THE EVOLUTIONARY POTENTIAL OF A PARASITE HOST: DIVERSITY, SYSTEMATICS AND PHYLOGEOGRAPHY OF THE FRESHWATER SNAILS GENUS *TAREBIA* H. & A. ADAMS, 1854

By
MISS Nuanpan VEERAVECHSUKIJ

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy BIOLOGY
Department of BIOLOGY
Graduate School, Silpakorn University
Academic Year 2018

วิวัฒนาการความเกี่ยวเนื่องระหว่างโฮสต์กับปรสิต: ความหลากหลาย ซิสเทมาติคส์ และชีวภูมิศาสตร์ ของหอยน้ำจืดสกุล *Tarebia* H. & A. Adams, 1854

โดย
นางสาวนวลพรรณ วีระเวชสุกิจ

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Title	The evolutionary potential of a parasite host: diversity, systematics and phylogeography of the freshwater snails genus *Tarebia* H. & A. Adams, 1854
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MISS NUANPAN VEERAVECHSUKIJ : THE EVOLUTIONARY POTENTIAL OF A PARASITE HOST: DIVERSITY, SYSTEMATICS AND PHYLOGEOGRAPHY OF THE FRESHWATER SNAILS GENUS *TAREBIA* H. & A. ADAMS, 1854 THESIS ADVISOR : ASSOCIATE PROFESSOR DUANGDUEN KRAILAS, Ph.D.

Tarebia sp. is widespread and abundant in various lentic and lotic water bodies in Southeast Asia, with its range extending onto islands in Indo-West-Pacific. This snail is one of snail in family Thiaridae, as one of the most frequent and major primary intermediate host, an important vector for digenic trematodes causing several animal and human diseases. In Thailand *Tarebia* has been reported with the occurrence of one species only and have highly variations of shell morphology, which makes it difficult to identify them using only shell morphology. In this study, the snail samples were collected from 90 locations in Thailand, between 2014-2016. They were separated by distint shell, three groups called morphs A, B and C here, without implying morphotypes in the sense of species under a respective species concept, but for convenience only and to facilitate further research into the potential correlation of phenotypical and genetic proprinquity. Thai specimens were compared with specimens from the Centre of Natural History (CeNak), Zoological Museum, Universität Hamburg, Germany (including 12 locations from Timor-Leste). The objective of this study was integrate evidence of phylogeographical analyses based on phenotypic variation (shell morphology, using biometry and geometrical morphometrics) and genotypic variation from the gene fragments cytochrome C oxidase I and ribosomal 16S (using several phylogenetic analyses, including haplotype networks and a dated molecular tree). The results showed that *Tarebia* snails were found variation of both phenotype and genotype. The biometric and geometric morphometric analyses and reproductive strategy of difference morphs and genetic clades were found similarity and widely overlap. The phylogenetic trees were found two genetically distinct clades (clade A and B) and hint at possible species differentiation within what has been traditionally considered as *T. granifera.* All specimens from Timor-Leste were included in clade A together with specimens mostly from the southern to southern-central parts of Thailand. The clade B specimens were more frequent in the northern part of Thailand. These two lineages started to split about 5 Mya. The collected snails were investigated for cercarial infections by using shedding and crushing methods. The infection rate was 5.80% (493/8,493). The cercariae were categorized into eleven species and seven types, viz. (i) virgulate xiphidiocercariae (*Loxogenoides bicolor* and *Loxogenes liberum*), (ii) armatae xiphidiocercariae cercariae (*Maritreminoides caridinae* and *Maritreminoides obstipus*); (iii) parapleurophocercous cercariae (*Haplorchis pumilio*, *Haplorchis taichui* and *Stictodora tridactyla*), (iv) pleurophocercous cercariae (*Centrocestus formosanus*), (v) megarulous cercariae (*Philophthalmus gralli*), (vi) Echinostome cercariae and (vii) Gymnocephalous cercariae. In addition, a phylogenetic marker (internal transcribed spacers II, ITS2) was employed in generic and infrageneric level classifications of these trematodes. Thus, this analysis combines the parasites data on morphology and geographical occurrence with molecular phylogeny, aiming to provide the groundwork for future studies looking into more details of the parasite-snail evolutionary relationships.

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CHAPTER I
Introduction

Animal Systematics

Systematics is the study of systems and the principles of taxonomy and classification, both past and present. For old systematics, only morphological species were employed such as Thiarid snails were reported to have 8 genera. The shells are variation in each other that must be careful examined to reveal their species, which can be distinguished by only shell characters. When new systematics was developed, molecular and ecological observations were employed to indicate the taxonomies. Recent studies of phylogenetic analyses using morphological and molecular data have revealed that *Brotia*, *Paracrostoma* and *Adamietta* were not members of family Thiaridae. All of them have distinctive features and adelphotaxon, which determine that they belong to the family Pachychilidae (Glaubrecht & Köhler, 2004; Strong et al., 2011). Michener et al. (1970) describe that the systematics can be divided into four major fields. The first field was taxonomy, often equated with systematics, is the discipline concerning discovery, description, and classification of organism groups, termed taxa. The second field was classification. It is the clustering of species into a hierarchical arrangement according to some criteria, usually an understanding of their relationships to other species. The third was phylogenetic analysis, an increasingly important aspect of systematics. It is the discovery of the historical, evolutionary relationships among species; this pattern of relationships is termed a phylogeny. The fourth component of systematics is biogeography, the study of species geographic distributions. Historical biogeography examines how species distributions have changed over time in relationship to the history of landforms and climate, as well as how those changes have contributed to the evolution of species living together in communities and ecosystems. In addition, Mayr (1970) gave 3 concepts (Morphospecies, Biological species, Phylogenetic species concept) for isolation of species. These concept include reproductive units, ecological units and genetic units.

Biodiversity in Thailand.

Biodiversity is the variety of life flora and fauna including levels of their organization from genes to populations, species and ecological systems existing throughout the world. Such diversity of life is a legacy of the evolutionary processes. The natural world of biological diversity is concentrated in tropical forests. Thailand is situated in one of the richest areas of the world with regard to biological resources (Baimai, 2010). Located in the centre of mainland Southeast Asia, it is situated in a hot and humid climatic zone of the wet tropics, which supports complex ecosystems as varied as rainforests and coral reefs, with numerous life forms. Although Thailand is a relatively small country, there are various kinds of limnic systems providing aquatic habitats that have gained less attention yet.

In the past, 70 percent of the total land area was covered with various kinds of tropical forest. These variations of forest type provide terrestrial and aquatic habitats for numerous life forms in complex ecosystems. Thailand's tropical forests support some 15,000 known species of animals and 12,000 species of vascular plants. For

example, Vertebrates were found 56 endemic species freshwater in Thailand. About 10 species of frogs were found endemic species (Chan–ard, 2003). For invertebrates, marine Shell-fish were found 1,538 species including 634 gastropods and 382 bivalves (Nabhitabhata, 1993). About 280 species of gastropods were found in fresh and brackish water areas (Brandt, 1974). Thus Thailand can claim to be situated in one of the richest areas of the world with regard to biological resources (Baimai, 2010). The gastropod species have proven their status as ideal model systems for speciation, systematics and evolution (Glaubrech et al., 2009; Glaubrecht, 2011; Glaubrecht & Podlacha, 2010). They showed a characteristic adaptation to habitats that was represented by variety of ecological and morphological characteristics. Still, the information about gastropods in adjacent river systems and lakes is scarce, and the lack of recently collected and museum materials might be the reason caused by lasting political complications. Therefore, gastropod should gain more attention and be taken in the focus of species diversity, in order that they may contribute to solving many fundamental questions and evolution diversity of fauna in Thailand.

Biogeography in Thailand

Biogeography represents a modern and lively zoological discipline concering the patterns of species distribution across geographical areas, which can usually be explained through a combination of historical factors such as speciation, extinction, variation of the sea levels, river routes and habitat. Biogeographers try to reconstruct and understand the evolutionary history of organisms in space and time, by which they try both to record and to explain the geographical distribution patterns of living organisms.

Thailand is located in the centre of the mainland of Southeast Asia, a relatively small country with a total area of about 513,000 km^2. To the north, it borders Myanmar and Laos; to the east, Laos and Cambodia; to the south, the Gulf of Thailand and Malaysia; and, to the west the Andaman Sea and the southern extremity of Myanmar. Its maritime boundaries include Vietnam in the Gulf of Thailand to the southeast, and Indonesia and India on the Andaman Sea to the southwest. Biologists have divided Thailand into two regions: the Indochinese region and the Sundaic region, separated by the Isthmus of Kra, a biogeographical barrier believed to be affected by a sea level change in the past (Bruyn et al., 2005; Dejtaradol et al., 2016; Parnell, 2013). For example, in contrast to those species among birds of the Northern Highlands with Chinese affinities, a number of species in the Southern Peninsula are related to those from the Sundaic region (Lekagul & Roung, 1991). However, the Thai peninsula not only forms a barrier to the distribution of several groups but also an important bridge in the biogeography of Southeast Asia, connecting taxa of northern and southern biotas.

Drainage and river systems of Thailand

Thailand can be divided into geographical regions based on distinct drainage systems; with those in the north, for example, forming the Chao Phraya drainage flowing into the Gulf of Thailand, those in the northeast as part of the Mekong River catchment area which eventually drains into the South China Sea, or the north-western region as part of the Salween River system. In contrast to these and other major river systems, in the south there are shorter rivers that either run east to the Gulf of Thailand or west to the Andaman Sea. These water bodies in Thailand form hotspots of aquatic biodiversity with various local endemism.

The National Committee on Hydrology separates Thailand into 25 distinct hydrological units or river basins (Fig. 1). There are regrouped into seven areas, each with specific characteristics, as follows (World, 2011);

1. The Central area: This is the most important area in Thailand. This is the area without large water sources. Therefore, it depends heavily on water from river basins upstream, such as the Chao Phraya River, which is the main river of Thailand. The Chao Phraya begins at the confluence of the Ping and Nan rivers (Northern area) in Nakhon Sawan Province. It flows north to south from the central plains through Bangkok into the Gulf of Thailand.
2. The Northern area: This area provides sources of water for the central area. For example, the Wang River flows north to south. This river has its source in Chiang Rai Province. One of the principal settlements along the river is Lampang, which is on the north bank of a curve in the river. From Lampang, the river flows southwards into Tak Province. It joins the Ping River near Mae Salit north of the town of Tak. The Ping River originates in Chiang Mai Province. After that, it flows through the provinces of Lamphun, Tak, and Kamphaeng Phet. The Nan River originates in Nan Province. The provinces along the river after Nan Province are Uttaradit, Phisanulok, and Phichit. The Yom River joins the Nan River at Chumsaeng district, Nakhon Sawam Province. When the Nan River joins together with the Ping River it becomes the Chao Phraya River.
3. The Northwestern area: This is a part of the Salween River system, which flows through Myanmar.
4. The Western area: This is part of the basin of Mae Klong River, which runs into the Gulf of Thailand.
5. The North-eastern area: This is part of the Mekong River basin's catchment area which drains into the South China Sea.
6. The Eastern area: The area is characterized by many short rivers.
7. The Southern area: Many short rivers and high annual rainfall characterize this area. There are a number of large water reservoirs.

So, the rivers are generally regarded as hotspots of aquatic biodiversity with local endemism and species of various groups. Especially, freshwater snails in the river are the most diverse in the country. The following map provides a clear picture and understanding of Thailand's river (Fig. 1).

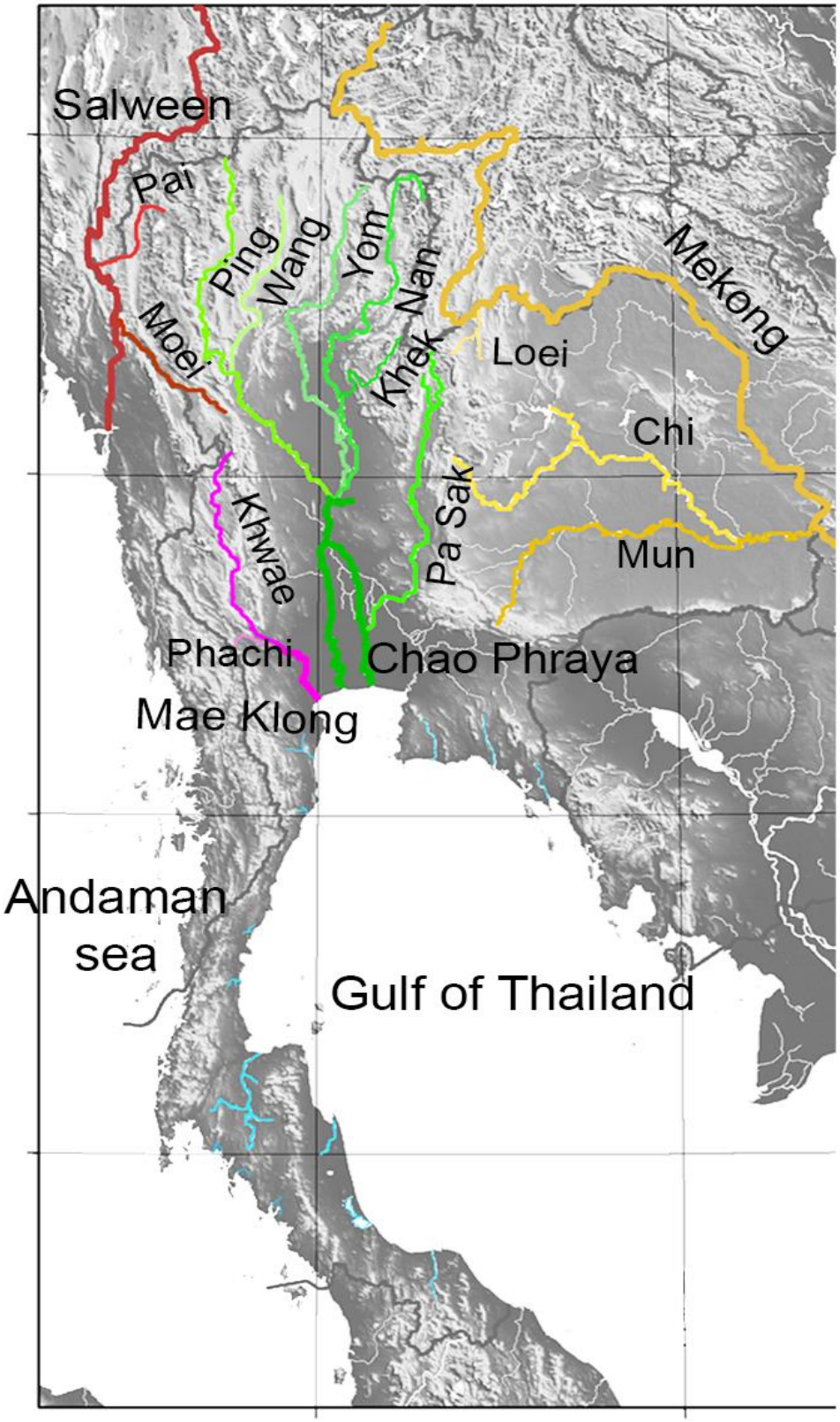

Figure 1. River systems map of Thailand.

Freshwater gastropod as a model of evolution

Among the aquatic biota, limnic molluscs feature the most prominent and diverse, as there are about 280 species of in freshwater and brackish-water habitats (Brandt, 1974). Although we should study and understand the origin of biodiversity and mechanisms of speciation in as many diverse model systems as possible, to date essentially vertebrates (mostly birds and fishes) are used, while other groups in particular among invertebrates remain widely untested. As shown by Schwenk et al. (2008) and Glaubrecht (1993, 1996, 2009, 2010, 2011), for example, mollusc and in particular freshwater gastropods hold the same chance for the study of evolutionary phenomena as other groups. Speciation should not only be reflected in the taxonomic description of any speciose group, but instead by the actual study of causation and underlying mechanisms of how species arise. Thus, instead of merely referring to "speciation", "adaptive radiation" or any "megadiverse" species assemblage for each and every speciose taxonomic group we should strive to investigate, with adequate methods and founded on solid theoretical ground, the underlying mechanisms of anagenetic versus cladogenetic change. This has been exemplified in the discussion of freshwater gastropods as model of speciation and evolutionary systematics by Glaubrecht (2006, 2011).

Accordingly, non-marine molluscs in Thailand should also gain more attention and be taken into focus of studies in order to look into their species diversity and contributing to solving fundamental questions and the evolution of faunal diversity. At the same time, any biological information about gastropods in Thailand's river systems and lakes is generally scarce and the often lacks recently collected material or available former museum collections, which results in hampering more in-depth studies. This has, in turn, become problematic, as several freshwater snails with their main occurrence in Southeast Asia have a considerable importance as first intermediate hosts for infections in humans and animals. Despite their proven medical importance, for example, the faunistic and systematic knowledge on Cerithioidean freshwater snails of the various families acting as one of the most important vectors for digenic human pathogens, is precarious. The Cerithiodea is ecologically and phylogenetically important, essentially as a marine caenogastropod group, with its freshwater members in SE Asia acting as first intermediate hosts of a wide array of diverse trematodes (Dechruksa et al., 2007; Krailas et al., 2014; Krailas et al., 2011; Ukong et al., 2007)

Family Thiaridae

Cerithioidean freshwater taxa were long subsumed under the historical concept of "melaniids", which was later uncritically replaced by the family assignment to the Thiaridae (Brandt, 1974; Brown, 1994). For a discussion of a more up-to-date concept of the freshwater Cerithioidea see reviews by Glaubrecht (1996, 1999, 2010, 2011), supplemented by comparative morphological as well as molecular phylogenetic studies corroborating these earlier findings (Lydeard et al., 2002; Strong et al., 2011). For example, molecular phylogenic analysis now allows the separation among the Thai malacofauna of those limnic gastropods of the genera *Brotia*, *Paracrostoma* and *Adamietta* to represent members of the Pachychilidae from members of the Thiaridae

sensu stricto, representing two independent invasions and colonisations of freshwater habitats in the tropics worldwide (Glaubrech et al., 2009; Glaubrecht, 1996; Glaubrecht, 2011; Glaubrecht & Köhler, 2004; Köhler & Glaubrecht, 2001; Köhler & Glaubrecht, 2006; Strong et al., 2011).

In Thailand, the Thiaridae are represented by several described species, mostly being conchologically highly variable, such as e.g. *Melanoides tuberculata* (Müller, 1774), *Mieniplotia scabra* (Müller, 1774), or *Tarebia granifera* (Lamarck, 1816), The latter is commonly referred to as the "Quilted Melania" in the aquarium industry. Accordingly, as is typical in thiarids, a plethora of species names has been applied, irrespective of the fact that their known polymorphic phenotype, in combination with their viviparity and mainly parthenogenetic reproduction, renders unequivocal species delimitation quite problematic (Dechruksa et al., 2013; Glaubrech et al., 2009; Glaubrecht, 2011; Glaubrecht & Podlacha, 2010; Maaß & Glaubrecht, 2012).

This holds true especially for species assigned to *Tarebia* H. & A. Adams, 1854, which are found in rivers, streams and lakes as well as canals and ponds throughout its authochthonous distributional range. It extends, according to literature records (Benthem-Jutting, 1937; Brandt, 1974; Rensch, 1934) and our analyses here (see Fig. 7), from India through the mainland and insular Southeast Asia, with northern occurrences in South China and Taiwan, to the Philippine Islands in the east, and further south and east throughout the Indonesian Archipelago (including Sumatra, Java, Bali and Lombok, Sumbawa, Sumba and Flores, as well as Borneo, Sulawesi and the Moluccas), and from New Guinea onto numerous islands of the Western Pacific; with the type locality of the nominal species *T. granifera* being in Timor.

In addition, this snail has become widely invasive in the tropics outside its native range, the spreading being attributed to the aquarium trade. As early as the 1950s, though, Abbott (1952) noted that the snail had been introduced in North, Central and South Americas. *T. granifera* was also first reported in South Africa in 1999, established in a concrete lined reservoir in Mandeni and northern KwaZulu-Natal (Appleton & Nadasan, 2002). It has since become widespread in the eastern part of South Africa, particularly in the provinces of KwaZulu-Natal and Mpumalanga (Appleton et al., 2009). Kruger National Park, South Africa's flagship national park, has also seen recent invasions with the spread of *T. granifera* increasing substantially between 2001 and 2006 (Wolmarans & Kock, 2006).

That way, this snail exhibits its potency as neozoon, in combination with its role as important vector for several diseases, supporting the life cycles of digenic parasites infecting humans as well as other animals. Throughout Southeast Asia and particularly in Thailand, *T. granifera* is known as a major first intermediate host and thus transmission vector for trematode parasites dangerous to humans, livestock and wild animals; among which are most prominently several species of the Heterophyidae and Opisthorchiidae reported as causing opportunistic infections in people (Dechruksa et al., 2007; Krailas et al., 2014; Krailas et al., 2011). As we have show in a parallel study, these trematodes with their larval stage (i.e. the cercariae) found in *T. granifera* occur in nearly every limnic habitat and ecological circumstance, including next to more or less natural streams, rivers, and lakes, as well as any water bodies that are subject to rapid environmental change in the increasingly human-dominated world.

Therefore, being able to ecologically adapt apparently to a broad range of different freshwater habitats, *Tarebia* is highly diverse, with quite polymorphic shells, which are mostly elongately ovate, turreted and strongly sculptured, with both spiral grooves and ridges formed by nodules or tubercles, resulting in a plethora of named shell phenotypes (see Fig. 8). In Thailand, this snail has been reported with only a single species by Brandt (1974), who assigned all forms to *T. granifera*. However, as we will show here specimens from various locations in Thailand traditionally identified as of this species exhibit a considerably high degree of variation in shell morphology, particularly in size, shape, sculpture, and coloration. Basically, there are two conchologically variable phenotypes or morphs: (i) with light brown to dark brown body whorls ornamented with tubercles, resembling quite closely the shells described and depicted as *granifera* by Lamarck (1816, 1822) and similar to the syntypes from Timor (MHNG 1093/72/1-4) (see Fig. 8 a-g); and, (ii) with characteristic rows of nodules or tubercles most distinctly arranged in undulating spiral ridges and often with brown to dark brown spiral lines, similarly to those in typical *lineata* as described by Gray (1828) (see Fig. 8 h-m).

In light of these phenotypical variations found in the shell morphology of *Tarebia*, a modern taxonomic-systematic revision, utilizing evidence from molecular phylogenetics and phylogeographical analyses, becomes desirable. However, as it is the case for most thiarids, this taxon also has not found more attention yet in intra- and interspecific species diversity, neither in Thailand nor elsewhere in adjacent regions. Here, results from our study of the morphological and molecular genetic variation in combination with the distributional and phylogenetic relationships are presented, including differences in the reproductive biology of thiarids, particularly in populations from the North, Central, Northeast and South of Thailand. We focus on the two phylogenetically highly informative and heterogeneous mitochondrial gene fragments of cytochrome C oxidase I gene (COI) and 16S. In addition, we have studied the progeny and ontogeny of representatives from populations throughout the geographical distribution in Thailand, i.e. the frequency of various ontogenetic stages of embryos and shelled juveniles in the females' brood pouch. Combining the study of morphological variation (using biometry and geometric morphometrics) with molecular genetic variation and reproductive biology analyses, we have compared the population of Thailand with special focus on topotypical samples recently collected from the type locality of Timor as reference.

Freshwater snail as the first intermediate hosts.

Freshwater snails have long been known as hosts for several parasites, including digenetic trematodes. Consequently, the distribution of freshwater snails accounts for the occurrence of different trematode taxa in a particular region. Despite the importance of the snail intermediate host(s) to the lifecycle of trematodes, the faunistic and biosystematic knowledge of these limnic molluscs is scarce in general. In Thailand, medically-important freshwater snails have been investigated since 1980 for trematode infections (Dechruksa et al., 2007; Krailas et al., 2008; Krailas et al., 2003; Krailas et al., 2014; Krailas et al., 2011; Sri-aroon et al., 2005; Ukong et al., 2007; Upatham et al., 1980). The distribution of trematodes depends on the presence of first and second intermediate host species, as well as the eating habit of local

people (Radomyos et al., 1998). Among freshwater snails, cerithioidean gastropods in the family Thiaridae Gill, 1871 are known to be important first intermediate hosts of trematodes. Pinto and Melo (2010) listed 37 species of cercariae and another 81 trematode larval forms that were categorized in the generic collective group *Cercaria* Müller, 1773 from *Melanoides tuberculata* Müller, 1774. Brandt (1974) listed five thiarid snails, i.e. *M. tuberculata*, *Melanoides jugicostis* Hanley & Theobald, 1876, *Sermyla riqueti* Grateloup, 1840, *Neoradina prasongi* Brandt, 1974 and *Tarebia granifera* Lamarck, 1816 in Thailand. They represented snail intermediate host of trematodes.(Chontananarth & Wongsawad, 2013; Krailas et al., 2011; McKoy et al., 2011; Namchote et al., 2015; Ukong et al., 2007). In Thailand, four species of cercariae were investigated from *M. tuberculata* and *M.* jugicostis. They were C. formosanus, H. taichui, Haplorchis pumilio, and Stictodora tridactyla (Dechruksa et al., 2017; Krailas et al., 2014)

Trematodes infecting humans and other mammals, especially liver and intestinal flukes, are highly prevalent in Southeast Asian countries (Chai et al., 2013; Krailas et al., 2014; Wongratanacheewin et al., 2001). In humans, these infections have a major public health impact and are also of economic importance in veterinary medicine. The prevalence of human trematode infections was the highest degree in the northern and northeastern regions of Thailand (Pungpak et al., 1998; Radomyos et al., 1998; Srisawangwong et al., 1997; Sukontason et al., 1999). The liver fluke *Opisthorchis viverrini*, for example, can cause chalangiocarcinoma, a kind of cancer in gall bladder, while the intestinal fluke *Haplorchis taichui* is a possible agent of irritable bowel syndrome-like symptoms and *Centrocestus formosanus* may cause epigastric pain and indigestion accompanied by occasional diarrhea (Chai et al., 2013; Sripa et al., 2010; Watthanakulpanich et al., 2010). However, Thai people have considerably underestimated these trematodes in the past by continually eating some locally-traditional food prepared from raw freshwater fish and snail (Chuboon et al., 2005). Hence, the prevalence of trematodes in Thailand has been a continual problem until now (Krailas et al., 2014).

Life cycle of trematodes

Trematodes are endoparasitic platyhelminths that often have very complex life cycles involving at least one, sometimes two or four, but usually three different hosts, of which the first is almost always a mollusc (Galaktionov & Dobrovolskij, 2003). Eggs are released by the definitive host and either the first larval stage, i.e. the miracidium, hatches from the egg in a suitable medium (usually water) being adapted for actively recognizing and penetrating the first intermediate host, or the miracidium remains embryonated within the egg and infects the first intermediate host through passive uptake and subsequent hatching and penetration within the host. The miracidium develops directly into a (mother) sporocyst that may produce daughter sporocysts or rediae (sometimes rediae also produce a second generation of rediae). Another larval form, i.e. the cercariae, then develops either within the sporocyst or within the redia in the first intermediate host and is typically released into the environment where it either actively searches and penetrates the host or is passively taken up. Within the second host cercariae encyst and develop into metacercariae. Through predation metacercariae are taken up by the definitive host and then develop

into the adult trematode completing the life cycle (Fig. 2). Deviations from this typical life cycle occur either in the number of different life cycle stages that actually develop in the number of hosts involved in the development (Galaktionov & Dobrovolskij, 2003).

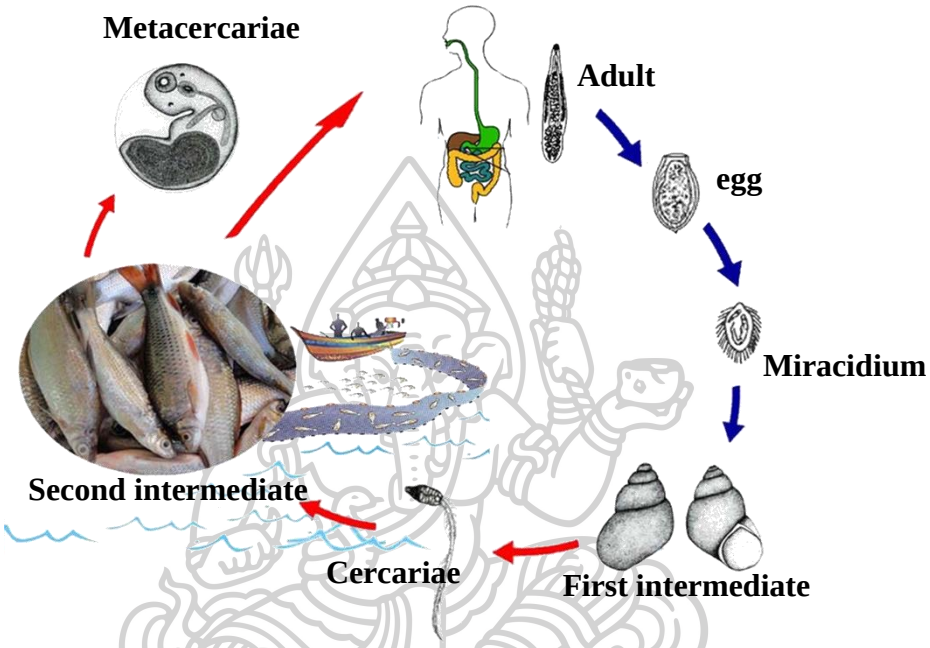

Figure 2. Life cycle of trematode (heterophyid).

Classification of Cercariae

Classification of digeneans is a complicated task, but the larval characteristics of the digeneans can also be used for identification (Brooks et al., 1985). Four types of cercariae are described depending on the position and number of suckers namely, monostome, amphistome, gasterostome, and distome; while 11 monotypes are described by the shape and size of the tail (Malek & Cheng, 1974; Schell, 1970; Yamaguti, 1975) (Fig. 3);

1. Amphistome: Ventral sucker is bigger than the oral sucker and located at posterior of the body.
2. Echinostome: Ventral sucker is located in the middle of the body. Collar is not well marked, and collar spines are difficult to observe.
3. Gymnocephalous: Small pear shaped body is connected to a long, simple tail. The ventral sucker is located at the posterior half of the body.
4. Gasterostome: The tail is forked tail and symmetry look like the horn.

5. Furcocercous: The long body terminates with a tapering end and a forked tail. A pair of large eyespots are located at the anterior. Ventral sucker is smaller than the oral sucker and is located at the posterior, about two-thirds of the body from the oral sucker.

6. Megalurous: The end of tail has adhesive gland cells.

7. Microcercous: Short tail, the tail look like cup-shape or knoblike.

8. Monostome: Oral sucker is small, with no ventral sucker. The tail is longer than the body.

9. Parapleurolophocercous: The body shape is oval. The tail is long, attached to the dorsal end of the body, with lateral finfolds nearby and a dorso-ventral finfold for the greater distal portion.

10. Pleurolophocercous: The oral sucker is large and conspicuous compared to the small, vestigial ventral sucker. Two small eyespots are located at the anterior. The tail is slender, with a very indistinct dorsal and ventral finfolds, both of which are more conspicuous in the distal half, with a tiny spike on the tip.

11. Xiphidiocercariae: This has a simple tail. The oral sucker has a stylet. The ventral sucker is located at the mid-region of the body.

Aside from traditional morphological methods, molecular genetic techniques were applied to delimit species of cercariae, i.e. sequencing of parts of the nuclear ribosomal RNA gene cluster, that have been shown to be efficient for the identification of different life stages of trematodes (Anucherngchai et al., 2016, 2017; Davies et al., 2015; Prasad et al., 2011; Skov et al., 2009)

Here, the morphological and molecular genetic variations are combined with the distributional and phylogenetic relationships, as well as differences in the reproductive biology and parasitology of thiarids, particular in the populations of *Tarebia* from the North, Central, Northeast and South of Thailand. This focus, as in the other studies, on the phylogenetically highly informative and heterogeneous gene fragments of mitochondrial cytochrome C oxidase I gene (COI) and 16S. In addition, the trematode infections in *Tarebia* were studied by using established methods (shedding and crushing methods). Also, the potential effect of parasites was analysed the infected female snails to the reproductive strategies of their progeny, i.e. the various stages of embryos and juveniles in the brood pouch. Viewed from the background of the molecular “backbone phylogeny” was able to analyse a suite of questions concerning the nature of cladogenesis, phylogeography, and reproductive biology in these snails in context with the evolution of infections by various trematodes, thus illuminating co-existence of human-infectious trematode parasites and their intermediate hosts.

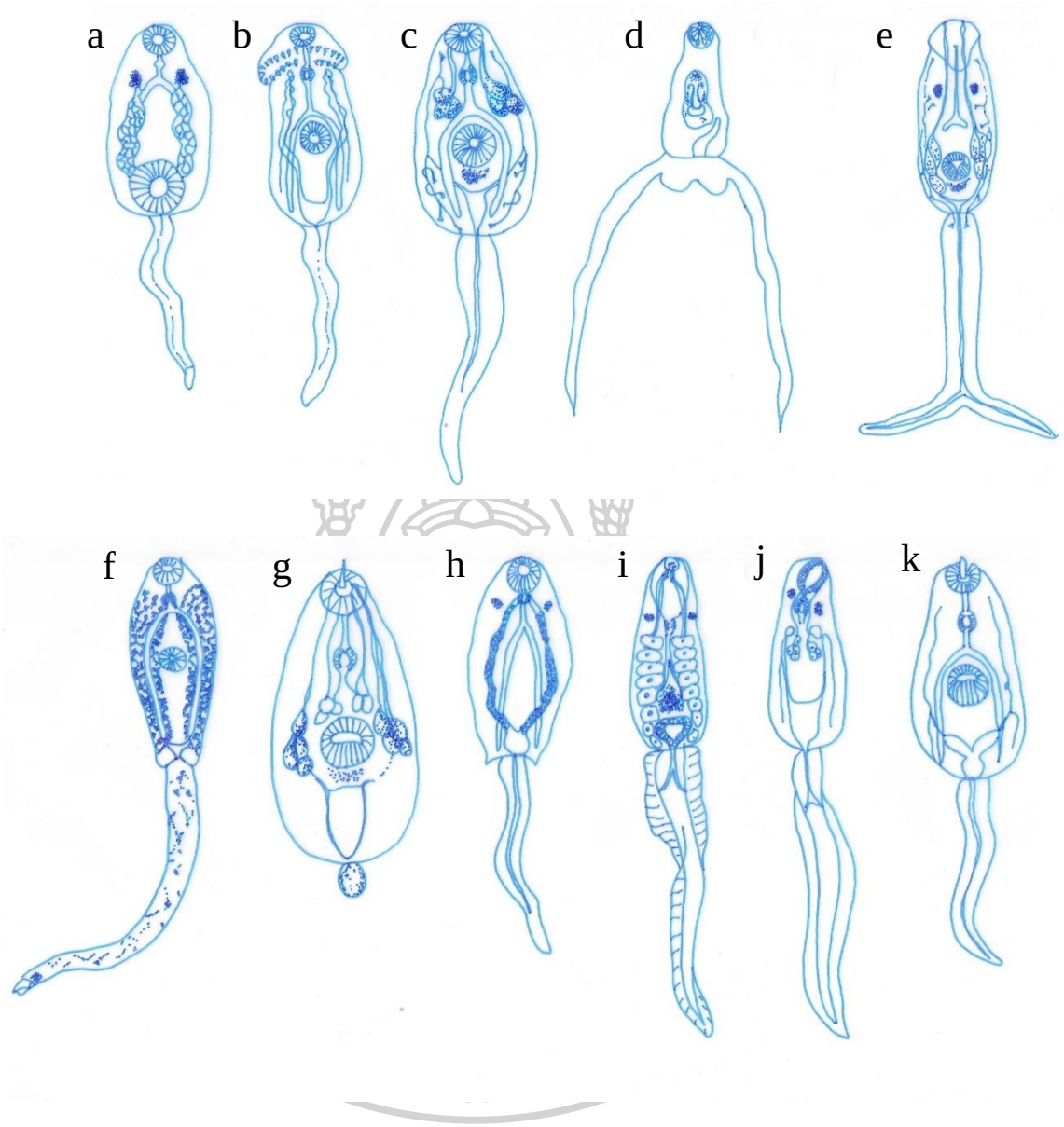

Figure 3. Morphology of the cercariae.
(a) Amphistome, (b) Echinostome, (c) Gymnocephalus, (d) Gasterostome, (e) Furcocercous, (f) Megalurous, (g) Microcercous, (h) Monostome, (i) Parapleurolophocercous, (j) Pleurolophocercous, (k) Xiphidiocercariae.

CHAPTER II
Objective

The evolutionary potential of a parasite host: diversity, systematics, and phylogeography of the freshwater snail genus *Tarebia* H. & A. Adams, 1854, was studied. The snail samples were collected from every region of Thailand. The current work focused on the diversity, systematics, and phylogeography revision of the snail genus *Tarebia* in Thailand by comparative analysis of shell morphology, radula, embryos, juveniles, and DNA analysis, based on a new taxonomic framework for the *Tarebia* in Thailand. In addition, the trematode infections of *Tarebia* was studied by using established methods (shedding and crushing methods). Also, the potential effect of parasites was analysed the infected female snails to the reproductive strategies of their progeny. The scope of the project were:

1. To study the diversity, systematics and phylogeography of the freshwater snail genus *Tarebia* in Thailand

2. To investigate the prevalence of cercarial infections of freshwater snail genus *Tarebia* populations in Thailand using morphological and molecular techniques.

3. To study the effect of parasite infections and to report on the reproductive strategy of freshwater snail genus *Tarebia* in Thailand

CHAPTER III
Materials and Methods

Snail sampling

Snail samples were collected from water resource such as streams, ponds, rivers, brooks, trenches and mountain creeks in North, South, East, Central and Northeast of Thailand. The snail were collected from 90 locations of Thailand between 2014-2016. The precise positions of the collection sites were obtained by GPS (Garmin PLUS III, Taiwan) (Table 1). The snails were collected using the counts per unit of time sampling method (Olivier & Schneiderman, 1956). Five researchers collected samples by handpicking and scooping every 10 minutes at each sampling site. The snails were transferred and studied in the laboratory of the Parasitology and Medical Malacology Research Unit, Silpakorn University, Nakhon Pathom, Thailand (PaMaSU: code SUT) The snails were identified according to their shell morphology, following essentially Brandt (1974). For reference, the snail samples were compared to snail samples of Timor-Leste from 12 locations (Table 2). All samples were preserved in 95 % ethanol. Voucher specimens are kept in the collection of the Center of Natural History (CeNak), Zoological Museum, Universität Hamburg, Germany (ZMH) and the collection of the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Thailand (SUT).

Table 1. Location of *Tarebia* sp. in Thailand.

