การประเมินอนุกรมวิธานของหอยทากจิ๋วปากแตรของไทยโดยใช้การวิเคราะห์สายวิวัฒนาการของลักษณะในระดับโมเลกุลและสัณฐานวิทยาของเปลือก

นางสาวปิโยรส ทองเกิด

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TAXONOMIC EVALUATION OF THAI PUPILLID MICRO LAND SNAILS USING PHYLOGENETIC ANALYSIS OF MOLECULAR AND SHELL MORPHOLOGICAL CHARACTERS

Ms. Piyoros Tongkerd

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ปิโยรส ทองเกิด : การประเมินอนุกรมวิธานของหอยทากจิ๋วปากแตรของไทยโดยใช้การวิเคราะห์สายวิวัฒนาการของลักษณะในระดับโมเลกุลและสัณฐานวิทยาของเปลือก (TAXONOMIC EVALUATION OF THAI PUPILLID MICRO LAND SNAILS USING PHYLOGENETIC ANALYSIS OF MOLECULAR AND SHELL MORPHOLOGICAL CHARACTERS) อาจารย์ที่ปรึกษา : รศ. ดร. สมศักดิ์ ปัญหา อาจารย์ที่ปรึกษาร่วม : Prof. Dr. John B. Burch จำนวน 120 หน้า ISBN 974-17-4004-2

หอยทากวงศ์ Pupillidae ของภูมิภาคเอเชียตะวันออกเฉียงใต้มักถูกเรียกกันว่า “หอยทากจิ๋ว” จัดเป็นหอยที่มีความโดดเด่นและเป็นที่รู้จักกันในระบบนิเวศหินปูน โดยเฉพาะในบริเวณแผ่นดินใหญ่ของประเทศเวียดนาม กัมพูชา พม่า มาเลเซีย และประเทศไทย เป็นกลุ่มหอยที่มีคุณสมบัติทางชีววิทยาที่หลากหลายรวมทั้งลักษณะทางสัณฐานวิทยาของเปลือกที่มีความจำเพาะและมีความจำเพาะถิ่นสูง งานอนุกรมวิธานของหอยทากจิ๋ววงศ์นี้ได้เริ่มขึ้น และพัฒนาจากงานของ Pilsbry (1916-1918a, 1935), Steenberg (1925) และ Baker (1935) โดยใช้ลักษณะของเปลือก และบางลักษณะทางกายวิภาคศาสตร์เป็นลักษณะสำคัญในการจัดจำแนก อย่างไรก็ตามการศึกษาในระดับสกุลของหอยทากจิ๋ววงศ์นี้ยังไม่เคยมีการวิเคราะห์สายวิวัฒนาการ โดยใช้ลักษณะสัณฐานวิทยาของเปลือก หรือใช้ลักษณะในระดับโมเลกุลมาก่อน การศึกษาในครั้งนี้ได้สร้างความสัมพันธ์ทางวิวัฒนาการ ของหอยทากจิ๋ววงศ์ Pupillidae โดยเน้นที่วงศ์ย่อย Gastrocoptinae ซึ่งเป็นกลุ่มเด่นในเขตเอเชียตะวันออกเฉียงใต้ ผลการศึกษาการวัดวิเคราะห์สัณฐานวิทยาของเปลือก โดยวิธี PCA (Principle Component Analysis) ของหอยทากจิ๋ว 2 สกุล (*Gyliotrachela* และ *Hypselostoma*) เพื่อหาลักษณะที่เหมาะสมในการจำแนก พบว่า การวิเคราะห์ลักษณะความกว้างของเปลือก (SWA) ไม่สามารถนำมาใช้ในการจำแนกได้ และผลการวิเคราะห์สายวิวัฒนาการโดยใช้ลักษณะของเปลือกแต่ละสกุลไม่พบความสัมพันธ์แบบ Monophyletic ทั้งนี้การเลือกลักษณะที่จะนำมาใช้ศึกษาโดยใช้สัณฐานวิทยาของเปลือกแต่เพียงอย่างเดียวทำให้มีความเป็นไปได้สูงที่จะแสดงความสัมพันธ์ทางวิวัฒนาการแบบ parallel หรือ convergent

จากการตรวจสอบและวิเคราะห์สายวิวัฒนาการในระดับโมเลกุลโดยใช้ยีน 16S Mitochondrial และ 28S Nuclear ดีเอ็นเอ พบว่าอนุกรมวิธานของปากเปลือกหอย มีความสัมพันธ์ไปในทางเดียวกันกับความสัมพันธ์ที่วิเคราะห์ด้วยยีนในหอยสกุล *Gyliotrachela, Hypselostoma* และ *Anauchen* อย่างไรก็ตามยังพบว่ามีความสัมพันธ์แบบ Paraphyletic อยู่ ส่วนหอยทากจิ๋วชนิดที่มีการลดรูปของฟันเปลือกเช่น *Aulacospira smaesarnensis* มีความสัมพันธ์ใกล้ชิดกับหอยในสกุล *Gyliotrachela* ที่มีการพัฒนาฟันเปลือกสมบูรณ์ ในกรณีเดียวกับ *Hypselostoma panhai* และ *H. erawan* ที่มีลักษณะของเปลือกที่แตกต่างกันมาก จึงสามารถอธิบายได้ว่าในบางกรณีบางสายพันธุ์ในพื้นที่หนึ่งๆที่อาศัยอยู่ร่วมกัน แต่มีถิ่นอาศัยที่แตกต่าง อาจมีการเปลี่ยนแปลงเกิดขึ้นอย่างรวดเร็วในลักษณะสัณฐานวิทยาของเปลือก ทำให้มีลักษณะแตกต่างกันมากทั้งที่มีความสัมพันธ์ใกล้ชิดกันในระดับโมเลกุล ดังนั้นการศึกษาชนิดของหอยทากจิ๋วจากถิ่นอาศัยที่หลากหลาย จะทำให้การศึกษาความสัมพันธ์ทางวิวัฒนาการของหอยกลุ่มนี้มีความสมบูรณ์ยิ่งขึ้น และจากการศึกษาในระดับโมเลกุลยังพบอีกว่า กลุ่มหอยที่มีความจำเพาะถิ่นสูงและมีความใกล้ชิดทางภูมิศาสตร์ แสดงความสัมพันธ์ใกล้ชิดกันในเชิงของบรรพบุรุษ วิวัฒนาการ และนิเวศวิทยา ส่วนบางชนิดเช่น *Krobylos maehongsonensis* มีการกระจายกว้าง แต่ยังคงแสดงความสัมพันธ์ใกล้ชิดกันในระดับชนิด ผลการวิจัยนี้ ไม่เพียงแต่จะทำให้เกิดความเข้าใจเกี่ยวกับสายวิวัฒนาการของหอยทากจิ๋วปากแตรของไทยเท่านั้น ยังสามารถเป็นแนวทางในการปรับปรุงเพิ่มเติมการจัดจำแนกที่อิงการศึกษาทางวิวัฒนาการได้ดียิ่งขึ้นอีกด้วย

สาขาวิชา วิทยาศาสตร์ชีวภาพ ลายมือชื่อนิสิต……………………………………………....

ปีการศึกษา 2546 ลายมือชื่ออาจารย์ที่ปรึกษา…………………………………….

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……………………………...

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KEY WORDS : Micro Land Snails / Pupillidae / Phylogenetic Analysis / Taxonomy / Systematics

PIYOROS TONGKERD : TAXONOMIC EVALUATION OF THAI PUPILLID MICRO LAND SNAILS USING PHYLOGENETIC ANALYSIS OF MOLECULAR AND SHELL MORPHOLOGICAL CHARACTERS.
THESIS ADVISOR: ASSOCIATE PROFESSOR SOMSAK PANHA, Ph.D., THESIS CO-ADVISOR: PROFESSOR JOHN B. BURCH, Ph.D., 120 pp. ISBN 974-17-4004-2

The Southeast Asian Pupillidae, commonly known as " micro snails ", are prominent and ubiquitous members of limestone ecosystems particularly on the main land countries like Vietnam Cambodia, Laos, Myanmar, Malaysia and Thailand. They exhibit various peculiar biological features, including the unique shell morphology, the highly endemism. Their taxonomy was primarily developed by Pilsbry (1916-1918a, 1935), Steenberg (1925) and Baker (1935), based on shell and some anatomical characters. Although the generic level within this micro land snail family was lack of a modern phylogenetic perspective and the use of shell morphology and molecular characters for phylogenetic utility has not been established yet. This dissertation addresses this shortcoming by conducting phylogenetic studies on the Pupillidae, focusing mainly on the subfamily Gastrocoptinae, a major subgroup of the family in Southeast Asian region. Chapter 1, 2 review the current understanding of pupillid taxonomy, classification and their unique shell morphological characters. All of materials and methods are defied in Chapter 3. Using morphological characters, Chapter 4 studies on morphometric analysis and tests phylogenetic relationships among Thai pupillid genera and, as a result, *Gyliotrachela and Hypselostoma* do not forming the distinctive clusters by SWA character analysis and a monophyly of each genus is not supported. However, using the shell morphology alone may contain more homoplastic and increase the risk that the similarity of the character observed is not the result of the common ancestry but rather of parallel or convergent evolution. The current taxonomy of the Pupillidae is also revised based on combined phylogenetic analyses of two different gene sequence data, mitochondrial ribosomal 16S, and nuclear ribosomal 28S (Chapter 5). Although characterized by high levels of genetic differentiation and homoplasy, the molecular dataset provided a number of novel insights into gastrocoptine evolution and systematics. Nominal conspecifics of three genera with replicate samples (*Gyliotrachela*, *Hypselostoma* and *Anauchen*) occupied contiguous sections of treespace, however all three were paraphyletic. Two inferred examples of reductive loss in apertural lamellae were encountered: *Aulacospira smaesarnensis*, was firmly nested within an otherwise exclusively *Gyliotrachela* tip clade; the leaf-litter-dwelling *Hypselostoma panhai*, exhibited striking conchological differentiation from its geographically proximate rock-dwelling sister taxon *H. erawan*. The results caution against the unquestioned use of apertural dentition characteristics as diagnostic generic characters, imply that ecological transitions can lead to rapid morphological change, and suggest that a comprehensive sampling of both rock and leaf-litter lineages is required to fully flesh out phylogenetic relationships among regional pupillid microsnails. This dissertation also emphasize the utility of geographically proximate gastrocoptine taxa to establishing sister relationships for locally endemic species, irrespective of apparent morphological similarity. However, not all Thai gastrocoptines have localized ranges; *Krobylos maehongsonensis*, has apparently experienced geographically extensive patterns of gene flow and colonization. The results of these evolutionary studies not only increase our understanding of the phylogeny of the Pupillidae, but also provide the way to conduct the precise systematic classification, an important in biological basic study.

Field of Study Biological Science Student's signature ....

Academic year 2003 Advisor's signature ....

Co-advisor's signature ....

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Table of Contents

Page

List of Tables

List of Figures

CHAPTER 1

INTRODUCTION TO THE PUPILLIDAE

The Pupillidae, are a large family of micro land snails, that are found in both terrestrial and arboreal habitats (Solem, 1979). They are the primary family in the superfamily Pupilloidea (Infraorder Orthurethra). Which is 1 of the 2 stylommatophoran superfamilies to have attained a world wide distribution with endemic representatives on all continents, excluding Antarctica (Pilsbry, 1935; Solem, 1979). The taxonomy of the Pupillidae was primarily developed by Pilsbry (1916-1918, 1935), Steenberg (1925) and Baker (1935) (Table 1.1), based on shell and some anatomical characters. Pilsbry (1935) and Baker (1935) partitioned the Pupillidae, into 5 subfamilies (Pupillinae, Gastrocoptinae, Vertigininae, Nesopupinae and Orculinae), and suggested the relationships among subfamilies as shown in Figure 1.1.Other authors (Steenberg, 1925; Thiele, 1929-1935; Zilch, 1959; Vaught, 1989) have expressed a range of differing interpretations concerning familial and sub-familial assignations (Table 1.2), although the latter 3 authors were mainly compilers.

Pupillidae are a family of small to minute snails, generally less than 5 mm in shell length. The shell are mostly pupa-shaped or cylindriform to conical, but most of the Southeast Asian species are trumpet shaped. Many of the species possess apertural barriers (“denticles” or “teeth”) (Figure 1.2). It is widely accept that, a number of land snails have evolved intricate apertural barriers effectively blocks off the shell’s aperture to many predators. Taxonomists, such as Pilsbry, relied heavily on patterns of apertural teeth to determine taxonomic relationships. The nomenclature for these barriers is examined from Pilsbry (1916). The plicae (singular, plica), also called folds, are denticles located on the outer lip. Lamellae (singular, lamella) are denticles located on the columellar lip and parietal wall. The primary denticles are columellar lamella (C), parietal lamella (P), angular lamella (A), upper palatal plica (upl), and lower palatal plica (lPl). Accessory to these, and either present or wanting, depending upon the species are twin lamella (a^1), basal plicae (B), infraparietal lamella (i^1), suprapalatal plica (i^2), interpalatal plica (i^3), infrapalatal plica (i^4) supracolumellar lamella (c^1) and subcolumellar lamella (c^2) (Figure 1.2). These apertural teeth are important characters in taxonomy and in species recognition. Table 1.3 shows the shell characters that are used to distinguish the 5 pupillid microsnail genera studied. The apertural barriers, there five genera are as follows. *Krobylos* always lacks apertural barriers, as do some of the *Aulacospira* posses. Both species have depressed helicoid shells, and do not deelop tubas. *Hypselostoma, Gyliotrachela* and *Anauchen* have turrited shells, either elongated or depressed, and the terminal whorls after from tubas. *Anauchen* lacks the angular lamella, both *Hypselostoma* and *Gyliotrachela* have both angular and parietal lamellae. Both lamellae are fused into one irregular lamella in *Hypselostoma* but they are independent and parallel in *Gyliotrachela*.

Pupillid snails occur in two very distinct habitats: the forest leaf litter and, in Southeast Asia, the more commonly, on the rock surfaces of limestone karst hills (Benthem Jutting, 1950). Obligate limestone rock dwellers frequently have highly modified adult shell morphologies in which the last body whorl is an uncoiled trumpet-shaped extension (tuba) separating the shell aperture from the main body whorls (Pilsbry, 1916-1918). Karst hill habitats in southeastern Asia are typically discontinuous and many individual hills, or regional groupings, are isolated geographically from their nearest neighbours (Figures 1.3-1.5). It is not surprising, therefore, to find that pupillid taxa are often restricted to specific hills or hill clusters and that isolated hill ranges typically display highly differentiated faunas (Benthem Jutting, 1950). There are some exceptions to this generality, such as *Gyliotrachela hungerfordiana* von Möllendorff, which has an extensive geographic range, possibly due to a proposed lack of exclusivity to karst hill habitats (Schilthuizen Vermeulen, Davison and Gittenberger, 1999).

This dissertation concerned with Thai representatives of the Pupillidae, focusing mainly on the subfamily Gastrocoptinae, a major subgroup of the Southeast Asian region. The taxonomy of Southeast Asian pupillids have been developed by a variety of workers (Blanford, 1863; Stoliczka, 1871, 1873; Möllendorff, 1881, 1894; Bavay and Dautzenberg, 1908, 1909, 1912; Pilsbry, 1916-1918; Tweedie, 1947; Benthem Jutting, 1949, 1950, 1960, 1961, 1962; Thompson and Dance, 1983). However, these studies have generally lacked a precise phylogenetic perspective. Thai pupillid snails were largely ignored until relatively recently, however this deficiency is being rapidly addressed by the publication of a growing series of studies (Thompson and Lee, 1988; Thompson and Upatham, 1997; Panha, 1997a, b, c; Panha and Burch, 1999a, b, c, 2000, 2001a, b, 2002; Burch and Panha, 2000; Burch, Panha and Tongkerd, 2002). Previous taxonomic hierarchies are primarily based on shell morphological characters: shape, sculpture, striation, apertural barriers and embryonic shell morphology, although generic-level distinctions are becoming increasingly tenuous as more taxa are included (Thompson and Dance, 1983). This shell-based taxonomic system has not been tested with an independent character set. I aimed to do so in this study by studying a cross section of Thai Pupillid taxonomic diversity, and constructing a phylogeny based on both shell characters and molecular data, the latter based on nuclear and mitochondrial ribosomal gene fragments.

1.1 Objectives

To test the previously proposed taxonomy of certain taxa within the micro land snail family Pupillidae, focusing mainly on the subfamily Gastrocoptinae, a major subgroup within the family, by using the phylogenetic analysis based on shell morphological characters and a combined mitochondrial and nuclear DNA sequence.

1.2 Anticipated Benefit of this Research

The combination of morphological and molecular phylogenetic analyses yiwlsa new insights into the evolutionary history of Thai pupillids, enables the revision of the conventional taxonomy, forms the basis for future in-depth study of their biodiversity, and will provide a letter perspective on this important element of the Thai terrestrial microfauna.

Table 1.1 Classification of the Pupillidae and geographic distribution of widely recognized pupillid subfamily and genera. The data presented are mostly based on Pilsbry (1916-1918a; 1918b-1920; 1935), Steenberg (1925) and Baker (1935)

Taxon	Geographic distribution
PHYLUM MOLLUSCA	
Class Gastropoda	
Subclass Pulmonata Cuvier 1817	
Order Stylommatophora Schmidt 1856	
Suborder Orthurethra Pilsbry 1900	
Superfamily Pupilloidea Turton 1831	
Family Pupillidae Turtun 1831	
Subfamily Pupillinae	
Genus *Pupilla*	America, Europe, Asia, Africa, Australia
Genus *Pupoides*	America, Oriental, Ethiopian, Australian
Subfamily Gastrocoptinae Pilsbry 1918	
Genus *Gastrocopta*	Nearly world-wide
Genus *Gibbulina*	South America
Genus *Chaenaxis*	North America
Genus *Bothriopupa*	Tropical America
Genus *Hypselostoma*	Oriental
Genus *Anauchen*	Oriental
Genus *Boysidia*	Oriental
Genus *Paraboysidia*	Oriental
Genus *Bensonella*	
Genus *Gyliauchen* (*Gyliotrachela*)	Oriental
Genus *Aulacospira*	Philippines
Genus *Systenostoma*	Indo-china
Genus *Abida*	Europe
Genus *Fauxulus*	South Africa
Genus *Odontocyclas*	Eastern Alpic region
Genus *Sandahlia*	Pyrenees
Genus *Granopupa*	Europe
Genus *Chondrina*	Europe, Morocco
Subfamily Vertigininae Pilsbry 1918	
Genus *Vertigo*	Atlantic island, Europe, Japan, Africa
Genus *Columella*	America, Japan, Europe, Northern Africa
Genus *Pupisoma*	America, Asia, Africa, Australia
Genus *Truncatellina*	Europe, Asia, Africa
Subfamily Orculinae	
Genus *Orcula*	Europe
Genus *Lauria*	Europe, Asia, Northern Africa
Subfamily Nesopupinae	
Genus *Nesopupa*	Pacific islands, Indian oceans,

Table 1.2 Comparison of Orthurethran higher taxonomy showing the differing interpretations concerning familial and sub-familial assignments by Steenberg (1925); Pilsbry (1935) and Baker (1935); Thiele (1935); Zilch (1959); and Vaught (1989).

Steenberg, 1925	Pilsbry, 1935 and Baker, 1935	Thiele, 1935	Zilch, 1959	Vaught, 1989
Suborder Orthurethra	**Suborder Orthurethra**	Strips Vertiginacea	**Suborder Orthurethra**	**Suborder Orthurethra**
Family Chondrinidae	**Family Pupillidae**	(Superfamily Vertiginoidea)	Superfamily Pupilloidea	Superfamily Pupilloidea
Family Orculidae	Subfamily Gastrocoptinae	**Family Amastridae**	(Vertiginoidea)	**Family Pupillidae**
Family Pupillidae	Subfamily Orculinae	Subfamily Leptachactininae	**Family Amastridae**	Subfamily Pupillinae
Subfamily Pupillinae	Subfamily Pupillinae	Subfamily Amastrinae	Subfamily Amastrinae	Subfamily Lauriinae
Subfamily Lauriinae	Subfamily Vertigininae	**Family Cochlicopidae**	**Family Cochlicopidae**	**Family Valloniidae**
Family Vertiginidae	Subfamily Nesopupinae	**Family Vertiginidae**	**(Cionellidae)**	**Family Vertiginidae**
Subfamily Nesopupinae	**Family Valloniidae**	Subfamily Vertigininae	**Family Pyramidulidae**	Subfamily Vertigininae
Subfamily Vertigininae	Subfamily Valloniinae	Subfamily Pupillinae	**Family Vertiginidae**	Subfamily Truncatellininae
Subfamily Truncatellininae	Subfamily Pleurodiscina	Subfamily Orculinae	Subfamily Truncatellininae	Subfamily Gastrocoptinae
Family Valloniidae	Subfamily Pyramidulinae	Subfamily Chondrininae	Subfamily Vertigininae	Subfamily Aulacospirinae
Subfamily Valloniinae	Subfamily Acanthinulinae	**Family Valloniidae**	Subfamily Nesopupinae	Subfamily Nesopupinae
Subfamily Pyramidulinae	**Family Strobilopsidae**	**Family Pleurodiscidae**	**Family Orculidae**	**Family Orculidae**
Subfamily Spelaeodiscinae		**Family Enidae**	**Family Chondrinidae**	**Family Pleurodiscidae**
Family Patulastridae		Subfamily Jaminiinae	Subfamily Chondrininae	**Family Strobilopsidae**
Family Strobilopsidae		Subfamily Eninae	Subfamily Gastrocoptinae	

Table 1.2 Continued

Steenberg, 1925	Pilsbry, 1935 and Baker, 1935	Thiele, 1935	Zilch, 1959	Vaught, 1989
Family Enidae		Subfamily Napaeinae	Subfamily Hyselostomatinae	**Family Pyramidulidae**
Family Pachnodidae		**Family Clausiliidae**	Subfamily Aulacospirinae	**Family Chondrinidae**
Family Partulidae		Subfamily Phaedusinae	**Family Pupillidae**	
Family Cochlicopidae		Subfamily Neniinae	Subfamily Pupillinae	
Family Amastridae		Subfamily Clausiliinae	Subfamily Lauriinae	
Family Achatinellidae		Subfamily Cochlodininae	**?Family [Pholeoterasidae]**	
Family Pacificellidae			**Family Valloniidae**	
Family Tornatellinidae			Subfamily Valloniinae	
Family Auriculellidae			Subfamily Acanthinulinae	
			?Subfamily Spelaeodiscinae	
			Subfamily Strobilopsinae	
			Family Pleurodiscidae	
			Family Enidae	
			Subfamily Chondrulinae	
			Subfamily Jamininae	
			Subfamily Eninae	
			Subfamily Spelaeoconchinae	
			Subfamily Cerastuinae	

Table 1.3 Shell characters used to distinguish the 5 pupillid micro snail genera after Pilsbry (1917) and Burch and Panha (2000).

Shell characters	**Taxa**				
	Hypselostoma	***Gyliotrachela***	***Anauchen***	***Aulacospira***	***Krobylos***
Conic spire either elevated or depressed Last whorl free, either straight or ascending	√	√			
Elongate, pyramidal spire Last whorl not free			√		
Helicoid Last whorl free, descending				√	
Helicoid, last whorl adnate Peristome present only at columellar margin					√
Aperture without barriers				√	√
Aperture with barriers	√	√	√	√	
Angular and parietal lamellae fused into one irregular lamella 	√				
Angular and parietal lamellae independent and parallel 		√			
Angular lamella absent			√	√	

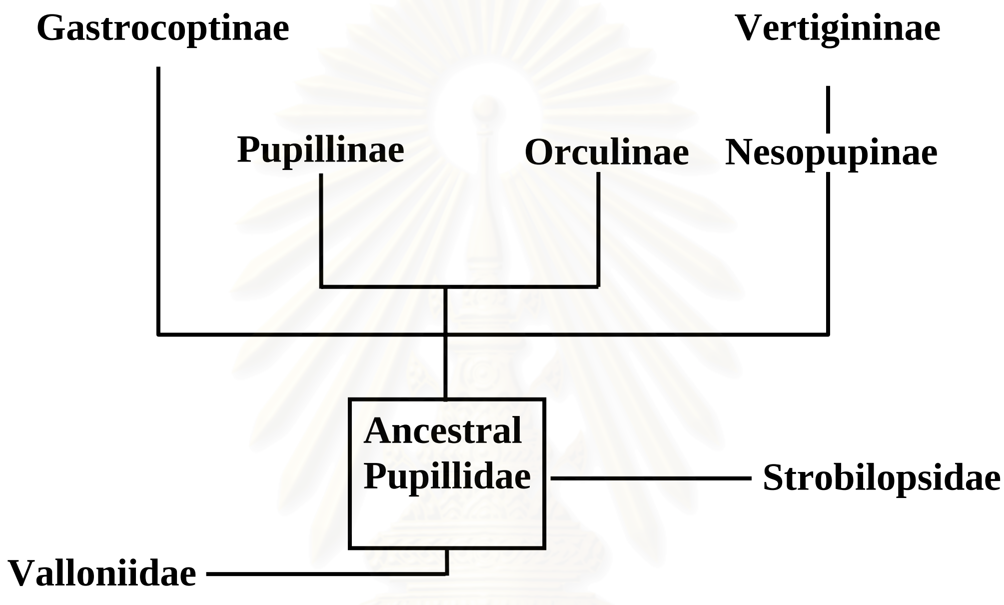


Figure 1.1 The inter-relation of subfamilies of Pupillidae after Pilsbry (1935).

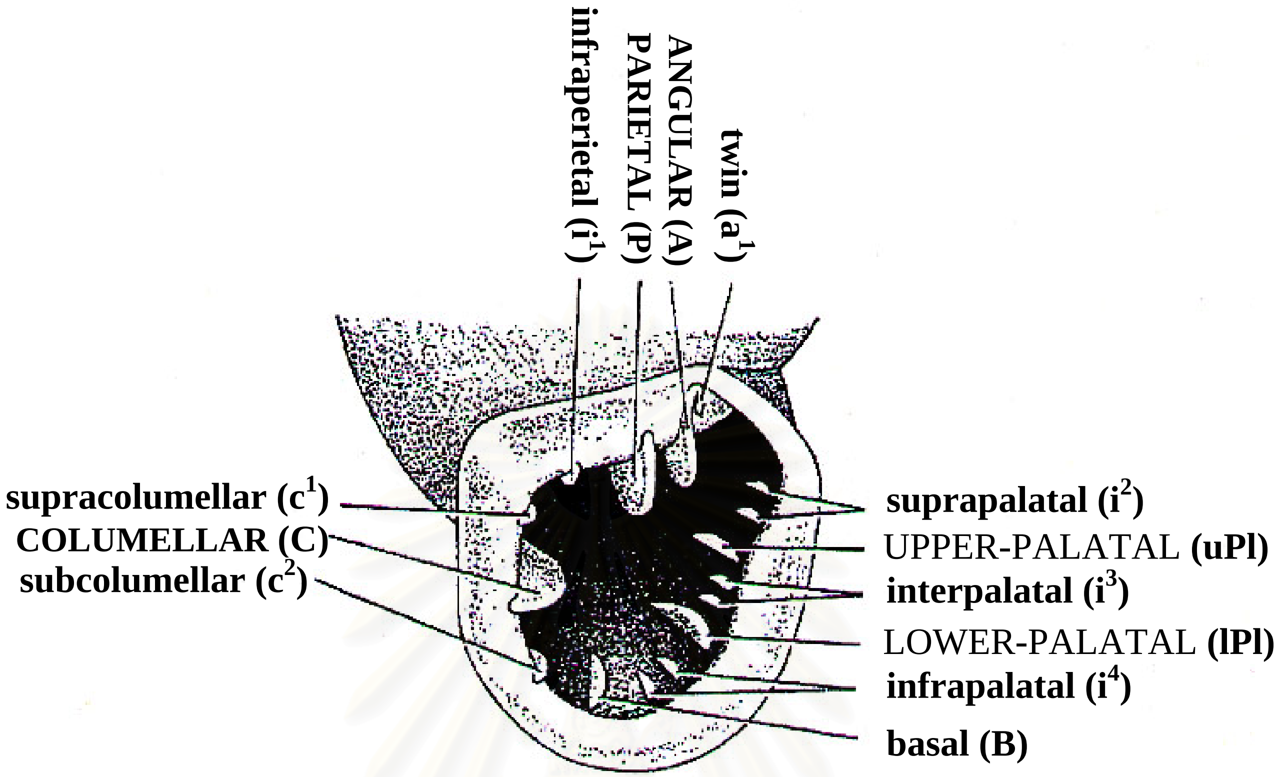


Figure 1.2 Terminology of the apertural barriers after Pilsbry (1916).

Parietal wall:	infraparietal lamella (i^1), parietal lamella (P), angular lamella (A), twin lamella (a^1).
Palatal wall:	infrapalatal plica (i^2), upper palatal plica (uPl), interpalatal plica (i^3), lower palatal plica (lPl), suprapalatal plica (i^4), basal plica (B).
Collumellar wall:	columellar lamella (C), supracolumellar lamella (c^1), subcolumellar lamella (c^2).

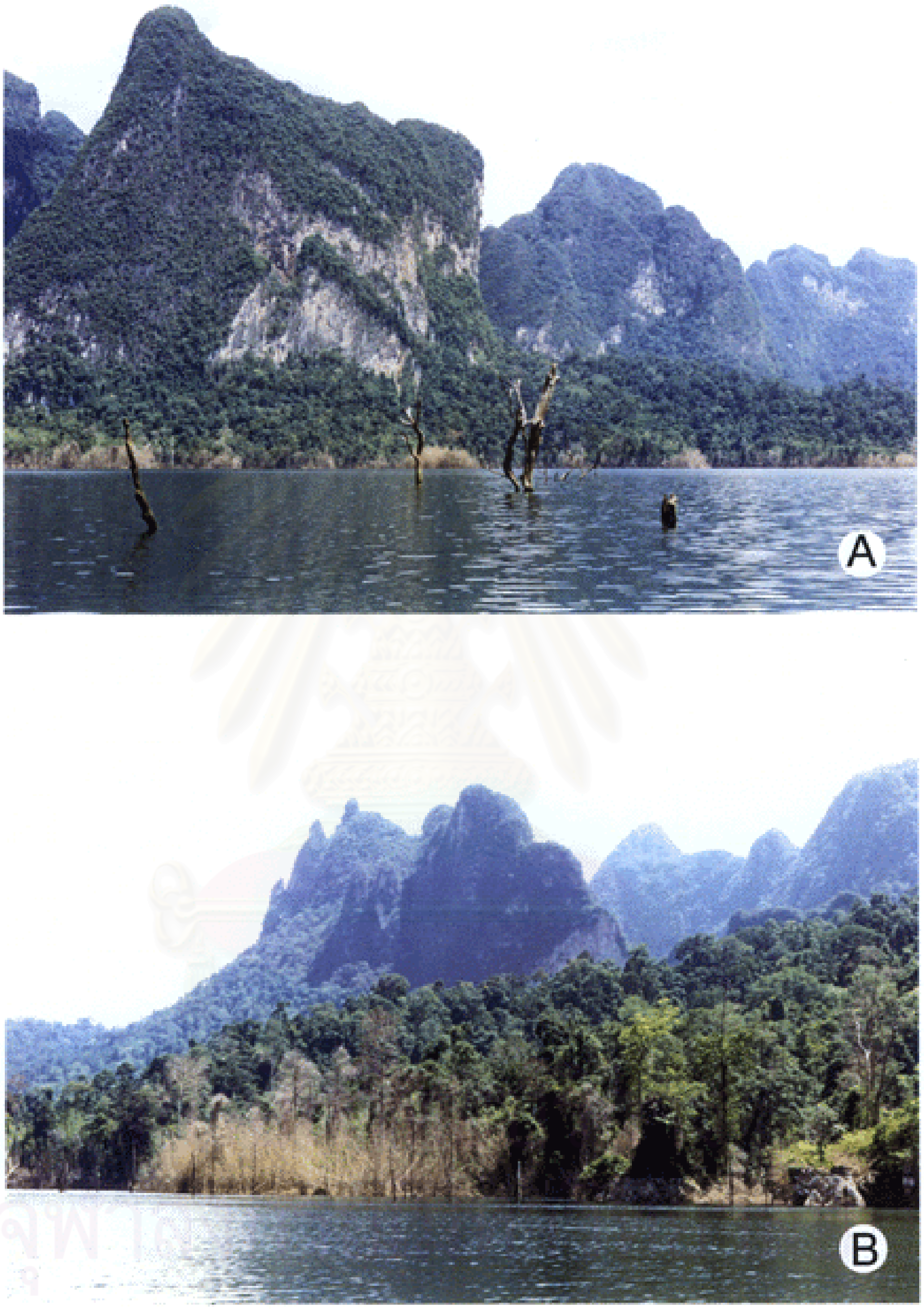


Figure 1.3 Collecting sites, A and B, karst in Klongsang Wildlife Sanctuary, Suratthani Province. (courtesy from S. Panha).

