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Molecular phylogeography and reproductive biology of the freshwater snail *Tarebia granifera* in Thailand and Timor (Cerithioidea, Thiaridae): morphological disparity versus genetic diversity

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Abstract

The freshwater thiarid gastropod *Tarebia granifera* (Lamarck, 1816), including taxa considered either congeneric or conspecific by earlier authors, is widespread and abundant in various lentic and lotic water bodies in mainland and insular Southeast Asia, with its range extending onto islands in the Indo-West-Pacific. This snail is, as one of the most frequent and major first intermediate host, an important vector for digenic trematodes causing several human diseases. As a typical thiarid *T. granifera* is viviparous and parthenogenetic, with various embryonic stages up to larger shelled juveniles developing within the female's subhemocoelic (i.e non-uterine) brood pouch. Despite the known conchological disparity in other thiarids as well as this taxon, in Thailand *Tarebia* has been reported with the occurrence of one species only. In light of the polytypic variations found in shell morphology of freshwater snails in general and this taxon in particular, the lack of a modern taxonomic-systematic revision, using molecular genetics, has hampered more detailed insights to date, for example, into the locally varying trematode infection rates found in populations of *Tarebia* from across its range in Thailand as well as neighboring countries and areas. Here, we integrate evidence from phylogeographical analyses based on phenotypic variation (shell morphology, using biometry and geometric morphometrics) with highly informative and heterogeneous mtDNA sequence data (from the gene fragments cytochrome c oxidase subunit 1 and 16 S rRNA). We evaluate both the morphological and molecular genetic variation (using several phylogenetic analyses, including haplotype networks and a dated molecular tree), in correlation with differences in the reproductive biology among populations of *Tarebia* from various water bodies in the north, northwest, central, and south of Thailand, supplementing our respective analyses of parasite infections of this thiarid by cercaria of 15 trematode species, reported in a parallel study. Based on the comparison of topotypical material from the island of Timor, with specimens from 12 locations as reference, we found significant, albeit not congruent variation of both phenotype and genotype in *Tarebia granifera*, based on 1,154 specimens from 95 Thai samples, representing a geographically wide-ranging, river-based cross-section of this country. Our analyses indicate the existence of two genetically distinct clades and hint at possible species differentiation within what has been traditionally considered

as *T. granifera*. These 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. Grounded on the site-by-site analysis of individual *Tarebia* populations, our country-wide chorological approach focussing on the conchologically distinct and genetically diverse lineages of *Tarebia* allows to discuss questions of this either reflecting subspecific forms versus being distinct species within a narrowly delimited species complex. Our results, therefore, provide the ground for new perspectives on the phylogeography, evolution and parasitology of Thai freshwater gastropods, exemplified here by these highly important thiarids.

Introduction

Thailand is situated in one of the most biodiverse areas of the world (e.g. Baimai 2010). Located in the center 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 little attention. Thailand is bordered to the north by Myanmar and Laos, to the east by Laos and Cambodia, to the south by the Gulf of Thailand and Malaysia, and to the west by 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 divide Thailand into two regions, viz. the Indochinese region and the Sundaic region separated by the Isthmus of Kra, a biogeographical barrier believed to be affected by sea level change in the past (e.g. Bruyn et al. 2005, Parnell 2013, Dejtaradol et al. 2016). For example, in contrast to those species among birds of the Northern Highland with Chinese affinities, a number of species in the Southern Peninsula are related to those from the Sundaic region (e.g. Lekagul and Round 1991). However, the Thai peninsula not only forms a barrier to the distribution of several groups, but is also an important bridge in the biogeography of Southeast Asia, connecting taxa of northern and southern biotas.

In addition, Thailand can be divided into geographical regions based on distinct drainage basins; 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 basin 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.

Among the aquatic biota, limnic molluscs are diverse, and include about 280 species of fresh and brackish water gastropods (Brandt 1974). Studies trying to elucidate the origin of biodiversity and mechanisms of speciation in diverse systems have focused primarily on vertebrates (mostly birds and fishes), while other groups, particularly invertebrates, remained widely untested. As shown by Schwenk et al. (2008) and Glaubrecht (1993, 1996, 2009, 2010, 2011, and literature therein), molluscs and in particular freshwater gastropods hold the same promise for studying 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; see e.g. the discussion of freshwater gastropods as model of speciation and evolutionary systematics in Glaubrecht (2006, 2009, 2010, 2011).

Accordingly, non-marine molluscs in Thailand should receive more attention and focus on studies looking into species diversity and contributing to solving fundamental questions and the evolution of faunal diversity. However, biological information on gastropods in Thai river systems and lakes is generally scarce and often lacks recently collected material or available former museum collections which hampers more in-depth studies. This is 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, in particular 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 Cerithioidea is an ecologically and phylogenetically important, albeit essentially marine caenogastropod group, with its freshwater members in Southeast Asia acting as first intermediate hosts of a wide array of diverse trematodes (see details and references e.g. in Dechruksa et al. 2007, Ukong et al. 2007, Krailas et al. 2011, 2012, 2014, Veeravechsukij et al. 2018).

Cerithioidean freshwater taxa were long subsumed under the historical concept of "melaniids", which was later uncritically replaced by the family assignment to the Thiaridae (see e.g. 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 and molecular phylogenetic studies corroborating these earlier findings (Lydeard et al. 2002, Strong et al. 2011). For example, molecular phylogenic analysis now supports the inclusion of the Thai genera *Brotia*, *Paracrostoma* and *Adamietta* into the Pachychilidae from members of the Thiaridae sensu stricto, representing two independent invasions and colonisations of freshwater habitats in the tropics worldwide (e.g. Glaubrecht 1996, 2011, Köhler and Glaubrecht 2001, 2006, Glaubrecht and Köhler 2004, Lydeard et al. 2002, Glaubrecht et al. 2009, Strong et al. 2011).

Thiaridae which are found mostly in tropical to subtropical regions worldwide, inhabit virtually all freshwater and brackish-water bodies, both in lotic (including springs, creeks, rivers and streams) and lentic habitats (lakes and ponds). They are essentially, and presumably originally, widely distributed throughout Southeast Asia and in Australia (see Glaubrecht 1996, 2011, Glaubrecht et al. 2009). With an estimated 60 to 200 species in about 12 genera, but many more named taxa (Glaubrecht unpublished data), the thiarids are yet in need of a comprehensive phylogenetic analysis as well as thorough systematic revision. The largely unresolved taxonomy of thiarids is characterized by a high frequency of redundancy (in the order of up to 70 % of all named species) due to the typological approach of naming each and every phenotype as distinct (morpho-)species, as was exemplarily shown for the Australian thiarid taxa (see e.g. Glaubrecht 2010, 2011, Glaubrecht et al. 2009).

To complicate matters, Thiaridae are both parthenogenetic, with many populations essentially representing clones of individual females, and viviparous, with various typical embryonic stages developing within the female's non-uterine, i.e. subhemocoelic brood pouch, and with distinct reproductive strategies to be found, viz. eu-viviparous vs. ovo-viviparous modes that are correlated with the amount of nourishment provided by the female (Glaubrecht 1996, 1999, 2006, 2011, Glaubrecht et al. 2009, Maaß and Glaubrecht 2012).

In Thailand, the Thiaridae are represented by several described species, mostly being conchologically highly variable, such as e.g. *Melanoides tuberculata* (O. F. Müller, 1774), *Mieniplotia scabra* (O. F. Müller, 1774) or *Tarebia granifera* (Lamarck, 1816), the latter being 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; see for detailed discussion e.g. Glaubrecht 2009, 2010, 2011, Glaubrecht et al. 2009, Maaß and Glaubrecht 2012, Dechruksa et al. 2013).

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 (e.g. Rensch 1934, Benthem-Jutting 1937, 1959, Brandt 1974, Starmühlner 1976) and our analyses here (see Fig. 1), 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 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 has been introduced in North, Central and South America. *T. granifera* was also first reported in South Africa in 1999, established in a concrete lined reservoir in Mandeni, northern KwaZulu-Natal (Appleton and 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 spread of *T. granifera* increasing substantially between 2001 and 2006 (Wolmarans and de 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 in particular in Thailand, *T. granifera* is known as 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 (e.g. Dechruksa et al. 2007, Krailas et al. 2011, 2012, 2014). As we show in a parallel study (see Veeravechsukij et al. 2018), 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 also those water bodies that are subject to rapid environmental change in an increasingly human-dominated world.