NO.	VOUCHER NUMBER	LOCATION	GPS
THE NORTH			
N1	SUT 0515083	Huai Pa Hung (Pai drainage, Salween river system), Pang Mapha District, Mae Hong Son Province	19°22’19.6” N 098°26’35.9” E Altitude 437 m
N2	SUT 0515081	Huay Nam Kong (Salween river system), Muang District, Mae Hong Son Province	19°28’33.6” N, 098°07’02.4” E Altitude 425 m
N3	SUT 0515077	Tham Pla (Pai drainage, Salween river system), Muang District, Mae Hong Son Province	19°25’31.1” N 097°59’27.2” E Altitude 300 m
N4	SUT 0515078	Pai river (Pai drainage, Salween river system), Muang District, Mae Hong Son Province	19°21’54.8” N 097°58’10.7” E Altitude 217 m
N5	SUT 0515079	Huay Sua Tao (Pai drainage, Salween river system), Muang District, Mae Hong Son Province	19°15’31.6” N 097°54’44.6” E Altitude 237 m
N6	SUT 0514052	Ban Mai Saraphi (Ping drainage,	18°16’26.1” N

		Chao Phraya river system), Chom Thong District, Chiang Mai Province	098°38’54.0” E Altitude 277 m
N7	SUT 0514051	Ban Mae Suai Luang (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province	18°17’04.4” N 098°39’15.0” E Altitude 268 m
N8	SUT 0514054	Mae Soy bridge (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province	18°17’23.0” N 098°39’3.6” E Altitude 271 m
N9	SUT 0514050	Ban Huay Phang (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province,	18°17’08.5” N 098°39’16.9” E Altitude 263 m
N10	SUT 0516119	Thansawan waterfall (Yom drainage, Chao Phraya river system), Chiang Muan District, Phayao Province	18°51’22.2” N 100°11’09.1” E Altitude 415 m
N11	SUT 0516117	Yom river (Yom drainage, Chao Phraya river system), Chiang Muan District, Phayao Province	18°54’39.7” N 100°16’27.7” E Altitude 266 m
N12	SUT 0516108	Mae Nam Saai kg 9 +457 bridge (Yom drainage, Chao Phraya river system), Muang District, Phrae Province	18°05’03.1” N 100°13’00.1” E Altitude 171 m
N13	SUT 0516113	Mae Marn reservoir (Yom drainage, Chao Phraya river system), Sung Men District, Phrae Province	18°00’50.6” N 100°08’22.6” E Altitude 205 m
N14	SUT 0514045	Wang river (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province	18°56’00.5” N 099°38’54.6” E Altitude 376 m
N15	SUT 0514044	Ban Thung Hang stream (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province	18°52’47.5” N 099°40’01.0” E Altitude 373 m
N16	SUT 0514046	Huay MaeYuak (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province	18°46’39.8” N 099°38’38.7” E Altitude 352 m
N17	SUT 0516124	km. 40+075 bridge (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province	18°42’14.8” N 099°35’31.7” E Altitude 330 m
N18	SUT 0515090	Wa river (Nan drainage, Chao	19°11’30.4” N

		Phraya river system), Bo Kluea District, Nan Province	101°12’13.2” E Altitude 713 m
N19	SUT 0516114	Huay Si Pun reservoir (Nan drainage, Chao Phraya river system), Ban Luang District, Nan Province	18°51’45.1” N 100°28’37.1” E Altitude 430 m
N20	SUT 0516109	Mae pool waterfall (Nan drainage, Chao Phraya river system), Laplae District, Uttaradit Province	17°43’42.3” N 099°58’49.6” E Altitude 123 m
N21	SUT 0516112	Kaeng Sai Ngam (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province	17°52’19.5” N 100°18’02.1” E Altitude 257 m
N22	SUT 0513019	Kaeng Wangwua (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province	17°52’29.5” N 100°18’25.6” E Altitude 231 m
N23	SUT 0513023	Huai Nam Re Noi (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province	17°52’51.3” N 100°16’14.9” E Altitude 269 m
N24	SUT 0516103	Tat Duen waterfall (Yom drainage, Chao Phraya river system), Si Satchanalai District, Sukhothai Province	17°33’16.2” N 099°29’48.2” E Altitude 135 m
N25	SUT 0516102	Si Satchanalai national park (Yom drainage, Chao Phraya river system), Si Satchanalai District, Sukhothai Province	17°33’07.7” N 099°29’28.8” E Altitude 147 m
N26	SUT 0515075	Cheek point near moei river (Moei drainage, Salween river system), Tha Song Yang District, Tak Province	17°13’23.4” N 098°13’34.2” E Altitude 130 m
N27	SUT 0515076	Mae Salit Luang harbour (Moei drainage, Salween river system), Tha Song Yang District, Tak Province	17°26’04.8” N 098°03’33.3” E Altitude 109 m
N28	SUT 0515073	Ban Wang Takhian (Moei drainage, Salween river system), Mae Sot District, Tak Province	16°42’38.5” N 098°30’22.2” E Altitude 196 m
N29	SUT 0515072	Thong Dee harbour (Moei drainage, Salween river system), Mae Sot District, Tak Province	16°41’39.3” N 098°31’04.4” E Altitude 206 m
N30	SUT 0515074	Ban Huay Muang (Moei drainage, Salween river system), Mae Sot District, Tak Province	16°40’58.4” N 098°31’06.9” E Altitude 199 m
N31	SUT 0516126	Ban Pak Huay Mae Tho (Ping drainage, Chao Phraya river	16°52’29.3” N 099°07’13.6” E

		system), Muang District, Tak Province	Altitude 106 m
N32	SUT 0516121	Kaeng Wang Nam Yen (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°37’23.8” N 100°54’0.5” E Altitude 710 m
N33	SUT 0516120	Rajapruek resort (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°36’01.3” N 100°54’29.9” E Altitude 707 m
N34	SUT 0516123	Huai Sa Dao Pong (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°34’24.1” N 100°59’23.6” E Altitude 322 m
N35	SUT 0515088	Kaeng Bang Ra Chan (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°32’51.7” N 100°54’03.2” E Altitude 599 m
N36	SUT 0516129	Sam Sip Khot waterfall (Pa Sak drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°32’25.6” N 101°04’58.4” E Altitude 386 m
N37	SUT 0514041	Ban Wang Ta Pak Moo 13 (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’54.2” N 101°14’8.1” E Altitude 120 m
N38	SUT 0514042	Huai Leng (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’52.2” N 101°13’54.4” E Altitude 117 m
N39	SUT 0514040	Ban Wang Tian (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’29.7” N 101°13’30.7” E Altitude 121 m
N40	SUT 0514043	Huay Range reservoir, Ban Wang Ta Pak (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’19.3” N 101°15’07.4” E Altitude 138 m
N41	SUT 0516130	Than Thip waterfall (Pa Sak drainage, Chao Phraya river system), Lom Sak District, Phetchabun Province	16°39’46.3” N 101°08’09.8” E Altitude 374 m
N42	SUT 0515087	Ban Kaeng Lat (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province	16°57’21.3” N 100°55’31.0” E Altitude 324 m

N43	SUT 0516118	Kaeng Sopha (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province	16°52’13.1” N 100°50’17.4” E Altitude 413 m
N44	SUT 0515067	Poi waterfall (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province	16°50’36.3” N 100°45’16.1” E Altitude 208 m
N45	SUT 0516105	Phunamkej Resort (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province	16°51’02.2” N 100°36’41.1” E Altitude 208 m
N46	SUT 0516111	Kaeng Nangkoi (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province	16°53’09.0” N 100°38’47.8” E Altitude 180 m
N47	SUT 0516106	Kaeng Hom (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province	16°52’20.8” N 100°50’46.8” E Altitude 185 m
N48	SUT 0515086	Huai Nam Sai (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province	17°01’07.6” N 100°55’36.0” E Altitude 217 m
THE NORTHEAST			
NE1	SUT 0516128	Tat Kok Tup waterfall (Loei drainage, Mekong river system), Phu Luang District, Loei Province	17°03’03.9” N 101°31’38.7” E Altitude 688 m
NE2	SUT 0515068	Pla Ba waterfall (Mekong river system), Phu Ruea District, Loei Province	17°23’24.7” N 101°22’27.3” E Altitude 664 m
NE3	SUT 0516125	km. 50+350 Loei river (Loei drainage, Mekong river system), Phu Luang District, Loei Province	17°04’38.0” N 101°29’20.6” E Altitude 675 m
NE4	SUT 0515064	Bueng Thung Sang (Chi drainage, Mekong river system), Muang District, Khon Kaen Province	16°34’45.6” N 102°50’22.5” E Altitude 160 m
NE5	SUT 0516131	Lamphraphloeng reservoir (Mun drainage, Mekong river system), Pak Thong Chai District, Nakhon Ratchasima Province	14°35’32.3” N 101°50’30.1” E Altitude 259 m
THE EAST			
E1	SUT 0516135	Mae Rumphueng Beach (Mae Rumphueng canal, Gulf of	12°37’50.0” N 101°20’35” E

		Thailand), Muang Rayong District, Rayong Province	Altitude 8 m
THE CENTRAL			
C1	SUT 0516127	Bung Boraphet (Chao Phraya river system), Muang District, Nakhon Sawan Province	15°40’59.6” N 100°14’59.3” E Altitude 32 m
C2	SUT 0516133	Dong Phaya Yen waterfall (Pa Sak drainage, Chao Phraya river system), Muak Lek District, Sara Buri Province	14°44’06.4” N 101°11’31.4” E Altitude 156 m
C3	SUT 0516132	Suanmaduea waterfall (Pa Sak drainage, Chao Phraya river system), Phatthana Nikhom District, Lop Buri Province	14°55’12.3” N 101°13’10.9” E Altitude 136 m
C4	SUT 0515055	Pond of Silpakorn University (Tha Chin river system), Muang District, Nakhon Pathom Province	13°49’01.2” N 100°02’27.9” E Altitude 79 m
C5	SUT 0515091	Hin dad hot spring (Khwae Noi drainage, Mae Klong river system), Thong Pha Phum District, Kanchanaburi Province	14°37’25.9” N 098°43’40.5” E Altitude 159 m
C6	SUT 0515092	Sai Yok Yai waterfall (Khwae drainage, Mae Klong river system), Sai Yok District, Kanchanaburi Province	14°26’03.0” N 098°51’14.7” E Altitude 104 m
C7	SUT 0515093	Sai Yok Noi waterfall (Khwae drainage, Mae Klong river system), Sai Yok District, Kanchanaburi Province	14°14’27.6” N 099°03’55.9” E Altitude 116 m
C8	SUT 0515061	Ban Thung Makham Tia (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province	13°54’18.1” N 099°23’07.8” E Altitude 45 m
C9	SUT 0515060	Ban Ta Pu (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province	13°51’17.7” N 099°22’58.9” E Altitude 56 m
C10	SUT 0515059	Ban Nong Phai (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province	13°46’44.8” N 099°25’26.7” E Altitude 72 m
THE SOUTH			
S1	SUT 0515066	Ban Purakom (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province	13°19’29.2” N 099°14’22.0” E Altitude 277 m

S2	SUT 0515069	Huay Nueng (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province	13°32’52.2” N 099°17’33.7” E Altitude 156 m
S3	SUT 0515070	Lum Nam Phachi (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province	13°32’54.2” N 099°21’42.3” E Altitude 110 m
S4	SUT 0515057	Ban Dan Thap Tako (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province	13°41’28.1” N 099°29’08.1” E Altitude 82 m
S5	SUT 0515058	Phachi river Bridge (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province	13°45’00.5” N 099°26’27.4” E Altitude 65 m
S6	SUT 0515056	Ban Pa Wai (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province	13°37’0.15” N 099°24’36.9” E Altitude 74 m
S7	SUT 0515071	Huai Ban Bor (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province	13°32’07.4” N 099°20’31.8” E Altitude 137 m
S8	SUT 0513032	Khlong Cha-am (Cha-am canal, Gulf of Thailand), Cha-am District, Phetchaburi Province	12°48’02.7” N 099°58’53.2” E Altitude 22 m
S9	SUT 0516146	Khlong Bueng reservoir (Bueng canal, Gulf of Thailand), Muang District, Prachuap Khiri Khan Province	11°55’29.1” N 099°42’40.9” E Altitude 72 m
S10	SUT 0514037	Khlong Huai Yang (Yang canal), Thap Sakae District, Prachuap Khiri Khan Province	11°36’50.0” N 099°40’07.9” E Altitude 53 m
S11	SUT 0514038	Kar on waterfall (Nongyaplong canal), Bang Saphan District, Prachuap Khiri Khan Province	11°26’14.4” N 099°26’33.0” E Altitude 53 m
S12	SUT 0516149	Krapo waterfall (Tha Sae canal), Tha Sae District, Chumphon Province	10°44’28.8” N 099°12’54.9” E Altitude 74 m
S13	SUT 0516137	Khlong Klai (Nong Noi canal, Ta Pi river system), Ban Na San District, Surat Thani Province	08°48’06.9” N 099°26’45.1” E Altitude 108 m
S14	SUT 0514048	Dat Fa waterfall (Lumpool canal, Ta Pi river system), Ban Na San District, Surat Thani Province	08°52’18.8” N 099°25’59.1” E Altitude 79 m

S15	SUT 0516142	Vibhavadi waterfall (Tha Thong canal), Don Sak District, Surat Thani Province	09°08’07.2” N 099°40’31.6” E Altitude 26 m
S16	SUT 0516147	Khlong Tha Sai (Takhoei canal, Gulf of Thailand), Tha Chang District, Surat Thani Province	09°12’39.8” N 099°11’55.7” E Altitude 8 m
S17	SUT 0516148	Ban Tung Ao (Ta Khoei canal, Gulf of Thailand), Phunphin District, Surat Thani Province	09°12’25.7” N 099°12’25.7” E Altitude 7 m
S18	SUT 0516145	Krung Ching waterfall (Klai canal), Nopphitam District, Nakhon Si Thammarat Province	08°43’17.3” N 099°40’14.8” E Altitude 195 m
S19	SUT 0516139	Khlong Prong (Klai canal), Nopphitam District, Nakhon Si Thammarat Province	08°47’23.0” N 099°38’13.2” E Altitude 98 m
S20	SUT 0515097	Khlong Sai (Khlong Sai canal, Andaman sea), Muang District, Krabi Province	08°10’20.8” N 098°47’37.6” E Altitude 23 m
S21	SUT 0515098	Wang Than Thip (Wang Than Thip canal, Andaman sea), Muang District, Krabi Province	08°09’49.2” N 098°47’50.9” E Altitude 21 m
S22	SUT 0515095	Khlong Palian (Palian canal), Yan Ta Khao District, Trang Province	07°22’11.0” N 099°40’47.9” E Altitude 19 m
S23	SUT 0516138	Khlong Tha Leung (Tha Nae canal), Si Banphot District, Phatthalung Province	07°42’48.3” N 099°51’33.6” E Altitude 70 m
S24	SUT 0516141	Khlong La reservoir (Utaphao canal, Gulf of Thailand), Khlong Hoi Khong District, Songkhla Province	06°52’29.3” N 100°19’48.4” E Altitude 60 m
S25	SUT 0516144	Khlong Sathing Mo (Songkhla lake, Gulf of Thailand), Singhanakhon District, Songkhla Province	07°13’36.6” N 100°31’41.8” E Altitude 10 m
S26	SUT 0516143	Khlong Cham Rai reservoir (Utaphao canal), Khlong Hoi Khong District, Songkhla Province	06°49’29.5” N 100°19’49.7” E Altitude 56 m

Table 2. Location of *Tarebia granifera* in Timor-Leste.

NO.	VOUCHER NUMBER	LOCATION	GPS
1	ZMH 119364	Manatuto district, W bank of Laclo river near Condae, ca. 4 km WSW of Manatuto	08°31’32” S 125°58’50” E Altitude 35 m
2	ZMH 119359	Manatuto district, south coast, 3.8 km N of Nancuro beach, 4.7 km SE of Natarbora	09°00’31” S 126°03’45” E Altitude 20 m
3	ZMH 119358	Manatuto district, south coast, 3.4 km N of Nancuro beach, 5 km SE of Natarbora	09°00’45” S 126°03’49” E Altitude 20 m
4	ZMH 119354	Manatuto district, south coast, 2.5 km N of Nancuro beach, 5.7 km SE of Natarbora	09°01’11” S 126°03’58” E Altitude 15 m
5	ZMH 119357	Baucau district, NE of Baucau, Watabo beach	08°26’36” S 126°28’11” E Altitude 20 m
6	ZMH 119356	Lautem district, Ira-Ara village, Lutu-Ira	08°20’32” S 127°01’08” E Altitude 100 m
7	ZMH 119353	Lautem district, near the Baucau/Lautem district border marker, 11.8 km NE of Laga	08°25’35” S 126°41’43” E Altitude 5 m
8	ZMH 119362	Bobonaro district, north coast, 0.5 km from the mouth, Large seasonal stream in Batugade	08°56’47” S 124°58’28” E Altitude 10 m
9	ZMH 119355	Viqueque district, Ossu subdistrict, near village Usu Decima, Wai-eu-Lau	08°44’36” S 126°22’50” E Altitude 670 m
10	ZMH 119360	Viqueque district, spring in the village, Loihuno	08°47’05” S 126°22’32” E Altitude 255 m
11	ZMH 119363	Viqueque district, spring in the village, Loihuno	08°47’05” S 126°22’32” E Altitude 255 m
12	ZMH 119361	Manufahi district, south coast, Fatuhcahi village, Wetetefuik creek	09°02’00” S 125°59’36” E Altitude 30 m

Geographic data and maps

Geographic coordinates (WGS84 datum) of sampling sites were determined with the global positioning system (GPS) (Garmin PLUS III, Taiwan).

Where GPS data for sampling sites were unavailable, coordinates were determined as accurately as possible from a map. Localities of the samples were mapped on a dot-by-dot basis on a public domain map (made with Natural Earth) with ArcMap 10.4.1 (Esri Inc., Redlands, CA, USA). Final maps were compiled using Photoshop CS6 (Adobe Systems Inc., San José, CA, USA). The spelling of localities (whenever possible) follows GeoNames (http://www.geonames.org). For climatic data, we used information from the climate of the world database (https://www.weatheronline.co.uk/reports/climate/Thailand).

Examination of the Shell Morphology and biometry

All available type specimens and the other examined material were photographed by remote shooting with EOS Utility (version 2.12.2.1 for Windows) and Digital Photo Professional (version 3.12.51.2 for Windows) using a digital camera (Canon EOS 5D MKII with Canon macro photograph lens MP-E 65 mm and Canon compact macro lens EF 50 mm, Canon, Tokyo, Japan). Shell orientation was fitted to a position where the aperture is in a 90° angle in relation to the camera and the columella in parallel to the background. Photo stacks were assembled in Helicon Focus (version 5.3.14.2 for Windows). The images were then edited with Photoshop CS6 (Adobe Systems Inc.). The following biometrical parameters of the adult shells were taken with a digital calliper (accuracy: 0.1 mm): height of shell (h), width of shell (w), length of aperture (la), width of aperture (wa), height of body whorl (hbw), height of the last three whorls (l3w) and number of whorls (nw) (Fig. 4). The analysis of the shells and the relationships formed from them, were performed with Microsoft excel for windows on the determination of the mean (M) and standard deviation (SD). Analyses of shell parameters were performed using the statistic software SPSS for Windows (version 20).

Geometric morphometrics

The method was used landmarks and takes the geometrical relationships among the landmarks into account for the shape of the organism is represented by parameters between *Tarebia* sp. in Thailand and specimens from the other locations. Maximum of 12 shells were measured in each location with three whorls that used for analysis. In this study used 15 landmark (LM) on the photos (Fig. 5). Shell variables were created using tpsUtil 1.21.0.1 (version 1.46) and tpsDig2 (version 2.16) (Rohlf, 2017). After placing the landmarks on each photo, the distances between the landmark were calculated and nonmetric multidimensional scaling plots were created using tpsRelw 1.49. The transformation of landmarks and statistical analysis were done by PAST version 2.10 (Hammer et al., 2001) using the principal components analysis (PCA) to compare different *T. granifera* in Thailand and Timor-Leste.

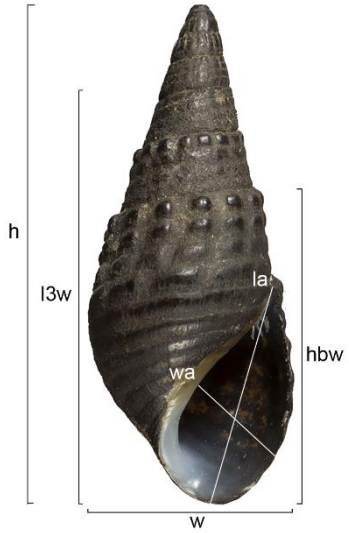

Figure 4. The biometrical parameters of the adult shells.
height of shell (h), width of shell (w), length of aperture (la), width of aperture (wa), height of body whorl (hbw) and height of last three whorl (l3w)

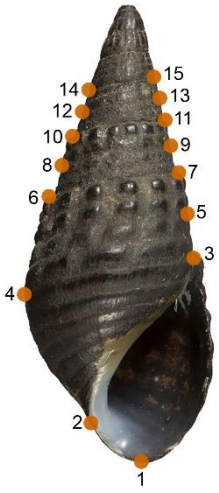

Figure 5. Landmark used in the morphometric analysis.

Brood pouch content

The content of the brood pouch was counted as best proxy for differences in the thiarid reproductive strategy following the method described in Glaubrech et al. (2009) and Maaß and Glaubrecht (2012). The shells were cracked with a small vice, and then cut off operculum from the posterior part of the foot using a scalpel and opened the soft body under a stereomicroscope. After opening the brood pouch, which is located in the neck region of the female, care was taken to count all embryos and shelled juveniles contained within the marsupial, according to the nine standard size classes established for Thiaridae before by Glaubrech et al. (2009): 1) early embryos, 2) late embryos, 3) juveniles up to 0.5 mm, 4) juveniles between 0.6 mm and 1.0 mm, 5) juveniles between 1.1 mm and 1.5 mm, 6) juveniles between 1.6 mm and 2.0 mm, 7) juveniles between 2.1 mm and 2.5 mm, 8) juveniles between 2.6 mm and 3.0 mm, and 9) juveniles > 3.0 mm. The number of juveniles in each stage was analysed by One-way ANOVA test of SPSS for Windows (version 20) value ≤ 0.05 was interpreted as significant/meaningful support.

Examination of the radula

The radula of snail samples will be removed from the head-foot organ, washed in water for a few minutes and transfered to 10% sodium hydroxide (NaOH) and cleaned with 2% hydrochloric acid (HCl) to neutralize the excess hydroxide. For radula image, the radula will be dried by air and then coated with gold-palladium in an ion-sputtering apparatus (Palaron CPD 7501, UK) for 3-4 minutes and examined in scanning electron microscope (Camscan MX 2000, UK; Hitachi S-2360N, Japan).

The radula pattern of snails in genus *Tarebia* was formula 2:1:1:1:2 (marginal: lateral: central: lateral: marginal). The cusps of the central teeth (C), lateral teeth (L), inner marginal cusps (M_1) and outer marginal cusps (M_2) will be counted using scanning electron micrographs (Fig. 6).

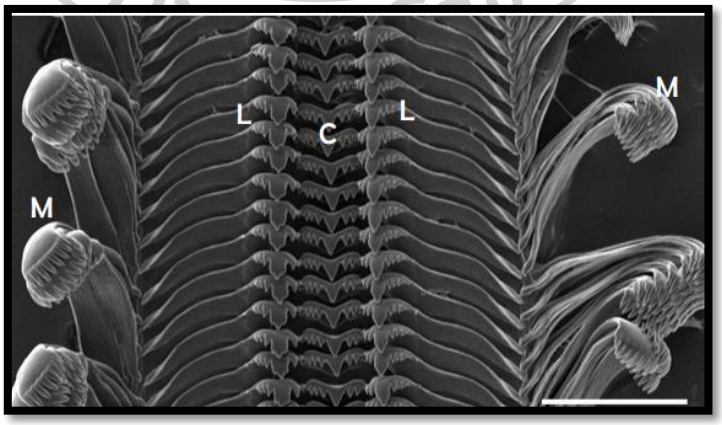

Figure 6. SEM micrographs of radula.
central teeth (C), lateral teeth (L), inner marginal cusps (M_1) and outer marginal cusps (M_2). (Glaubrech et al., 2009; Winnepenninck et al., 1993)

Molecular Genetics

DNA Extraction

Sequences from 131 specimens of *T. granifera* populations in Thailand and 12 specimens from 11 populations in Timor-Leste were generated. Two specimens of *Thiara amarula* (Linnaeus 1748) was selected as outgroup. The tissue from head-foot region of snails were cut and extracted using CTAB protocol (Winnepenninck et al., 1993). 300 µl of CTAB buffer was added to tissue with 0.6 µl β-mercaptoethanol and 15 µl Proteinase K and incubated at 55^0C for 1 hours. Then, 500 µl chloroform-isoamy-alcohol (24:1) were added and mix by shaking tubes for 2 minutes at room temperature. After a centrifugation step for 15 minutes at 12,000 rpm (4°C). Pipette the supernatant into a new tubes and added 500 µl chloroform-isoamy-alcohol (24:1) and mixed by shaking tubes for 2 minutes at room temperature. After a centrifugation step for 15 minutes at 12,000 rpm (4°C) for removing protein. Then, pipette the supernatant into the tubes contain cold 25 µl 3 M ammonium acetate and 600 µl 70% ethanol. The supernatant included DNA which was precipitated in this step. Incubation at -20^0C, overnight. After, Centrifuging at 12,000 rpm for 10 min (4°C). Pipette off the liquid carefully don't touch the DNA pellet. Then, 250 µl cold 70% ethanol were added and mix for wash the DNA pellet. Centrifuging at 12,000 rpm for 10 min (4°C). The supernatant was discarded by a micropipette. After air drying in the incubator for 10 minutes at 60°C. DNA pellet were dissolved in 50 µl TE buffer and RNase A (50 µl TE+0.5 µl RNase 10mg/ml) and incubated at 37 °C for 30 minutes. Genomic DNA sample were preserved in -20°C.

DNA Amplification

The polymerase chain reaction (PCR) was used for amplification of mitochondrial gene fragments, 780 base pairs (bp) of 16S ribosomal DNA and 658 bp. of Cytochrome Oxidase (COI) gene of mitochondrial DNA. Amplifications were conducted in 25 µl volumes containing, 2.5 µl 10× DreamTaq Green Buffer (Thermo Fisher Scientific, Waltham, MA, USA), 1.0 µl dNTP mix (5 mM each), 1.0 µl of each primer (10 µM), 0.2 µl of DreamTaq DNA polymerase (Thermo Fisher Scientific), 1.0 µl DNA template and 18.3 ddH_2O. The PCR Thermo cycle was programmed as following:

Initial denaturation	94 °C	3	min	
Denaturation	94 °C	30	sec	
Annealing	50 °C	45	sec	35 cycles
Extension	72 °C	1	min	
Final extension	72 °C	10	min	

Table 3. Primer sequences.
Y = C or T, W = A or T, K = G or T and R = A or G. (Boeden, 1985)

Primers	Sequence (5'-3')	Source
16S_F_Thia2	CTTYCGCACTGATGATAGCTAG	Rintelen, unpulished; Gimnich (2015)
H3059	CCGGTYTGAACTCAGATCATGT	Wilson et al. (2004)
LCO-1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
HCO2198var	TAWACTTCTGGGTGKCCAAARAAT	Rintelen et al. (2004)

Prior to sequencing, PCR products were enzymatically cleaned by adding 0.65 µl thermosensitive alkaline phosphatase (Thermo Fisher Scientific) and 0.35 µl exonuclease I (Thermo Fisher Scientific) to a 5 µl aliquot of the PCR reaction followed by an incubation step at 37°C for 15 min and enzyme inactivation at 85°C for 15 min. Both strands of the amplified products were sequenced at Macrogen Europe Laboratory (Amsterdam, The Netherlands).

Sequencing Both DNA Strands (Forward and Reverse Strand)

Alignments of forward and reverse strands were conducted using Geneious 10.1.3

The Alignment and Phylogenetic Analyses Phylogeny of COI and 16S ribosomal RNA gene

The sequences of COI gene 16S gene and Concatenated creates aligned with MUSCLE algorithm (Edgar, 2004) with 1,000 bootstrap value. Phylogenetic analyses was analyzed by Neighbor joining (NJ) method with automatic parameters of MEGA 6.0 program (Tamura et al., 2013). Bayesian Inference (BI), Maximum likelihood (ML) and maximum parsimony (MP) approaches were used to reconstruct the phylogenetic relationships. The sequence data set was initially divided into four partitions for the nucleotide model-based ML and BI approaches: 1) 1st codon positions of *cox1*, 2) 2nd codon positions of *cox1*, 3) 3rd codon positions of *cox1*, and 4) 16S. To select an appropriate partitioning scheme and/or evolutionary models for the mitochondrial sequences, the data set was analysed with PartitionFinder 2.1.1 (Lanfear et al., 2012) conducting an exhaustive search and allowing for separate estimation of branch lengths for each partition using the Bayesian information criterion as recommended by Luo et al. (2010) for model selection. Models to choose from were restricted to those available in MrBayes 3.2.6 (Ronquist et al., 2012) as well as in Garli 2.1 (Zwickl, 2006). As best-fit partitioning scheme, the PartitionFinder analysis suggested to combine all predefined partions into a single partition, with the HKY+G model as best-fit model under the Bayesian information criterion.

The BI analysis was performed using MrBayes 3.2.6. Metropolis-coupled Monte Carlo Markov chain (MC^3) searches were run with four chains in two separate runs for 50,000,000 generations with default priors, trees and parameters sampled

every 1,000 generations under default heating using the best-fit model as suggested by PartitionFinder. Diagnostic tools in MrBayes, including estimated sample size (ESS) values ≥ 200, were used to ensure that the MC^3 searches had reached stationarity and convergence. The first 5,000,000 generations were discarded as burn-in.
Heuristic ML analysis was performed with Garli using the best-fit models as suggested by PartitionFinder. Support values were computed by bootstrapping with 1,000 replications.

Heuristic MP searches were carried out with PAUP v4.0b10 (Swofford, 2002) using 100 random-addition-sequence replicates and TBR branch swapping. Support values were computed by bootstrapping with 1,000 replications.
Bayesian posterior probabilities (PP) values ≥ 0.95 and bootstrap (BS) values ≥ 70% and were interpreted as significant/meaningful support. BS values from the ML and MP analyses were mapped onto the Bayesian 50% majority rule consensus tree with SumTrees 3.3.1, which is part of the Dendropy 3.8.0 package (Sukumaran & Holder, 2010).

Molecular species delimitation

General Mixed Yule-coalescent (Pons et al., 2006) was used for Bayesian implementation (bGMYC) (Reid & Carstens, 2012) and the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al., 2012) with p-distances for DNA sequence-based species delimitation. The bGMYC method allows for taking phylogenetic uncertainty into account by basing the analysis on several ultrametric trees sampled from the same posterior distribution. Ultrametric trees were constructed for the concatenated 16S and *cox1* data set with Beast 2.4.1 (Bouckaert et al., 2014) assuming a strict clock and the same evolutionary model as in the Bayesian and ML analyses (root age was set to one using a lognormal prior). Chains were run for 10,000,000 generations discarding the first 50% of the generations as burn-in and sampling every $50{,}000^{th}$ tree resulting in a set of 100 ultrametric trees which were used in the bGMYC analyses. For each of the 100 ultrametric trees in the 16S and *cox1* data set, the Markov-chain Monte Carlo sampler implemented in the bGMYC R package (Reid & Carstens, 2012) was run for 100,000 generations, discarding the first 90,000 generations as burn-in and sampling every 100 generations.

Examination of trematode infections

Snail samples from 90 sampling sites were investigated for trematode infections by using shedding and crushing methods. The emerged cercariae were studied unstained or vitally stained with 0.5% neutral red. Sample measurements (average size) in micrometers were taken from 10 specimens fixed in 10% formalin. Details of the cercariae were drawn using a *camera lucida* and identified according to Krailas et al. (2014); Schell (1970); Yamaguti (1975). Some cercariae belonging to all identified species were then preserved in 95% ethanol for further DNA analysis.

Molecular study of cercariae

The emergence cercariae were studied for molecular classifications at Universität Hamburg, Center for Natural History (CeNak), Zoological Museum, Department of Animal Diversity, Germany. Genomic DNA from the cercariae was extracted using the DNeasy blood and animal tissue kit (QIAGEN, Venlo, The Netherlands). Amplification by polymerase chain reaction (PCR) of the nuclear internal transcribed spacers 2 (ITS2) region were performed with the following primers ITS2-F (5'-CTT GAACGC ACA TTG CGG CCA TGG G-3') and ITS2-R: (5'-GCG GGT AAT CACGTC TGA GCC GAG G-3') (Sato et al., 2009). Reactions were set up in 20 µl volumes containing 1.0 µl dNTPs (2 mM each), 2.0 µl 10× mM DreamTaq^{TM} Green buffer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 0.3 µl GreenTaq^{TM} DNA polymerase (5 U/µl, Thermos Fisher Scientific), 1.0 µl of each primer (10 µM) and 14.7 µl ddH_2O. The DNA samples were initially denatured at 94 °C for 4 min followed by 35 cycles (denaturation at 94 °C for 1 min, annealing at 60 °C for 30 s, and elongation at 72 °C for 2 min; see Sato et al. 2009) and a final elongation step at 72 °C for 7 min. The PCR products were purified according to the protocol for enzymatic PCR product clean-up with exonuclease I (20 U/µl, Thermo Fisher Scientific) and FastAP thermosensitive alkaline phosphatase (1 U/µL, Thermo Fisher Scientific). Purified PCR products were sequenced at Macrogen Europe Lab. (Amsterdam, The Netherlands).

The Alignment and Phylogenetic Analyses

Alignments of forward and reverse strands were conducted using Geneious 10.1.3 (Biomatters Ltd., Auckland, New Zealand). The ITS2 consensus sequences were aligned in MEGA 7 (Kumar et al., 2016) using MUSCLE (Edgar, 2004) under default settings. A Neighbor joining (NJ) analysis was performed based on p-distances with 1,000 bootstrap replicates.

CHAPTER IV
Results

Part I: Systematic results of *Tarebia granifera* in Thailand.

Characterization of *Tarebia* H. & A. Adams, 1854

Taxonomic remark: The genus *Tarebia* existed in the past different terms for the type species. For example, *Melania semigranosa* (Busch, 1842) and *Tarebia lineata* (Gray, 1828) are synonyms. Brandt (1974) gave a description that *Melania semigranosa* (Busch, 1842) is now considered a synonym of *Melania granifera* (Lamarck, 1822). This species was already included in the list of species attributed by H. & A. Adams (1854). Because of the synonymy there is no change in the choice of a type species as it was designated by later authors.
Diagnosis: The shells has a medium size (12-44 mm). The shell shape are elongated ovate-conical or turreted, but shorter than genus *Melanoides* and rather thick. The color of shell is greenish or brownish. The body whorl being greater in length than half the entire length of the shell. The spire is usually sharp. The whorls are almost flat in the spire. The sculpture consists of spiral grooves and tubercles on the whorl. The shape of the aperture is oval with sharp peristome and curved columella. The umbilicus is enclosed.

Systematics and Classification of *Tarebia granifera* Lamarck, 1816

Common name: Quilted melanis
Class: Gastropoda
Subclass: Caenogastropoda
Order: Cerithiomorpha
Superfamily: Cerithioidea
Family: Thiaridae
Genus: *Tarebia* H. Adams and A. Adams, 1854
Type species: *Melania granifera* Lamarck, 1816
Synonyms:

1822 *Melania granifera* – Lamarck, Hist. Anim. S. vert., 6 (2): p. 167 (Ile de Timor).
1828 *Helix lineata* – Gray in Wood, Index test., Suppl.: p. 24, fig. 68 (Ganges).
1834 *Melania celebensis* – Quoy & Gaimard, Voy. Astrolabe, Zool., 3: p. 152, pl. 56, fig. 26-29 (Cèlébes).
1836 *Melania lirata* – Benson, J. asiat. Soc. Bengal, 5: p. 782 (River Hooghly near Calcutta).
1842 *Melania semigranosa* – Bush in Philippi, Abb. Beschr., 1: 2, pl.1 fig. 13 (Java).
1843 *Melania coffea* – Philippi, Abb. Beschr., 1: p. 60, pl. 2, fig. 4 (Java?).
1843 *Melania batana* – Gould, Proc. Boston Soc. nat. Hist., 1: p. 144 (Tavoy, Burma).

1844 *Melania flavida* – Dunker in Philippi, Abb. Beschr., 1: p. 164, pl. 3, fig. 15 (Teria Ghat, Java).
1844 *Melania* verrucosa – Hinds, Ann. Mag. Nat. Hist., 14: p. 9 (New Ireland).
1850 *Melania lateritia* – Lea, Proc. zool. Soc. London, 1850: p. 184 (Philippines).
1850 *Melania rudis* – Lea, Proc. zool. Soc. London, 1850: p. 185 (Ceylon, Amboyina).
1850 *Melania microstoma* – Lea, Proc. zool. Soc. London, 1850: p. 185 (Colombo, Ceylon).
1850 *Melania crenifera* – Lea, Proc. zool. Soc. London, 1850: p. 192 (Java).
1857 *Melania granospira* – Mousson, J. de Conch., p. 6, pl.161 (Java).
1858 *Vibex (Tarebia) granifera* – Adams & Adams, p. 304.
1859 *Melania broti* – Reeve, Conch. Icon., p. 12, pl. 22, fig. 160 (Ceylon).
1859 *Melania lyrata* – Reeve, Conch. Icon., p. 12, pl. 22, fig. 170 (Java).
1860 *Melania chocolatum* – Brot, Rev. Zool., 1860: pl. 16, fig. 2 (Ceylon).
1860 *Melania granospiralis* – Zollinger, Natuurk. Tijdschr. Nederl. Ind., 18: 424 (Java).
1868 *Melania asperula* – Brot, Matér. Mélan., 2: 30, pl. 1, fig. 11 (non Lamarck, 1822) (Java).
1879 *Melania junghuhni* – Martin, Tertiärsch. Java: 89, pl. 14, fig. 20 (Java).
1904 *Melania lateritia* – Fischer & Dautzenberg, Miss. Pavie, 3: 418 (Riviére Ménam Pin á Xien – Mai, Laos occidental).
1905 *Melania tjariangensis* – Martin, Samml. Geol. Reichsmus. Leiden, (NF) 1: 23 (Java).
1905 *Melania kritjianensis* – Martin, Samml. Geol. Reichsmus. Leiden, (NF) 1: 23 (Tjariang; Kritjian, Java).
1914 *Melania tjibodasensis* – Leschke, Mitt. Naturh. Mus. Hamburg, 31: 219 (Tjibodas, Java).
1914 *Melania margaritana* – Leschke, Mitt. Naturh. Mus. Hamburg, 31: 258, fig. 12 (Tjibodas, Java).
1935 *Melania martini* – Oostingh, Wet. Meded. Dienst Mijnb. Nederl. Ind., 26: 25 (non Schepman, 1898).
1950 *Melanoides lateritia* – Suvatti, Fauna Thailand: 62 (Klong Ranode off Tale Sap).
1952 *Thiara (Tarebia) granifera* – Abbott, Proc. U. S. nation. Mus., 102: 72, 113, pl. 8, fig. 1-2 (Guam Island; Naujan River, Mindoro Island; Lithia Spring, Florida).
1955 *Melanoides granifera* – Benthem Jutting, p.52. Benthem Jutting, 1956: vol. 23, p. 404, fig. 90. Benthem Jutting, 1959: p. 98f.
1963 *Melanoides graniferus graniferus* – Benthem-Jutting, p. 468–469.
1963 *Melanoides graniferus laevis* – Benthem-Jutting, p. 468–469.
1974 *Tarebia granifera* – Brandt, p. 167, pl. 16, fig. 14–18. Starmühlner, 1976: p. 569, pl. 16, fig. 175-179. Starmühlner, 1984: p. 183f.

Type material: 4 syntypes (MHNG 1093/72/1-4) (Fig. 8).
Type locality: Originally given as "Timor" by Lamarck (1822). This island, of which the western part is today a province of Indonesia (the eastern part, in contrast, forms the recently independent state of East Timor, or Timor-Leste), was an important stop-over for major expeditions of discovery in the Indowest-Pacific and Australia in particular (Glaubrech, 2002). However, at that time and the time of collecting, around 1800, all expeditions known to us have anchored at the natural harbor of Kupang. Thus, we here restrict the type locality on this island to the vicinity of its western part (see Fig. 7). Nevertheless, we regard material collected recently by Vince Kessner elsewhere on this island of Timor and used in the present study as reference and for comparison as to qualify as topotypical material.
Taxonomy: Lamarck (1816) depicted for the first time shells of this thiarid, creating the name *Melania granifera*, however without any further description. Later, described this new species and its shell morphology in more detail; see also Mermod (1952). Adam & Adam (1854) transferred *granifera* to its own genus *Tarebia*. Many subsequent authors, though, referring to Lamarck (1822) continue to use the generic allocation as "*Melania" granifera*; see e.g. Brot (1874) in his widely used monography that was followed by most authors for nearly a century. However, the generic allocation remains vage, as e.g. Benthem-Jutting (1937) either used *Thiara* while she later employed *Melanoides* (W.S.S. Benthem-Jutting, 1959). Starmühler (1976), in his thorough faunistic revision, provided an extensive list of synonyms for this taxon.

In addition, some authors employed "*Melania" lineata* for shells found to exhibit spiral ridges and/or dark bands on its body whorls. Accordingly, Rensch (1934) divided *Tarebia* into two subspecies, namely "*Melania" granifera granifera* and "*Melania" granifera lineata.* In contrast, Brandt (1974) considered and employed *Tarebia granifera* as the only congeneric species to exist in Thailand; as was also done by Glaubrecht (1996).
Diagnosis: Shells are highly polymorphic, elongately ovate-conoidal or turreted and strongly sculptured with both spiral grooves and nodules along the axial ribs. There is no umbilicus. The operculum is oval and paucispiral operculum with an eccentric nucleus.

Biogeography

The distributional range of *Tarebia granifera* (Fig. 7) extends from mainland Southeast Asia, with Thailand and Vietnam at its northern most margin, to the island of Taiwan and the Philippines. It also comprises, from the Malay Peninsula south and east, the region of the entire Sunda shelf area, with occurrences on the larger Sunda islands Sumatra, Java and Borneo, as well as the islands of Nusa Tenggara (or Lesser Sunda islands), i.e. from Bali east to Timor. The species is also abundant in Wallacea, i.e. on Sulawesi and on several islands of the Moluccas (e.g. Halmahera, Ceram, Ambon). From there, it extends east into the Indowest Pacific, with occurrences in western and eastern New Guinea and the Bismarck Archipelago.

In Thailand, this species occurs in most lentic and lotic water bodies ranging throughout the various regions, provinces and river systems. There, *T. granifera* was found in both natural and artificial water bodies on a variety of substrate, such as e.g.

sand, mud, rock (and, alternatively, concrete bridge foundations, concrete walls), on bottoms of reservoirs, irrigation canals and ornamental ponds. This species is usually found together with other thiarids, most often with *M. tuberculata* and *Mieniplotia scabra*. We were not able to correlate any consistent ecological features that clearly distinguish either a particular locations or specific habitat and/or population where *T. granifera* was found to occur. Thus, the ecological requirements of this taxon, in particular contrasting those to that of other thiarids, remain insufficiently known.