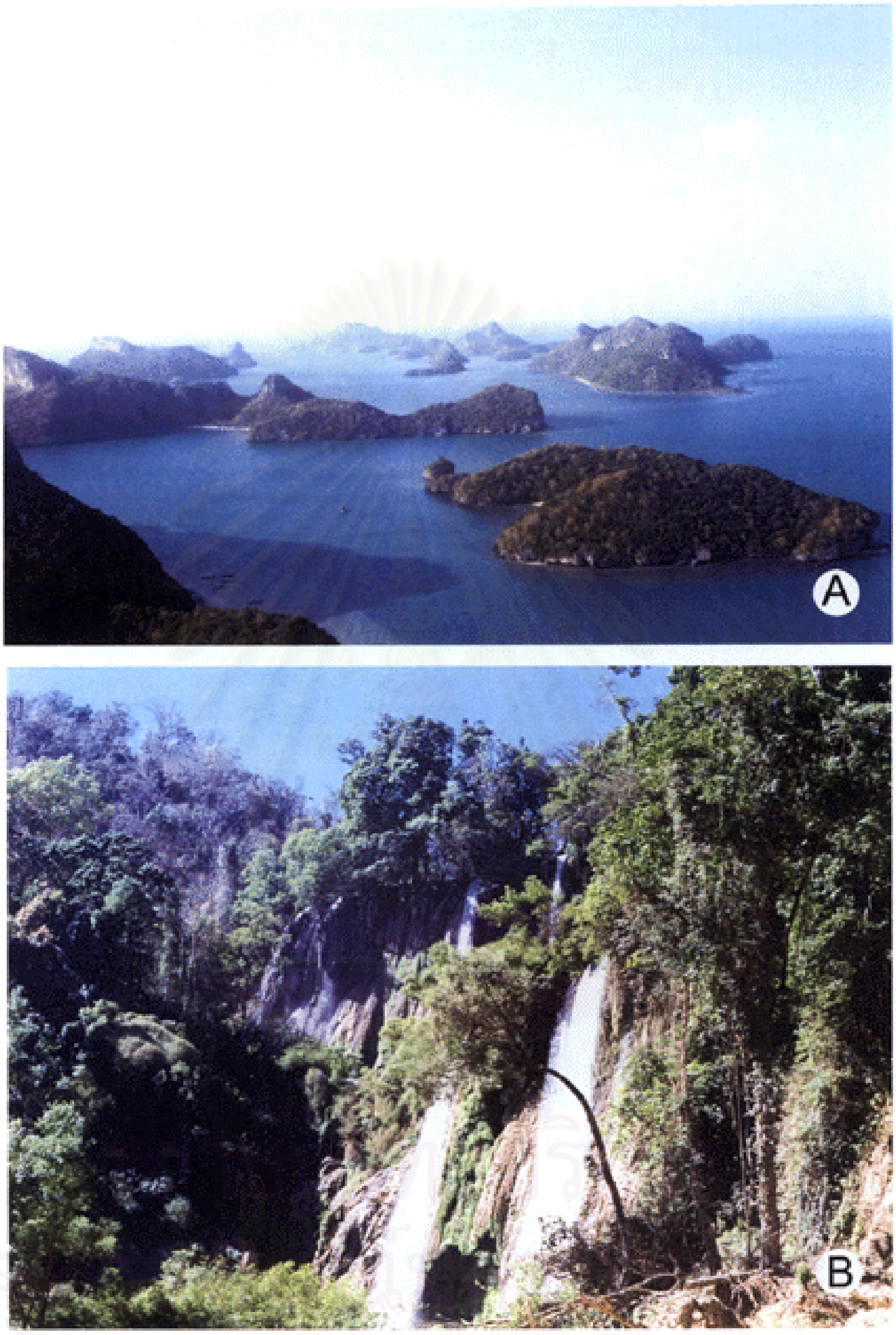


Figure 1.4 Collecting sites, A, Angthong Marine National Park exhibit various small limestone islands. B, A soft limestone mountain at Tee Law Zoo Waterfall, Tak Province. (courtesy from S. Panha).

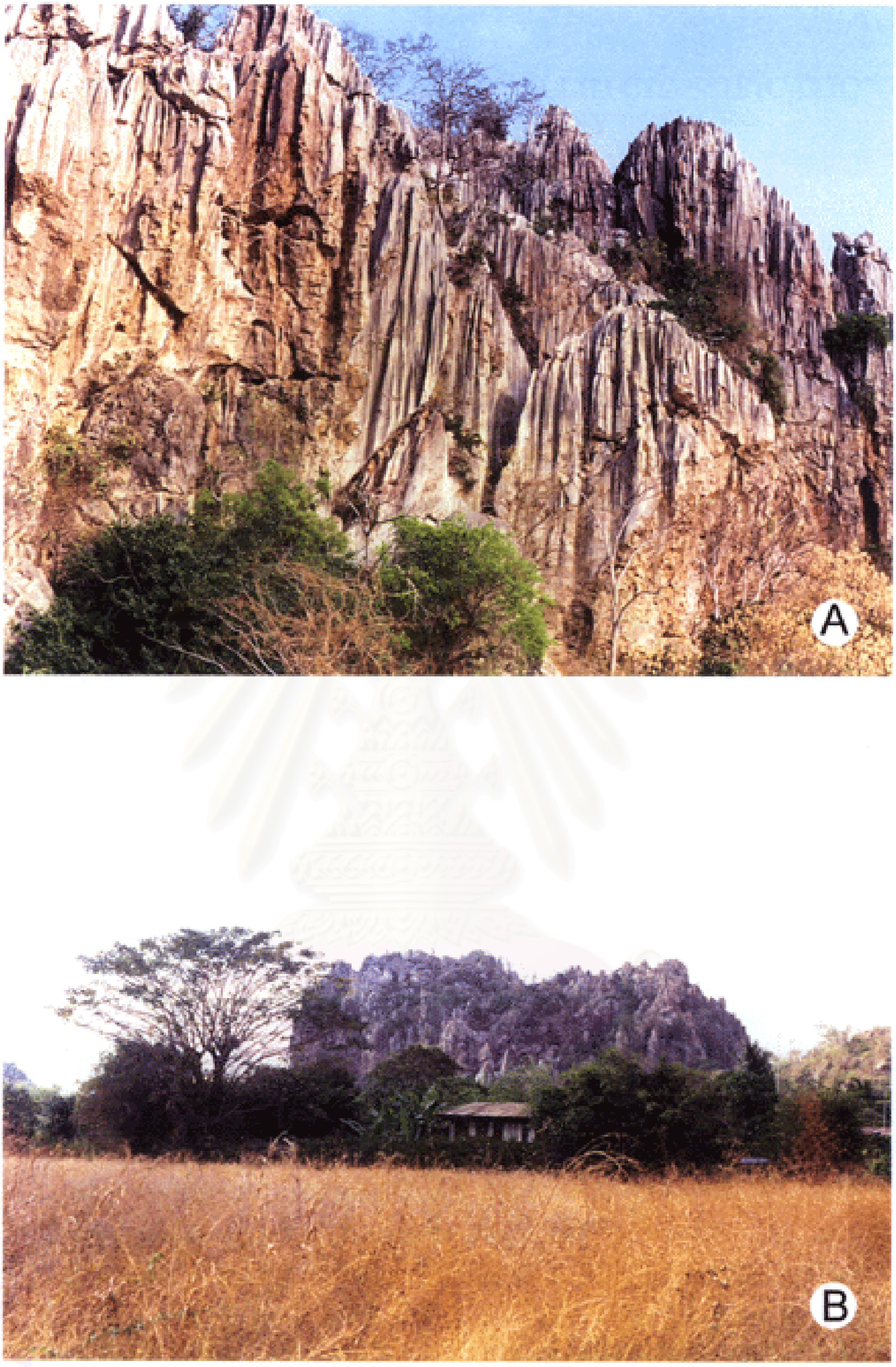


Figure 1.5 Collecting sites, A, A limestone hill in Nakornsawan area showing highly eroded condition. B, A small hill at Ta-kli District, Nakornsawan Province, surrounding with rice field. (courtesy from S. Panha).

CHAPTER 2

LITERATURE REVIEW ON PUPILLID CLASSIFICATION AND PHYLOGENY

The pupillid micro land snails are distributed worldwide and are comprised of multiple taxa at various levels in the taxonomic hierarchy. The Pupillidae belong to the Subclass Pulmonata, which is characterized by (1) the development of a lung with a pneumostome and (2) the development of a procerebrum combined with a cerebral gland (Barker, 1999). The Pupillidae belong to the Order Stylommatophora, a numerically dominant group of strictly terrestrial pulmonate gastropods. The Stylommatophora contain 28 superfamilies and more than 80 families (Vaught, 1987), including the family of minute snails, the Pupillidae. Among pupilloid landsnails classification, even at supraspecific levels, needs reevaluation. Pilsbry (1917) placed the Asian pupillids in the subfamily Gastrocoptinae. He divided this subfamily into 2 major groups, the Hypselostoma-Boysidia group: *Hypselostoma* Benson 1856, *Boysidia* Ancey 1881 and *Gyliauchen* Pilsbry 1917 (preoccupied = *Gyliotrachela* Tomlin 1930), which show pupiform, conic or depressed shell shape and the tangential last whorl; and the Aulacospira group: *Aulacospira* Möllendorff 1890 and *Systenostoma* Bavay and Dautzenberg 1909 (helicoids-shaped and lack of tangential last whorl). Steenberg (1925) studied the anatomy of a diverse group of pupilloid snails and, based on his observations and using a less conservative approach to classification, divided the pupilloids into numerous families (Table 1.2). Pilsbry (1935) and Baker (1935) evaluated the anatomical evidence and concluded that raising so many groups to familial rank was not yet warranted, and Pilsbry (1935) retained his earlier more conservative familial/subfamilial groupings, supported by the anatomical information and opinion of Baker (1935). Thiele (1935) following Steenberg in part, recognized 7 families in his “Stirps Vertiginacea” (= Superfamily Vertiginoidea: Amastrinidae, Cochlicopidae, Vertiginidae, Vallonidae, Pleurodiscidae, Enidae and Clausiliidae). He placed the Southeast Asian pupilloids in the family Vertiginidae: subfamily Vertigininae (which include the genus *Nesopupa* Pilsbry 1900) and subfamily Chondrininae (with include the genera

Anauchen Pilsbry 1917, *Hypselostoma*, *Boysidia*, *Gyliotrachela*, *Aulacospira* and *Systenostoma*). Zilch (1959), also influenced by Steenberg, recognized 10 families in the superfamily Pupillacea [= Pupilloidea: Amastrinidae, Cochlicopidae, Pyramidulidae, Vertiginidae, Orculidae, Chondrinidae, Pupillidae, Vallonidae, Pleurodiscidae and Enidae]. He excluded the Southeast Asian pupilloids from the Vertiginidae (except for the genus *Nesopupa*), but kept them in the Chondrinidae, subfamily Hypselostomatinae (which include the genera *Hypselostoma*, *Anauchen, Boysidia*, *Gyliotrachela*, *Aulacospira* and *Systenostoma*). Solem (1981) used the family name Vertiginidae for *Gyliotrachela* in Australia, but later, in 1988, decided against such a division of the pupilloids and followed Pilsbry's and Baker's more conservative taxonomy, returning to the single family Pupillidae for the Australian pupilloids. For the latter, he used the subfamily names Pupillinae and Gastrocoptinae.

For Thailand, the taxonomic publications until the present day on the Pupillidae are those of Möllendorff (1894), Pilsbry (1917, 1920), Thiele (1931), Zilch (1959, 1982, 1984), Thompson and Lee (1988), Thompson and Upatham (1997); Panha (1997a, b, c); Panha and Burch (1999a, b, c; 2000; 2001a, b; 2002); Burch and Panha (2000) and Burch *et al.* (2002). Thompson and Lee (1988) used "pupillid" in the title of their article for one species of the genus *Hypselostoma* in Thailand. Panha (1996; 1997a, b), following Thiele (1931), used the family name Vertiginidae, and included two genera (*Hypselostoma* and *Gyliotrachela*) but later after critically revised from literatures especially in Pilsbry (1900). The classification was rearranged in the family Pupillidae and the several genera are gradually identified, i.e., *Aulacospira*, *Boysidia* and *Systenostoma* (Panha and Burch, 1999a, b, c; 2000; 2001a, b; 2002; Burch and Panha, 2000; Burch *et al.*, 2002). Thompson and Upatham (1997) put Thai pupilloids in the Vertiginidae, subfamilies Nesopupinae Steenberg, 1925 (which include the genus *Nesopupa*) and Gastrocoptinae Pilsbry, 1918 (which include the genera *Gyliotrachela* and *Systenostoma*), and additionally named the new genus *Acinolaemus* Thompson and Upatham, 1997. Even though the classification at the supraspecific level of Southeast Asian pupilloids is still confusing and unstable, some malacologists construct the dichotomous keys in order to facilitate their identification. Baker (1935) presented the following subfamily key (Pupillinae, Orculinae, Gastrocoptinae, Nesopupinae and Vertigininae) by using shell, radula and anatomical characters as follows:

1a Shell more pupilliform (than in Valloninae, Acanthinulinae, Pyramidulinae and Pleurodiscinae), usually with pupilloid teeth; radula with fundamentally bicuspid laterals and marginals but last with ectoconal accessories; penis usually strong; inferior tentacles usually present……………………………………………….……2

2a Oviducal cul-de-sac absent; prostate very short; penis and retractor bifid, although epiphallar arm weakly demarcated; shell with few extravagantly developed teeth……………………………………………….…..…Pupillinae

2b Oviducal cul-de-sac short; prostate long; penial retractor (at least) undivided; penis with modified apex prolonged epiphallar entrance; shell with angular lamella (when present) short………………………………….….Orculinae

2c Oviducal cul-de-sac long; prostate fairly long; penis and retractor undivided, with epiphallar entrance near or at apex, which is not modified; shell usually with longer angular lamella……………………………...….Gastrocoptinae

1b Shell more pupilliform (than in Valloninae, Acanthinulinae, Pyramidulinae and Pleurodiscinae), but usually with prominent epidermis; radula with fundamentally tricuspid teeth and with interstitials prevalent throughout; penis usually weak; prostate very short; inferior tentacles (always?) obsolete………………………....3

3a Penis with appendix and with bifid retractor……………..…..…Nesopupinae

3b Penis without appendix and with undivided retractor………..…Vertiginidae

Pilsbry (1917) used shell characters, especially the apertural barriers as the important characters of the key to genera given below (slightly modified) in the Hypselostoma-Boysidia group of the subfamily Gastrocoptinae.

1a Angular and parietal lamella concrescent into one sinuous or lobed lamella ……..…2

2a Last whorl adnate to the preceding………………………………...…*Boysidia*

2b Last whorl becoming free, projecting or ascending……………*Hypselostoma*

1b Angular and parietal lamellae distinct and parallel…………………….…………....3

3a Last whorl adnate to the preceding……………………..…………..……...4

4a Columellar lamella horizontally entering…………………..…………..5

5a Teeth ending outwardly in hooks……………….……..*Bensonella*

5b Teeth simple, not hooked……………………….…..*Paraboysidia*

[4b Columellar lamella descending inward……..….*Boysidia strophostoma*]

3b Last whorl becoming free and projecting……………………..…*Gyliotrachela*

1c No angular lamella, the parietal standing alone; last whorl adnated to the preceding ……………………………………………………………………..*Anauchen*

[1d No teeth in the aperture;last whorl becomimg free in front..*Hypselostoma edentulum*]

Thompson and Upatham (1997) constructed a key using embryonic whorl sculpture, as well as apertural teeth characters, apertural shape, whorl characteristics, and shell size for pupillid snails in Southeast Asia.

1a Embryonic whorls with raised spiral threads. Posterior corner of aperture forming a distinct bay nearly separated from the main area by the angular lamella and the upper palatal fold. Angular lamella most strongly developed of apertural teeth. Size minute, less than 2 mm high or wide……………………………………………….…..2

2a Aperture with teeth on parietal, palatal and columellar walls…...*Acinolaemus*

2b Teeth absent in aperture, or a low fold may be present on parietal wall…… ……………………………………………………………….…*Systenostoma*

1b Embryonic whorls without spiral sculpture. Posterior corner of aperture without a conspicuous bay formed by angular lamella and upper palatal fold. Parietal lamella most strongly developed of apertural teeth. Size variable, but generally greater than 2 mm high or wide………………………………..……….……………………...3

3a Angular lamella absent……………………………………….………..*Anuachen*

3b Angular lamella present……………………………………………...…………4

4a Parietal and angular lamellae separated…………………………….….5

5a Part of the last whorl free from the proceeding whorl……………. ………………………….......................................*Gyliotrachela*

5b Last whorl attached to the proceeding whorl throughout its length ………………………………………………………*Paraboysidia*

4b Parietal and angular lamella concrescent ………………………...……6

6a Part of the last whorl free from the proceeding whorl……………. …………………………………………………...*Hypselostoma*

6b Last whorl attached to the proceeding whorl throughout its length. …………………………………………..……….…....*Boysidia*

Since, the first publication has been recorded the pupillid micro land snails in Thailand by Möllendorff (1894), the following papers included Pilsbry (1917, 1920), Thiele (1931), Zilch (1959, 1982, 1984), Thompson and Lee (1988), Thompson and Upatham (1997), Panha (1997a, b, c), Panha and Burch (1999a, b, c; 2000; 2001a, b; 2002), Burch and Panha (2000) and Burch *et al.* (2002). Collectively, their papers have described 47 species, belonging to 12 genera [*Acinolaemus* (5 spp.), *Anauchen* (7 spp.), *Antroapiculus* (1 sp.), *Aulacospira* (2 spp.), *Boysidia* (2 spp.), *Gyliotrachela* (10 spp.), *Hypselostoma* (6 spp.), *Krobylos* (2 spp.), *Montapiculus* (1 sp.), *Nesopupa* (1 sp.), *Paraboysidia* (6 spp.) and *Systenostoma* (4 spp.)] see Table 2.1 for details of nomenclature, type localities and the map of each species in Thailand is shown in Figure 2.1.

Table 2.1 List of 47 described pupillid species in Thailand and collection sites. (C: central; E: Eastern; N: Northern; S: Southern; W: Western; NE: North-Eastern)

Species	Locality
Family Pupillidae	
Subfamily Gastrocoptinae	
1. *Acinolaemus colpodon* Thompson and Upatham, 1997	Rayong Province (E)
2. *Ac. ptychochilus* Thompson and Upatham, 1997	Chiangmai Province (N)
3. *Ac. rhamphodon* Thompson and Upatham, 1997	Chachaengsao Province (E)
4. *Ac. sphinctinion* Thompson and Upatham, 1997	Prachuap Khiri Khan Province (S)
5. *Ac. stenopus* Thompson and Upatham, 1997	Chantaburi Province (E)
6. *Anauchen chedi* (Panha, 1997)	Nakornsawan Province (C)
7. *Ana. angthongense* Burch and Panha, 2000	Angthong Islands, Gulf of Thailand (S)
8. *Ana. huaykhakang* Burch and Panha, 2000	Utaithani Province (C)
9. *Ana. utaithaniensis* Burch and Panha, 2000	Utaithani Province (C)
10. *Ana. khaochongpan* Panha and Burch, 2002	Ratchaburi Province (C)
11. *Ana. sichang* Panha and Burch, 2002	Sichang Island, Gulf of Thailand (E)
12. *Ana. chatnareeae* Burch and Panha, 2002	Suphanburi Province (C)
13. *Antroapiculus pendulus* Panha and Burch, 1999	Tak Province (W)
14. *Aulacospira lampangensis* Panha and Burch, 2001	Lampang Province (N)
15. *Au. smaesarnensis* Panha and Burch, 2001	Chonburi Province (E)
16. *Boysidia chiangmaiensis* Panha and Burch, 1999	Chiangmai Province (N)
17. *B. tholus* Panha and Burch, 1999	Phrae Province (N)
18. *Gyliotrachela striolata* (Möllendorff, 1894)	Samui Island, Gulf of Thailand (S)
19. *G. transitans transitans* (Möllendorff, 1894)	Samui Island, Gulf of Thailand (S)
20. *G. adela* Thompson and Upatham, 1997	Suratthani Province (S)
21. *G. burchi* Panha, 1997	Pattalong Province (S)
22. *G. khaochongensis* Panha, 1997	Trang Province (S)
23. *G. diarmaidi* Burch and Panha, 2002	Chonburi Province (E)
24. *G. khaochakan* Burch and Panha, 2002	Srakeaw Province (E)
25. *G. kohrin* Burch and Panha, 2002	Rin Island, Gulf of Thailand (E)
26. *G. saraburiensis* Burch and Panha, 2002	Saraburi Province (C)
27. *G. surakiti* Burch and Panha, 2002	Nongbualumpoo Province (NE)
28. *Hypselostoma holimamae* Thompson and Lee, 1988	Kanchanaburi Province (W)
29. *H. cucumensis* Panha, 1997	Petchburi Province (S)
30. *H. khaowongensis* Panha, 1997	Nakornsawan Province (C)
31. *H. erawan* Burch and Panha, 2002	Kanchanaburi Province (W)
32. *H. panhai* Burch and Tongkerd, 2002	Kanchanaburi Province (W)
33. *H. taehwani* Burch and Panha, 2002	Petchburi Province (S)
34. *Krobylos maehongsonensis* Panha and Burch, 1999	Maehongson Province (N)
35. *K. pomjuk* Panha and Burch, 1999	Maehongson Province (N)
36. *Montapiculus proboscidea* Panha and Burch, 1999	Nakornsawan Province (C)
37. *Paraboysidia tamphathai* Panha and Burch, 2000	Lampang Province (N)
38. *P. muaklekensis* Panha and Burch, 2001	Saraburi Province (C)
39. *P. nabhitabhatai* Panha and Burch, 2001	Chaiyapum Province (NE)
40. *P. pangmapaensis* Panha and Burch, 2001	Maehongson Province (N)
41. *P. phupaman* Panha and Burch, 2001	Petchaboon Province (C)
42. *P. tarutao* Panha and Burch, 2001	Tarutao Island, Undamun Sea, Satul Province (S)
43. *Systenostoma concava* Thompson and Upatham, 1997	Nakhonratchasima Province (NE)
44. *S. elevata* Thompson and Upatham, 1997	Chiangmai Province (N)
45. *S. edentatum* Panha and Burch, 1999	Phrae Province (N)
46. *S. tamlod* Panha and Burch, 1999	Maehongson Province (S)
Subfamily Nesopupinae	
47. *Nesopupa malayana samuiana* (Möllendorff, 1894)	Samui Island, Gulf of Thailand (S)

Despite the large body of taxonomic work that has been published on pupilloids, only a few phylogenetic analyses have been concluded. A number of recent studies have aimed to resolve relationship and systematic classification schemes in with the Order Stylommatophora using morphological and molecular data (e.g., Tillier, 1989; Emberton, Kuncio, Davis, Phillips, Monderewicz and Guo, 1990; Emberton, 1991; Emberton and Tillier, 1995). The initial systematics work on stylommatophorans which generally use nowadays, originated from Pilsbry (1900). The structure and position of excretory system was the key character. The ordinal group Orthuretra which possessing an orthureter on the distal part of the nephridium was nominated. This character has been assumed as a plesiomorphic character in Stylommatophora because it also occurs in mangrove and various aquatic pulmonates species (Nordsieck, 1985; Tillier, 1989). However, some authors have been explicit the monophyly of the orthurethrans (Solem, 1985; Tillier, 1989; Emberton and Tillier, 1995). In general, the retention of the orthureter among stylommatophorans has been recognized only in the superfamily Pupilloidea, Chondrinoidea and Partuloidea.

Tillier (1989) investigated shell morphology and the anatomy of the digestive, nervous and excretory systems, considering the monophyly of the superfamily Pupilloidea (which included: Pupillidae, Pyramidulidae, Achatinellidae, Vallonidae, Chondrinidae, Cochlicopidae and Vertiginidae). Emberton *et al*. (1990) suggested a different cladogram topology by using ribosomal RNA sequence data, did not show orthurethran monophyly. Baker (1999) developed a more complete morphological phylogenetic study. Recently, Wade, Morton and Clarke (2001) undertook a large-scale molecular phylogenetic analysis of stylommatophoran taxa using 28S ribosomal RNA sequences. They proposed the strong support for the monophyletic lineage Orthurethra (which included: Pupillidae, Pyramidulidae, Amastridae, Vallonidae, Chondrinidae, Cochlicopidae, Buliminidae, Orculidae, Cerastidae and Vertiginidae).

Generic level systematics within the Pupillidae was lack of a modern phylogenetic perspective and the phylogenetic utility of shell morphology and molecular characters have not been established yet. It is widely accepted that the resolution of classification problems as well as the determination of phylogenetic relations among certain taxa could be facilitated by the parallel use of molecular and morphological approaches, although congruence between the two approaches, is not guaranteed (Patterson, Williams and Humphries, 1993). However, Douris, Rodakis, Giokas, Mylonas and Lecanidou (1995) successfully utilized the combined method in the clausiliid genus *Albinaria* in Crete. In the present study, the similar approaches was focused on pupillid micro land snails using samples from various localities in Thailand, and some specimens from Japan, Europe and United State.

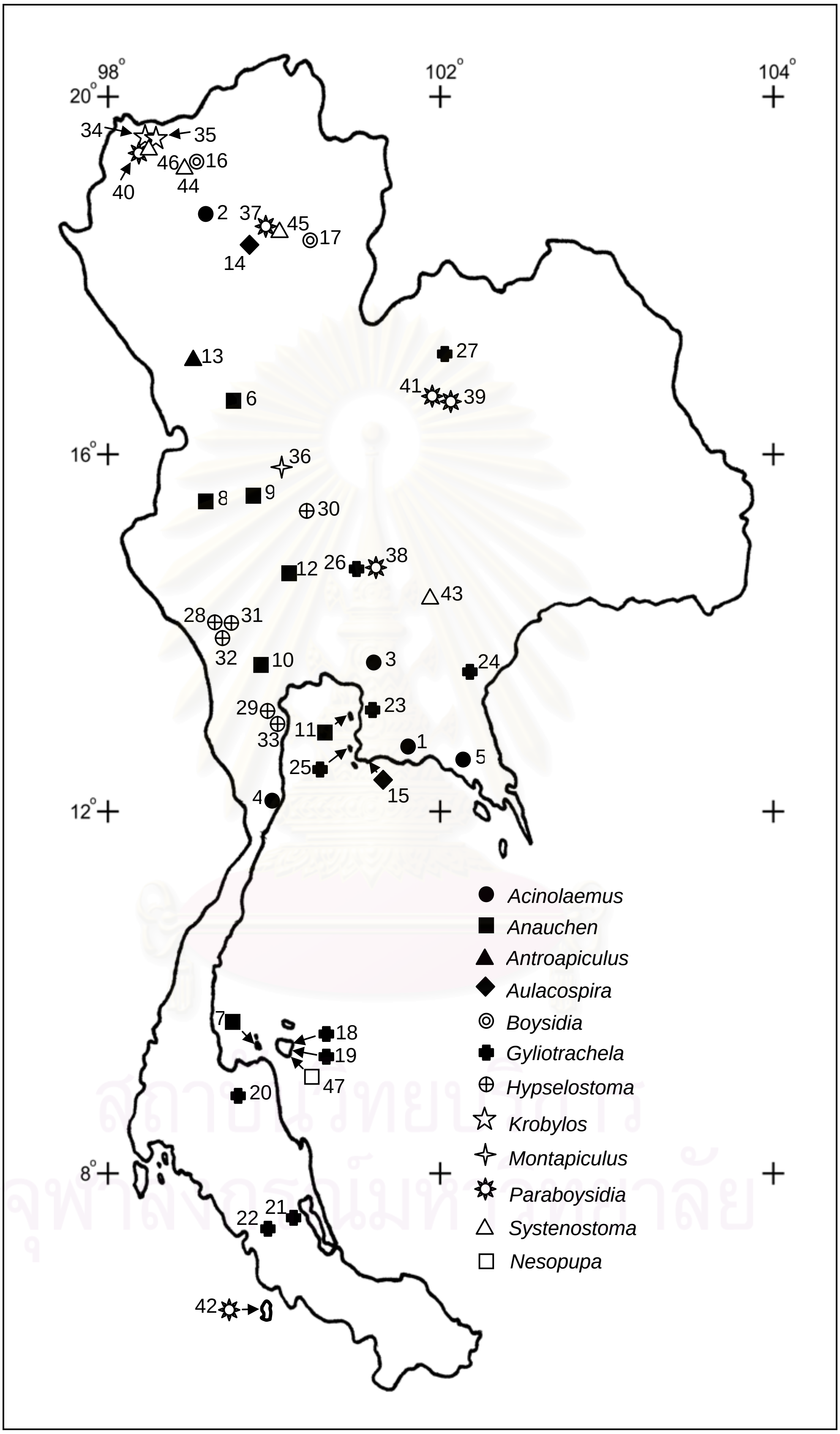


Figure 2.1 Map showing type localities of 47 described species in Thailand.

CHAPTER 3

MATERIALS AND METHODS

3.1 A PHYLOGENETIC ANALYSIS OF THAI PUPILLID GENERA BASED ON SHELL MORPHOLOGY

3.1.1 Sample collection

Pupillid micro land snails were collected from limestone hills throughout Thailand from both from mainland and islands, and some specimens were obtained from Japan, Europe and the United State. The position of each locality was recorded by GPS. Of the twelve pupillid genera occurred in Thailand, 1 to s7 representative species of ten Thai genera were chosen for analysis (*Anauchen*, *Antroapiculus*, *Aulacospira*, *Boysidia*, *Gyliotrachela*, *Hypselostoma*, *Krobylos*, *Montapiculus*, *Paraboysidia* and *Systenostoma*).

Twenty-four species selected were in subfamily Gastrocoptinae, 1 species in the subfamily Pupillinae and 2 species in Vertigininae. Species in the families Valloniidae and Strobilopsidae were included as outgroups in order to root the pupillid phylogeny (Tables 3.1, 3.5). The Valloniidae are closest to the Pupillidae in Tillier's (1989) classification. Valloniidae and Strobilopsidae possessing both the nephridium and orthureter (usually with only the proximal part of the ureter develop as a groove) as presented in Pupillidae (Pilsbry, 1900). Indeed, the possesstion of an orthureter on the distal part of the nephridium is probably plesiomorphic morphological character in Stylommatophora because this configuration occurs in basal pulmonate groups (Nordsieck, 1985 and Tillier, 1989).