Therefore, being able to ecologically adopt 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. 2). In Thailand, this snail has been reported with only a single species by Brandt (1974), though, 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

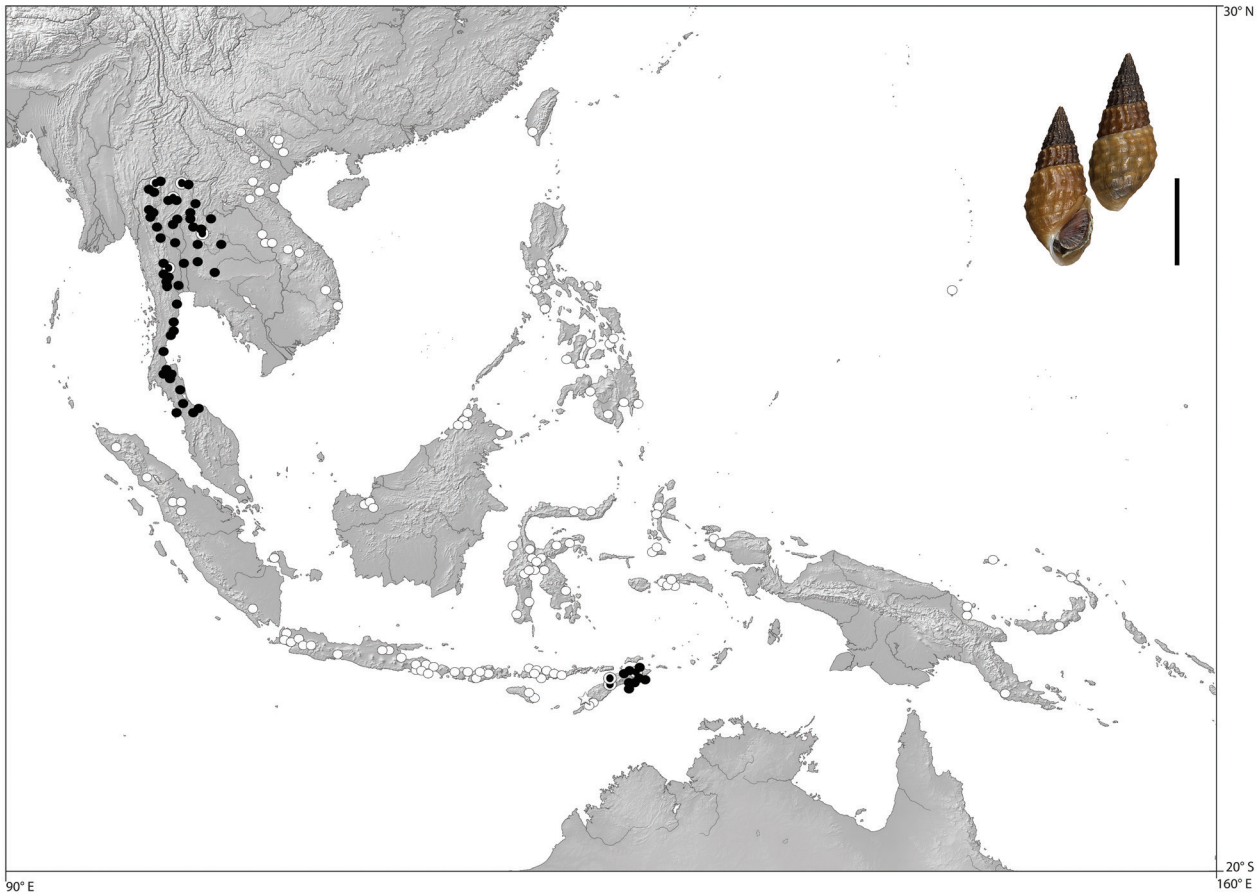

Figure 1. Distribution of the freshwater thiarid snail *Tarebia granifera* (Lamarck, 1816) across its range in Southeast Asia, with the focus on occurrences in Thailand, contrasted with type and topotypical material from the island of Timor. Asteriks: type locality of "*Melania*" *granifera* Lamarck, 1816, reconstructed to originate from near Kupang in western Timor (see text for more details); black dots: sequenced material used in this study; white dots: shell material from museum collections analysed and literature records; white dots with black dot inside: wet material preserved in ethanol.

a considerably high degree of variation in shell morphology, particularly in size, shape, sculpture, and colouration. 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. 2a–g); (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. 2h–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 as to intra- and interspecific species diversity, neither in Thailand nor elsewhere in adjacent regions. Here, we present results from our study of the morphological and molecular genetic variation in combination with the distributional and phylogenetic relationships as well as differences in the reproductive biology of thiarids, in particular in populations from the North, Central, Northeast and South of Thailand. We focus on the two phylogenetically highly informative and heterogeneous mitochondrial gene fragments cytochrome c oxidase subunit 1 and 16 S rRNA genes. 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 compared the populations of Thailand as our special focus to topotypical samples recently collected from the type locality Timor as reference.

Viewed from the background of a molecular backbone phylogeny we are, finally, able to analyse a suite of questions concerning the nature of cladogenesis, phylogeography and reproductive biology in these snails, in context with the infections by various trematodes, eventually hoping to elucidate the interrelationship and co-existence of human-infectious trematode parasites and their first intermediate snail hosts.

Figure 2. 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, SUT0516142). **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.

Material and methods

Drainage and river systems of Thailand

The National Committee on Hydrology separates Thailand into 25 distinct hydrological units or river basins, which are used in this study as an established geographical reference. These units comprise the following rivers and drainage systems: Salween, Mekong, Kok, Shi, Moon, Ping, Wang, Yom, Nan, Chao Phraya, Sakaekrang, Pasak, Tha Chin, Mae Klong, Prachinburi, Bang Pakong, Tonle Sap, Peninsular East Coast, Phetchaburi, Peninsular West Coast, Southeast Coast, Tapi, Songkhla Lake, Pattani and Southwest Coast. These catchment and drain-

age systems are re-grouped here into seven areas, each with specific characteristics; refer to Figs 1, 4a, 8 for a graphical overview:

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

Sampling

Specimens of *Tarebia granifera* were collected throughout Thailand. For reference, we compare with samples available to MG from Timor Leste through the courtesy of Vince Kessner, who collected there recently. 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).

Geographic data and maps

To reconstruct in detail the distributional range, in addition to own collecting activities in most parts of the region, material was analysed in several major museum collections, as well as literature records which were sufficiently verifiable as to the species identity (in general documented by descriptions and, even better, figures of shells collected).

Geographic coordinates of newly collected material were taken with a GPS device at the sampling site (WGS84 datum). 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 (NaturalEarth, www.naturalearthdata.com) 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).

Shell morphology and biometry

The snails identified as belonging to *Tarebia* were grouped according to their morphological characteristics and geographic origin in four preliminary classes or morphs (see Fig. 2): 1.) specimens from Timor corresponding morphologically to *T. granifera*, i.e. without spiral pattern of narrow brown bands (Timor), 2.) specimens from Thailand corresponding to *T. granifera* (morph A), 3.) specimens from Thailand corresponding to *T. lineata*, i.e. specimens with a pattern of narrow brown spiral ridges (morph B), and 4.) *Tarebia* specimens from Thailand with broad brown spiral bands (morph C). In a second approach, specimens were grouped according to mitochondrial clades for morphological comparisons and analyses (see below).

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. 3a). We were able to obtain these measurements from a total of 1,154 specimens. Analyses of shell parameters were performed using RStudio (RStudio Team 2016), with packages "ade4" (Chessel et al. 2004), "lawstat" (Hui et al. 2008), "agricolae" (Mendiburu 2010) "dunn.test" (Dinno 2017), and "car" (Fox and Weisberg 2011). For further testing the data set was partitioned in different predefined groups. These were: (a) four different morphs (see above) and (b) two different mitochondrial clades based on our molecular genetic analyses (see below). These different subdivisions of the data required slightly different approaches with regard to statistical testing. For the four different morphs, we first tested for normal distribution. Hence, we conducted the Shapiro-Wilk test for each subgroup individually. In case all measured variables for each group were normally distributed (Shapiro-Wilk-test $p > 0.05$), we performed an analysis of variance (ANOVA). If significant, it was followed by a Bonferroni-corrected LSD-Test. If at least one test for normal

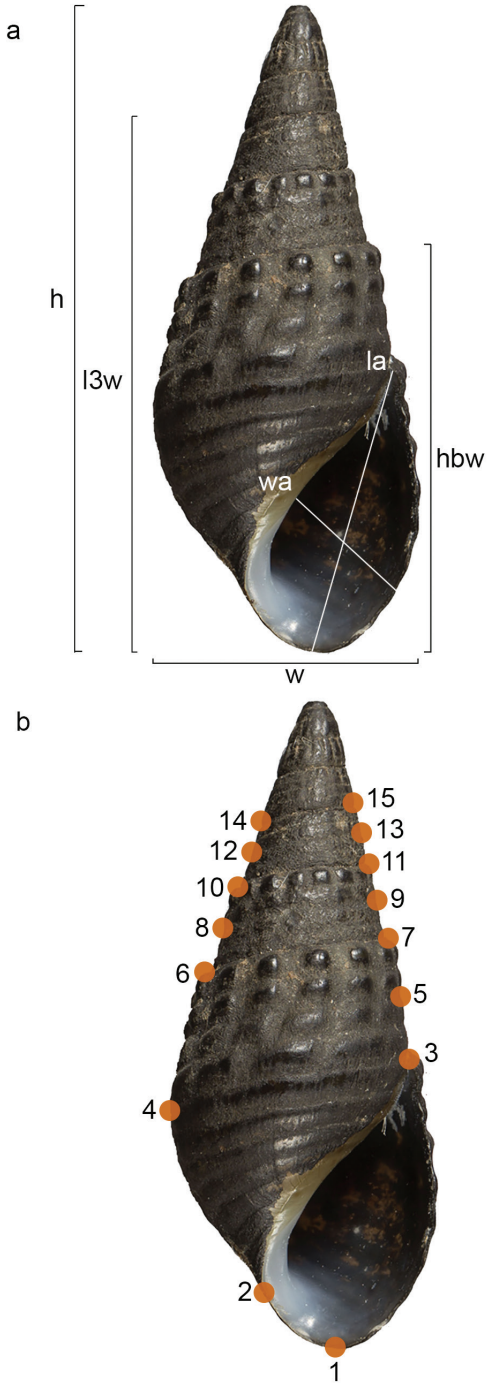

Figure 3. Biometrical parameters (**a**) and position of landmarks (**b**). Abbreviations: height of shell (h), width of shell (w), length of aperture (la), width of aperture (wa), height of body whorl (hbw) and height of last three whorls (l3w).

distribution (Shapiro-Wilk-test $p < 0.05$) was not significant, we instead deployed a Kruskal-Wallis-rank sum test. If the latter was found to be significant, a Bonferroni-corrected Dunn-test was conducted subsequently.

