Morphological and Ecological Plasticity of Land Snails in the Philippines

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氏名（本籍地）	Emmanuel Ryan C. de Chavez
学位の種類	博士（生命科学）
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博士論文審査委員	（主査）　教　授　千葉　聡 教　授　牧　雅之 助　教　牧野　渡

論文内容の要旨

CHAPTER 1. INTRODUCTION

Variation within lineages based various selective pressures reveal fundamental processes that generate and maintain biodiversity (McArthur and Wilson, 1963; 1967). Observed patterns may lead to insights regarding the nature of speciation, the temporal and spatial occurrence of barriers to gene flow and the appropriate partitioning of diversity into taxonomic units (Esseltyn and Brown, 2009). Island species diversity, a product of accumulation through time by inter-island and intra-island speciation brought about selective different selective forces such as habitat change, and predation and parasitism pressure. In the Philippines, an archipelago formed by unique geological and climatic events, however, no study has yet examined the adaptive strategy for invertebrates, particularly land snails. Thus, this research was designed to examine the ecomorphological plasticity of land snails against anthropogenic disturbance and predation pressure.

CHAPTER 2. LAND SNAIL COMMUNITY STRUCTURE ACROSS THE FOREST DISTURBANCE GRADIENT IN MOUNT MAKILING, PHILIPPINES

The Philippines supports many endemic land snails which are threatened by habitat destruction and exotic species invasion. In order address this, I determined how anthropogenic-induced disturbance affect the community structure of native and invasive land snails. Quadrat sampling was conducted for four years (2007, 2010-2012) across a forest disturbance gradient (old-growth, secondary, plantation forests and former slash and burn sites) on Mount Makiling in Luzon Island. Biotic (number of trees, diameter of trees, canopy cover and forest litter) and abiotic (elevation and soil pH) environmental variables that could influence snail population were also measured. Disturbance score was computed for each species while generalized linear mixed-effect modelling (GLMM) was performed to select the best predictor of species richness and abundance. Native pulmonates and prosobranchs have lower disturbance scores compared to the invasive snails. In old-growth forests, only native pulmonates and prosobranchs were present. Diversity was highest in secondary forests having species representing various ecological successional stages. In plantation and former slash and burn sites, tolerant native pulmonates and invasive species thrive while native prosobranchs were absent. GLMM revealed that forest disturbance was the main

factor in predicting land snail species richness while abundance was influenced by multiple variables.

The community structure of land snails in Mt. Makiling is primarily affected by historic forest disturbance. The stochastic response in species richness in relation to variables, and the large proportion of rare species with few common species reflected the complex system occurring in a tropical forest ecosystem. However, most of the ecological interpretations presented here were biased towards macro land snails and the need to include micro-snails is very necessary for a more conclusive study.

CHAPTER 3. A NEW *HELICOSTYLA* SPECIES (BRADYBAENIDAE:HELICOSTYLINAE) FROM PATNANUNGAN ISLAND, PHILIPPINES

A new species of land snail belonging to Subfamily Helicostylinae, Family Bradybaenidae is described from a native dipterocarp forest in Patnanungan Island, Polillo Group of Islands (PGI), the Philippines. *Helicostyla amagaensis* new species differs from other congeners by its ovate-globose, cream white color shell with single chestnut brown band. Aperture is ovate and moderately oblique with reddish-brown streak around the aperture outer lip. Penis long with inner oblique striations terminating towards a leaf-like stimulator. Vas deferens is short and flagellum is absent. This new species is consistent with the diagnostic features for the genus *Helicostyla*. Conchologically, *H. amagaensis* is most similar to the shell shape and banding pattern of *H. hydrophana* from Mindoro Island. However, the former is twice smaller in shell size and has broader band extending faintly around the outer peristomial lip outline. The later has straw-yellow shell color covered with hydrophanous cuticle forming thin film of oblique shreds. The collumelar band along the umbilical region is prominent in *H. hydrophana* compared to *H. amagaensis*.