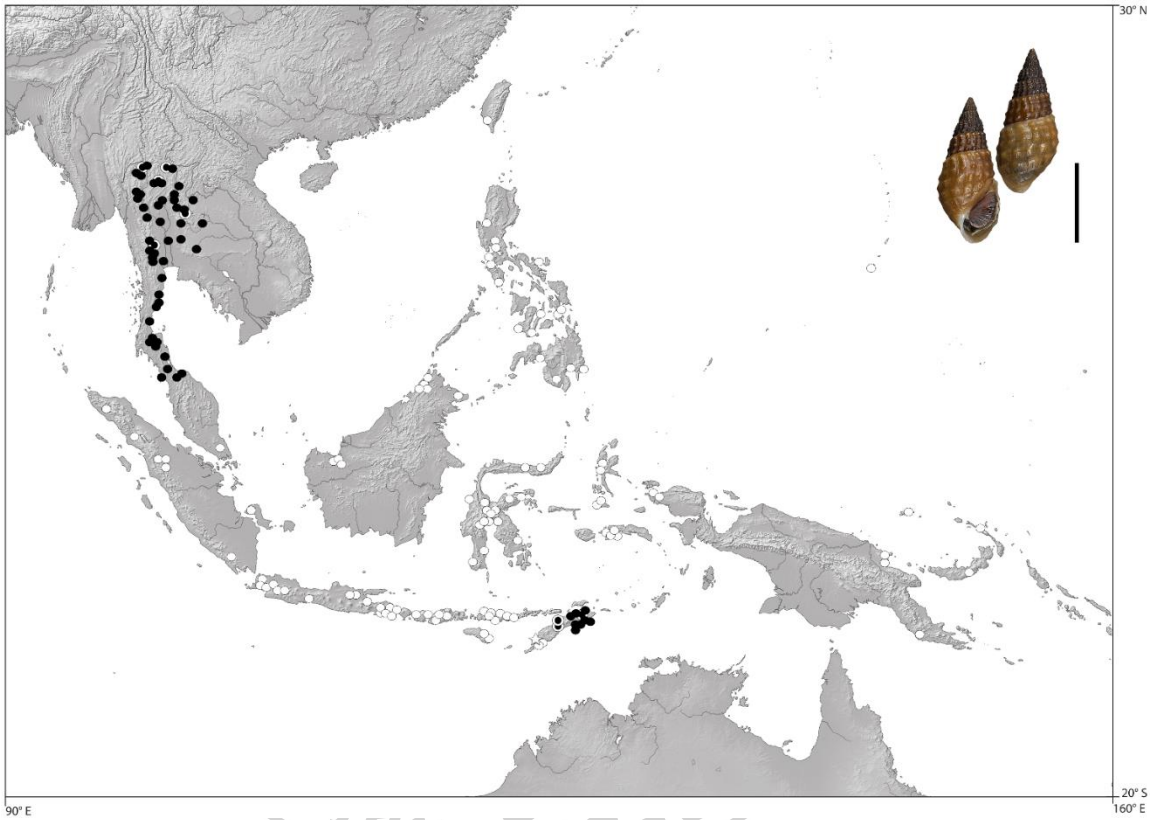

Figure 7. Distribution of the Thiarid snail *Tarebia granifera* (Lamarck, 1816) across its range in Southeast Asia.
The focus on occurrences in Thailand, contrasted with type and topotypical material from the island of Timor. Asteriks: type locality of "*Melania" grainfera* Lamarck, 1816, reconstructed to originated from near Kupang in western Timor (see text for more details); black dot: sequenced material used in this study; white dot: shell material from museum collections analysed and literature records; white dot with black dot inside: wet material preserved in ethanol. Scale bar: 10 mm

Shell morphology

The shells of *Tarebia granifera* (Fig. 8), which are often of greenish or brownish color, are medium-sized, with 12 to 44 mm, of elongately ovate-conoidal or turreted shape, much shorter than *Melanoides* and rather thick, the body whorl being greater in length than half the entire length of the shell. The spire is usually sharp, the whorls are not much convex, almost flat in the spire. The sculpture consists of spiral grooves and tubercles on the whorl. The shape of the aperture is oval with sharp peristome and curved columella; the umbilicus is enclosed.

As shown in Fig. 8, *Tarebia granifera* exhibits a wide phenotypical spectrum of shell morphology, which varies with respect to size and shape and in particular in sculpture and coloration including banding patterns. We separated, based on superficial "Gestaltwahrnehmung" of morphologically distinct shells, three groups called morphs A, B and C here, without implying morphotypes in the sense of species under a respective species concept, but for convenience only and to fasciliate further research into the potential correlation of phenotypical and genetic proprinquity.

Starting off from the type series of *T. granifera* from Timor (Fig. 8a) and comparing to topotypical material collected in Timor-Leste (Fig. 8t-y) we distinguished based on phenotype only three major morphologies, comprising a combination of several distinct features, which taken together allows to differentiate the three morphs. The first (morph A) is similar to and characteristic by shell features also visible in the Timor types (Fig. 8b-g), with shell shape ovate-conoidal to moderately turreted and rather thick; the apex is pointed and often eroded; the color is highly variable, ranging from yellowish-brown to dark brown and even nearly black. The number of whorls is mostly between 3 and 7, with a high spire and regularly increasing size. The body whorl is large and measures about half the length of the shell. The sculpture consists of spiral grooves and tubercles on the whorl, the suture is shallow. Next we separated those shells as morph B which agree to features similar to the description of *T. lineata* (Gray, 1828), as shown in Fig. 8 (h-m), with the shell being moderately thick and elongately or ovate-conoidal, with 3-9 whorls and the body whorl being two-thirds of the shell. The color is mostly yellowish-brown to dark brown. The sculpture of these shell were found to have small brown spiral ridges on the whorl, sometimes built as rows of tubercles. Morph C is represented by shells which combine features from both of the former morphs, but were differentiated here primarily due to the pronounced banding pattern (Fig. 8n-s).

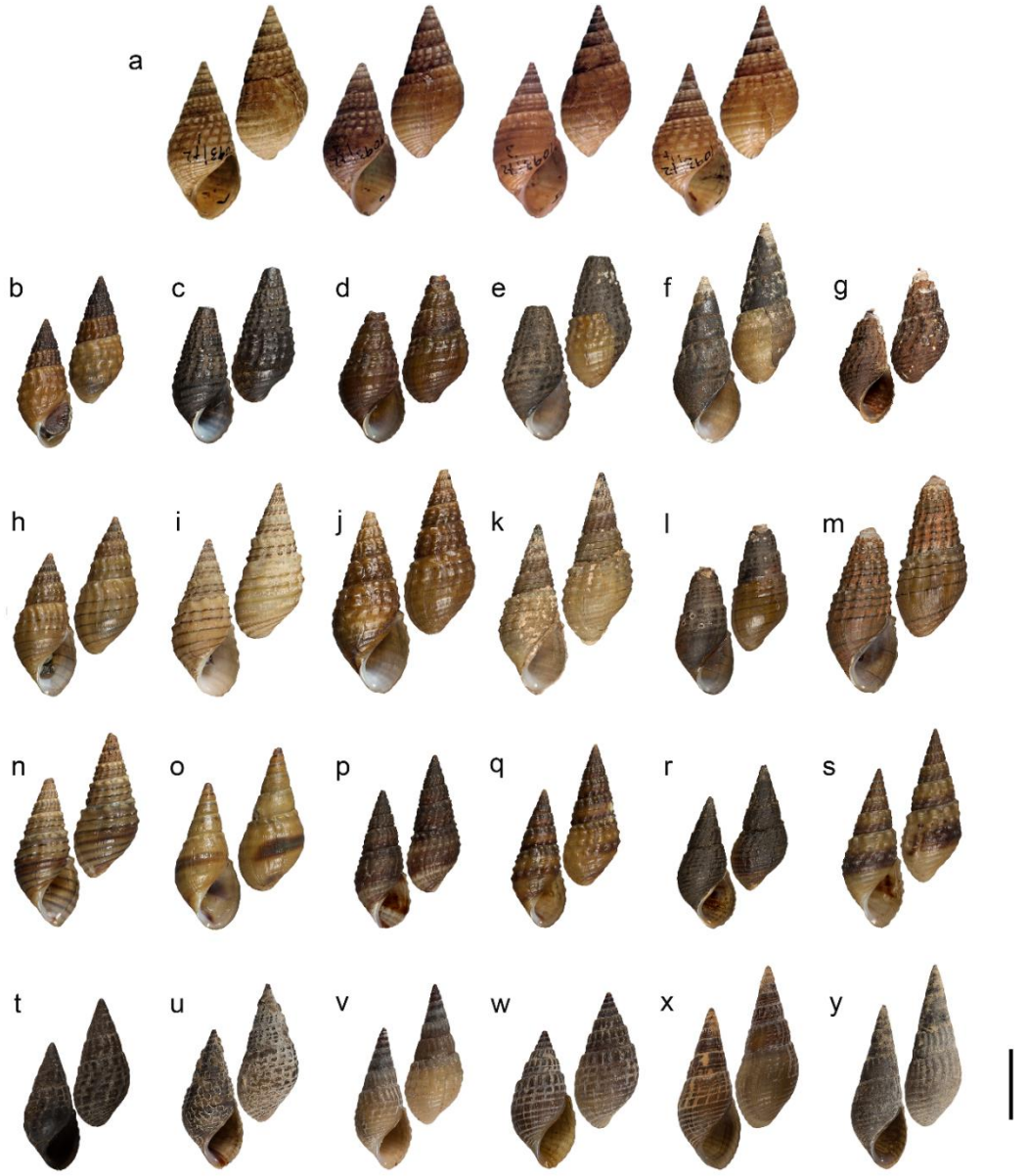

Figure 8. Shells of *Tarebia granifera* (Lamarck, 1816) from Timor and Thailand. a. Syntypes (MHNG 1093/72/1-4) from Timor. b-g. Morph A, i.e. specimens from Thailand corresponding to T. granifera (SUT 0514044, SUT 0516123, SUT 0515088, SUT 0515068, SUT 0515059, SUT 0516144). h-m. Morph B, i.e. specimens from Thailand corresponding to named "T. lineata" (Gray, 1828) (SUT 0515081, SUT 0514046, SUT 0516129, SUT 0515092, SUT 0515095, SUT 0516143). n-s. Morph C from Thailand (SUT 0515079, SUT 0516126, SUT 0515055, SUT 0515091, SUT 0516147, SUT 0516142). t-y. Shells of *T. granifera* from Timor-Leste (ZMH 119364, ZMH 119359, ZMH 119357, ZMH 119353, ZMH 119363, ZMH 119361). For locality data, see the material list in the main part of the text. Scale bar: 10 mm.

Radula

Snail samples were studied radula characters. In the nominal species of *T. granifera* the taenioglossan radula is slender, between 74 and 118 rows of teeth (for n=30 specimens of 3 morphs), with the length and width of the radula being correlated with the shell height or size of the snail. The snails were found the central teeth or rachidian has a large central cusp, which is flanked by two to three slender denticles on both sides (Fig. 9-11 a,c,e). Three morph of snails have different the number cusps of central teeth. There were two patterns that comprised of 2:1:2 and 3:1:3 (Table 4). Two lateral teeth nearly in pattern of the central teeth, bearing also 2-3:1:2 with long central cusp flanked by sharper cusps (Fig. 9-11 a,c,e) (Table 4). Marginal teeth long and spatulated, both inner and outer marginal teeth are two pairs of parallel rows, with inner and outer marginal teeth have 7-9 cusps of dentricles teeth (Fig 9-11 b,d,f).

Table 4. The pattern and number of cups of radula teeth from *Tarebia granifera* in Thailand.

Radula teeth	Shell Characteristics		
	Morph A	Morph B	Morph C
Number row of radula	80-114 mean: 92.08	74-118 mean: 93.00	76-109 mean: 87.67
Length of radula (mm)	1.06-2.96 mean: 1.84	1.07-2.93 mean: 2.09	1.00-2.51 mean: 1.99
Width of radula (mm)	0.49-0.85 mean: 0.64	0.49-0.82 mean: 0.67	0.43-0.82 mean: 0.66
Number of central cusps	2:1:2 (20%), 3:1:3 (80%)	2:1:2 (10%), 3:1:3 (90%)	2:1:2 (80%), 3:1:3 (20%)
Number of lateral cusps	2:1:2	2:1:2 (90%), 3:1:2 (10%)	2:1:2
Number of inner marginal cusps	8-9	8-9	7-9
Number of outer marginal cusps	7-8	7-9	7-9
Number of snails	10	10	10

Juvenile shell

The shell of the juveniles in the brood pouch from snails in Thailand (Fig. 13-15) was compared with topotypical material collected in Timor-Leste (Fig. 12). The juvenile was typical for eu-viviparous thiarids the sculpture of the initial cap is wrinkled (Fig 12-15 d,h,l), with axial elements and growth lines starting on the second whorl. On the third whorl spiral lines develop and more pronounced sculpture commences. After the fourth whorl the axial ribs become most pronounced. The increasing number of whorls the step-like appearance of the shell also markedly increases, due to subsutural angulation. The comparison of the lateral view of the apical whorls presented (Fig. 12-15 b,f,j). The juvenile shell of Thailand resemble Timor-Leste in the sculpture dominated by one apical and growth lines on each whorl. While the axial ridges from Thailand and some location in Timor-Leste was pronounced the strong axial ribs, except the specimen from Timor-Leste as Manatuto district, south coast, 3.8 km N of Nancuro beach, 4.7 km SE of Natarbora was found unclear axial ribs (Fig. 12 a,c).

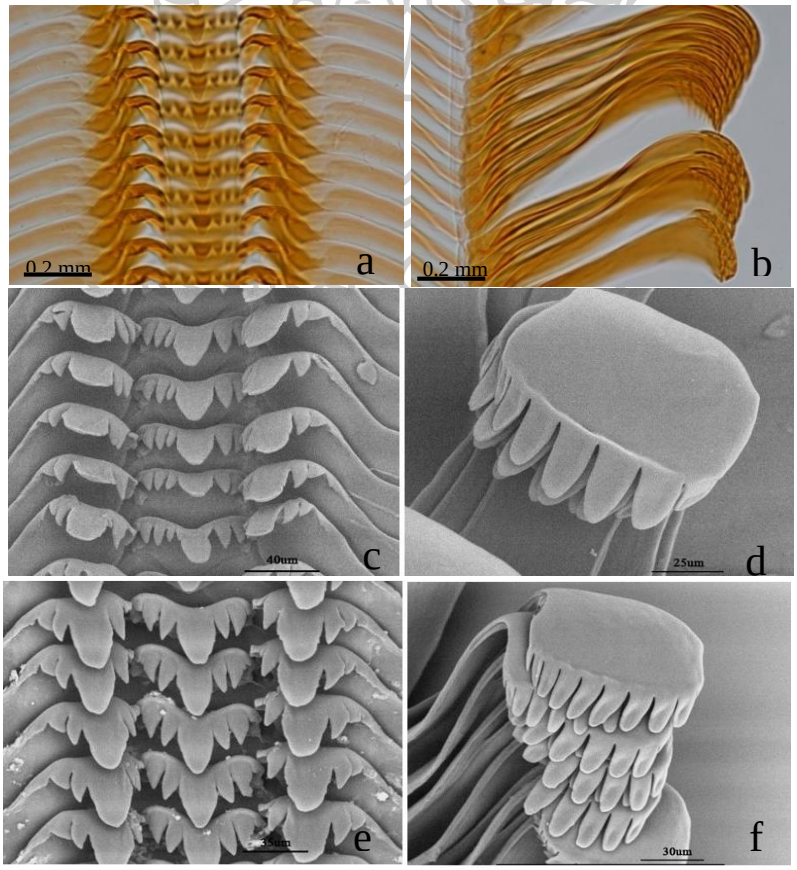

Figure 9. Radula of *Tarebia granifera* (Morph A).
a-b. Ban Thung Hang stream (SUT 0514044) stained with 4% Orange G; c-d. Huai Sa Dao Pong (SUT 0516123); e-f. Kaeng Bang Ra Chan (SUT 0515088); a,c,f. lateral and central teeth; b,d,f. marginal teeth.

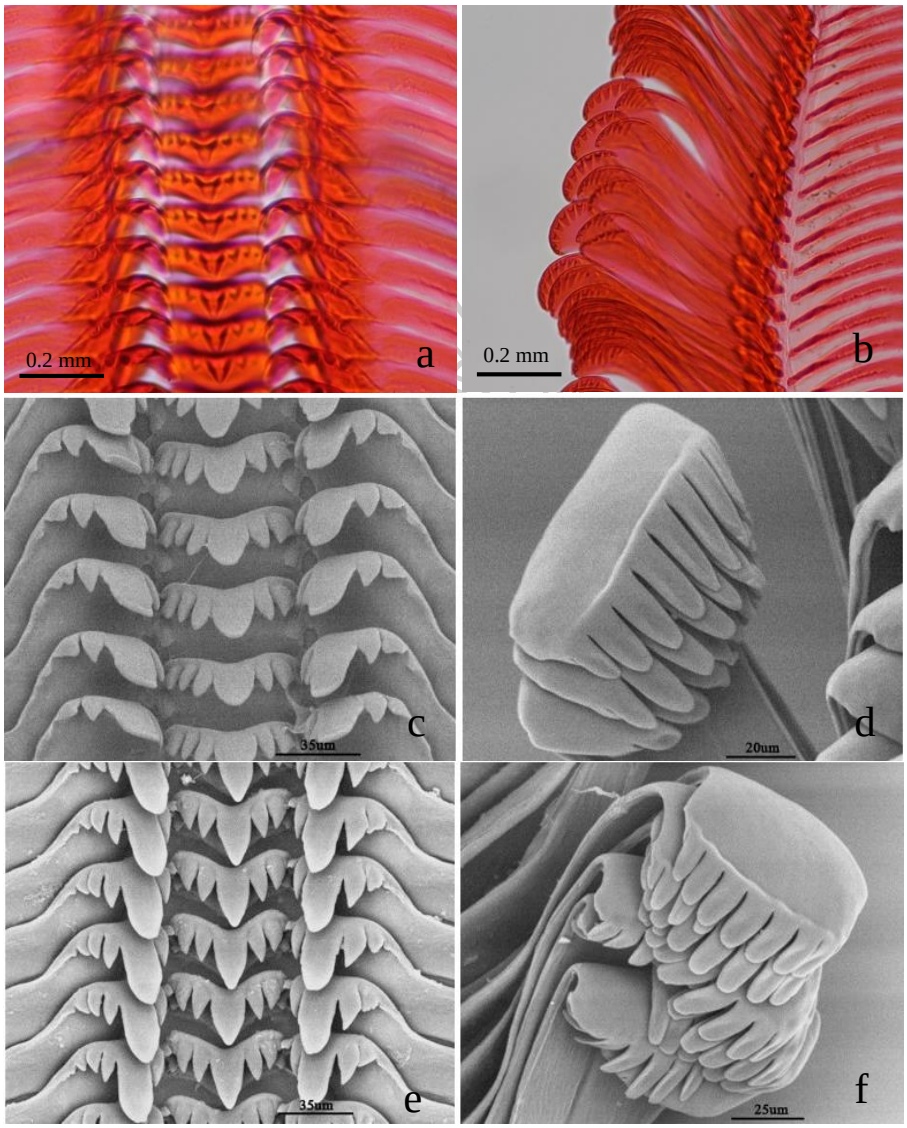

Figure 10. Radula of *Tarebia granifera* (Morph B).
a-b. Huay Nam Kong (SUT 0515081) stained with 4% Eosin Y; c-d. Sam Sip Khot waterfall (SUT 0516129); e-f. Sai Yok Yai waterfall (SUT 0515092); a,c,f. lateral and central teeth; b,d,f. marginal teeth.

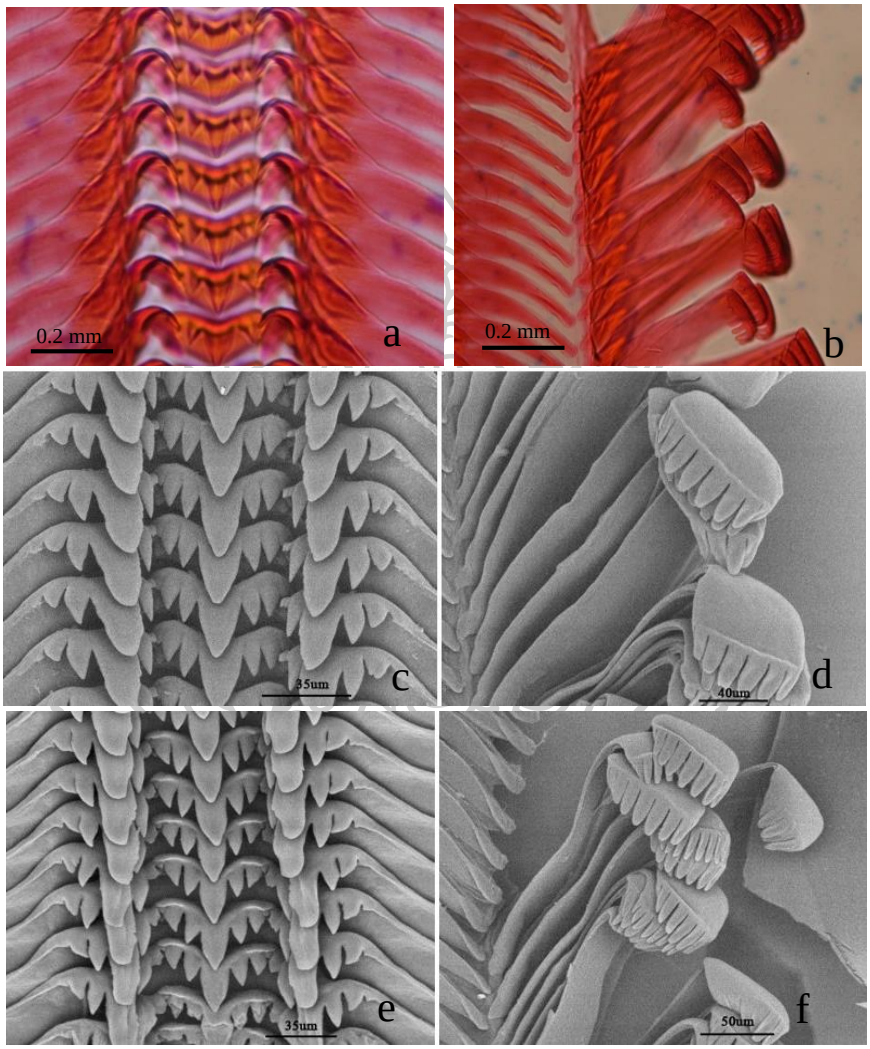

Figure 11. Radula of *Tarebia granifera* (Morph C).
a-b. Huay Sua Tao (SUT 0515079) stained with 4% Eosin Y; c-d. Ban Pak Huay Mae Tho (SUT 0516126); e-f. Vibhavadi waterfall (SUT 0516142); a,c,f. lateral and central teeth; b,d,f. marginal teeth.

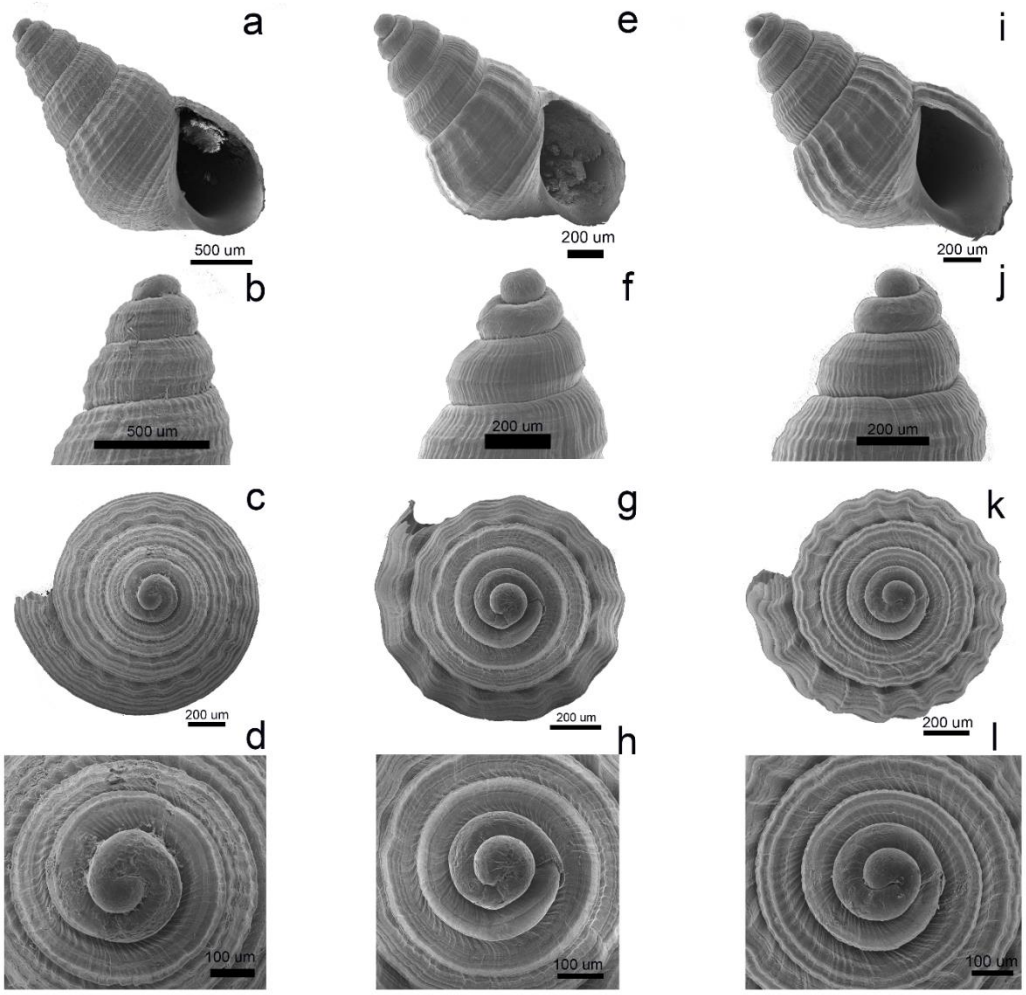

Figure 12. Juvenile shell of *Tarebia granifera* from Timor-Leste.
a-d. 4.7 km SE of Natarbora (ZMH 119359); e-h. Lutu-Ira (ZMH 119356); i-l. Watabo beach (ZMH 119357); a,e,i. lateral view; b,f,j. apical whorls; c,g,k. apical view; d,h,l. details of protoconch.

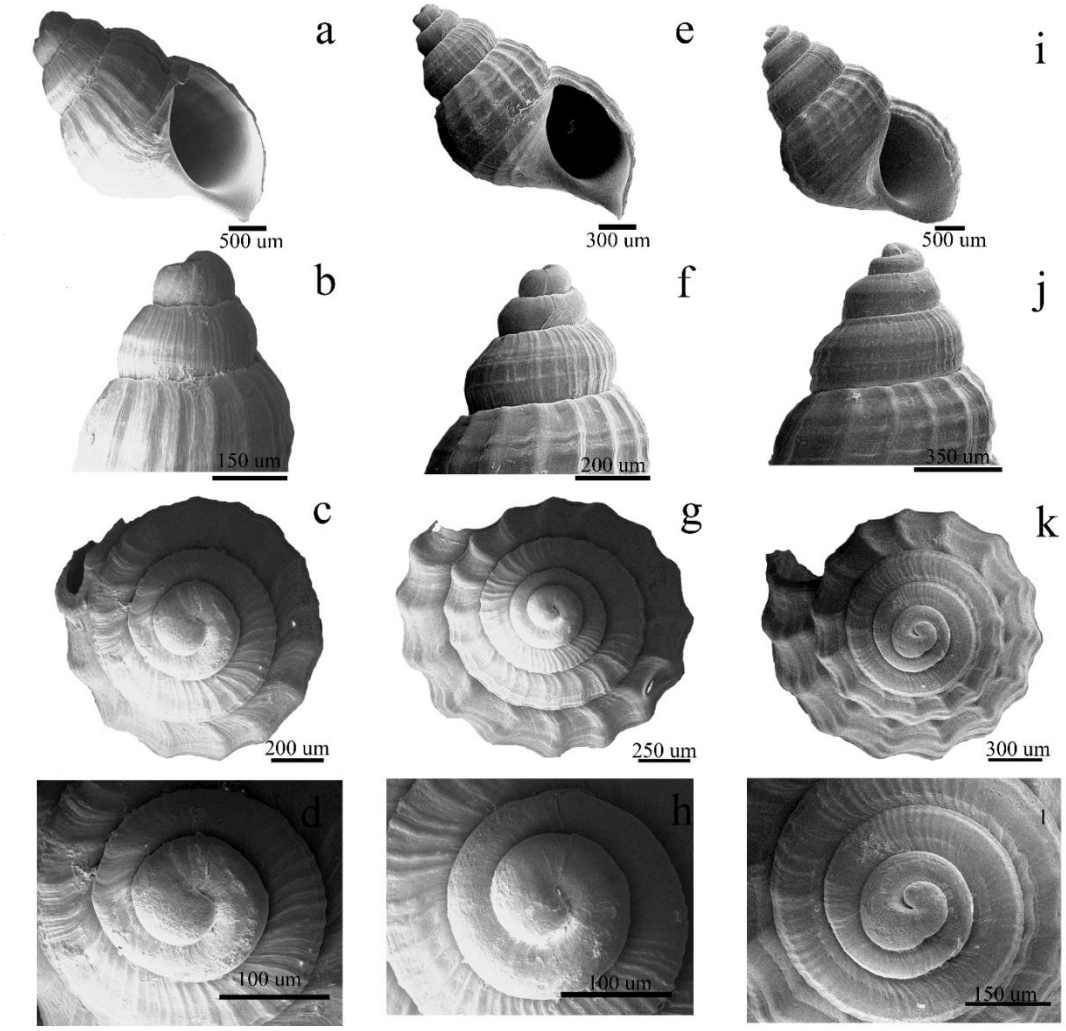

Figure 13. Juvenile shell of *Tarebia granifera* from Thailand (Morph A).
a-d. Khlong Cha-am (SUT 0513032); e-h. Huai Leng (SUT 0514042); i-l. Kaeng Bang Ra Chan (SUT 0515088); a,e,i. lateral view; b,f,j. apical whorls; c,g,k. apical view; d,h,l. details of protoconch.

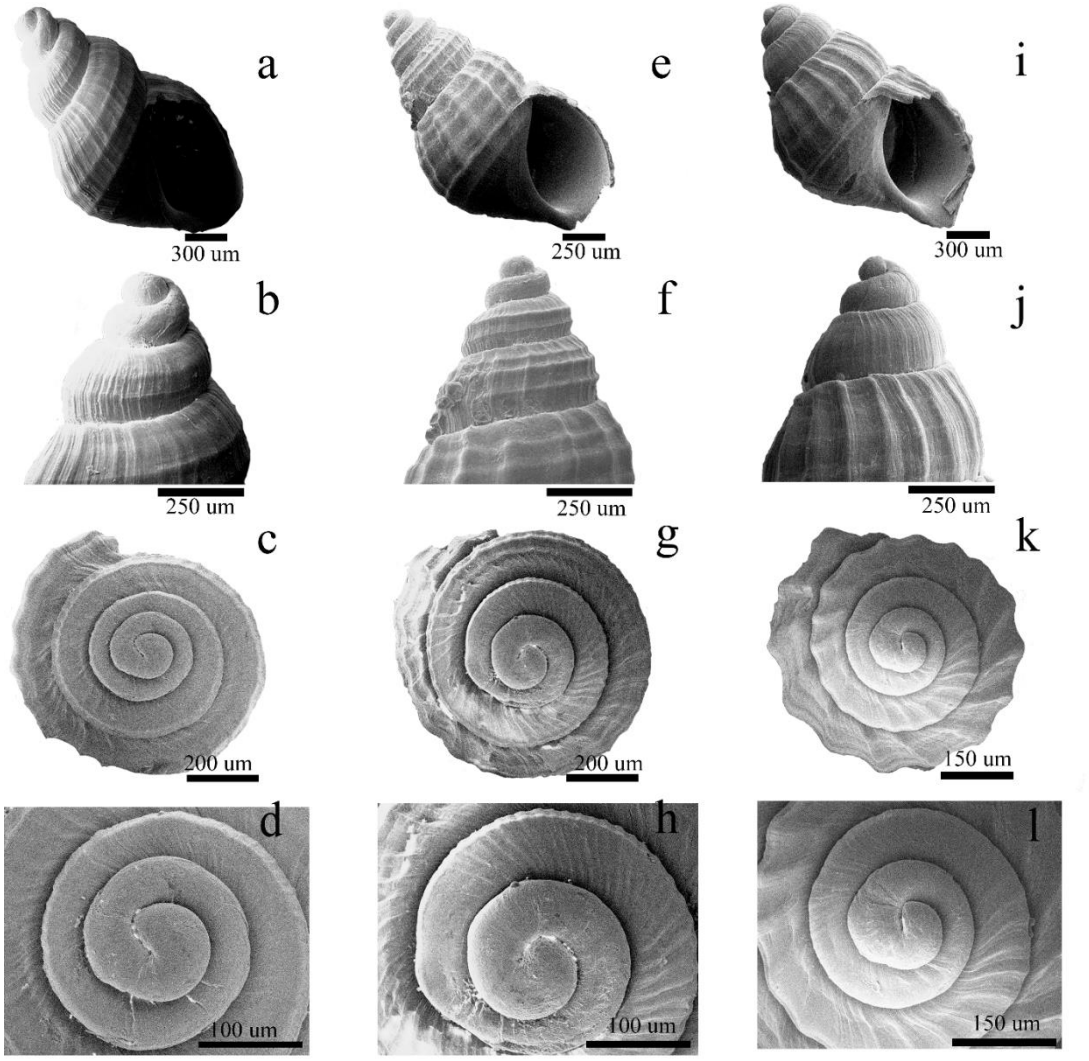

Figure 14. Juvenile shell of *Tarebia granifera* from Thailand (Morph B).
a-d. Huay Nam Kong (SUT 0515081); e-h. Sam Sip Khot waterfall (SUT 0516129); i-l. Sai Yok Yai waterfall (SUT 0515092); a,e,i. lateral view; b,f,j. apical whorls; c,g,k. apical view; d,h,l. details of protoconch.

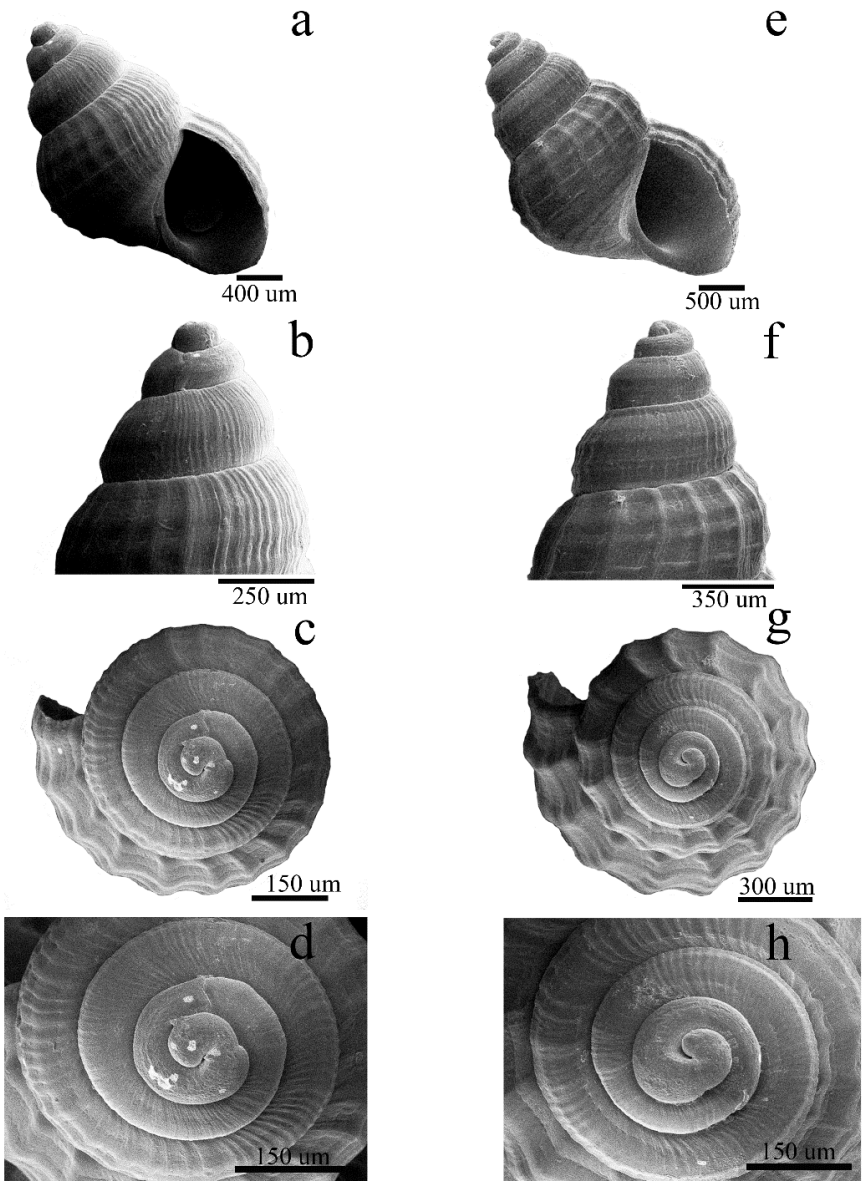

Figure 15. Juvenile shell of *Tarebia granifera* from Thailand (Morph C).
a-d. Pond of Silpakorn University (SUT 0515055); e-h. Vibhavadi waterfall (SUT 0516142); a,e,i. lateral view; b,f,j. apical whorls; c,g,k. apical view; d,h,l. details of protoconch.

Phylogenetic analyses

The final alignment of the *cox1* sequences had a length of 658 base pairs (bp) and that of the 16S sequences 781 bp. Genetic p-distances for *cox1* sequences of specimens determined as *T. granifera* from Thailand ranged from 0% to 14.7%, whereas all *cox1* sequences obtained from specimens from Timor-Leste were identical.

For 16S sequences, p-distances among specimens from Thailand ranged from 0% to 10.4% and for Timor-Leste, pairwise p-distance between specimens were very low, ranging from 0% to 0.1%.

All three phylogenetic analyses recovered two deeply divergent clades of specimens assigned to *T. granifera* (clades A and B, Fig. 16), with high to very high support (clade A, PP: 1.00, BS (ML): 95, BS (MP): 100; clade B, PP: 1.00, BS (ML): 90, BS (MP): 100). Genetic p-distances between these two clades were distinctly higher than p-distances within either clade A or clade B, 13.8% for *cox1* and 10% for 16S sequences. Genetic p-distances within clade A were with 0% to 3.34% for *cox1* and 0% to 1.44% for 16S sequences rather low.

All specimens from Timor-Leste were included in clade A together with specimens mostly from the southern to southern-central parts of Thailand (Fig. 16), viz. those from the provinces Songkhla, Trang, Krabi, Nakhon Si Thammarat, Surat Thani, Chumphon, Prachuap Khiri Khan, Phetchaburi, Ratchaburi, Kanchanaburi, Nakhon Pathom, Sara Buri and Nakhon Sawan. But this clade include also specimens from the northern part of the country, viz. Chang Mai, Lampang, Phrae and Phitsanulok, and specimens from Nakhon Ratchasima and Rayong in northeast to eastern Thailand. Within clade A, relationships among specimens were generally not well-supported (Fig. 16). However, there is a general pattern that Thai specimens of *T. granifera* assigned to clade A were more frequent in the southern part of the country.

In contrast, specimens of *T. granifera* assigned to clade B were more frequent in the northern part of Thailand, i.e. the majority of specimens in this clade origin from the northern to northeast Thai provinces, such as Chang Mai, Mueang Mae Hong Son, Phayao, Lampang, Nan, Uttaradit, Tak, Sukhothai, Phitsanulok, Phetchabun and Loei, while only few specimens in this clade are from the southern-central Thai provinces Phatthalung, Nakhon Si Thammarat, Surat Thani, Ratchaburi, Kanchanaburi and Lop Buri. Almost all specimens assigned to clade B were placed in a polytomy in the tree shown in Fig. 16. Corresponding to the results of the phylogenetic analyses, genetic p-distances within clade B were very low, with 0% to 0.46% for *cox1* and 0% to 0.52% for 16S sequences.

When analysed by drainage systems, all specimens from the north-western part of Thailand, which is drained through the Salween river system into the Andaman Sea, were included in clade B. Likewise, specimens from the headwaters of the Ping, Wang, Yom and Nan rivers belonging to the Chao Phraya system, with few exceptions, were assigned to clade B in the phylogenetic analyses. In the lower courses of northern to northern-central Thai drainages, such as e.g. the Chao Phraya

and Mae Klong drainages that run into the Gulf of Thailand, specimens assigned to both clades are present.

Similarly, specimens belonging to both mitochondrial clades are present in the Mekong drainage, whereas specimens assigned to clade A predominate in the smaller rivers in the Thai parts of the Malay Peninsula to the north and south of the Isthmus of Kra that either drain into the Gulf of Thailand or the Andaman Sea (Fig. 16). Noteworthy are a few populations from the somewhat more elevated parts of the provinces Surat Thani (SUT 0516137), Nakhon Si Thammarat (SUT 0516139) and Phatthalung (SUT 0516138) on the Malay Peninsula that were assigned to clade B (Fig. 16).

In contrast to this geographical pattern in *Tarebia granifera*, with broadly speaking an essentially southern clade A and an essentially northern clade B, we found no correspondence of specimens from the three morphotypes with the two genetically differentiated clades as outlined above as all morphs were present in both clades.

Haplotype networks, molecular species delimitation and dating

Evolutionary relationships among haplotypes were inferred applying a median-joining network approach that showed the two mitochondrial clades A and B to be separated by > 60 steps (*cox1* and 16S; Fig. 17a, b), while within these clades haplotypes were separated by usually only a few steps (Fig. 17a, b).

The ABGD approach suggested that the *T. granifera* clades A and B could be classified as two species for prior intraspecific divergences (*d*) of the combined *cox1* and 16S data set of $d \geq 0.0077$. The bGMYC analysis (Fig. 18) recovered a probability of conspecifity of less than 0.05 for specimen pairs belonging to both, the mitochondrial clades A and B. For specimen pairs assigned to clade A in the phylogenetic analyses a probability of conspecificity of more than 0.7 was recovered, with most pairs having a probability of conspecificity of more than 0.95. All specimen pairs assigned to clade B in the phylogenetic analyses were assigned a probability of conspecificity of more than 0.95 in the bGMYC analysis.

The results of the BEAST analysis assuming a strict molecular clock and a divergence rate of 1% per million years (Fig. 19) suggests, following the split of *Tarebia (granifera)* from *Thiara (amarula)* at about 7.1 million years ago (Mya), a separation of the mitochondrial clades A and B at about 5.3 Ma BP (95% highest posterior density interval (HPD): c. 6.5–4.0 Mya). The diverification within clade A is suggested to have started c. 0.65 Mya (95% HPD: 0.95–0.45 Ma BP), while the slitting within clade B occurred presumable c. 0.33 Mya (95% HPD: 0.50–0.25 Mya).

