bornean *Georissa* into 6 species, while PTP divided the species into at least 15 species. These results were obtained based on the molecular data of what I argue are in fact 11 species of the “scaly” *Georissa*. Therefore, I could conclude that it is not advisable to identify a species of the Bornean *Georissa* based on molecular data alone. Moreover, it is widely known that many land snails have a highly fragmented population structure (see Liew et al., 2014; Rundell, 2008; Tongkerd et al., 2004), which increases the possibility of cryptic diversity of the *Georissa*. For this reason, I further refrained from algorithm-based species delimitation in the “non-scaly” *Georissa* (**Chapter 3**).

Despite the many challenges in species delimitation in the Bornean *Georissa*, the approaches that I took eventually provided a comprehensive and pragmatic arrangement into species. Therefore, I would suggest that future species delimitation of *Georissa* would best be done by complementing morphological assessment and biogeographic data with molecular analysis.

Morphological variation

The Bornean *Georissa* show two distinct shell surface sculpture types. First, there is the "scaly group", which comprises species with scale-like protuberances on the shell surface, and second, the "non-scaly group" which comprises species without such scales; instead, there may be raised and/or weak radial and/or spiral sculpture in this latter group.

Georissa species vary based on their morphological features. This could be observed even within small populations. For this reason, I conducted a molecular study to understand the shell variation of *Georissa* in a small-scale geographical area (**Chapter 4**). Interestingly, I found that the seemingly high shell variation of *Georissa silaburensis* is in fact due to a morphological parallelism in two sympatric species (i.e., *G. silaburensis* + *G.* "sp. Silabur"). These sympatric species are derived from ancestors from the outer part of the cave (**Chapter 4**). This finding is similar to Haase and Schilthuizen (2007), who found a sister species (*Georissa filiasaulae*) of *Georissa saulae*, which had strongly diverged from a "scaly" to a smooth (non-scaly) shell. In addition, the findings also highlight the fact that locally endemic species may render the ancestral species paraphyletic (Schilthuizen et al., 2005).

Shell character evolution

Based on shell morphology, the two informal groups of the Bornean *Georissa* show distinct shell characteristics, as stated earlier in this chapter. The phylogenetic approach with which I studied the shell evolution of *Georissa* (**Chapter 5**) showed that both groups to a large extent correspond with monophyletic groups (the "scaly" and "non-scaly" groups). The only exception is *Georissa saulae*, the only "scaly" species that is phylogenetically contained within the "non-scaly" clade of *Georissa*.

Furthermore, the phylogenetic reconstruction (**Chapter 5**) shows little geographic structure, with species from Sabah, Sarawak, and non-Bornean species mixed across the tree. This further indicates their long history of dispersal. However, some shell traits seem to be geographically restricted. For example, I found *Georissa hadra* and *Georissa niahensis* are the largest forms of Bornean *Georissa* (adult shell height > 2.5 mm) and they are both found in the northern part of Sarawak (Miri district). On the other hand, *Georissa xesta*, *Georissa nephrostoma*, *Georissa flavescens*, and *Georissa bangueyensis* are the smallest Bornean species with shell heights around 1.0 mm or less. These species are mainly distributed in the Kinabatangan region (Sabah).

Additionally, I found that shell coloration of *Georissa* varies widely, even among individuals of the same species (as discussed in the taxonomy sections in **Chapters 2 and 3**). The cause of this variation remains unknown. Future research could possibly investigate associations between shell coloration and the environment to unravel this variability, which may be genetic or phenotypically plastic. I did, however, find that *Georissa* which inhabit the hypogean environment show less pigmentation as compared to the epigean species. For example, the hypogean *G. filiasaulae* is white in color, while the ancestral species *G. saulae* is orange to red. A similar situation is seen in epigean *G. pyrrhoderma* with orange to red shells and the hypogean *G. silaburensis* with less pigmentation, rendering it light orange (**Chapter 4**). In both cases, the hypogean species also have larger shell size as compared to the epigean species. Such shifts in shell color and size in cave snails might be due to the effects of a combination of ecological factors, such as the abiotic environment (Baur, 1988; Cameron and Cook, 1989; Chiba, 2004; Chiu et al., 2002; Goodfriend, 1986; Haase and Misof, 2009), predation pressure (Moreno-Rueda, 2009; Schilthuizen et al., 2006), and physical restrictions (Okajima and Chiba, 2009). It is also not known by what developmental means *Georissa* shell color can change. Recent papers on snail shell coloration provide indications of the chemical compounds that could play a role (e.g., Jordaens et al., 2006; Schilthuizen et al., 2018).

Future prospects

Haase and Schilthuizen (2007) described the genitalia structures of *G. saulae* and *G. filiasaulae*, and this is the only complete description of a *Georissa*'s genitalia. Given the genus's uniformity in shell shape, an additional set of soft anatomy characters could certainly help improving the taxonomy. Moreover, a focus on the evolution of their genitalia might give indications of reproductive isolating mechanisms in sympatric species. Additionally, molecular studies that focus on the genus *Georissa* at a larger geographical scale could improve our understanding on their phylogeographic pattern and mode of dispersion.

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Summary

Samenvatting

Summary

This thesis started with a general overview on the evolutionary process of animals due to ecological changes. In **Chapter 1**, I introduced my model organism and stated the aims of this PhD project. I included preliminary findings on the ontogenetic measurement of *Georissa* shells. Also, I reconstructed a time-calibrated phylogeny, which is then used in **Chapter 5** for the ancestral state reconstruction of selected characters of *Georissa*.