For the two different mitochondrial clades, the Shapiro-Wilk-test was performed on all measured variables for each group individually to test for normal distribution. If at least one group was not normally distributed, we conducted a Wilcoxon signed rank test with continuity correction, to test for significant differences between clades. If the data for both groups were normally distributed, a Levene-test based on absolute deviations from the mean was performed to check for homoscedasticity. In case homoscedasticity was detected, we tested different groups using a two-sample t-test. Otherwise, we performed Welch's heteroscedastic t-test.

Geometric morphometrics

All available type specimens and the other examined material was photographed by remote shooting with EOS Utility 2.12.2.1 for Windows (Canon Inc., Tokyo, Japan) and Digital Photo Professional 3.12.51.2 for Windows (Canon Inc.) using a digital camera (EOS 5D MKII with Canon macro photograph lens MP-E 65 mm and compact macro lens EF 50 mm, Canon Inc.). Shell orientation was adjusted so that the apertural plane of the shell was perpendicular in relation to the optical axis of the camera and the shell's columella parallel to the background. Photo stacks were assembled in Helicon Focus 5.3.14.2 for Windows (Helicon Soft Ltd., Kharkiv, Ukraine). The images were then edited with Photoshop CS6 (Adobe Systems Inc.).

A total of 1,169 standardized images of adult, unbroken shells could be included in our geometric morphometrics data set. Using tpsUtil version 1.74 (Rohlf 2017a), a tps-file including all specimens was assembled. We placed 15 landmarks (see Fig 3b for landmark positions) with tpsDig2 version 2.30 (Rohlf 2017b). Data were analysed in RStudio, with "geomorph" (Adams and Otárola-Castillo 2013) and all packages listed for our biometry data analysis. After performing a Procrustes superimposition, a principal component analysis (PCA) was conducted to identify major axes of variance and to reduce dimensionality. Only axes with a relevant proportion of variance (> 0.05) were included. Following this procedure, we analysed the data set with partitions and tests as described above for the analyses biometric measurements (for each partition and principal component).

Reproductive biology – 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 Glaubrecht et al. (2009) and Maaß and Glaubrecht (2012). The shells were cracked with a small vice, the operculum cut off from the posterior part of the foot using a scalpel and the soft body opened under a stereo microscope. 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 marsupium, according to the nine standard size classes established for Thiaridae before by Glaubrecht 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.) ju-

veniles between 2.6 mm and 3.0 mm, and 9.) juveniles > 3.0 mm. We compared brood pouch contents for the different predefined morphological/geographic groups as described above, the main mitochondrial clades and for the different river systems in Thailand.

Molecular Phylogeny

Sequences from 131 specimens of *T. granifera* from 95 populations in Thailand and 12 specimens from 11 populations in Timor Leste were generated (see Table 1). Two specimens of *Thiara amarula* (Linnaeus, 1748) were selected as outgroup. Total genomic DNA was extracted from ethanol-preserved foot tissue using a CTAB protocol (Winnepenninckx et al. 1993). For phylogenetic analyses fragments of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*; 658 bp) gene using the primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3', Folmer et al. 1994) and HCO2198var (5'-TAW ACT TCT GGG TGK CCA AAR AAT-3', Rintelen et al. 2004) and the 16 S rRNA (16S; c. 780 bp aligned) gene using the primers 16S_F_Thia2 (5'-CTT YCG CAC TGA TGA TAG CTA G-3', Rintelen, unpublished data, see Gimnich 2015) and H3059 (5'-CCG GTY TGA ACT CAG ATC ATG T-3', Wilson et al. 2004) were amplified by PCR. 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. After an initial denaturation step of 3 min at 94 °C, 35 cycles of 30 s at 94 °C, 60 s at 45–62 °C and 60–120 s at 72 °C were performed, followed by a final extension step of 5 min at 72 °C. 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).

Forward and reverse strands were assembled using the program Geneious (Biomatters Limited, Auckland, New Zealand) and corrected by eye. The protein coding *cox1* sequences were aligned with MUSCLE (Edgar 2004) as implemented in MEGA7 (Kumar et al. 2016) under default settings. The 16S sequences were aligned with MAFFT (Katoh and Standley 2013) using the Q-INS-i iterative refinement algorithm and otherwise default settings, because this algorithm has been described to perform better for the alignment of sequence data sets that may contain deletions and insertions than alternative multiple sequence alignment methods (Golubchik et al. 2007).

For information on vouchers and GenBank accession numbers, see Table 1. Pairwise genetic p-distances for the *cox1* and 16S data sets were calculated with MEGA7.

Phylogenetic analyses

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 partitions 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 and Holder 2010).

Molecular species delimitation and dating

We used the General Mixed Yule-coalescent (Pons et al. 2006) in its Bayesian implementation (bGMYC) (Reid and 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. We constructed ultrametric trees for the concatenated 16S and *cox1* data set

Table 1. Collection voucher numbers, geographic coordinates of sampling sites and GenBank accession numbers for specimens of *Tarebia granifera* (Lamarck, 1816) used in the molecular analyses.