3.1.2 Shell Morphological study

3.1.2.1 Identification

All 27 species (Table 3.1) were identified by S. Panha and P. Tongkerd, and confirmed by J. B. Burch. Specimens were prepared in both ways by (1) scanning electron microscopy (SEM), removing external debris using a fine (#0) paint brush, dehydration in 95% ethanol followed by air drying and gold-coating steps prior to viewing with JEOL (JSM-5410 LV) scanning electron microscope (SEM) at The Scientific and Technological Research Equipment Centre (STREC), Chulalongkorn University, (2) an OLYMPUS-SZX9 stereomicroscopic light microscope with SZX-DA camera lucida attachment.

3.1.2.2 Morphometric analysis

Fifteen specimens of each species were taken the pictures by using a wild M5 stereomicroscopic light microscope in tree positions: front view, top view and the umbilicus view. Six shell characteristics were chosen for measurement (Appendix I): Shell height, including aperture lip, or tuba if present (SHA), Height of body whorl and spire (BSH), Shell width, including aperture lip, or tuba if present (SWA), Width of body whorl (to where tuba begins) (SW), Length of tuba (TL) and Width of umbilicus (UW). Shells were measured by using the Micro Image 4.0 program and state micrometer was also used as a standard. The morphometric analysis using multivariate analysis was performed. The result of shell measurement analysis was used to classify character states of some characters in the cladistic study.

3.1.2.3 Character determined

Character states for each species were determined from the examination of dry cleaned shell and alcohol specimens deposited in the Chulalongkorn University, Museum of Zoology (see catalog numbers in Table 3.1). Only the diagnostic characters accepted widely by pupillid systematists were coded. Character diagnoses are listed in Appendix II, and the data-matrix used in the analysis were shown in Table 3.2.

3.1.2.4 Data analysis

The data were analyzed using PAUP* 4.10 (Swofford, 2002) under the maximum parsimony optimality criterion. Analyses were performed as heuristic searches using equal character weighting. Of 27, 6 multi-state characters were treated as ordered, because of the obvious succession of their states (Appendix II). *Vallonia costata* were designated as the outgroup, and pupillid taxa were forced to be monophyletic in order to root the phylogeny. Also unrooted analysis of ingroup taxa was conducted and the results were compared with outgroup root topology.

Character transformation series were determined on one of the equally parsimonious trees using MacClade 4.03 (Maddision and Maddision, 2001) and PAUP*. Branch support levels were calculated with bootstrapping (100 replications, heuristic searches, 10 random additions each) using PAUP*.

3.2 PHYLOGENETIC RELATIONSHIP OF THE CERTAIN THAI GASTROCOPTINAE (STYLOMMATOPHORA; PUPILLIDAE) INFERRED FROM MITOCHONDRIAL AND NUCLEAR RIBOSOMAL DNA SEQUENCES

3.2.1 Taxa Examined

The seventeen pupillids (15 Gastrocoptinae, 1 Pupillinae and 1 Vertigininae), are listed in Table 3.3, including details of sampling localities and habitats. Replicate sets of voucher specimens have been deposited in the collections of the Chulalongkorn University Museum of Zoology (CUMZ 000101-24) and in the Mollusc Division of the University of Michigan's Museum of Zoology (UMMZ 300052-75). All samples were collected by S. Panha and the author. Species identifications were determined by S. Panha and P. Tongkerd, and confirmed by J. B. Burch. Taxa were chosen because of their availability and to test the monophyly of gastrocoptinid genera. However, the member of the *Antroapiculus*, *Boysidia*, *Montapiculus*, *Paraboysidia* and *Systenostoma* in Thailand were not available for the present analysis. In most case, at least 2 individuals were conducted for each species. Whenever the specimens were collected from the different localities, and/or continents, several individuals from multiple localities were sequenced. Various other pupilloid family samples were included in the analysis. Two Valloniid species, *Vallonia costata* from two different localities in Michigan and *Zoogenetes harpa*, and one Strobilopsid species, *Strobilops labyrinthica*, were selected as outgroups (Table 3.3).

3.2.2 Polymerase Chain Reaction (PCR) and DNA Sequencing

Molecular analyses were performed at the University of Michigan, Museum of Zoology (UMMZ). Total genomic DNA was isolated from 95% ethanol-preserved foot tissue, or the whole animal, using a DNeasy™ Tissue Kit (Qiagen, Chatsworth, CA.) according to the manufactorer's instructions. Fragments of two ribosomal genes, the mitochondrial large subunit (16S, *circa* 500 nt) and the nuclear large subunit (28S, *circa* 650 nt), were amplified for each species. Primer sequences are given in Table 3.4

(1) A fragment of the mitochondrial ribosomal large subunit gene (16S) was amplified using primers 16Sar and 16Sbr (Kessing, Croom, Martin, McIntosh, McMillan and Palumbi, 1989) with *Taq* DNA Polymerase (Promega, Madison, WI, Buffer A) for all species.
(2) Domain 2 of the large nuclear ribosomal subunit was amplified using primers 28SF4 and 28SR5 (Morgan, DeJong, Jung,

Khallaayoune, Kock, Mkoji and Loker, 2002), and FastStart *Taq* DNA Polymerase (Roche Applied Science, Indianapolis, IN).

A common touchdown protocol (Palumbi, 1996) was used for all PCR reactions [after 4 min denaturation at 94ºC, the initial annealing temperature of 65°C was decreased by 2°C/cycle (40 sec denaturing at 94ºC, 40 sec annealing and 1.5 min extension at 72ºC) until the final annealing temperature (45-50°C for 16S and 50-55°C for 28S) was reached and subsequently maintained for an additional 30 cycles (all denaturation was performed at 95ºC when FastStart *Taq* was used)] was utilized and a negative control (no template) was included in each amplification run. PCR products were gel-purified (1% agarose), excised over UV light, and sequencing templates were prepared using a QIAEX QXII Gel Extraction Kit (Qiagen). Direct, cycle sequencing reactions were performed using BigDye Terminator Cycle Sequencing Ready Reaction (Perkin-Elmer Applied Biosystems, Polo Alto, CA) with the respective original PCR primers (annealing temperature 45°C for 16S and 50°C for 28S) for both strands of amplified products. Excess dyes were removed from sequencing reaction products using Centri-sep spin columns (Princeton Separations) loaded with G-50 Sephadex (Sigma). Sequencing products were either electrophoresed on an UMMZ ABI 377 automated DNA sequencer (Perkin-Elmer Inc.) or sent to the University of Michigan Sequencing Core Facility.

3.2.3 Phylogenetic Analyses

Resulting chromatograms were edited manually by comparing both strands for all taxa using Sequence Navigator 1.0.1 (Applied Biosystems). The 16S mitochondrial and 28S nuclear ribosomal DNA fragments were compiled with Sequence Monkey ver. 2.9.0 (Graf, 2000; available from http://www.monkeysoftwerk.com) and aligned using Clustal_X (Thompson Gibson, Plewniak, Jeanmougin and Higgins, 1997; available from http://ncbi.nlm.nih.gov). The alignment was refined manually where necessary. Both alignments show in the APPENDIX III.

All of the gene fragments utilized in this study have been deposited in GenBank: accession numbers AY187566-89 for mt 16S and AY189454-77 for nuclear 28S sequences (Table 3.5). In order to test if each data set has a hierarchical structure, the degree of skewness (g_1; Hillis and Huelsenbeck, 1992) was calculated (100,000 randomly sampled trees) and permutation tail probability (PTP; Archie, 1989) analyses were conducted (1000 replicates) using PAUP* ver. 4.0b10 (Swofford, 2002).

To assess the degree of saturation, pairwise base difference values for each gene fragment were compared with respective maximum parsimony (MP) branch lengths (Philippe, Sörhannus, Baroin, Perasso, Gasse and Adoutte, 1994). MP steps for both 16S and 28S were counted over the topology of the combined (16S+28S) phylogeny. The pairwise base difference and branch length were determined using PAUP*, and the Branch Swinger 1.0.1 (Graf, 2001) was used to generate text files containing all pairwise comparisons. In addition, the incongruence length difference (ILD) test (Farris *et al*.,1994) was performed (1000 random replications) using PAUP* to assess character congruence between 16S and 28S data sets.

The 16S and 28S datasets were analyzed as either individual or combined (16S + 28S) matrices. Phylogenetic analyses were conducted on each partition and on the combined matrix under the maximum parsimony (MP) as well as maximum likelihood (ML) optimality criteria using PAUP*, and the two vallonids and one strobilopsid were designated as outgroup taxa. MP analyses were performed using heuristic search option with 100 random stepwise additions and tree bisection-reconnection (TBR) branch-swapping. Characters were unordered and equally weighted, and inferred sequence gaps were considered as missing data. Branch support levels were estimated with bootstrapping (Felsenstein, 1985) (1000 replications, heuristic searches, 10 random additions each), and also with Bremer decay index values (Bremer, 1994) calculated using TreeRot ver. 2 (Sorenson, 1999), which generates a constraint file for PAUP*.

A MP tree was used to estimate the log-likelihood scores using PAUP* and then, the best-fit ML model for each partition was determined by hierarchical likelihood ratio tests (hLRTs) using Modeltest 3.06 (Posada and Crandall, 1998). ML analyses were conducted using a heuristic search option in which the parameter values under the best-fit model were fixed and a MP tree was used as a starting point for TBR branch swapping. Several iterations of model parameter re-estimation on the resulting tree and subsequent heuristic search were performed until the parameter values were stabilized. Bootstrap estimates for ML topology were assessed with 100 replicates using the “fast” stepwise-addition option for heuristic searches.

Table 3.1 Catalog of the studied taxa, sampling localities and voucher specimen information (CUMZ: University of Chulalongkorn University, Museum of Zoology) [Thailand (C: Central, E: Eastern, N: North, W: Western, NE: North-Eastern and S: Southern)]

Species	Locality	Catalog No.
Gastropoda, Stylommatophora		
Family Pupillidae		
Subfamily Gastrocoptinae		
1. *Anauchen angthongense*	Angthong Islands, Gulf of Thailand (S)	CUMZ00128
2. *Ana. chatnareeae*	Suphanburi Province (C)	CUMZ00129
3. *Ana. chedi*	Nakornsawan Province (C)	CUMZ00130
4. *Ana. utaithaniensis*	Utaithani Province (C)	CUMZ00131
5. *Antroapiculus pendulus*	Tak Province (W)	CUMZ00132
6. *Aulacospira smaesarnensis*	Chonburi Province (E)	CUMZ00133
7. *Boysidia chiangmaiensis*	Phrae Province (N)	CUMZ00134
8. *Gyliotrachela adela*	Suratthani Province (S)	CUMZ00135
9. *G. diarmaidi*	Chonburi Province (E)	CUMZ00136
10. *G. khaochakan*	Srakeaw Province (E)	CUMZ00137
11. *G. khaochongensis*	Trang Province (S)	CUMZ00138
12. *G. kohrin*	Rin Island, Gulf of Thailand (E)	CUMZ00139
13. *G. saraburiensis*	Saraburi Province (C)	CUMZ00140
14. *G. surakiti*	Nongbualumpoo Province (NE)	CUMZ00141
15. *Hypselostoma cucumensis*	Petchburi Province (S)	CUMZ00142
16. *H. erawan*	Kanchanaburi Province (W)	CUMZ00143
17. *H. holimamae*	Kanchanaburi Province (W)	CUMZ00144
18. *H. panhai*	Kanchanaburi Province (W)	CUMZ00145
19. *H. taehwani*	Petchburi Province (S)	CUMZ00146
20. *Krobylos maehongsonensis*	Maehongson Province (N)	CUMZ00147
21. *Montapiculus proboscidea*	Utaithani Province (C)	CUMZ00148
22. *Paraboysidia phupaman*	Petchaboon Province(C)	CUMZ00149
23. *Systenostoma tamlod*	Maehongson Province (N)	CUMZ00150
Subfamily Pupillinae		
24. *Pupilla muscorum* (Linnaeus)	Ann Arbor, Mich., USA	CUMZ00151
Subfamily Vertigininae		
25. *Vertigo gouldi*	Ann Arbor, Mich., USA	CUMZ00152
26. *Ve. shimochii*	Japan	CUMZ00153
Family Valloniidae		
27. *Vallonia costata* (Müller)	Ann Arbor, Mich., USA	CUMZ00154

Table 3.2 Data matrix used in the shell morphological phylogenetic analysis. Characters and states are described in Appendix II.

Taxon/Node	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Anauchen angthongense	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0
Ana. chatnareeae	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0
Ana. chedi	0	0	1	1	0	1	1	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1	0	1	0	0
Ana. utaithaniensis	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	1	0	0
Antroapiculus pendulus	0	0	0	0	0	1	2	1	0	1	0	0	1	1	0	1	0	0	1	0	1	0	0	0	1	0	0
Aulacospira smaesarnensis	0	1	1	0	0	1	0	1	0	1	0	1	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0
Boysidia chiangmaiensis	0	0	1	0	0	1	0	0	0	0	1	1	1	0	1	1	1	0	1	0	1	0	0	0	1	1	0
Gyliotrachela adela	0	2	1	1	0	1	2	1	0	1	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0
G. diarmaidi	0	2	0	1	0	1	2	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0
G. khaochakan	0	0	1	1	0	1	2	1	0	0	0	0	1	0	1	1	1	0	1	0	1	0	1	0	1	0	0
G. khaochongensis	0	1	1	1	0	1	2	1	0	0	0	0	1	0	1	1	1	0	1	1	1	1	0	1	1	1	0
G. kohrin	0	2	1	1	0	1	2	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0
G. saraburiensis	0	1	0	1	0	1	2	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0
G. surakiti	0	2	1	0	0	1	2	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0
Hypselostoma cucumensis	0	2	0	1	0	1	2	1	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0
H. erawan	0	2	1	1	0	1	2	1	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1	1	1	0	0
H. holimanae	0	1	0	1	0	1	2	1	0	0	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0
H. panhai	0	0	1	1	0	1	0	0	0	0	1	0	1	0	0	1	0 1	0	0	0	0 1	0	0	0	0 1	0	1
H. taehwani	0	1	0	1	0	1	2	1	0	1	0	0	1	0	1	1	1	0	1	0	1	0	1	0	1	0	0
Krobylos maehongsonensis	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Montapiculus proboscidea	0	1	2	0	0	1	2	1	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Paraboysidia phupaman	0	0	1	0	0	1	2	1	0	0	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	0
Systenostoma tamlod	0	1	2	1	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Pupilla muscorum	1	0	1	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0 1	0	0	0	1
Vertigo gouldi	1	0	1	0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	1	0	1	0	0	0	1	1	1
Ve. shimochii	1	0	2	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0	1	0	0	0	1	1	1
Vallonia costata	0	2	2	0	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 3.3 Sampling location data for the study taxa, habitat and voucher specimen information (LR: Limestone-Rock habitat; LL: Leaf-Litter habitat; CUMZ: Chulalongkorn University, Museum of Zoology, Thailand; UMMZ: University of Michigan, Museum of Zoology, USA.)

Taxa	Collection locality	Habitat	Catalog No.
Gastropoda, Stylommatophora			
Family Strobilopsidae			
1. *Strobilops labyrinthica* (Say)	Pellston, Mich., USA	LL	CUMZ 000101 UMMZ 300052
Family Vallonidae			
2. *Vallonia costata* (Müller)	(A) Ann Arbor, Mich., USA	LL	CUMZ 000102 UMMZ 300053
3. *Va. costata*	(B) Pellston, Mich., USA	LL	CUMZ 000103 UMMZ 300054
4. *Zoogenetes harpa* (Say)	Pellston, Mich., USA	LL	CUMZ 000104 UMMZ 300055
Family Pupillidae			
Subfamily Pupillinae			
5. *Pupilla muscorum* (Linnaeus)	(A) Ann Arbor, Mich., USA	LL	CUMZ 000105 UMMZ 300056

Table 3.3 Continued.

Taxa	Collection locality	Habitat	Catalog No.
6. *P. muscorum*	(B) Pellston, Mich., USA	LL	CUMZ 000106
			UMMZ 300057
7. *P. muscorum*	(E) Vienna, Austria	LL	CUMZ 000107
			UMMZ 300058
Subfamily Vertigininae			
8. *Vertigo gouldi*	Ann Arbor, Michigan, USA	LL	CUMZ 000108
			UMMZ 300059
Subfamily Gastrocoptinae			
9. *Anauchen chedi*	Tebpratan Nature Reserve, Kampangpet, Thailand	LR	CUMZ 000109
			UMMZ 300060
10. *Ana. chatnareeae*	Tamsae Hill, Supanburi, Thailand	LR	CUMZ 000110
			UMMZ 300061
11. *Ana. utaithaniensis*	Patavi Mt., Uthaithani, Thailand	LR	CUMZ 000111
			UMMZ 300062
12. *Aulacospira smaesarnensis*	Smaesarn, Chonburi, Thailand	LR	CUMZ 000112
			UMMZ 300063

Table 3.3 Continued.

Taxa	Collection locality	Habitat	Catalog No.
13. *Gyliotrachela adela*	Tamkhaoplu Hill, Chumporn, Thailand	LR	CUMZ 000113
			UMMZ 300064
14. *G. diarmaidi*	Pluangthong Mt., Chonburi, Thailand	LR	CUMZ 000114
			UMMZ 300065
15. *G. khaochakan*	Chakan Mt., Srakaew, Thailand	LR	CUMZ 000115
			UMMZ 300066
16. *G. kohrin*	Rin Is., Chonburi, Thailand	LR	CUMZ 000116
			UMMZ 300067
17. *G. saraburiensis*	Teppitak Hill, Saraburi, Thailand	LR	CUMZ 000117
			UMMZ 300068
18. *G. surakiti*	Phajoa Hill, Nongbualampoo, Thailand	LR	CUMZ 000118
			UMMZ 300069
19. *Hypselostoma cucumensis*	KhaoYoi Hill, Petchaburi, Thailand	LR	CUMZ 000119
			UMMZ 300070
20. *H. erawan*	Chongkhaokad Hill, Kanchanaburi, Thailand	LR	CUMZ 000120
			UMMZ 300071

Table 3.3 Continued.

Taxa	Collection locality	Habitat	Catalog No.
21. *H. holimanae*	Agricultural College, Kanchanaburi, Thailand	LR	CUMZ 000121
			UMMZ 300072
22. *H. panhai*	Chongkhaokad Hill, Kanchanaburi, Thailand	LL	CUMZ 000122
			UMMZ 300073
23. *Krobylos maehongsonensis*	(1) Tamphatai Hill, Phare, Thailand	LR	CUMZ 000123
			UMMZ 300074
24. *K. maehongsonensis*	(2) Tamweruwan Hill, Supanburi, Thailand	LR	CUMZ 000124
			UMMZ 300075

Table 3.4 Primers used to amplify fragments of mitochondrial large ribosomal subunit (16S) and nuclear large ribosomal subunit (28S) for each species studied. (F): forward primer. (R): reverse primer.

Gene	**Code**	**Sequence (5’⟶3’)**	**References**
16S	16Sar (F)	CGCCTGTTTATCAAAAACAT	Kessing *et al.* (1989)
	16Sbr (R)	CCGGTCTGAACTCAGATCACGT	Kessing *et al.* (1989)
28S	28SF4 (F)	AGTACCGTGAGGGAAAGTTG	Morgan *et al.* (2002)
	28SR5 (R)	HCGRGACGGGCCGGTGGTGC	Morgan *et al.* (2002)

H = ACT; R = AG

Table 3.5 GenBank accession numbers for the taxa used in this study.

Taxon	**16S**	**28S**
Strobilops labyrinthica	AY187566	AY189454
Vallonia costata (A)	AY187567	AY189455
Va. costata (B)	AY187568	AY189456
Zoogenetes harpa	AY187569	AY189457
Pupilla muscorum (A)	AY187570	AY189458
P. muscorum (B)	AY187571	AY189459
P. muscorum (E)	AY187572	AY189460
Vertigo gouldi	AY187573	AY189461
Anauchen chedi	AY187574	AY189462
Ana. chatnareeae	AY187575	AY189463
Ana. utaithaniensis	AY187576	AY189464
Aulacospira smaesarnensis	AY187577	AY189465
Gyliotrachela adela	AY187578	AY189466
G. diarmaidi	AY187579	AY189467
G. khaochakan	AY187580	AY189468
G. kohrin	AY187581	AY189469
G. saraburiensis	AY187582	AY189470
G. surakiti	AY187583	AY189471
Hypselostoma cucumensis	AY187584	AY189472
H. erawan	AY187585	AY189473
H. holimanae	AY187586	AY189474
H. panhai	AY187587	AY189475

Table 3.5 Continued.

Taxon	**16S**	**28S**
Krobylos maehongsonensis (1)	AY187588	AY189476
K. maehongsonensis (2)	AY187589	AY189477

(A) = Ann Arbor, Mich., USA

(B) = Pellston, Mich., USA

(E) = Vienna, Austria

(1) = Tamphatai Hill, Phare Province, Thailand

(2) = Tamweruwan Hill, Supanburi Province, Thailand

CHAPTER 4

MORPHOMETRIC AND PHYLOGENETIC ANALYSIS OF THAI PUPILLID GENERA BASED ON SHELL MORPHOLOGY

4.1 Result and discussion on Morphometric analysis

Six shell characteristics were chosen for measurement (Appendix I): shell height, including aperture lip, or tuba if present (SHA), height of body whorl and spire (BSH), shell width, including aperture lip, or tuba if present (SWA), width of body whorl (to where tuba begins) (SW), length of tuba (TL) and width of umbilicus (UW). A total of 390 shell from 26 species was measured from the collections held in the Natural History Museum of Chulalongkorn Universiry, Thailand (Appendix I, Table 3.1). All statistical computations employed the statistical package SPSS v11.0. All average (X) and SE of six measurements and their ratios for each species were showed in Table 4.1. The previous studied by Gould and Woodruff (1978) developed a more objective approach to characterization of complex shell form. Gould's method involves the measurement simple shell features and their ratios, by multivariate analysis.

The pupullid micro land snail conventional taxonomy mostly based on apertural dentition characters. Visual examination under light microscope of the different of the various genera in southeast Asia, *Gyliotrachela* and *Hypselostoma* species, suggests that neither shell height nor shell width are key characters in differentiating species or genera. Principal Components Analysis (PCA) was employed (using Pearson correlation coefficient with VARIMAX rotation) to isolate the characters that account for most variation between species.

Shell of *Gyliotrachela* and *Hypselostoma* species were ordinated using all six measurements and their ratios. In the PCA analysis results of all 7 *Gyliotrachela* and 5 *Hypselostoma* species, the most useful characters of six measurement was SWA, explained by the KMO and Bartlett's Test more than 0.5 (Kaiser-Meeyer-Olkin Measure of sampling Adequacy = 0.506). According to the result, the most variance factor contained 28.08%. Figure

4.1 showed the three component plot, which four significant groups were separated: 1.) hcc, hhm and gdm; 2.) htw, gkr and gkc; 3.) hph, gkk and gad and 4.) her, gsr and gsb. I can conclude that these two genera never formed the distinctive clusters by the characters or morphometric method that described in this study.

4.2 Results of phylogenetic analysis

Twenty-seven characters were coded, including those from the shell characters (1-10), Peristome characters (11-14), Apertural barriers (15-26) and habitat types (27). Within each pupillid genus, all representative species have the parietal teeth, except *Krobylos maehongsonensis*, which completely lack teeth in the aperture. (Character 27; Appendix II). To facilitate a through analysis, one character set from each genus was included into the data matrix (Table 3.2). Of 27 total, 25 characters were found to be parsimony-informative and only 2 were uninformative character. Six equally most-parsimonious trees of 77 steps (CI = 0.390, RI = 0.710) were obtained from the analysis of the data matrix including outgroup taxa, and a strict consensus was recovered (Figure 4.2). The following observations can be made on that tree figure:

a.) The five species of genus Gyliotrachela formed a monophyletic group with 61 bootstrap value and it was sister to *Hypselostoma erawan.*

b.) Two subgroups are obvious in both group: one containing 5 genera from 3 subfamilies (Gastrocoptinae: *Boysidia, Hypselostoma* and *Krobylos*; Pupillinae: *Pupilla*; Vertigininae: *Vertigo*), and the second subgroup consisting of the polyphyletic of 8 gastrocoptinid subfamilies (*Anauchen, Antroapiculus*, *Aulacospira*, *Gyliotrachela, Hypselostoma, Paraboysidia, Montapiculus* and *Systenostoma*). Character transformations were depicted on one of the most parsimonious cladograms obtained from the outgroup rooted analysis (Figure 4.3).

4.3 Discussion of phylogenetic analysis

The present morphological analyses did not clearly show a sister-relationship between subfamilies Gastrocoptinae, Pupillinae and Vertigininae (Figures 4.2 and 4.3). According to the outgroup rooted analysis, the forming unreslove polytomy of *Gyliotrachela diarmaidi*, *G. kohrin*, *G. khaochongensis*, *G. saraburiensis* and *G. surakiti* clade is supported by bootstrap value 69 and diagnosed by at least apertural barrier characters: suprapalatal (character 18), interpalatal (character 20), supracolumellar (character 24) (Appendix II; Figure 4.2). This result is congruent with molecular phylogenies based on combined nuclear and mitochondrial ribosomal DNA data sets in this dissertation (Chapter 5, Figure 5.1). *Boysidia chiangmaiensis* and two *Vertigo* species forming clade (68 bootstrap value) which four palatal and columellar Apertural teeth synapomophyes: upper-palatal (character 19), lower-palatal (character 21), columellar (character 25) and subcolumellar (character 26) (Appendix II; Figure 4.3). However, many of these shell characters, including the informative ones, appear to be either controversial or poorly coded. For example, of tree synapomorphies supporting the *Gyliotrachela-Hypselostoma* clade, Character "lack or present of the suprapalatal Apertural barrier" is also the reversal character in *Gyliotrachela khaochongensis* species. Even the multi-state characters were treated as ordered, because of the obvious succession of their state, the polarized transformation series of each character is also difficult. The carelessly defined character states and omitted characters seriously undermine the accuracy of the phylogeny. However, using shell morphology alone may contain more homoplastic and increase the risk that the similarity of the character observed is not the result of the common ancestry but rather of parallel or convergent evolution (Schander and Sundberg, 2001). In this study, the high level of homoplasy, unsolved polytomies and unstable topologies (> 50 bootstrap value) were observed in Pupillid taxa. Probably, this was due to the large number of species studies, or it may reflect more complex evolutionary phenomena. Utilizing DNA sequences, combined analyses of DNA sequences and anatomical data is needed to complete a comprehensive phylogeny of the Pupillidae.

Table 4.1 Measurement shell characters for each Pupillid species. See Figure I.1 in Appendix I for explanation of characters.

Characters Species	n		SHA	BSH	SWA	SW	TL	UW	UW/ SW	SHA/ SWA	BSH/ SW
Anauchen angthongense	15	X	3.47	3.28	3.20	2.30	0.66	0.65	0.28	1.08	1.43
		±SE	0.0079	0.0061	0.0049	0.0061	0.0080	0.0032			
Ana. Chatnareeae	15	X	2.94	2.85	2.80	2.42	0.25	0.72	0.30	1.05	1.18
		±SE	0.0066	0.0062	0.0044	0.0068	0.0043	0.0027			
Ana. chedi	15	X	2.86	2.61	2.36	1.57	0.68	0.42	0.27	1.21	1.67
		±SE	0.0065	0.0045	0.0052	0.0064	0.0036	0.0040			
Ana. utaithaniensis	15	X	3.18	2.70	2.56	1.64	0.72	0.43	0.26	1.24	1.64
		±SE	0.0062	0.0081	0.0105	0.0062	0.0059	0.0048			
Antroapiculus pendulus	15	X	3.45	1.78	3.45	2.64	2.41	1.93	0.73	1.00	0.68
		±SE	0.0047	0.0038	0.0056	0.0062	0.0035	0.0042			
Aulacospira smaesarnensis	15	X	2.01	1.54	2.63	1.93	0.83	0.21	0.11	0.76	0.80
		±SE	0.0049	0.0064	0.0068	0.0055	0.0045	0.0053			
Boysidia chiangmaiensis	15	X	4.15	4.01	3.82	3.71	0.00	0.21	0.06	1.09	1.08
		±SE	0.2003	0.0116	0.0193	0.0154	0	0.0027			
Gyliotrachela adela	15	X	1.81	1.17	2.03	1.81	0.41	0.57	0.32	0.90	0.65
		±SE	0.0360	0.0333	0.0506	0.0606	0.0038	0.0063			
G. diarmaidi	15	X	2.38	1.66	3.44	1.99	1.23	1.08	0.54	0.70	0.83
		±SE	0.0360	0.0545	0.0838	0.0267	0.0107	0.0068			
G. khaochakan	15	X	3.12	2.99	3.00	2.05	0.43	0.76	0.37	1.04	1.46
		±SE	0.0179	0.0171	0.0070	0.0148	0.0052	0.0069			
G. khaochongensis	15	X	1.94	1.91	2.67	1.48	0.96	0.70	0.47	0.73	1.29
		±SE	0.0090	0.0082	0.0193	0.0114	0.0061	0.0076			
G. kohrin	15	X	1.77	1.64	2.92	1.85	0.53	0.71	0.39	0.61	0.89
		±SE	0.0088	0.0076	0.0106	0.0079	0.0038	0.0036			
G. saraburiensis	15	X	2.24	2.08	2.82	1.84	0.54	0.66	0.36	0.80	1.13
		±SE	0.0098	0.0065	0.0172	0.0089	0.0060	0.0067			
G. surakiti	15	X	2.50	1.88	4.44	2.27	1.82	1.03	0.46	0.56	0.83
		±SE	0.0134	0.0072	0.0175	0.0075	0.0165	0.0058			

Table 4.1 Continued.

