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A molecular phylogeny of *Geotrochus* and *Trochomorpha* species (Gastropoda: Trochomorphidae) in Sabah, Malaysia reveals convergent evolution of shell morphology driven by environmental influences.

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There are currently eleven *Geotrochus* and four *Trochomorpha* species in Sabah. The primary diagnostic character that separates the two genera is the intensity of sculpture on the shell upper surface. All *Trochomorpha* species have a coarse nodular sculpture while *Geotrochus* species has a non-nodular sculpture or smooth shell. However, it is known that shell characters are often evolutionary labile with high plasticity in response to environmental factors. Hence, identified the phylogenetic and ecological determinants for the shell characters will shed light on the shell-based taxonomy. This study aims to estimate the phylogenetic relationship between *Geotrochus* and *Trochomorpha* species in Sabah based in two mitochondrial genes (COI, 16S) and one nuclear gene (ITS- and also to examine the influence of temperature, elevation and precipitation on the coarseness of shell upper surface sculpture and shell sizes of the species of both genera. Besides, we also investigated the phylogenetic signal of the shell characters. The phylogenetic analysis showed that *Geotrochus* and *Trochomorpha* species are not reciprocally monophyletic. The phylogenetic signal test suggested that shell size and upper surface sculpture are homoplastic and these shell traits are strongly influenced by elevation and precipitation. The highland species of both genera have a coarser shell surface than lowland species. The shell and aperture width decrease with increasing elevation and precipitation. In the view of finding above, the current taxonomy of *Geotrochus* and *Trochmorpha* in Sabah and elsewhere that based on shell characters need to be revised.

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Abstract

There are currently eleven *Geotrochus* and four *Trochomorpha* species in Sabah. The primary diagnostic character that separates the two genera is the intensity of sculpture on the shell upper surface. All *Trochomorpha* species have a coarse nodular sculpture while *Geotrochus* species has a non-nodular sculpture or smooth shell. However, it is known that shell characters are often evolutionary labile with high plasticity in response to environmental factors. Hence, identified the phylogenetic and ecological determinants for the shell characters will shed light on the shell-based taxonomy. This study aims to estimate the phylogenetic relationship between *Geotrochus* and *Trochomorpha* species in Sabah based in two mitochondrial genes (COI, 16S) and one nuclear gene (ITS) and also to examine the influence of temperature, elevation and precipitation on the coarseness of shell upper surface sculpture and shell sizes of the species of both genera. Besides, we also investigated the phylogenetic signal of the shell characters. The phylogenetic analysis showed that *Geotrochus* and *Trochomorpha* species are not reciprocally monophyletic. The phylogenetic signal test suggested that shell size and upper surface sculpture are homoplastic and these shell traits are strongly influenced by elevation and precipitation. The highland species of both genera have a coarser shell surface than lowland species. The shell and aperture width decrease with increasing elevation and precipitation. In the view of finding above, the current taxonomy of *Geotrochus* and *Trochmorpha* in Sabah and elsewhere that based on shell characters need to be revised.

Introduction

Geotrochus and *Trochomorpha* are two land snail genera that with similar shell forms belonging to the family Trochomorphidae (Fig. 1). The species of the two genera are ground-dwelling snails typically spotted on the understory vegetation and with overlapping distribution ranges in the region of Oceania and Southeast Asia. A recent revision of both genera reveals a total of eleven *Geotrochus* species and four *Trochomorpha* species in Sabah (Vermeulen *et al*. 2015). *Trochomorpha* species are endemic to montane forest and subalpine forest between 1400 m and 3500 m on Mount Kinabalu and Crocker Range in Sabah, while *Geotrochus* species are widespread in Sabah occur from lowland forest at sea level to highland until 3200m (Vermeulen *et al*., 2015).

Taxonomy of *Geotrochus* and *Trochomorpha* in Sabah has been mainly based on shell and anatomical characters (Tillier & Boucher, 1988; Vermeulen *et al*., 2015). *Trochomorpha rhysa* is the first species of *Trochomorpha* species described from Sabah (Tillier & Boucher, 1988) from Mount Kinabalu between 3000 m and 3500 m. This new species was placed under *Trochomorpha* based on the genitalia and radula characters. After that, more new species of *Trochomorpha* and *Geotrochus* were described solely based on the shell characters (Vermeulen et al., 2015). Vermeulen et al. (2015) noted that these species of the two genera have a similar shell, but *Trochomorpha* species have a coarser nodular sculpture on the upper surface of the shell.

Taxonomy of land snails based on anatomy and shell characters are not without its weakness because many of these characters are evolutionary labile (Pfenninger *et al*., 1996; Liew *et al.*, 2009; Holznagel *et al*., 2010; Hyman & Ponder, 2010; Hirano *et al*., 2014; Dowle *et al*., 2015; Köhler & Criscione, 2015). This open a question to what extent the shell upper surface sculpture is phylogenetically informative in *Geotrochus* and *Trochomorpha* as shell surface sculpture is known to evolve rapidly and in parallel or convergently in response to environmental conditions (Pfenninger & Magnin, 2001; Schilthuizen *et al*., 2006; Liew *et al*., 2009). Therefore, it is vital to examine the phylogenetic relationship among *Trochomorpha* and *Geotrochus* species and the influences of habitat climatic factors to clarify the taxonomy of the two genera in Sabah as a way forward to improve the taxonomy of the two genera in Oceania and Southeast Asia in general. Hence, this study aims to estimate the molecular phylogenetic relationship of selected species of *Geotrochus* and *Trochomorpha* species in Sabah by using two mitochondrial genes (COI and 16S) and one nuclear gene (ITS-1). After that, we examined the association of the shell size and shell upper surface sculptures with several environmental variables in their habitats. Lastly, the phylogenetic signal of the shell characters was tested.

Materials & Methods

Samples

All the eleven *Geotrochus* and four *Trochomorpha* species from Sabah are available in the BORNEENSIS Mollusca collection of Institute of Tropical Biology and Conservation in Universiti Malaysia Sabah. However, not all specimens of the species were suitable for phylogenetic and morphological analysis (Table 1). A total of six *Geotrochus* species, namely, *G. meristotrochus*, *G. kinabaluensis*, *G. paraguensis*, *G. oedobasis*, *G. kitteli*, and *G. whiteheadi*; and three *Trochomorpha* species, namely, *T. haptoderma*, *T. rhysa*, and *T. thelecoryphe* were selected phylogenetic analysis. For morphological analysis, a total of 155 specimens of eight *Geotrochus* and three *Trochomorpha* species with intact shells were chosen to obtain quantitative and qualitative measurements. As there is no good quality specimen in the collection for *Trochomorpha trachus*, *Geotrochus conicoides, Geotrochus spilokeiria* and *Geotrochus scolops*, these species were not included in the present study.

Shell Characters Measurement

A total of five primary diagnostic shell characters that used for delimitation of the species in *Geotrochus* and *Trochomorpha* were measured qualitatively and quantitatively (Fig. 2). The types of shell upper surface sculptures for the adult and subadult specimens with at least three whorls were recorded based on the four categories (S1 – S4) of coarseness that are visible at 8× magnification. Sculpture S1 - Densely placed, more or less regularly spaced radial riblets and between 11-19 spiral threads that form nodes over the radial sculpture; S2 – Raised and distinct radial growth lines and 15 thin spiral threads; S3 – Indistinct radial growth lines and inconspicuous riblets and between 6 - 23 thin or very thin spiral threads; and S4 – Inconspicuous growth lines and between 4 - 25 low and thin spiral threads.

In addition, four quantitative measurements of shell size, namely, shell height (SH), shell width (SW), aperture height (AH) and aperture width (AW) were measurement to nearest 0.1 mm from the photograph of the shell apertural view with the aid of Leica Stereo Microscope M205.