Voucher Number	Latitude	Longitude	GenBank accession number	
			cox1	16 S rRNA
SUT 0514050	18°17'08.5"N	098°39'16.9"E	MK000303	MK025577
SUT 0514051	18°17'04.4"N	098°39'15.0"E	MK000304	–
SUT 0514054 (A)	18°17'23.0"N	098°39'03.6"E	MK000307	MK025580
SUT 0514054 (B)	18°17'23.0"N	098°39'03.6"E	–	MK025581
SUT 0514052 (B)	18°16'26.1"N	098°38'54.0"E	MK000305	MK025578
SUT 0514052 (C)	18°16'26.1"N	098°38'54.0"E	MK000306	MK025579
SUT 0515081 (B_1)	19°28'33.6"N	098°07'02.4"E	MK000331	–
SUT 0515081 (B_9)	19°28'33.6"N	098°07'02.4"E	–	MK025609
SUT 0515077	19°25'31.1"N	097°59'27.2"E	–	MK025606
SUT 0515083	19°22'19.6"N	098°26'35.9"E	MK000332	MK025610
SUT 0515078	19°21'54.8"N	097°58'10.7"E	MK000329	MK025607
SUT 0515079 (C_3)	19°15'31.6"N	097°54'44.6"E	MK000330	–
SUT 0515079 (C_5)	19°15'31.6"N	097°54'44.6"E	–	MK025608
SUT 0516119	18°51'22.2"N	100°11'09.1"E	MK000350	MK025628
SUT 0514045 (B_1)	18°56'00.5"N	099°38'54.6"E	MK000300	–
SUT 0514045 (B_2)	18°56'00.5"N	099°38'54.6"E	–	MK025574
SUT 0514044 (A)	18°52'47.5"N	099°40'01.0"E	MK000298	MK025572
SUT 0514044 (B_1)	18°52'47.5"N	099°40'01.0"E	MK000299	–
SUT 0514044 (B_2)	18°52'47.5"N	099°40'01.0"E	–	MK025573
SUT 0514046	18°46'39.8"N	099°38'38.7"E	MK000301	MK025575
SUT 0516124	18°42'14.8"N	099°35'31.7"E	MK000353	MK025631
SUT 0515090	19°11'30.4"N	101°12'13.2"E	MK000336	MK025614
SUT 0516114	18°51'45.1"N	100°28'37.1"E	MK000348	MK025625
SUT 0516108	18°05'03.1"N	100°13'00.1"E	–	MK025621
SUT 0516113 (B)	18°00'50.6"N	100°08'22.6"E	MK000346	MK025623
SUT 0516113 (C_1)	18°00'50.6"N	100°08'22.6"E	–	MK025624
SUT 0516113 (C_2)	18°00'50.6"N	100°08'22.6"E	MK000347	–
SUT 0516112 (B_2)	17°52'19.5"N	100°18'02.1"E	MK000345	–
SUT 0516112 (B_3)	17°52'19.5"N	100°18'02.1"E	–	MK025622
SUT 0513019 (A)	17°52'29.5"N	100°18'25.6"E	MK000292	–
SUT 0513019 (B)	17°52'29.5"N	100°18'25.6"E	–	MK025563
SUT 0513023	17°52'51.3"N	100°16'14.9"E	–	MK025564
SUT 0516109	17°43'42.3"N	099°58'49.6"E	MK000344	–
SUT 0515075 (B_1)	17°13'23.4"N	098°13'34.2"E	MK000327	–
SUT 0515075 (B_2)	17°13'23.4"N	098°13'34.2"E	–	MK025604
SUT 0515076 (B_1)	17°26'04.8"N	098°03'33.3"E	MK000328	–
SUT 0515076 (B_2)	17°26'04.8"N	098°03'33.3"E	–	MK025605
SUT 0516126 (C_1)	16°52'29.3"N	099°07'13.6"E	MK000355	–
SUT 0516126 (C_2)	16°52'29.3"N	099°07'13.6"E	–	MK025633
SUT 0515073	16°42'38.5"N	098°30'22.2"E	MK000326	MK025602
SUT 0515072	16°41'39.3"N	098°31'04.4"E	MK000325	MK025601
SUT 0515074	16°40'58.4"N	098°31'06.9"E	–	MK025603
SUT 0516103 (B_1)	17°33'16.2"N	099°29'48.2"E	MK000343	–
SUT 0516103 (B_2)	17°33'16.2"N	099°29'48.2"E	–	MK025620
SUT 0515086 (A_1)	17°01'07.6"N	100°55'36.0"E	MK000333	–
SUT 0515086 (A_2)	17°01'07.6"N	100°55'36.0"E	–	MK025611
SUT 0515087	16°57'21.3"N	100°55'31.0"E	MK000334	MK025612
SUT 0516118 (A)	16°52'13.1"N	100°50'17.4"E	MK000349	MK025626
SUT 0516118 (B)	16°52'13.1"N	100°50'17.4"E	–	MK025627
SUT 0515067	16°50'36.3"N	100°45'16.1"E	MK000319	MK025595
SUT 0516130	16°39'46.3"N	101°08'09.8"E	–	MK025637
SUT 0516121	16°37'23.8"N	100°54'00.5"E	–	MK025629
SUT 0516120	16°36'01.3"N	100°54'29.9"E	MK000351	–
SUT 0516123	16°34'24.1"N	100°59'23.6"E	MK000352	MK025630
SUT 0515088 (A_1)	16°32'51.7"N	100°54'03.2"E	MK000335	–
SUT 0515088 (A_2)	16°32'51.7"N	100°54'03.2"E	–	MK025613
SUT 0516129 (B_2)	16°32'25.6"N	101°04'58.4"E	MK000358	–
SUT 0516129 (B_3)	16°32'25.6"N	101°04'58.4"E	–	MK025636
SUT 0514041	15°47'54.2"N	101°14'08.1"E	–	MK025570
SUT 0514042	15°47'52.2"N	101°13'54.4"E	MK000296	–
SUT 0514040 (B)	15°47'29,7"N	101°13'30,7"E	–	MK025568
SUT 0514040 (C)	15°47'29,7"N	101°13'30,7"E	–	MK025569
SUT 0514043 (B_1)	15°47'19.3"N	101°15'07.4"E	MK000297	–
SUT 0514043 (B_2)	15°47'19.3"N	101°15'07.4"E	–	MK025571

Voucher Number	Latitude	Longitude	GenBank accession number	
			cox1	16 S rRNA
SUT 0515068	17°23'24.7"N	101°22'27.3"E	MK000320	MK025596
SUT 0516125	17°04'38.0"N	101°29'20.6"E	MK000354	MK025632
SUT 0516128 (B_4)	17°03'03.9"N	101°31'38.7"E	MK000357	–
SUT 0516128 (B_5)	17°03'03.9"N	101°31'38.7"E	–	MK025635
SUT 0515064 (B_4)	16°34'45.6"N	102°50'22.5"E	MK000317	–
SUT 0515064 (B_5)	16°34'45.6"N	102°50'22.5"E	–	MK025593
SUT 0516131 (B)	14°35'32.3"N	101°50'30.1"E	MK000359	MK025638
SUT 0516131 (C)	14°35'32.3"N	101°50'30.1"E	MK000360	MK025639
SUT 0516135	12°37'50.0"N	101°20'35.0"E	MK000362	MK025642
SUT 0516127 (B_1)	15°40'59.6"N	100°14'59.3"E	MK000356	–
SUT 0516127 (B_2)	15°40'59.6"N	100°14'59.3"E	–	MK025634
SUT 0516132	14°55'12.3"N	101°13'10.9"E	MK000361	MK025640
SUT 0516133	14°44'06.4"N	101°11'31.4"E	–	MK025641
SUT 0515055 (C_1)	13°49'01.2"N	100°02'27.9"E	MK000308	–
SUT 0515055 (C_2)	13°49'01.2"N	100°02'27.9"E	–	MK025582
SUT 0515091 (C_1)	14°37'25.9"N	098°43'40.5"E	MK000337	–
SUT 0515091 (C_2)	14°37'25.9"N	098°43'40.5"E	–	MK025615
SUT 0515092 (B_1)	14°26'03.0"N	098°51'14.7"E	MK000338	–
SUT 0515092 (B_2)	14°26'03.0"N	098°51'14.7"E	–	MK025616
SUT 0515093	14°14'27.6"N	099°03'55.9"E	MK000339	–
SUT 0515061 (B)	13°54'18.1"N	099°23'07.8"E	–	MK025591
SUT 0515061 (C)	13°54'18.1"N	099°23'07.8"E	MK000316	MK025592
SUT 0515060 (B_1)	13°51'17.7"N	099°22'58.9"E	MK000315	–
SUT 0515060 (B_2)	13°51'17.7"N	099°22'58.9"E	–	MK025590
SUT 0515059 (A_1)	13°46'44.8"N	099°25'26.7"E	MK000313	–
SUT 0515059 (A_2)	13°46'44.8"N	099°25'26.7"E	–	MK025588
SUT 0515059 (B)	13°46'44.8"N	099°25'26.7"E	MK000314	MK025589
SUT 0515058	13°45'00.5"N	099°26'27.4"E	MK000312	MK025587
SUT 0515057 (B_1)	13°41'28.1"N	099°29'08.1"E	MK000311	–
SUT 0515057 (B_2)	13°41'28.1"N	099°29'08.1"E	–	MK025586
SUT 0515056 (A)	13°37'00.15"N	099°24'36.9"E	–	MK025583
SUT 0515056 (B)	13°37'00.15"N	099°24'36.9"E	MK000309	MK025584
SUT 0515056 (C)	13°37'00.15"N	099°24'36.9"E	MK000310	MK025585
SUT 0515070 (B_1)	13°32'54.2"N	099°21'42.3"E	MK000322	–
SUT 0515070 (B_2)	13°32'54.2"N	099°21'42.3"E	–	MK025598
SUT 0515070 (C)	13°32'54.2"N	099°21'42.3"E	MK000323	MK025599
SUT 0515069	13°32'52.2"N	099°17'33.7"E	MK000321	MK025597
SUT 0515071	13°32'07.4"N	099°20'31.8"E	MK000324	MK025600
SUT 0515066	13°19'29.2"N	099°14'22.0"E	MK000318	MK025594
SUT 0513032	12°48'02.7"N	099°58'53,2"E	MK000293	MK025565
SUT 0516146 (B_3)	11°55'29.1"N	099°42'40.9"E	MK000372	–
SUT 0516146 (B_7)	11°55'29.1"N	099°42'40.9"E	–	MK025652
SUT 0516146 (C)	11°55'29.1"N	099°42'40.9"E	MK000373	MK025653
SUT 0514037 (A_1)	11°36'50.0"N	099°40'07.9"E	–	MK025566
SUT 0514037 (A_7)	11°36'50.0"N	099°40'07.9"E	MK000294	–
SUT 0514038	11°26'14.4"N	099°26'33.0"E	MK000295	MK025567
SUT 0511149	10°44'28,8"N	099°12'54.9"E	MK000291	MK025562
SUT 0516137 (B_1)	08°48'06.9"N	099°26'45.1"E	MK000363	–
SUT 0516137 (B_2)	08°48'06.9"N	099°26'45.1"E	–	MK025643
SUT 0514048	08°52'18.8"N	099°25'59.1"E	MK000302	MK025576
SUT 0516147	09°12'39.8"N	099°11'55.7"E	MK000374	MK025654
SUT 0516148	09°12'25.7"N	099°12'25.7"E	MK000375	MK025655
SUT 0516142 (B)	09°08'07.2"N	099°40'31.6"E	MK000367	MK025647
SUT 0516142 (C)	09°08'07.2"N	099°40'31.6"E	MK000368	MK025648
SUT 0516139	08°47'23.0"N	099°38'13.2"E	MK000365	MK025645
SUT 0516145 (B_1)	08°43'17.3"N	099°40'14.8"E	MK000371	–
SUT 0516145 (B_2)	08°43'17.3"N	099°40'14.8"E	–	MK025651
SUT 0515097 (A_1)	08°10'20.8"N	098°47'37.6"E	MK000341	–
SUT 0515097 (A_2)	08°10'20.8"N	098°47'37.6"E	–	MK025618
SUT 0515098	08°09'49.2"N	098°47'50.9"E	MK000342	MK025619
SUT 0515095	07°22'11.0"N	099°40'47.9"E	MK000340	MK025617
SUT 0516138	07°42'48.3"N	099°51'33.6"E	MK000364	MK025644
SUT 0516144 (A_1)	07°13'36.6"N	100°31'41.8"E	–	MK025650
SUT 0516144 (A_2)	07°13'36.6"N	100°31'41.8"E	MK000370	–
SUT 0516141 (B_1)	06°52'29.3"N	100°19'48.4"E	MK000366	–
SUT 0516141 (B_2)	06°52'29.3"N	100°19'48.4"E	–	MK025646