There were not any correlation of shell morphology with molecular genetic cluster as described above, or any other geographical or ecological factor matching these distinct phenotypes in *Tarebia granifera.*

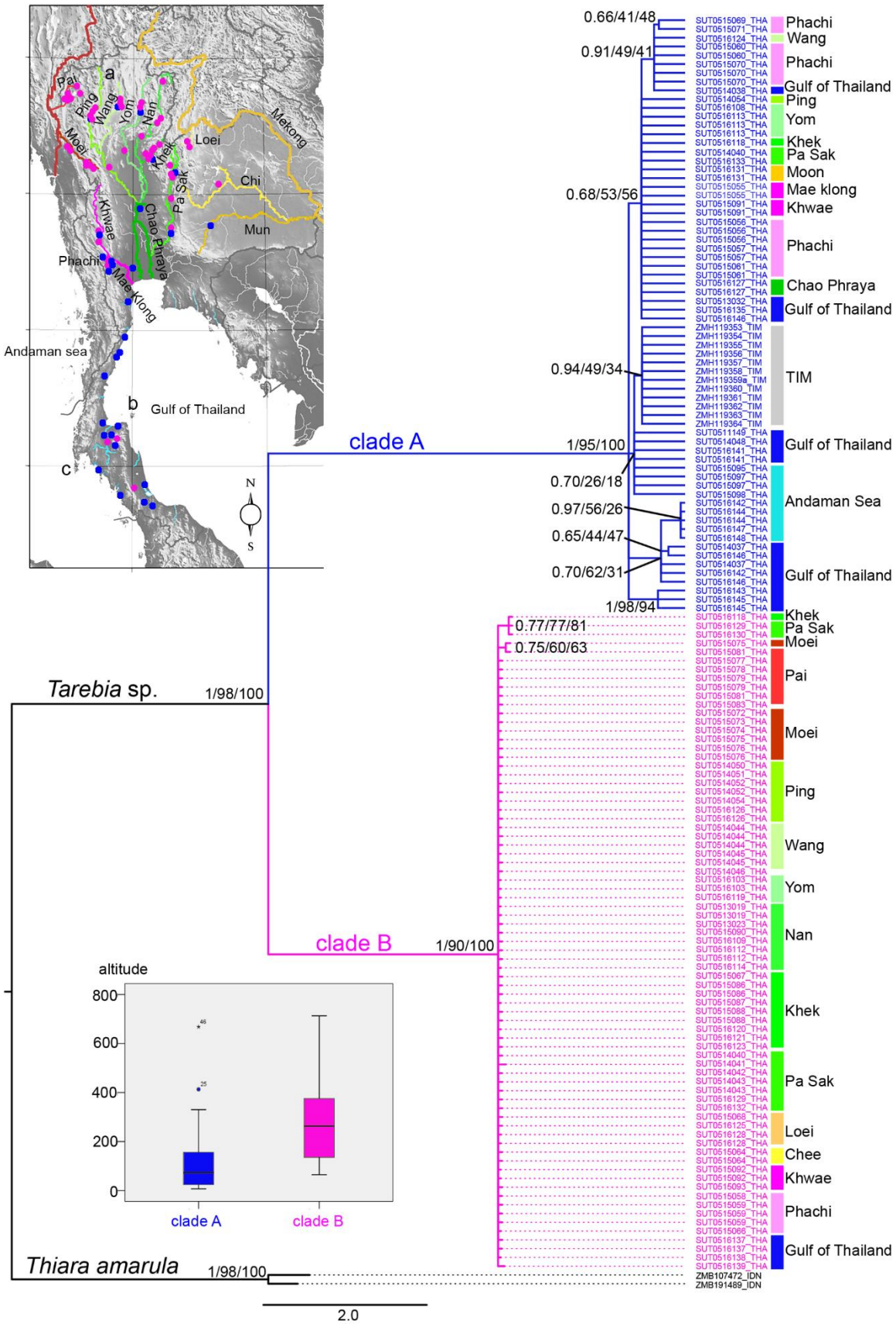
a
b
c
Pai
Ping
Wang
Yom
Nan
Mekong
Loei
Khek
Pa Sak
Chi
Mun
Moei
Khwae
Chao Phraya
Mae Klong
Phachi
Andaman sea
Gulf of Thailand
N
S
clade A
clade B
Tarebia sp.
Thiara amarula
1/98/100
1/90/100
1/98/100
0.66/41/48
0.91/49/41
0.68/53/56
0.94/49/34
1/95/100
0.70/26/18
0.97/56/26
0.65/44/47
0.70/62/31
1/98/94
0.77/77/81
0.75/60/63
Phachi
Wang
Phachi
Gulf of Thailand
Ping
Yom
Khek
Pa Sak
Moon
Mae klong
Khwae
Phachi
Chao Phraya
Gulf of Thailand
TIM
Gulf of Thailand
Andaman Sea
Gulf of Thailand
Khek
Pa Sak
Moei
Pai
Moei
Ping
Wang
Yom
Nan
Khek
Pa Sak
Loei
Chee
Khwae
Phachi
Gulf of Thailand
altitude
800
600
400
200
0
clade A
clade B
2.0

Figure 16. Bayesiam 50% majority-rule consensus tree showing two major mitochondrial clades in *Tarebia granifera* (Lamarck, 1816).
Numbers at the nodes correspond to posterior probabilities (left), maximum likelihood (middle) and maximum parsimony (right) bootstrap values. At the tips of the tree voucher numbers (see material list in the main part of the text), country codes (THA: Thailand; TIM: Timor-Leste) and the river where specimens were collected are indicated. The inset map shows the distribution of mitochondrial clades in Thailand (clade A: blue dots; clade B: pink dots) and major river systems. The letters a–c in the map refer to localities, for which climatic data were available (see also Fig. 26). The inset with box plots shows the altitudinal distribution of mitochondrial caldes A and B, respectively.

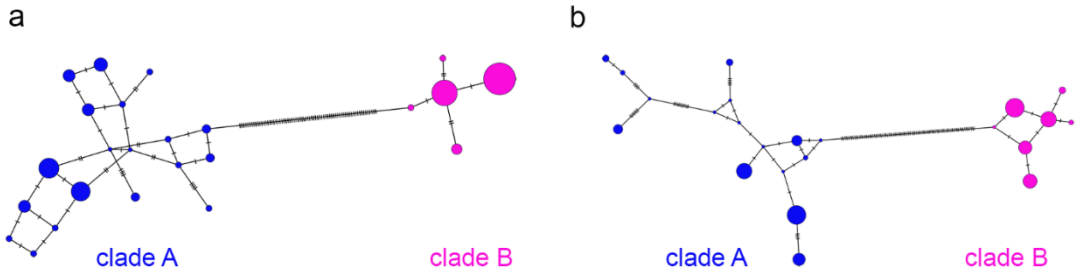

Figure 17. Molecular analysis of *Tarebia*. a-b. Median-joining haplotype networks based on 16S (a) and cox1 (b) sequence data of *Tarebia granifera* (Lamarck, 1816).
The size of each circle represents the frequency of a haplotype and the color refers to main mitochondrial clades obtained from the phylogenetic analyses (Fig. 16; blue: clade A, pink: clade B). Tick marks between circles represent evolutionary steps.

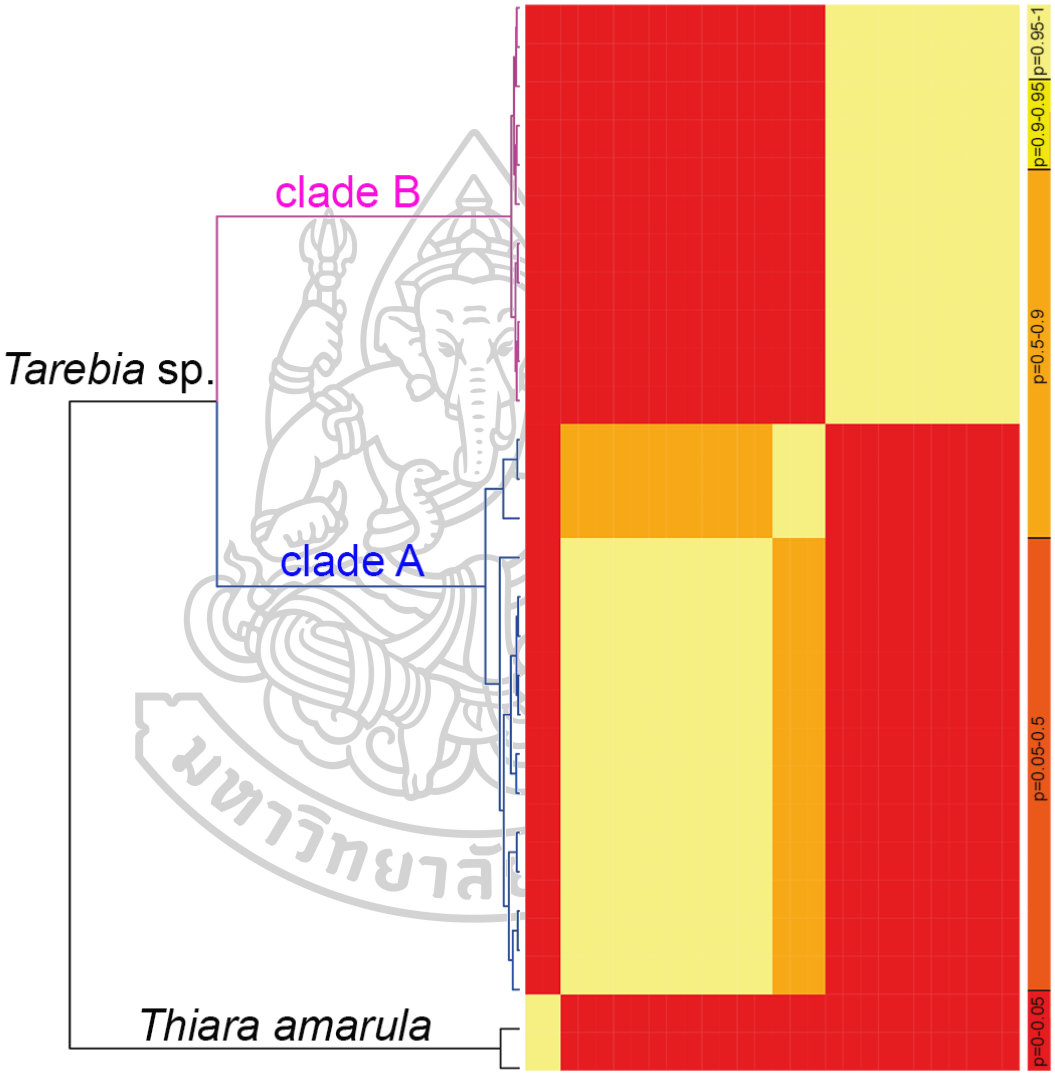

Figure 18. Results of the bGMYC analysis.
Coloration of the matrix cells represents pairwise probabilities of conspecificity.

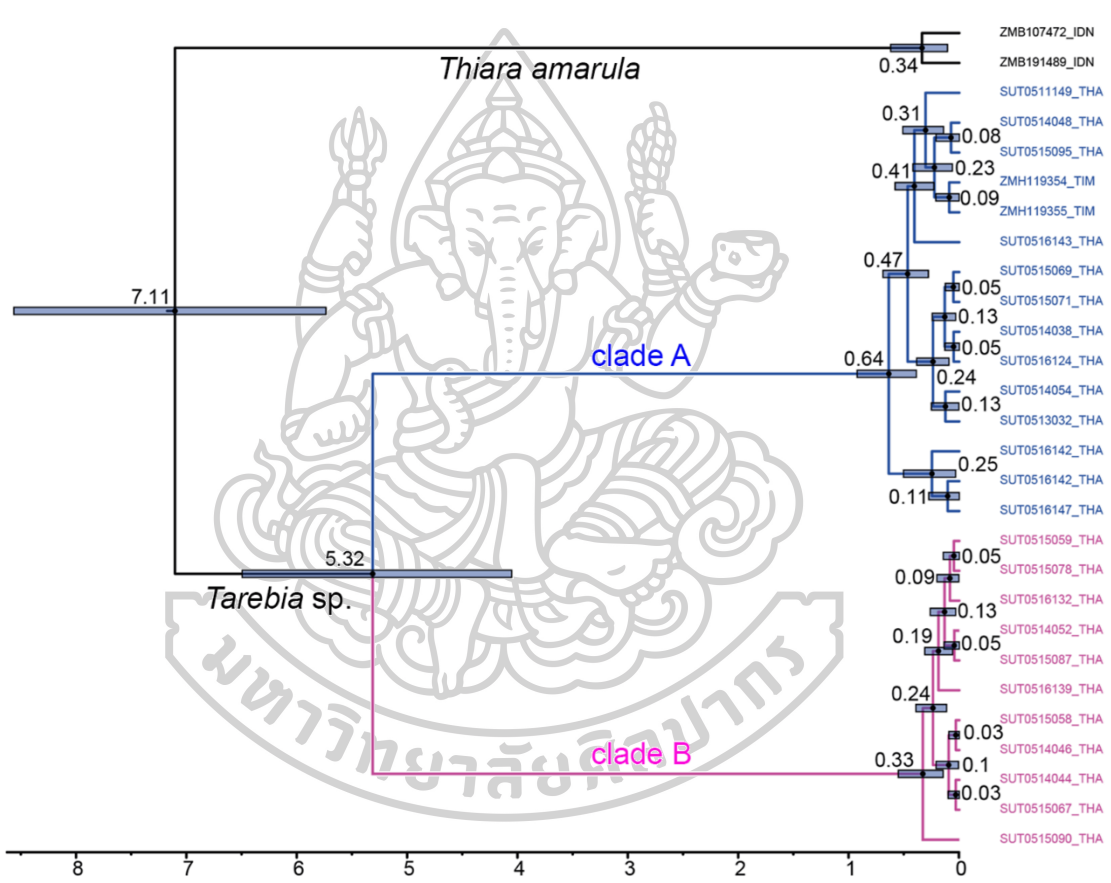

Figure 19. Dated molecular tree (only unique haplotypes were included). Numbers at the nodes are node ages in Ma, bars represent 95% highest posterior probabilitiy intervals.

Biometric and Geometric morphometric analyses

The biometrical measurements showed that shell varies in *T. granifera* from Thailand and Timor-Leste. For ranges and mean values of measured shell parameters for the different predefined groups, i.e. shell morphs/geographic groups or genetic clades.

T. granifera materials were grouped base on the morphology of shell. The results were shown the shell height of Morph A_THA, Morph B_THA, Morph C_THA and Timor-Leste is 9.29 to 29.83, 8.56 to 32.38, 10.53 to 26.88 and 11.67 to 28.53 mm, respectively (Fig. 20a). The smallest and the tallest of shell were found in Morph B_THA including SUT 0514038 from Prachuap Khiri Khan Province and SUT 0516142 from Surat Thani Province, respectively. The width of shell from Morph A_THA, Morph B_THA, Morph C_THA and Timor-Leste is 3.73 to 13.28, 3.49 to 14.46, 4.39 to 11.58 and 5.04 to 12.18 mm, respectively (Fig. 20b). The thinnest sample was Morph B_THA from Tak Province (SUT 0515073). The fattest shell was Morph B_THA from Nakhon Si Thammarat Province (SUT 0516139). The height of last three whorl was used for some specimen is eroded of the apex. The height of last three whorl of three morph from Thailand and Timor-Leste is 7.93 to 26.43, 7.73 to 28.74, 9.20 to 21.34 and 9.46 to 23.89 mm, respectively (Fig. 20c). The smallest were found Timor-Leste from Viqueque District (ZMH 119360). The tallest of snail were Morph B_THA from Nakhon Si Thammarat Province (SUT 0516139). The size index represented the shell shape that was indicated by the ratio of height of last three whorl and width of shell (L3W/W) which depicting similar pattern (Fig. 20d). The size index of sample from Morph A_THA, Morph B_THA, Morph C_THA and Timor-Leste was ranging 1.27 to 2.54, 1.22 to 2.53, 1.39 to 2.65 and 1.66 to 2.28, respectively. The statistical analysis of four parameter showed that size index of three morph from Thailand and Timor-Leste were no significant differences ($\alpha > 0.05$). But height, width and last three whorl were statistically different ($\alpha=0.013$, $\alpha=0.035$, $\alpha=0.013$). The height was found significant differences ($p=0.18$) between the means of morph A and C, while width and last three whorl was found significant differences ($p=0.024$, $p=0.008$) between the means of morph B and C (Appendix D-F). It has to be noted, however, that the ranges of all measured shell parameters widely overlap and, therefore, do not qualify as diagnostic characteristics (see boxplots in Fig. 20a–d). Between genetic clades, the results showed the shell height of clade A and B is 8.56 to 32.38 and 9.45 to 30.67 (Fig. 21a). The width of shell is 3.73 to 13.28 and 3.49 to 14.46 mm (Fig. 21b). The height of last three whorl of three is 7.93 to 26.22 and 7.73 to 28.74 mm (Fig. 21c). The size index was ranging 1.27 to 2.65 and 1.22 to 2.38 (Fig. 21d). The statistical analysis of genetic clades showed that mean of clade B have width, height of last three whorl and size index more than clade A with statistical analysis found significant different ($\alpha < 0.05$) but the height of clade A and B no significant differences ($\alpha > 0.05$) (Appendix G-H). However, similar to the situation when comparing the different shell morphs/geographical groups, it has to be noted that the ranges of all measured shell parameters widely overlap and, therefore, do not allow to derive diagnostic characteristics for the two main clades found in the phylogenetic analyses (see boxplots in Fig. 21a–d).

Geometric morphometrics (GM) analysis were observed between samples of the same species with a principle component analysis (PCA) was carried out employing Palaeontological Statistics (PAST) version 2.10. The result shows all of *T. granifera* have been highly variable conchology of shells in Thailand comparison with Timor-Leste. The PCA show quite nicely clustered point groups for *T. granifera* from Thailand similarity with Timor-Leste. The Geometric morphometrics (GM) analysis were found shell shape of difference morphs and genetic clades have similarity and widely overlap, which indicates that a clear separation is not possible on the basis of shell shape (Fig. 20-21e).

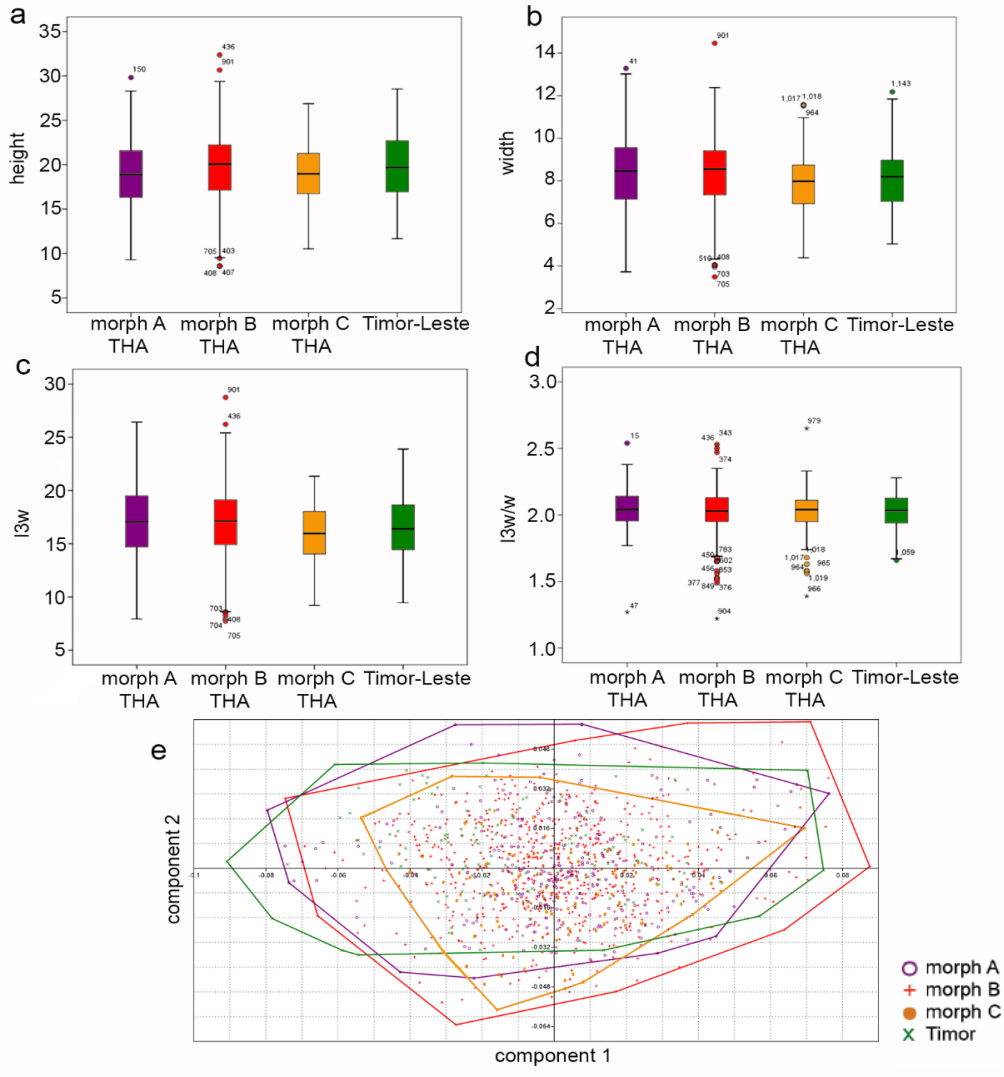

Figure 20. Results of biometric (a-d) and geometric morphometrics study (e), for four different morphs (A,B,C,Timor) of *Tarebia granifera* (Lamarck, 1816).
Boxplots of a. shell height; b. shell width; c. height of the last three whorls; d. index of height of last three whorls agaianst shell width; e. relative variance in shell shape along PC1 and PC2. Color corresponding planes indicate the spread of each morph in the data set.

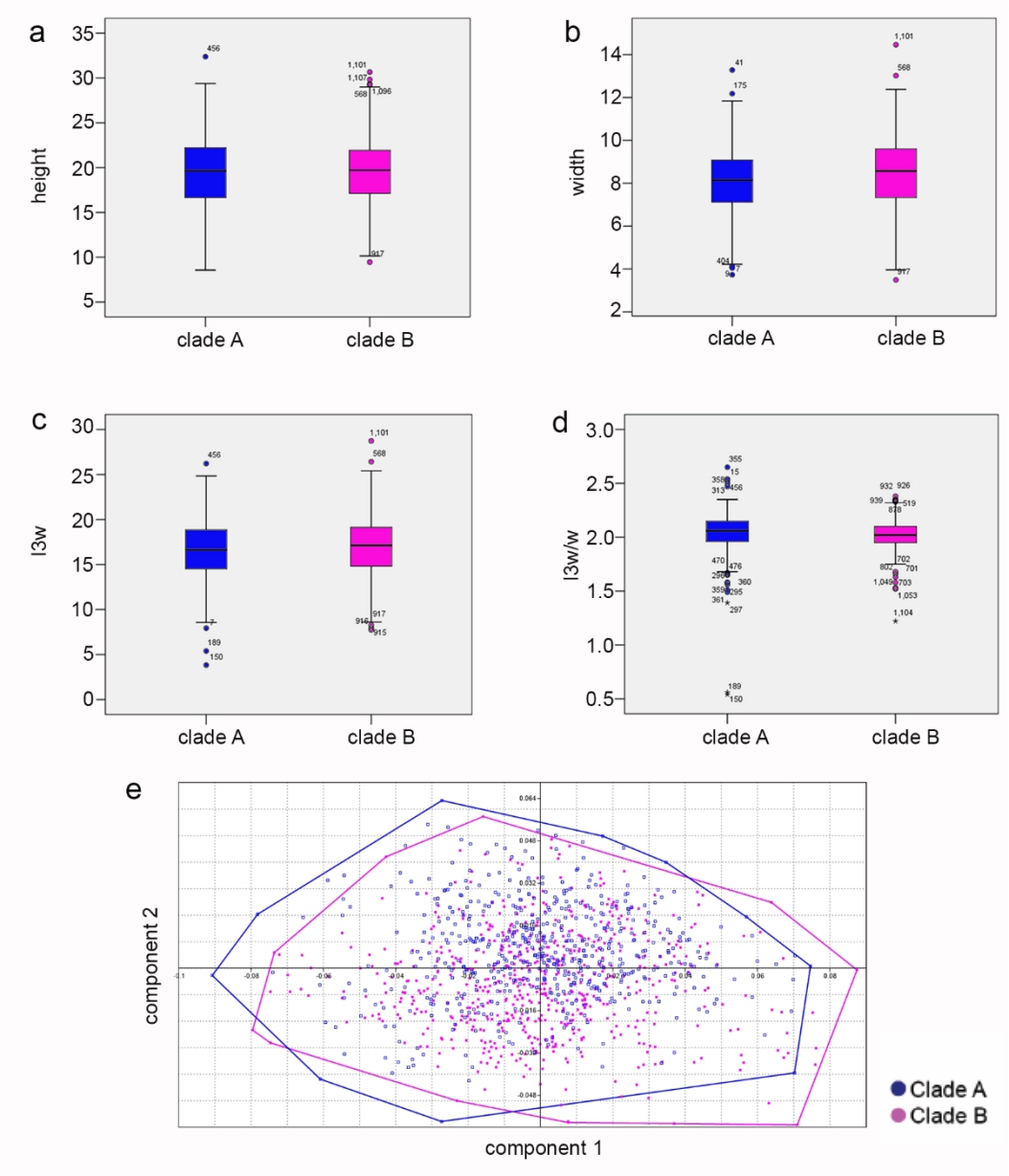

Figure 21. Results of biometric (a-d) and geometric morphometrics study (e), for the two mitochondiral clades of *Tarebia granifera* (Lamarck, 1816) found in this study. Boxplots of a. shell height; b. shell width; c. height of the last three whorls; d. index of height of last three whorls agaianst shell width; e. relative variance in shell shape along PC1 and PC2. Color corresponding planes indicate the spread of each morph in the data set.

Brood pouch content

Females of *Tarebia granifera* were found to contain embryos and shelled juveniles in their "marsupium", or subhemocoelic brood pouch, situated in the neck region as in other thiarids studied so far. They usually release crawling juveniles with shells comprising several whorls that are built before hatching from the brood pouch. In this study, we found the snails to possess brood pouch filled with all ontogenetic stages, ranging from early to late embryos and six additional size classes of juveniles, with shells measuring between less than 0.5 to more than 3 mm (see Fig. 22-24).

The frequency of these different size classes in the subhemocoelic brood pouch of the total of n = 1,107 dissected females of *Tarebia granifera* from a total of 107 populations from Thailand (n = 95) and Timor-Leste (n = 12) is shown as to their geographic occurrence for the two mitochondrial clades A (n = 42) and B (n = 53) as well as the predefined morphs A, B and C in Fig. 22 and 23 a-c. Although the content of the brood pouch varied considerably among individuals and populations, no geographic pattern could be observed, neither for the populations within Thailand nor for those from Timor-Leste. There were not to find any specific pattern in the distribution of the eight ontogenetic stages in correlation with the two genetic clades A and B or for the different predefined shell morphs (Fig. 22-23).

In all examined populations, the number of early and late embryonic stages was above 50%, in most cases even above 75%; see Fig. 24 a,b for the composition of the brood pouch contents according to the three morphs A-C, and see Fig. 24c,d for those of the two mitochondrial clades. Nevertheless, in nearly all populations shelled juveniles of the size between less than 0.5 to more than 3.0 mm were present in the female's brood pouches; with the only exception for females (n = 1 and 9) from two populations of morph A and C, both in locations in the south in streams draining to the Gulf of Thailand (see Fig. 23a,b).

When considering the overall distribution of different size classes in the different morphs/geographic clusters or mitochondrial clades, the resulting histograms (Fig. 24a,c) all show essentially the same composition of ontogenetic stages, which suggests the presence of the same reproductive strategy in all investigated grouping. The overall ratio of non-gravid vs. gravid specimens was 164:943 (= 17.4%). Among the 255 dissected specimens assigned to morph A, 21 snails were found to be non-gravid (= 8.2%), while among the 652 dissected snails assigned to morph B, in 123 of these no offspring was observed (= 18.9%). For morph C, the ratio of gravid vs. non-gravid specimens was 11:128 (= 8.6%) and that ratio for specimens from Timor-Leste was 9:72 (= 12.5%) (Fig. 24b). Considering the two main mitochondrial clades, similar values were observed (Fig. 24d), with the proportion of gravid females well above 85%.

We also compared the size class composition of offspring in the subhemocoelic brood pouches of *Tarebia* populations from different drainage systems. Although considerable variation was present among the rivers and streams of the 17 drainage systems in Thailand (Fig. 25a), clear differences could not be observed. There was, however, one possible exception, i.e. females of *T. granifera* from the Moei River in the Northwest of Thailand, where a very low amount of early

embryonic stages and less later embryonic stages were found, while there were the largest proportion of larger shelled juveniles. Also, there was a slight trend for populations in streams and rivers in the south of Thailand, both draining into the Gulf of Thailand and the Andaman Sea, to exhibit higher proportions of the earliest embryonic stages.

The distribution of gravid vs. non-gravid specimens according to the 17 rivers systems exhibit some variation (Fig. 25b), albeit with usually (far) more gravid specimens present in all populations; but again with the exception of females from populations in the Northwest of Thailand, in particular from the rivers Moei, Ping and Pai. The populations in Moei River were in this respect exceptional because only there were found more non-gravid than gravid specimens. Conversely, all females from populations in the rivers Chao Phraya, Loei, Chee, Moon, Khwae, Mae Klong and from streams of the Andaman Sea were found to be gravid, with no non-gravid specimens at all detected in our samples.

Whether reproduction is seasonal, or whether there is any influence of the month of collecting on the data, can currently not be answered with certainty. In an attempt to correlate reproduction (i.e. the frequency of gravid vs. non-gravid females) with climatic effects such as, for example, rainy season resulting in high water levels in rivers and streams, meteorological data (e.g. minimum/maximum temperature and precipitation) were used for stations representing the different climatic regions of Thailand, viz. Chiang Mai for northern inland region, Ko Samui for the Gulf of Thailand and Phuket for the Andaman Sea localities (see map in Fig. 22 for these locations). As is evident from Fig. 26, specimens collected in populations from inland places were to a high proportion gravid females at the end of winter (January-February) and into the summer season (March-June). During this first half of the year the proportion of gravid females somehow reflect percipitation in so far, as there is a trend to be high when it is dry (see Fig. 26a); also the proportion of non-gravid females increases towards the rainy season in the North of Thailand (April/May). At localities in the Gulf of Thailand region, high numbers of specimens with brood pouch content were found both during the little (May-June) and great (Oct.-Nov.) rainy season; however, in the dry season did not have the collecting data (Fig. 26b). For the Andaman Sea region, only specimens collected during the rainy season were available, reflecting in general the picture from the Gulf region, though; with ~25% non-gravid specimens at the beginning and only gravid specimens shortly after the peak of the rainy season (Fig. 26c).

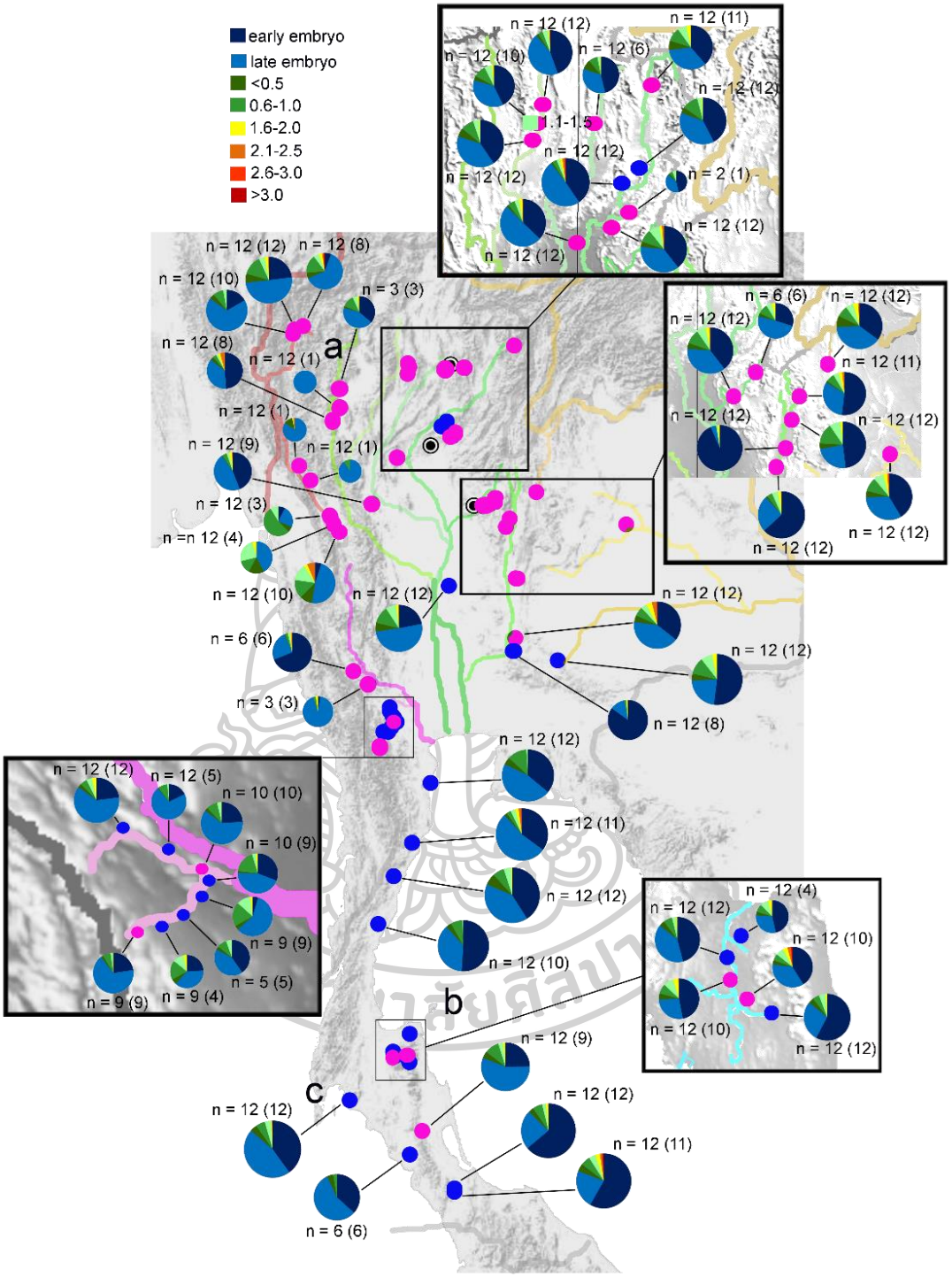

Figure 22. Frequency of ontogenetic stages in the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (morph B) depending on occurrence in Thailand.

Blue dots: mitochondrial clade A; Pink dots: mitochondrial clade B. Size classes are assigned different colors in the pie charts (see legend) and rivers are colored according to drainage systems; numbers at the pie charts refer to the total number of dissected specimens and the number of gravid females (in parentheses).

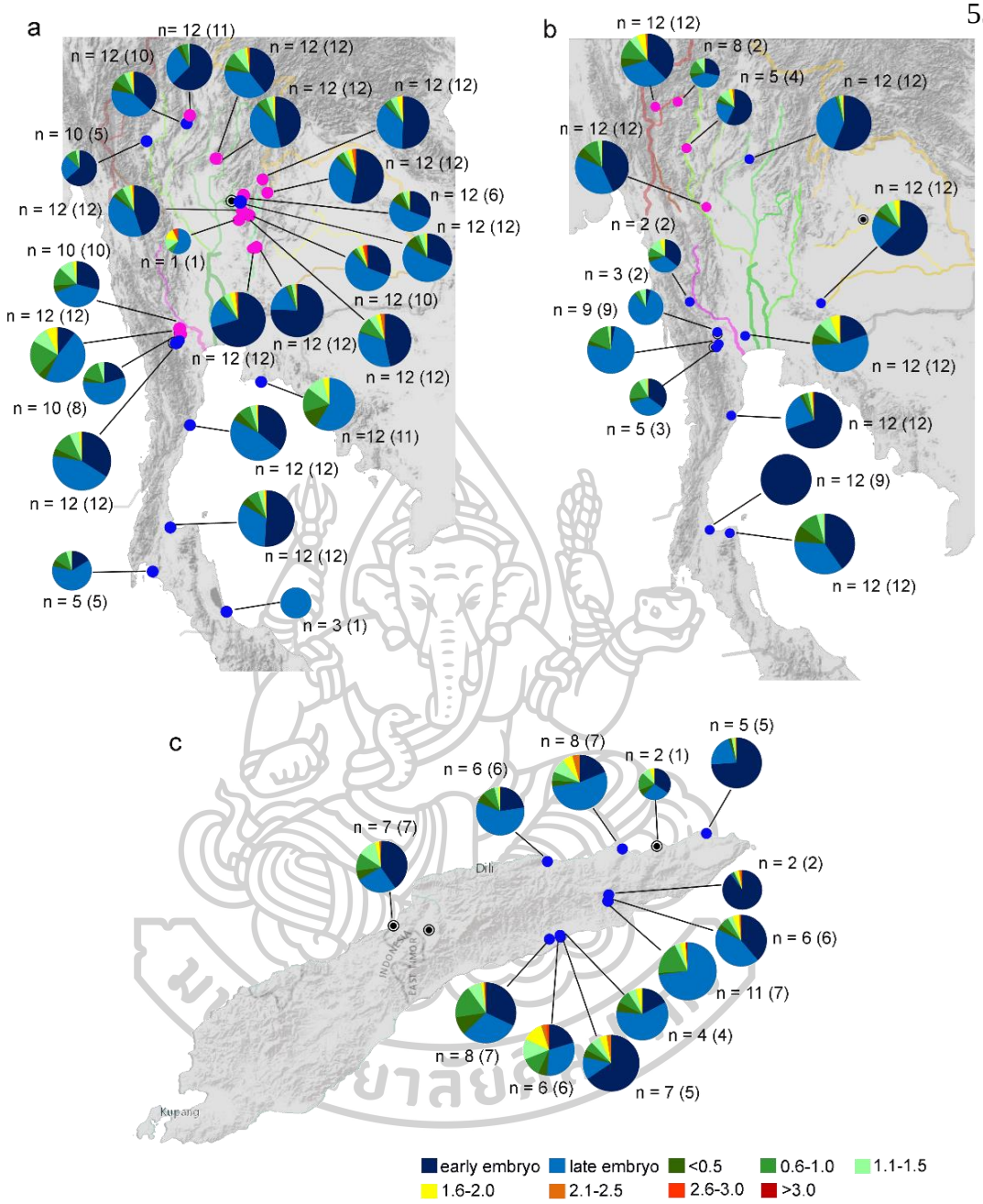

Figure 23**.** Frequency of ontogenetic stages in the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) depending on occurrence in Thailand and Timor-Leste.

a. Morph A in Thailand; b. Morph C in Thailand; c. Timor-Leste. Blue dots: mitochondrial clade A; Pink dots: mitochondrial clade B. Size classes are assigned different colors in the pie charts (see legend) and rivers are colored according to drainage systems; numbers at the pie charts refer to the total number of dissected specimens and the number of gravid females (in parentheses).

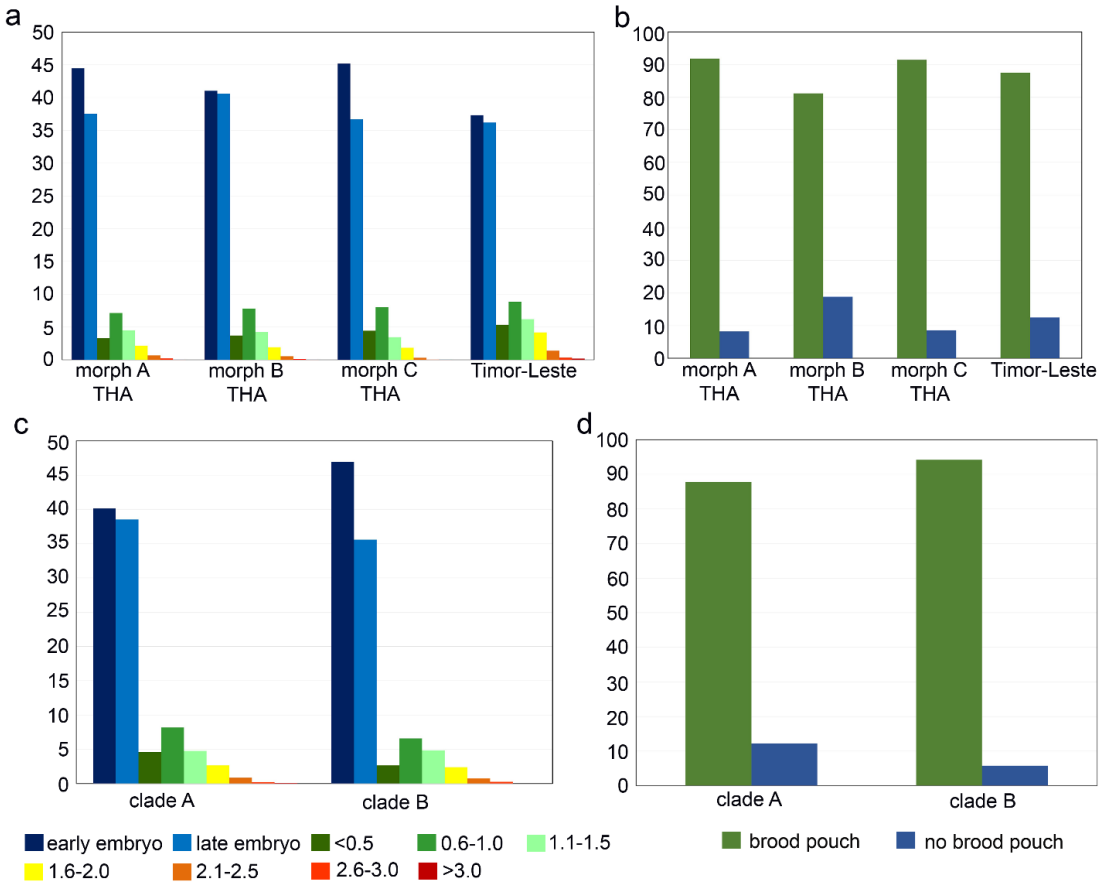

Figure 24**.** Composition of contents of the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (a, c) and proportions of gravid animals. i.e. those with filled brood pouch, versus non-gravid specimens (b, d) from Thailand and Timor-Leste.

a. Composition of contents of the brood pouches for morph A, B and C from Thailand (THA) and specimens from Timor-Leste. b. Proportion of gravid vs. non-gravid specimens for morph A, B and C from Thailand and specimens from Timor-Leste. c. Composition of contents of the brood pouches for mitochondrial clades A and B, respectively. d. Proportion of gravid vs. non-gravid specimens for mitochondrial clades A and B, respectively. For color coding, see the inset legends.