Characters Species	n		SHA	BSH	SWA	SW	TL	UW	UW/ SW	SHA/ SWA	BSH/ SW
Hypselostoma cucumensis	15	X	2.10	1.78	3.27	1.90	0.75	1.03	0.54	0.64	0.94
		±SE	0.0097	0.0047	0.0094	0.0068	0.0079	0.0065			
H. erawan	15	X	1.57	1.56	2.72	1.75	0.34	1.06	0.60	0.58	0.89
		±SE	0.0092	0.0087	0.0129	0.0074	0.0069	0.0071			
H. holimamae	15	X	2.81	2.81	3.65	2.20	0.73	0.82	0.37	0.73	1.28
		±SE	0.0071	0.0071	0.0207	0.0304	0.0052	0.0072			
H. panhai	15	X	2.43	2.41	1.96	1.89	0.00	0.32	0.17	1.24	1.28
		±SE	0.0078	0.0077	0.0084	0.0049	0	0.0054			
H. taehwani	15	X	2.55	2.35	3.44	2.06	1.05	0.75	0.37	0.74	1.14
		±SE	0.0083	0.0074	0.0080	0.0102	0.0074	0.0049			
Krobylos maehongsonensis	15	X	4.15	3.73	3.46	3.05	0.00	0.17	0.06	1.20	1.22
		±SE	0.0067	0.0066	0.0077	0.0055	0	0.0029			
Montapiculus proboscidea	15	X	1.33	1.08	1.53	1.28	0.93	0.50	0.39	0.87	0.85
		±SE	0.0058	0.0046	0.0058	0.0056	0.0052	0.0039			
Paraboysidia phupaman	15	X	4.34	4.05	4.05	2.70	1.10	1.01	0.37	1.07	1.50
		±SE	0.0059	0.0064	0.0051	0.0042	0.0056	0.0052			
Systenostoma tamlod	15	X	1.27	1.05	1.06	0.91	0.13	0.24	0.27	1.19	1.15
		±SE	0.0055	0.0042	0.0041	0.0034	0.0032	0.0043			
Pupilla muscorum	15	X	3.23	2.98	1.85	1.66	0.00	0.13	0.08	1.74	1.80
		±SE	0.0073	0.0064	0.0066	0.0071	0	0.0025			
Vertigo gouldi	15	X	1.88	1.64	1.22	1.14	0.00	0.13	0.11	1.55	1.43
		±SE	0.0068	0.0042	0.0036	0.0060	0	0.0026			
Ve. shimochii	15	X	2.14	1.83	1.05	1.05	0.00	0.12	0.12	2.03	1.75
		±SE	0.0050	0.0048	0.0043	0.0045	0.0034				

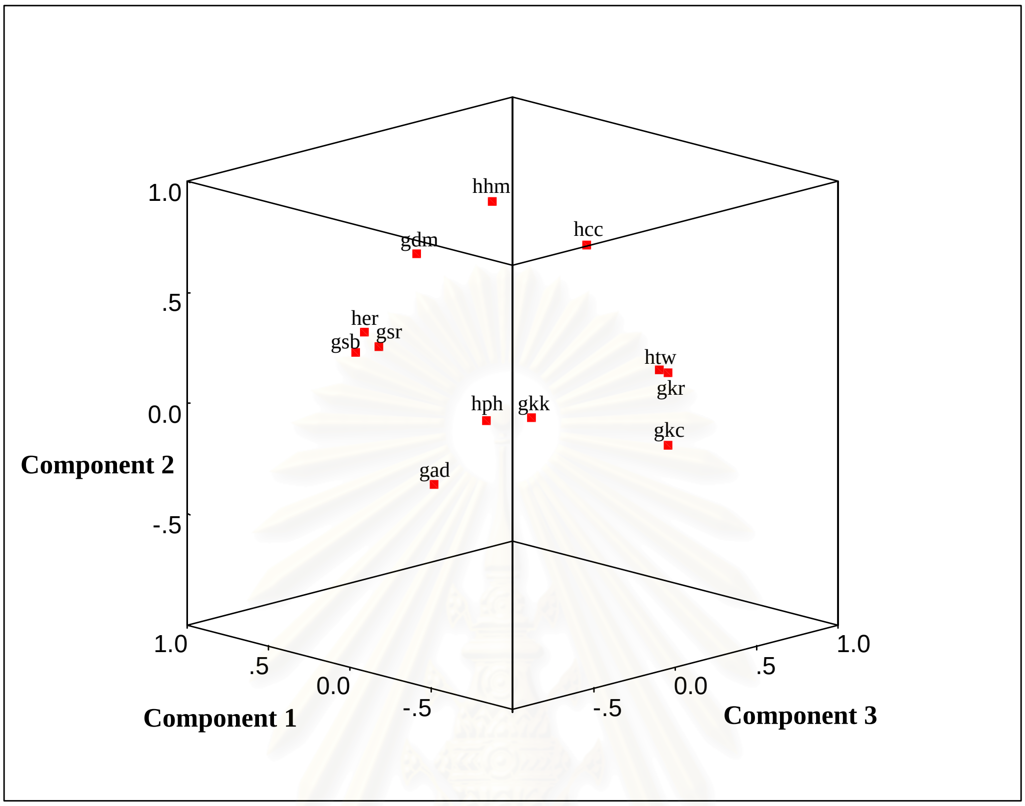


Figure 4.1 Component Plot in Rotated Space for *Gyliotrachela* and *Hypselostoma* species. Species are denoted 'gad' for *Gyliotrachela adela*, 'gdm' for *G. diarmaidi*, 'gkk' for *G. khaochakan*,'gkc' for *G. khaochongensis*,'gkr' for *G. kohrin*,'gsb' for *G. saraburiensis*,'gsr' for *G. surakiti*, 'hcc' for *Hypselostoma cucumensis*, 'her' for *H. erawan*, 'hhm' for *H. holimamae*, 'hph' for *H. panhai*, 'htw' for *H. taehwani*.

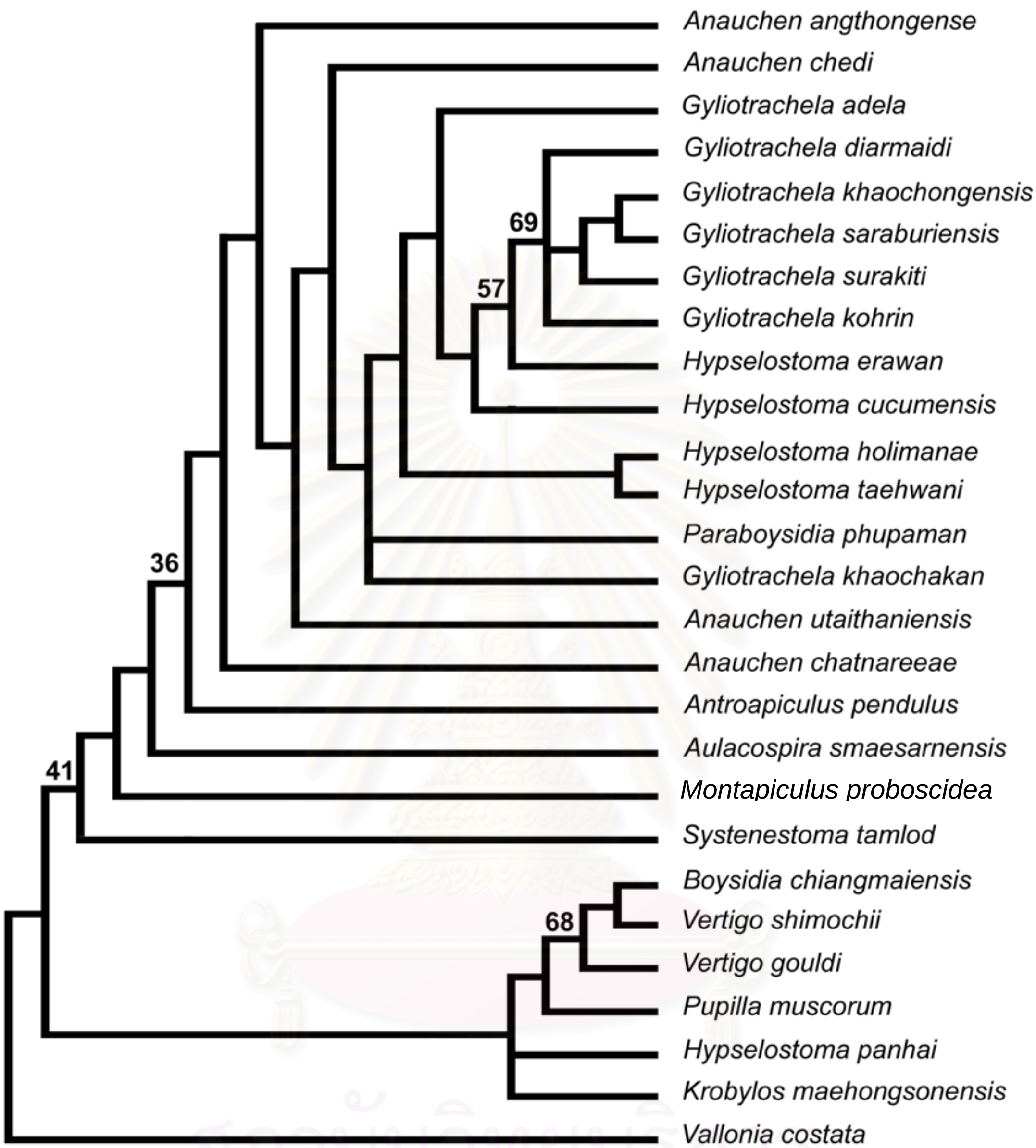


Figure 4.2 Strict concensus of 6 equally parsimonious trees (L =77, CI = 0.390, RI = 0.710) obtained from the analysis including outgroup taxa. Number above internodes are bootstrap values.

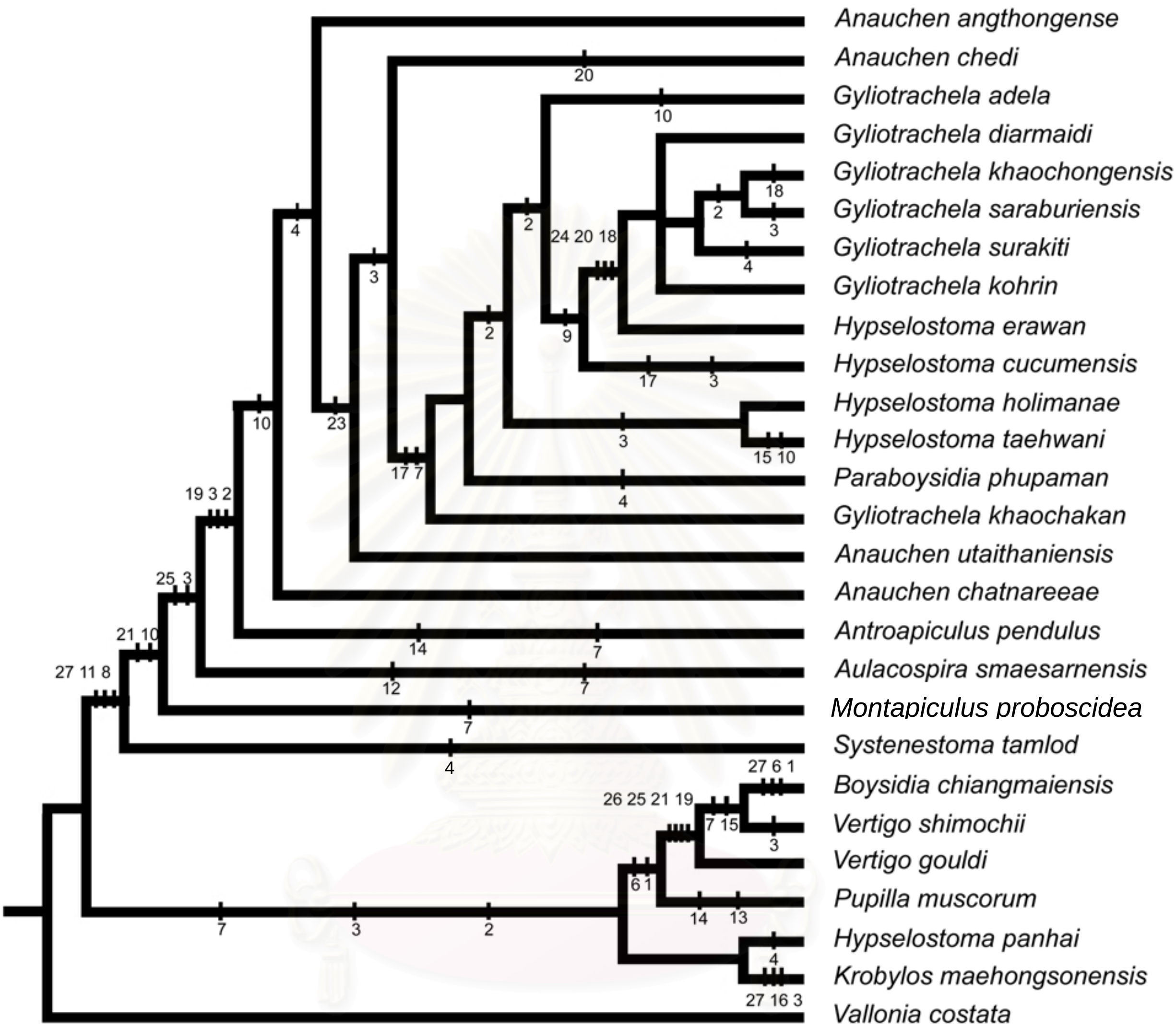


Figure 4.3 One of 6 equally pasimonious trees root by outgroup showing character transformations. The number at the bars denote the character number changing along that internode. Character numbers refer to those listed in Appendix II.

CHAPTER 5

PHYLOGENETIC RELATIONSHIPS OF THE CERTAIN THAI GASTROCOPTINAE (STYLOMMATOPHORA; PUPILLIDAE) INFERRED FROM MITOCHONDRIAL AND NUCLEAR RIBOSOMAL DNA SEQUENCES

5.1 Results

Data Characteristics

Seventeen pupillid taxa, including 2 valloniid and 1 strobilopsid species, were examined and 24 different hapotypes of two different gene fragments were obtained (Table 2.1). With the introduction of gaps, the ribosomal 16S data set contained 519 aligned sites, and of these 233 characters were parsimony-informative. All of the 725 nt target fragment of nuclear ribosomal 28S data were analyzed, and the number of parsimony-informative characters was 81 (Table 5.1). Considerable sequence length variation was observed in the combined 16S and 28S data sets (Appendix III). The average base frequencies for each gene fragment are summarized in Table 5.2. As expected, a strong AT bias is observed in mitochondrial gene. In the other hand, 28S nuclear ribosomal showed a low GC base frequencies.

Phylogenetic Analyses of combined data sets

Qualitative assays of both 16S and 28S gene fragments indicated that significant hierarchical information was contained in the two datasets. Highly skewed [g_1 = -0.947 ($p<0.01$) and g_1 = -0.517 ($p<0.01$) respectively] distributions were obtained for 10^5 randomly sampled trees (Hillis and Huelsenbeck, 1992) and all of 1000 random permutation trials (PTP test) resulted in higher tree lengths (Archie, 1989). Nevertheless, phylogenetic trees generated from these datasets were characterized by a preponderance of poorly supported nodes as determined by low bootstrap and decay-index values. To explore this issue further, Ratio of pairwise divergence values were compared to estimated maximum parsimony steps for both gene fragments to an idealized plot expected in the absence of multiple hits (Figure 5.1). For both 16S and 28S datasets, close agreement among observed and idealized values was obtained only for a small fraction of pairwise comparisons among the least diverged genotypes (Figure 5.1). This strongly indicates that phylogenetic signal in both mitochondrial and nuclear datasets has been diluted by considerable homoplasy.

Partition-Homogeneity (ILD; Farris *et al*., 1994) analysis of both mitochondrial and nuclear ribosomal datasets indicated that they were not significantly incongruent (p = 0.294) and all datasets were amalgamated to perform combined phylogenetic analyses. Tree statistics obtained from MP and ML analyses on individual as well as combined datasets are shown in Table 5.3. Figure 5.2 shows the ML tree topology obtained for the combined dataset. The Thai micro snail topological relationship generated by MP analysis differed slightly in that 2 relatively weak nodes, present in the maximum likelihood tree, collapsed (indicated by *s in Figure 5.2). A notable feature of the combined tree topology is that although included 15 Thai gastrocoptine species, only 2 terminal sister-species relationships were well supported by both ML and MP bootstrap values: *Hypselostoma erawan* and *H. panhai*; *Gyliotrachela kohrin* and *G. diarmaidi*. The other two well-supported interspecific nodes among the Thai taxa are the stem node for the entire Thai clade and the stem node for the predominently *Gyliotrachela* clade (*G. saraburiensis*(*G. surakiti*(*Aulacospira smaesarensis*(*G. diarmaidi*, *G. kohrin*)))). Subsampling of *Gyliotrachela* and *Hypselostoma*, the 2 most speciose Southeast Asian gastrocoptine genera, did not recover either as monophyletic, although all 11 nominal members of both genera sampled formed a weakly-supported paraphyletic clade. (Figure 5.2). The remaining 4 Thai gastrocoptine taxa formed a less than robust paraphyletic *Anauchen-Krobylos* clade.

A number of interesting geographical associations are evident in the phylogenetic tree topology (Figure 5.2). *Krobylos maehongsonensis* is atypical in that it has an extensive regional distribution and replicate samples from limestone hill ranges approximately 500 km apart (Figure 5.3), were robustly monophyletic (Figure 5.2). The remaining species that were sampled are characterized by much more restricted geographic ranges and many tip clades (Figure 5.2) were composed of inferred sister-relationships among geographically proximate taxa (Figure 5.3). These include *Anauchen chedi* and *A. utaithaniensis* in central Thailand, *Gyliotrachela diarmaidi*, *G. kohrin* and *Aulacospira smaesarnensis*, in south-central Thailand and nearby islands, *Gyliotrachela adela* and *Hypselostoma cucumensis* in southwestern Thailand and *Hypselostoma holimanae*, *H. panhai* and *H. erawan* in west-central Thailand.

Possibly the most intriguing result centres on the west-central tip clade of *Hypselostoma holimanae*, *H. panhai* and *H. erawan* (Figure 5.2). *H. panhai* was the only leaf litter dwelling species in Thai gastrocoptine survey and it was sampled within 10 meters of the limestone rock surface habitat from which the specimens of its inferred sister species *H. erewan* were obtained. In terms of general shell morphology, *H. panhai* is distinct from the other 10 members of the *Gyliotrachela*/*Hypselostoma* clade in that it has a pyramidal spire, an adnate terminal whorl and poorly developed or missing apertural barriers (Figure 5.4). In terms of traditional classification *panhai* lacks the diagnostic characterization of either *Gyliotrachela* or *Hypselostoma*, however, sculptural details of both the embryonic and adult shell exteriors (Figure 5.4), together with gene tree topological placement (Figure 5.2; confirmed by sequencing multiple *H. panhai* individuals for both 16S and 28S), support its sister status with the geographically proximate (Figure 5.3) *Hypselostoma erawan* and *H. holimanae*.

5.2 <u>Discussion</u>

The Thai gastrocoptinae molecular dataset is characterized by high levels of genetic differentiation and homoplasy (Figure 5.1) which results in low levels of nodal support throughout much of the resulting phylogenetic treespace (Figure 5.2). This pattern is by no means unique among land snail molecular phylogenetic studies, being also evident in a number of parallel mitochondrial datasets (Thomaz, Guiller and Clarke, 1996; Douris, Cameron, Rodakis and Lecanidour, 1998; Thacker and Hadfield, 2000). Why this should be the case is not yet clear. Thomaz *et al.* (1996) favored the hypothesis that land snail population structure (large numbers of demes with low inter-deme migration rates) could act to greatly prolong coalescence times (Slatkin, 1991), thereby allowing the persistence of ancient, genetically diverse, haplotypes. Accelerated molecular evolution, however cannot be ruled out and estimates of interspecific mitochondrial sequence divergences for many land snail taxa are up to 20 times faster (≥10% per million years) than those of other animal lineages (Chiba, 1999; Thacker and Hadfield, 2000; Hayashi and Chiba, 2000).

This study has no independent temporal reference points to calibrate observed Thai gastrocoptine divergence data. However, unrooted maximum likelihood trees of the mitochondrial dataset (Figure 5.5) demonstrate that members of tip Thai gastrocoptine clades can exhibit equivalent levels of topological definition to interfamilial taxonomic comparisons. This is consistent with the hypothesis that rates of molecular evolution among Thai gastrocoptine 16S mt rDNA are sufficiently high to have largely overwritten the phylogenetic signal in this gene fragment. In an effort to compensate for this, a fragment of nuclear 28S was additionally sequenced, which typically accrues changes more slowly than mt 16S (Hillis and Dixon, 1991), for the study taxa. Unfortunately, the 28S data also showed compelling evidence for saturation (Figure 5.1), did not significantly strengthen internal nodes in the Thai gastrocoptine topology (Figure 5.2), and indicated that the processes underlying rapid loss of phylogenetic signal in land snail molecular evolution may also apply to the nuclear genome. It would seem therefore that molecular phylogeneticists wishing to comprehensively study regional pupillid microsnails face distinct challenges, although the scope for resolving micro-evolutionary questions may be quite promising.

The primary goal of this study was to test the currently accepted shell-based taxonomy of Thai gastrocopines with molecular phylogenetic gene trees and, in this regard, which obtained mixed results. Although the fit of conchological taxonomy to gene tree topology is compromised to some degree by the preponderance of weakly supported nodes in the latter (Figure 5.2), the diagnostic apertural lamellar morphologies of the three genera with replicate samples (*Gyliotrachela*, *Hypselostoma* and *Anauchen*), are moderately successful in predicting molecular phylogenetic relationships. Nominal conspecifics of these three genera occupy contiguous sections of treespace, however all three genera are paraphyletic (Figure 5.2). It is perhaps a little surprising that the reciprocally monophyletic *Gyliotrachela* and *Hypselostoma* clades were not recovered. Pilsbry (1916-1918a) was strongly of the opinion that the two genera (distinguished primarily by parietal and angular lamellar morphology) were "independent derivatives from boysidioid ancestors, their resemblance in shape being due to parallel modifications in both series". The sampling strategy of this study was too limited to explicitly test this genealogical hypothesis, however the data indicate that not only shell shape, but also parietal and angular lamellar morphology are capable of extensive (especially reductive) evolutionary plasticity. For instance, *Aulacospira smaesarensis* is firmly nested within an otherwise exclusively *Gyliotrachela* tip clade (Figure 5.2), however in addition to a distinctive shell morphology, it contains a highly simplified set of apertural lamellae and completely lacks an angular lamella (Panha and Burch, 2001a).

Chiba (1999) found incongruence among molecular phylogenetic relationships and shell-based taxonomy for the land snail *Mandarina* and concluded that it stemmed from multiple evolutionary transitions in habitat resulting in convergent adaptive changes in shell morphology. This study have one apparent example of this process in Thai gastrocoptine dataset. *Hypselostoma panhai* occurs in leaf-litter habitats and is robustly sister to the geographically adjacent limestone rock dweller *H. erawan* (Figure 5.2). Although sharing protoconch and teloconch sculptural details, these sister taxa differ markedly in shell shape and in apertural lamellar repertoires (Figure 5.4). The placement of *H. panhai* in the gene trees (Figure 5.2) is consistent with an evolutionary transition from an ancestral limestone rock dweller having a free last whorl and well-developed apertural lamellae including wholly concrescent angular and parietal lamellae. Loss of these ancestral shell characters may have been driven by distinct selective pressures in the new leaf litter habitat and may be still ongoing for apertural lamellae. The sample of *H. panhai* exhibited considerable intrapopulational variation in apertural outline and lamellar development, including individuals bearing single parietal, palatal and columelar lamellae (Figure 5.4B2), those having single parietal and palatal lamellae (Figure 5.4B4), and (most commonly) specimens having a single reduced parietal lamella (Figure 5.4B5). Although the parietal lamella in some *H. panhai* specimens (Figure 5.4B4) bears traces of concresence (a possible vestige of the typical *Hypselostoma* condition?), the unambiguously singular parietal found in most specimens would, according to Pilsbry's (1917) taxonomic hierarchy, place these specimens in the genus *Anauchen*.

Although interesting, it is difficult to evaluate the general significance of the inferred ancestral *H. panhai* ecological transition, and associated morphological change, for southeast Asian gastrocoptine taxonomy and systematics. Is this a singular event of limited relevance, or have there been many such cryptic transitions, and if so, are such transitions uni- or bi-directional? The results do imply however that it could be seriously misleading to focus exclusively on the more easily sampled limestone rock gastrocoptine taxa and that a comprehensive sampling of leaf litter lineages is also required to fully flesh out phylogenetic relationships among regional pupillid microsnails.

There is a striking geographical dichotomy among studied taxa. Most of the species appear to be endemic to single hills or adjacent hill ranges (Thompson and Lee, 1988; Burch and Panha, 2000; Panha and Burch, 2001; Burch *et al*, 2002) and to have geographically proximate sister taxa from which they show considerable molecular, as well as morphological, definition (Figures 5.2, 5.3). This pattern is consistent with tight spatial restrictions on gene flow over cladogenically significant time periods. In sharp contrast, *Krobylos maehongsonensis* exhibited no significant morphological distinction, and maintained robust molecular monophyly (Figure 5.2), among discrete limestone hill populations approximately 500 kilometeres apart (Figure 5.3). Its type locality is in Mae Hong Son Province, adjacent to the Myanmar border (Panha and Burch, 1999a), which gives *K. maehongsonensis* a known collective north/south range within Thailand of approximately 700 km. A similar pattern of morphological (Benthem Jutting, 1950) and molecular (Schilthuizen *et al*., 1999) cohesiveness over an extensive geographic range has been documented for *Gyliotrachela hungerfordiana* in the karst hills of Malaysia. It would appear that these two species experience fundamentally different patterns of gene flow, colonization and cladogenesis from the bulk of regional karst hill microsnails, although the underlying mechanism(s) [a lack of exclusivity to karst hill habitats? (Schilthuizen *et al*., 1999)] remain obscure.

This study was hampered by considerable molecular homoplasy, its restriction to a Thai subsample of southeast Asian gastrocoptine lineages, and the absence of type species, however some useful pointers emerged for future research on these compelling regional pupillid microsnails. Although Pilsbry's (1917) taxonomic characters have phylogenetic relevance, the results caution against the unquestioned use of apertural lamellar morphology as diagnostic generic characters. This study findings also emphasize the utility of geographically proximate taxa for establishing sister relationships, especially concerning locally endemic species (irrespective of apparent morphological similarity). Widely used mitochondrial and nuclear gene fragments may be of limited utility for broad phylogenetic questions concerning these taxa, but could prove to be very insightful for microevolutionary questions. Finally, researchers of regional gastrocoptines should be aware of the potential for ecological transitions followed by profound morphological change. The serious efforts be made to include a comprehensive sampling of both leaf litter and limestone-rock-dwelling species within the geographic area of interest, should be considered the future study.

Table 5.1 Molecular character statistics for each partition

	Number of Characters			
Partition	Total	Constant	Uninformative	Informative
16S	519	234	52	233
28S	725	540	104	81
16S+28S	1244	774	156	314

Table 5.2 Average base frequencies for each original and combined data sets

	Base			
Data sets	A	C	G	T
16S	35.6	12.0	16.4	36.0
28S	17.8	31.3	35.0	15.8
16S+28S	25.3	23.1	27.2	24.4

Table 5.3 Tree statistics obtained for individual and combined molecular datasets.

	Maximum Parsimony (MP)				Maximum Likelihood (ML)		
Partition	No. of trees	Tree length	CI	RI	Best-fit model	No. of trees	-Ln
16S	1	1045	0.474	0.500	TVM+I+Γ	3	4816.8161
28S	4	360	0.708	0.655	TrN+I+Γ	1	2820.3150
Combined	4	1420	0.528	0.523	TVM+I+Γ	1	7964.8196

CI: Consistency Index

RI: Retention Index

TVM: f_A, f_C, f_G, f_T, *r*AC, *r*AT, *r*CG, *r*GT, *r*AG = *r*CT (Posada and Crandall, 2001), a submodel of GTR (Rodríguez, Oliver, Marín and Medina, 1990)

TrN: f_A, f_C, f_G, f_T, *r*AC = *r*AT = *r*CG = *r*GT, *r*AG, *r*CT (Tamura and Nei, 1993)

I: proportional invariant sites

Γ: gamma-distributed heterogeneity of the substitution rate across site (Yang 1994)

Figure 5.1 Plots of pairwise sequence difference and inferred MP steps for 16S (A) and 28S (B) datasets of land snail study taxa (Table 5.3). In each chart, the pairwise sequence differences (*Y* axis) were plotted against the respective calculated maximum parsimony branch lengths (*X* axis). Parsimony branch lengths were determined from one of four most parsimonious combined (16S+28S) trees obtained. Assuming that the analysis recovered the correct phylogeny, those pairwise comparisons free of saturation (multiple substitutions at the same site) are expected to overlay the respective dotted diagonals (Philippe *et al*., 1994). In both gene fragments, this expectation is met by only a small minority of the least divergent comparisons.

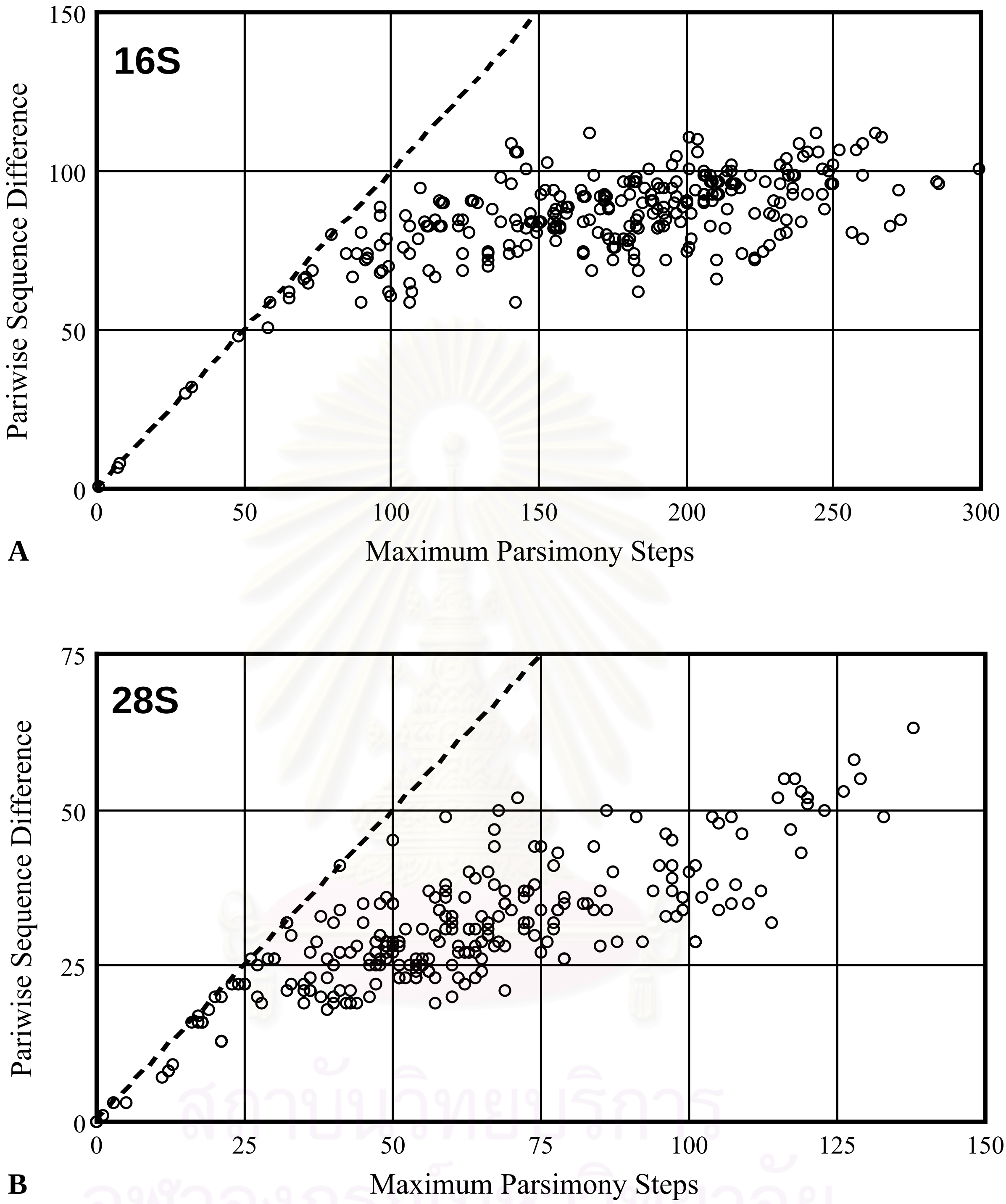
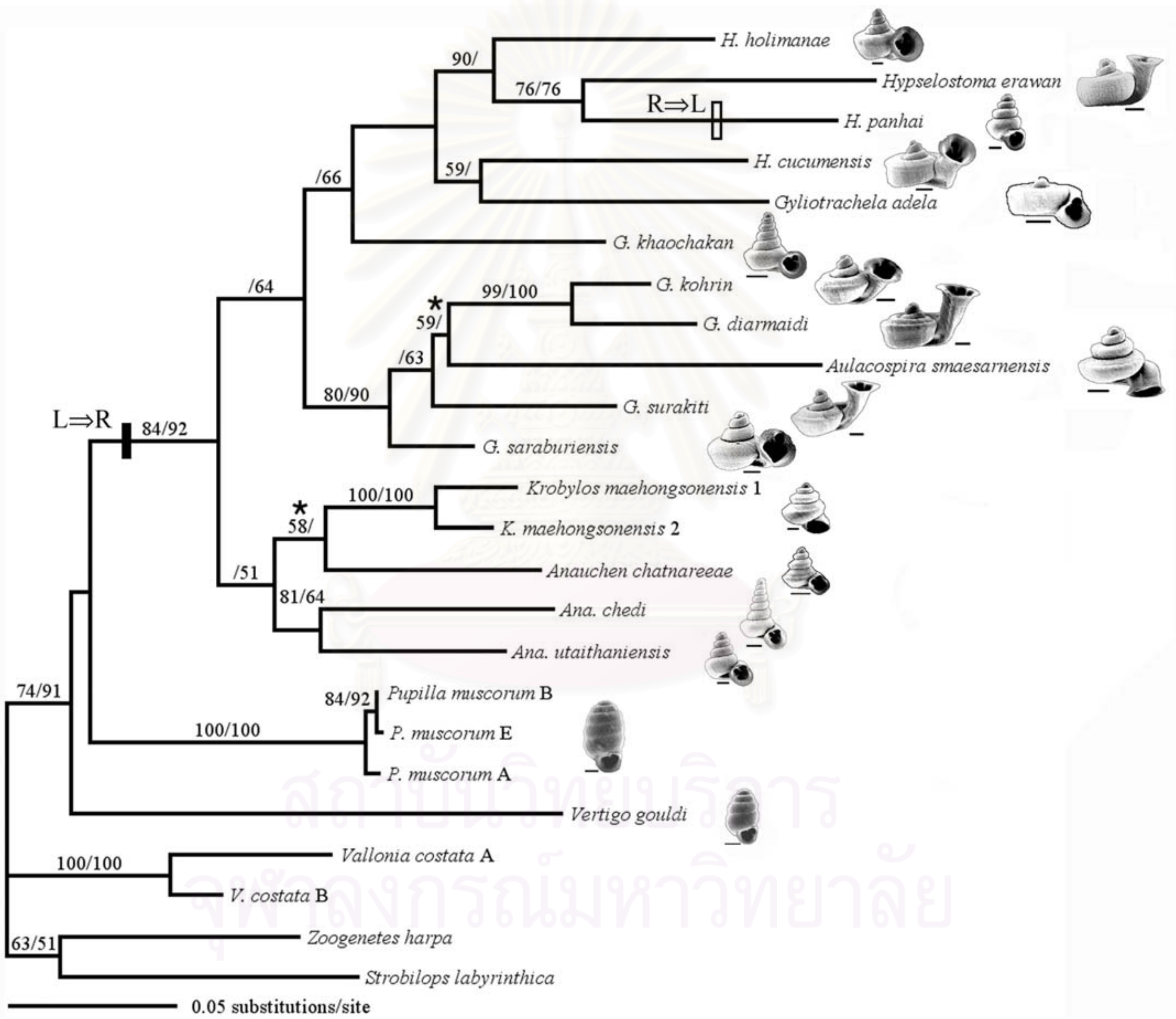

16S
Pariwise Sequence Difference
0
50
100
150
0
50
100
150
200
250
300
Maximum Parsimony Steps
A
28S
Pariwise Sequence Difference
0
25
50
75
0
25
50
75
100
125
150
Maximum Parsimony Steps
B

Figure 5.2 Maximum-likelihood phylogram (lnL = -7964.8196) of the combined mt 16S and nuclear 28S land snail dataset in which the non-pupillid taxa [*Strobilops labyrinthica* (Strobilopsidae), *Vallonia costata* and *Zoogenetes harpa* (Vallonidae)] were designated as outgroups. Numbers above internal branches show the estimated bootstrap support values (>50; ML/MP respectively) for each node. The ML and MP topologies were largely congruent except that two nodes, indicated by *s, collapsed in the latter analysis. Inferred evolutionary transitions among leaf litter and limestone rock habitats are indicated: leaf litter to rock (L⇒R) on the stem branch supporting the Thai gastrocoptine clade; reversal to leaf litter habitat (R⇒L) on the terminal *Hypselostoma panhai* branch. See Table 2 to identify the respective multiple sampling locations of *Vallonia costata*, *Pupilla muscorum* and *Krobylos maehongsonensis*.