Voucher Number	Latitude	Longitude	GenBank accession number	
			cox1	16 S rRNA
SUT 0516143	06°49'29.5"N	100°19'49.7"E	MK000369	MK025649
ZMH 119364	08°31'32.3"S	125°58'50.0"E	–	MK025664
ZMH 119359	09°00'30.6"S	126°03'45.0"E	MK000382	MK025661
ZMH 119358	09°00'44.8"S	126°03'49.2"E	MK000381	MK025660
ZMH 119354	09°01'11.4"S	126°03'58.3"E	MK000377	MK025656
ZMH 119357	08°26'36.3"S	126°28'11.4"E	MK000380	MK025659
ZMH 119356	08°20'32.1"S	127°01'07.9"E	MK000379	MK025658
ZMH 119353	08°25'34.6"S	126°41'42.5"E	MK000376	–
ZMH 119362	08°56'47.1"S	124°58'28.4"E	MK000385	–
ZMH 119355	08°44'36.4"S	126°22'49.7"E	MK000378	MK025657
ZMH 119360	08°47'05.0"S	126°22'32.0"E	MK000383	MK025662
ZMH 119363	08°47'05.0"S	126°22'32.0"E	MK000386	–
ZMH 119361	09°01'59.6"S	125°59'35.9"E	MK000384	MK025663

Outgroup: *Thiara amarula*: ZMB (Museum für Naturkunde, Berlin, Germany) 107472, Indonesia, Ambon Island (*cox1*: MK000289; 16S: MK025560) and ZMB 191489, Indonesia, Obi Island (*cox1*: MK000290; 16S: MK025561).

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 50,000th 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 and Carstens 2012) was run for 100,000 generations, discarding the first 90,000 generations as burn-in and sampling every 100 generations.

We dated the divergence times for the main clades of *Tarebia* included in this study using the Bayesian algorithm implemented in Beast 2 based on the concatenated mitochondrial data assuming a strict molecular clock as the test implemented in MEGA 7 (Kumar et al. 2016) rejected a strict molecular clock at $\alpha = 0.05$. The same partitioning scheme and nucleotide substitution models as in the MrBayes analysis described above were used. As tree prior the Yule speciation model was chosen. In the absences of fossil calibration points, a constant substitution rate of 1% per Ma was assumed as has previously been done by Köhler and Glaubrecht (2010) for related freshwater cerithioideans in the Pachychilidae. The Beast 2 analysis was run for 10,000,000 generations with a sampling frequency set to 10,000. Tracer 1.7 (Rambaut et al. 2018) was used to assess convergence of runs and to check whether effective sample sizes for all estimated parameters were above 200. A maximum clade credibility tree with median node heights was calculated with Treeannotator v1.8.2 from the BEAST 2 package discarding 10% of generations as burn-in.

Results

Tarebia granifera Lamarck, 1816

Type material. 4 syntypes (MHNG 1093/72/1-4).

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 Indo-West-Pacific and Australia in particular (see Glaubrecht 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. 1). 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, Lamarck (1822) described this new species and its shell morphology in more detail; see also Mermod (1952: 75, fig. 137). Adams and Adams (1854) transferred *Melania 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 (1874–1879) 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* (see Benthem-Jutting 1956). Starmühlner (1976), in his thorough faunistic revision, provided an extensive list of synonyms for this taxon.

In addition, in the past 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, for Thailand, Brandt (1974) considered and employed *Tarebia granifera* as the only congeneric species to exist there; as was also done by Glaubrecht (1996).

Biogeography

The distributional range of *Tarebia granifera* (Fig. 1) 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 Indo-West 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 substrata, 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 at particular locations or specific habitat and/or populations 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.

Material examined

In the following we document here in detail the geographical origin of material studied from Thailand, in comparison with the syntypes as well as topotypical material from Timor as reference (see above). Data on other localities indicated in Fig. 2 to depict the extension of the entire distribution range of the species, will be provided and analysed elsewhere (Glaubrecht et al., in prep.).

Thailand:1

Pai drainage (Salween river system): Mae Hong Son province: Pang Mapha district, Huai Pa Hung, 19°22’20”N, 098°26’36”E, 435 m (SUT 0515083, 03. V. 2015); Mueang Mae Hong Son district, Huay Nam Kong, 19°28’34”N, 098°07’02”E, 425 m (SUT 0515081, 03. V. 2015); Tham Pla, 19°25’31”N, 097°59’27”E, 300 m (SUT 0515077, 02. V. 2015); Pai River, 19°21’55”N, 097°58’11”E, 215 m (SUT 0515078, 02. V. 2015); Huay Sua Tao, 19°15’32”N, 097°54’45”E, 235 m (SUT 0515079, 02. V. 2015).

Moei drainage (Salween river system): Tak province: Tha Song Yang district, check point near Moei River, 17°13’23”N, 098°13’34”E, 130 m (SUT 0515075, 02. V. 2015); Mae Salit Luang harbour, 17°26’05”N, 098°03’33”E, 110 m (SUT 0515076, 01. V. 2015); Mae Sot district, Ban Wang Takhian, 16°42’39”N, 098°30’22”E, 195 m (SUT 0515073, 30. IV. 2015); Thong Dee harbour, 16°41’39”N, 098°31’04”E, 205 m (SUT 0515072, 30. IV. 2015); Ban Huay Muang, 16°40’58”N, 098°31’07”E, 200 m (SUT 0515074, 30. IV. 2015).

Ping drainage (Chao Phraya river system): Chiang Mai province: Chom Thong district, Mae Soy bridge, 18°17’23”N, 098°39’04”E, 270 m (SUT 0514054, 24. VI. 2014); Ban Huay Phang, 18°17’09”N, 098°39’17” E, 260 m, SUT 0514050, 25. VI. 2014; Ban Mae Suai Luang, 18°17’04”N, 098°39’15”E, 270 m (SUT 0514051, 25. VI. 2014); Ban Mai Saraphi, 18°16’26”N, 098°38’54”E, 275 m (SUT 0514052, 25. VI. 2014); Tak province: Mueang Tak district, Ban Pak Huay Mae Tho, 16°52’29”N, 099°07’14”E, 105 m (SUT 0516126, 10. III. 2016).

Wang drainage (Chao Phraya river system): Lampang province: Chae Hom district, Wang river, 18°56’01”N, 099°38’55”E, 375 m (SUT 0514045, 23. IV. 2014); Ban Thung Hang stream, 18°52’48”N, 099°40’01”E, 375 m (SUT 0514044, 23. IV. 2014); Huay MaeYuak, 18°46’40”N, 099°38’39”E, 350 m (SUT 0514046, 22. IV. 2014); km. 40 + 075 bridge, 18°42’15”N, 99°35’32”E, 330 m (SUT 0516124, 09. III. 2016).

Yom drainage (Chao Phraya river system): Phayao province: Chiang Muan district, Thansawan waterfall, 18°51’22”N, 100°11’09”E, 230 m (SUT 0516119, 08. III. 2016); Phrae province: Mueang Phrae district, Mae Nam Saai km 9/457 bridge, 18°05’03”N, 100°13’00”E, 170 m (SUT 0516108, 07. III 2016); Sung Men district, Mae Marn reservoir, 18°00’51”N, 100°08’23”E, 205 m (SUT 0516113, 07. III 2016); Sukhothai province: Si Satchanalai district, Tat Duen waterfall, 17°33’16”N, 099°29’48”E, 135 m (SUT 0516103, 06. III. 2016).

Nan drainage (Chao Phraya river system): Nan province: Bo Kluea district, Wa river, 19°11’30”N, 101°12’13”E, 715 m (SUT 0515090, 11. VI. 2015); Ban Luang district, Huay Si Pun reservoir, 18°51’45”N, 100°28’37”E, 430 m (SUT 0516114, 08. III. 2016); Uttaradit province: Tha Pla district, Kaeng Sai Ngam, 17°52’20”N, 100°18’02”E, 255 m (SUT 0516112, 07. III 2016); Kaeng Wang Wua, 17°52’30”N, 100°18’26”E, 230 m (SUT 0513019, 28. VI. 2013); Huai Nam Re Noi, 17°52’51”N, 100°16’15”E, 270 m (SUT 0513023, 28. VI. 2013); Laplae district, Mae pool waterfall, 17°43’42”N, 099°58’50”E, 125 m (SUT 0516109, 07. III. 2016).