Collection of ecological data

To investigate the correlation between shell size and upper surface sculpture and the environmental variables, we obtained the elevation, precipitation and temperature of the location where the specimens were collected. The elevation of the location were extracted from SRTM DEM 30-meter resolution (http://earthexplorer.usgs.gov/), and the annual precipitation and annual average temperature were extracted from global average temperature and annual precipitation layers of 30 arc-seconds (~1 km) resolution of WorldClim v1.4 database (www.worldclim.org) using point sampling tool of QGIS v2.60 (QGIS Development Team, 2019). As expected, the annual average temperature confounding with the elevation. Hence, we explored the influence of the elevation and annual precipitation to the shell sizes and shell surface sculptures as suggested by Goodfriend (1986).

Statistical analysis

We examined the collinearity of among the four shell size measurements. The results showed that aperture width (AW) is strongly correlated with shell width (SW) (r = 0.99), while the pairwise correlations among the other measurements are weaker with correlation coefficient values (r) range between 0.65 and 0.71. Hence, only SH, SW and AH measurements were retained for further analysis. All the three measurements were not normally distributed as reveal by Shapiro-Wilk test (Shapiro & Wilk, 1965). Therefore, Spearman's correlation tests (Spearman, 1904) were employed to examine the relationships between each of two environmental variables with the three shell measurements. Kruskal-Wallis tests were used to determine if there are statistically significant differences of the species with four different shell upper surface sculptures in term of the elevation and precipitation of their habitat or types (Kruskal & Wallis, 1952). Both analyses were performed in RStudio 1.1.4 (RStudio Team, 2015).

DNA extraction, amplification and sequencing

Foot muscle with about two mm^3 was excised from the preserved land snails using a sterilised scalpel. Genomic DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen Inc., Hilden, Germany) following the standard procedure of the manual. Two mitochondrial genes fragments (COI and 16S) and one nuclear gene fragment (ITS-1) were amplified using the pairs primer listed in Table 2 at the following thermal-cycling profile: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at a locus-specific temperature for each primer for 45s (Table 2), extension at 72°C for 1 min and a final extension at 72°C for 5 min. Positive PCR products were then sent to MyTACG Bioscience Enterprise for sequencing by using the forward and reverse primers that were used during PCR.

Sequence alignment and molecular phylogenetic reconstruction

Resulting forward and reverse sequences were assembled and aligned in Bioedit 7.2.6 (Hall, 1999). The sequences were deposited in GenBank (Table 3). Before the phylogenetic analysis, the sequences of the three genes (COI, 16S and ITS-1) were concatenated. Then, the best-fitted model of nucleotide's substitution for each of the gene partition (Table 4) was estimated by using jModelTest v2.1.6 (Darriba *et al*., 2012) via CIPRES Science Gateway (https://www.phylo.org/portal2/; Miller *et al*., 2010). Next, we used Bayesian Inference (BI) and Maximum Likelihood (ML) approached to reconstruct the phylogenetic trees by using MrBayes v3.2.6 (Huelsenbeck & Ronquist, 2001) and RAxML v 8.2.10 (Stamatakis, 2014) respectively. The BI analysis was run for 1000000 generations along four chains with sample frequency set to 100 and a burn-in of 2500 (25%) while Maximum Likelihood analyses (ML) was calculated using GAMMAI model estimation. The phylogenetic trees generated from the two approaches were then viewed and edited using TreeGraph 2.14 (Stöver & Müller, 2010). *Everettia klemmantanica* (Dyakiidae) was selected as an outgroup because this species was the sister taxa of the Trochomorphidae (Bouchet *et al*., 2017).

Phylogenetic signal analysis

To investigate the influence of phylogeny on the evolution of shell upper surface sculpture and shell size, phylogenetic signal of these shell characters were assessed with Pagel's Lambda (Pagel, 1999) and Blomberg's K (Blomberg *et al*., 2003) by using "geiger" package (Harmon *et* *al*., 2008) and "phytol" package (Revell, 2012) respectively in the environment of RStudio 1.1.4 (RStudio Team, 2015) following the method of Phung *et al*. (2017). For the qualitative shell trait, all tip in the phylogenetic tree were retained but for the quantitative shell traits, the tips represented by juvenile specimen were excluded.

Results

Molecular phylogeny of *Trochomorpha* and *Geotrochus* species in Sabah

The final DNA alignment data matrix consists of 34 taxa and 1918 characters (16S: 1– 461bps; COI: 462 – 1112; and ITS-1:1113 - 1918). The phylogenetic relationship of *Geotrochus* and *Trochomorpha* species was shown in Figure 3. Analysis of ML and BI yielded a phylogenetic tree with an identical topology that with > 70% bootstrap values for ML and > 0.92 posterior probability values for the four major clades. Both ML and BI analyses showed that *Geotrochus* and *Trochomorpha* species are not monophyletic. *Geotrochus kitteli* is sister taxa to *Trochomorpha rhysa* (Clade D), and *T. thelecoryphe* is nested in the *T. haptoderma* (Clade A). *Geotrochus paraguensis* from Banggi and Balambangan Island is paraphyletic with *G. kinabaluensis* (Clade C). Clade B contained *Geotrochus meristotrochus*.

Association between shell morphology and environmental variables

The *Geotrochus* and *Trochomorpha* species that have coarser shell surface sculpture (i.e. Type S1 and S2) tend to occupy habitats at higher elevation (above 2000 m) and annual precipitation between 2400 mm and 2500 mm. ($p<0.00$, Fig. 4). Meanwhile, the shell width of the *Geotrochus* and the *Trchomorpha* species was negatively correlated with elevation ($r = -0.42$, $p < 0.001$, Fig. 5) and precipitation ($r = -0.41$, $p < 0.001$, Fig. 6).

Phylogenetic signal

The result from these two approaches showed that all diagnostic shell characters of *Geotrochus* and *Trochomorpha* considered in this study exhibited a weak phylogenetic signal (λ, $K < 1$; $P > 0.05$) (Fig. 7 and Table 5).

Discussion

Phylogeny of *Geotrochus* and *Trochomorpha* and its implication to taxonomy

The phylogenetic analysis showed that *Geotrochus* and *Trochomorpha* are not reciprocal monophyly (Fig. 3). This result is contrary to the current taxonomy of the two genera that based on the shell characters, especially the shell upper surface sculpture. The confusing taxonomy of the two genera goes back to the description of *Geotrochus* by van Hasselt (1823, but published in 1824) based on the specimens from Java Island, Indonesia, and the description of *Trochomorpha* by Albers (1850) base on several *Geotrochus*-like species from Southeast Asia and Pacific Islands. After that, von Martens (1867) questioned the validity of the description of the genus *Geotrochus* by van Hasselt (1823, published in 1824) as there is not type assigned to the genus. Hence, von Martens (1867) concluded that the *Geotrochus* is morphologically similar to *Trochomorpha* and he used *Trochomorpha* instead of *Geotrochus* as a valid genus for the land snails from Borneo. Later, Issel (1874) used only *Trochomorpha* for the species recorded in Borneo with no mention of *Geotrochus* at all. Until the year 1935, Pilsbry (1935) validated the genus *Geotrochus* based on the Opinions no. 46 rendered by the International Commission on Zoological Nomenclature. Solem (1964) used only *Geotrochus* for the checklist of land snails in Sabah.

The first detailed description of the species of the two genera was the *Trochomorpha rhysa* from Mount Kinabalu when it was first described by Tillier & Bouchet (1988). Although the shell morphology, genitalia character and radula were described in detail, there was no comparison made to the known *Geotrochus* species or *Trochomorpha* species from other regions. In fact, *Geotrochus* was not mentioned at all in Tillier & Bouchet (1988). The first comprehensive revision on *Geotrochus* and *Trochomorpha* is by Vermeulen et al. (2015) for the species in Sabah based on the shell morphology. There are four *Trochomorpha* species of which three are new, and 11 *Geotrochus,* of which six are new were included in the revision (Vermeulen et al., 2015).