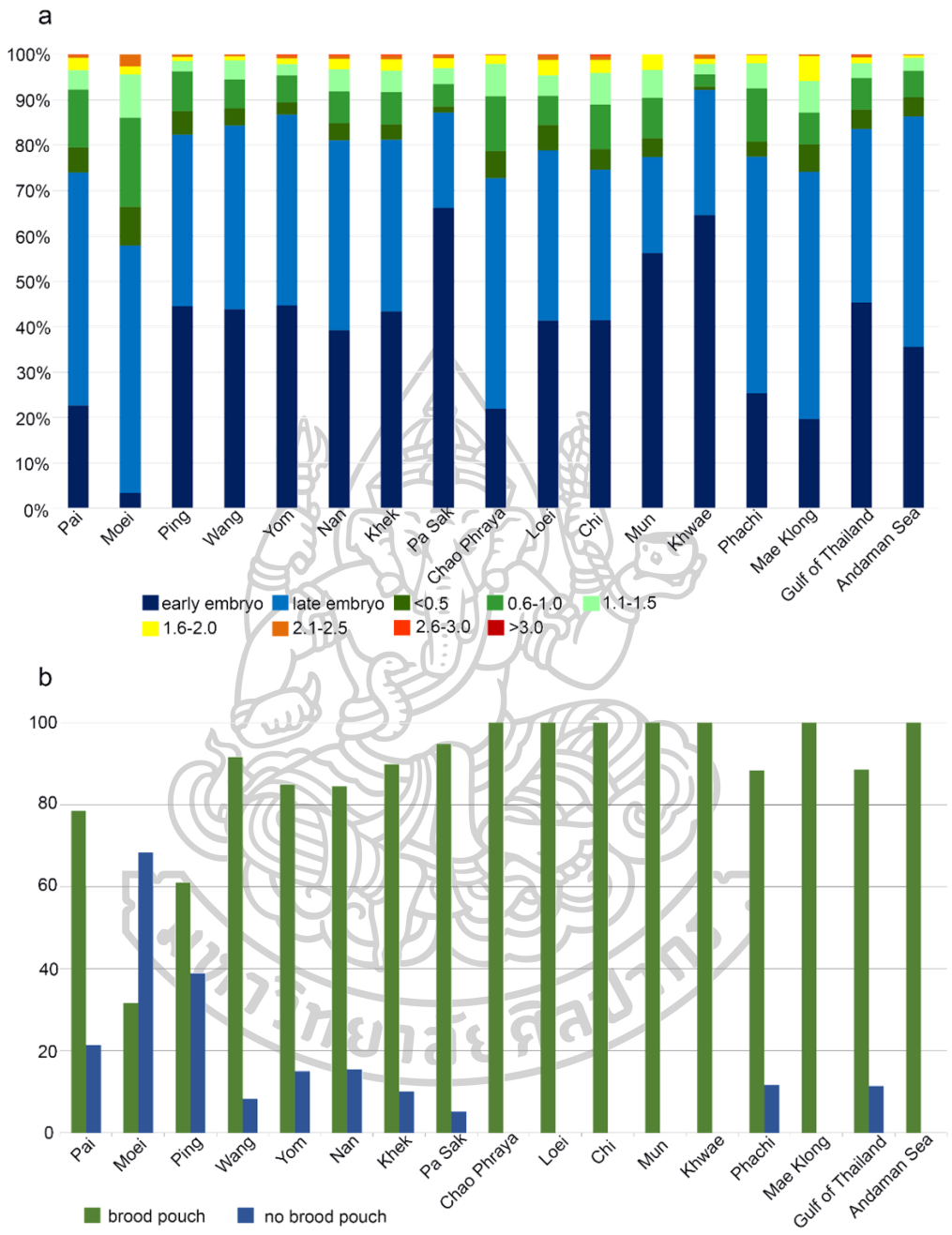

Figure 25**.** Composition of contents of the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (a) and proportions of gravid animals (b). i.e. those with brood pouch containing juveniles or other stages, and non-gravid specimens (b) from Thailand grouped according to rivers. For color coding, see the inset legends.

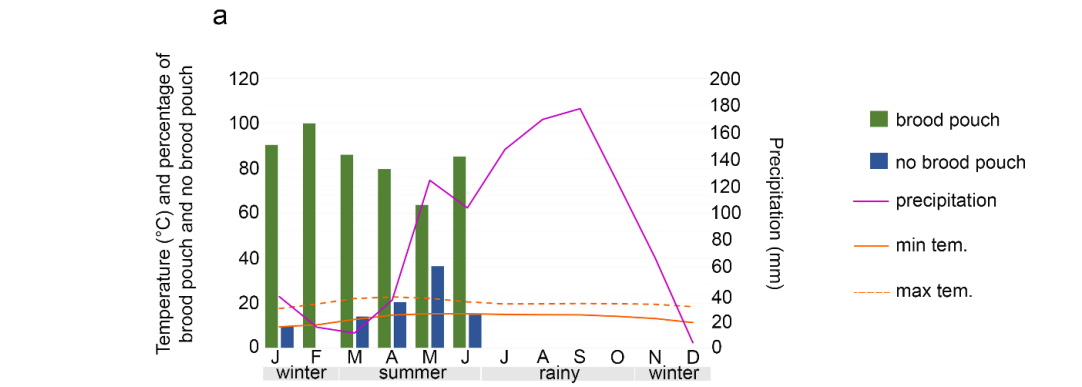

Figure 26**.** Proportions of gravid vs. non-gravid specimens of *Tarebia granifera* (Lamarck, 1816) collected in different months within a given year, plotted on climate charts for localities that are representative for different climatic regimes in Thailand. (a) Chiang Mai for inland locations; (b) Ko Samui for the Gulf of Thailand; (c) Phuket for the Andaman Sea. For color coding, see the inset legend.

Part II: The larval stages of pathogen digenic trematodes in their thiarid snail host *Tarebia granifera* in Thailand.

Geographical origin of collected snails.

Specimens of *Tarebia granifera* were found at 90 sampling sites in five regions of Thailand. The infected snails were reported from 51 sampling sites. For information on sampling sites including geographic coordinates and the number of infected snails, see Table 5.

Occurrence of trematodes obtained from *Tarebia granifera* in Thailand.

The various of cercariae distinguished and described in more detail below exhibit a certain geographical pattern within the various water bodies in Thailand. Only two among the fifteen trematode species found in the thiarid snail *T. granifera*, viz. *Loxogenoides bicolor* and *Stictodora tridactyla*, were recorded in the present study from almost all major river systems in Thailand (Fig. 27).

In contrast, several species exhibit a more restricted distribution. For example, *Haplorchis taichui* was only detected in *T. granifera* samples from the Nan River (Chao Phraya river system) and the Loei River (Mekong river system), whereas *Philophthalmus gralli* and gymnocephalous cercaria were only detected in the Phachi River (Mae Klong river system). Echinostome cercaria were only present in the *T. granifera* population from the Khek River (Chao Phraya river system).

Cercariae of *Loxogenes liberum*, *Centrocestus formosanus* and *Maritreminoides obstipus* had again a somewhat wider distribution in Thai *T. granifera* populations, being present in several rivers of the Chao Phraya, Mae Klong and Gulf of Thailand drainages (Fig. 27).

Cercarial diversity and infection rates

A total of 8,493 snails of *T. granifera* were collected and examined for trematode infections. The infection rate was 5.80%. The obtained cercariae were classified into a total of eleven species from seven morphologically distinguishable types representing at least seven distinct trematode families, viz. (i) virgulate xiphidiocercariae (*Loxogenoides bicolor*, and *Loxogenes liberum*), (ii) armatae xiphidiocercariae (*Maritreminoides caridinae* and *Maritreminoides obstipus*), (iii) parapleurophocercous cercariae (*Haplorchis pumilio*, *Haplorchis taichui* and *Stictodora tridactyla*), (iv) pleurophocercous cercariae (*Centrocestus formosanus*), (v) megarulous cercariae (*Philophthalmus gralli*), (vi) echinostome cercariae, and (vii) gymnocephalous cercariae.

The parapleurophocercous cercariae were the dominant cercarial type infecting snails (2.72%), while infections with other cercarial types were found at rates of 2.52%, 0.26%, 0.14%, 0.04%, 0.12%, and 0.01%, respectively (Table 6).

In this study, neither double trematode infections nor triple trematode infections of collected *Tarebia granifera* were found.

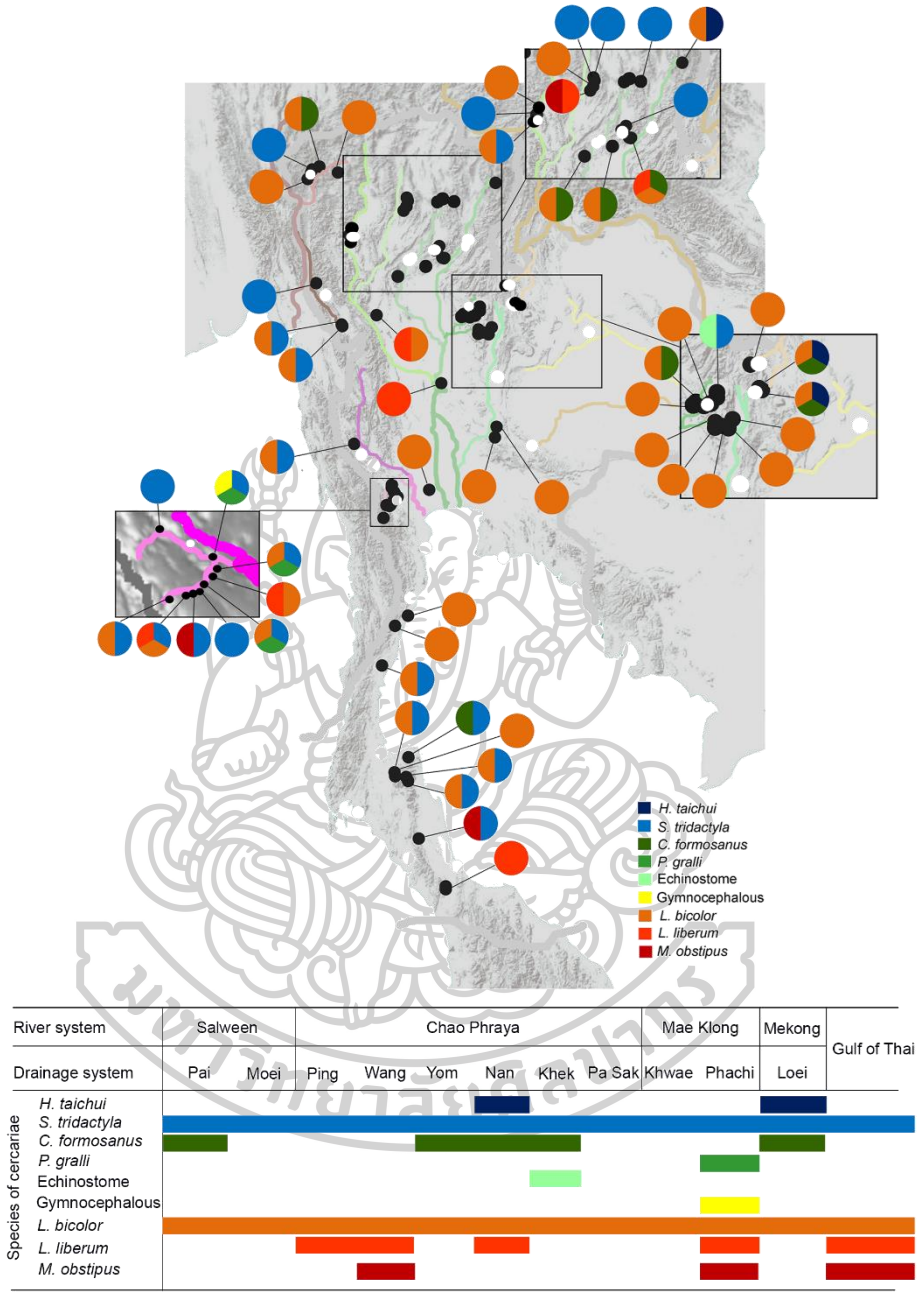

Figure 27. Distribution of *Tarebia granifera* and trematodes in different river systems in Thailand.
a. Distribution map. b. Comparative table of the occurrence of trematode cercariae in different river systems in Thailand. Black dots with attached pie charts in the map represent sampling sites where trematode infected specimens of *T. granifera* were found; white dots represent sampling sites where no infections were observed. Colors in the pie charts and the comparative table refer to trematode species/types.

Table 5. Localities, number of collected snails, number of infected snails and trematodes obtained from collected snails.

No.	Voucher Number	Location	GPS	THE PREVIOUS STUDY (Recorded by PaMaSU) 2004–2009			IN THIS STUDY 2014–2016		
				No. of collected snails	No. of infected snails	Infection rates (%)	No. of collected snails	No. of infected snails	Infection rates (%)
THE NORTH									
N1	SUT 0515083	Huai Pa Hung (Pai drainage, Salween river system), Pang Mapha District, Mae Hong Son Province	19°22’19.6” N 098°26’35.9” E Altitude 437 m	*	*	*	179	1 *L. bicolor* (1)	0.56
N2	SUT 0515081	Huay Nam Kong (Salween river system), Muang District, Mae Hong Son Province	19°28’33.6” N, 098°07’02.4” E Altitude 425 m	*	*	*	24	0	0
N3	SUT 0515077	Tham Pla (Pai drainage, Salween river system), Muang District, Mae Hong Son Province	19°25’31.1” N 097°59’27.2” E Altitude 300 m	185	144 *L. bicolor* (34) *A. hitaense* (25) *H. pumilio* (68) *C. formosanus* (7) *C. alseae* (5) *T. laruei* (5)	77.84	179	8 *L. bicolor* (3) *H. pumilio* (5)	4.47
N4	SUT 0515078	Pai river (Pai drainage, Salween	19°21’54.8” N 097°58’10.7” E	*	*	*	64	1 *S. tridactyla* (1)	1.56

		river system), Muang District, Mae Hong Son Province	Altitude 217 m						
N5	SUT 0515079	Huay Sua Tao (Pai drainage, Salween river system), Muang District, Mae Hong Son Province	19°15’31.6” N 097°54’44.6” E Altitude 237 m	574	98 *L. bicolor* (52) *A. hitaense* (38) *H. pumilio* (5) *T. laruei* (3)	17.07	153	2 *L. bicolor* (2)	1.31
N6	SUT 0514052	Ban Mai Saraphi (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province	18°16’26.1” N 098°38’54.0” E Altitude 277 m	*	*	*	162	11 *L. bicolor* (6) *S. tridactyla* (5)	6.79
N7	SUT 0514051	Ban Mae Suai Luang (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province	18°17’04.4” N 098°39’15.0” E Altitude 268 m	*	*	*	23	2 *S. tridactyla* (2)	8.70
N8	SUT 0514054	Mae Soy bridge (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province	18°17’23.0” N 098°39’3.6” E Altitude 271 m	*	*	*	70	5 *L. bicolor* (5)	7.14
N9	SUT 0514050	Ban Huay Phang (Ping drainage, Chao Phraya river system), Chom Thong District, Chiang Mai Province,	18°17’08.5” N 098°39’16.9” E Altitude 263 m	*	*	*	103	0	0

N10	SUT 0516119	Thansawan waterfall (Yom drainage, Chao Phraya river system), Chiang Muan District, Phayao Province	18°51’22.2” N 100°11’09.1” E Altitude 415 m	219	2 *A. hitaense* (1) *A. mustelae* (1)	0.91	17	1 *S. tridactyla* (1)	5.88
N11	SUT 0516117	Yom river (Yom drainage, Chao Phraya river system), Chiang Muan District, Phayao Province	18°54’39.7” N 100°16’27.7” E Altitude 266 m	*	*	*	30	0	0
N12	SUT 0516108	Mae Nam Saai kg 9 +457 bridge (Yom drainage, Chao Phraya river system), Muang District, Phrae Province	18°05’03.1” N 100°13’00.1” E Altitude 171 m	*	*	*	143	0	0
N13	SUT 0516113	Mae Marn reservoir (Yom drainage, Chao Phraya river system), Sung Men District, Phrae Province	18°00’50.6” N 100°08’22.6” E Altitude 205 m	*	*	*	52	0	0
N14	SUT 0514045	Wang river (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province	18°56’00.5” N 099°38’54.6” E Altitude 376 m	*	*	*	49	12 *S. tridactyla* (12)	24.49
N15	SUT 0514044	Ban Thung Hang stream (Wang	18°52’47.5” N 099°40’01.0” E	*	*	*	165	11 *S. tridactyla* (11)	6.67

		drainage, Chao Phraya river system), Chae Hom District, Lampang Province	Altitude 373 m						
N16	SUT 0514046	Huay MaeYuak (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province	18°46’39.8” N 099°38’38.7” E Altitude 352 m	*	*	*	44	1 *L. bicolor* (1)	2.27
N17	SUT 0516124	km. 40+075 bridge (Wang drainage, Chao Phraya river system), Chae Hom District, Lampang Province	18°42’14.8” N 099°35’31.7” E Altitude 330 m	*	*	*	59	4 *L. liberum* (3) *M. obstipus* (1)	6.78
N18	SUT 0515090	Wa river (Nan drainage, Chao Phraya river system), Bo Kluea District, Nan Province	19°11’30.4” N 101°12’13.2” E Altitude 713 m	*	*	*	159	16 *L. bicolor* (6) *H. taichui* (10)	10.06
N19	SUT 0516114	Huay Si Pun reservoir (Nan drainage, Chao Phraya river system), Ban Luang District, Nan Province	18°51’45.1” N 100°28’37.1” E Altitude 430 m	*	*	*	108	0	0
N20	SUT 0516109	Mae pool waterfall (Nan drainage, Chao Phraya river system), Laplae District,	17°43’42.3” N 099°58’49.6” E Altitude 123 m	137	43 *L. bicolor* (29) *A. hitaense* (5) *H. pumilio* (6)	31.39	91	10 *L. bicolor* (4) *L. liberum* (4) *C. formosanus* (2)	10.99

		Uttaradit Province		*C. formosanus* (3)				
N21	SUT 0516112	Kaeng Sai Ngam (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province	17°52’19.5” N 100°18’02.1” E Altitude 257 m	*	*	*	32	0	0
N22	SUT 0513019	Kaeng Wangwua (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province	17°52’29.5” N 100°18’25.6” E Altitude 231 m	*	*	*	292	4 *S. tridactyla* (4)	1.37
N23	SUT 0513023	Huai Nam Re Noi (Nan drainage, Chao Phraya river system), Tha Pla District, Uttaradit Province	17°52’51.3” N 100°16’14.9” E Altitude 269 m	*	*	*	155	0	0
N24	SUT 0516103	Tat Duen waterfall (Yom drainage, Chao Phraya river system), Si Satchanalai District, Sukhothai Province	17°33’16.2” N 099°29’48.2” E Altitude 135 m	300	141 *L. bicolor* (71) *A. hitaense* (36) *H. pumilio* (8) *C. formosanus* (19) *A. mustelae* (7)	47	137	0	0
N25	SUT 0516102	Si Satchanalai national park (Yom drainage, Chao Phraya river system), Si Satchanalai District,	17°33’07.7” N 099°29’28.8” E Altitude 147 m	749	262 *L. bicolor* (85) *A. hitaense* (35) *H. pumilio* (11) *C. formosanus*	34.98	147	1 *C. formosanus* (1)	0.68

		Sukhothai Province			(116) *A. mustelae* (15)				
N26	SUT 0515075	Cheek point near moei river (Moei drainage, Salween river system), Tha Song Yang District, Tak Province	17°13’23.4” N 098°13’34.2” E Altitude 130 m	*	*	*	55	9 *S. tridactyla* (9)	16.36
N27	SUT 0515076	Mae Salit Luang harbour (Moei drainage, Salween river system), Tha Song Yang District, Tak Province	17°26’04.8” N 098°03’33.3” E Altitude 109 m	*	*	*	25	0	0
N28	SUT 0515073	Ban Wang Takhian (Moei drainage, Salween river system), Mae Sot District, Tak Province	16°42’38.5” N 098°30’22.2” E Altitude 196 m	*	*	*	17	0	0
N29	SUT 0515072	Thong Dee harbour (Moei drainage, Salween river system), Mae Sot District, Tak Province	16°41’39.3” N 098°31’04.4” E Altitude 206 m	*	*	*	304	21 *L. bicolor* (3) *S. tridactyla* (18)	6.91
N30	SUT 0515074	Ban Huay Muang (Moei drainage, Salween river system), Mae Sot District, Tak Province	16°40’58.4” N 098°31’06.9” E Altitude 199 m	*	*	*	300	21 *L. bicolor* (1) *S. tridactyla* (20)	7.00

N31	SUT 0516126	Ban Pak Huay Mae Tho (Ping drainage, Chao Phraya river system), Muang District, Tak Province	16°52’29.3” N 099°07’13.6” E Altitude 106 m	*	*	*	150	3 *L. bicolor* (1) *L. liberum* (2)	2.00
N32	SUT 0516121	Kaeng Wang Nam Yen (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°37’23.8” N 100°54’0.5” E Altitude 710 m	*	*	*	9	8 *L. bicolor* (8)	88.89
N33	SUT 0516120	Rajapruek resort (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°36’01.3” N 100°54’29.9” E Altitude 707 m	*	*	*	52	28 *L. bicolor* (28)	53.85
N34	SUT 0516123	Huai Sa Dao Pong (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°34’24.1” N 100°59’23.6” E Altitude 322 m	*	*	*	31	0	0
N35	SUT 0515088	Kaeng Bang Ra Chan (Khek drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	16°32’51.7” N 100°54’03.2” E Altitude 599 m	*	*	*	71	6 *L. bicolor* (6)	8.45
N36	SUT 0516129	Sam Sip Khot waterfall (Pa Sak	16°32’25.6” N 101°04’58.4” E	*	*	*	47	18 *L. bicolor* (18)	38.30

		drainage, Chao Phraya river system), Khao Kho District, Phetchabun Province	Altitude 386 m						
N37	SUT 0514041	Ban Wang Ta Pak Moo 13 (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’54.2” N 101°14’8.1” E Altitude 120 m	*	*	*	312	0	0
N38	SUT 0514042	Huai Leng (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’52.2” N 101°13’54.4” E Altitude 117 m	*	*	*	84	0	0
N39	SUT 0514040	Ban Wang Tian (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’29.7” N 101°13’30.7” E Altitude 121 m	*	*	*	212	0	0
N40	SUT 0514043	Huay Range reservoir, Ban Wang Ta Pak (Pa Sak drainage, Chao Phraya river system), Wichian Buri District, Phetchabun Province	15°47’19.3” N 101°15’07.4” E Altitude 138 m	*	*	*	128	0	0
N41	SUT 0516130	Than Thip waterfall (Pa Sak drainage,	16°39’46.3” N 101°08’09.8” E	*	*	*	41	16 *L. bicolor* (16)	39.02

		Chao Phraya river system), Lom Sak District, Phetchabun Province	Altitude 374 m						
N42	SUT 0515087	Ban Kaeng Lat (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province	16°57’21.3” N 100°55’31.0” E Altitude 324 m	*	*	*	14	5 *L. bicolor* (5)	35.71
N43	SUT 0516118	Kaeng Sopha (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province	16°52’13.1” N 100°50’17.4” E Altitude 413 m	282	72 *L. bicolor* (33) *A. hitaense* (24) *C. formosanus* (15)	25.53	30	2 *L. bicolor* (2)	6.67
N44	SUT 0515067	Poi waterfall (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province	16°50’36.3” N 100°45’16.1” E Altitude 208 m	*	*	*	83	9 *L. bicolor* (6) *M. caridinae* (1) *H. pumilio* (2)	10.84
N45	SUT 0516105	Phunamkej Resort (Khek drainage, Chao Phraya river system), Wang Thong District, Phitsanulok Province	16°51’02.2” N 100°36’41.1” E Altitude 208 m	*	*	*	73	0	0
N46	SUT 0516111	Kaeng Nangkoi (Khek drainage, Chao Phraya river system), Wang Thong District,	16°53’09.0” N 100°38’47.8” E Altitude 180 m	*	*	*	15	0	0

		Phitsanulok Province							
N47	SUT 0516106	Kaeng Hom (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province	16°52’20.8” N 100°50’46.8” E Altitude 185 m	*	*	*	95	0	0
N48	SUT 0515086	Huai Nam Sai (Khek drainage, Chao Phraya river system), Nakhon Thai District, Phitsanulok Province	17°01’07.6” N 100°55’36.0” E Altitude 217 m	*	*	*	93	38 *S. tridactyla* (28) Echinostome (10)	40.86
THE NORTHEAST									
NE1	SUT 0516128	Tat Kok Tup waterfall (Loei drainage, Mekong river system), Phu Luang District, Loei Province	17°03’03.9” N 101°31’38.7” E Altitude 688 m	*	*	*	45	12 *L. bicolor* (10) *H. taichui* (1) *C. formosanus* (1)	26.67
NE2	SUT 0515068	Pla Ba waterfall (Mekong river system), Phu Ruea District, Loei Province	17°23’24.7” N 101°22’27.3” E Altitude 664 m	53	1 *A. hitaense* (1)	1.89	178	3 *L. bicolor* (3)	1.69
NE3	SUT 0516125	km. 50+350 Loei river (Loei drainage, Mekong river system), Phu Luang District, Loei Province	17°04’38.0” N 101°29’20.6” E Altitude 675 m	*	*	*	55	13 *L. bicolor* (9) *H. taichui* (3) *C. formosanus* (1)	23.64
NE4	SUT 0515064	Bueng Thung Sang (Chi drainage,	16°34’45.6” N 102°50’22.5” E	*	*	*	20	0	0

		Mekong river system), Muang District, Khon Kaen Province	Altitude 160 m						
NE5	SUT 0516131	Lamphraphloeng reservoir (Mun drainage, Mekong river system), Pak Thong Chai District, Nakhon Ratchasima Province	14°35’32.3” N 101°50’30.1” E Altitude 259 m	*	*	*	36	0	0
THE EAST									
E1	SUT 0516135	Mae Rumphueng Beach (Mae Rumphueng canal, Gulf of Thailand), Muang Rayong District, Rayong Province	12°37’50.0” N 101°20’35” E Altitude 8 m	*	*	*	150	0	0
THE CENTRAL									
C1	SUT 0516127	Bung Boraphet (Chao Phraya river system), Muang District, Nakhon Sawan Province	15°40’59.6” N 100°14’59.3” E Altitude 32 m	*	*	*	42	1 *L. liberum* (1)	2.38
C2	SUT 0516133	Dong Phaya Yen waterfall (Pa Sak drainage, Chao Phraya river system), Muak	14°44’06.4” N 101°11’31.4” E Altitude 156 m	371	1 *L. bicolor* (1)	0.27	27	1 *L. bicolor* (1)	3.70

		Lek District, Sara Buri Province							
C3	SUT 0516132	Suanmaduea waterfall (Pa Sak drainage, Chao Phraya river system), Phatthana Nikhom District, Lop Buri Province	14°55’12.3” N 101°13’10.9” E Altitude 136 m	358	5 *L. bicolor* (5)	1.40	48	0	0
C4	SUT 0515055	Pond of Silpakorn University (Tha Chin river system), Muang District, Nakhon Pathom Province	13°49’01.2” N 100°02’27.9” E Altitude 79 m	381	2 *L. bicolor* (2)	0.52	30	0	0
C5	SUT 0515091	Hin dad hot spring (Khwae Noi drainage, Mae Klong river system), Thong Pha Phum District, Kanchanaburi Province	14°37’25.9” N 098°43’40.5” E Altitude 159 m	39	5 *L. bicolor* (1) *H. pumilio* (3) *S. tridactyla* (1)	12.82	2	0	0
C6	SUT 0515092	Sai Yok Yai waterfall (Khwae drainage, Mae Klong river system), Sai Yok District, Kanchanaburi Province	14°26’03.0” N 098°51’14.7” E Altitude 104 m	*	*	*	49	0	0
C7	SUT 0515093	Sai Yok Noi waterfall (Khwae drainage, Mae	14°14’27.6” N 099°03’55.9” E	*	*	*	29	0	0

		Klong river system), Sai Yok District, Kanchanaburi Province	Altitude 116 m						
C8	SUT 0515061	Ban Thung Makham Tia (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province	13°54’18.1” N 099°23’07.8” E Altitude 45 m	*	*	*	42	1 *S. tridactyla* (1)	2.38
C9	SUT 0515060	Ban Ta Pu (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province	13°51’17.7” N 099°22’58.9” E Altitude 56 m	*	*	*	99	0	0
C10	SUT 0515059	Ban Nong Phai (Phachi drainage, Mae Klong river system), Dan Makham Tia District, Kanchanaburi Province	13°46’44.8” N 099°25’26.7” E Altitude 72 m	*	*	*	118	5 *S. tridactyla* (3) *P. gralli* (1) Gymnocephalous (1)	4.24
THE SOUTH									
S1	SUT 0515066	Ban Purakom (Phachi drainage, Mae Klong river system), Suan Phueng District,	13°19’29.2” N 099°14’22.0” E Altitude 277 m	*	*	*	280	30 *L. bicolor* (29) *S. tridactyla* (1)	10.71

		Ratchaburi Province							
S2	SUT 0515069	Huay Nueng (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province	13°32’52.2” N 099°17’33.7” E Altitude 156 m	832	94 *L. bicolor* (30) *S. tridactyla* (64)	11.30	272	23 *L. liberum* (2) *S. tridactyla* (21)	8.46
S3	SUT 0515070	Lum Nam Phachi (Phachi drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province	13°32’54.2” N 099°21’42.3” E Altitude 110 m	*	*	*	242	5 *S. tridactyla* (5)	2.07
S4	SUT 0515057	Ban Dan Thap Tako (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province	13°41’28.1” N 099°29’08.1” E Altitude 82 m	*	*	*	240	11 *L. bicolor* (3) *L. liberum* (8)	4.58
S5	SUT 0515058	Phachi river Bridge (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province	13°45’00.5” N 099°26’27.4” E Altitude 65 m	*	*	*	292	16 *L. bicolor* (1) *M. caridinae* (10) *S. tridactyla* (4) *P. gralli* (1)	5.48
S6	SUT 0515056	Ban Pa Wai (Phachi drainage, Mae Klong river system), Chom Bueng District, Ratchaburi Province	13°37’0.15” N 099°24’36.9” E Altitude 74 m	*	*	*	111	11 *L. bicolor* (3) *M. caridinae* (4) *S. tridactyla* (3) *P. gralli* (1)	9.91
S7	SUT	Huai Ban Bor (Phachi	13°32’07.4” N	*	*	*	196	21	10.71

	0515071	drainage, Mae Klong river system), Suan Phueng District, Ratchaburi Province	099°20’31.8” E Altitude 137 m					*M. obstipus* (1) *S. tridactyla* (20)	
S8	SUT 0513032	Khlong Cha-am (Cha-am canal, Gulf of Thailand), Cha-am District, Phetchaburi Province	12°48’02.7” N 099°58’53.2” E Altitude 22 m	*	*	*	72	0	0
S9	SUT 0516146	Khlong Bueng reservoir (Bueng canal, Gulf of Thailand), Muang District, Prachuap Khiri Khan Province	11°55’29.1” N 099°42’40.9” E Altitude 72 m	*	*	*	92	0	0
S10	SUT 0514037	Khlong Huai Yang (Yang canal), Thap Sakae District, Prachuap Khiri Khan Province	11°36’50.0” N 099°40’07.9” E Altitude 53 m	961	1 *L. bicolor* (1)	0.10	22	0	0
S11	SUT 0514038	Kar on waterfall (Nongyaplong canal), Bang Saphan District, Prachuap Khiri Khan Province	11°26’14.4” N 099°26’33.0” E Altitude 53 m	685	5 *L. bicolor* (5)	0.73	39	0	0
S12	SUT 0516149	Krapo waterfall (Tha Sae canal), Tha Sae District, Chumphon	10°44’28.8” N 099°12’54.9” E Altitude 74 m	223	181 *L. bicolor* (32) *S. tridactyla* (149)	81.17	30	0	0

		Province							
S13	SUT 0516137	Khlong Klai (Nong Noi canal, Ta Pi river system), Ban Na San District, Surat Thani Province	08°48’06.9” N 099°26’45.1” E Altitude 108 m	*	*	*	104	4 *L. bicolor* (4)	3.85
S14	SUT 0514048	Dat Fa waterfall (Lumpool canal, Ta Pi river system), Ban Na San District, Surat Thani Province	08°52’18.8” N 099°25’59.1” E Altitude 79 m	*	*	*	144	2 *L. bicolor* (1) *S. tridactyla* (1)	1.39
S15	SUT 0516142	Vibhavadi waterfall (Tha Thong canal), Don Sak District, Surat Thani Province	09°08’07.2” N 099°40’31.6” E Altitude 26 m	*	*	*	107	24 *S. tridactyla* (17) *C. formosanus* (7)	22.43
S16	SUT 0516147	Khlong Tha Sai (Takhoei canal, Gulf of Thailand), Tha Chang District, Surat Thani Province	09°12’39.8” N 099°11’55.7” E Altitude 8 m	*	*	*	20	0	0
S17	SUT 0516148	Ban Tung Ao (Ta Khoei canal, Gulf of Thailand), Phunphin District, Surat Thani Province	09°12’25.7” N 099°12’25.7” E Altitude 7 m	*	*	*	35	0	0
S18	SUT 0516145	Krung Ching waterfall (Klai canal), Nopphitam District,	08°43’17.3” N 099°40’14.8” E Altitude 195 m	157	12 *L. bicolor* (5) *A. hitaense* (2)	7.64	22	4 *L. bicolor* (4)	18.18

		Nakhon Si Thammarat Province			*S. tridactyla* (5)				
S19	SUT 0516139	Khlong Prong (Klai canal), Nopphitam District, Nakhon Si Thammarat Province	08°47’23.0” N 099°38’13.2” E Altitude 98 m	*	*	*	50	11 *L. bicolor* (1) *S. tridactyla* (10)	22.00
S20	SUT 0515097	Khlong Sai (Khlong Sai canal, Andaman sea), Muang District, Krabi Province	08°10’20.8” N 098°47’37.6” E Altitude 23 m	*	*	*	5	0	0
S21	SUT 0515098	Wang Than Thip (Wang Than Thip canal, Andaman sea), Muang District, Krabi Province	08°09’49.2” N 098°47’50.9” E Altitude 21 m	*	*	*	42	0	0
S22	SUT 0515095	Khlong Palian (Palian canal), Yan Ta Khao District, Trang Province	07°22’11.0” N 099°40’47.9” E Altitude 19 m	77	15 *L. bicolor* (2) *S. tridactyla* (11) *C. alseae* (2)	19.48	15	4 *S. tridactyla* (4)	26.67
S23	SUT 0516138	Khlong Tha Leung (Tha Nae canal), Si Banphot District, Phatthalung Province	07°42’48.3” N 099°51’33.6” E Altitude 70 m	*	*	*	36	14 *M. obstipus* (5) *S. tridactyla* (9)	38.89
S24	SUT 0516141	Khlong La reservoir (Utaphao canal, Gulf of Thailand), Khlong Hoi Khong District, Songkhla Province	06°52’29.3” N 100°19’48.4” E Altitude 60 m	*	*	*	35	0	0

S25	SUT 0516144	Khlong Sathing Mo (Songkhla lake, Gulf of Thailand), Singhanakhon District, Songkhla Province	07°13’36.6” N 100°31’41.8” E Altitude 10 m	*	*	*	3	0	0
S26	SUT 0516143	Khlong Cham Rai reservoir (Utaphao canal), Khlong Hoi Khong District, Songkhla Province	06°49’29.5” N 100°19’49.7” E Altitude 56 m	*	*	*	139	3 *L. liberum* (3)	2.16
TOTAL				**6,583**	**1,084**	**16.47**	**8,493**	**493**	**5.80**

Table 6. Distribution of trematodes obtained from *Tarebia granifera* (A total of 8,493 snails) in Thailand.
(N = North, NE = Northeast, E = East, C = Central, S = South).

Type and species of trematodes	The previous study (Recorded by PaMaSU) 2004-2009 No. infected snails					In this study 2014-2016 No. infected snails					Total	Infection rate (%) (infected snail / no. of the total collected snails = 8,483)
	N	**NE**	**E**	**C**	**S**	**N**	**NE**	**E**	**C**	**S**		
Type 1. Virgulate xiphidiocercariae cercariae												
1. *Loxogenoides bicolor*	304	0	0	9	75	122	22	0	1	46	191	2.25
2. *Loxogenes liberum*	0	0	0	0	0	9	0	0	1	13	23	0.27
3. *Acanthatrium histaense*	164	1	0	0	2	0	0	0	0	0	0	0
Total	**468**	**1**	**0**	**9**	**77**	**131**	**22**	**0**	**2**	**59**	**214**	**2.52**
Type 2. Armatae xiphidiocercariae cercariae												
1. *Maritreminoides caridinae*	0	0	0	0	0	1	0	0	0	14	15	0.18
2. *Maritreminoides obstipus*	0	0	0	0	0	1	0	0	0	6	7	0.08
Total	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**20**	**22**	**0.26**
Type 3. Parapleurophocercous cercariae												
1. *Haplorchis pumilio*	98	0	0	3	0	7	0	0	0	0	7	0.08
2. *Haplorchis taichui*	0	0	0	0	0	10	4	0	0	0	14	0.16
3. *Stictodora tridactyla*	0	0	0	1	229	111	0	0	4	95	210	2.47
Total	**98**	**0**	**0**	**4**	**229**	**128**	**4**	**0**	**4**	**95**	**231**	**2.72**
Type 4. Pleurophocercous cercariae												
1. *Centrocestus formosanus*	160	0	0	0	0	3	2	0	0	7	12	0.14
Total	**160**	**0**	**0**	**0**	**0**	**3**	**2**	**0**	**0**	**7**	**12**	**0.14**
Type 5. Megarulous cercariae												
1. *Philophthalmus gralli*	0	0	0	0	0	0	0	0	1	2	3	0.04

Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**		**1**	**2**	**3**	**0.04**
Type 6. Furcocercous cercariae												
1. *Cardicola alseae*	5	0	0	0	2	0	0	0	0	0	0	0
2. *Alaria mustelae*	23	0	0	0	0	0	0	0	0	0	0	0
3. *Transversotrema laruei*	8	0	0	0	0	0	0	0	0	0	0	0
Total	**36**	**0**	**0**	**0**	**2**	0	0	0	0	0	0	0
Type 7. Echinostome cercariae												
1. Echinostome cercariae	0	0	0	0	0	10	0	0	0	0	10	0.12
Total	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**0**	**0**	**10**	**0.12**
Type 8. Gymnocephalous cercariae												
1. Gymnocephalous cercariae	0	0	0	0	0	0	0	0	1	0	1	0.01
Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0.01**

Morphology of infecting cercariae

The cercariae were categorized by their morphology and organ characters, using as reference previous morphological descriptions (Frandsen & Christensen, 1984; Krailas et al., 2014; Schell, 1970; Yamaguti, 1975). They are described in the following for the eight distinct morphological cercarial types known and found to date, attributable to at least seven distinct trematode families.

Type 1. Virgulate xiphidiocercariae cercariae

Lecithodendriidae Lühe, 1901 (sensu Odhner 1910)

1.1 *Loxogenoides bicolor* (Krull, 1933) (sensu Kaw 1945)
(Fig. 28)
Body oval; throughout with granules. Oral sucker bigger than ventral sucker; globular in shape and with stylet. Virgulate organ in the anterior part of the body. Pharynx small; an esophagus was not observed. Three pairs of penetration glands present located at about two thirds of the body, two anterior pairs with fine granules and a posterior pair with rather coarse, dark granules. Genital primordial C-shaped; excretory bladder U-shaped. Tail shorter than body; spinose at its tip.
The cercariae develop within sporocysts.

The infection rate was 2.25% (191/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	53–88 μm (mean: 72 μm) × 105–138 μm (mean: 117 μm)
Stylet	5–8 μm (mean: 6 μm) × 20–40 μm (mean: 30 μm)
Oral sucker	23–40 μm (mean: 33 μm) × 23–33 μm (mean: 29 μm)
Pharynx	8–12 μm (mean: 10 μm) × 5–8 μm (mean: 8 μm)
Ventral sucker	13–25 μm (mean: 18 μm) × 8–20 μm (mean: 16 μm)
Excretory bladder	18–55μm (mean: 33 μm) × 10–35 μm (mean: 20 μm)
Tail	10–28 μm (mean: 21 μm) × 25–88 μm (mean: 44 μm)

1.2 *Loxogenes liberum* Seno, 1907
(Fig. 29)
Body oval. Oral sucker at the anterior end of body, with stylet. Virgulate organ present. Ventral sucker roundish, smaller than oral sucker. Pharynx very small, a prepharynx, an esophagus and ceca were not observed. Four pairs of penetration glands present, located near the middle of the body; the two anterior pairs with fine granules and the two posterior pairs with coarse granules. Excretory bladder V-shaped. Tail shorter than body, rather slender and spinose at its tip.
The cercariae develop within sporocysts.

The infection rate was 0.27% (23/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	65–93 μm (mean: 81 μm) × 95–120 μm (mean: 108 μm)
Stylet	3–3 μm (mean: 3 μm) × 10–23 μm (mean: 16 μm)
Oral sucker	13–30 μm (mean: 24 μm) × 10–28 μm (mean: 20 μm)
Pharynx	5–15 μm (mean: 10 μm) × 8–10 μm (mean: 8 μm)
Ventral sucker	8–33 μm (mean: 18 μm) × 13–28 μm (mean: 19 μm)
Excretory bladder	13–35 μm (mean: 27 μm) × 13–48 μm (mean: 37 μm)
Tail	15–25 μm (mean: 20 μm) × 40–90 μm (mean: 72 μm)

1.3 *Acanthatrium histaense* Koga, 1953
(Fig. 30)
Body oval. Oral sucker with stylet, virgulate organ near oral sucker. Pharynx round and short, esophagus absent. Ventral sucker smaller than oral sucker. Two pairs of penetration glands present, one anterior pair with fine granules and one posterior pair with coarse granules. Excretory bladder near posterior end of body. Tail short, spinose at its end.
The cercariae develop within sporocysts.