Khek drainage (Chao Phraya river system): Phitsanulok province: Nakhon Thai district, Huai Nam Sai, 17°01’08”N, 100°55’36”E, 215 m (SUT 0515086, 20. V. 2015); Ban Kaeng Lat, 16°57’21”N, 100°55’31”E, 325 m (SUT 0515087, 20. V. 2015); Wang Thong district, Kaeng Sopha, 16°52’13”N, 100°50’17”E, 415 m (SUT 0516118, 08. III. 2016); Poi waterfall, 16°50’36”N, 100°45’16”E, 200 m (SUT 0515067, 08. II. 2015); Khao Kho district, Kaeng Wang Nam Yen, 16°37’24”N, 100°54’01”E, 710 m (SUT 0516121, 09. III. 2016); Rajapruek resort, 16°36’01”N, 100°54’30”E, 705 m (SUT 0516120, 09. III. 2016); Phetchabun province: Khao Kho district, Huai Sa Dao Pong, 16°34’24”N, 100°59’24”E, 320 m (SUT 0516123, 10. III. 2016); Kaeng Bang Ra Chan, 16°32’52”N, 100°54’03”E, 600 m (SUT 0515088, 21. V. 2015).

Pa Sak drainage (Chao Phraya river system): Phetchabun province: Lom Sak district, Than Thip waterfall, 16°39’46”N, 101°08’10”E, 375 m (SUT 0516130, 11. III.

2016); Khao Kho district, Samsipkhot waterfall, 16°32’26”N, 101°04’58”E, 385 m (SUT 0516129, 11. III. 2016); Wichian Buri district, Ban Wang Ta Pak Moo 13, 15°47’54”N, 101°14’08”E, 120 m (SUT 0514041, 27. VI. 2014); Huai Leng, 15°47’52”N, 101°13’54”E, 115 m (SUT 0514042, 27. VI. 2014); Ban Wang Tian, 15°47’30”N, 101°13’31”E, 120 m (SUT 0514040, 27. VI. 2014); Huay Range reservoir at Ban Wang Ta Pak, 15°47’19”N, 101°15’07”E, 140 m (SUT 0514043, 27. VI. 2014); Lop Buri province: Phatthan Nikhom district, Suanmaduea waterfall, 14°55’12”N, 101°13’11”E, 135 m (SUT 0516132, 26. IV. 2016); Sara Buri province: Muak Lek district, Dong Phaya Yen waterfall, 14°44’06”N, 101°11’31”E, 155 m (SUT 0516133, 26. IV. 2016). Nakhon Sawan province: Mueand Nakhon Sawan district, Bungboraped, 15°41’00”N, 100°14’59”E, 30 m (SUT 0516127, 10. III. 2016).

Loei drainage (Mekong river system): Loei province: Phu Ruea district, Pla Ba waterfall, 17°23’25”N, 101°22’27”E, 665 m (SUT 0515068, 07. II. 2015); Phu Luang district, km. 50/350 at Loei River, 17°04’38”N, 101°29’21”E, 675 m (SUT 0516125, 10. III. 2016); Tatkoktup waterfall, 17°03’04”N, 101°31’39”E, 690 m (SUT 0516128, 10. III. 2016).

Chee drainage (Mekong river system): Khon Kaen province: Mueang Khon Kaen district, Bueng Thung Sang, 16°34’46”N, 102°50’23”E, 170 m (SUT 0515064, 05. II. 2015).

Moon drainage (Mekong river system): Nakhon Ratchasima province: Pak Thong Chai district, Lamphraphloeng reservoir, 14°35’32”N, 101°50’30”E, 260 m (SUT 0516131, 22. III. 2016).

Khwae drainage (Mae Klong river system): Kanchanaburi province: Thong Pha Phum district, Hindad hot spring, 14°37’26”N, 098°43’41”E, 160 m (SUT 0515091, 27. VI. 2015); Sai Yok district, Sai Yok Yai waterfall, 14°26’03”N, 098°51’15”E, 105 m (SUT 0515092, 27. VI. 2015); Sai Yok Noi waterfall, 14°14’28”N, 099°03’56”E, 115 m (SUT 0515093, 27. VI. 2015).

Phachi drainage (Mae Klong river system): Kanchanaburi province: Dan Makham Tia district, Ban Thung Makham Tia, 13°54’18”N, 099°23’08”E, 45 m (SUT 0515061, 17. III. 2015); Ban Ta Pu, 13°51’18”N, 099°22’59”E, 55 m (SUT 0515060, 17. III. 2015); Ban Nong Phai, 13°46’45”N, 099°25’27”E, 70 m (SUT 0515059, 17. III. 2015); Ratchaburi province: Chom Bueng district, Phachi River bridge, 13°45’01”N, 099°26’27”E, 65 m (SUT 0515058, 17. III. 2015); Ban Dan Thap Tako, 13°41’28”N, 099°29’08”E, 80 m (SUT 0515057, 17. III. 2015); Ban Pa Wai, 13°37’00”N, 099°24’37”E, 75 m (SUT 0515056, 17. III. 2015); Suan Phueng district, Lum Nam Phachi, 13°32’54”N, 099°21’42”E, 110 m (SUT 0515070, 23. I. 2015); Huai Ban Bor, 13°32’07”N, 099°20’32”E, 135 m (SUT 0515071, 23. I. 2015); Huay Nueng, 13°32’52”N, 099°17’34”E, 155 m (SUT 0515069, 23. I. 2015); Suan Phueng district, Ban Purakom, 13°19’29”N, 099°14’22”E, 275 m (SUT 0515066, 23. I. 2015).

Mae Klong river system: Nakhon Pathom province: Mueang Nakhon Pathom district, pond on campus of Silpakorn University, 13°49’01”N, 100°02’28”E, 80 m (SUT 0515055, 13. I. 2015).

Gulf of Thailand: Rayong province: Mueang Rayong district, Mae Rumphueng beach (Mae Rumphueng canal), 12°37’50”N, 101°20’35”E, 10 m (SUT 0516135, 28. IV. 2016); Phetchaburi province: Cha-am district, Khlong Cha-am (Cha-am canal), 12°48’03”N, 099°58’53”E, 20 m (SUT 0513032, 16. X. 2013); Prachuap Khiri Khan province: Mueang Prachuap Khiri Khan district, Khlong Bueng reservoir, 11°55’29”N, 099°42’40.9” E, 70 m (SUT 0516146, 11. V. 2016); Huai Yang district, Khlong Huai Yang (Yang canal), 11°36’50”N, 099°40’08”E, 55 m (SUT 0514037, 23. XI. 2014); Bang Saphan district, Kar on waterfall, 11°26’14”N, 099°26’33”E, 55 m (SUT 0514038, 23. XI. 2014); Chumphon province: Tha Sae district, Krapo waterfall, 10°44’29”N, 099°12’55”E, 75 m (SUT 0511149, 2. VII. 2011); Surat Thani province: Tha Chang district, Khlong Tha Sai (Takhoei canal), 09°12’40”N, 099°11’56”E, 10 m (SUT 0516147, 04. VI. 2016); Phunphin district, Ban Tung Ao (Ta Khoei canal), 09°12’26”N, 099°12’26”E, 5 m (SUT 0516148, 04. VI. 2016); Don Sak district, Vibhavadi waterfall (Tha Thong canal), 09°08’07”N, 099°40’32”E, 25 m (SUT 0516142, 09. V. 2016); Ban Na San district, Dat Fa waterfall, 08°52’19”N, 099°25’59”E, 80 m (SUT 0514048, 22. XI. 2014); Khlong Klai (Nong Noi canal), 08°48’07”N, 099°26’45”E, 110 m (SUT 0516137, 9. V. 2016); Nakhon Si Thammarat province: Nopphitam district, Khlong Prong (Klai canal), 08°47’23”N, 099°38’13”E, 100 m (SUT 0516139, 09. V. 2016); Krung Ching waterfall, 08°43’17”N, 099°40’15”E, 195 m (SUT 0516145, 09. V. 2016); Phatthalung province: Si Banphot district, Khlong Tha Leung (Tha Nae canal), 07°42’48”N, 099°51’34”E, 70 m (SUT 0516138, 08. V. 2016); Songkhla province: Singhanakhon district, Khlong Sathing Mo (Songkhla lake), 07°13’37”N, 100°31’42”E, 10 m (SUT 0516144, 08. V. 2016); Khlong Hoi Khong district, Khlong La reservoir, 06°52’29”N, 100°19’48”E, 60 m (SUT 0516141, 07. V. 2016); Khlong Cham Rai reservoir, 06°49’30”N, 100°19’50”E, 55 m (SUT 0516143, 07. V. 2016).

Andaman Sea: Krabi province: Mueang Krabi district, Khlong Sai (Khlong Sai canal), 08°10’20.8’’ N, 098°47’38’’ E, 25 m (SUT 0515097, 30. X. 2015); Wang Than Thip (Wang Than Thip canal), 08°09’49”N, 098°47’51”E, 20 m (SUT 0515098, 30. X. 2015); Trang province: Yan Ta Khao district, Khlong Palian (Palian canal), 07°22’11”N, 099°40’48”E, 20 m (SUT 0515095, 29. X. 2015).