The taxonomy history of the two genera in Sabah that lead to their confusing taxonomy is not merely an isolated case but reflects the taxonomy problem of the two genera on a large scale. The two genera have been used interchangeably as seen in the records of the two genera in the museum worldwide (File S3). As revealed by the GBIF data, there is large extend of the overlapping in the distribution ranges of the two genera. This pattern could represent a real situation or could be resulted from the misidentification of the species or genera given the fact that the shells of the species in the two genera are very similar. Schileyko (2002a, 2002b) recognised current taxonomy of *Trochomorpha* is still unresolved, and he placed *Trochomorpha* in the Family Trochomorphidae whereas *Geotrochus* in the Family Helicarionidae. Our results indicate that more comprehensive taxonomy study on *Trochomorpha* and *Geotrochus* are needed, not only for the Sabah taxa but for the entire distribution ranges of the two genera. However, the fact that *Trochomorpha rhysa* is more genetically closely related to the *Geotrochus* species implies that its putative taxonomy position was likely misled by the parallelism in genital character as documented occasionally occurred in other groups of land snails (e.g. Davison *et al*., 2005; Hirano *et al*., 2014). Moreover, the coarse nodular upper surface sculpture was also taxonomically uninformative as the shell character has found evolved independently in this study.

Regarding taxonomy at the species level, this study confirmed the existence of the eight genetically distinct species that classified by Vermeulen et al. (2015), except *Trochomorpha thelecoryphe* and T. *haptoderma*. The two *Trochomorpha* species are very similar in shell but *T. thelecoryphe* has a flatter spire than *T. haptoderma* (Vermeulen *et al*., 2015). It is possible the type specimen of *T. thelecoryphe* in Vermeulen et al. (2015) was a juvenile shell. Hence, more good condition specimens are needed for further clarification s in a future study.

Evolution of shell surface sculpture coarseness and shell sizes of *Geotrochus* and *Trochomorpha*

Polyphyly of the genus *Trochomorpha* indicated that the diagnostic shell upper surface sculpture is a homoplasy character. Our results show that the characters are strongly influenced by environments of the habitat, and phylogenetic closely related species do not tend to resemble each other in the shell size and shell upper surface sculpture. Hence, these shell traits of *Geotrochus* and *Trochomorpha* are evolutionary labile that are not suitable to be served as diagnostic characters at the genus level.

Convergence of the shell traits is instead a common phenomenon among land snails that occupying similar ecological niches (Emberton, 1995; Phung *et al*., 2017) as the physical shell is deemed to be the by-product of adaptation to their environmental attributes (Goodfriend, 1986; Pfenninger *et al*., 2005; Baur & Raboud, 1988, Proćków *et al*., 2017; Proćków *et al*., 2018). The unsmooth surface of the shell helps land snail interact with the water in their habitats, for example, ribbed shells retain more water on the shell surface (Giokas et al., 2014); hairy shell increase the snails' adherence wet surface of the plants in a more humid high-elevated area (Pfenninger *et al*., 2005; Proćków *et al*., 2018, but see Shyydka et al., 2019); and coarser granular-like surface sculpture on shell helps in reducing the water retention on the surface (Nosonovsky & Bhushan, 2008; Maeda et al., 2019). Thus, the coarser shell surface helps *Trochomorpha* and *Geotrochus* species at highland elevation habitat dwell through fallen wet leaves by reducing the adhesiveness to its surrounding.

The relationships between shell size and two significant environmental variables, namely, elevation and precipitation, are well documented (Goodfriend, 1986; Baur & Raboud, 1988; Pfenninger & Magnin, 2001; Glass & Darby, 2009; Anderson *et al*., 2007; Proćków *et al.*, 2017). Our results show that the shell width and aperture width of the two genera are negatively correlated with elevation and precipitation. As the temperature is cofounding with elevation, it also means that the shell size of the species in both genera follows converse Bergmann's rule (Baur & Raboud, 1988; Anderson *et al*., 2007; Proćków *et al.*, 2017). It was hypothesised that the colder environment induces highland land snail to reach sexual maturity faster than those living in the warmer area. Hence, shells of the highland land snails are often smaller as the growth of the land snails is limited after maturity (Proćków *et al.*, 2017).

It is known that there is a positive relationship between high precipitation and shell size of land snails because humid habitat promotes the growth and expansion rate of shell whorls (Goodfriend, 1986). However, this may not be the case for montane species (Goodfriend, 1986; Proćków *et al.*, 2017). Our results show that *Geotrochus* and *Trochomorpha* species from sites with lower precipitation has a larger shell size. The negative correlation could probably due to the favourable effect of moisture on shell size has been compensated by the lower temperature on the high elevation that generally has a negative effect on shell size (Goodfriend, 1986, Baur & Raboud, 1988, Anderson *et al*., 2007). Besides, decreasing in aperture size with the altitudinal gradient has generally been interpreted as an adaptation to the lower humidity at lower elevational area (Goodfriend, 1986) as smaller apertures tend to lose proportionately more water per unit aperture area (Goodfriend, 1986).

Conclusions

This study presents the first molecular phylogeny study on the genus *Geotrochus* and *Trochomorpha*. The phenotypically identified *Geotrochus* and *Trochomorpha* do not congruent with the phylogenetic relationships. This incongruency is due to the homoplasy of upper surface sculpture which is used as the diagnostic character of the two genera. The coarser shell character may be an adaption of the land snails to highland habitat with a more humid condition in the area. Besides, species at the lower elevation habitat tend to has a smaller shell. From the finding above, we concluded that the upper shell sculpture and shell size cannot be used for the delimitation of *Geotrochus* and *Trochomorpha*. Hence, the current taxonomy of the two genera need further revision and the future attempt should consider more samples that cover the entire distribution of the two genera.

Acknowledgements

We would like to thank Cornelius Peter for his assistance in molecular work.

References

Albers, J. C. 1850. Die Heliceen, nach natürlicher Verwandtschaft systematisch geordnet. Berlin.

Anderson, R. P., Lew, D., & Peterson, A. T. 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. *Ecological modelling*, 162(3), 211-232.

Baur, B., & Raboud, C. 1988. Life history of the land snail Arianta arbustorum along an altitudinal gradient. *The Journal of Animal Ecology*, 71-87.

Blomberg, S. P., Garland Jr, T., & Ives, A. R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, 57(4), 717-745.

Bouchet, P., Rocroi, J. P., Hausdorf, B., Kaim, A., Kano, Y., Nützel, A., Parkhaev, P., Schrödl, M & Strong, E. E. 2017. Revised classification, nomenclator and typification of gastropod and monoplacophoran families. Malacologia, 61(1-2), 1-527.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature methods*, 9(8), 772-772.

Davison, A., Wade, C. M., Mordan, P. B., & Chiba, S. 2005. Sex and darts in slugs and snails (Mollusca: Gastropoda: Stylommatophora). *Journal of Zoology*, 267(4), 329-338.

Dowle, E. J., Morgan-Richards, M., Brescia, F., & Trewick, S. A. 2015. Correlation between shell phenotype and local environment suggests a role for natural selection in the evolution of Placostylus snails. *Molecular Ecology*, 24(16), 4205-4221.

Emberton, K. C. 1995. Sympatric convergence and environmental correlation between two land-snail species. *Evolution*, 49(3), 469-475.

Folmer, O., Black, M., Hoeh, W., Lutz, R.A., & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3,294–299.

Giokas, S., Páll-Gergely, B., & Mettouris, O. 2014. Nonrandom variation of morphological traits across environmental gradients in a land snail. *Evolutionary Ecology*, 28, 323–340.