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	54–93 μm (mean: 78 μm) × 80–110 μm (mean: 100 μm)
Stylet	9–14 μm (mean: 11 μm) × 12–14 μm (mean: 12 μm)
Oral sucker	26–33 μm (mean: 31 μm) × 35–41 μm (mean: 38 μm)
Pharynx	11–16 μm (mean: 14 μm) × 13–25 μm (mean: 21 μm)
Ventral sucker	15–17 μm (mean: 17 μm) × 16–19 μm (mean: 18 μm)
Excretory bladder	9–13 μm (mean: 10 μm) × 21–47 μm (mean: 39 μm)
Tail	18–26 μm (mean: 24 μm) × 27–76 μm (mean: 69 μm)

Type 2. Armatae xiphidiocercariae cercariae

Microphallidae Ward, 1901 (sensu Travassos 1921)

2.1 *Maritreminoides caridinae* (Yamaguti & Nisimura, 1944) (sensu Chen 1957)
(Fig. 31)
Body oval, rather small. Stylet present, but virgulate organ absent. Pharynx small, esophagus Y-shaped. Ventral sucker poorly developed. Two pairs penetration glands present, located near the middle of the body. Excretory bladder thin-walled, located in the posterior part of the body. Tail long and round.
The cercariae develop within sporocysts.

The infection rate was 0.18% (15/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	78–98 μm (mean: 89 μm) × 105–133 μm (mean: 113 μm)
Stylet	3–3 μm (mean: 3 μm) × 10–18 μm (mean: 15 μm)
Oral sucker	18–30 μm (mean: 25 μm) × 20–30 μm (mean: 23 μm)
Pharynx	5–10 μm (mean: 8 μm) × 5–10 μm (mean: 9 μm)
Ventral sucker	15–20 μm (mean: 19 μm) × 15–20 μm (mean: 18 μm)
Excretory bladder	30–40 μm (mean: 34 μm) × 15–18 μm (mean: 16 μm)
Tail	13–20 μm (mean: 16 μm) × 85–125 μm (mean: 106 μm)

2.2 *Maritreminoides obstipus* (Van Cleave & Mueller, 1932) (sensu Rankin 1939)
(Fig. 32)

Body oval, rather small. Oral and ventral sucker of approximately equal size. Oral sucker with long stylet, virgulate organ absent. Pharynx rather large, esophagus short and slender, bifurcating, located between oral and ventral sucker. Genital primordium located just posterior of ventral sucker. Four pairs of penetration glands grouped together near anterior margin of ventral sucker. Excretory bladder thin-walled. Tail shorter than body and round, not spinose at its tip.

The cercariae develop within sporocysts.

The infection rate was 0.08% (7/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	73–103 μm (mean: 89 μm) × 85–128 μm (mean: 106 μm)
Stylet	3–3 μm (mean: 3 μm) × 13–18 μm (mean: 16 μm)
Oral sucker	20–30 μm (mean: 25 μm) × 13–30 μm (mean: 24 μm)
Pharynx	8–13 μm (mean: 9 μm) × 5–13 μm (mean: 9 μm)
Ventral sucker	13–20 μm (mean: 16 μm) × 10–20 μm (mean: 15 μm)
Excretory bladder	18–35 μm (mean: 28 μm) × 13–23 μm (mean: 16 μm)
Tail	15–28 μm (mean: 20 μm) × 65–113 μm (mean: 82 μm)

Type 3. Parapleurophocercous cercariae

Heterophyidae (Leiper, 1909) (sensu Odhner 1914)

3.1 *Haplorchis pumilio* (Looss, 1896) (sensu Looss 1899)
(Fig. 33)

The cercarial body is pear-shaped. It has a circular oral sucker that is located near the proximal end of the body. The mouth is equipped with transverse rows of spines. The small ventral sucker is located approximately at two-thirds of the body length measured from the front. The small pharynx is situated in the anterior part of the body

just distal of the oral sucker between the two distinct eyespots; an esophagus is absent. There are seven pairs of penetration glands, which are arranged laterally in two longitudinal rows in the posterior two thirds of the body. The excretory bladder has an oval shape and is dark pigmented. A genital primordium is present, located between the ventral sucker and the excretory bladder. The tail is longer than the body and rather slender, and is equipped with lateral finfolds proximally and a dorsoventral finfold along the longer distal portion.

The cercariae develop within rediae.

The infection rate was 0.08% (7/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	91–141 μm (mean: 125 μm) × 169–296 μm (mean: 258 μm)
Oral sucker	28–49 μm (mean: 37 μm) × 28–49 μm (mean: 36 μm)
Pharynx	9–11 μm (mean: 10 μm) × 13–20 μm (mean: 16 μm)
Ventral sucker	15–25 μm (mean: 19 μm) × 15–24 μm (mean: 18 μm)
Excretory bladder	29–41 μm (mean: 35 μm) × 29–41 μm (mean: 35 μm)
Tail	11–37 μm (mean: 31 μm) × 466–529 μm (mean: 491 μm)
Lateral finfolds	9–18 μm (mean: 14.75 μm) × 70–129 μm (mean: 111 μm)

3.2 *Haplorchis taichui* (Nishigori, 1924) (sensu Witenberg 1930)
(Fig. 34)
Body is oval shape. Oral sucker is located at the anterior of body. Mouth aperture has transverse rows of spines. A pair of pigment eyespots and pharynx are presented. Seven pairs of penetration glands extend from the pharynx to posterior end of the body. Cystogenous cell is arranged in lateral fields from level of pharynx to posterior end of the body. Excretory bladder has saccular and thick-wall. Tail is longer than the body. There are lateral finfolds at one-third of tail tunk and a dorso-ventral finfolds widen distal portion.
The cercariae develop within rediae.

The infection rate was 0.16% (14/8,493) (Table 6)

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	43–83 μm (mean: 61 μm) × 105–140 μm (mean: 120 μm)
Oral sucker	20–30 μm (mean: 25 μm) × 23–35 μm (mean: 28 μm)
Ventral sucker	15–33 μm (mean: 23 μm) × 18–30 μm (mean: 25 μm)
Pharynx	8–20 μm (mean: 14 μm) × 8–25 μm (mean: 12 μm)
Excretory bladder	10–50 μm (mean: 26 μm) × 20–35 μm (mean: 26 μm)
Tail	20–30 μm (mean: 26 μm) × 263–355 μm (mean: 311 μm)
Lateral finfolds	8–15 μm (mean: 13 μm) × 75–125 μm (mean: 103 μm)
Dorsal finfolds	5–23 μm (mean: 13 μm) × 183–253 μm (mean: 218 μm)

3.3 *Stictodora tridactyla* Martin & Kuntz, 1955
(Fig. 35)
Body is oval shape. Oral sucker is located at the anterior of body. There are three transverse row of oral spines. Seven pairs of penetration glands in four groups of 3:4:4:3 that are situated between pharynx and excretory bladder. A pairs of pigment eyespots and pharynx are presented. Ventral sucker was poorly developed. Excretory bladder is V-shape and thick-wall. Tail is longer than the body. There are dorsal-ventral finfold with a bilaterial finfold and a dorso-ventral finfold.
The cercariae develop within rediae.

The infection rate was 2.47% (210/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	80–118 μm (mean: 99 μm) × 168–207 μm (mean: 202 μm)
Oral sucker	28–38 μm (mean: 34 μm) × 30–50 μm (mean: 41 μm)
Eye spots	5–15 μm (mean: 9 μm) × 5–15 μm (mean: 9 μm)
Pharynx	10–22 μm (mean: 17 μm) × 10–28 μm (mean: 19 μm)
Ventral sucker	13–35 μm (mean: 23 μm) × 15–45 μm (mean: 27 μm)
Excretory bladder	43–90 μm (mean: 64 μm) × 20–55 μm (mean: 39 μm)
Tail	20–33 μm (mean: 26 μm) × 405–495 μm (mean: 458 μm)
Lateral finfold	10–25 μm (mean: 18 μm) × 74–148 μm (mean: 108)

Type 4. Pleurophocercous cercariae

Heterophyidae (Leiper, 1909) (sensu Odhner 1914)

4.1 *Centrocestus formosanus* (Nishigori, 1924) (sensu Price 1932)
(Fig. 36)
Body is oval shape. Oral sucker has oral spines or rostellar hooks like tapeworm on the dorsal wall of the mouth aperture. A pair of eyespots are located above prenetration glands the same level as the pharynx. There are seven pairs of penetration glands. The genital primordial is elongated-triangular and located between the ventral sucker and the excretory bladder. The excretory bladder has dark granules and thin-wall. The tail is slender and longer than body. It is equipped with very narrow finfolds.
The cercariae develop within rediae.

The infection rate was 0.14% (12/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	45–73 μm (mean: 65 μm) × 83–121 μm (mean: 118 μm)
Oral sucker	17–27 μm (mean: 25 μm) × 18–30 μm (mean: 26 μm)
Pharynx	8–10 μm (mean: 9 μm) × 9–11 μm (mean: 10 μm)

Ventral sucker	13–17 μm (mean: 15 μm) × 14–18 μm (mean: 16 μm)
Excretory bladder	25–31 μm (mean: 29 μm) × 39–53 μm (mean: 46 μm)
Tail	15–18 μm (mean: 15 μm) × 70–93 μm (mean: 83 μm)

Type 5. Megarulous cercariae

Philophthalmidae (Looss, 1899) (sensu Travassos 1918)

5.1 *Philophthalmus gralli* Mathis & Léger, 1910
(Fig. 37)
Body is elongate pear-shaped and distinctly granulose. Eyespots are absent. The pharynx is large and extends into an esophagus that is bifurcating (Y-shape) into two blind ending intestinal caeca that almost reach the posterior end of the body. The ventral sucker is bigger than the oral sucker. The excretory bladder is rather small. The tail is about as long as the body and relatively slender. There is an adhesive gland present at its tip.
The cercariae encyst rapidly after developing within rediae.

The infection rate was 0.04% (3/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	143–175 μm (mean: 153 μm) × 438–470 μm (mean: 453 μm)
Oral sucker	50–68 μm (mean: 60 μm) × 63–73 μm (mean: 68 μm)
Pharynx	15–23 μm (mean: 20 μm) × 28–38 μm (mean: 34 μm)
Ventral sucker	60–78 μm (mean: 67 μm) × 48–80 μm (mean: 6 μm)
Excretory bladder	43–48 μm (mean: 45 μm) × 33–40 μm (mean: 36 μm)
Tail	40–50 μm (mean: 45 μm) × 463–475 μm (mean: 469 μm)

Type 6. Furcocercous cercariae

Sanguinicolidae Graff, 1907

6.1 *Cardicola alseae* Meade & Pratt, 1965
(Fig. 38)
Body is elongate-oval, slightly bent. Eyespots, a pharynx, an esophagus, intestinal caeca and a ventral sucker are absent. There is narrow dorsal fanfold in the middle part of the body. The penetration gland is located in the anterior part of the body. The excretory bladder is small and thin-walled, located at the posterior end of the body. The tail is forked. The stem of the tail is rather thick and longer than the furcae. Finfolds are present along the margins of the furcae.
The cercariae develop within sporocysts.

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	19–40 μm (mean: 30 μm) × 73–112 μm (mean: 96 μm)
Anterior organ	12–16 μm (mean: 14 μm) × 15–22 μm (mean: 19 μm)
Excretory bladder	4–8 μm (mean: 6 μm) × 12–37 μm (mean: 23 μm)
Tail stem	16–32 μm (mean: 28 μm) × 155–199 μm (mean: 187 μm)
Tail furcal	8–12 μm (mean: 10 μm) × 29–56 μm (mean: 52 μm)
Dorso-median finfold	6–15 μm (mean: 11 μm)

Diplostomidae Poirier, 1886

6.2 *Alaria mustelae* Bosma, 1931
(Fig. 39)
Body is elongate-oval in shape. A pairs of eyespots are unpigmented. Prepharynx is presented but rather short. Pharynx is small and roundish in shape. The esophagus is long, bifurcating into two intestinal caeca that are shorter than half the length of the esophagus. The oral sucker is larger than the ventral sucker. There are two pairs of penetration glands, filled with dark granules that are located around ventral sucker. There is a Y-shaped excretory bladder located medially close to the posterior end of the body. The tail is longer than the body and divided into two furcae. The tail stem is slender and about as long as the furcae.
The cercariae develop within sporocysts.

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	106–155 μm (mean: 139 μm) × 186–282 μm (mean: 257 μm)
Oral sucker	29–41 μm (mean: 37 μm) × 29–42 μm (mean: 38 μm)
Pharynx	12–16 μm (mean: 14 μm) × 15–20 μm (mean: 17 μm)
Ventral sucker	16–38 μm (mean: 26 μm) × 16–32 μm (mean: 23 μm)
Tail	49–62 μm (mean: 57 μm) × 221–311 μm (mean: 275 μm)
Fork-tail	40–65 μm (mean: 61 μm) × 241–321 μm (mean: 286 μm)

Transversotrematidae Yamaguti, 1954

6.3 *Transversotrema laruei* Velasquez, 1958
(Fig. 40)
Body is a bowl-liked shape. The surface of the body is covered with spines that have the appearance of fish scales. The genital pore of the seminal vesicle is located in the anterior part of the body. Eyespots are present. The mouth is located near the ventral sucker. The esophagus is narrow and the intestinal caeca form a ring. There is one pair of testes present, and an ovary is located anterolateral to the left of the testes. The excretory bladder is small and short, and is situated close to the posterior end of the body. The tail is longer than the body and possesses spatulate furcae. At the base of the tail a pair of bilaterally symmetrical appendages is present, each equipped with an adhesive pad at its distal end.
The cercariae develop within rediae.

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	460–600 µm (mean: 533 µm) × 280–430 µm (mean: 362 µm)
Genital pore	20–40 µm (mean: 31 µm) × 20–50 µm (mean: 34 µm)
Ventral sucker	50–110 µm (mean: 76 µm) × 50–120 µm (mean: 77 µm)
Testis	30–120 µm (mean: 88 µm) × 40–120 µm (mean: 85 µm)
Excretory bladder	20–70 µm (mean: 40 µm) × 40–90 µm (mean: 57 µm)
Tail	120–180 µm (mean: 146 µm) × 620–800 µm (mean: 686 µm)
Tail stem	120–180 µm (mean: 146 µm) × 390–530 µm (mean: 467 µm)
Tail furcal	80–150 µm (mean: 111 µm) × 180–290 µm (mean: 219 µm)
Appendages	40–70 µm (mean: 58 µm) × 120–150 µm (mean: 138 µm)

Type 7. Echinostome cercariae
(Fig. 41)
Body is elongate pear-shaped. Eyespots are absent. Oral sucker is circular in shape and is equipped with collar spines. Prepharynx is long. Esophagus is shorter than the prepharynx, bifurcating into two intestinal caeca that almost reach to the posterior end of the body. The relatively large ventral sucker is located approximately at two-thirds of the body length measured from the front. Penetration glands are absent. The excretory bladder is small and triangular in shape, its two main collecting tube beginning at the level of the esophagus. The tail is slender and almost of the same length as the body.
The cercariae develop within rediae.

The infection rate was 0.012% (10/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	150–163 µm (mean: 151 µm) × 243–325 µm (mean: 270 µm)
Oral sucker	38–48 µm (mean: 44 µm) × 38–48 µm (mean: 44 µm)
Ventral sucker	40–73 µm (mean: 62 µm) × 55–63 µm (mean: 60 µm)
Pharynx	13–18 µm (mean: 14 µm) × 20–30 µm (mean: 24 µm)
Excretory bladder	18–55 µm (mean: 38 µm) × 18–55 µm (mean: 33 µm)
Tail	28–40 µm (mean: 34 µm) × 195–313 µm (mean: 240 µm)

Type 8. Gymnocephalous cercariae
(Fig 42)
Body is oval and covered with spines. The terminal oral sucker is equipped with minute spines. Eyespots are absent. The pre-pharynx is long and thin. The pharynx is rather large and of a round shape. The esophagus is short but rather wide, bifurcating into two intestinal caeca that extend towards the posterior part of the body. There are 4–5 penetration glands present that are located laterally of the caeca between the level of the pharynx and the ventral sucker. The ventral sucker is of about the same size as the oral sucker. The excretory bladder is roundish, with a thin wall and located medially near the posterior end of the body. Two thin, undulating excretory tubules that begin just anterior of the pharynx insert into the excretory bladder. The tail is

longer than the body, with the opening duct of the excretory bladder located at its end. The groups of 3–5 distinct pigment granules present in the tail but flame cells cannot observed.
The cercariae develop within rediae.

The infection rate was 0.01% (1/8,493) (Table 6).

Size range and average size (in micrometers, calculated from 10 cercariae):

Body	115–160 μm (mean: 134 μm) × 150–195 μm (mean: 176 μm)
Oral sucker	30–40 μm (mean: 33 μm) × 28–40 μm (mean: 36 μm)
Pharynx	8–20 μm (mean: 13 μm) × 13–28 μm (mean: 22 μm)
Ventral sucker	35–48 μm (mean: 41 μm) × 33–45 μm (mean: 41 μm)
Excretory bladder	28–45 μm (mean: 39 μm) × 25–43 μm (mean: 31 μm)
Tail	23–35 μm (mean: 27 μm) × 183–223 μm (mean: 199 μm)

Molecular analysis

In the present study, ITS2 sequences from seven distinct cercarial types of a total of eleven trematode species found in Thai populations of *Tarebia granifera* could be amplified by PCR and sequenced. The ITS2 sequences of the virgulate xiphidiocercariae and the armatae xiphidiocercariae had a length of approximately 320 bp, while the ITS2 sequences of the parapleurophocercous cercariae and the pleurophocercous cercariae had a length of approximately 380 bp. The ITS2 sequences of the remaining cercarial types, i.e. megarulous cercariae, echinostome cercariae and gymnocephalous cercariae, had a length of approximately 500 bp.

The phylogenetic tree obtained from the neighbor-joining analysis (Fig. 43) was rooted with *Angiostrongylus cantanensis* (Chen, 1935) (GenBank accession number: HQ540551.1, Table 7). All trematode species from Thai populations of *T. granifera* that were distinguished on the basis of cercarial morphology and for which more than one sequence was obtained, formed well supported groups in the phylogenetic analysis. These are highlighted in the following:

- Specimens of *S. tridactyla*, *C. formosanus*, *Centrocestus* sp., *H. taichui*, *O. viverrini*, *O. felineus* (Rivolta, 1884) and *H. pumilio*, which all have cyprinoid fish as a second intermediate host, were grouped together with relatively high support.
- The sequences of the echinostome cercaria and the gymnocephalous cercaria obtained from *T. granifera* were grouped together with relatively high support.
- This latter clade in turn formed a well-supported clade together with *P. gralli* and *Fasciola hepatica* Linnaeus, 1758 and *Fasciola gigantica* Cobbold, 1856 (for which we obtained data from previously published sequences).
- A group of species with arthropods as second intermediate hosts, i.e. *L. bicolor*, *L. liberum*, *Lecithodendrium spathulatum* (Ozaki, 1929), *Lecithodendrium linstowi* Dollfus, 1931 and *M. obstipus*, formed a moderately supported group in the phylogenetic analysis. The relationships of species within this clade, however, could not be resolved robustly.

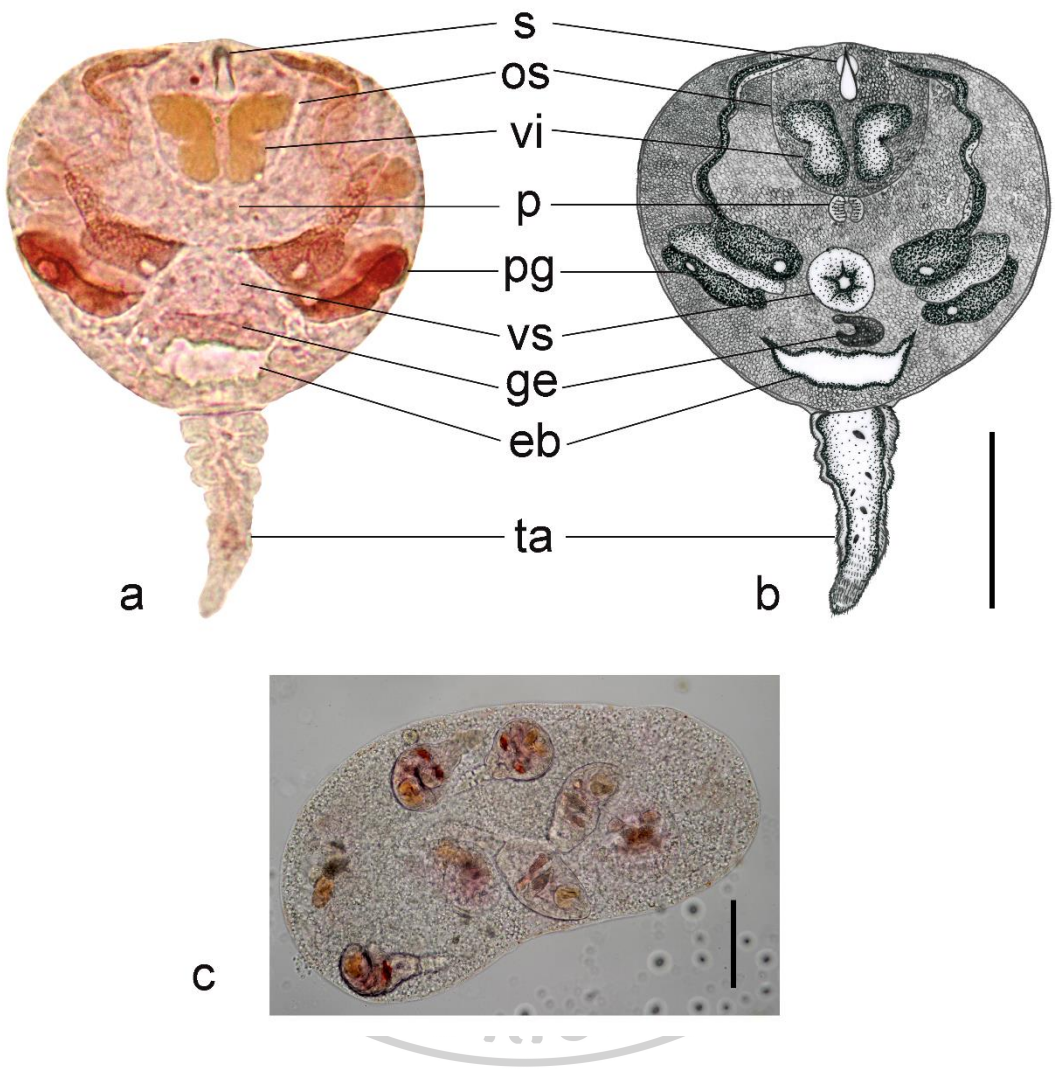

Figure 28. Images of *Loxogenoides bicolor* (Krull, 1933).
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ge: genital primordium; p: pharynx; pg: penetration gland; os: oral sucker; s: stylet; ta: tail; vi: virgulate organ; vs: ventral sucker. scale bars: 50 µm.

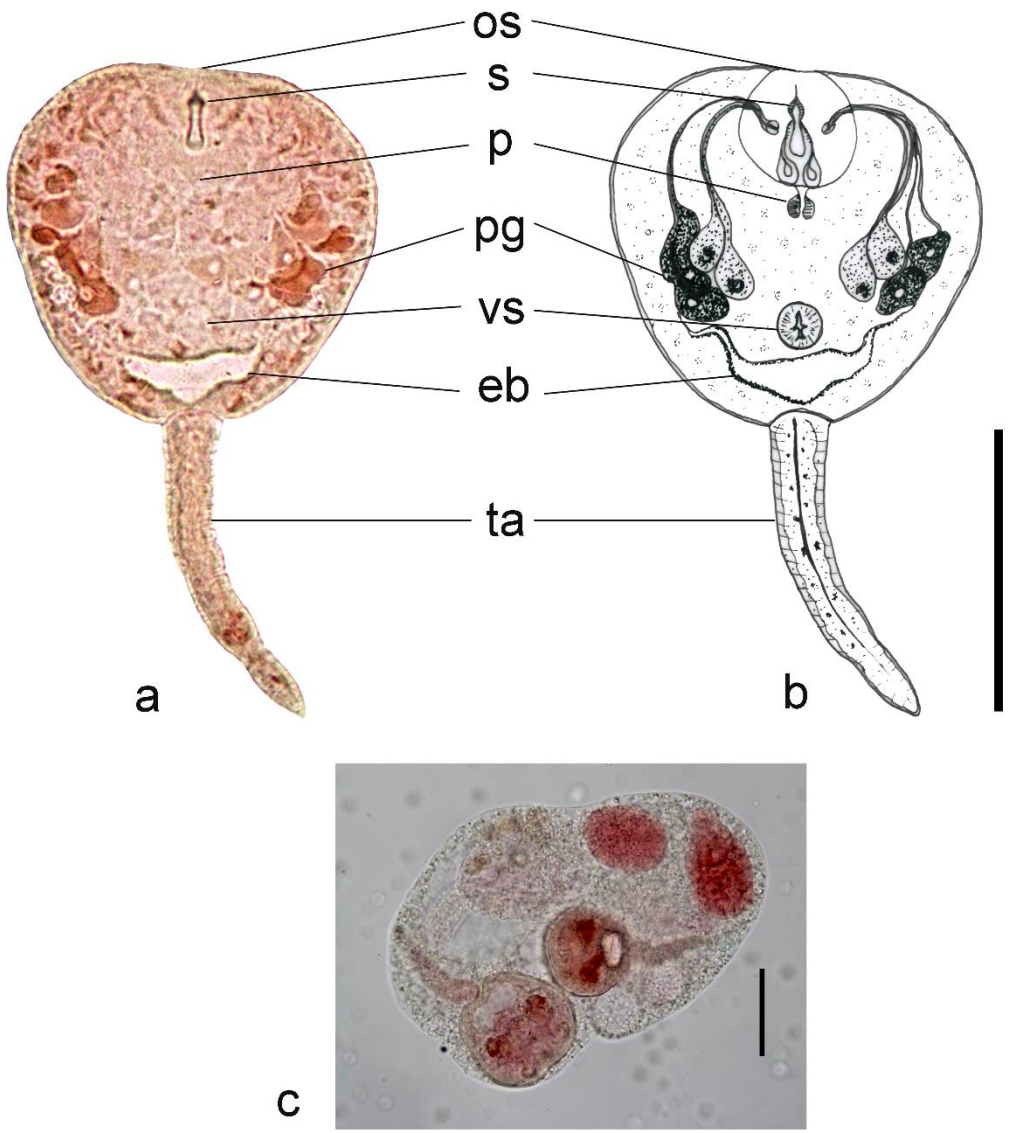

Figure 29. Images of *Loxogenes liberum* Seno, 1907.
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; os: oral sucker, p: pharynx, pg: penetration gland, s: stylet; ta: tail; vs: ventral sucker. scale bars: 50 µm.

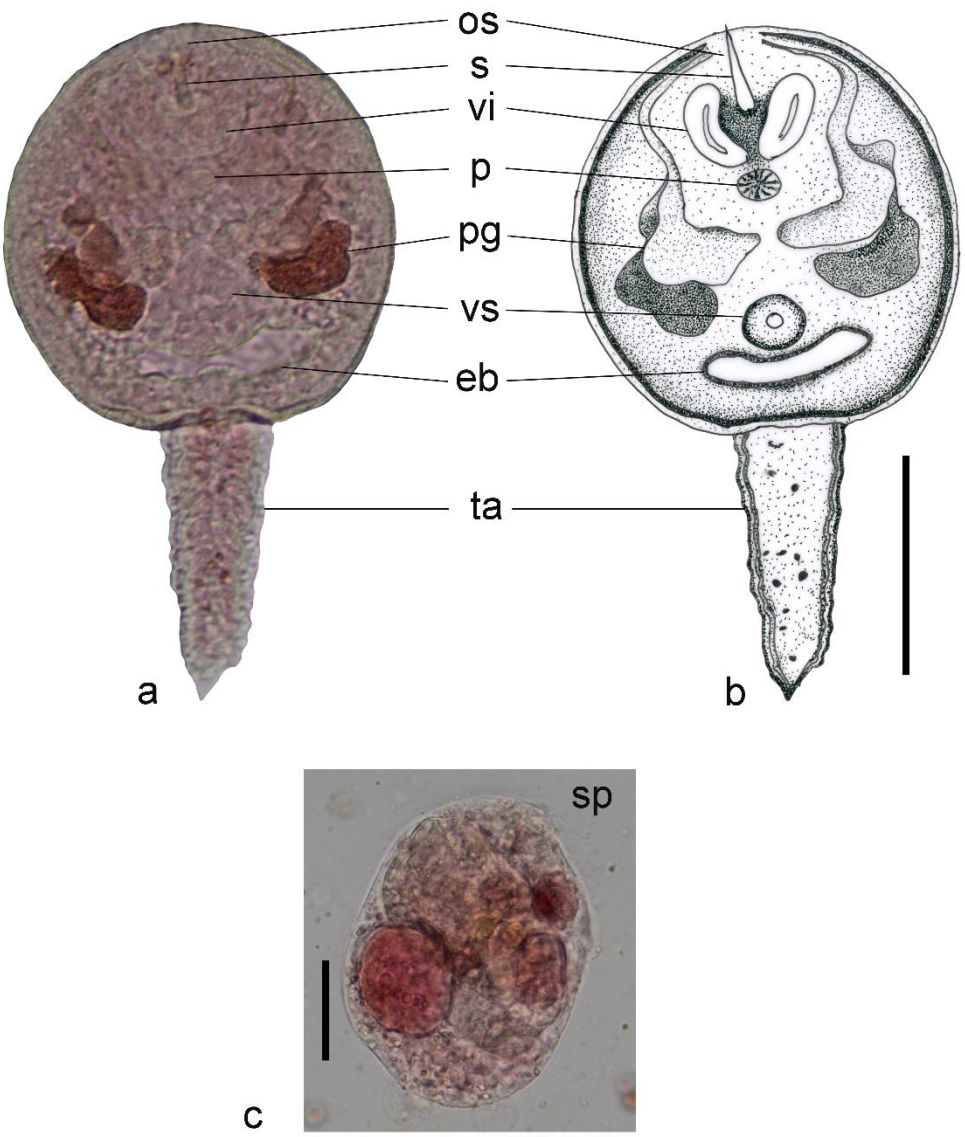

Figure 30. Images of *Acanthatrium histaense* Koga, 1953.
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; os: oral sucker; s: stylet; p: pharynx; pg: penetration gland; ta: tail; vi: virgulate organ; vs: ventral sucker. scale bars: 50 µm.

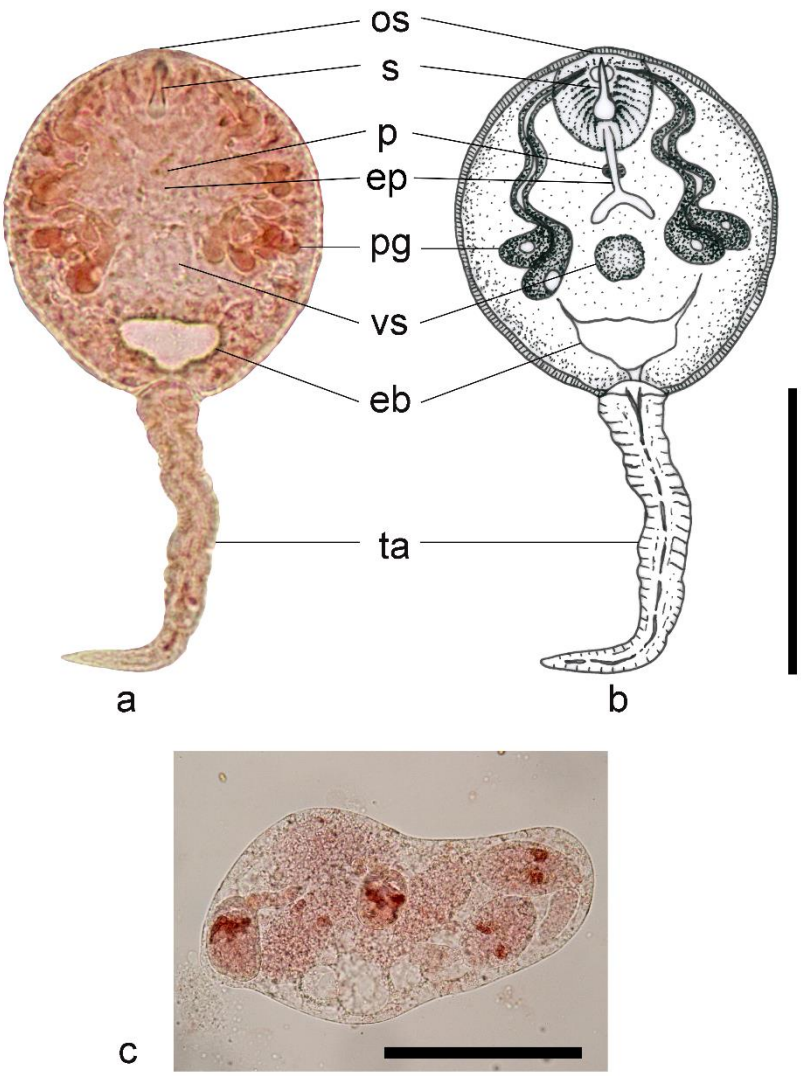

Figure 31. Images of *Maritreminoides caridinae* (Yamaguti & Nisimura, 1944).
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; os: oral sucker; p: pharynx; pg: penetration gland; s: stylet; ta: tail; vs: ventral sucker. scale bars: 50 µm.

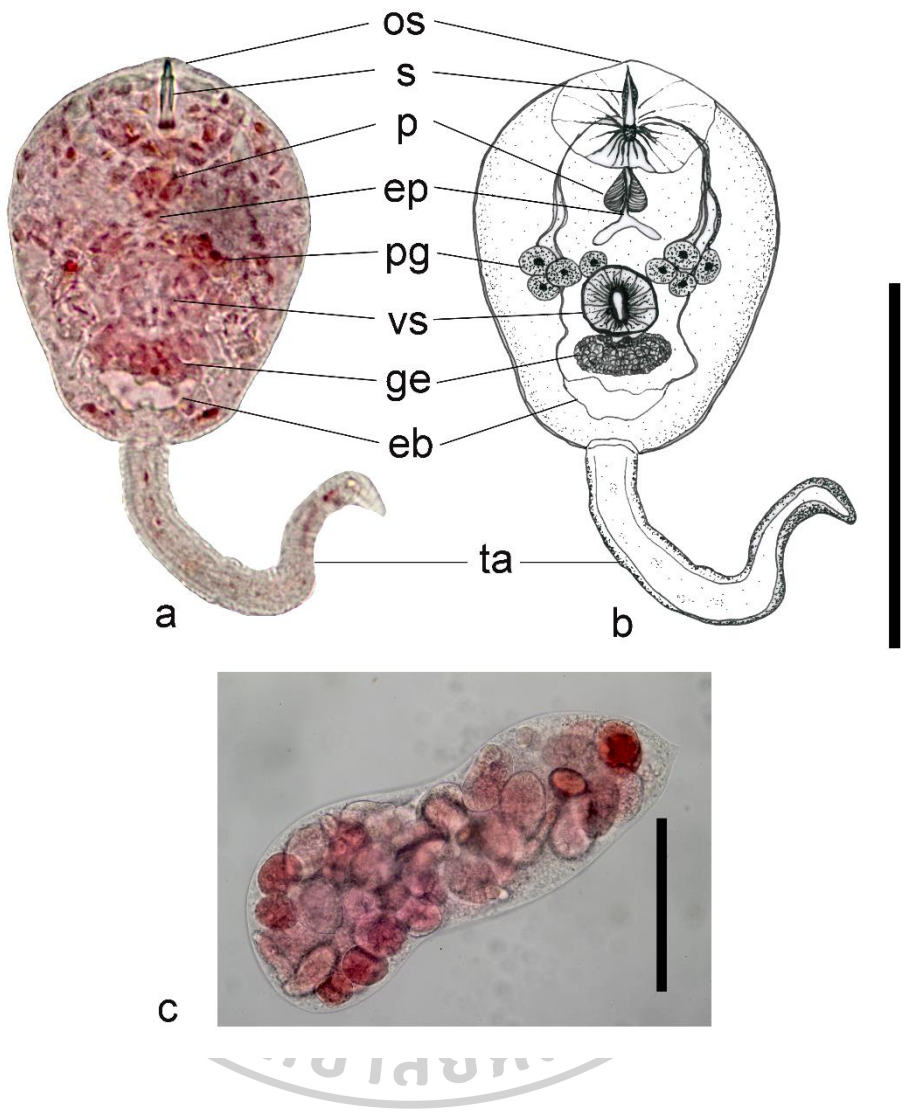

Figure 32. Images of Maritreminoides obstipus (Van Cleave & Müller, 1932).

a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; ge: genital primordium; os: oral sucker; p: pharynx; pg: penetration gland; s: stylet; ta: tail; vs: ventral sucker. scale bars: 50 µm.

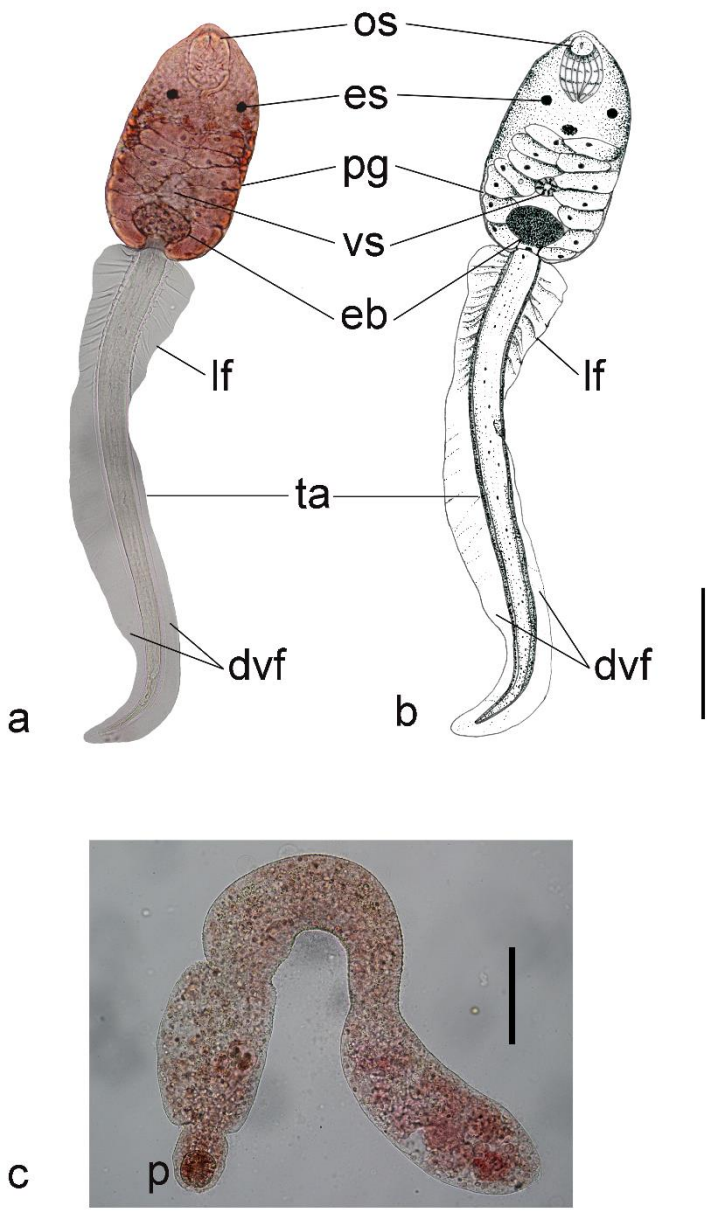

Figure 33. Images of *Haplorchis pumilio* (Looss, 1896).
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. redia stained with 0.5% neutral red. Abbreviations – dvf: dorsoventral finfold; eb: excretory bladder; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta – tail; vs: ventral sucker. scale bars: 50 µm.

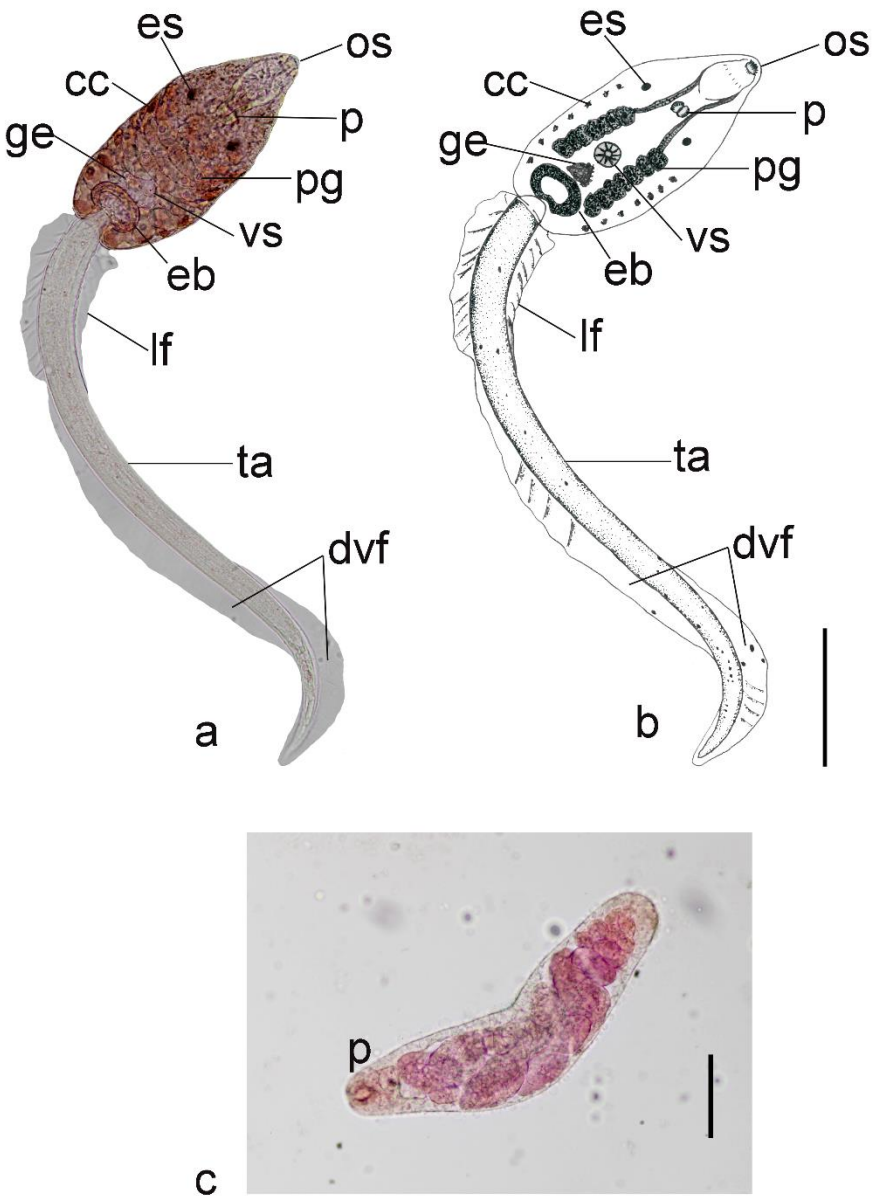

Figure 34. Images of *Haplorchis taichui* (Nishigori, 1924).
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. redia stained with 0.5% neutral red. Abbreviations – cc: cystogenous cells; dvf: dorsoventral finfold; eb: excretory bladder; es: eyespot; ge: genital primordium; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. scale bars: 50 µm.