Timor Leste: Manatuto district, W bank of Laclo river near Condae, ca. 4 km WSW of Manatuto, 08°31’32”S, 125°58’50”E, 35 m (ZMH 119364, 21 VI. 2012); south coast, 3.8 km N of Nancuro beach, 4.7 km SE of Natarbora, 09°00’31”S, 126°03’45”E, 20 m (ZMH 119359, 13. XI. 2011); 3.4 km N of Nancuro beach, 5 km SE of Natarbora, 09°00’45”S, 126°03’49”E, 20 m (ZMH 119358, 13. XI. 2011); 2.5 km N of Nancuro beach, 5.7 km SE of Natarbora, 09°01’11”S, 126°03’58”E, 15 m (ZMH 119354, 13. XI. 2011); Baucau district, NE of Baucau, Watabo beach, 08°26’36”S, 126°28’11”E, 20 m (ZMH 119357,

9. XI. 2011); Lautem district, Ira-Ara village, Lutu-Ira, 08°20'32"S, 127°01'08"E, 100 m (ZMH 119356, 23. V. 2011); near the Baucau/Lautem district border marker, 11.8 km NE of Laga, 08°25'35"S, 126°41'43"E, 5 m (ZMH 119353, 10. XI. 2011); Bobonaro district, north coast, 0.5 km from the mouth, Large seasonal stream in Batugade, 08°56'47"S, 124°58'28"E, 10 m (ZMH 119362, 20. V. 2012); Viqueque district, Ossu subdistrict, near village Usu Decima, Wai-eu-Lau, 08°44'36"S, 126°22'50"E, 670 m (ZMH 119355, 13. V. 2011); spring in the village, Loihuno, 08°47'05"S, 126°22'32"E, 255 m (ZMH 119360, 11. XI. 2011); spring in the village, Loihuno, 08°47'05"S, 126°22'32"E, 255 m (ZMH 119363, 17.V. 2012); Manufahi district, south coast, Fatuhcahi village, Wetetefuik creek, 09°02'00"S, 125°59'36"E, 30 m (ZMH 119361, 12 XI. 2011).

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. 4), 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. 4), 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 included 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. 4). 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 originate from the northern to north east 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. 4. 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, we found that 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. 4). 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. 4).

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 (data not shown).

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. 5a, b), while within these clades haplotypes were separated by usually only a few steps (Fig. 5a, 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. 5c) 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 conspecifity of more than 0.7 was recovered, with most pairs having a probability of conspecifity of more than 0.95. All specimen pairs assigned to clade B in the phylogenet-

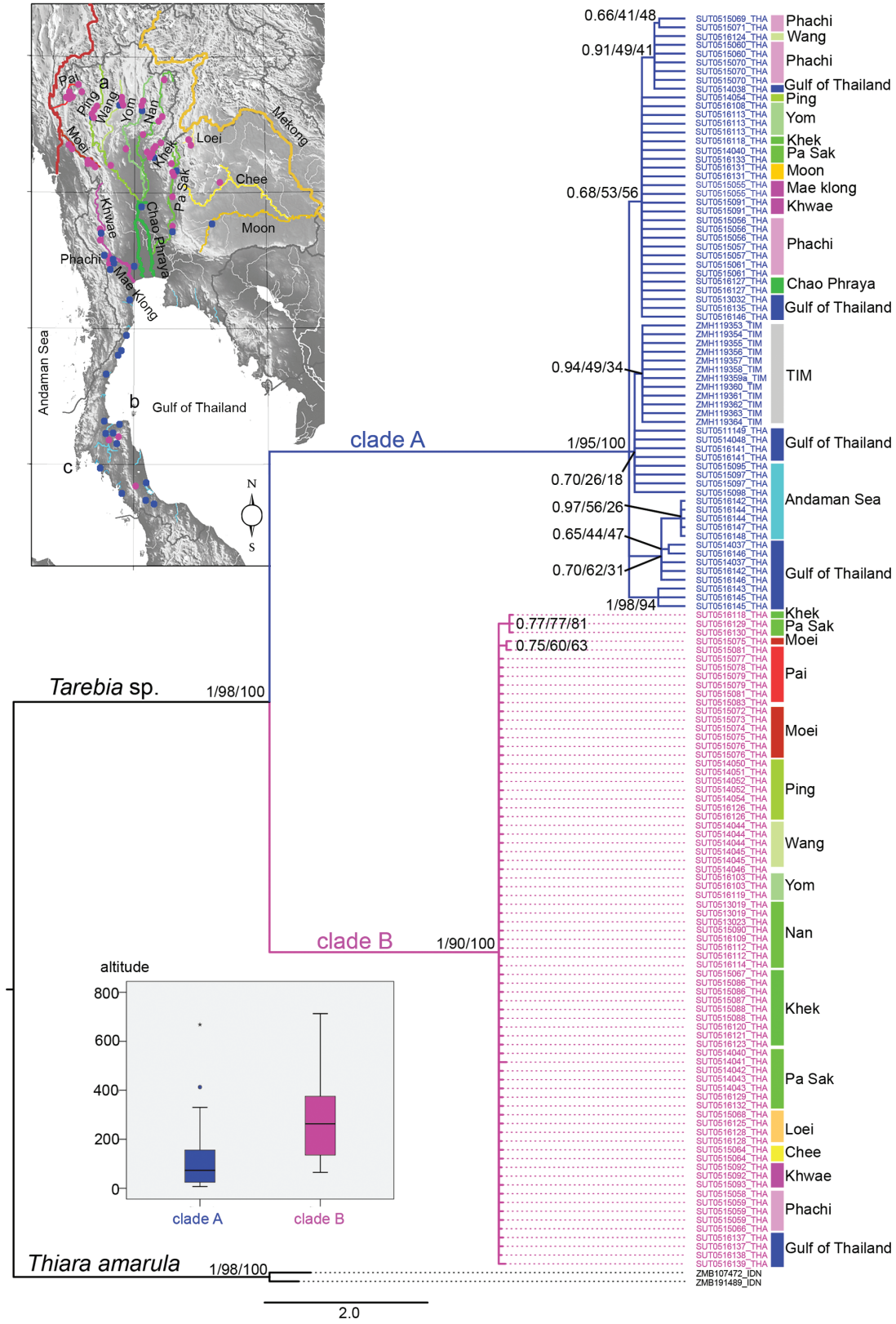

Figure 4. Bayesiam 50% majority-rule consensus tree showing two major mitochondrial clades in *Tarebia granifera* (Lamarck, 1816). Numbers at the nodes correspond to posterior probabilities (left), maximum likelihood (middle) and maximum parsimony (right) bootstrap values. At the tips of the tree voucher numbers (see material list in the main part of the text), country codes (THA: Thailand; TIM: Timor Leste; IDN: Indonesia) 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: magenta dots) and major river systems. The letters a–c in the map refer to localities, for which climatic data were available (see also Fig. 12). The inset with box plots shows the altitudinal distribution of mitochondrial caldes A and B, respectively.

ic 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. 5d) 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 presumably c. 0.33 Mya (95% HPD: 0.50–0.25 Mya).

Shell morphology

The shells of *Tarebia granifera* (Fig. 2), which are often of greenish or brownish colour, 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 closed.

As shown in Fig. 2 *Tarebia granifera* exhibits a wide phenotypical spectrum of shell morphology, which varies with respect to size and shape and in particular in sculpture and colouration 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. 2a) and comparing to topotypical material collected in Timor Leste (Fig. 2t–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. 2b–g), with shell shape ovate-conoidal to moderately turreted and rather thick; the apex is pointed and often eroded; the colour 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. 2(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 colour is mostly yellowish-brown to dark brown. The sculpture of these shells 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. 2n–s).

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

Biometry

For ranges and mean values of measured shell parameters for the different predefined groups, i.e. shell morphs/geographic groups or genetic clades, see Table 2. For all but one of the shell parameters tested, at least one group was present that was not normally distributed (Shapiro-Wilk-test, $p < 0.05$). The exception was the length of the last three whorls (l3w). Here, normally distributed data was found in every tested shell morph/geographic group (Shapiro-Wilk-test, $p > 0.05$). Hence, we conducted an ANOVA, scoring significant ($p < 0.05$) followed by a Bonferroni-corrected LSD-test. The latter found significant differences ($p < 0.025$) between the means of morph B and C. For all other parameters we performed a Kruskal-Wallis-rank sum test, significant ($p < 0.05$) for shell height and width, but not for the index of lw3/w ($p > 0.05$). Hence, the latter was found to contain no differences between groups. For shell height, a subsequent Bonferroni-corrected Dunn-test identified significant differences between the means of morph A and B ($p < 0.025$). The same test identified significant differences of means in shell width between morph B and C ($p < 0.025$). 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. 6a–d).

Between genetic clades at least one of the groups was found to be not normally distributed (Shapiro-Wilk-test, $p < 0.05$) for shell width and l3w/w. By contrast normal distribution was found for lw3 and shell height. Subsequent Levene-testing identified the height and l3w data sets as homoscedastic ($p > 0.05$), hence a two-sample t-test was performed, identifying significant differences ($p < 0.025$) between the means for the two clades for lw3 and no significant differences for shell height. For shell width and l3w/w a Wilcoxon signed rank test was performed, revealing significant differences ($p < 0.025$) for the mean of both shell parameters. 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. 7a–d).