My study has revealed that the Bornean *Georissa* are more diverse than previously anticipated. In **Chapters 2 and 3**, dedicated to the taxonomic revision of the genus *Georissa*, I discussed the morphological features, phylogenetic relationships, and the biogeographic distribution of *Georissa*. I examined material from various natural history museum and personal collections (as listed in **Chapters 2 and 3**). Furthermore, this approach has benefited me in the process of species delimitation, which, at the beginning of our research, was still in a state of confusion. As a result, I concluded that there are at least 29 species of minute land snails of the genus *Georissa* occurring in Malaysian Borneo.

In **Chapter 4**, I focused on the morphological and genetic variation of a pair of *Georissa* species sympatric in a small geographical region. One species is epigean, the other one cavernicolous. The morphological variation among these populations were surprisingly high. Therefore, I investigated the genetic relationships and morphological characters among these populations. Interestingly, I found that the hypogean species is a descendant of the above ground species, which is similar to the case of shell divergence in other *Georissa* species near Sepulut, Sabah.

In **Chapter 5**, I studied the shell character evolution of the Bornean *Georissa* in a phylogenetic framework. Based on the phylogeny, I found convergence in the formation of scales. Additionally, I discussed the differentiation of size and coloration of the Bornean *Georissa* in dependence on different habitats.

Finally, in **Chapter 6**, I summarised the research findings from my study. I suggested prospective research related to the evolutionary study of Bornean *Georissa* that could improve our understanding and knowledge on the evolution of terrestrial shelled organisms.

Samenvatting

Deze dissertatie begint met een algemeen overzicht van de evolutionaire processen die ervoor zorgen dat ecologische omstandigheden de lichaamsbouw van dieren doen veranderen. In **Hoofdstuk 1** introduceer ik mijn modelorganisme en zet ik de doelen van dit promotieonderzoek uiteen. Ik geef voorlopige resultaten van ontogenetische metingen aan de slakkenhuisjes van *Georissa*. Verder presenteer ik in dit hoofdstuk een aan de tijd gecalibreerde fylogenetische reconstructie, die verder wordt gebruikt in **Hoofdstuk 5** voor het reconstrueren van de voorouderlijke toestanden van bepaalde kenmerken bij *Georissa*.

Mijn studie laat zien dat het genus *Georissa* in Borneo veel diverser is dan eerder werd gedacht. In **Hoofdstukken 2 en 3**, die gewijd zijn aan taxonomische revisies van het genus, bediscussieer ik morfologische eigenschappen, fylogenetische verwantschappen en de biogeografische verspreidingen van *Georissa*-soorten. Hiervoor maak ik gebruik van materiaal uit diverse natuurhistorische collecties, zowel in musea als van privépersonen. Deze aanpak heeft geholpen bij het beter afgrenzen van de verschillende soorten, iets wat aan het begin van mijn studie nog in een tamelijk verwarde toestand verkeerde. Uiteindelijk heb ik kunnen vaststellen dat er tenminste 29 verschillende soorten *Georissa* in het Maleisische deel van Borneo te vinden zijn.

In **Hoofdstuk 4** besteed ik aandacht aan de morfologische en genetische variabiliteit bij een tweetal *Georissa*-soorten die symptraisch leven in een klein geografisch gebied. De ene soort leeft bovengronds, terwijl de andere ondergronds in een grottencomplex te vinden is. De morfologische variatie binnen deze populaties was opvallend groot. Daarom onderzocht ik nauwkeurig hun genetische verwantschappen en morfologische eigenschappen en ontdekte dat de ondergrondse soort een directe afstammeling is van de bovengrondse, vergelijkbaar met een situatie die we aantreffen bij twee andere soorten van *Georissa* nabij Sepulut in Sabah.

In **Hoofdstuk 5** gaat het over de evolutie van schelpkenmerken bij de *Georissa* van Borneo binnen een fylogenetische context. Op basis van de fylogenie ontdekte ik convergentie in het ontstaan van de 'schubben' op het huisje.

Daarnaast bespreek ik ook de diversifiëring in lichaamsgrootte en kleur binnen de Borneose *Georissa* afhankelijk van verschillende milieus.

Tenslotte vat ik in **Hoofdstuk 6** mijn onderzoeksresultaten samen. Ik suggereer toekomstig onderzoek aan de evolutiebiologie van Borneose *Georissa* waarmee ons begrip en kennis van de evolutie van landmollusken kan worden verbeterd en vergroot.

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Curriculum vitae

Biography

Mohd Zacaery bin Khalik was born on February 25th, 1986, in Kuching, Sarawak, Malaysia. He received primary and secondary education at SK Merpati Jepang and SK Gita (primary schools), and SMK Batu Lintang (secondary school). Later, he went to Labuan Matriculation College (2004-2005) in a biology stream class. After completing his matriculation, he continued with BSc (Resource Chemistry) at Universiti Malaysia Sarawak (2005-2008), and MSc (Forensic Science) at Universiti Teknologi Malaysia (2010-2012).

In December 2008, Zac joined the Department of Zoology, Universiti Malaysia Sarawak (UNIMAS), as a research assistant. During his early career, he assisted the faculty members with their research. This was the point where Zac started to build his interest in the diversity of animals, especially in Malaysian Borneo. In February 2012, after obtaining his master degree, Zac was appointed as a lecturer and junior researcher at the same department.

In September 2015, Zac started his PhD at Naturalis Biodiversity Center and Leiden University in the field of evolutionary biology, under the supervision of Prof Dr Menno Schilthuizen and Dr Martin Haase (University of Greifswald, Germany). After completion of his PhD, Zac will continue his work at the Department of Zoology, UNIMAS. His main research interest is to study the behaviour of land snails that might substantially contribute to shape their species diversity. Also, Zac is interested to do community outreach activities in the effort to disseminate the importance of habitat conservation.

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