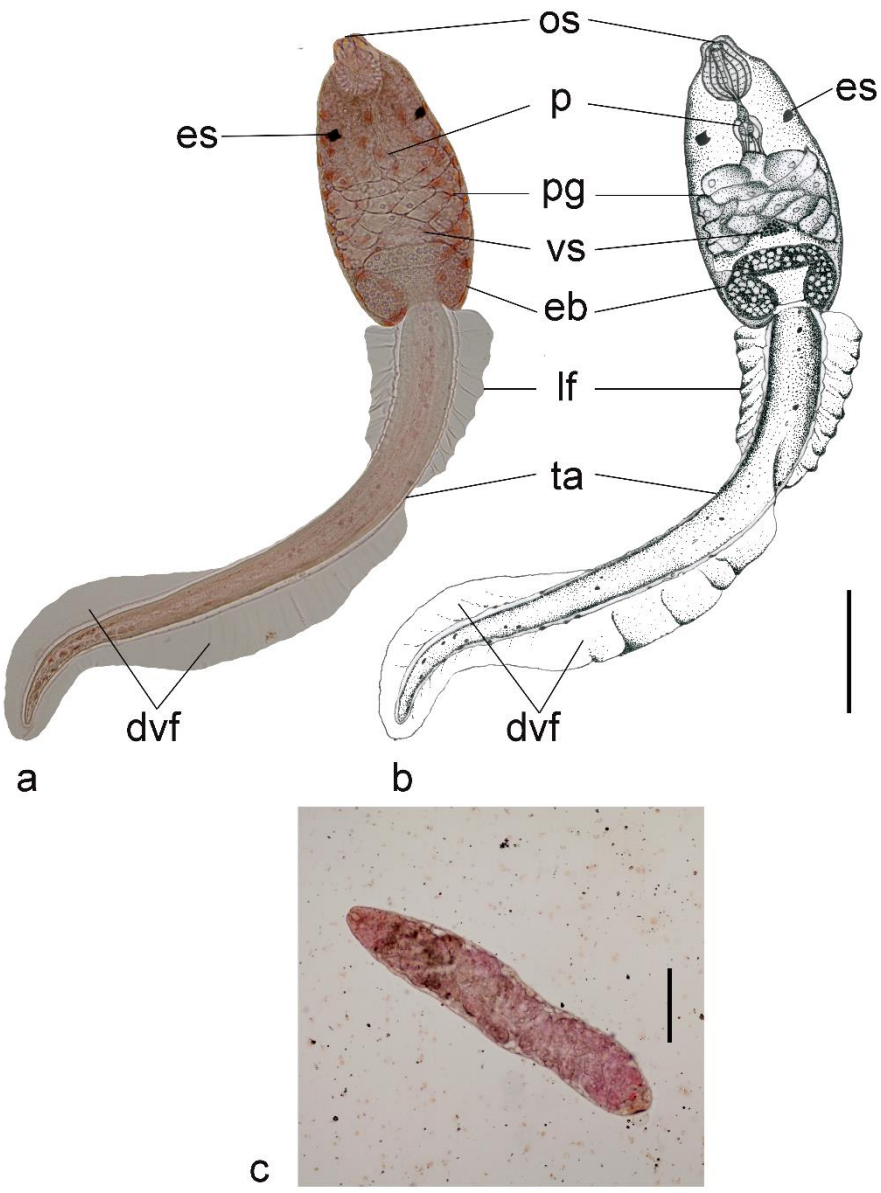

Figure 35. Images of *Stictodora tridactyla* Martin & Kuntz, 1955.
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. redia stained with 0.5% neutral red. Abbreviations – dvf: dorsal finfold; eb: excretory bladder; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. scale bars: 50 µm.

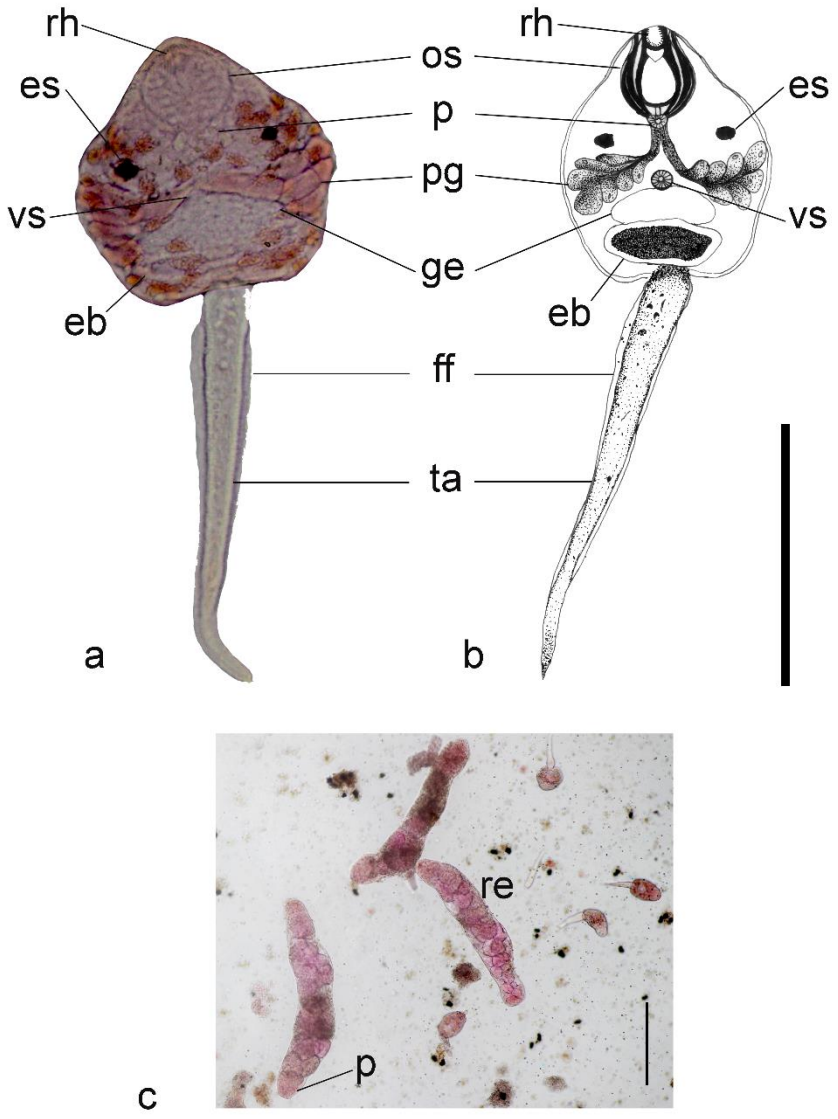

Figure 36. Images of *Centrocestus formosanus* (Nishigori, 1924).
a. Specimen stained with 0.5% neutral red. b. Drawing of cercaria. c. Redia stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; es: eyespot; ff: finfold; ge: genital primordium; os: oral sucker; p: pharynx; pg: penetration gland; rh: rostellar hooks; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

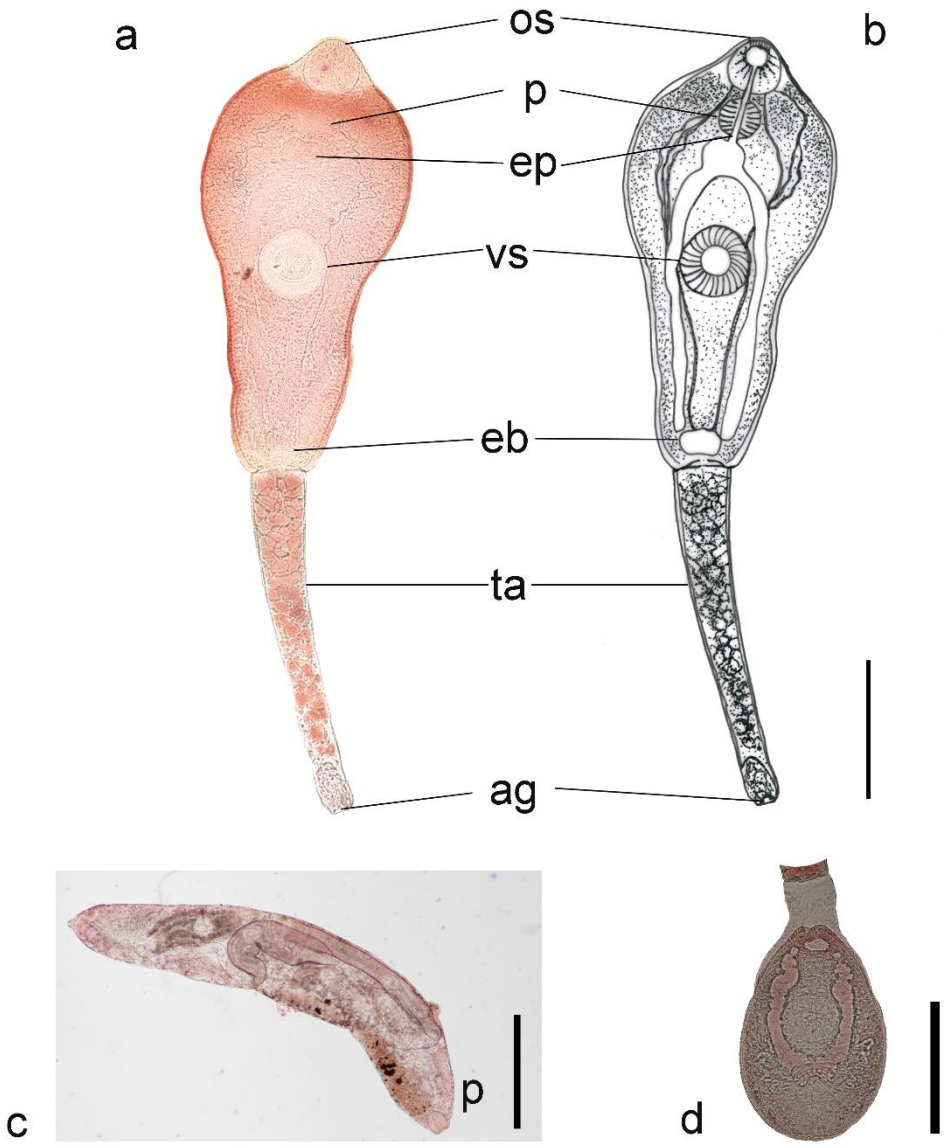

Figure 37. Images of *Philophthalmus gralli* Mathis & Léger, 1910
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. redia stained with 0.5% neutral red. d. metacercaria stained with 0.5% neutral red. Abbreviations – ag: adhesive gland; eb: excretory bladder; ep: esophagus; os: oral sucker; p: pharynx; ta: tail; vs: ventral sucker. scale bars: 50 µm.

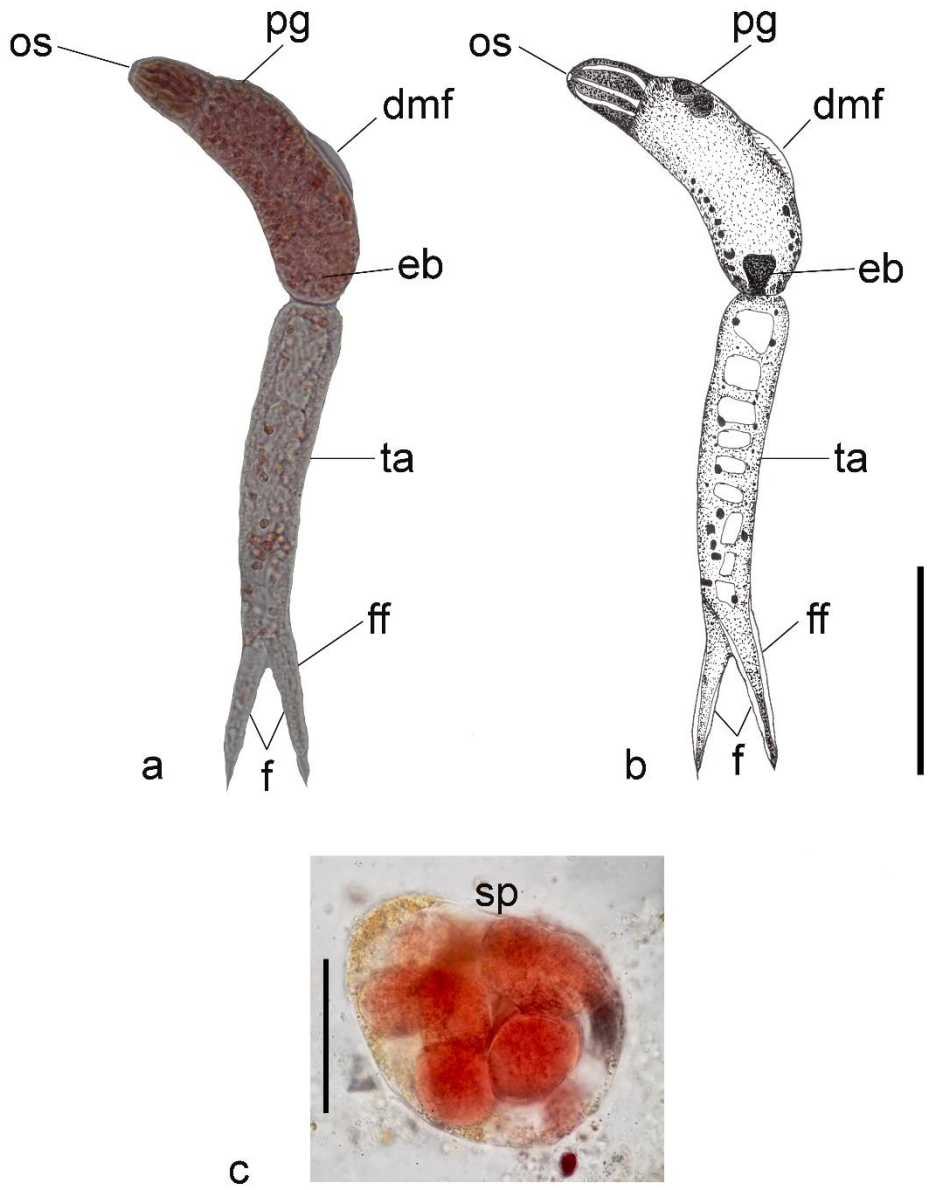

Figure 38. Images of *Cardicola alseae* Meade & Pratt, 1965.
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. sporocyst stained with 0.5% neutral red. Abbreviations – dmf: dorso-median finfold; eb: excretory bladder; f: furca; ff: furcal finfold; os: oral sucker; pg: penetration gland; ta: tail. scale bars: 50 µm.

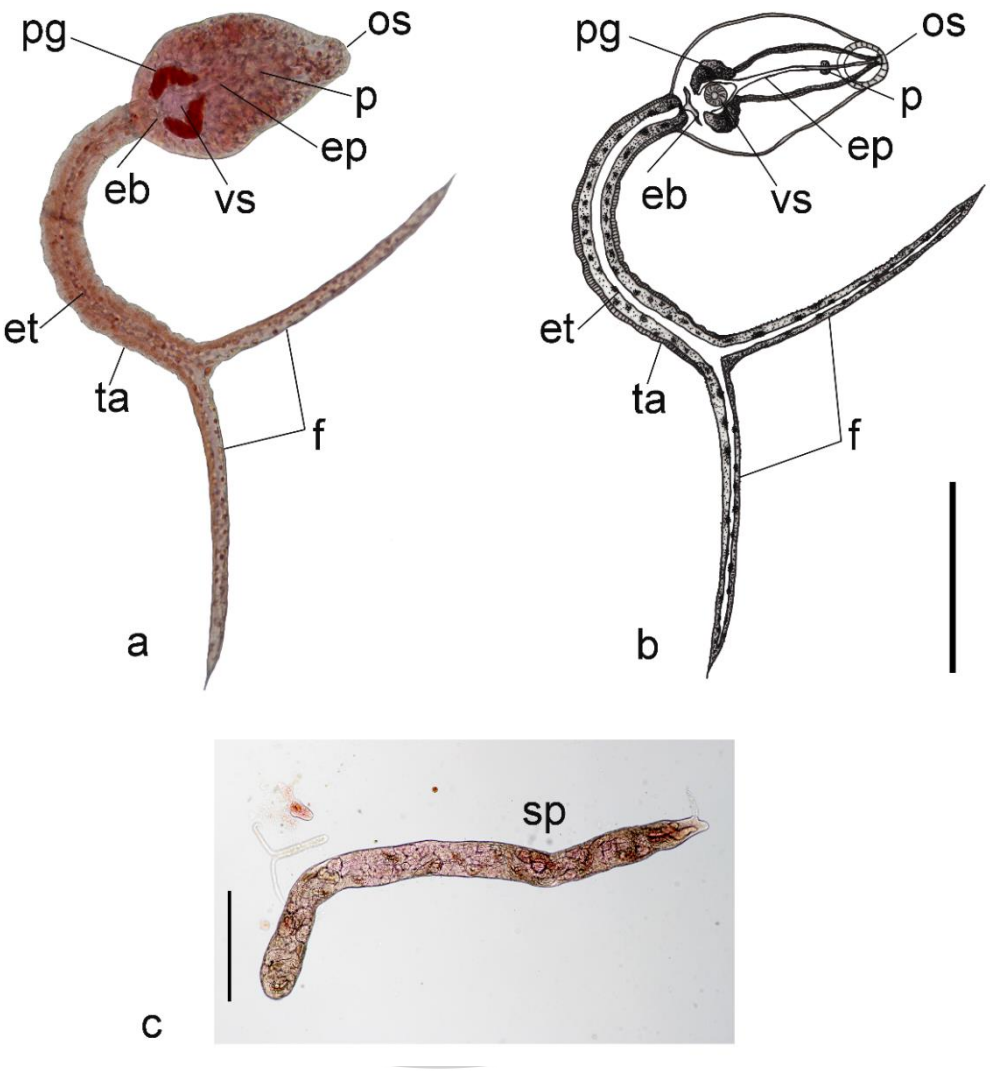

Figure 39. Images of *Alaria mustelae* Bosma, 1931.
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. redia stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; et: excretory tubule; f: furca; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. scale bars: 50 µm.

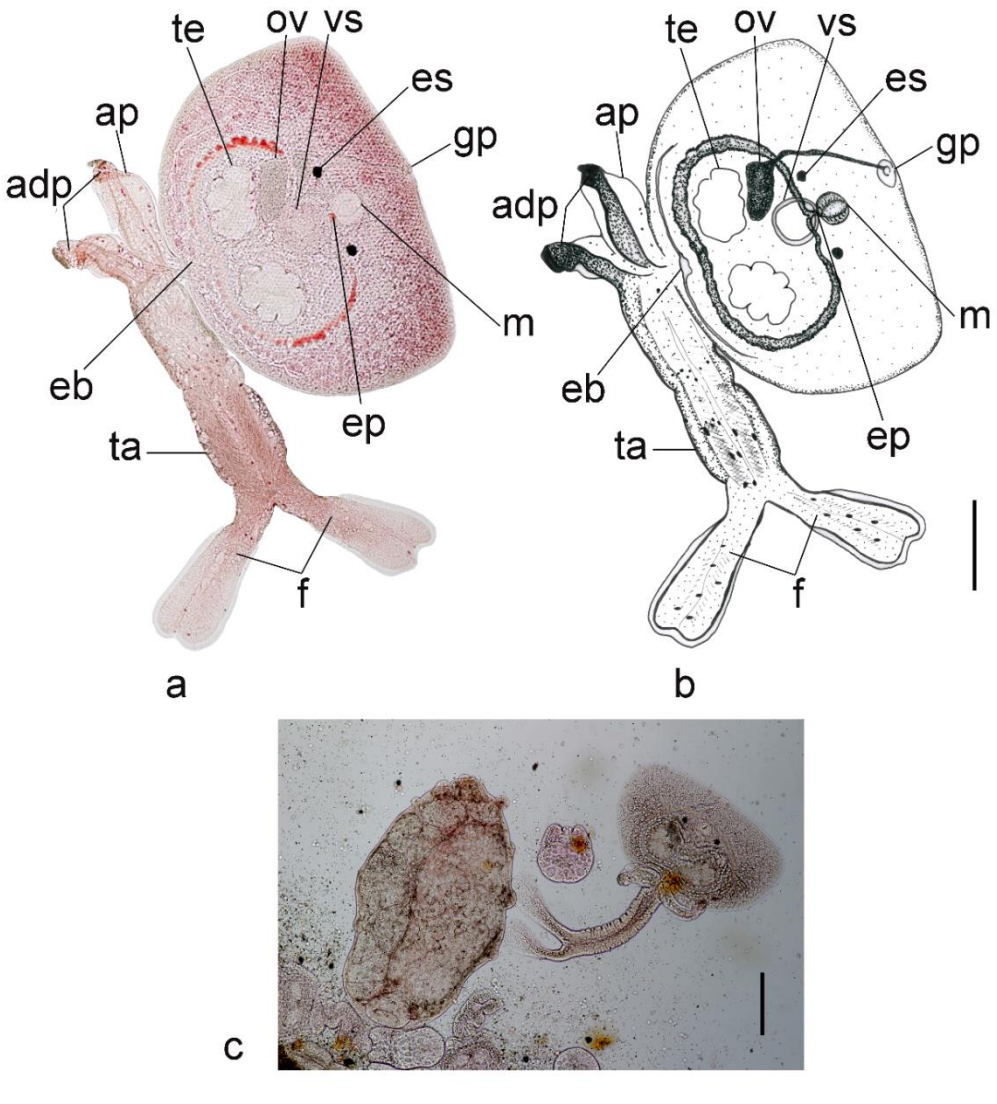

Figure 40. Images of *Transversotrema laruei* Velasquez, 1958.
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. redia (left) and cercaria (right) stained with 0.5% neutral red. Abbreviations – adp: adhesive pad; ap: appendages; eb: excretory bladder; ep: esophagus; es: eyespot; f: furca; gp: genital pore; m: mouth; ov: ovary; ta: tail; te: testes; vs: ventral sucke. scale bars: 50 µm.

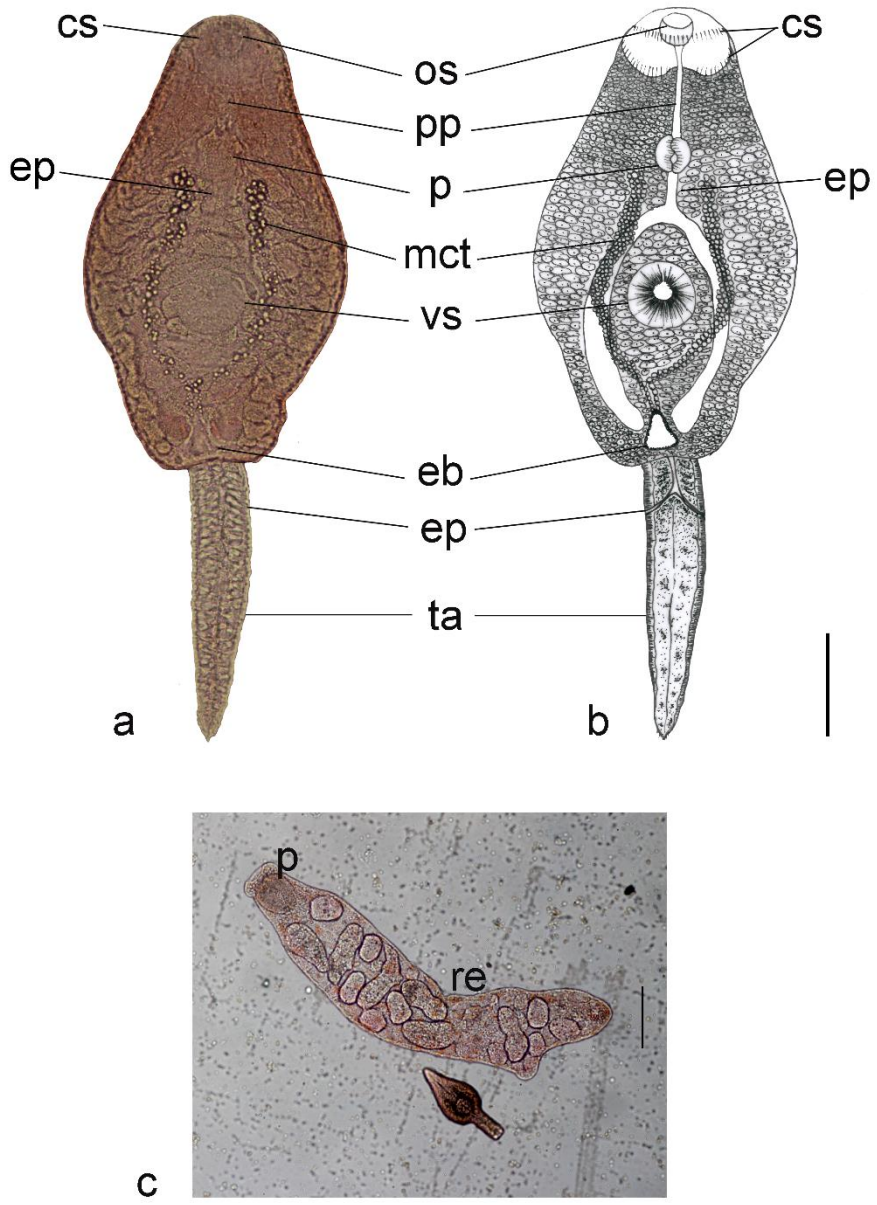

Figure 41. Images of Echinostome cercaria.
a. specimen stained with 0.5% neutral red. b. drawing of cercaria. c. redia stained with 0.5% neutral red. Abbreviations – cs: collar spines; eb: excretory bladder; ep: esophagus; mct: main collecting tube; os: oral sucker; p: pharynx; pp: prepharynx; ta: tail; vs: ventral sucker. scale bars: 50 µm.

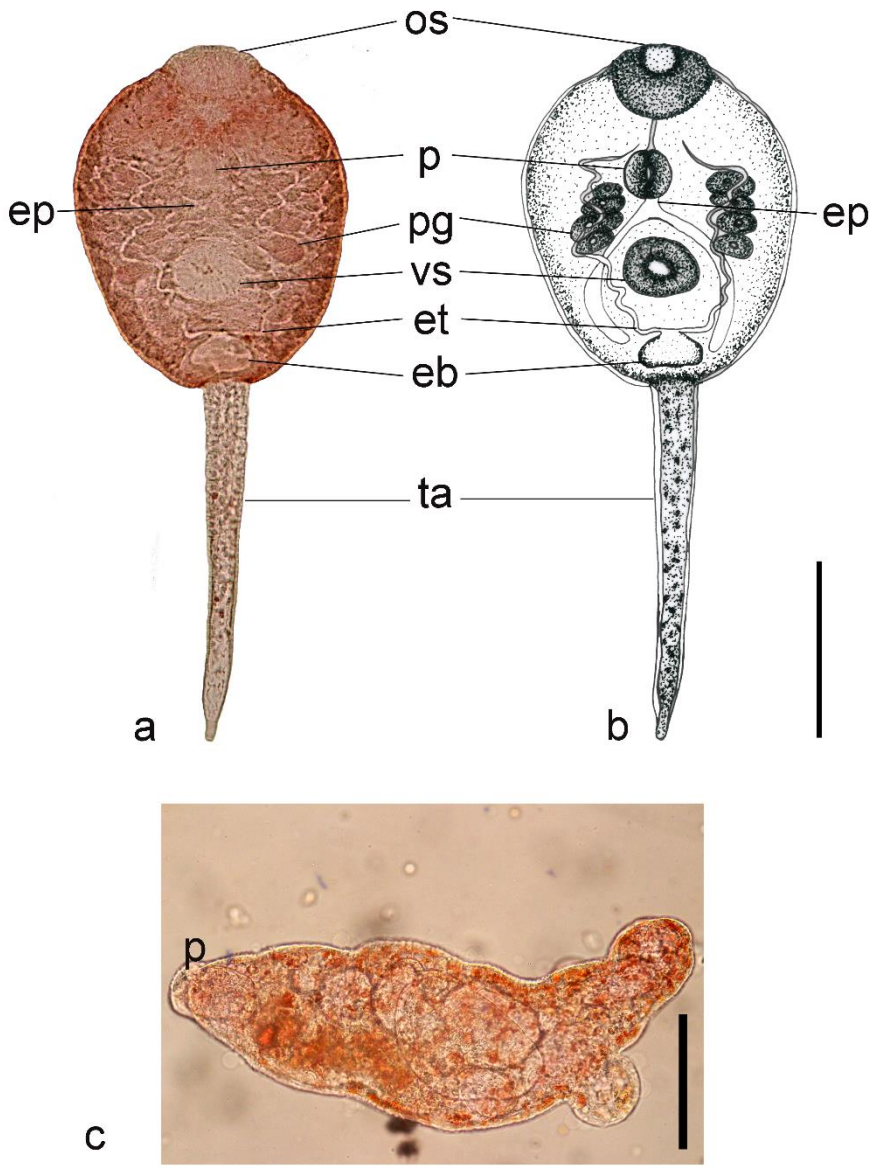

Figure 42. Images of Gymnocephalous cercaria.
a. Specimen stained with 0.5% neutral red. b. Drawing of cercaria. c. Redia stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; ep: esophagus; et: excretory tubule; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker. Scale bars: 50 µm.

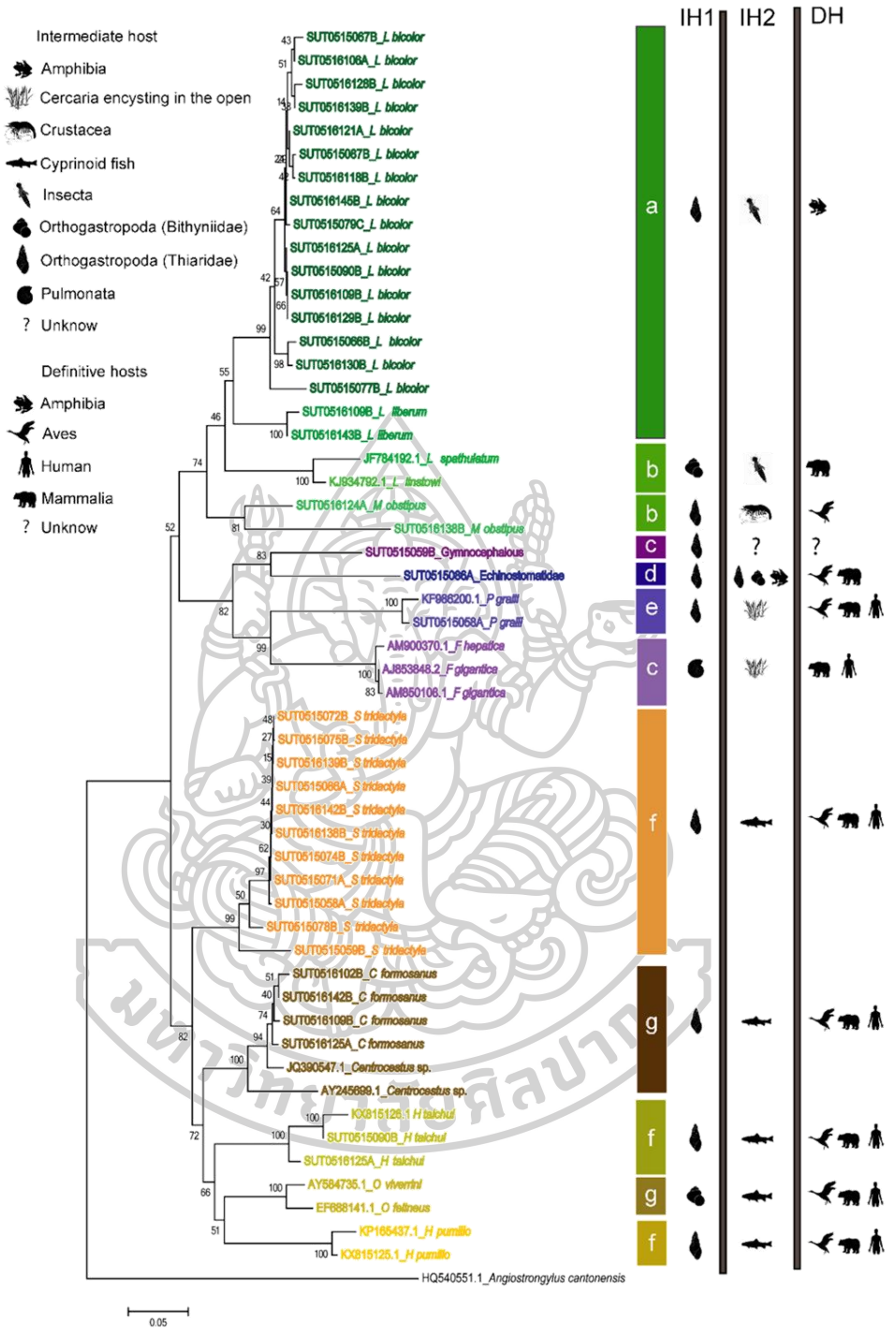

Figure 43. Neighbor-joining tree on the basis of ITS2 sequences of cercarial species. Taxon names and voucher or GenBank accession numbers are provided at the tips of the tree (see also Table 7). (DH: definitive host: IH1: first intermediate host; IH2: second intermediate host). Cercarial types – a: virgulate xiphidiocercariae; b: armatae xiphidiocercariae; c: gymnocephalous cercariae; d: echinostome cercariae; e: megarulous cercariae; f: parapleurophocercous cercariae; g: pleurophocercous cercariae.

Table 7. List of ITS2 sequences used for the phylogenetic analysis.

Species of cercariae	Type of cercariae	References
Lecithodendrium spathulatum	Xiphidiocercariae cercariae	JF784192.1
Lecithodendrium linstowi	Xiphidiocercariae cercariae	KJ934792.1
Haplorchis pumilio	Parapleurophocercous cercariae	KP165437.1
Haplorchis pumilio	Parapleurophocercous cercariae	KX815125.1
Haplorchis taichui	Parapleurophocercous cercariae	KX815126.1
Centrocestus formosanus	Pleurophocercous cercariae	JQ390547.1
Centrocestus formosanus	Pleurophocercous cercariae	AY245699.1
Opisthorchis viverrini	Pleurophocercous cercariae	AY584735.1
Opisthorchis felineus	Pleurophocercous cercariae	EF688141.1
Philophthalmus gralli	Megarulous cercariae	KF986200.1
Fasciola hepatica	Gymnocephalous cercariae	AM900370.1
Fasciola gigantica	Gymnocephalous cercariae	AJ853848.2
Fasciola gigantica	Gymnocephalous cercariae	AM850108.1

Parasite effect with reproductive system of snail.

Soft body-Snail is coiling in 3 whorls. The head-foot is light brown to dark brown, while the mantle is green with gray to black line in serrated marginal edge. The mantel papillae are visible on the ventral side of the mantle edge. The body has green color. The snout is broad. Cephalic tentacle is about 2 mm in length. The location of eyes is at the base of tentacle (Fig. 44a,c). In the females, we found all ontogenetic stages of early embryos to large shelled juveniles in the brood pouch situated in the dorsal part of the head-foot (Fig. 44b). The digestive gland of snail located at the posterior end of the body (Fig. 44c). In this study, the snails infected with trematode displayed digestive glands, compared with nonparasitized snails, with were characterized by firm bodies, dark brown, coiled digestive glands, and distinct branched ovaries. The snails infected displayed mottled, white and brown patches comprising the digestive gland. The white areas signified the presence of larval stages (rediae, sporocysts and cercariae); brown areas represented the remains of the digestive gland (Fig. 44d-f). Rediae, sporocysts and cercariae of either trematode occurred initially within the digestive gland of the snails, but spread to the ovary in cases of heavy infections.

In addition, the trematodes can influence the growth of snail host, especially they effect with reproductive system. A pattern of reproductive strategies of uninfected snails were observed. There were embryos and juveniles in brood pouches of uninfected snails from Thailand and Timor-Leste see in Fig. 22-24. The number of larval stage of uninfected snails was more than infected snails. The snails were infected trematodes including *Loxogenoides bicolor*, *Loxogenes liberum*, *Maritreminoides caridinae* and *Maritreminoides obstipus* showed lower larval stages than uninfected snails but the snails were infected 7 species of trematodes, there are *Centrocestus formosanus*, *Stictodora tridactyla*, *Haplorchis taichui*, *Haplorchis*

pumilio, *Philophthalmus gralli*, Echinostome cercariae, and Gymnocephalous cercariae, were not found larval stage in their brood pouchs (Fig. 45).

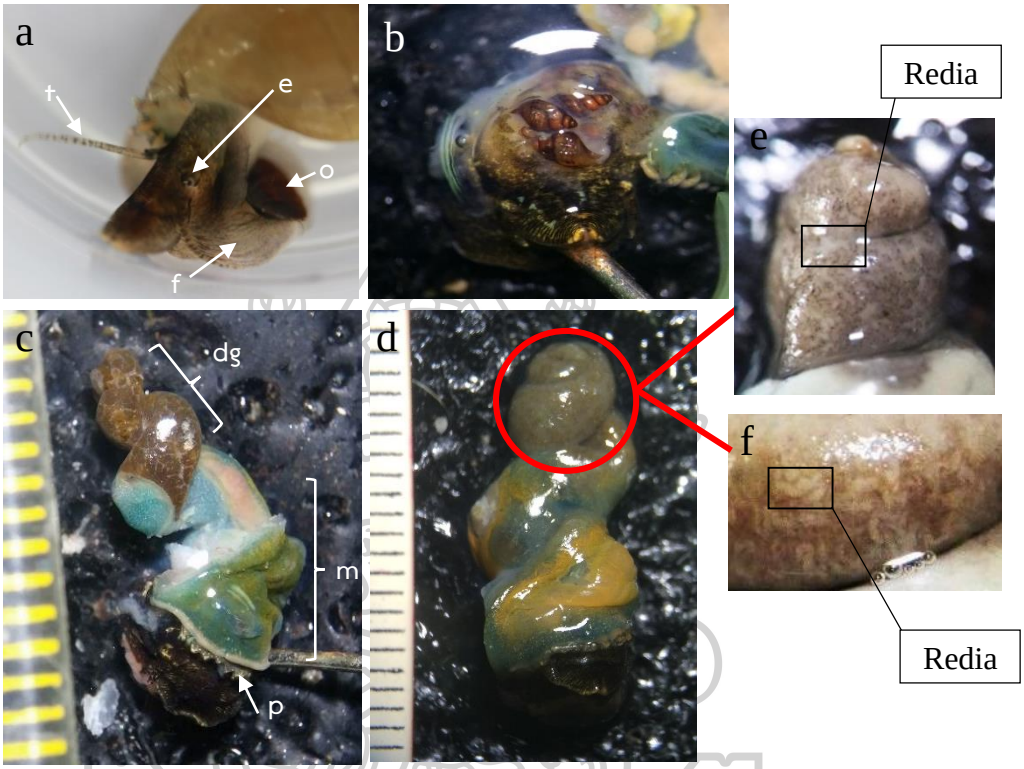

Figure 44. External view of *Tarebia granifera*.
a. the head; b. the brood pouch located in neck part of the head; c. soft body of nonparasitized snail; d. soft body of infected snail; e-f. digestive gland of infected snail. (dg: digestive gland; e: eye; f: food; m : mantle; o: operculum; p: papilla; t : tentacle)

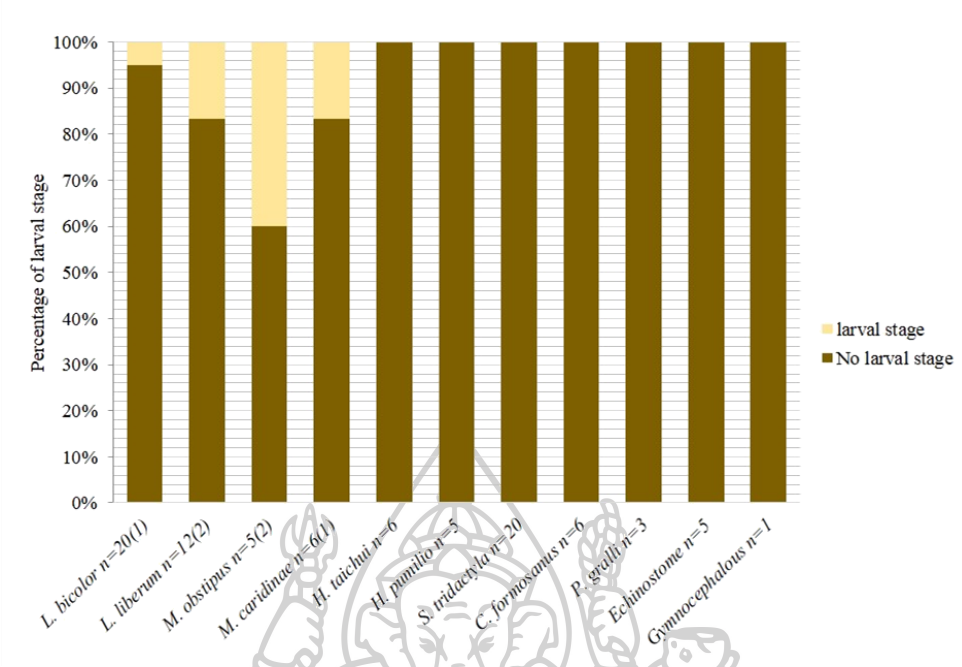

Figure 45. The percentage of larval stage were found in the brood pouch of infected snails.