H. holimanae
Hypselostoma erawan
H. panhai
R⇒L
H. cucumensis
Gyliotrachela adela
G. khaochakan
G. kohrin
G. diarmaidi
Aulacospira smaesarnensis
G. surakiti
G. saraburiensis
Krobylos maehongsonensis 1
K. maehongsonensis 2
Anauchen chatnareeae
Ana. chedi
Ana. utaithaniensis
Pupilla muscorum B
P. muscorum E
P. muscorum A
Vertigo gouldi
Vallonia costata A
V. costata B
Zoogenetes harpa
Strobilops labyrinthica
L⇒R
90/
76/76
59/
/66
/64
99/100
59/
/63
80/90
84/92
100/100
58/
/51
81/64
74/91
84/92
100/100
100/100
63/51
0.05 substitutions/site

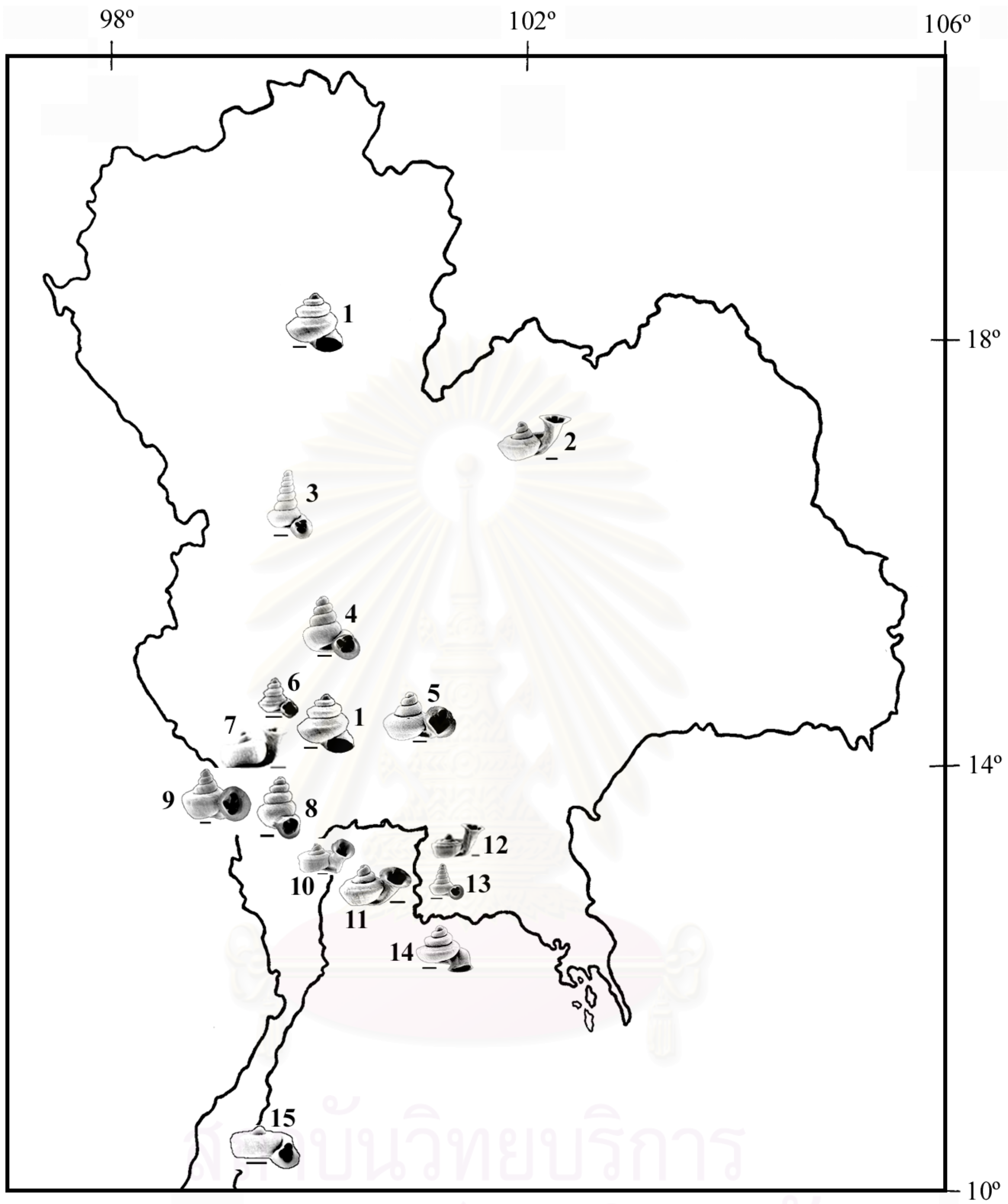


Figure 5.3 Distribution map indicating approximate sampling locations of gastrocoptine study taxa within Thailand; see Table 3.3 for precise locations. Key to species identification: 1. *Krobylos maehongsonensis, 2. Gyliotrachela surakiti*, 3. *Anauchen chedi*, 4. *Ana. utaithaniensis*, 5. *G. saraburiensis*, 6. *Ana. chatnareeae*, 7. *Hypselostoma erawan*, 8. *H. panhai*, 9. *H. holimanae*, 10. *H. cucumensis*, 11. *G. kohrin*, 12. *G. diarmaidi*, 13. *G. khaochakan*, 14. *Aulacospira smaesarnensis*, 15. *G. adela*.

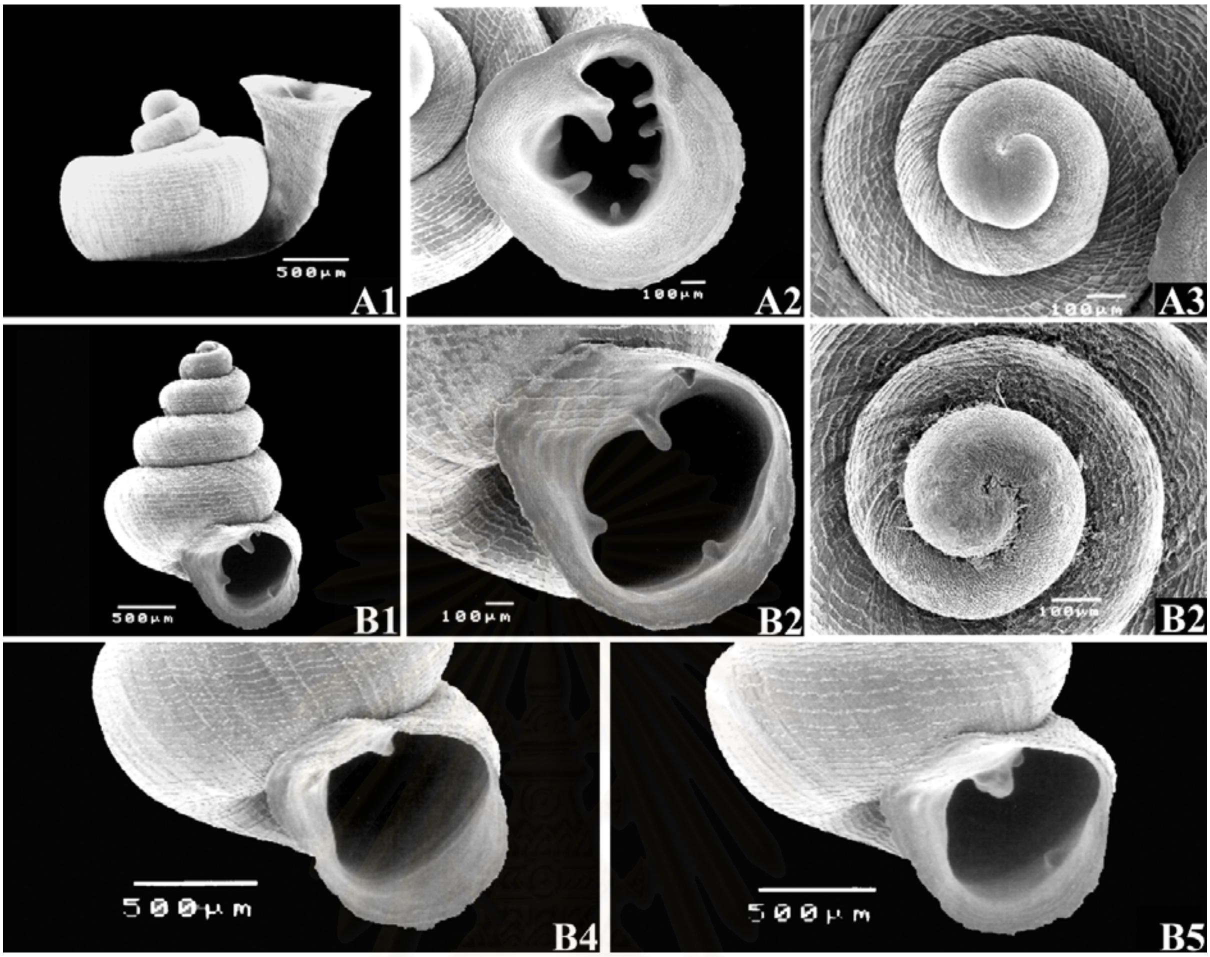


Figure 5.4 Scanning electron micrographs showing conchological details of two *Hypselostoma* sister species (Figures 5.2, 5.5), sampled within meters of each other on Chongkhaokad Hill, Kanchanaburi, Thailand. A1-A3: Views of the limestone-rock-dweller *H. erawan*'s gross shell morphology (A1); apertural lamellae (A2); apex (A3). B1-B3: Views of the leaf-litter species *H. panhai*'s gross shell morphology (B1); apertural lamellae (B2); apex (B3). Note the sculptural similarity among the two species in which the predominant detail is the presence of raised spiral threads that are relatively uniformly distributed over the shell surface, a condition also found in the co-clustering (Figs 5.2, 5.5), Kanchanaburi co-endemic, *H. holimanae* (Thompson and Lee, 1988). Considerable among-individual variation in apertural outline and lamellar development was observed in *H. erawan* sample (B2, B4, B5), although the condition depicted in B4 was the one most commonly encountered.

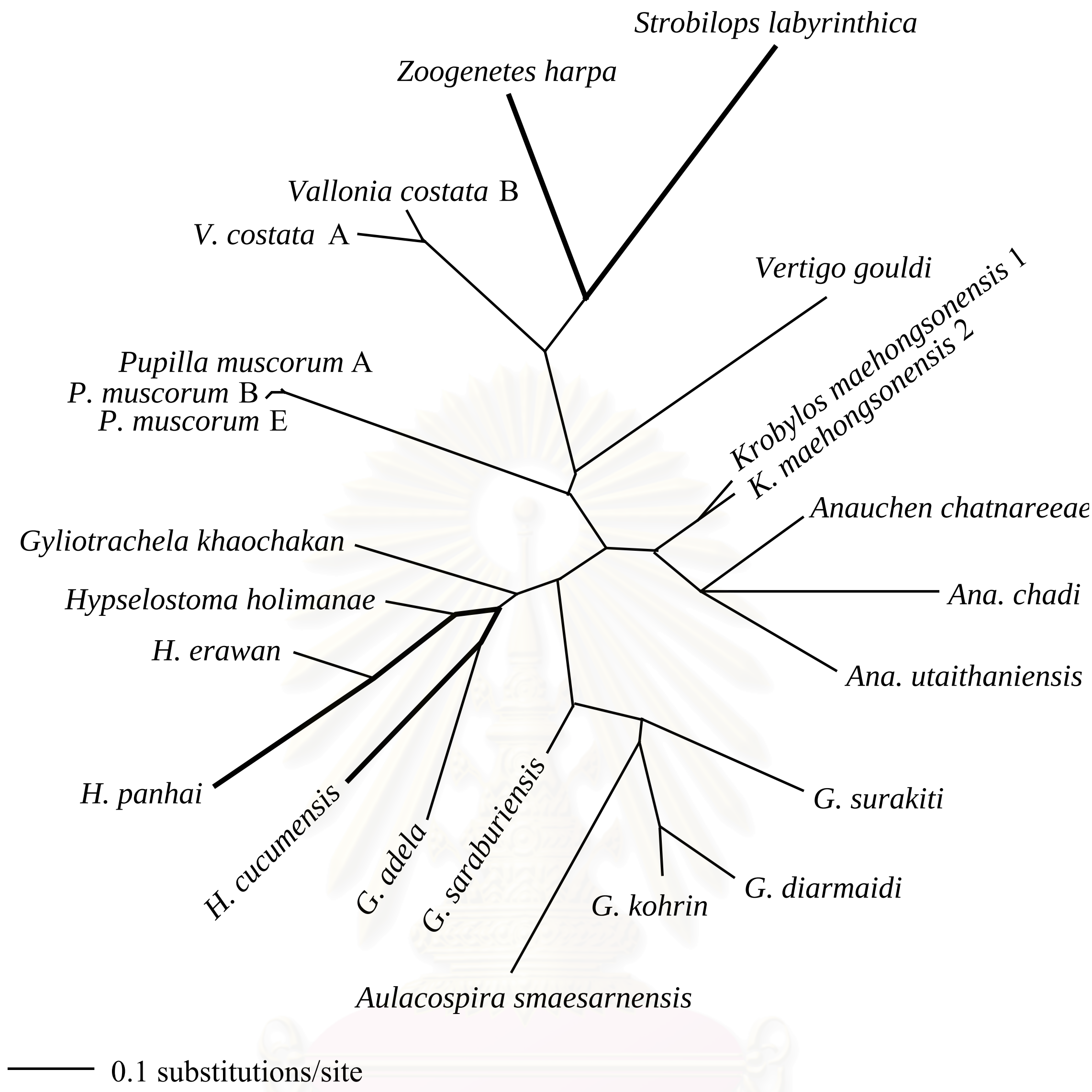


Figure 5.5 Unrooted maximum-likelihood phylogram (lnL = -4816.8161) of the land snail mt 16S rDNA dataset. Note that some of the branch lengths (in bold) connecting taxa placed in separate families [*e.g.*, the outgroup taxa *Strobilops labyrinthica* (Strobilopsidae), *Zoogenetes harpa* (Vallonidae)] are approximately equivalent to a subsample of branches joining geographically proximate (Fig. 3) tip clade Thai gastrocoptines, *e.g.*, *Hyselostoma panhai* and *H. cucumensis*.

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APPENDICES

APPENDIX I

SHELL MORPHOLOGICAL CHARACTERS FOR MORPHOMETRIC ANALYSIS OF THAI PUPILLID GENERA

Shell Measurement Characters

1. Shell height, including aperture lip, or tuba if present (SHA)
2. Height of body whorl and spire (BSH)
3. Shell width, including aperture lip, or tuba if present (SWA)
4. Width of body whorl (to where tuba begins) (SW)
5. Length of tuba (TL)
6. Width of umbilicus (UW)

The width of the umbilicus was measured from where the coil of the body whorl diverges from the regular coiling direction of the shell to the opposite side of the umbilicus. For those species with a sulcus (groove) in the umbilicus wall, two measurements were taken, one from suture to opposing suture, and, the other from the edge of the sulcus on one side of the umbilicus to the edge of the opposing sulcus on the opposite side of the umbilicus. The latter measurement was taken as the width of the umbilicus. All measurement terminology showed in figure I.1.

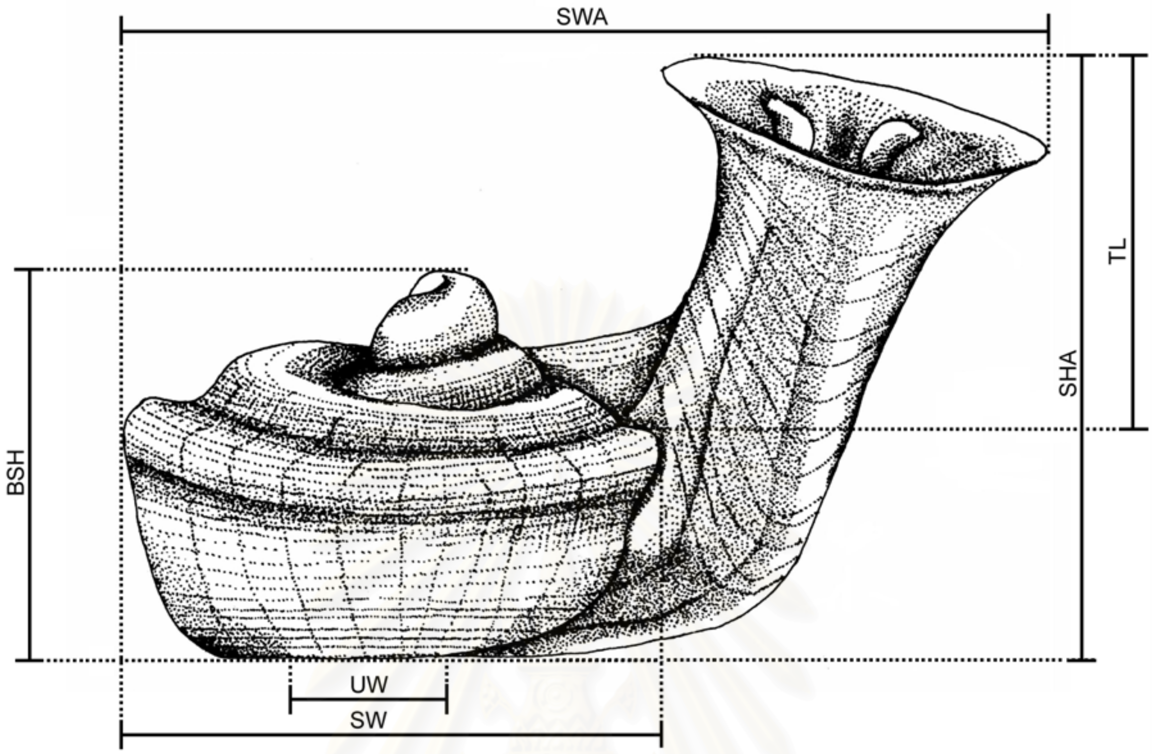

Figure I.1 Shell measurement terminology (Type from *Gyliotrachela diarmaidi*)

- Shell height, including aperture lip, or tuba if present (SHA)
- Height of body whorl and spire (BSH)
- Shell width, including aperture lip, or tuba if present (SWA)
- Width of body whorl (to where tuba begins) (SW)
- Length of tuba (TL)
- Width of umbilicus (UW)

Table I.1 Shell measurement of 15 individuals of *Anauchen angthongense*

Individual	*Anauchen angthongense*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	3.49	3.26	3.19	2.28	0.63	0.64
2	3.52	3.31	3.18	2.25	0.67	0.65
3	3.48	3.27	3.21	2.33	0.63	0.66
4	3.45	3.25	3.20	2.31	0.69	0.65
5	3.44	3.30	3.19	2.34	0.71	0.64
6	3.40	3.32	3.22	2.29	0.63	0.63
7	3.51	3.27	3.24	2.27	0.60	0.67
8	3.49	3.28	3.20	2.28	0.69	0.65
9	3.47	3.25	3.21	2.30	0.68	0.66
10	3.47	3.29	3.20	2.30	0.67	0.64
11	3.46	3.30	3.19	2.31	0.67	0.67
12	3.44	3.32	3.17	2.32	0.70	0.67
13	3.44	3.28	3.17	2.31	0.64	0.65
14	3.48	3.26	3.20	2.28	0.65	0.65
15	3.48	3.29	3.22	2.29	0.65	0.64

Table I.2 Shell measurement of 15 individuals of *Anauchen chatnareeae*

Individual	*Anauchen. chatnareeae*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.97	2.86	2.79	2.39	0.23	0.72
2	2.98	2.89	2.82	2.45	0.24	0.73
3	2.90	2.85	2.77	2.42	0.25	0.71
4	2.96	2.88	2.81	2.37	0.28	0.72
5	2.94	2.88	2.80	2.41	0.26	0.71
6	2.91	2.84	2.80	2.41	0.27	0.71
7	2.91	2.85	2.81	2.43	0.25	0.72
8	2.93	2.86	2.78	2.44	0.25	0.70
9	2.95	2.86	2.77	2.46	0.28	0.74
10	2.93	2.83	2.78	2.39	0.27	0.72
11	2.94	2.84	2.80	2.40	0.24	0.71
12	2.98	2.89	2.82	2.40	0.25	0.73
13	2.92	2.82	2.81	2.44	0.27	0.73
14	2.92	2.81	2.79	2.45	0.24	0.72
15	2.95	2.85	2.78	2.41	0.23	0.71

Table I.3 Shell measurement of 15 individuals of *Anauchen chedi*

Individual	*Anauchen. chedi*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.87	2.63	2.36	1.59	0.69	0.40
2	2.91	2.59	2.33	1.54	0.68	0.45
3	2.84	2.63	2.39	1.61	0.70	0.43
4	2.85	2.61	2.38	1.56	0.71	0.42
5	2.83	2.61	2.36	1.56	0.67	0.41
6	2.84	2.61	2.34	1.59	0.68	0.43
7	2.90	2.59	2.35	1.55	0.67	0.40
8	2.87	2.63	2.35	1.53	0.69	0.42
9	2.87	2.58	2.37	1.54	0.70	0.41
10	2.86	2.64	2.39	1.57	0.66	0.42
11	2.83	2.62	2.33	1.57	0.68	0.44
12	2.85	2.62	2.38	1.60	0.67	0.43
13	2.90	2.60	2.36	1.54	0.67	0.43
14	2.87	2.61	2.37	1.56	0.69	0.42
15	2.88	2.63	2.34	1.59	0.68	0.45

Table I.4 Shell measurement of 15 individuals of *Anauchen utaithaniensis*

Individual	*Anauchen. utaithaniensis*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	3.18	2.64	2.51	1.60	0.70	0.44
2	3.21	2.73	2.63	1.67	0.72	0.46
3	3.14	2.69	2.53	1.61	0.73	0.45
4	3.17	2.67	2.61	1.63	0.74	0.43
5	3.20	2.70	2.57	1.65	0.71	0.41
6	3.19	2.71	2.55	1.63	0.69	0.45
7	3.19	2.74	2.60	1.62	0.75	0.45
8	3.18	2.76	2.61	1.68	0.71	0.41
9	3.17	2.70	2.56	1.66	0.70	0.43
10	3.20	2.69	2.56	1.67	0.73	0.40
11	3.21	2.68	2.53	1.66	0.74	0.44
12	3.23	2.75	2.62	1.64	0.75	0.42
13	3.19	2.69	2.54	1.67	0.70	0.41
14	3.16	2.71	2.52	1.63	0.69	0.44
15	3.15	2.70	2.52	1.65	0.68	0.45

Table I.5 Shell measurement of 15 individuals of *Antroapiculus pendulus*

Individual	*Antroapiculus pendulus*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	3.45	1.78	3.46	2.61	2.41	1.93
2	3.48	1.80	3.44	2.63	2.42	1.91
3	3.44	1.79	3.45	2.66	2.44	1.93
4	3.42	1.76	3.48	2.60	2.40	1.94
5	3.44	1.78	3.49	2.64	2.41	1.96
6	3.47	1.77	3.44	2.65	2.43	1.93
7	3.45	1.77	3.42	2.60	2.43	1.93
8	3.47	1.79	3.43	2.67	2.40	1.92
9	3.43	1.76	3.43	2.65	2.41	1.91
10	3.45	1.80	3.47	2.64	2.42	1.90
11	3.45	1.81	3.48	2.63	2.41	1.91
12	3.43	1.77	3.45	2.60	2.43	1.92
13	3.48	1.79	3.43	2.66	2.40	1.92
14	3.45	1.79	3.45	2.66	2.40	1.94
15	3.46	1.78	3.47	2.65	2.40	1.95

Table I.6 Shell measurement of 15 individuals of *Aulacospira smaesarnensis*

Individual	*Aulacospira smaesarnensis*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.99	1.53	2.63	1.90	0.83	0.21
2	2.05	1.53	2.67	1.93	0.82	0.23
3	2.01	1.55	2.64	1.90	0.80	0.20
4	2.02	1.56	2.63	1.92	0.83	0.22
5	2.02	1.52	2.66	1.95	0.85	0.20
6	2.04	1.54	2.62	1.94	0.84	0.19
7	1.98	1.55	2.64	1.96	0.86	0.16
8	1.99	1.54	2.64	1.90	0.82	0.21
9	2.02	1.59	2.63	1.91	0.83	0.21
10	2.01	1.51	2.61	1.95	0.81	0.20
11	2.03	1.50	2.63	1.95	0.84	0.25
12	2.01	1.58	2.56	1.93	0.82	0.22
13	2.00	1.57	2.66	1.94	0.85	0.22
14	2.02	1.54	2.66	1.92	0.86	0.20
15	2.02	1.53	2.64	1.90	0.84	0.19

Table I.7 Shell measurement of 15 individuals of *Boysidia chiangmaiensis*

Individual	*Boysidia chiangmaiensis*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	4.18	4.05	3.84	3.69	0.00	0.22
2	4.01	3.94	3.83	3.66	0.00	0.23
3	4.26	4.05	3.87	3.68	0.00	0.21
4	4.10	3.97	3.74	3.65	0.00	0.22
5	4.14	4.01	3.69	3.58	0.00	0.20
6	4.20	4.01	3.81	3.72	0.00	0.21
7	4.28	4.10	3.91	3.80	0.00	0.22
8	4.05	3.98	3.76	3.70	0.00	0.21
9	4.11	3.96	3.77	3.69	0.00	0.20
10	4.15	4.02	3.88	3.72	0.00	0.20
11	4.21	4.07	3.89	3.73	0.00	0.19
12	4.23	4.03	3.82	3.74	0.00	0.21
13	4.06	3.96	3.76	3.67	0.00	0.20
14	4.13	3.99	3.81	3.77	0.00	0.21
15	4.19	4.02	3.98	3.81	0.00	0.20

Table I.8 Shell measurement of 15 individuals of *Gyliotrachela adela*

Individual	*Gyliotrachela adela*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.81	1.28	1.91	1.62	0.43	0.59
2	1.72	1.03	1.86	1.54	0.41	0.56
3	1.58	1.05	1.87	1.60	0.40	0.55
4	2.00	1.25	2.41	2.25	0.45	0.57
5	1.67	1.08	1.83	1.70	0.41	0.58
6	1.83	1.09	1.93	1.67	0.40	0.59
7	1.94	1.18	1.98	1.69	0.39	0.60
8	1.63	1.01	1.87	1.64	0.41	0.54
9	1.77	1.08	1.91	1.72	0.42	0.55
10	1.68	1.15	1.97	1.74	0.43	0.58
11	1.82	1.29	1.92	1.64	0.40	0.59
12	1.98	1.45	2.27	2.01	0.41	0.61
13	1.97	1.28	2.13	2.08	0.41	0.54
14	1.78	1.02	2.36	2.18	0.41	0.53
15	1.99	1.24	2.23	2.05	0.41	0.58

Table I.9 Shell measurement of 15 individuals of *Gyliotrachela diarmaidi*

Individual	*Gyliotrachela diarmaidi*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.28	1.45	3.14	1.82	1.18	1.07
2	2.21	1.47	3.56	1.97	1.23	1.05
3	2.10	1.65	3.78	2.15	1.32	1.10
4	2.54	1.57	3.18	1.96	1.21	1.08
5	2.41	1.93	3.99	2.20	1.17	1.05
6	2.56	1.84	3.28	2.04	1.25	1.07
7	2.48	1.49	3.06	1.87	1.22	1.12
8	2.43	1.74	3.81	2.09	1.28	1.11
9	2.17	1.16	2.98	1.93	1.22	1.10
10	2.31	1.92	3.43	2.04	1.19	1.08
11	2.54	1.65	3.24	2.01	1.28	1.09
12	2.38	1.61	3.43	1.97	1.27	1.12
13	2.45	1.73	3.12	1.93	1.23	1.05
14	2.38	1.82	3.84	1.89	1.22	1.04
15	2.44	1.87	3.69	2.04	1.21	1.09

Table I.10 Shell measurement of 15 individuals of *Gyliotrachela khaochakan*

Individual	*Gyliotrachela khaochakan*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.92	2.83	2.96	1.98	0.43	0.76
2	3.18	3.06	3.01	2	0.48	0.74
3	3.11	3.01	3	2.15	0.44	0.78
4	3.15	2.96	3.03	2.06	0.42	0.75
5	3.18	2.99	3.01	2.04	0.45	0.79
6	3.09	2.84	2.95	1.99	0.41	0.81
7	3.18	3.02	3.02	2.04	0.42	0.74
8	3.17	3.01	3	2.03	0.43	0.73
9	3.12	3.03	3.01	2.11	0.43	0.78
10	3.11	3.01	3.04	2.1	0.41	0.77
11	3.1	3.01	3.03	2.09	0.4	0.73
12	3.05	3.02	3.03	2.14	0.45	0.71
13	3.15	3.04	3.01	2.05	0.43	0.77
14	3.17	2.99	2.98	1.99	0.43	0.78
15	3.18	2.98	2.97	1.98	0.41	0.75