Glass, N.H., & Darby, P.C. 2009. The effect of calcium and pH on Florida apple snail, Pomacea paludosa (Gastropoda: Ampullariidae), shell growth and crush weight. *Aquatic Ecology*, 43(4), 1085.

Goodfriend, G.A. 1986. Variation in land-snail shell form and size and its causes: a review. *Systematic Biology*, 35(2), 204-223.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Harmon, L.J., Jason, T.W., Chad, D.B., Richard, E.G., & Wendell, C. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics*, 24, 129-131.

Hillis, D.M., & Dixon, M.T. 1991. Ribosomal DNA: Molecular Evolution and Phylogenetic Inference. *The Quartely Review of Biology*, 66, 411-453.

Hirano, T., Kameda, Y., & Chiba, S. 2014. Phylogeny of the land snails Bradybaena and Phaeohelix (Pulmonata: Bradybaenidae) in Japan. *Journal of Molluscan studies*, 80(2), 177-183.

Holznagel, W.E., Colgan, D.J., & Lydeard, C. 2010. Pulmonate phylogeny based on 28S rRNA gene sequences: A framework for discussing habitat transitions and character transformation. *Molecular Phylogenetics and Evolution*, 57(3), 1017-1025.

Huelsenbeck, J.P., Ronquist, F.R., Nielsen, R., & Bollback, J.P. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, 294, 2310-2314.

Hyman, I. T., & Ponder, W. F. 2010. A morphological phylogenetic analysis and generic revision of Australian Helicarionidae (Gastropoda: Pulmonata: Stylommatophora), and an assessment of the relationships of the family. *Zootaxa*, 2462(1), 1-148.

Issel, A. (1874) *Molluschi Borneensi. Illustrazione delle specie terrestri e d'acqua dolce raccolte nell'isola di Borneo. Dai Signori G. Doria e O. Beccari.* Tipografia del R. Instituto Sordo-Muti, Genova.

Kessing, B., Croom, H., Martin, A., McIntosh, C., McMillan, W.O., & Palumbi, S. 1989. *The simple fool's guide to PCR*. Honolulu: Department of Zoology, University of Hawaii, 1-23.

Köhler, F., & Criscione, F. 2015. A molecular phylogeny of camaenid land snails from north-western Australia unravels widespread homoplasy in morphological characters (Gastropoda, Helicoidea). *Molecular Phylogenetics and Evolution*, 83, 44-55.

Kruskal, W.H., & Wallis, W.A. 1952. Use of ranks in one-criterion variance analysis. *Journal of the American statistical Association*, 47(260), 583-621.

Liew, T. S., Schilthuizen, M., & Vermeulen, J. J. 2009. Systematic revision of the genus Everettia Godwin-Austen, 1891 (Mollusca: Gastropoda: Dyakiidae) in Sabah, northern Borneo. *Zoological Journal of the Linnean Society*, 157(3), 515-550.

Maeda, H., Yamagishi, R., Ishida, E. H., & Kasuga, T. (2019). Wettability and dynamics of water droplet on a snail shell. *Journal of colloid and interface science*, 547, 111-116.

von Martens, E. C. 1867. *Die preussische Expedition nach Ost-Asien: nach amtlichen Quellen. Zoologischer Theil/bearb. von Eduard v. Martens* (Vol. 2). Verlag der Königlichen geheimen ober-hofbuchdruckerei (R. v. Decker).

Miller, M.A., Pfeiffer, W., & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *In 2010 gateway computing environments workshop (GCE)*, 1-8.

Nosonovsky, M., & Bhushan, B. 2008. Roughness-induced superhydrophobicity: a way to design non-adhesive surfaces. *Journal of Physics: Condensed Matter*, 20(22), 225009.

Pagel M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology*, 48(3), 612-622.

Pfenninger, M., Hrabáková, M., Steinke, D., & Dèpraz, A. 2005. Why do snails have hairs? A Bayesian inference of character evolution. *BMC Evolutionary Biology*, 5(1), 59.

Pfenninger, M., & Magnin, F. 2001. Phenotypic evolution and hidden speciation in Candidula unifasciata ssp. (Helicellinae, Gastropoda) inferred by 16S variation and quantitative shell traits. *Molecular Ecology*, 10, 2541–2554.

Pfenninger, M., Bahl, A., & Streit, B. 1996. Isolation by distance in a population of a small land snail Trochoidea geyeri: evidence from direct and indirect methods. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 263(1374), 1211-1217.

Phung, C.C., Heng, P.S., & Liew, T.S. 2017. Land snails of Leptopoma Pfeiffer, 1847 in Sabah, Northern Borneo (Caenogastropoda: Cyclophoridae): an analysis of molecular phylogeny and geographical variations in shell form. *PeerJ*, 5, p. e3981. https://doi.org/10.7717/peerj.3981

Pilsbry, H. A., 1935, The status of *Geotrochus* van Hasselt. *The Nautilus*, 49(2), 67.

Proćków, M., Proćków, J., Błażej, P., & Mackiewicz, P. 2018. The influence of habitat preferences on shell morphology in ecophenotypes of Trochulus hispidus complex. *Science of the Total Environment*, 630, 1036-1043.

Proćków, M., Kuźnik-Kowalska, E., & Mackiewicz, P. 2017. The Influence of climate on shell variation in *Trochulus striolatus* (C. Pfeiffer, 1828) (Gastropoda: Hygromiidae) and its implications for subspecies taxonomy. In *Annales zoologici* (Vol. 67, No. 2, pp. 199-221). Museum and Institute of Zoology, Polish Academy of Sciences.

QGIS Development Team, 2019. QGIS Geographic Information System. Open Source Geospatial Foundation. URL http://qgis.org.

R Core Team. 2015. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at http://www.R-project.org/.

Revell, L.J. 2012. Phytools: an R package for phylogenetic comparative biology (and other things), *Methods in Ecology and Evolution*, 3, 217-223.

Schileyko, A.A. 2002a. Treatise on Recent Terrestrial Pulmonate Molluscs, Part 8: Punctidae, Helicodiscidae, Discidae, Cystopeltidae, Euconulidae, Trochomorphidae. *Ruthenica*, Supplement 2, 1035-1163.

Schileyko, A.A. 2002b. Treatise on Recent Terrestrial Pulmonate Molluscs, Part 9: Helicarionidae, Gymnarionidae, Rhysotinidae, Ariophantidae. *Ruthenica*, Supplement 2, 1167-1302.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52(3-4), 591-611.

Shvydka, Z., Kovalev, A., & Gorb, S. N. 2019. The frictional and adhesive properties of the shells of terrestrial hairy snails. *Journal of Zoology*.

Solem, A. 1964. A collection of non-marine mollusks from Sabah. *Sabah Society Journal*, 11, 1–40.

Spearman, C. 1904. The proof and measurement of association between two things. *American journal of Psychology*, 15(1), 72-101.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30(9), 1312-1313.

Stöver, B.C., & Müller, K.F. 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *BMC bioinformatics*, 11(1), 7.

Tillier, S., & Bouchet, P. 1988. Land snails from the upper montane zone of Mt. Kinabalu (Sabah,Borneo), with descriptions of new species and genera. *Indo-Malayan Zoology*, 5, 255–293.

Van Hasselt, J. C. 1824. Extrait d'une letter de sur les mollusques de l'île de Java. *Bulletin Sciences Naturelles, Paris*, 3, 81-87.

Vermeulen, J. J., Liew, T. S., & Schilthuizen, M. 2015. Additions to the knowledge of the land snails of Sabah (Malaysia, Borneo), including 48 new species. *ZooKeys*, 531, 1-139.