CHAPTER V
Discussion

The prosobranch *Tarebia granifera* is viviparous and parthenogenetic snail that has occurred in many places of Southeast Asian. In addition, *T. granifera* have been report as the intermediate host of trematodes, thus supporting the life cycles of vectors infecting humans as well as other animals with widespread diseases. In the present study, phenotypically distinguishable shell morphs of *Tarebia granifera* were examined, in reference to samples from Timor-Leste as known type locality of the nominal species, using radula, juvenile shell, biometry and geometric morphometric in combination with phylogeographical analyses of molecular genetics and reproductive strategy. Furthermore, the trematode infections in *T. granifera* were studied by using established methods (shedding and crushing methods) and confirmed species of trematodes by using molecular methods. Also, the potential effect of parasites were analysed for infecting female snails to the reproductive strategies of their progeny. They were summary and discussion some of the relevant aspect and description here.

Shell morphology

In this study, *Tarebia* snails were found widespread in almost all freshwater bodies throughout Thailand, with a wide range of conchological variants or morphs, of which some closely resemble the types and topotypical material of *granifera* collected on Timor. While in Thailand *Tarebia* has been reported with only one species by Brandt (1974), distinct shell morphologies allow to distinguish phenotypically disparate morphs. This study shows that shell morphology of *T. granifera* from Thailand, there are conchological variability within species greater than *T. granifera* from Timor-Leste. The shell morphology was described that were found tubercles and sometime presence spiral lines on the body whorl. Furthermore, *T. granifera* of Morph B_THA and Morph C_THA were found brown spiral lines on the whorl that the shell morphology look like *T. lineata*, Grar 1828 (Fig 8). So, some of these have even been formally named as distinct species, based on ornamental features such as tubercles and/or nodules as well as the formation of elevated spiral ridges prominent in particular on the last body whorls. For example, Subba (1989) discussed that *T. lineata* was often synonymised with *T. granifera*, or treated as its variety (Benthem-Jutting, 1959), although it is readily distinguished from the latter by the presence of the distinct spiral ridges or spiral lines. Also, Appleton et al. (2009) for invading populations of *T. granifera* in South Africa described two distinct morphological variants found at different locations, among them also one with pronounced spiral ridges. In addition, the genetic basis of phenotypical variation are important, such as *Oncomelania hupensis* that Davis and Ruff (1973) were able to show that apparently a single mutation in only one gene is sufficient for producing axially ribbed shells in a smooth-shelled population. So, freshwater gastropods were found to exhibit a pronounced individual conchological variability, which has been

attributed to the environmental conditions of their habitats that widely fluctuate on a temporal and spatial scale (Dillon, 2000; Glaubrecht, 1993; Rensch, 1929, 1934).

Applying a drainage-based phylogeographical as well as a biometrical approach, there were unable to find for the populations in Thailand any correlation of the morphs distinguished in this study based on discernible shell features. However, in the absence of any of the discussed parameters or factors to be causally correlated with these morphological differences we are left with the hypothesis that they either qualify for reflecting phenotypical plasticity correlated with ecological variables in the habitat of the individual populations studied, and/or, alternatively, being correlated with the parthenogenetic reproduction.

Radula

The radula of all *T. granifera* are Taenioglossa according to typical of Thiarid snails. Although the variations of radula were observed that was not enough to use in species distinction (Glaubrech et al., 2009).

Juvenile shell

The sculpture of the initial cap of juvenile shell is wrinkled, with axial elements and growth lines starting on the second whorl. On the third whorl spiral lines develop and more pronounced sculpture commences. After the fourth whorl the axial ribs become most pronounced. While *Melanoides jugicostis* show the axial and spiral elements resulting in a more or less reticulate pattern (Dechruksa et al., 2013).

Biometry and geometric morphometrics

Biometric analyses are found useful tools for the study of characteristics that shape morphologically distinct entities, thus allowing to look into evolutionary pattern (Bocxlaer & Schultheiß, 2010; Maaß & Glaubrecht, 2012). Geometric analyses are used in addition to traditional morphometrics in order to compare in detail different populations and relationships among variable groupings (Sheets et al., 2006).

The measurement of shell height of *T. granifera* showed that they are within the size range previously reported as to vary between 6 to 44 mm (Abbott, 1952; Brandt, 1974). Also Bradstreet and Rogowski (2012) reported on specimens of *T. granifera* to exhibit the same overall shape with an elongately or ovate-conoidal shell with the size index (L3W/W) in the order of 0.54-2.65 mm (Fig. 20d). Isnaningsih et al. (2017) found the shape of *T. granifera* from the Indonesia islands of Lombok, as well as from Banten and Maros, to be for the ratio of shell height to width 1.29-3.02 mm. The dimension or size of shell actually shows spatial and temporal variations that follow the influence of different ecological conditions (Haase & Bouchet, 2006). Although there are some differences in the biometric parameters and in the geometric morphology of Thai *Tarebia*, it is generally impossible to delimit distinct entities based on these features, as all of them largely overlap (Fig. 20-21 a-d). Thus, our morphometric data do not support the distinction of *T. lineata* or other morphs from the nominal *T. granifera*, based on shell size and/or form. In addition, the same holds

true for the two distinct molecular clades separated by mitochrondrial DNA sequences used in our study, for which we failed to find any diagnostic features in shell morphology or other phenotypical characteristics and biometric parameters.

Geometric analyses were used traditional morphometrics in order to compare different populations or relationships among the variable groups (Sheets et al., 2006). The results of geometric morphometrics revealed the overall shell shape of *T. granifera* from Thailand to be very similar to, and virtually undistinguishable from, conspecifics from Timor-Leste (Fig. 20-21e). Thus, although *T. granifera* exhibit shell polymorphism this intra- and interpopulational variability in its shell characteristics does not allow for species-specific differentiation, as it was found, for example, in the thiarid *Melanoides* (Genner et al., 2004; Yousif et al., 2009)

Phylogenetic analyses

Based on molecular genetics, the phylogenetic tree were separated into two clades that there are different distribution in *Tarebia* from Thailand, contrast with shell morphology (morphs A-C, or *lineata* vs. *granifera* phenotypes) (Fig. 16-19). Therefore, our analyses would potentially allow for a more narrow species delimitation within what has been to date traditionally treated in Thailand as *T. granifera* only (Brandt, 1974). At the same time, the two clades correspond with a geographical separation into a northern and southern group. This is also reflected in ecology insofar, as both show a preference in altitude (Fig 16). In contrast, the two genetically distinct lineages do neither match with features in shell morphology or biometry nor with differences in their reproductive strategies.

However, the p-distance of 13.8 % for *cox1* and 10 % for 16S sequences has to be considered relatively high, hinting potentially at the existence of two genetically distinct species. However, a definite decision as to this species question in *Tarebia* in Thailand should remain open until the geographical distribution of genetically characterized populations of *T. granifera* and other congeneric forms is completely resolved and better understood within the entire autochthonous range in the Oriental region.

Biogeography

While we found representatives of clade A in the northern tributaries of rivers such as the Chao Phrara and Mae Klong that run into the Gulf of Thailand, with only few others occurring at some localities in the south of Thailand, those in clade B were found in the Salween River and the headwaters of Ping, Wang, Yom and Nan River (Fig. 16, 22-23). Accordingly, *Tarebia* snails from clade A are more frequent in the central to southern part of the country, whereas those from clade B are more frequent in the northern part. This overall geographic picture allows to attribute clade A as an element of the Sundaic region, given that it extends even further south and also comprises the Timor group (thus rendering it the nominal *granifera*), while clade B is mainly distributed in the Indochinese region (Fig. 7, 16).

However, some representatives of clade B occur in more southern locations, such as in the province Surat Thani (SUT 0516137), Nakhon Si Thammarat (SUT

0516139) and Phatthalung (SUT 0516138). We anticipate that this might reflect occurrences of passive dispersal, potentially via aquatic plant or other material or even transport by birds, rather than vicariance via the influence of sea level or tidal flow of drainage system. The results of the median-joining haplotypes network and bGMYC analysis (Fig. 17-18) reveal that clade A and B exhibit many steps separating these two groups, and have low probability for distinguishing between clade A and B (p=0-0.05). As we found in our molecular analyses this major slit of clade A and B in Thai *Tarebia* to be as old as most likely 5.32 million year ago (Fig. 19), it is worthwhile to look for a possible biogeographic explanation of the above distribution. For the distributional pattern found in *Tarebia* in Thailand, a vicariant hypothesis can be formulated using a major biogeographic transition zone between the Sundaic and Indochinese biota, located just north of the Isthmus of Kra. Parnell (2013) examined, based on the relevant geological, geographical, climatic, biogeographic and sea-level data, the available evidence on the Isthmus of Kra as being a significant biogeographic divide on the Thai–Malay Peninsula of mainland Southeast Asia. In general, distinct faunal and floral assemblages are biogeographically restricted by barriers to dispersal such as characteristic geomorphological boundaries, even when individual taxa among each of the biota on either side often vary and may not all reflect the same discrete pattern. As Dejtaradol et al. (2016) reported that population boundaries in birds did not coincide with the Isthmus of Kra, but instead were located north of the Thai-Malay Peninsula in Central Thailand, while only one of four divides represented an Indochinese-Sundaic transition. They supposed that different phylogeographical patterns among target species were presumably shaped by different ecological preferences. They found in bulbuls which they hypothesized that it coincides with strong vegetational changes on the Peninsula shaping two phylogeographical transitions. As distribution limits of bird species roughly coincide with these transition zones, the avifaunal Thai-Malay transition represents apparently a broad zone rather than a sharp boundary.

The molecular and distributional data of *T. granifera* (Fig. 16, 19) suggest, with its two lineages in the north and south along the Thai peninsular mainland, to roughly correlate with a Late Miocene/Early Pliocene event (5.5–4.5 Mya). Thus, the separation of clade A and B can be hypothesized as resulting from a later marine transgression in the area to the north of today's Isthmus of Kra that may have produced high sea-level stands with a seaway that dissected the Thai-Malay Peninsula for durations longer than one million years (Bruyn et al., 2005).

The distributional boundaries of the two *Tarebia* populations in clade A and B do not coincide exactly with the position of the Isthmus of Kra, but are instead placed further to the north, could in this case be attributed to later palaeo-drainage differentiation in connection with orogenesis or other tectonic events in the mountainous central and northern regions of Thailand, as it was discussed using relevant geological and available biogeographical data, for example, from fishes and gastropods (Glaubrecht and Kohler (2004). Thus, although being today located north of the Thai-Malay Peninsula in Central Thailand, the Isthmus of Kra and late Miocen/early Pliocene marine transgression might have caused in the freshwater

thiarids of this region the separation of the Indochinese and Sundaic lineage within what has been regarded as *Tarebia granifera* to date.

Reproductive biology

Tarebia snails are viviparous and parthenogenesis with females brooding their juveniles in subhemocoelic brood pouch, located at the back of the head in the female's body (Glaubrech et al., 2009). For *T. granifera*, Glaubrecht (1996) described an eu-viviparous strategy that progeny were developed in the subhemocoelic "marsupium" from early to late embryos and subsequently build their multi-whorled shells before hatching as crawling juveniles. This strategy, also known as typical for other thiarids such as e.g. *Melanoides* (Dechruksa et al., 2013; Glaubrech et al., 2009; Maaß & Glaubrecht, 2012).

In the Thai populations of *Tarebia*, as well as those from Timor, we found most if not all ontogenetic stages contained at the same time in the female's marsupium, from early embryos to late embryos and shelled juveniles, in all morphs (A-C), both molecular genetic clades (A and B) and specimens from all drainage systems, without differentiation of this reproductive strategy. In particular, the ontogeny of *T. granifera* in Thailand is not obviously correlated to specific drainage systems, no matter where these water bodies eventually drain. Therefore, we conclude that *Tarebia* throughout its distributional range covered here is eu-viviparous, with only very few representatives in some populations that were found to only possess late and/or even early embryonic stages, respectively as two populations of morph A (SUT 0516144) and C (SUT 0516147), both in locations in the south in streams draining to the Gulf of Thailand (Fig. 23). As same as Thai thiarid *Melanoides jugicostis*, that was found to lack viviparous populations at least in some geographical regions and during some time of the year (Dechruksa et al., 2013).

As in this later case, it could be hypothesized that any environmental factor might affect the reproductive strategy also in *Tarebia*. However, our analysis of representative climatic charts for the two parameters temperature and precipitation revealed no clear regional pattern of brood pouch content, as no correlation with the various ontogenetic stages were found across all locations in Thailand where *T. granifera* was sampled (Fig. 22-26). Some populations of rivers in the northwest (Pai, Moei, Ping), that were sampled essentially in the first half of the year (i.e. particularly early in the rainy season from April to June) exhibit a considerable amount of non-gravid specimens. The same might be true for some populations sampled during the early rainy season (April-July) in the Gulf of Thailand drainages, and to a lesser extent, too, in samples collected in May in the Andaman Sea drainages (Fig. 25b, 12a). In contrast to this temporal (spatial) hypothesis, do not explain the frequency of non-gravid specimens as being indicative of the varying existence of males. The *Tarebia* apparently lack males in most populations. Parthenogenetic reproduction has gained much interest in the past in evolutionary biology, not only with respect to the origin of sex. Clonal reproduction in natural populations has obviously many advantages over sexual modes, with growth rates in the former often being much accelerated over the latter, as all individuals within the population are able to contribute (Smith, 1978). In addition, these clones are considered instrumental in fast

colonization of new habitats and areas, as even a single female can give rise to a new population (Baker, 1955). Nevertheless, most faunas are dominated by sexually reproducing species, with asexual organisms being in the minority (Bell, 1982).

Also in malacology there are some classical case studies, such as the New Zealand freshwater hydrobiid *Potamopyrgus antipodarum* (Jokela et al., 2003) or the thiarid *Melanoides tuberculata* (Ben-Ami & Heller, 2005; Berry & Kadri, 1974; Jacob, 1957, 1958). However, in both cases reproduction is not exclusively parthenogenetic. In populations of *Melanoides tuberculata,* for example, the frequency of males was found to vary between 40 % in the French West Indies (Samadi et al., 1998) and up to 66 % in Israel (Heller & Farstey, 1990; Livshits & Fishelson, 1983). It would be expect a similar phenomenon of *T. granifera* in Thailand and Timor-Leste here from the varying frequencies (with up to 17.40 %) of non-gravid specimens. None feature such as e.g. shell morphology between male and female could be differentiated in cerithiodea. So, in the present study we assumed not only any brood pouch-bearing snail to be female but also those without brood pouch as being non-gravid females rather than being rare males, for the reasons discussed above in connection with regional and/or climatic differences.

The prevalence of trematodes obtained from *Tarebia granifera*

Tarebia granifera have frequently been reported as first intermediate hosts of trematodes affecting the respiratory, intestinal and hepatic systems in humans and some domestic animals. As outlined in the Introduction, this represents a serious threat to public health as these thiarids transmit also the parasites of native birds, fishes or mammals. For example, thiarid snails such as *Melanoides tuberculata, Mieniplotia scabra* and *Sermyla riqueti* have been reported as the intermediate hosts of a wide array of diverse trematodes, such as *Haplorchis pumilio, H. taichui, Loxogenoides bicolor, Centrocestus formosanus, Acanthatrium hitaense, Haematoloechus similes, Cloacitrema philippinum, Transversotrema laruei, Stictodora tridactyla, Apatemon gracilis, Mesostephanus appendicalatus, Cardicola alseae* and *Alaria mustelae* (Krailas et al., 2014; Krailas et al., 2011; Ukong et al., 2007).

Tarebia granifera host is common in many Thai freshwater systems, inhabiting rivers, lakes, streams and ponds (Hyslop, 2003). Pillay and Perissinotto (2008) recorded that *T. granifera* was also able to colonize moderately saline habitats (brackish water). Without doubt, therefore, this thiarid is well established as an intermediate host for several species of trematodes.

Only three species of trematodes, viz. *L. bicolor, S. tridactyla, C. formosanus,* were found to commonly occur in *Tarebia granifera* from most river systems and regions in Thailand. They were also found during all seasons, thus independent of the time of the year the snails were collected. By re-visiting during the years 2014 to 2016 the same locations of the first collecting period five to ten years earlier (2004-2009, recorded by PaMaSU), and recording infected snails in 18 of these sampling sites, they also found that these trematode infections in the populations of the snail host are apparently long-lasting, despite seasonal variation in the abundances of plants and animals in general (Shimadzu et al., 2013). Among the total of 15 species in 8 types

of cercariae recorded in this study, the previously study found only half of them (i.e. 8 species in 4 types); whereas 11 species in 7 types were found in the present study (Table 5-6). Thus, with the new study period and with collecting at various other and thus new locations all over Thailand we were able to expand our knowledge with respect to the taxonomical and geographical aspects of this analysis.

Epidemiology of cercarial stage in *Tarebia granifera*

Parapleurophocercous cercariae and pleurophocercous cercariae were reported to be commonly found also in other freshwater snail in Thailand, such as e.g. *Melanoides tuberculata*. In this study, three species of parapleurophocercous cercariae and one species of pleurophocercous cercariae were found in *T. granifera*. Some species of pleurophocercous cercariae of the intestinal trematodes Heterophyidae, such as *H. taichui, H. pumilio, S. tridactyla* and *C. formosanus*. This parasite has an aquatic life cycle, using freshwater snails as the first and cyprinid fish as the second intermediate host, with definitive hosts being fish-eating mammals and humans (Krailas et al., 2014; Krailas et al., 2011; Nithikathkul & Wongsawad, 2008; Ukong et al., 2007). Especially, the snail infection by the minute intestinal fluke of *S. tridactyla* (2.47%) showed a high level of prevalence in this study. In addition, *H. taichui* is important for public health, as was shown in several studies. For example, Kumchoo et al. (2005) reported high prevalence of fish as being the second intermediate host (91.4%) of *H. taichui* from Mae Taeng district of Chiang Mai province. Also, in the PDR Laos many patients have been infected by *H. taichui*, as cases were reported with mucosal ulceration, chronic inflammation and fibrosis of submucosa (Sohn et al., 2014; Sukontason et al., 2005). Chai et al. (2013) reported for seven patients who were infected by *C. formosanus* in Laos that they had abdominal pain, indigestion and diarrhea. Chung et al. (2011) reported the first case in Korea for patients being infected by *H. pumilio*. This heterophyid trematode is an important and continuing public health problem in many countries, as there are case reports not only from Southeast Asia but also from other Asia countries.

In contrast, known as parasites to animal only, xiphidiocercariae can be distinguished by their stylet organ in the mouth part of the cercariae. They can are divided into two morphological types, the first type being the virgulate xiphidiocercariae, and the second type the armatae xiphidiocercariae. The virgulate xiphidiocercariae has a virgular organ present in the region of the oral sucker. For this group, the present study reported three species of parasites from the Lecithodendriidae, viz. *L. bicolor* and *L. liberum* , for which the hosts are amphibians (Brooks et al., 1985). It should be noted that *L. bicolor* have the highest prevalence, with an infection rate of 2.25 %, and distributed in every water body, river system and region of Thailand. For armatae xiphidiocercariae, the cercaria was not found virgular organ. We reported two species of bird's parasite, *M. caridinae* and *M. obstipus* in family Microphallidae.

Megarulous cercariae have been morphologically characterized as belonging to *Philophthalmus*. This parasite is commonly known as the oriental avian eyefluke and it had been reported in connection with human accidental infections (Derraik, 2008; Waikagul et al., 2006). Nollen and Murray (1978) reported that *P. gralli*

parasitized the conjunctival sac of various galliform and anseriform birds. This fluke was also found in ostriches, causing conjunctivitis. In general, the cercariae can be found in *Melanoides tuberculata* as intermediate host (Kalatan et al., 1997; Krailas et al., 2014; Pinto & Melo, 2010). In this study, we found *P. gralli* now also in the thiarid *Tarebia granifera* from the Phachi River. The river is a river in western Thailand. It originates in the Tenasserim mountain range and tributes to the Mae Klong river system.

Furcocercous cercariae are generally from trematodes of the Sanguinicolidae; and they develop to cercariae in brackish-water and freshwater snails, while the adult stages were found in fishes. The others furcocercous cercariae, such as Transversotrematidae, were found with metacercariae in brackish and freshwater fishes. The adult stages of these flukes inhibit the small intestine of their bird hosts (Smith & Hickman, 1983) . In this study, we found cercariae of three species, viz. *C. alseae, A. mustelae* and *T. laruei*, to parasitize *Tarebia granifera* as intermediate host. Cercariae of all three trematode species were also found in other thiarid snails, as they were reported in *Melanoides tuberculata* (Krailas et al., 2014).

Echinostome cercariae are distributed throughout Southeast Asia (Chai, 2009). Most species mainly parasitize avian hosts, such as migratory birds, but sometimes also infect mammals including humans. The echinostome trematodes are associated with the ingestion of raw snails and amphibians that transmit metacercariae as the infective stage (Esteban & Muñoz-Antoli, 2009). In the present study, echinostome cercariae was found in *Tarebia granifera* populations from the north of Thailand only; which corroborates the report by Nithikathkul et al. (2008) that echinostomiasis cases have been commonly found in the north and northeast of Thailand. The north and northeast of Thailand have main of the river system and serves as a source of freshwater fish. In case of humans, the people have life-style and resorted to fisheries, thus increasing the risk of helminthiasis by eating raw or improperly prepared fish (Carney, 1991)

Gymnocephalous cercariae are small larval stages of trematodes, in general attributed to the Fasciolidae (Schell, 1970). In this study, were found only one snail infection with cercariae that morphologically are obviously attributable to *Fasciola* cercariae. However, the molecular identification showed that these cercariae were actually neither *F. gigantica* nor *F. hepatica.* Instead, the phylogenetic analyses indicate a closer affinity of these sequences to those from cercariae with echinostoma type. By morphology the echinostome cercariae are clearly distinguishable by being elongated spinose with a reniform collar, armed with a single or double row of spines surrounding the dorsal and lateral margins of the oral sucker (Anucherngchai et al., 2016; Ayoubi et al., 2017). Thus, the study here revealed one case of obvious conflict between the morphologically based identification and the molecular indication of affinity, which clearly is in need to be studied further.

In the previous report, the gymnocephalous cercariae was produced by trematodes of the family Fasciolidae. They were found from *Biomphalaria* sp., *Bulinus* sp., *Ceratophallus* sp., *Gabbiella* sp., *Gyraulus* sp., *Lymnaea* sp., and *Melanoides* sp. (Frandsen & Christensen, 1984). However, thiarid snails never reported the fasciolidae trematodes infection in Thailand. Even though, the

morphology of gymnocephalous cercariae was obviously to be *Fasciola* cercariae. The sequence of DNA was shown in the same group of echinostome cercariae.

Molecular analyses of cercaria and their host correlations

Morphology and molecular studies of cercariae can be confirmed the prevalent of trematodes in this study. ITS2 marker allowed to distinguish a total of nine trematode species, with the cercariae attributable to seven of the morphologically distinguishable types, viz. the parapleurophocercous cercariae, pleurophocercous cercariae, the virgulate xiphidiocercariae and armatae xiphidiocercariae, megarulous cercariae, as well as echinostome cercariae and gymnocephalous cercariae; only the furcocercous cercariae were not available for molecular studies.

For available molecular identifications, the genetic characters of ITS2 were shown the dendrogram construction into two groups that were reported in case of zoonotic parasites and human pathogens (Fig. 43).

The first group with parapleurophocercous and pleurophocercous cercariae, respectively (marked f and g in Fig. 43), i.e. *S. tridactyla*, *C. formosanus*, *Centrocestus* sp., *H. taichui*, *H. pumilio*, *O. viverrini*, *O. felineus*, all have cyprinoid fish as second intermediate host, while birds and mammals, including in particular humans, are the definite host. Note that the latter two trematode species have a bithyniid instead a thiarid snail as first intermediate host.

In a second group cluster trematode species with virgulate xiphidiocercariae and armatae xiphidiocercariae, respectively (marked a and b in Fig. 43), i.e. *L. bicolor*, *L. liberum*, *Lecithodendrium spathulatum*, *L. linstowi* and *M. obstipus*, which all have arthropods (Insecta or Crustacea) as second intermediate hosts while amphibians, birds and mammals, but with the exclusion of humans, are the definitive hosts.

In addition, also the sequences of trematode species with echinostome cercaria and the gymnocephalous cercaria obtained from *T. granifera* grouped together with relatively high support.

However, no clear picture as to a correlation with their second intermediate hosts and definitive hosts is visible to date, as we lack knowledge on the later in particular for the gymnocephalous cercaria. Nevertheless, the latter two form a well-supported clade together with *P. gralli*, *F. hepatica* and *F. gigantica*, which all have gymnocephalous, echinostome or megarulous cercariae (Fig. 43, c,d,e). However, note that the latter two are known to have an eupulmonate instead a third snail host. Interestingly, in this latter monophyletic clade, formed by *P. gralli* together with *F. hepatica* and *F. gigantica*, only those trematodes are known to be human pathogens as definite hosts.

The effect of parasite with reproductive strategies from snails.

The trematodes can influence the reproductive strategies of snails. The number of larval stage of uninfected snails was more than infected snails (Fig. 24 and 45). The

snails infected displayed mottled, white and brown patches comprising the digestive gland. The white areas signified the presence of larval stages (rediae, sporocysts and cercariae); brown areas represented the remains of the digestive gland (Fig. 44 d-f). In addition, rediae, sporocysts and cercariae can spread to the ovary in cases of heavy infections. Sorensen and Minchella (2001) described that the trematodes with rediae or sporocysts stages cause severe mechanical damage to host tissue. In the present study, rediae or sporocysts of all trematodes appeared the destruction of the digestive gland and, finally, the reproductive strategies of *T. granifera*, with approximately 6.74% (6:89) of total infected snails possessing eggs, embryos, or juveniles in the brood pouch, compared with 85.19% (943:1,107) of uninfected snails. Because the ovary appears not to be targeted by the early stages of infection by parasites, some snails are probably still able to reproduce (as observed in 6.74% of total infected snails); however, once the ovary is destroyed, reproduction is inhibited. While, James (1965) found *Littorina saxatilis tenebrosa* indicated the digestive gland of healthy (uninfected) snails to be dark brown. So the presence of trematode larvae resulted in changes in coloration and destruction of the visceral hump comprising the digestive gland and gonad. In the present study, the digestive gland of *T. granifera* was a reliable indicator of trematode infections. The coiled and dark brown appearance of digestive gland of uninfected snails indicate that it is rich in nutrients (glycogen) required for growth and reproduction of the snail while the trematode larvae result in loss of nutrients (Cheng & Snyder, 1962; Fretter & Graham, 1994).

CHAPTER VI
Conclusion

These studies were found variations of both phenotype and genotype of *Tarebia granifera*. *T. granifera* were found distinct shell morphologies allow to distinguish phenotypically disparate morphs. But the biometric and geometric morphometric analyses and reproductive strategy of difference morphs and genetic clades were found similarity and widely overlap, which indicates that a clear separation is not possible on the basis of shell shape and reproductive system. The result of genotype was shown that the phylogenetic trees were found two genetically distinct clades (clade A and B). All specimens from Timor-Leste were included in clade A together with specimens mostly from the southern to southern-central parts of Thailand. While, specimens of clade B were more frequent in the northern part of Thailand. The two lineages started to split about 5 mya, possibly related to marine transgressions forming what became known as biogeographical barrier north of the Isthmus of Kra. For epidemiology of cercarial stage in *T. granifera* were found infection rate to be 5.80%, which infected with eleven species from seven types, viz. (i) virgulate xiphidiocercariae (*Loxogenoides bicolor*, *Loxogenes liberum* and *Acanthatrium histaense*), (ii) armatae xiphidiocercariae cercariae (*Maritreminoides caridinae* and *Maritreminoides obstipus*); (iii) parapleurophocercous cercariae (*Haplorchis pumilio*, *Haplorchis taichui* and *Stictodora tridactyla*), (iv) pleurophocercous cercariae (*Centrocestus formosanus*), (v) megarulous cercariae (*Philophthalmus gralli*), (vi) Echinostome cercariae and (vii) Gymnocephalous cercariae. In addition, the trematodes can influence the reproductive system. The result was shown that the number of larval stage of uninfected snails was more than infected snails.

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Appendices

Appendix A: Reagents for staining / SEM of radulae

- 10% sodium hydroxide (NaOH)
  Sodium hydroxide 10 g
  Add distilled water to a final volume of 100 ml
- 2% hydrochloric acid (HCl)
  Hydrochloric acid 2 ml
  Add distilled water to a final volume of 100 ml
- 4% Orange G
  Orange G 4 g
  95% Ethanol 100 ml
- 4% Eosin Y
  Eosin Y 4 g
  95% Ethanol 100 ml

Appendix B: Reagents for DNA extraction

- Ethylene Diamine Tetra Acetic acid (EDTA, 0.5 M)
  EDTA ($C_{10}H_{14}N_2O_8Na_2.2H_2O$) (MW = 372.2) 186.1 g
  The pH of the solution was adjusted to 8.0 using in NaOH.
  Add distilled water to a final volume of 1000 ml and the solution was autoclaved.
- Sodium chloride (NaCl, 5M)
  NaCl 292.2 g
  Add distilled water to a final volume of 1000 ml
  The solution was autoclaved prior to use.
- Tris-HCl buffer (pH 8.0, 1M)
  Tris base 121.1 g
  The pH of the solution was adjusted to 8.0 using 1 N NaCl.
  Add distilled water to a final volume of 1000 ml
  The solution was autoclaved prior to use.
- 2X CTAB
  CTAB (Cetyltrimethyl ammonium bromide) 2 g
  1.4 M NaCl 28 ml of 5 M
  1 mM EDTA (pH 8) 4 ml of 0.5 M
  100 mM Tris-HCl 10 ml of 1 M
  Add distilled water to a final volume of 100 ml
  Autoclaved before used.
- CTAB buffer
  0.2% of 2-mercaptoethanol add 2X CTAB
- Proteinase K
  Proteinase K 20 mg/ml 20.0 µl
  Distilled water 980.0 µl
  Mixed well and stored -20ºC

- Sodium Acetate solution (3M, pH 5.6)
  Sodium Acetate 30.75 g
  The pH of the solution was adjusted to 5.6 with glacial acetic acid and volume made upto 50 ml. The solution was autoclaved and stored till use.
- Chloroform: Isoamyl alcohol (24:1) mixture 96 ml of chloroform was mixed with 4 ml of isoamyl alcohol. It was stored in amber coloured bottle.
- 70% Ethanol
  Ethanol 70 ml Add distilled water to a final volume of 100 ml
- TE buffer 1 mM Tris-HCl (pH 8.0) 1.0 ml of 1.0 M
  1 mM EDTA (pH 8.0) 200 µl of 0.5M

Appendix C: Reagents for DNA Electrophoresis

- 1X TAE buffer
  Distilled water 490 ml
  50X TAE buffer 10 ml
- 2% Agarose gel
  Agarose gel 2 g
  1X TBE buffer 100 ml

Appendix D: Table shows the descriptive Statistics between three morphs of Thai specimens (Morph A, B, C) with specimens from Timor-Leste (TIM).

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Height	Morph A	255	18.9211	3.71398	.23258	18.4630	19.3791	9.29	29.83
	Morph B	664	19.7304	3.87082	.15022	19.4355	20.0254	8.56	32.38
	Morph C	134	19.0330	3.00980	.26001	18.5187	19.5473	10.53	26.88
	TIM	100	19.6762	3.74706	.37471	18.9327	20.4197	11.67	28.53
	Total	1153	19.4657	3.74815	.11038	19.2491	19.6822	8.56	32.38
Width	Morph A	255	8.2763	1.71998	.10771	8.0642	8.4884	3.73	13.28
	Morph B	664	8.3499	1.61219	.06257	8.2270	8.4727	3.49	14.46
	Morph C	134	7.9219	1.31214	.11335	7.6977	8.1461	4.39	11.58
	TIM	100	8.1507	1.42404	.14240	7.8681	8.4333	5.04	12.18
	Total	1153	8.2666	1.59380	.04694	8.1745	8.3587	3.49	14.46
L3W	Morph A	255	16.8643	3.31164	.20738	16.4559	17.2727	7.93	26.43
	Morph B	664	16.9266	3.28100	.12733	16.6765	17.1766	7.73	28.74
	Morph C	134	15.9672	2.47575	.21387	15.5441	16.3902	9.20	21.34
	TIM	100	16.5556	3.12110	.31211	15.9363	17.1749	9.46	23.89
	Total	1153	16.7691	3.20208	.09430	16.5841	16.9541	7.73	28.74
L3W/ W	Morph A	255	2.0471	.13445	.00842	2.0305	2.0637	1.27	2.54
	Morph B	664	2.0327	.14585	.00566	2.0216	2.0438	1.22	2.53
	Morph C	134	2.0248	.16028	.01385	1.9974	2.0522	1.39	2.65
	TIM	100	2.0294	.13204	.01320	2.0032	2.0556	1.66	2.28
	Total	1153	2.0347	.14402	.00424	2.0264	2.0430	1.22	2.65

Appendix E: Table shows the relationships between three morphs of Thai specimens with specimens from Timor-Leste by one way ANOVA.

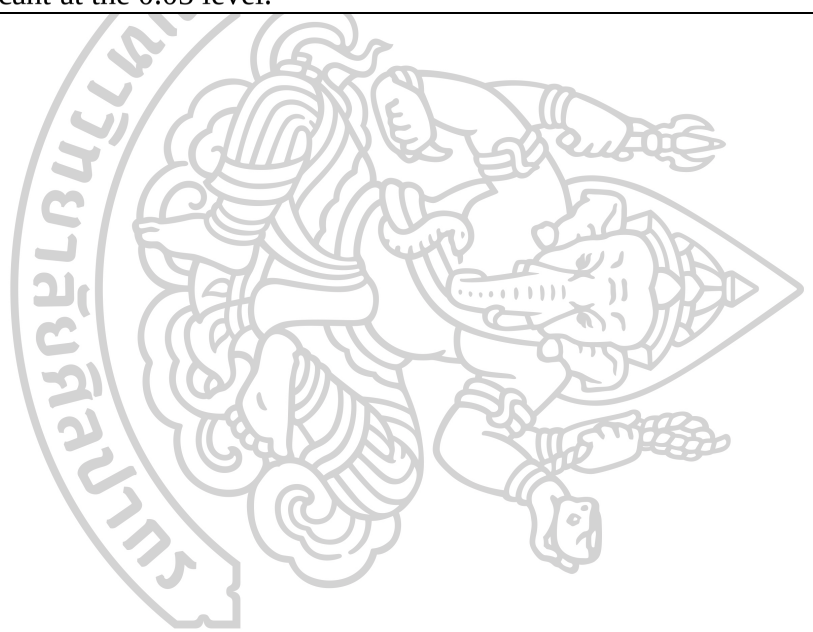
ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Height	Between Groups	151.695	3	50.565	3.624	.013
	Within Groups	16032.306	1149	13.953		
	Total	16184.001	1152			
Width	Between Groups	21.892	3	7.297	2.887	.035
	Within Groups	2904.415	1149	2.528		
	Total	2926.307	1152			
L3W	Between Groups	109.505	3	36.502	3.584	.013
	Within Groups	11702.348	1149	10.185		
	Total	11811.853	1152			
L3W/W	Between Groups	.058	3	.019	.929	.426
	Within Groups	23.838	1149	.021		
	Total	23.895	1152			

Appendix F: Table shows the relationships between three morphs of Thai specimens with specimens from Timor-Leste by Turkey HSD method.

Multiple Comparisons							
Tukey HSD							
Dependent Variable	(I) Morph	(J) Morph	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Height	Morph A	Morph B	-.80936*	.27520	.018	-1.5174	-.1013
		Morph C	-.11193	.39856	.992	-1.1373	.9135
		TIM	-.75514	.44074	.317	-1.8891	.3788
	Morph B	Morph A	.80936*	.27520	.018	.1013	1.5174
		Morph C	.69744	.35376	.199	-.2127	1.6076
		TIM	.05422	.40068	.999	-.9767	1.0851
	Morph C	Morph A	.11193	.39856	.992	-.9135	1.1373
		Morph B	-.69744	.35376	.199	-1.6076	.2127
		TIM	-.64321	.49362	.561	-1.9132	.6268
	TIM	Morph A	.75514	.44074	.317	-.3788	1.8891
		Morph B	-.05422	.40068	.999	-1.0851	.9767
		Morph C	.64321	.49362	.561	-.6268	1.9132
Width	Morph A	Morph B	-.07358	.11713	.923	-.3749	.2278
		Morph C	.35437	.16964	.157	-.0821	.7908
		TIM	.12561	.18759	.909	-.3570	.6082
	Morph B	Morph A	.07358	.11713	.923	-.2278	.3749
		Morph C	.42795*	.15057	.024	.0406	.8153

		TIM	.19919	.17054	.647	-.2396	.6380
	Morph C	Morph A	-.35437	.16964	.157	-.7908	.0821
		Morph B	-.42795*	.15057	.024	-.8153	-.0406
		TIM	-.22876	.21010	.696	-.7693	.3118
	TIM	Morph A	-.12561	.18759	.909	-.6082	.3570
		Morph B	-.19919	.17054	.647	-.6380	.2396
		Morph C	.22876	.21010	.696	-.3118	.7693
L3W	Morph A	Morph B	-.06228	.23511	.993	-.6672	.5426
		Morph C	.89711*	.34051	.042	.0211	1.7732
		TIM	.30867	.37655	.845	-.6601	1.2775
	Morph B	Morph A	.06228	.23511	.993	-.5426	.6672
		Morph C	.95939*	.30223	.008	.1818	1.7370
		TIM	.37095	.34233	.700	-.5098	1.2517
	Morph C	Morph A	-.89711*	.34051	.042	-1.7732	-.0211
		Morph B	-.95939*	.30223	.008	-1.7370	-.1818
		TIM	-.58844	.42173	.503	-1.6735	.4966
	TIM	Morph A	-.30867	.37655	.845	-1.2775	.6601
		Morph B	-.37095	.34233	.700	-1.2517	.5098
		Morph C	.58844	.42173	.503	-.4966	1.6735
	TIM	Morph A	.10677*	.02669	.000	.0381	.1754
		Morph B	.04122	.02426	.325	-.0212	.1036
		Morph C	-.00399	.02989	.999	-.0809	.0729
L3W/W	Morph A	Morph B	.01437	.01061	.528	-.0129	.0417
		Morph C	.02232	.01537	.467	-.0172	.0619
		TIM	.01770	.01699	.725	-.0260	.0614
	Morph B	Morph A	-.01437	.01061	.528	-.0417	.0129
		Morph C	.00795	.01364	.937	-.0271	.0430
		TIM	.00333	.01545	.996	-.0364	.0431
	Morph C	Morph A	-.02232	.01537	.467	-.0619	.0172

		Morph B	-.00795	.01364	.937	-.0430	.0271
		TIM	-.00462	.01903	.995	-.0536	.0443
	TIM	Morph A	-.01770	.01699	.725	-.0614	.0260
		Morph B	-.00333	.01545	.996	-.0431	.0364
		Morph C	.00462	.01903	.995	-.0443	. 0536
*. The mean difference is significant at the 0.05 level.							



Appendix G: Table shows the descriptive statistics based on genetic results

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Height	Clade A	540	19.2408	3.85931	.16608	18.9146	19.5671	8.56	32.38
	Clade B	613	19.6637	3.63908	.14698	19.3751	19.9524	9.45	30.67
	Total	1153	19.4657	3.74815	.11038	19.2491	19.6822	8.56	32.38
Width	Clade A	540	8.0451	1.50640	.06483	7.9177	8.1724	3.73	13.28
	Clade B	613	8.4618	1.64363	.06639	8.3314	8.5921	3.49	14.46
	Total	1153	8.2666	1.59380	.04694	8.1745	8.3587	3.49	14.46
L3W	Clade A	540	16.4934	3.22163	.13864	16.2211	16.7658	7.93	26.22
	Clade B	613	17.0120	3.16755	.12794	16.7607	17.2632	7.73	28.74
	Total	1153	16.7691	3.20208	.09430	16.5841	16.9541	7.73	28.74
HW	Clade A	540	2.3948	.22160	.00954	2.3761	2.4135	1.50	3.13
	Clade B	613	2.3416	.23226	.00938	2.3232	2.3600	1.22	2.93
	Total	1153	2.3665	.22878	.00674	2.3533	2.3797	1.22	3.13
L3WW	Clade A	540	2.0527	.15533	.00668	2.0396	2.0658	1.27	2.65
	Clade B	613	2.0188	.13136	.00531	2.0084	2.0292	1.22	2.38
	Total	1153	2.0347	.14402	.00424	2.0264	2.0430	1.22	2.65

Appendix H: Table shows the relationships based on genetic results by one way ANOVA.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Height	Between Groups	51.350	1	51.350	3.664	.056
	Within Groups	16132.650	1151	14.016		
	Total	16184.001	1152			
Width	Between Groups	49.848	1	49.848	19.946	.000
	Within Groups	2876.459	1151	2.499		
	Total	2926.307	1152			
L3W	Between Groups	77.193	1	77.193	7.571	.006
	Within Groups	11734.660	1151	10.195		
	Total	11811.853	1152			
HW	Between Groups	.812	1	.812	15.721	.000
	Within Groups	59.484	1151	.052		
	Total	60.296	1152			
L3WW	Between Groups	.330	1	.330	16.094	.000
	Within Groups	23.566	1151	.020		
	Total	23.895	1152			

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