Table I.11 Shell measurement of 15 individuals of *Gyliotrachela khaochongensis*

Individual	*Gyliotrachela khaochongensis*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.91	1.89	2.77	1.43	0.98	0.71
2	1.94	1.92	2.74	1.42	0.96	0.70
3	1.96	1.93	2.54	1.51	0.93	0.68
4	1.95	1.91	2.76	1.54	0.97	0.74
5	1.94	1.91	2.69	1.41	0.99	0.65
6	1.98	1.95	2.65	1.48	0.94	0.68
7	2.00	1.98	2.67	1.56	0.93	0.73
8	1.98	1.95	2.72	1.53	0.97	0.72
9	1.93	1.90	2.74	1.48	0.96	0.69
10	1.91	1.89	2.71	1.47	0.97	0.65
11	1.89	1.87	2.68	1.50	0.94	0.73
12	1.87	1.86	2.63	1.51	0.93	0.72
13	1.94	1.91	2.59	1.46	0.98	0.69
14	1.96	1.94	2.56	1.45	0.97	0.67
15	1.95	1.91	2.58	1.50	0.91	0.73

Table I.12 Shell measurement of 15 individuals of *Gyliotrachela kohrin*

Individual	*Gyliotrachela kohrin*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.78	1.68	2.96	1.87	0.54	0.71
2	1.76	1.65	2.94	1.88	0.55	0.71
3	1.72	1.63	2.90	1.90	0.56	0.69
4	1.75	1.59	2.98	1.85	0.53	0.70
5	1.79	1.68	2.93	1.84	0.54	0.73
6	1.80	1.69	2.99	1.89	0.55	0.71
7	1.76	1.64	2.91	1.80	0.51	0.70
8	1.70	1.61	2.89	1.81	0.52	0.73
9	1.81	1.67	2.93	1.84	0.54	0.71
10	1.74	1.64	2.93	1.86	0.52	0.74
11	1.76	1.63	2.91	1.87	0.54	0.72
12	1.78	1.64	2.89	1.86	0.53	0.71
13	1.79	1.61	2.84	1.83	0.51	0.70
14	1.73	1.63	2.87	1.81	0.52	0.73
15	1.82	1.67	2.88	1.82	0.53	0.72

Table I.13 Shell measurement of 15 individuals of *Gyliotrachela saraburiensis*

Individual	*Gyliotrachela saraburiensis*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.32	2.11	2.72	1.81	0.52	0.67
2	2.23	2.04	2.73	1.80	0.54	0.64
3	2.21	2.09	2.87	1.87	0.58	0.69
4	2.25	2.05	2.84	1.84	0.52	0.63
5	2.27	2.07	2.81	1.82	0.51	0.61
6	2.30	2.05	2.79	1.83	0.54	0.68
7	2.24	2.07	2.83	1.81	0.55	0.64
8	2.23	2.06	2.84	1.81	0.53	0.67
9	2.21	2.08	2.87	1.87	0.53	0.67
10	2.22	2.07	2.85	1.84	0.54	0.68
11	2.25	2.04	2.72	1.83	0.57	0.69
12	2.26	2.11	2.76	1.80	0.55	0.64
13	2.24	2.12	2.78	1.82	0.56	0.66
14	2.25	2.08	2.90	1.88	0.51	0.63
15	2.16	2.09	2.94	1.92	0.58	0.69

Table I.14 Shell measurement of 15 individuals of *Gyliotrachela surakiti*

Individual	*Gyliotrachela surakiti*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.45	1.87	4.31	2.23	1.92	1.01
2	2.48	1.86	4.39	2.28	1.71	1.04
3	2.40	1.89	4.40	2.27	1.81	1.03
4	2.60	1.93	4.53	2.31	1.82	1.04
5	2.54	1.88	4.55	2.29	1.85	1.06
6	2.53	1.89	4.44	2.26	1.84	1.03
7	2.49	1.91	4.46	2.30	1.89	1.05
8	2.48	1.87	4.48	2.28	1.86	1.02
9	2.51	1.86	4.49	2.25	1.74	1.03
10	2.47	1.91	4.36	2.23	1.79	1.06
11	2.53	1.90	4.39	2.22	1.91	1.04
12	2.57	1.83	4.38	2.24	1.83	1.03
13	2.49	1.89	4.41	2.28	1.87	1.01
14	2.55	1.92	4.45	2.27	1.79	0.99
15	2.45	1.85	4.51	2.31	1.74	1.08

Table I.15 Shell measurement of 15 individuals of *Hypselostoma cucumensis*

Individual	*Hypselostoma cucumensis*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.08	1.79	3.29	1.91	0.75	1.02
2	2.05	1.77	3.23	1.85	0.73	1.03
3	2.06	1.78	3.33	1.93	0.78	1.04
4	2.09	1.81	3.34	1.94	0.76	1.02
5	2.08	1.80	3.32	1.93	0.79	1.01
6	2.07	1.76	3.25	1.87	0.74	1.03
7	2.14	1.78	3.24	1.88	0.78	1.04
8	2.16	1.77	3.28	1.91	0.75	1.09
9	2.05	1.74	3.24	1.90	0.71	1.06
10	2.13	1.79	3.30	1.86	0.70	1.07
11	2.11	1.76	3.28	1.89	0.69	1.02
12	2.08	1.77	3.25	1.92	0.74	1.01
13	2.07	1.77	3.28	1.91	0.78	1.05
14	2.14	1.78	3.23	1.89	0.77	0.98
15	2.15	1.80	3.25	1.90	0.75	1.03

Table I.16 Shell measurement of 15 individuals of *Hypselostoma erawan*

Individual	*Hypselostoma erawan*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.56	1.54	2.64	1.76	0.31	1.01
2	1.58	1.58	2.63	1.70	0.34	1.05
3	1.53	1.52	2.68	1.73	0.38	1.08
4	1.59	1.58	2.73	1.80	0.33	1.06
5	1.55	1.55	2.78	1.79	0.36	1.08
6	1.57	1.56	2.76	1.77	0.38	1.09
7	1.56	1.56	2.74	1.74	0.32	1.02
8	1.58	1.57	2.75	1.78	0.33	1.08
9	1.59	1.57	2.69	1.73	0.34	1.07
10	1.63	1.61	2.73	1.76	0.39	1.05
11	1.60	1.60	2.67	1.75	0.31	1.00
12	1.53	1.51	2.67	1.75	0.32	1.08
13	1.64	1.62	2.74	1.70	0.31	1.06
14	1.51	1.51	2.79	1.76	0.33	1.06
15	1.58	1.57	2.75	1.75	0.35	1.07

Table I.17 Shell measurement of 15 individuals of *Hypselostoma holimanae*

Individual	*Hypselostoma holimanae*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.82	2.82	3.77	1.99	0.73	0.77
2	2.78	2.78	3.79	2.22	0.72	0.79
3	2.84	2.84	4.02	2.34	0.74	0.84
4	2.79	2.79	3.75	1.96	0.73	0.86
5	2.85	2.85	4.00	2.21	0.70	0.83
6	2.80	2.80	3.95	2.33	0.69	0.81
7	2.77	2.77	0.79	2.09	0.74	0.79
8	2.81	2.81	3.90	2.14	0.75	0.78
9	2.77	2.77	3.81	2.20	0.74	0.84
10	2.85	2.85	3.85	2.33	0.71	0.85
11	2.84	2.84	3.87	2.30	0.72	0.81
12	2.81	2.81	3.81	2.25	0.74	0.80
13	2.81	2.81	3.78	2.30	0.76	0.84
14	2.79	2.79	3.77	2.19	0.73	0.83
15	2.79	2.79	3.84	2.22	0.70	0.79

Table I.18 Shell measurement of 15 individuals of *Hypselostoma panhai*

Individual	*Hypselostoma panhai*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.50	2.48	1.95	1.89	0.00	0.31
2	2.44	2.43	1.91	1.88	0.00	0.32
3	2.42	2.39	1.96	1.89	0.00	0.30
4	2.40	2.40	1.98	1.87	0.00	0.36
5	2.44	2.41	1.94	1.86	0.00	0.33
6	2.40	2.38	1.95	1.88	0.00	0.31
7	2.38	2.37	1.95	1.89	0.00	0.29
8	2.42	2.39	1.91	1.91	0.00	0.30
9	2.45	2.38	1.90	1.93	0.00	0.31
10	2.48	2.42	1.96	1.87	0.00	0.32
11	2.40	2.38	1.99	1.90	0.00	0.31
12	2.41	2.40	2.01	1.88	0.00	0.31
13	2.46	2.44	1.99	1.91	0.00	0.33
14	2.47	2.44	1.98	1.87	0.00	0.36
15	2.44	2.41	1.98	1.87	0.00	0.34

Table I.19 Shell measurement of 15 individuals of *Hypselostoma taehwani*

Individual	*Hypselostoma taehwani*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.56	2.36	3.48	2.10	1.06	0.75
2	2.54	2.37	3.46	2.08	1.07	0.72
3	2.52	2.32	3.44	2.04	1.04	0.75
4	2.58	2.34	3.43	2.07	1.01	0.73
5	2.61	2.35	3.40	2.06	1.08	0.78
6	2.53	2.38	3.46	2.11	1.05	0.74
7	2.62	2.41	3.45	2.13	1.06	0.76
8	2.56	2.30	3.48	2.09	1.09	0.74
9	2.54	2.33	3.39	2.04	1.01	0.76
10	2.53	2.32	3.49	2.01	1.00	0.75
11	2.52	2.33	3.43	2.10	1.01	0.78
12	2.51	2.37	3.44	2.00	1.03	0.75
13	2.55	2.36	3.41	2.04	1.05	0.78
14	2.54	2.35	3.40	2.03	1.05	0.77
15	2.53	2.38	3.45	2.02	1.08	0.73

Table I.20 Shell measurement of 15 individuals of *Krobylos maehongsonensis*

Individual	*Krobylos maehongsonensis*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	4.15	3.73	3.51	3.09	0.00	0.16
2	4.14	3.71	3.48	3.04	0.00	0.17
3	4.12	3.69	3.45	3.03	0.00	0.15
4	4.15	3.74	3.41	3.07	0.00	0.18
5	4.15	3.69	3.46	3.06	0.00	0.19
6	4.18	3.73	3.46	3.05	0.00	0.16
7	4.12	3.75	3.43	3.04	0.00	0.17
8	4.11	3.74	3.45	3.06	0.00	0.17
9	4.17	3.74	3.43	3.04	0.00	0.17
10	4.14	3.73	3.48	3.03	0.00	0.19
11	4.18	3.72	3.49	3.06	0.00	0.16
12	4.20	3.78	3.49	3.03	0.00	0.17
13	4.13	3.76	3.41	3.01	0.00	0.17
14	4.13	3.71	3.44	3.04	0.00	0.18
15	4.17	3.70	3.46	3.08	0.00	0.18

Table I.21 Shell measurement of 15 individuals of *Montapiculus proboscidea*

Individual	*Montapiculus proboscidea*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.34	1.08	1.52	1.28	0.90	0.51
2	1.36	1.06	1.53	1.29	0.91	0.52
3	1.33	1.09	1.51	1.27	0.96	0.50
4	1.31	1.09	1.54	1.23	0.94	0.49
5	1.33	1.11	1.51	1.28	0.93	0.46
6	1.36	1.07	1.52	1.26	0.91	0.50
7	1.38	1.10	1.55	1.29	0.96	0.51
8	1.33	1.12	1.58	1.30	0.93	0.51
9	1.30	1.08	1.56	1.32	0.90	0.49
10	1.33	1.07	1.53	1.27	0.93	0.49
11	1.32	1.07	1.51	1.28	0.94	0.48
12	1.35	1.10	1.51	1.28	0.94	0.49
13	1.32	1.06	1.57	1.30	0.95	0.49
14	1.30	1.08	1.53	1.28	0.91	0.50
15	1.34	1.09	1.53	1.25	0.92	0.51

Table I.22 Shell measurement of 15 individuals of *Paraboysidia phupaman*

Individual	*Paraboysidia phupaman*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	4.34	4.02	4.03	2.68	1.10	1.03
2	4.32	4.00	4.01	2.69	1.13	1.01
3	4.36	4.06	4.06	2.72	1.12	1.01
4	4.38	4.06	4.05	2.71	1.11	1.02
5	4.32	4.04	4.05	2.70	1.10	0.99
6	4.33	4.07	4.08	2.70	1.13	1.00
7	4.33	4.05	4.03	2.72	1.14	0.96
8	4.35	4.03	4.05	2.69	1.08	0.98
9	4.32	4.04	4.06	2.69	1.09	1.02
10	4.31	4.06	4.08	2.70	1.07	1.02
11	4.34	4.08	4.07	2.73	1.12	1.00
12	4.34	4.09	4.06	2.68	1.12	0.98
13	4.30	4.07	4.06	2.68	1.08	1.02
14	4.37	4.06	4.05	2.72	1.08	1.03
15	4.36	4.02	4.03	2.70	1.10	1.01

Table I.23 Shell measurement of 15 individuals of *Systenostoma tamlod*

Individual	*Systenostoma tamlod*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.26	1.04	1.06	0.91	0.12	0.26
2	1.29	1.05	1.04	0.90	0.15	0.24
3	1.24	1.03	1.09	0.93	0.13	0.23
4	1.26	1.05	1.06	0.92	0.12	0.25
5	1.27	1.06	1.07	0.90	0.14	0.25
6	1.27	1.05	1.07	0.89	0.15	0.27
7	1.29	1.04	1.06	0.90	0.15	0.23
8	1.31	1.06	1.04	0.91	0.13	0.23
9	1.28	1.08	1.05	0.93	0.11	0.22
10	1.30	1.06	1.05	0.93	0.12	0.23
11	1.27	1.03	1.08	0.91	0.12	0.25
12	1.24	1.04	1.08	0.92	0.13	0.27
13	1.25	1.04	1.07	0.92	0.13	0.24
14	1.25	1.07	1.09	0.91	0.14	0.26
15	1.28	1.08	1.06	0.93	0.13	0.22

Table I.24 Shell measurement of 15 individuals of *Pupilla muscorum*

Individual	*Pupilla muscorum*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	3.23	2.98	1.86	1.65	0.00	0.14
2	3.26	2.97	1.83	1.62	0.00	0.15
3	3.20	2.95	1.84	1.67	0.00	0.13
4	3.21	2.96	1.87	1.70	0.00	0.13
5	3.19	2.94	1.89	1.69	0.00	0.12
6	3.24	2.94	1.82	1.68	0.00	0.14
7	3.24	3.01	1.80	1.65	0.00	0.13
8	3.19	3.02	1.86	1.63	0.00	0.13
9	3.25	2.99	1.84	1.63	0.00	0.14
10	3.26	2.98	1.85	1.67	0.00	0.13
11	3.26	2.99	1.87	1.63	0.00	0.12
12	3.20	3.00	1.83	1.62	0.00	0.13
13	3.21	2.98	1.87	1.69	0.00	0.13
14	3.27	3.01	1.86	1.67	0.00	0.12
15	3.25	2.97	1.89	1.68	0.00	0.15

Table I.25 Shell measurement of 15 individuals of *Vertigo gouldi*

Individual	*Vertigo gouldi*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	1.89	1.65	1.20	1.14	0.00	0.13
2	1.91	1.64	1.22	1.13	0.00	0.12
3	1.87	1.63	1.21	1.15	0.00	0.14
4	1.93	1.62	1.23	1.18	0.00	0.13
5	1.83	1.63	1.20	1.12	0.00	0.11
6	1.84	1.64	1.20	1.11	0.00	0.12
7	1.89	1.66	1.22	1.10	0.00	0.13
8	1.90	1.62	1.20	1.12	0.00	0.14
9	1.91	1.61	1.23	1.15	0.00	0.14
10	1.90	1.61	1.23	1.15	0.00	0.13
11	1.87	1.63	1.24	1.17	0.00	0.13
12	1.89	1.65	1.21	1.17	0.00	0.11
13	1.88	1.66	1.20	1.16	0.00	0.12
14	1.88	1.65	1.21	1.15	0.00	0.13
15	1.87	1.63	1.23	1.13	0.00	0.14

Table I.26 Shell measurement of 15 individuals of *Vertigo shimochii*

Individual	*Vertigo shimochii*					
	SHA (mm)	**BSH (mm)**	**SWA (mm)**	**SW (mm)**	**TL (mm)**	**UW (mm)**
1	2.13	1.81	1.07	1.06	0.00	0.12
2	2.14	1.83	1.04	1.04	0.00	0.11
3	2.12	1.84	1.04	1.03	0.00	0.14
4	2.15	1.83	1.03	1.03	0.00	0.12
5	2.17	1.86	1.06	1.05	0.00	0.13
6	2.13	1.82	1.06	1.05	0.00	0.14
7	2.18	1.85	1.05	1.05	0.00	0.14
8	2.16	1.84	1.07	1.06	0.00	0.13
9	2.11	1.82	1.08	1.08	0.00	0.11
10	2.12	1.85	1.06	1.05	0.00	0.10
11	2.14	1.86	1.08	1.08	0.00	0.12
12	2.14	1.83	1.05	1.05	0.00	0.12
13	2.15	1.81	1.03	1.02	0.00	0.10
14	2.13	1.80	1.04	1.04	0.00	0.12
15	2.13	1.82	1.04	1.03	0.00	0.13

APPENDIX II

SHELL MORPHOLOGICAL CHARACTERS AND THEIR STATES USED IN THE PHYLOGENETIC ANALYSIS OF THAI PUPILLID GENERA

The following is a synopsis of the morphological characters and their states used in the phylogenetic analysis of pupilloid taxa.

Shell Characters

1. Pupa-shaped: 0 = absent; 1 = present
2. Shell spire: 0 = high spire; 1 = depressed; 2 = very depressed
3. Shell color: 0 = dark brown; 1 = light brown; 2 = white
4. Body whorl spiral striae: 0 = absent; 1 = present
5. Body whorl transverse rib: 0 = absent; 1 = present
6. Sutures: 0 = shallow; 1 = well impressed (deep)
7. Umbilicus: 0 = narrow; 1 = moderated; 2 = wide

8-10. Tuba: 0 = absent; 1 = horizontal; 2 = bends upward; 3 = bends downward (non-additive)

The terms “upward” and “downward” are in reference to the shell when it is observed with the apex up, the base down, and the shell aperture toward to the observer. (Figure II.1)

Peristome Characters

11. Peristome with the body whorl: 0 = free, 1 = adnate
12. Peristome lips: 0 = thin; 1 = thick
13. Peristome expanded: 0 = absent; 1 = present
14. Peristome reflection: 0 = absent; 1 = present

Apertural barriers

Parietal lamellae

15. infraparietal: 0 = absent; 1 = present
16. parietal: 0 = absent; 1 = present
17. angular : 0 = absent; 1 = present

Palatal plicae or folds

18.suprapalatal: 0 = absent; 1 = present

19.upper-palatal: 0 = absent; 1 = present

20.interpalatal: 0 = absent; 1 = present

21.lower-palatal: 0 = absent; 1 = present

22.infrapalatal: 0 = absent; 1 = present

Basal plica

23.basal: 0 = absent; 1 = present

Columellar lamellae

24.supracolumellar: 0 = absent; 1 = present

25.columellar: 0 = absent; 1 = present

26.subcolumellar: 0 = absent; 1 = present

Habitat type

27.Habitat: 0 = rock; 1 = Leaf Litter

The shell and apertural barrier characters for each species studies were showed in Figure II.1 – II.9.

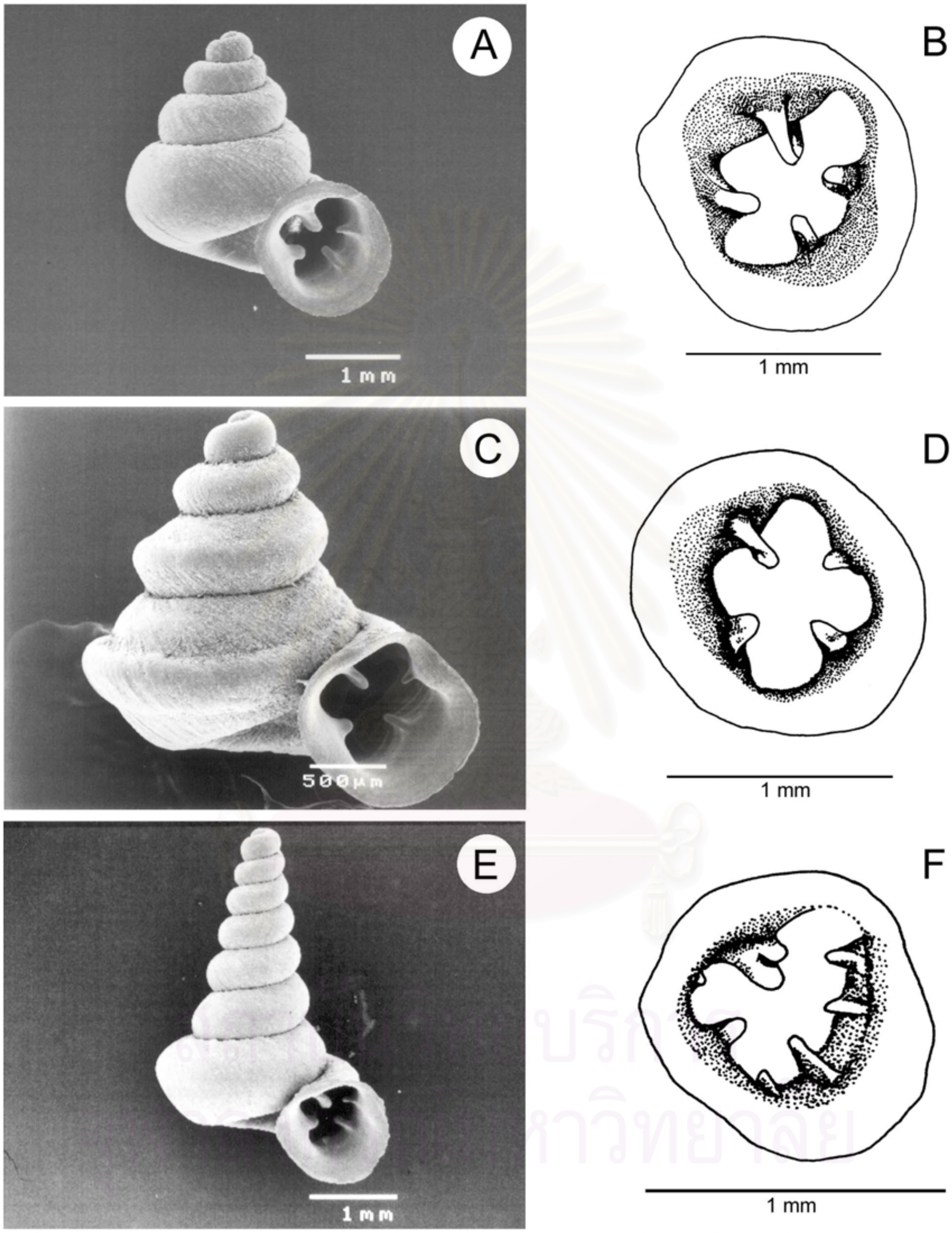

Figure II.1 A, B. *Anauchen angthongense* (A, Shell; B, Aperture), C, D. *Ana. chatnareeae* (C, Shell; D, Aperture), E, F. *Ana. chedi* (E, Shell; F, Aperture).

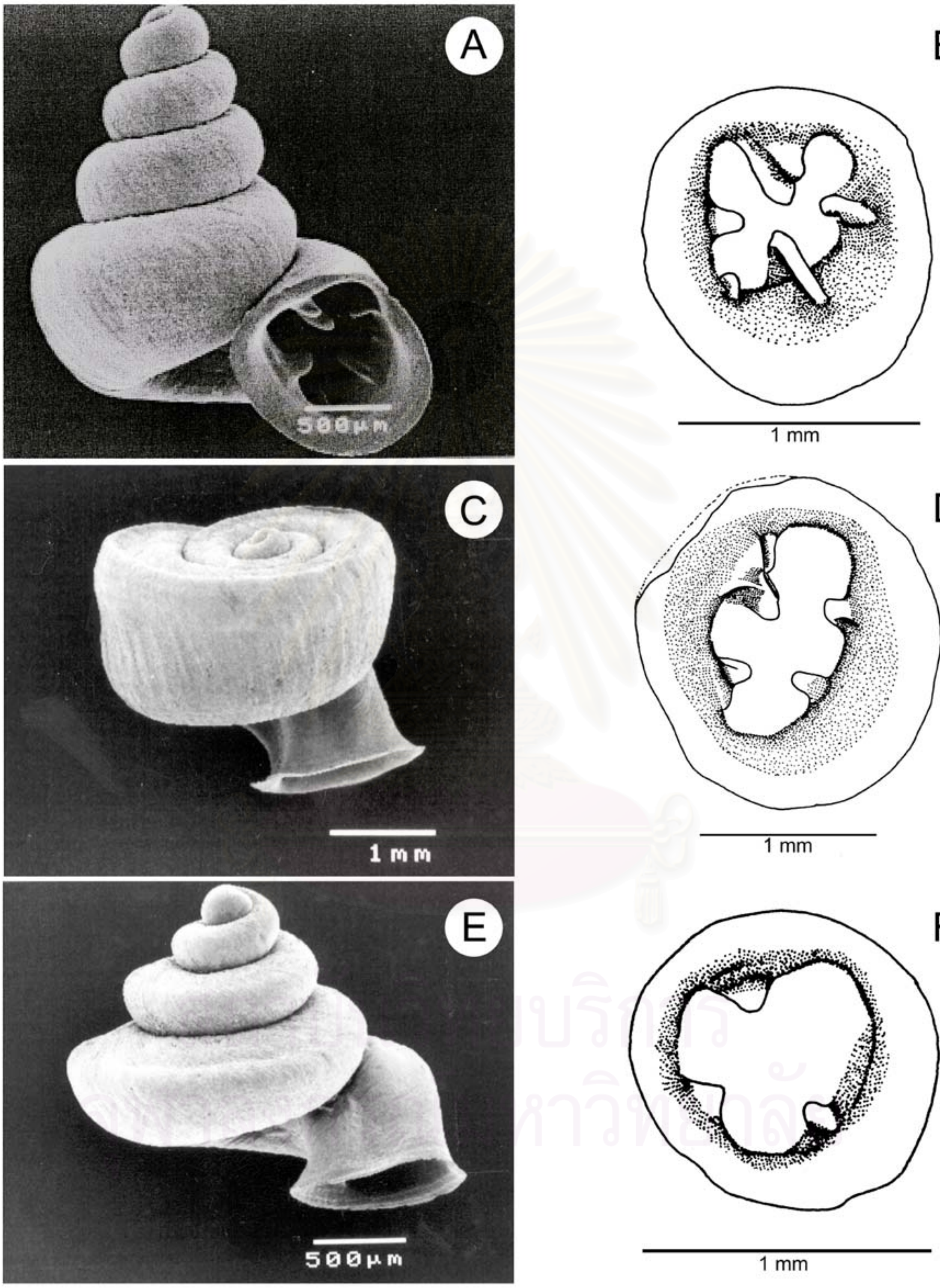

Figure II.2 A, B. *Anauchen. utaithaniensis* (A, Shell; B, Aperture), C, D. *Antroapiculus pendulus* (C, Shell; D, Aperture), E, F. *Aulacospira smaesarnensis* (E, Shell; F, Aperture).

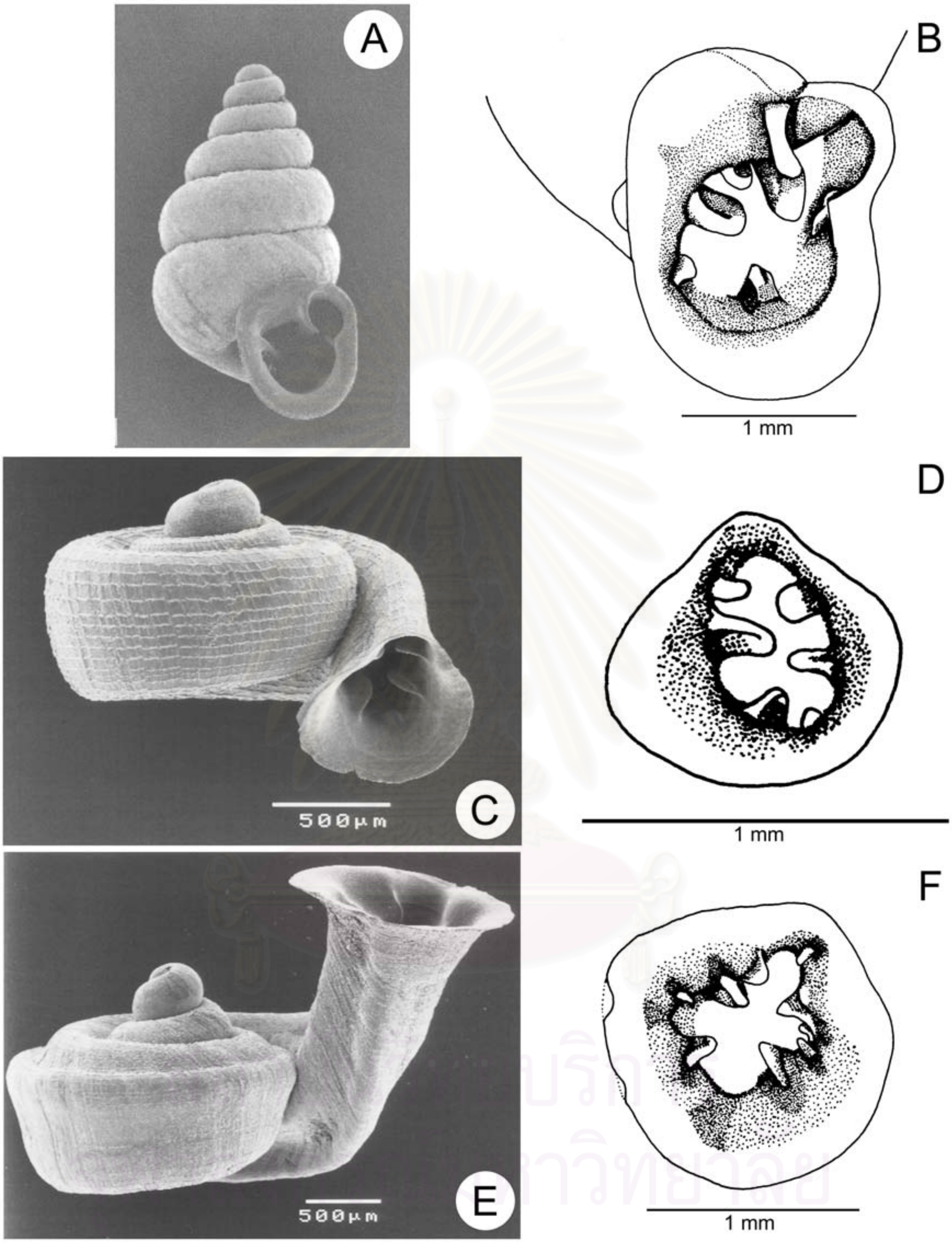

Figure II.3 A, B. *Boysidia chiangmaiensis* (A, Shell; B, Aperture), C, D. *Gyliotrachela adela* (C, Shell; D, Aperture), E, F. *G. diarmaidi* (E, Shell; F, Aperture).