Table 1(on next page)

Number of specimens of *Geotrochus* and *Trochomorpha* species included in shell morphological analysis and phylogenetic analysis.

Species	Genetic	Quantitative	Qualitative
Geotrochus kinabaluensis (E.A. Smith, 1895)	2	4	4
Geotrochus kitteli Vermeulen, Liew & Schilthuizen, 2015	1	2	4
Geotrochus labuanensis (Pfeiffer, 1863)	NA	16	16
Geotrochus meristotrochus Vermeulen, Liew & Schilthuizen, 2015	5	27	27
Geotrochus oedobasis Vermeulen, Liew & Schilthuizen, 2015	3	6	6
Geotrochus paraguensis (E.A. Smith, 1893)	8	10	10
Geotrochus subscalaris Vermeulen, Liew & Schilthuizen, 2015	NA	10	10
Geotrochus whiteheadi (E.A. Smith, 1895)	1	1	1
Trochomorpha haptoderma Vermeulen, Liew & Schilthuizen, 2015	8	7	43
Trochomorpha rhysa Tillier & Bouchet, 1988	6	5	26
Trochomorpha thelecoryphe Vermeulen, Liew & Schilthuizen, 2015	1	0	8
	35	88	155

Table 2(on next page)

Primer and annealing temperature for each gene

Gene	Primer	Sequences (5’-3’)	References	Annealing temperature
COI	L1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994	54°C
	H2198	TAAACTTCAGGGTGACCAAAAAATCA		
16S	16Sar	CGCCTGTTTATCAAAAACAT	Kessing et al. 1989	47°C
	16Sbr	CCGGTCTGAACTCAGATCACGT		
ITS-1	5.8c	GTGCGTTCGAAATGTCGATGTTCAA	Hillis and Dixon, 1991	55°C
	18d	CACACCGCCCGTCGCTACTACCGATTG		

Table 3(on next page)

Detail of the specimens used in phylogenetic analysis and the Genbank accession number of the successfully sequenced genes.

BORNENSIS	Taxon	Location	Sequence		
			COI	16S	ITS-1
6347	*Trochomorpha rhysa*	Mt Kinabalu at 3024m	MK779474	MK334188	MK335437
6350	*Trochomorpha rhysa*	Mt Kinabalu at 3088m	MK779475	MK334190	MK335439
6353	*Trochomorpha rhysa*	Mt Kinabalu at 2944m	MK779477	MK334191	N/A
6354	*Trochomorpha rhysa*	Mt Kinabalu at 2944m	MK779479	N/A	MK335440
6407	*Trochomorpha rhysa*	Mt Kinabalu at 3221m	MK779478	MK334195	MK335444
6411	*Trochomorpha rhysa*	Mt Kinabalu at 3119m	MK779476	MK334196	MK335446
6312	*Trochomorpha haptoderma*	Mt Kinabalu at 2775m	N/A	MK334185	MK335433
6349	*Trochomorpha haptoderma*	Mt Kinabalu at 2896m	MK779473	MK334189	MK335438
6356	*Trochomorpha haptoderma*	Mt Kinabalu at 2800m	MK779472	MK334192	MK335441
6408	*Trochomorpha haptoderma*	Mt Kinabalu at 2484m	MK779471	N/A	N/A
6409	*Trochomorpha haptoderma*	Mt Kinabalu at 2526m	MK779470	N/A	MK335445
6412	*Trochomorpha haptoderma*	Mt Kinabalu at 2500m	MK779469	MK334197	MK335447
6413	*Trochomorpha haptoderma*	Mt Kinabalu at 2404m	MK779468	N/A	MK335448
6417	*Trochomorpha haptoderma*	Mt Kinabalu at 2896m	MK779467	N/A	MK335449
6335	*Trochomorpha thelecoryphe*	Mt Kinabalu at 2700m	MK779480	N/A	MK335434
6342	*Geotrochus oedobasis*	Mt Kinabalu (Mesilau)	MK779461	MK334186	MK335435
6404	*Geotrochus oedobasis*	Mt Kinabalu at 2200m	MK811549	MK334193	MK335442
6343	*Geotrochus oedobasis*	Tambuyukon at 2080m	MK811548	N/A	N/A
6344	*Geotrochus whiteheadi*	Tambuyukon	MK811544	MK334187	MK335436

6406	*Geotrochus kitteli*	Mt Kinabalu at 2300m	MK779460	MK334194	MK335443
12670	*Geotrochus kinabaluensis*	Mahua	MK811543	N/A	MK335450
13017	*Geotrochus kinabaluensis*	Mahua	MK811542	N/A	N/A
13016	*Geotrochus meristotrochus*	Inikea	MK811545	MK334198	MK335451
13323	*Geotrochus meristotrochus*	Imbak Canyon	MK811547	MK334204	MK335459
13325	*Geotrochus meristotrochus*	Imbak Canyon	MK811546	MK334205	MK335460
13373	*Geotrochus meristotrochus*	Maliau	N/A	N/A	MK335461
13376	*Geotrochus meristotrochus*	Maliau	N/A	N/A	MK335462
13061	*Geotrochus paraguensis*	Banggi Island	MK811550	MK334198	MK335452
13176	*Geotrochus paraguensis*	Banggi Island	MK811552	MK334200	MK335454
13177	*Geotrochus paraguensis*	Banggi Island	MK811551	MK334201	MK335455
13223	*Geotrochus paraguensis*	Banggi Island	MK779464	MK334202	MK335456
13224	*Geotrochus paraguensis*	Banggi Island	MK779465	N/A	MK335457
13225	*Geotrochus paraguensis*	Banggi Island	MK779463	MK334203	MK335458
13068	*Geotrochus paraguensis*	Balambangan Island	MK779462	N/A	MK335453
13084	*Geotrochus paraguensis*	Balambangan Island	MK779466	N/A	N/A

3

Table 4 (on next page)

Length of alignment and the best fit model for each gene.

Gene	**Length of alignment**	**Model of sequence evolution**
CO1 1^{st} codon	218	TVM+G
COI 2^{nd} codon	217	TIM2
COI 3^{rd} codon	217	TrN+I
16S	461	TVM+I+G
ITS-1	805	TrNef+G

Table 5(on next page)

Result of the phylogenetic signal test using Pagel's λ method and Blomberg's *K* method.

Shell traits	Lambda (λ)	p-value	K	p-value
Upper surface sculpture	0.881	0.218	0.991	0.043
Maximum shell height	0.484	0.651	0.751	0.185
Maximum shell width	0.847	0.343	0.851	0.121
Maximum aperture height	0.00	1	0.550	0.437
Maximum aperture width	0.701	0.575	0.753	0.200

Figure 1

The variation of shell forms of 11 *Geotrochus* species and 4 *Trochomorpha* species in Sabah.

(A) *Geotrochus conicoides* (BOL/MOL 2431). (B) *G. paraguensis* (BOL/MOL 13061). (C) *G. kinabaluensis* (BOL/MOL 13020). (D) *G. labuanensis* (BOL/MOL 904). (E) *G. oedobasis* (BOL/MOL 908). (F) *G. subscalaris* (BOL/MOL 2430). (G) *G. meristotrochus* (BOL/MOL 13833). (H) *G. whiteheadi* (BOL/MOL 4110). (I) *G. kitteli* (BOL/MOL 4109). (J) *G. spilokeiria* (image from Vermeulen *et al*., 2015). (K) *G. scolops* (image from Vermeulen *et al*., 2015). (L) *Trochomorha trachus* (BOL/MOL 2959). (M) *T. haptoderma* (BOL/MOL 6312). (N) T. *rhysa* (BOL/MOL 3986). (O) *T. thelecoryphe* (BOL/MOL 6334).