DNA barcoding using *cytochrome c oxidase* I subunit (COI) revealed *H. amagaensis* has closest sequence affinity with *H. woodiana.* Neighbor joining and maximum parsimony trees showed 100% bootstrap support for the inclusion of this new species as a member of the genus *Helicostyla*. The COI sequence showed no clear relationship between *H. amagaensis* with other *Helicostyla* from the PGI and other islands based

on geographic proximity. In comparing with *H. amagaensis,* the *Helicostyla* from the adjacent Luzon have greater mean p-distances than those species found in the farther Cebu. The constructed NJ tree rooted on *Eulota mighelsiana* and *Bradybaena similaris* confirmed the phylogenetic position of the new land snail species marked by its clustering within the *Helicostyla* taxa with 99% NJ and 100% MP bootstrap supports. *H. woodiana* did not form a monophyletic group, with two individuals clustering with *H. amagaensis* with 100% NJ and MP bootstraps. Both these taxa differed from *H. daphnis* from Cebu with 99% NJ and 100% MP bootstrap supports. Although clearly morphologically different from one another, there was an inclusion of one group of *H. woodiana* with *H. amagaensis* in NJ and MP trees. This could be attributed to two possible reasons. First, the genetically distantly related *H. woodiana* populations might be derived independently, and one of these was derived from the common ancestor with *H. amagaensis*. Secondly, hybridization could have occurred between *H. amagaensis* and one population of *H. woodiana*, and mtDNA haplotype of this *H. woodiana* was replaced by that of *H. amagaensis*. Surveys using nuclear DNA are needed in future study to solve this issue; however, the above interpretations do not refute the conclusion that *H. amagaensis* is a new and distinct species.

CHAPTER 4. VARANID LIZARD PREDATION DRIVES HABITAT-USE AND SHELL SHAPE SHIFTS IN THE TROPICAL ISLAND LAND SNAIL, *HELICOSTYLA PORTEI* (BRADYBAENIDAE: HELICOSTYLINAE)

Evolution of anti-predator adaptation, which can lead to speciation, varies from morphological to behavioral modifications. I demonstrated how the varanid lizard, *Varanus olivaceus,* affected the ecomorphology of the island endemic land snail, *Helicostyla portei*. Land snails were surveyed from Polillo (with predator lizard), and Patnanungan (without predator lizard) Islands in the Philippines. Shells were analyzed using geometric morphometrics based on 14 landmarks, while general shell size, shell and aperture indices were determined. Habitat-use was discriminated by the sitting position (on tree trunk, leaves or vines) of the snail. The relationship among shell morphology, habitat-use and other environmental variables (island, altitude, tree density, canopy cover, leaf litter depth) were tested using generalized linear mixed model. Land snails on the island where predator is present have larger shell size and wider body whorl with narrower aperture. More snails were found on leaves, followed

by vines and rarely on tree trunk. Land snails on the island without the lizard have more extensive size range and narrower body whorl but wider aperture. Habitat-use among snails shifted in the absence of the predator, wherein tree trunk became the most preferred, followed by leaves and vines. This suggested that predation induced habitat-use variation resulting in change in shell shape. Allometric shell growth was also observed between juvenile and adult shells. The significant relationship between habitat utilization and shell form indicated that *H. portei* exposed to predator selection favored characters more adapted to sites inaccessible to the lizard, whereas in the absence of the predator, shells showed relaxed phenotype more adapted to tree trunk. The lack of relationship between habitat-use with other environmental variables further supported the hypothesis.

To test the observed predator-induced differences among land snails from the two islands constitute genetic divergence and possible signal for speciation, a total of 28 individuals of *H. portei* as well as two individuals of *H. daphnis* and five individuals of *H. rufogaster* as outgroup taxa were used for the molecular analyses. Three gene fragments (ITS2 16S rRNA and COI genes) were then amplified using polymerase chain reaction. Phylogenetic trees were constructed using the ML and NJ methods. ML and NJ trees revealed a distinct split among *H. portei* samples from different location. *H. portei* from the southern region of Polillo, Palasan and Patnanungan Islands formed a separate clade with those *H. portei* collected in the northern Polillo Island (bootstrap support of ML-both 98%; NJ- 93 and 95%, respectively). Median-joining network also revealed similar pattern wherein the *H. portei* from the northern Polillo Island were separated by around 14 mutation distance from the southern Polillo, Palasan and Patananungan group. Haplotype frequency was 21.43% among the northern group, and 78.57% in the southern Polillo, Palasan and Patanungan group. The geological history of the PGI could have contributed to the separation. Southern section of Polillo originated by the sliding-off the Luzon carrying very ancient substrate components ranging from Early Cretaceous to Late Miocene, while Palasan and Patnanungan arose through ocean floor uplift during the Miocene. However, the northern Polillo is a recent landmass which uplifted during the Pleistocene. It could be inferred that since the southern Polillo, Palasan and Patnanungan are older, there will be longer time for the establishment of native rainforests capable of supporting *H. portei* population, as well as its haplotype diversification. During the last Pleistocene period, the entire PGI were connected to

Luzon, forming the Pleistocene Aggregate Island Complex. It is hypothesized that it was during this moment that *V. olivaceus* invaded the PGI with an already genetically diversified *H. portei* population. Thus the anti-predator adaptations were recent (around 10,000 YA), showing remarkable plasticity of the endemic land snails.