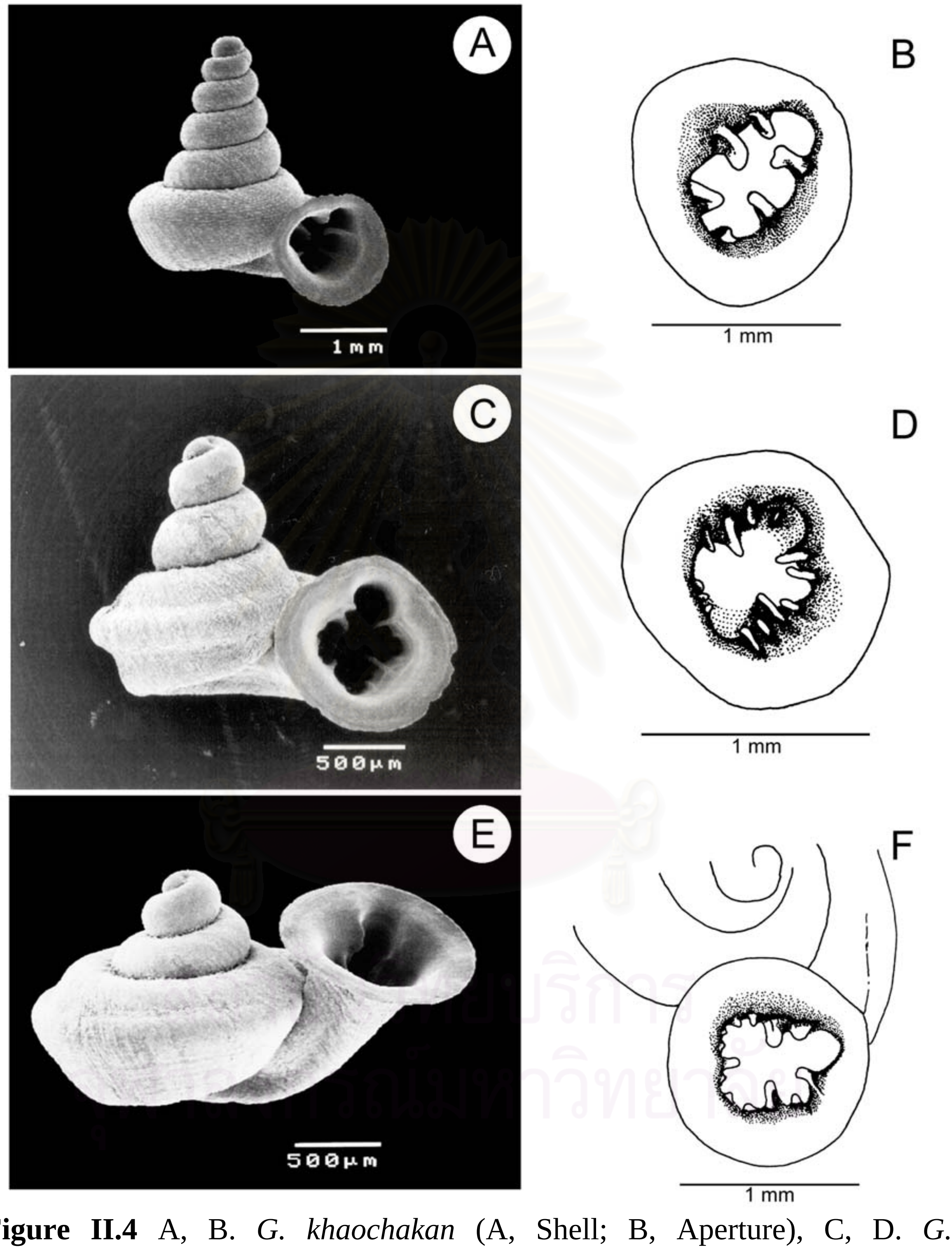

Figure II.4 A, B. *G. khaochakan* (A, Shell; B, Aperture), C, D. *G. khaochongensis* (C, Shell; D, Aperture), E, F. *G. kohrin* (E, Shell; F, Aperture).

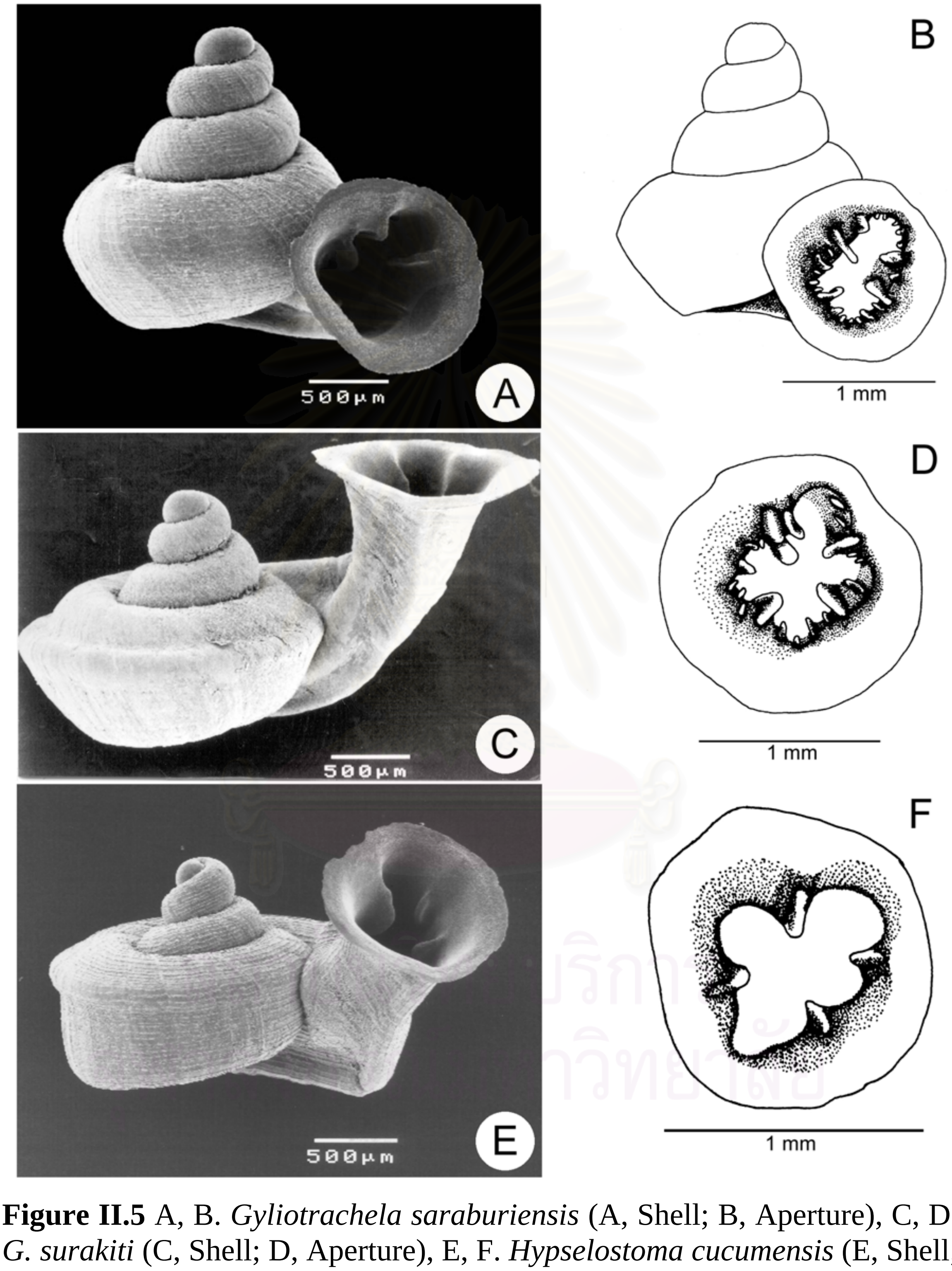

Figure II.5 A, B. *Gyliotrachela saraburiensis* (A, Shell; B, Aperture), C, D. *G. surakiti* (C, Shell; D, Aperture), E, F. *Hypselostoma cucumensis* (E, Shell; F, Aperture).

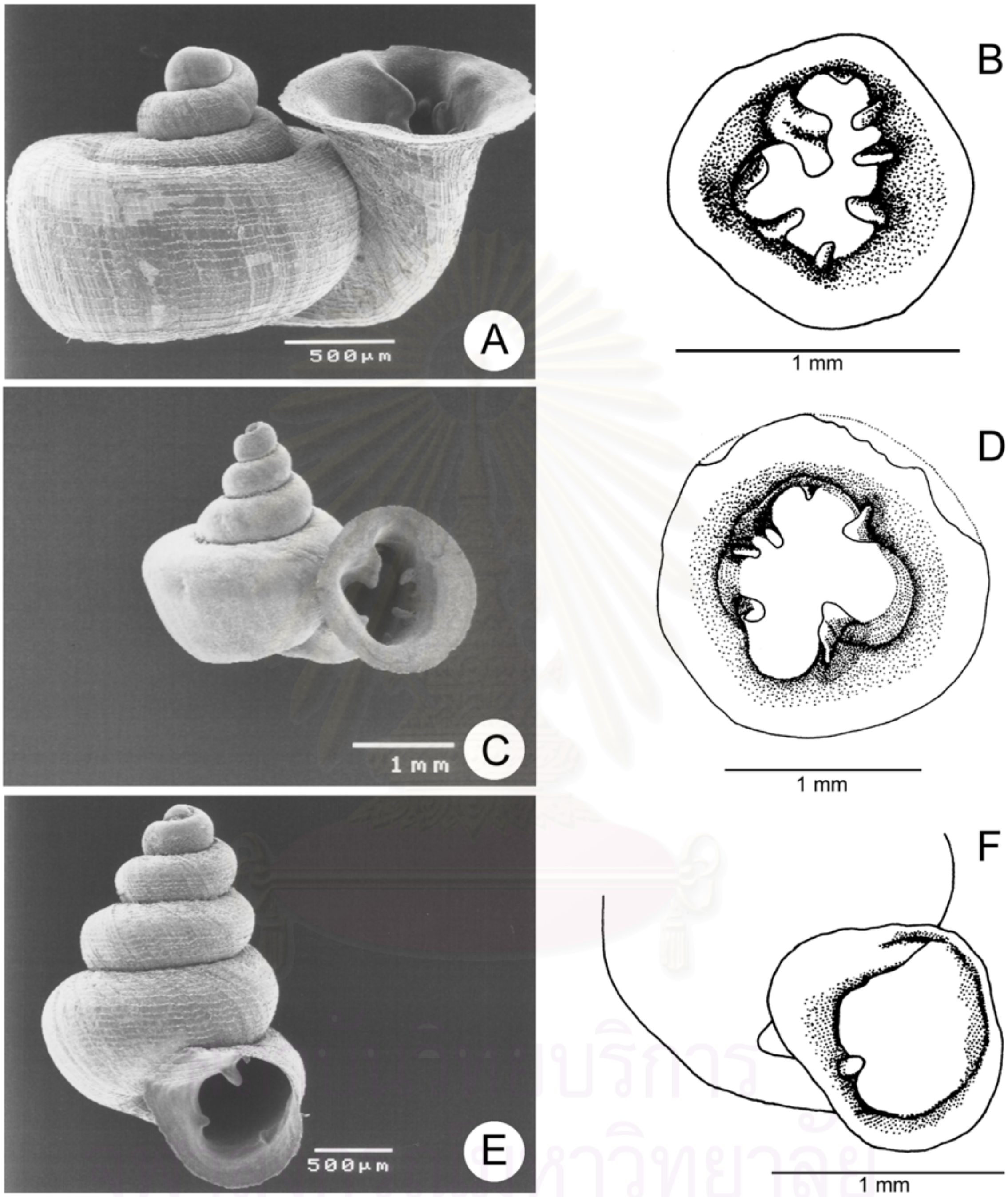

Figure II.6 A, B. *Hypselostoma erawan* (A, Shell; B, Aperture), C, D. *H. holimanae* (C, Shell; D, Aperture), E, F. *H. panhai* (E, Shell; E, Aperture).

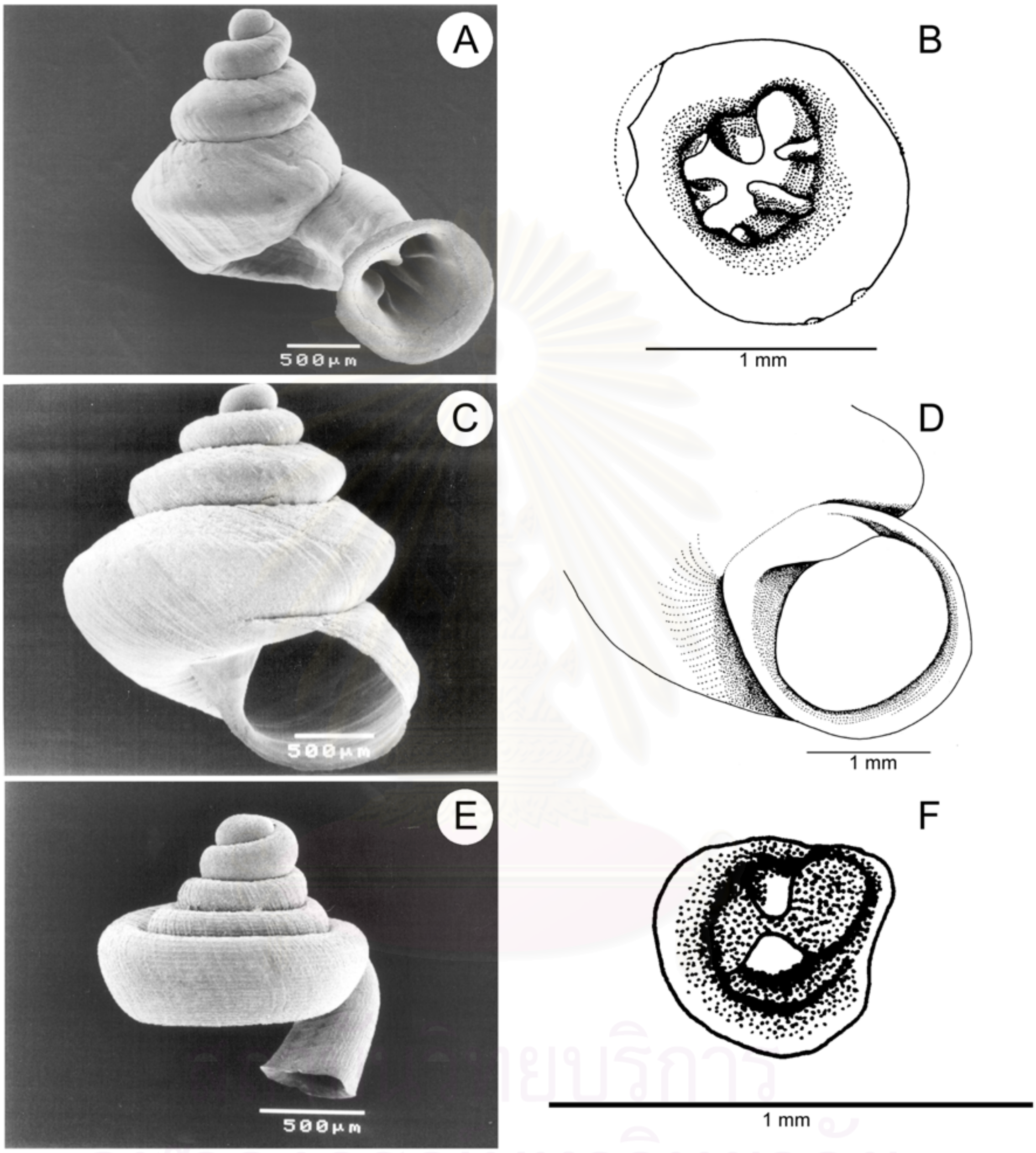

Figure II.7 A, B. *Hypselostoma taehwani* (A, Shell; B, Aperture), C, D. *Krobylos maehongsonensis* (C, Shell; D, Aperture), E, F. *Montapiculus proboscidea* (E, Shell; F, Aperture).

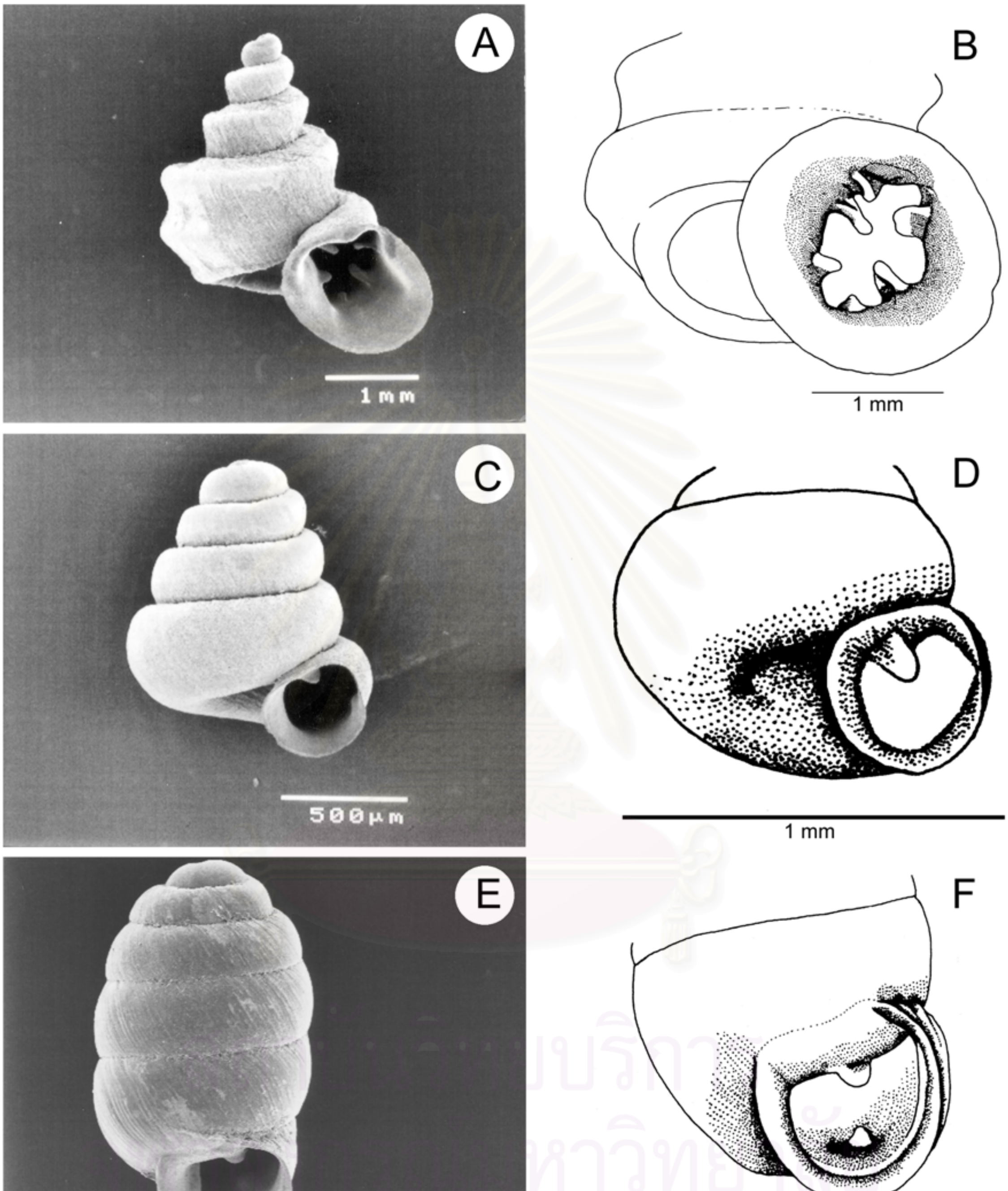

Figure II.8 A. *Paraboysidia phupaman* (A, Shell; B, Aperture), C, D. *Systenostoma tamlod* (C, Shell; D, Aperture), E, F. *Pupilla muscorum* (E, Shell; F, Aperture).

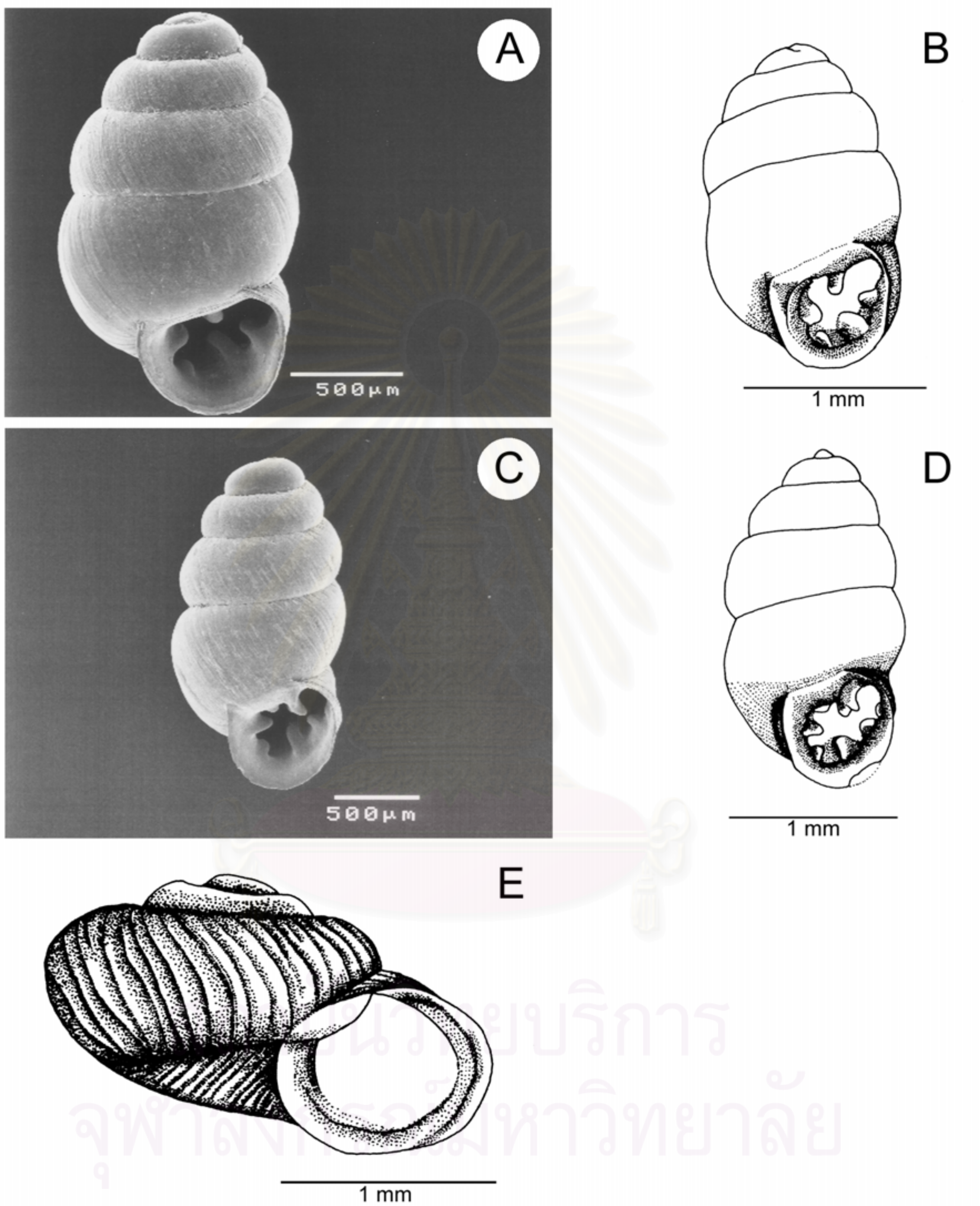

Figure II.9 A, B. *Vertigo gouldi* (A, Shell; B, Aperture), C, D. *Ve. shimochii* (C, Shell; D, Aperture), E. *Vallonia costata* (E, Shell).

APPENDIX III

ALLIGNED 16S MITOCHONDRIAL AND 28S NUCLEAR RIBOSOMAL DNA FRAGMENTS OF THE PUPILLIDAE

Twenty 16S mitochondrial and 28S nuclear ribosomal DNA sequences obtained from 17 pupillid species, 2 valloniid and 1 strobilopsid outgroup species employed in this study were compiled with Sequence Monkey ver. 2.9.0 and aligned using Clustal_X with various gap open and gap extension penalties. Then the resulting alignment was refined manually where necessary. A dash represents a gap and dots indicate nucleotide identity to the first sequence presented (*Anauchen chedi*).

```
                              1........|.........|.........|.........|.........|........60

Anauchen chedi                aaagaactttgaagagagagttcaagagtacgtgaaaccgcccagaggtaaacgggtgga
A. chatnareeae                ............................................................
A. utaithaniensis             ............................................................
Aulacospira smaesarnensis     ............................................................
Gyliotrachela adela           ............................................................
G. diarmaidi                  ............................................................
G. khaochakan                 ............................................................
G. kohrin                     ............................................................
G. saraburiensis              ............................................................
G. surakiti                   ............................................................
Hypselostoma cucumensis       ............................................................
H. erawan                     ............................................................
H. holimanae                  ............................................................
H. panhai                     ...........................................................g
Krobylos maehongsornensis 1   ............................................................
K. maehongsornensis 2         ............................................................
Pupilla muscorum (A)          .........................................t..................
P. muscorum (B)               .........................................t..................
P. muscorum (E)               .........................................t..................
Vertigo gouldi                ............................................................
Vallonia costata (A)          ............................................................
V. costata (B)                .........................................g..................
Zoogenetes harpa              ............................................................
Strobilops labyrinthica       ............................................................
```

	61.......|.........|.........|.........|.........|.......120
Anauchen chedi	tccgc-----------------------aaagtcggtccgcggaattcagcgcgg-cgcg
A. chatnareeae	.....-----------------------........c..................-....
A. utaithaniensis	.....-----------------------...........................-....
Aulacospira smaesarnensis	.....-----------------------...........................-....
Gyliotrachela adela	.....-----------------------........c..................-....
G. diarmaidi	.....-----------------------...........................-....
G. khaochakan	.....-----------------------...........................-....
G. kohrin	.....-----------------------...........................-....
G. saraburiensis	.....-----------------------...........................-....
G. surakiti	.....-----------------------...........................-....
Hypselostoma cucumensis	.....-----------------------...........................-....
H. erawan	.....cagaggtaaacgggtggatccgc........c..................-....
H. holimanae	.....-----------------------..t........................-....
H. panhai	.....-----------------------...........................-....
Krobylos maehongsornensis 1	.....-----------------------...........................-....
K. maehongsornensis 2	.....-----------------------...........................-....
Pupilla muscorum (A)	.....-----------------------...........................-....
P. muscorum (B)	.....-----------------------...........................-....
P. muscorum (E)	.....-----------------------...........................-....
Vertigo gouldi	.....-----------------------...........................-..a.
Vallonia costata (A)	.....-----------------------........................--.-...c
V. costata (B)	.....-----------------------...........................-....
Zoogenetes harpa	.....-----------------------...........................-....
Strobilops labyrinthica	.....-----------------------...........................-....

	121......|.........|.........|.........|.........|.......180
Anauchen chedi	cggcccggggctt-------cgccgtg--gtttcgggatcccta--gtgaaccgaccgc-
A. chatnareeae	..........t..-------t....g.gatc.cg......-..c--.c.g.......c.-
A. utaithaniensis	.............-------.......--..c...........c--.............-
Aulacospira smaesarnensis	.....t....t..t------t.....c--...-..........c--...........t.t
Gyliotrachela adela	..........t..-------t.....c--...-.......g..c--...........t.-
G. diarmaidi	.....t....t..t------t.....c--...-.......g..c--...........t.-
G. khaochakan	..........t..tt-----t.....c--...-..........ctc...g.......t.-
G. kohrin	.....t....t..t------t.....c--...-.......g..c--...........t.-
G. saraburiensis	.....t....t..-------t......-g...-.......-..c--...........t.-
G. surakiti	.....t....t..-------t.....c--...-.......-..c--...........t.-
Hypselostoma cucumensis	...t......t..-------......ttg...-.......g..c--...........t.-
H. erawan	..........t..-------t....ct--...-.......g..c-gt........a.c.-
H. holimanae	.............-------gtt...---...-.......g..c-gaa.......a.t.-
H. panhai	.............-------g....c.--...-.......g..c-gt........a.t.-
Krobylos maehongsornensis 1	..........t..tctttt-g......-g..gga.........c--...g....c..a.-
K. maehongsornensis 2	..........t..tcttttgg......-g..gga.........c--...g....c..a.-
Pupilla muscorum (A)	..........t---------.......--...-.......-..t--.............-
P. muscorum (B)	..........t---------.......--...-.......-..t--.............-
P. muscorum (E)	..........t---------.......--...-.......-..t--.............-
Vertigo gouldi	..........t---------.......--...-.......-..tt-.a...........-
Vallonia costata (A)	..........t---------.....c.--...-.......-..t--.........t...-
V. costata (B)	..........t---------.....c.--t..-.......-..t--.........t...-
Zoogenetes harpa	..........t---------.......--...-.......-.ct--.............-
Strobilops labyrinthica	..........t---------.....c.--...-.......-..t--.............-

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                             181......|.........|.........|.........|.........|.......240

Anauchen chedi               ggtagcctcgccgggttccgccgc-gtgcac-tttccgcgggcagagagccacaaccggt
A. chatnareeae               ...............c........-......-............................
A. utaithaniensis            .....t..................-......-...............t............
Aulacospira smaesarnensis    ...............cc.....a.a......c...........g................
Gyliotrachela adela          ...............cc.......-......-............................
G. diarmaidi                 ...............cc.......-......-............................
G. khaochakan                ...............cc.......-......-...........g................
G. kohrin                    ...............cc.......-......-............................
G. saraburiensis             ...............cc.......-......-............................
G. surakiti                  ...............cc.......-......-............................
Hypselostoma cucumensis      ...............cc.......-......-............................
H. erawan                    ...............cc.......-......-.........-.g................
H. holimanae                 .c.............cc.......-......-...........g................
H. panhai                    ................c.......-......-...........g................
Krobylos maehongsornensis 1  ...............c........-......-............................
K. maehongsornensis 2        ...............c........-......-.........a..................
Pupilla muscorum (A)         ...-....a......c........-......-.........a..................
P. muscorum (B)              ...-....a......c........-......-.........a..................
P. muscorum (E)              ...-....a......c........-......-.........a..................
Vertigo gouldi               ...gt..ga.......c.......-......-.........a..................
Vallonia costata (A)         ...-...ga.t....cc.......-......-......t..a..................
V. costata (B)               ...-...ga......c........-......-.........a..................
Zoogenetes harpa             ...-...ga......c........-......-.........a..................
Strobilops labyrinthica      ...-...ga......c........-......-.........a.g................