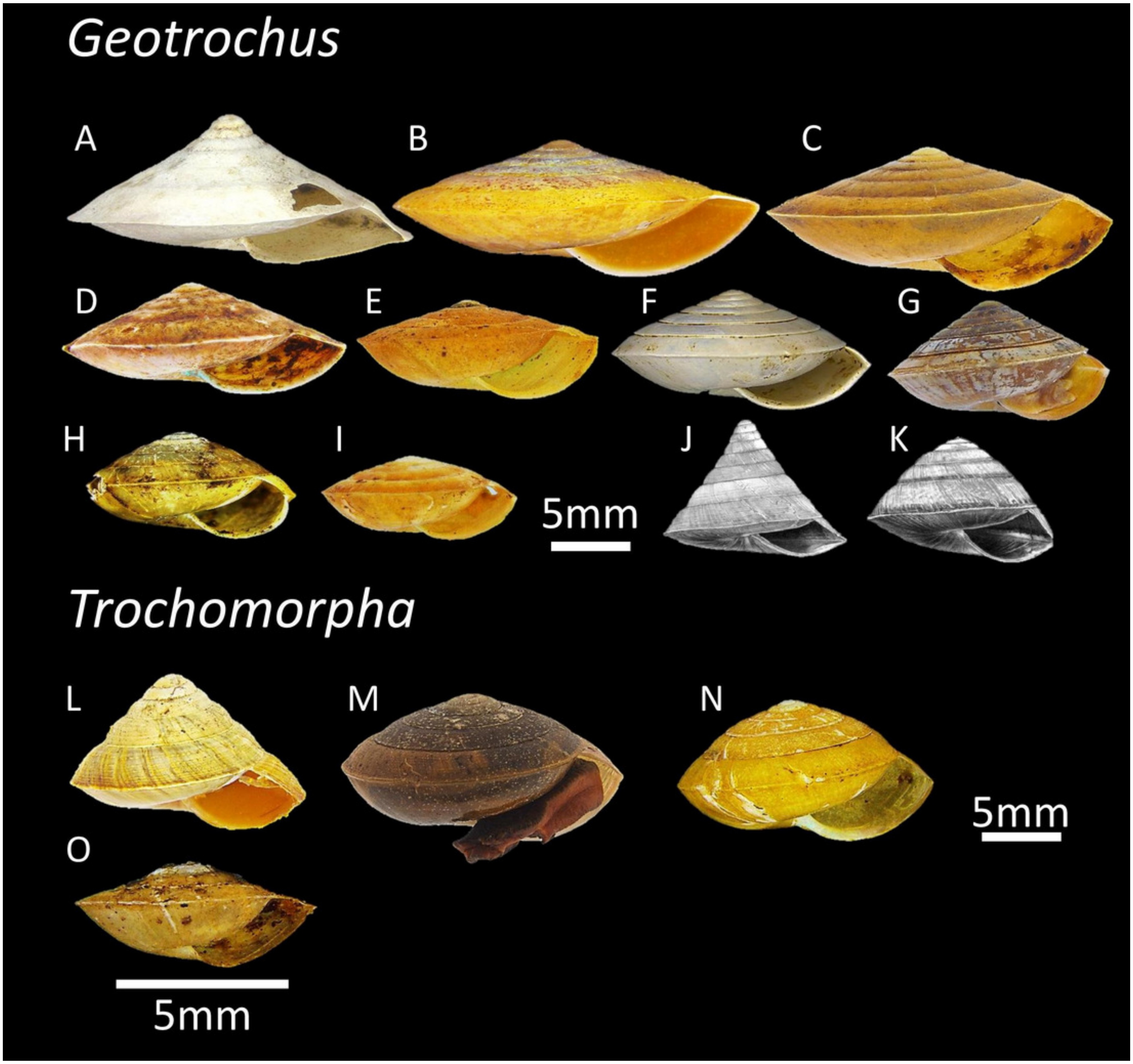
Geotrochus
A
B
C
D
E
F
G
H
I
5mm
J
K
Trochomorpha
L
M
N
5mm
O
5mm

Figure 2

Upper surface sculptures and quantitative shell traits included in this study.

(A) Shell upper surface sculpture pattern. S1: Sculpture with spiral threads form nodes over radial sculpture(BOL/MOL 6312); S2: Sculpture with raised and distinct radial growth lines and thin spiral threads (BOL/ MOL 6406); S3: Sculpture with indistinct radial growth lines and inconspicuous riblets and thin or very thin spiral threads (BOL/ MOL 13061) and S4: Sculpture with inconspicuous growth lines and low and thin spiral threads (BOL/ MOL 890). (B) Four quantitative shell measurements: SH, Shell height; SW, Shell width; AH, Aperture height, AW, Aperture width.

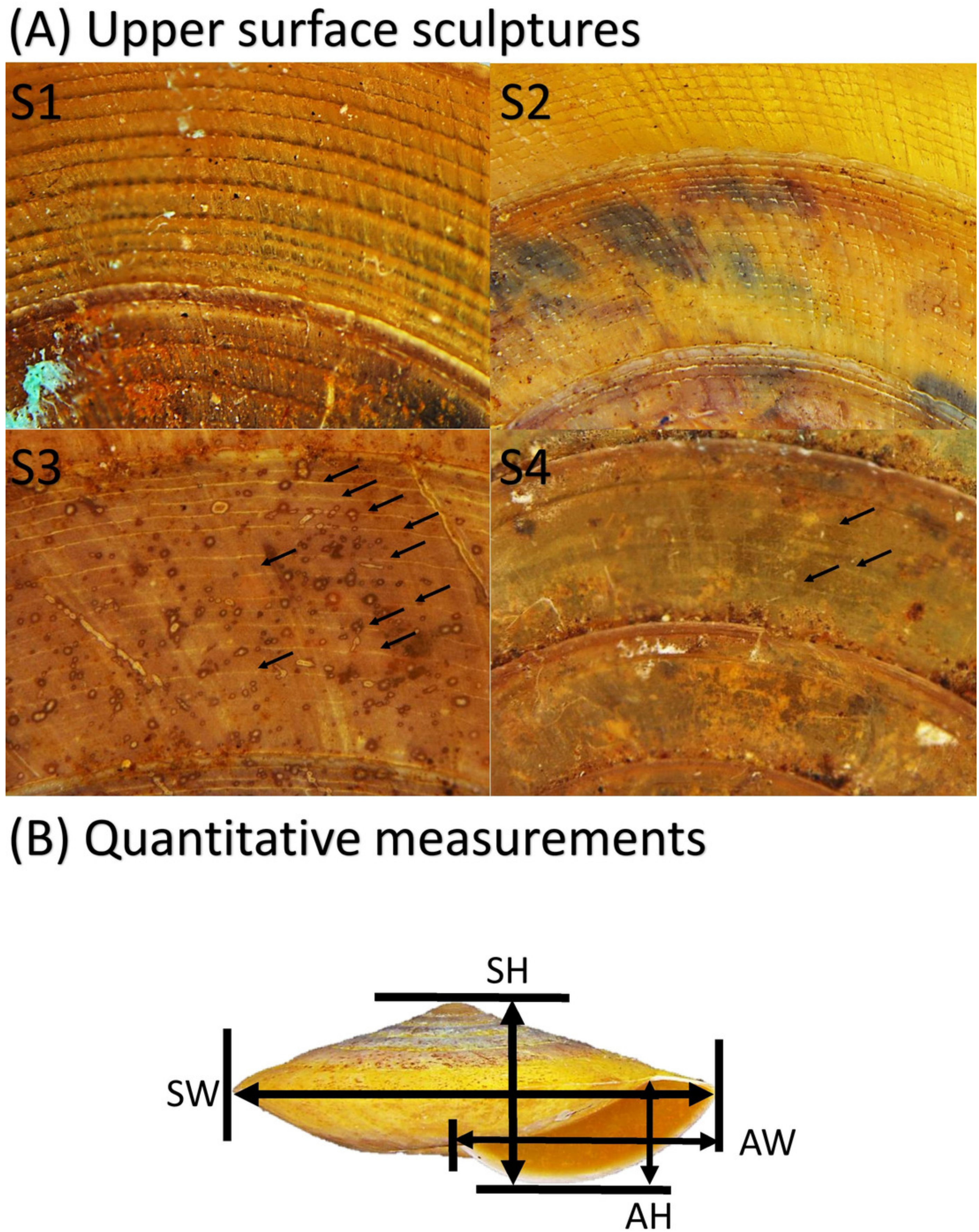
(A) Upper surface sculptures
S1
S2
S3
S4
(B) Quantitative measurements
SH
SW
AW
AH

Figure 3

Concatenated MI and BI tree rooted to *Everettia klemmantanica* based on the combined analysis of COI, 16S and ITS-1 datasets.