CHAPTER 5. ANTI-PREDATORY ROLE OF SHELL COLOR AND BANDING PATTERN DIVERGENCE IN THE ISLAND ENDEMIC LAND SNAIL, *HELICOSTYLA PORTEI* (BRADYBAENIDAE: HELICOSTYLINAE)

Selection pressure by predators and microhabitat differentiation are known to generate character divergence among island species. In this study, I examined how the arboreal malacophagus varanid lizard, *Varanus olivaceus,* affected the shell coloration and banding patterns in the island endemic land snail, *Helicostyla portei*. Snails were surveyed from Polillo (with predator lizard), and Patnanungan (without predator lizard) Islands in the Philippines. Shell pigments and intensity were quantified and expressed as decimal pixel values, while the number and frequency of whorl bands were determined. The relationship between shell color and banding pattern, island, microhabitat-use (leaves, vines and tree trunk) and island were tested using generalized linear mixed-effect model. Polillo land snails exhibited shell vegetative cryptic coloration, brighter shells with more bands adapted to leaf and vine microhabitat. Patnanungan land snails have duller and darker shells with less banding blending to their tree trunk habitat. Habitat-use among snails shifted in the absence of the lizard predator. This suggested that predation induced habitat-use variation resulting in change in shell coloration.

Feeding strategy and dentition development in the arboreal lizard, *Varanus olivaceus* were also examined. Feeding experiments were conducted among zoo-kept lizards while preserved heads were analyzed using x-ray and computed tomography. Feeding experiments showed higher predation success among land snails from the island without the lizard. Significant blunting of the teeth associated with malacophagous diet was also observed among adult lizards as compared to the juveniles. This study revealed a possible model of co-evolution of morphological and behavioral traits between the varanid lizard and the land snail in a tropical island ecosystem.

論文審査結果の要旨

本論文はフィリピンにおける陸産貝類をモデル系として、熱帯地域における種多様性の形成、維持機構について、特に人為的な環境攪乱と捕食―被食の相互作用の面から明らかにしたものである。

本論文では、まず熱帯雨林が広域に残存するフィリピン・ルソン島のマキリン山系において、陸貝の種構成と森林の攪乱状況との関係について、野外調査を中心として研究を行った。その結果、攪乱のレベルと種多様性の間には、明瞭な負の相関が認められ、人為的な攪乱が陸貝の種多様性に大きく影響を与えていることを明らかにした。熱帯雨林において、陸貝の種多様性におよぼす人為的な攪乱の影響を考察した研究はこれが 2 例目であり、特に人為的な群集構造へのインパクトを明瞭に示した研究はこれが最初である。

次に本研究では、ポリロ諸島において、陸貝食のトカゲと陸貝の相互作用が生態や形態に及ぼす影響を、野外調査、形態解析、操作実験、および分子系統解析によって解析した。その結果、トカゲの有無が陸貝の生活様式変化させるとともに、形態や色彩の変化をもたらすことを示した。またトカゲの成長とともに果実食から陸貝食への食性の変化に伴って、トカゲの頭骨や歯の形状が変化することを示した。本研究は貝食性トカゲの存在が、陸貝の進化的変化をもたらすとともに、生活様式の変化を介して種分化をもたらしうることを示した最初の研究である。

本研究では、上記とあわせて未知な点の多いポリロ諸島の陸貝相の研究を進め、分子系統解析と形態解析により従来未知であった種を検出し、新種として記載した。これはフィリピンにおける陸貝で最初の DNA バーコーディングの適用例であり、今後のフィリピン地域における生物多様性の実態解明を推進する端緒となりうる成果である。

以上のような一連の成果から見て Emmanuel Ryan Chavez 氏は、自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，Emmanuel Ryan Chavez 提出の論文は，博士（生命科学）の博士論文として合格と認める。