                             241......|.........|.........|.........|.........|.......300

Anauchen chedi               tcggc----gag-c-------t--gcggcgacaggccgggagggttgtaggtgggggcgc
A. chatnareeae               .....----.c.-.aca-------....................................
A. utaithaniensis            .....----...---------.--....t...............................
Aulacospira smaesarnensis    .....----.c.-.gcttgc-.aa....................................
Gyliotrachela adela          .....----.c.-.tctaaa---c....................................
G. diarmaidi                 .....----.c.-.tc--------....................................
G. khaochakan                .....----.c.caccttct-.--....................................
G. kohrin                    .....----.c.-.tcatc-----....................................
G. saraburiensis             .....----.c.-.tc--------....................................
G. surakiti                  .....----.c.-.tc--------....................................
Hypselostoma cucumensis      ....g----.tt-tg-tttt-.gc....................................
H. erawan                    .t...----.c.gttctttt-.-c..t.-----.---.a.....................
H. holimanae                 .....gtcgat.-aactg---.gc....................................
H. panhai                    .....--ag.t--tgcttt------------.-...........................
Krobylos maehongsornensis 1  .....----.c.-.acaacca.--....................................
K. maehongsornensis 2        .....----.c.-.acaacca.--....................................
Pupilla muscorum (A)         ....------t.-agc-----.--....t...............................
P. muscorum (B)              ....------t.-agc-----.--....t...............................
P. muscorum (E)              ....------t.-agc-----.--....t...............................
Vertigo gouldi               .a...----.---atc-----.--....tc..........................t..t
Vallonia costata (A)         ....------t.-agct----.--...a.........ac.....................
V. costata (B)               ....------t.-agct----.--....................................
Zoogenetes harpa             ....------t.-agc-----.--....................................
Strobilops labyrinthica      .t..------t.-.gc-----.--....................................
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                            301.....|.........|.........|.........|.........|.......360

Anauchen chedi              tcg--cgtccctaccagccttccccg----gttagccgccgccggaccgagggaccgccg
A. chatnareeae              ...--.....................----..............................
A. utaithaniensis           ...-t-....................----..............................
Aulacospira smaesarnensis   ...c-......c..............----..............................
Gyliotrachela adela         ...-t.....................----.........g...................t
G. diarmaidi                ...-t......c..............----..............................
G. khaochakan               ...c-......c..............----.........g....................
G. kohrin                   ...-t......c..............----..............................
G. saraburiensis            ...-t......c..............----..............................
G. surakiti                 c..-t......c..............----..............................
Hypselostoma cucumensis     ...c-.....................----.........g...................t
H. erawan                   ...c-.a....c........ct....tcag..c.....t...........a.a.---..t
H. holimanae                ...ct...g..c..............----.............................t
H. panhai                   ...c-......c..............----.............................t
Krobylos maehongsornensis 1 ...c-.....................----.............................a
K. maehongsornensis 2       ...c-.....................----......................a......a
Pupilla muscorum (A)        ...--.....................----..g..........................a
P. muscorum (B)             ...--.....................----..g..........................a
P. muscorum (E)             ...--.....................----..g..........................a
Vertigo gouldi              ...--.t...................----.a...........tt..........aa..a
Vallonia costata (A)        ...--.....................----..........t...................
V. costata (B)              ...--.....................----..............................
Zoogenetes harpa            ...--.....................----.............................a
Strobilops labyrinthica     c..--..............c......----..........a...................


                            361.....|.........|.........|.........|.........|.......420

Anauchen chedi              cgcgc-atcgaggccaccct----cccctccgggcgagtccgactgggagagactgggca
A. chatnareeae              .....-t...........t.cccc...............t....................
A. utaithaniensis           .....-t.............----...............t....................
Aulacospira smaesarnensis   .....-t.............----...............t.c....at............
Gyliotrachela adela         .....c-...........t.----...............t..................t.
G. diarmaidi                .....-t.............----...............t....................
G. khaochakan               .....c-.............----...............t....................
G. kohrin                   .....-t.............----...............t....................
G. saraburiensis            .....-t.............----...............t....................
G. surakiti                 .....-t.............----...............t....................
Hypselostoma cucumensis     .....c-...........t.----...............t....................
H. erawan                   ---..c-........g..t----c...................................g
H. holimanae                .....ct...........t.tccc...............t...................g
H. panhai                   .....ct...........t.----....................................
Krobylos maehongsornensis 1 .....-t...........t.----...............t....a...............
K. maehongsornensis 2       .....-t...........t.----...............t....................
Pupilla muscorum (A)        .....-t.............----..........t....t....................
P. muscorum (B)             .....-t.............----..........t....t....................
P. muscorum (E)             .....-t.............----..........t....t....................
Vertigo gouldi              .....-t.............----..........a....t....................
Vallonia costata (A)        .....-t.........t...----..........t...gt....a...............
V. costata (B)              .....-t.............----..........t....t....................
Zoogenetes harpa            .....-t........g....----..........t....t....................
Strobilops labyrinthica     .....-t.............----...............a....................
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                              421......|.........|.........|.........|.........|.......480

Anauchen chedi                accgtgtctcccgaccgctcgcccgcgatcggcgccggggctggccgtgcgcg-aa----
A. chatnareeae                ...................................t...........g.....-.cc---
A. utaithaniensis             ...................................t...........g....t-t.----
Aulacospira smaesarnensis     ...............................................g.....-c-----
Gyliotrachela adela           .........t........................tt...-.......g.t...a.----g
G. diarmaidi                  ...................................t...........g.....-.-----
G. khaochakan                 ..................................tt...........g..-..c.-----
G. kohrin                     ...................................t...........g.....-.-----
G. saraburiensis              ...................................t...........g.....-.-----
G. surakiti                   ...................................t...........g.....-.-----
Hypselostoma cucumensis       ...................................a...-.......g.....-c-----
H. erawan                     ..........................---------a..------..cc.....-gcg-aa
H. holimanae                  ...................................t...........g.....-.-----
H. panhai                     ...................................a...-.......g.....-..acaa
Krobylos maehongsornensis 1   ...................................t...........g.....-.-----
K. maehongsornensis 2         ...................................t...........g.....-.-----
Pupilla muscorum (A)          ...........t........a..............a...-.......g.....-..a-aa
P. muscorum (B)               ...........t........a..............a...-.......g.....-..a-aa
P. muscorum (E)               ...........t........a..............a...-.......g.....-..a-aa
Vertigo gouldi                ...................................t...-.......g.....-.---aa
Vallonia costata (A)          ...........t........a.............-t.....c.....g.....-.-----
V. costata (B)                ....................a.............-t...........g.....-.-----
Zoogenetes harpa              ....................a..............-...........g.....a.---ca
Strobilops labyrinthica       ...............................................g.....-.---ga


                              481......|.........|.........|.........|.........|.......540

Anauchen chedi                ----------aagctttga-------g-agggtttgtggcaa-tctgtcggcattccacc
A. chatnareeae                ----------------g.t-------.c......g.......g.................
A. utaithaniensis             ----------ctt-.g-.t-------.-..............g.................
Aulacospira smaesarnensis     ---------ctgc.aaa.t-------.-t.............a.................
Gyliotrachela adela           ccagcaagattc.t....ttggcggt.-......g.......g.................
G. diarmaidi                  --------tc.-tg.c-.t-------.-t.....a.......g.................
G. khaochakan                 --------gc.-..ac-ac---------......g.......g.................
G. kohrin                     --------tc.-tg.c-.t-------.-t.....a.......g.................
G. saraburiensis              --------tt.-tg.c..t-------.-......a.......g.................
G. surakiti                   --------ta..tg.c-.t-------.-......a.......g.................
Hypselostoma cucumensis       -------gcc..t-....tac-----.-......a.......g.................
H. erawan                     ag------------c.a.c-------.-......g.......g.................
H. holimanae                  -----------ccgag----------.-......a.......g.................
H. panhai                     agagagtcgc.tttc..tgggt----.-......a.......g.................
Krobylos maehongsornensis 1   ---agag-agt---------------.-..............g.................
K. maehongsornensis 2         ---agag-agt---------------.-..............g.................
Pupilla muscorum (A)          ata-----------------------.-..............g.................
P. muscorum (B)               ata-----------------------.-..............g.................
P. muscorum (E)               ata-----------------------.-..............g.................
Vertigo gouldi                ttt-----------------------.-......a.......g.................
Vallonia costata (A)          ttga----------------------.-..............g.................
V. costata (B)                ttga----------------------.-..............g.................
Zoogenetes harpa              gt------------------------.-..............g..........c......
Strobilops labyrinthica       gtga----------------------.c......a.......g.................
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                              541......|.........|.........|.........|.........|.......600

Anauchen chedi                cgacccgtcttgaaacacggaccaaggagtctaacatgcgcgcgagtcattgggcggtac
A. chatnareeae                ............................................................
A. utaithaniensis             ........................................................t...
Aulacospira smaesarnensis     ............................................................
Gyliotrachela adela           ............................................................
G. diarmaidi                  ............................................................
G. khaochakan                 ............................................................
G. kohrin                     ............................................................
G. saraburiensis              ............................................................
G. surakiti                   ............................................................
Hypselostoma cucumensis       ............................................................
H. erawan                     ...................................................aa.......
H. holimanae                  ............................................................
H. panhai                     ..............................t.............................
Krobylos maehongsornensis 1   ............................................................
K. maehongsornensis 2         ............................................................
Pupilla muscorum (A)          ............................................................
P. muscorum (B)               ............................................................
P. muscorum (E)               ............................................................
Vertigo gouldi                ............................................................
Vallonia costata (A)          ............................................................
V. costata (B)                ............................................................
Zoogenetes harpa              ............................................................
Strobilops labyrinthica       ............................................................


                              601......|.........|.........|.........|.........|.......660

Anauchen chedi                gaaacccaaaggcgcagtgaaagcgagggccgtctccgagcggcccaggtgggatcctct
A. chatnareeae                .............................t..............................
A. utaithaniensis             .............................t..............................
Aulacospira smaesarnensis     ............................................................
Gyliotrachela adela           ............................................................
G. diarmaidi                  .............................t..c..ta......t................
G. khaochakan                 .............................t..c...t.......................
G. kohrin                     .............................t..c..ta......t................
G. saraburiensis              ............................................................
G. surakiti                   ................................-...........................
Hypselostoma cucumensis       .............................t..............................
H. erawan                     .............................t..c...........................
H. holimanae                  ............................................................
H. panhai                     .............................t..............................
Krobylos maehongsornensis 1   .............................t..............................
K. maehongsornensis 2         .............................t..............................
Pupilla muscorum (A)          .............................t..............................
P. muscorum (B)               .............................t..............................
P. muscorum (E)               .............................t..............................
Vertigo gouldi                .............................t..c...-...t...................
Vallonia costata (A)          .............................t...t...........t..............
V. costata (B)                .............................t..............................
Zoogenetes harpa              .............................t...gaa--......................
Strobilops labyrinthica       .............................t..c...........................
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                                 661.....|.........|.........|.........|.........|.......720

Anauchen chedi                   cc-tcc---------aggagca--a-tc--------------cga---------cgaggg
A. chatnareeae                   ..-...ga-------c......--g-..--------------..gcg---c---......
A. utaithaniensis                ..-...---------.......--.-..--------------...---------......
Aulacospira smaesarnensis        ..-...c-------tc.....g--.-..--------------..gcg-------a.....
Gyliotrachela adela              ..-..------------....---------------------tc----------......
G. diarmaidi                     ..-..t---------.......--.-..--------------..g---------......
G. khaochakan                    ..-...c--------.......--.-..--------------...---------......
G. kohrin                        ..-...c--------.......--.-..--------------..g-g-------......
G. saraburiensis                 ..-...---------.......--.-..--------------..g---------......
G. surakiti                      ..-...---------.......--.-..--------------..g---------......
Hypselostoma cucumensis          ..-..taaacttccctca.a..gc.t..acgtgttgg-cttg..ggggggccgg.....a
H. erawan                        t--...cc--------ctt..cc---..g-----cgggcttgt.---------tg.g..a
H. holimanae                     ..-...---------.c-.t..--.-c.--------------.t----------g.....
H. panhai                        ..c..t----------c.c.ag--.------------------.----------t.-...
Krobylos maehongsornensis 1      ..-...---------.......--.-..--------------..g---------......
K. maehongsornensis 2            ..-...---------.......--.-..--------------..g---------......
Pupilla muscorum (A)             ..-..tc---------c.....--.-..--------------t.----------t.....
P. muscorum (B)                  ..-..tc---------c.....--.-..--------------t.----------t.....
P. muscorum (E)                  ..-..tc---------c.....--.-..--------------t.----------t.....
Vertigo gouldi                   ..-..t---------.t.....--.-..--------------t.-----------....a
Vallonia costata (A)             ..-..t---------g......--.-..--------------.-----------t.....
V. costata (B)                   ..-...---------.......--.-..--------------.-----------......
Zoogenetes harpa                 ..-..tc---------c.....--.-..--------------t.----------t.....
Strobilops labyrinthica          ..-..tc---------c.....--.-..--------------t.----------t.....


                                 721.....|.........|.........|.........|.........|.......780

Anauchen chedi                   gtggcagctttaat-----------gaacatatttaaagttttttctgcccggtga----
A. chatnareeae                   .......tc....g--------caattta........ga.g.aa.....t.a....----
A. utaithaniensis                .............g------------.gtc.............ac...........----
Aulacospira smaesarnensis        ........c.at.ga------gggt.t----...at.g..g.ag.......aa...----
Gyliotrachela adela              ........c....ga-------tttt-----......g..aaaac......a....----
G. diarmaidi                     .............ga------aagtat----.........ga.g............----
G. khaochakan                    ..........a..ga-ctta--tgt------....t.g...aaac......a....----
G. kohrin                        ........c....gg------aataat----......g..ga.g............----
G. saraburiensis                 .............g---tta-atttat----.........ggaa.......a....----
G. surakiti                      .............gc--tta---ttac----......g..a.ag.......a....----
Hypselostoma cucumensis          .............gattttc--tttt-----.........aaaac...........----
H. erawan                        .............gg------agta------..........aag.......a....----
H. holimanae                     .............g-------attttt----......g...aaa............----
H. panhai                        .............ga-------tttttt---.........gaagc......a....----
Krobylos maehongsornensis 1      .............ga------------ta..............ac...........----
K. maehongsornensis 2            .............gt------------.a..............ac...........----
Pupilla muscorum (A)             ........c.a..g-----------atct......t.g..gagac....t.aa...agat
P. muscorum (B)                  ..........a..g-----------tt-t......t.g..gagac....t.aa...agat
P. muscorum (E)                  ..........a..g-----------tt-t......t.g..gagac....t.aa...agat
Vertigo gouldi                   .g...........g-----------attt..............ac..........g----
Vallonia costata (A)             ..........a..g-----------t..t......t.....agac......a....----
V. costata (B)                   ..........a.g.-----------at.-............aagc......a....----
Zoogenetes harpa                 ..........attga----------a-.-....aat.....aag.......a....----
Strobilops labyrinthica          ...........ttgatttt------at.-.-----.....gaa........a....----
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                              781.....|.........|.........|.........|.........|.......840

Anauchen chedi                --gtc-tat-tttaacg-gccgcagtactttgactgtgctaaggtagcataataaattga
A. chatnareeae                --t.t-----......a-.........................................g
A. utaithaniensis             --tat-----..a....-.........................................g
Aulacospira smaesarnensis     --t-a--gag..g..t.-..........................................
Gyliotrachela adela           --t.t----a.......-..........................................
G. diarmaidi                  --t.a--..a.......-..........................................
G. khaochakan                 --t.t--t.t.......-.........................................g
G. kohrin                     --tca--..a.......-..........................................
G. saraburiensis              --t.a--t.a.......-..........................................
G. surakiti                   --t-a--..a.......-..........................................
Hypselostoma cucumensis       --tat----t.......-..............g..........................g
H. erawan                     --t.t-----.......-.........................................g
H. holimanae                  -at.t--..t.......-.........................................g
H. panhai                     --t.g--.-a.......-.........................................g
Krobylos maehongsornensis 1   --t.t-----.......-.........................................g
K. maehongsornensis 2         --aat-----..c....-.........................................g
Pupilla muscorum (A)          gtaga-.tat..a..ta-.........................................g
P. muscorum (B)               gtaga-.tat..a..ta-.........................................g
P. muscorum (E)               gtaga-.tat..a..ta-.........................................g
Vertigo gouldi                --t-a-.t--c......-..........................................
Vallonia costata (A)          gta.t-.tac.......-.........................................g
V. costata (B)                gt----.t.c.......-.........................................g
Zoogenetes harpa              -aa.a-.t--.......-.........................................g
Strobilops labyrinthica       ---.a-.tca.......-..........ac.............................g


                              841.....|.........|.........|.........|.........|.......900

Anauchen chedi                cttttaattaaagtctagtatgaatgga-tttttggaaaat-aactgtctctgtta--at
A. chatnareeae                ...........g......a........g-ag.a..a...g.-g.......a.a.a.--.a
A. utaithaniensis             ...........g......a.....a...-aa....a.g...-......t..aa.a.--.a
Aulacospira smaesarnensis     .c.....a.ggg.c....g..a..a...a.aa.g.a...t.--t......t.t...-gga
Gyliotrachela adela           .........g........a.....c..g-aa.....gg.ga-c.......taaaa.--.a
G. diarmaidi                  .cc......ggg.c....a.....c...-.aa..aa...t.-g.......t.a..--t.a
G. khaochakan                 .c.......ggg............c..g-ag.....gg...-.g......t.ta..--.g
G. kohrin                     .c.......g.g.c....a.....a...-.aa..aa...t.-gt......t.a..--t.a
G. saraburiensis              ..c......ggg.c....a.....a..g-aaa..aa.....-........t.t...--.a
G. surakiti                   .........g.g......a.....a...aaaa...ag..t--.g........taa.--.a
Hypselostoma cucumensis       .........g........g.........-aaa...aggg.g-........t.taa.--.a
H. erawan                     .........g..................-.a.....gg..a-.g..t...tat.at--ca
H. holimanae                  .........ggg....t.........a.-.a.....gg..a-........tgtaa.--.a
H. panhai                     .c.......g.g............a...-a.....ag...a-.t..t....ataat-t..
Krobylos maehongsornensis 1   .........g.g....g.a.....a..g-aga.......ga-..........aaa.--.g
K. maehongsornensis 2         ........................a..g-ag........gg-..........aaa.--.g
Pupilla muscorum (A)          .........g......g.a.....a.-gtaaa..t.....a-g.t.......aa.tag.-
P. muscorum (B)               .........g......g.a.....a.-gtaaa..t.....a-g.t.......aa.tag.-
P. muscorum (E)               .........g......g.a.....a.-gtaaa..t.....a-g.t.......aa.tag.-
Vertigo gouldi                .........g.....gg.......a..g--a...t.g..gat.........aaa..tata
Vallonia costata (A)          .........t.....gg.a.....a..gt-.aa...gg.---g.t.c.g...tacttc.a
V. costata (B)                .........t.....gg.a.....a..g-c.aa...gg.---..t.c.g...tacttt.a
Zoogenetes harpa              .......a.g.....gg.a.....a.a.-ccca......ga-.t.......-aa.tat.a
Strobilops labyrinthica       .........gg....gg.......a...-ac.a..a...ta-........a-aa..at.g
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                             901.....|.........|.........|.........|.........|.......960

Anauchen chedi               ct--tttaaaaatttact-tatt-aggtgaaaatacctatattta--ttaaataagacga
A. chatnareeae               ..-a.a.....-......-....-.................aa.t--...c.........
A. utaithaniensis            a.-a.a.....-....t.t....-.................aactt-..tc.........
Aulacospira smaesarnensis    t--aaaa.t..-......-..g.-g.........t..c.c.a.---....c..t......
Gyliotrachela adela          t.-aaa..t..-c..c..-a.a.-..........t.....ga..t-a......t......
G. diarmaidi                 t.-taa.....-......-....-..........t....-.a..tt-c..c.........
G. khaochakan                a.-aa......-......-..aa-..............t..g..t--.............
G. kohrin                    t.-taa.....-......-....-..........t....-.ac..t-c..c.........
G. saraburiensis             t.-aaa.....-......-..a.-.................a..t--...c.........
G. surakiti                  t.-taac....-c.....-a...-..........t....--a..tt-...c.........
Hypselostoma cucumensis      a.-a.......-......-....-................-a..t--......t......
H. erawan                    a.-aa......-......-..a.-.................aaa.--a..c..t......
H. holimanae                 ..-aaa.....-....a.-..a.-..........g.......aa---...c.........
H. panhai                    a.--.......-c...a.-....-..........g.....t...---...t..t......
Krobylos maehongsornensis 1  a.-a..-..t........-....-g.........t..c...caag--a..c..t......
K. maehongsornensis 2        a.-a.......-......-..a.-g.........t..c...ga.t--...c..t......
Pupilla muscorum (A)         ..ta..---...c.....-..a.-..........t.....g...taca..-..-......
P. muscorum (B)              t.ta..---g..c.....-..a.-..........t.....g...ttca..-..-......
P. muscorum (E)              t.ta..---g..c.....-..a.-..........t.....g...ttca..-..-......
Vertigo gouldi               t.-a...t--......a.-..a.-g.........t.-.catac.tac...c..t......
Vallonia costata (A)         t--aa..t..........-..a.-..........t....taaatata.g-..-......
V. costata (B)               .--ta..t..........-..a.-..........t.........tt-a.g-..-......
Zoogenetes harpa             g--ta..ttg..a...a.--.a.t.a........t.-.-..ag..aaa..t.........
Strobilops labyrinthica      g-aaaa..t-..g.....-..aa-..............t..aa.tt-a.g--.t......


                             961.....|.........|.........|.........|.........|......1020

Anauchen chedi               gaagaccctaagaattattaataa-aa-taact-t--atttttaat---att---ttttt
A. chatnareeae               ....................-...----...a.a.-tt.a.-....---..ctta.....
A. utaithaniensis            ..................-.....-.--.t.t.t.ttc.aa-....---...c-g.....
Aulacospira smaesarnensis    ....................c...----gc.gaactt.c.g..gt.---ta.--g.....
Gyliotrachela adela          .................c.gt...-..-att.ct.tagag.---t.---.aac---....
G. diarmaidi                 ..........t.........t...----a.tgac.ccc.c.c.c..---ta--tt.....
G. khaochakan                ....................-...-c.-a..aat.aata...ctt.---ta---t-....
G. kohrin                    ..........t.........t...----ac-ga-.ttt...c.cg.---ta--at....-
G. saraburiensis             ..........t.......a.t...----...a.a.-t...-g..t.---t.-taa.....
G. surakiti                  ..........t.......a.t..c----acta.gcttt.gc.....---.aa---.....
Hypselostoma cucumensis      .................cagtg..t..t..ga.t.ttt..a---t.---taa--ac....
H. erawan                    ........................----a.gact.aatg.--..t.---...---.....
H. holimanae                 ..................a.t...----..---t.-at...aa.t.---..g---.....
H. panhai                    ..................a....g-ttta.-t.t.aa.aa--a.t.---.a.tt-.....
Krobylos maehongsornensis 1  ....................-...----...a.a.-g.a.....t.---ggc--g.....
K. maehongsornensis 2        ....................c-..----...a.aatg.aa..-gt.---.ac--g.....
Pupilla muscorum (A)         .....................a..----...g.--tt...-...c.t--taat--.....
P. muscorum (B)              .....................a..----...g.--tt...-...c.t--taat--.....
P. muscorum (E)              .....................a..----...g.--tt...-...c.t--taat--.....
Vertigo gouldi               .............g....a.tc..----g-.tgaa-tt.a...c..---..atca.....
Vallonia costata (A)         ....................t..t---g..tt------a.-...t.taata.tca.....
V. costata (B)               ....................t..c---a..tt-----.a.-...t.tagtaatca.....
Zoogenetes harpa             .......................t---a..taa---t.a.a...t.--ata-tct.....
Strobilops labyrinthica      a.................a.t...----..taaa--t.a.a...t.ta-caat-a.....
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                             1021....|.........|.........|.........|.........|......1080

Anauchen chedi               gttggggcaacaaattta--aaattt--ta-aat--taattaatt------aattt--ta
A. chatnareeae               ............-taa.t--gg.a..--.-t..c--c..--t.a---tat-..---..
A. utaithaniensis            ............-..a.t--gg.a..--..-.-c--c...tt.---tat.g..----
Aulacospira smaesarnensis    ............-..g.t--gg.a.ataagt..c--c..a-.aac---ttt.-----at
Gyliotrachela adela          ............-t.g.t--g..a..-a---.c--c..ca.t-.----att.gaa----
G. diarmaidi                 ............-gcg.t--gg.a---agta..c--c..cggcaa---ag..gg-----
G. khaochakan                ............-..a.t--gg.aa.--..t..c--c...a...---aatt.aa-----
G. kohrin                    ............-.cg.t--gg.g---a.tt..c--c..cg.tga--aatt.a------
G. saraburiensis             ............-..g.t--gg.caat----..--c..ca.c.a--tatt.c.---c.
G. surakiti                  ............-.cg.t--gg.a.at-..--gc--....c..cg--gaaat..a---a.
Hypselostoma cucumensis      ............-t.g.t--tgg.aa------..--c..ca.ta.at-aag..g.-----
H. erawan                    ............-..g.t--gg.-.a-a---.c--c..cacta.-ttattt.-----g.
H. holimanae                 ............-t.g.t--gg..a.-a-.-..c--c..ca.t..----cat.a.g----
H. panhai                    ............-..g.t--gg.-.a-a-.g..c--c...a-ta.attatat------a.
Krobylos maehongsornensis 1  ............-g.g.t--gg..a.--.-..c--c..-...a----tt..---..
K. maehongsornensis 2        ............-..g.t--gg...--.-..c--c..-...a----c....--..
Pupilla muscorum (A)         ............-.c..t--g..ca.-a-.-..c--c..-agggc----t..aga----
P. muscorum (B)              ............-.c..t--g..ca.-a-.-..c--c..-aggg.----t..aga----
P. muscorum (E)              ............-.c..t--g..ca.-a-.-..c--c..-aggg.----t..aga----
Vertigo gouldi               ............-.a----g.c.gg-a..-..----.ccgtca.----t..gaaac--
Vallonia costata (A)         ............-tag.t--ggt.aa-cc--..c--...-ac------tat.aa-----
V. costata (B)               ............-tag.t--gg.aaa-cc--..c--...-ac.-------.aa.t---
Zoogenetes harpa             ............-tag.t--ggt--.taacc..c------..-a---ttat..a-----
Strobilops labyrinthica      ............-.a..c--.g.aa.taa.t..c--.g.......---atct-.aa---t


                             1081....|.........|.........|.........|.........|......1140

Anauchen chedi               a-tgt---gtca----atta----tta-taagaat-ataa-ttaattaccttagggataa
A. chatnareeae               tt.t.aa-.a..----.a..----..t-.....a-.att-...................
A. utaithaniensis            -t.t.---.......----.a.t----..t-.......-.a..-...................
Aulacospira smaesarnensis    ta.a.aaat-..-----..t----.a.t..-at.aa.att-.a-................
Gyliotrachela adela          --ga.------.atat...tactaa.tg....ga-...-g.a................
G. diarmaidi                 tt.a.aaac-..----.a.tt---a.ttc.-at.a--a.c-.a-...............
G. khaochakan                --aa.gtac.-.tca-...t----..-a.t.t...-ta.c-.a-...............
G. kohrin                    .a.t.aagt-..----.c.ct---a.ttc.-a..a--a..-.a................
G. saraburiensis             ca.c.a--t-..-----..t----...t..-at.aa.a.--.a................
G. surakiti                  ta.a.ttat-..-----..t---t...t..tat.-a.a.--.a-...............
Hypselostoma cucumensis      --aa.ac----.tca-..c.-----.ta..c.g.a--attt..................
H. erawan                    ttaa.aa----.-att.g-----a..tc...tt.a-.a..-.a................
H. holimanae                 -aaa.gt----.tca-.c.t----..g-...at.a-.a..-.a................
H. panhai                    tt.acat----.t-ttg..-----..tac..tt..-gag.-.a................
Krobylos maehongsornensis 1  t-ca.---.......----.gat----..t-....t.a-ga..-.a................
K. maehongsornensis 2        ttca.a---......----.aa-----..tt.....a-ga..-.a................
Pupilla muscorum (A)         --.t.taa.a..----.a.----t...t.tt...--.a..a.................
P. muscorum (B)              --.tctaa.a..----.a.----t...t.tt...--.a..a.................
P. muscorum (E)              --.tctaa.a..----.a.----t...t.tt...--.a..a.................
Vertigo gouldi               --.t.aacaaa.ccag.ac-----..c-.t.a..--.g..a.a................
Vallonia costata (A)         tt.tattt.a-.---ag-.----t...tc.t...--tag.t.................
V. costata (B)               --.tatat.a-.---ag-.----t...tc.t...--ta.tt.................
Zoogenetes harpa             ta.t.ttt.a..a---.a..----a.tt.......-.acta.a...............
Strobilops labyrinthica      .aatacag.a..----.a..----...t...tt..-ga.-t.................
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                             1141.....|.........|.........|.........|.........|......1200

Anauchen chedi               cagca-taatttataa-attagattgtgacctcgatgttggattaaggac--tttattta
A. chatnareeae               .....-.....atactt..-.....a......................t.--...ta.a.
A. utaithaniensis            .....-.....a-g..t...............................g.--....a...
Aulacospira smaesarnensis    .....-.....a-at.tt...........................g.a.g--....a...
Gyliotrachela adela          .....-.....a.attat..............................g.--....ac.g
G. diarmaidi                 .....-......t-t.tt.a..c...........................--....c...
G. khaochakan                .....-.....a-at.t............................g....--....g..g
G. kohrin                    .....-.....at-tgtt....t...........................--....a...
G. saraburiensis             .....-.....atat.t-................................--....a...
G. surakiti                  .....-.....at-t.tt...........................g....--.c.tg...
Hypselostoma cucumensis      .....-.....a-gtttt..............................c.--....a...
H. erawan                    .....-.....g-attt.................................--....a...
H. holimanae                 .....a.....a-a-.tt................................--...ta...
H. panhai                    .....-.....a-atta.................................--....c...
Krobylos maehongsornensis 1  .....-.....a.g.ttt................................--....a...
K. maehongsornensis 2        .....-.....a.attt-................................--....a...
Pupilla muscorum (A)         .....-......tct-tt................................ccc...gg..
P. muscorum (B)              .....-......tct-tt................................ccc...gg..
P. muscorum (E)              .....-......tct-tt................................ccc...gg..
Vertigo gouldi               .....-.....a..t--t................................--........
Vallonia costata (A)         .....-.....a..t-t.........................c.......--a...a.a.
V. costata (B)               .....-.....a..t-t.........................c.......--a...a.a.
Zoogenetes harpa             .....-.....at-ttat........................c....a..--...t..a.
Strobilops labyrinthica      .....-....-gt.ttt.aa......................c..g....--.a..a.ag


                             1201.....|.........|.........|.........|1244

Anauchen chedi               ctagccgtaattaaagatggttctgttcgaacttttaga-cctt
A. chatnareeae               t.....a...........a..............-.a.t.-....
A. utaithaniensis            t.....a.t..a......a..............-..tc.-....
Aulacospira smaesarnensis    t..............ac.a.................ttt-....
Gyliotrachela adela          t.....ac..........a.............a-.a.t.-....
G. diarmaidi                 t.....a...........a..............g.a.a.-....
G. khaochakan                t.....ac.g........a.................ta.-....
G. kohrin                    t...a.a.........c.a..............a.gta.-....
G. saraburiensis             t..a..a......g....a..............g...a.-....
G. surakiti                  t.....a....cgg....aa...........-....ta.-..c.
Hypselostoma cucumensis      tc...aa.....g.....t.............a-.a.a.-....
H. erawan                    t..a..a...........t.............a-aa.a.-....
H. holimanae                 t.....a...........a.............a-a..t.-....
H. panhai                    tc....a...........a.............a-aagt.-....
Krobylos maehongsornensis 1  t.....a.t.........a..............-.a.c.-....
K. maehongsornensis 2        t.....a.t.........a..............-...t.-....
Pupilla muscorum (A)         --....acttaag.ga..a..............-...t.t....
P. muscorum (B)              --....acttaag.ga..a..............-...t.t....
P. muscorum (E)              --....acttaag.ga..a..............-...t.c....
Vertigo gouldi               ..........a.c.....a..............g.a.t.-....
Vallonia costata (A)         ........t.....t..................-..tt.-....
V. costata (B)               t.......tg....t...a..............-..tt.a....
Zoogenetes harpa             t.....a.t.aa.g....a..............-..tt.ct...
Strobilops labyrinthica      ta....a.t....t....a..............-..ttga..c.
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BIOGRAPHY

Miss Piyoros Tongkerd was born on the 31st of March 1977. She graduated her Bachelor's Degree of Science from Department of Biology, Faculty of Science, Chulalongkorn University in 1998. In 1999, she was awarded the scholarship by the Royal Golden Jubilee (Ph.D. Program) of the Thailand Research Fund for her Ph.D. study in Biological Science Program at Faculty of Science Chulalongkorn University.