Posterior probability (above the branch) from Bayesian inference and bootstrap support values (below the branch) from maximum likelihood analysis are indicated at the nodes with support values less than 0.7 of PP and 70% of BS were not shown in the figure. The number annotated in front of the species name was the BORNEENSIS collection number.

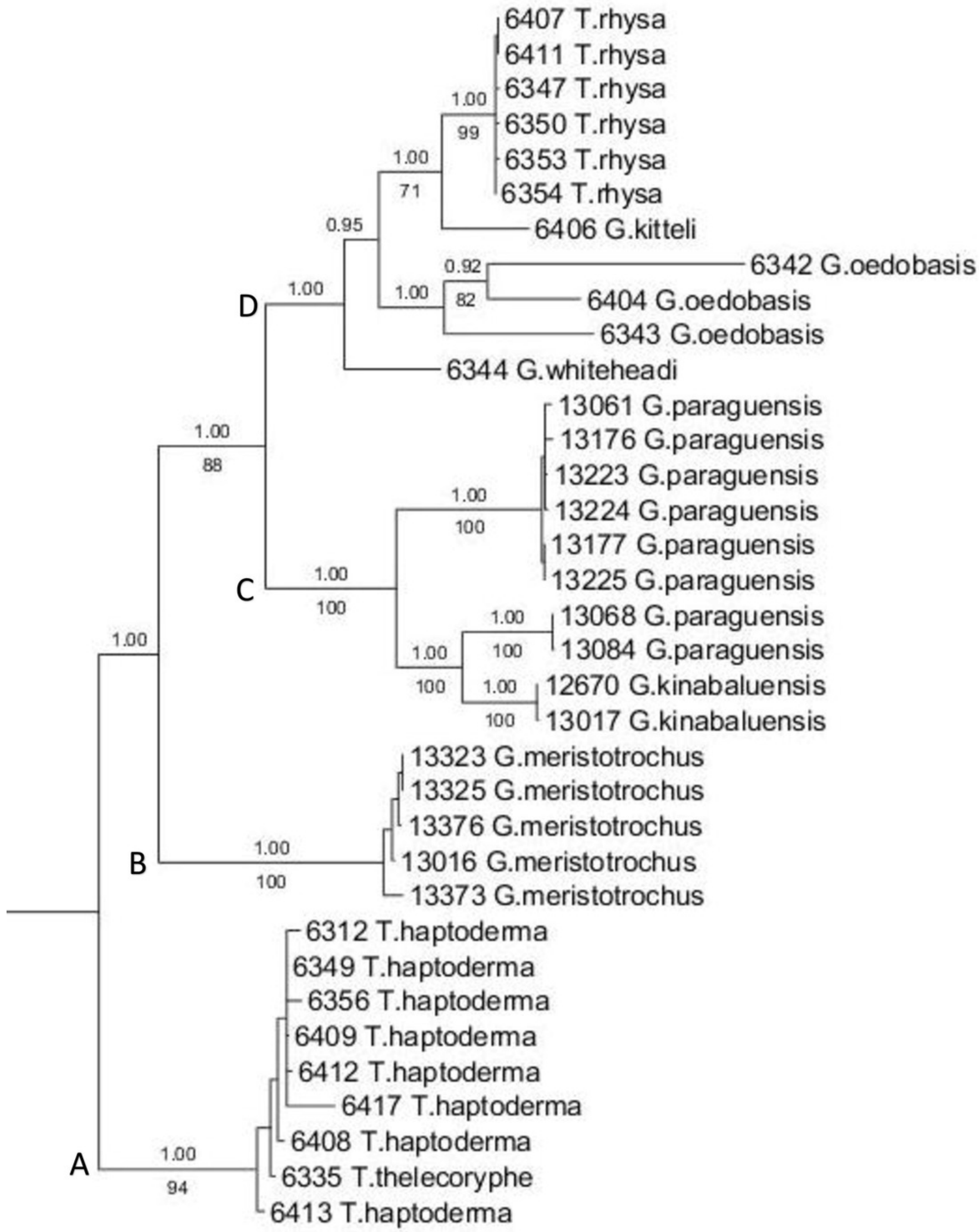
6407 T.rhysa
6411 T.rhysa
6347 T.rhysa
6350 T.rhysa
6353 T.rhysa
6354 T.rhysa
6406 G.kitteli
6342 G.oedobasis
6404 G.oedobasis
6343 G.oedobasis
6344 G.whiteheadi
13061 G.paraguensis
13176 G.paraguensis
13223 G.paraguensis
13224 G.paraguensis
13177 G.paraguensis
13225 G.paraguensis
13068 G.paraguensis
13084 G.paraguensis
12670 G.kinabaluensis
13017 G.kinabaluensis
13323 G.meristotrochus
13325 G.meristotrochus
13376 G.meristotrochus
13016 G.meristotrochus
13373 G.meristotrochus
6312 T.haptoderma
6349 T.haptoderma
6356 T.haptoderma
6409 T.haptoderma
6412 T.haptoderma
6417 T.haptoderma
6408 T.haptoderma
6335 T.thelecoryphe
6413 T.haptoderma
1.00
99
1.00
71
0.95
0.92
82
1.00
1.00
D
1.00
88
1.00
100
1.00
100
C
1.00
100
1.00
100
1.00
100
1.00
1.00
100
B
1.00
94
A

Figure 4

Association between shell upper surface sculpture pattern and environmental variables.

(A): Elevation. (B): Precipitation.

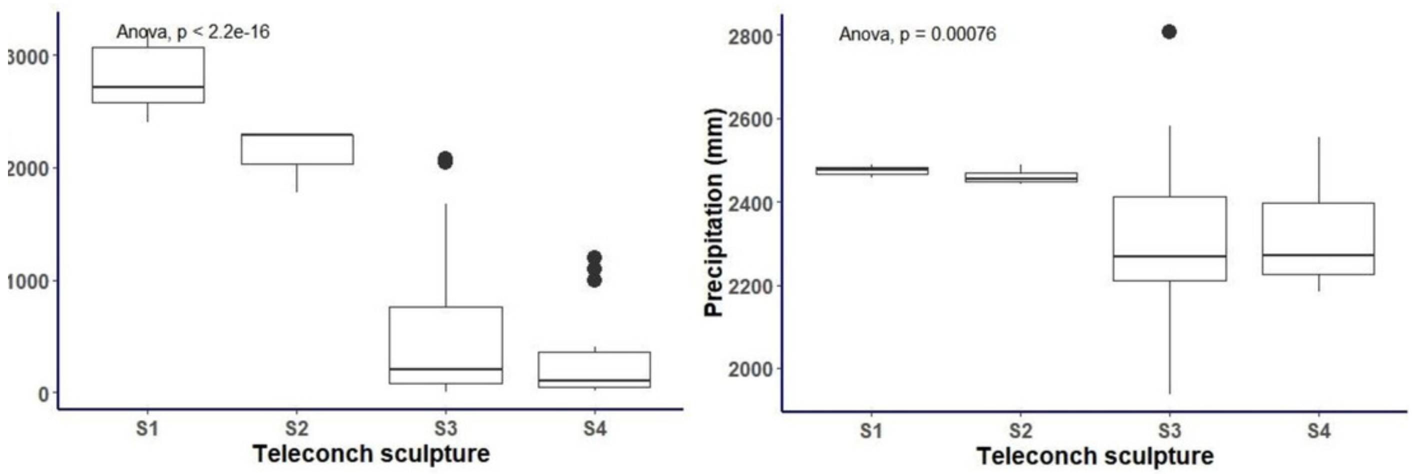

Figure 5

Correlation relationship between quantitative shell traits (A): SH; (B): SW; (C): AH and elevation. P-value with a symbol (*) indicated a significant correlation between the shell traits and elevation.

P-value with a symbol (*) indicated a significant correlation between the shell traits and elevation.

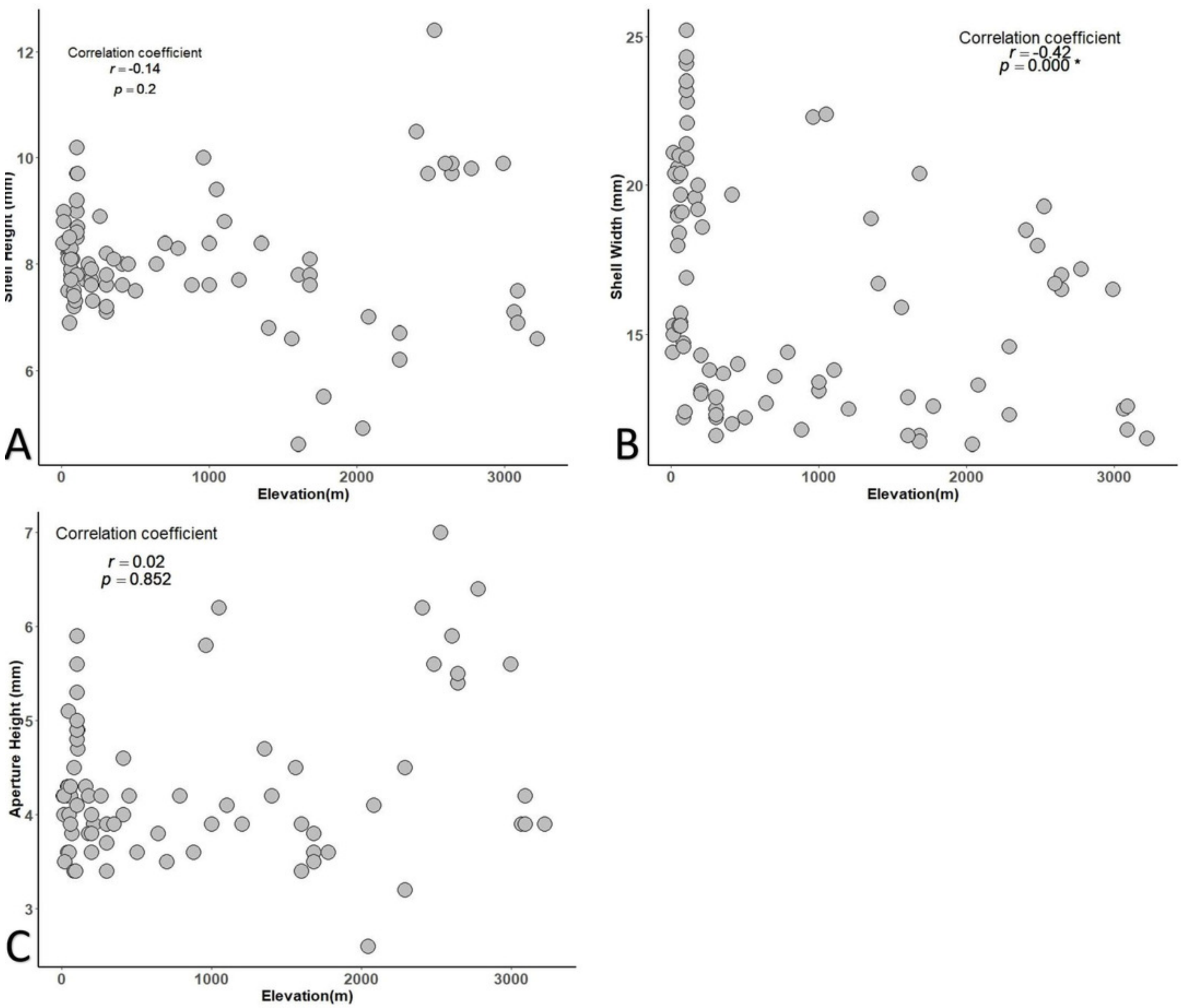

Figure 6

Correlation relationship between quantitative shell traits (A): SH; (B): SW; (C): AH and precipitation.

P-value with a symbol (*) indicated a significant correlation between the shell traits and precipitation.

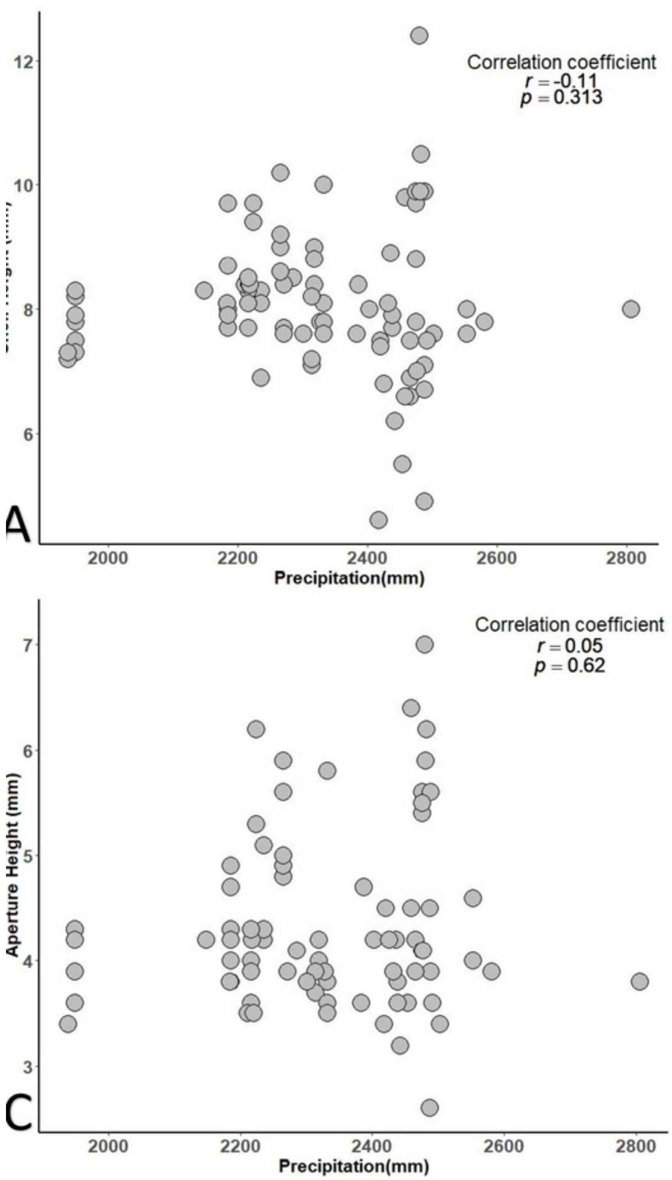

Figure 7

Figure 6 Visualization of the shell upper surface sculpture pattern (left) and the four quantitative shell traits (right) on the phylogenetic tree.