Geometric morphometrics

A principal component analysis (PCA) identified the first six major axes to account for a relevant proportion of

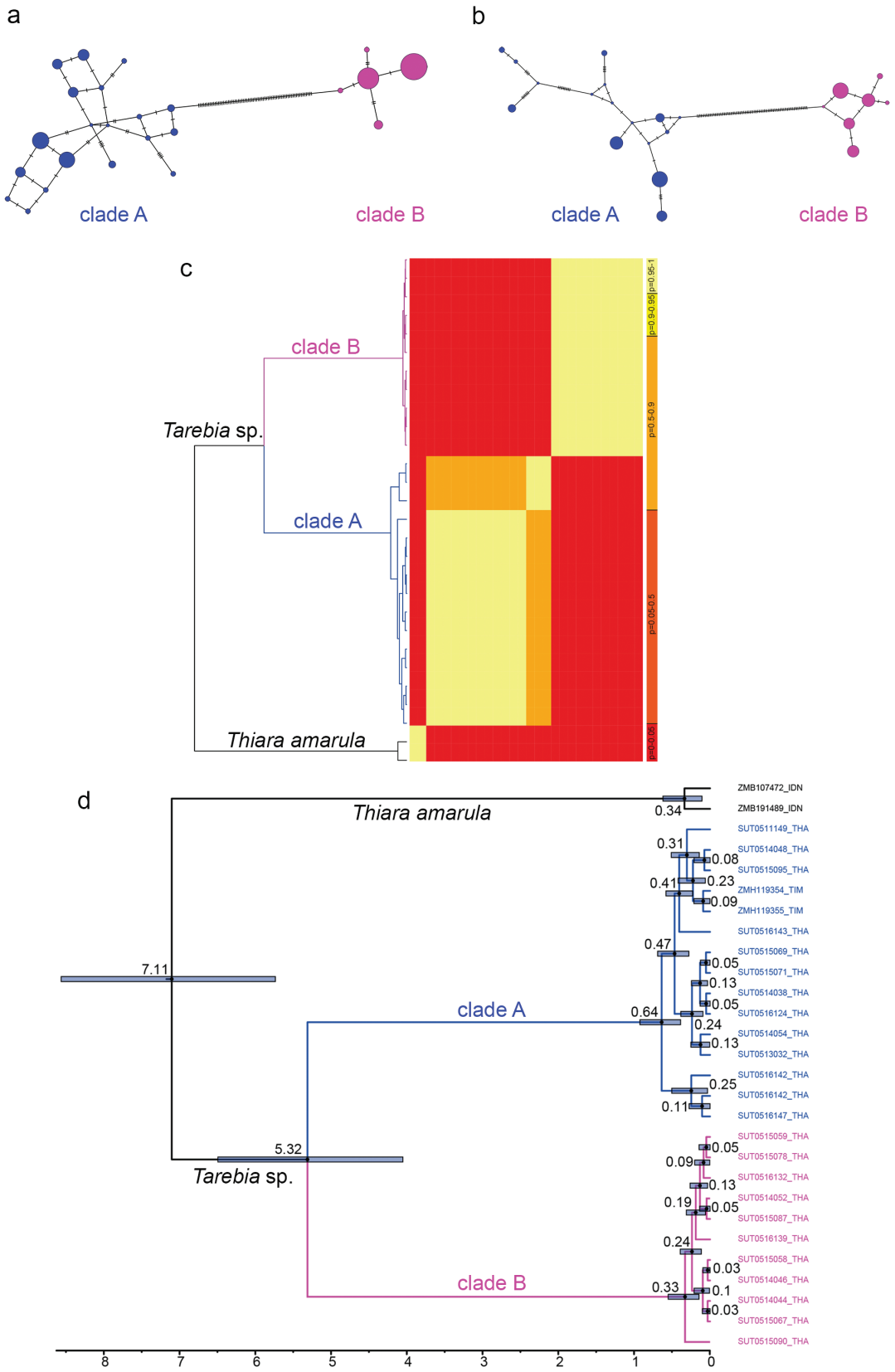

Figure 5. Molecular analysis of *Tarebia*. **a–b**. Median-joining haplotype networks based on 16S (**a**) and *cox1* (**b**) sequence data of *Tarebia granifera* (Lamarck, 1816). The size of each circle represents the frequency of a haplotype and the colour refers to main mitochondrial clades obtained from the phylogenetic analyses (Fig. 4; blue: clade A, magenta: clade B). Tick marks between circles represent evolutionary steps. **c**. Results of the bGMYC analysis. Colouration of the matrix cells represents pairwise probabilities of conspecificity. **d**. Dated molecular tree (only unique haplotypes were included). Numbers at the nodes are node ages in Ma, bars represent 95% highest posterior probabilitiy intervals.

variance ($p > 0.05$) (PC1: 0.303; PC2: 0.181; PC3: 0.117; PC4: 0.090; PC5: 0.058; PC6: 0.052), explaining a cumulative proportion of 0.801 of variance.

Principal components (PC) 1–6 had all at least one group that proved to be not normally distributed (Shapiro-Wilk-test, $p < 0.05$). Subsequent Kruskal-Wallis-testing was significant ($p < 0.05$) in PC1–5 and not significant in PC6. Hence, no further testing was done for PC6. The Bonferroni-corrected Dunn-test identified the mean value for specimens from Timor to be significantly different ($p > 0.025$) from all other morphs on PC1. By contrast, examining PC2 and PC4 with the same test, proved morph A and B to be the only groups not significantly different (with regard to mean values) from one another. Finally, on PC3 and PC5 the Bonferroni-corrected Dunn-Test revealed the mean value of morph C not to be significantly different from all other groups, but the means of morph A and B to be significantly different to that of the specimens from Timor.

Finally, when morph C was integrated into morph B (since these were only differentiated on the basis of slight differences in banding pattern), PC1–5 supported only the group consisting of specimens from Timor to have significantly different means from all other specimens (data not shown). The scatter plot in Fig. 6e shows the distribution of PC1 vs. PC2, illustrating that all predefined groups widely overlap, which indicates that a clear separation is not possible on the basis of shell shape.

For PC1 and PC3–6 at least one of the groups (clade A/clade B) was not normally distributed (Shapiro-Wilk-test, $p > 0.05$). Hence, we conducted Wilcoxon singed rank tests for all these PC, with none showing significant differences between groups ($p > 0.05$). By contrast, in PC2 both groups showed normally distributed data. Therefore, Levene-testing based on deviations from the mean followed and was found significant ($p < 0.05$). Accordingly, we conducted Welch's two sample t-test, revealing significant differences between the means of the two clades on PC2. The scatter plot in Fig. 7e shows the distribution of PC1 vs. PC2, illustrating that the clusters of specimens assigned either to clade A or clade B widely overlap, which indicates that a clear separation is not possible on the basis of shell shape.

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 pouches 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 Figs 8–10a).

The frequency of these different size classes in the subhemocoelic brood pouch of the total of n = 1,007 dissected

Table 2. Biometric data for different shell morphs/geographic groups (see also Figs 2, 8, 9) and mitochondrial clades (see Figs 4, 8, 9) of *Tarebia granifera* (Lamarck, 1816).

	Min	Max	Mean	Median	Standard deviation
Height					
Morph A	9.29	29.83	18.93	18.90	3.69
Morph B	8.56	32.38	19.73	20.06	3.87
Morph C	10.53	26.88	19.03	18.94	3.02
Morph B+C	8.56	32.38	19.62	19.81	3.76
Timor	11.67	28.53	19.68	19.69	3.75
Clade A	8.56	32.38	19.24	19.52	3.86
Clade B	9.45	30.67	19.66	19.68	3.64
Width					
Morph A	3.73	13.28	8.26	8.44	1.71
Morph B	3.49	14.46	8.35	8.55	1.61
Morph C	4.39	11.58	7.94	7.98	1.32
Morph B+C	3.49	14.46	8.28	8.39	1.58
Timor	5.04	12.18	8.15	8.20	1.42
Clade A	3.73	13.28	8.05	8.13	1.51
Clade B	3.49	14.46	8.46	8.61	1.64
Aperture height					
Morph A	4.38	14.39	9.23	9.31	1.84
Morph B	4.23	15.35	9.31	9.46	1.75
Morph C	4.94	18.96	9.06	8.98	1.72
Morph B+C	4.23	18.96	9.27	9.38	1.74
Timor	5.16	13.6	9.13	9.12	1.62
Clade A	4.38	14.39	9.06	9.10	1.72
Clade B	4.23	18.96	9.41	9.50	1.77
Aperture width					
Morph A	1.63	8.92	4.30	4.29	0.87
Morph B	1.68	8.91	4.25	4.25	0.90
Morph C	2.42	8.41	4.31	4.23	1.01
Morph B+C	1.68	8.91	4.26	4.25	0.92
Timor	2.40	6.07	4.09	4.14	0.71
Clade A	1.63	8.92	4.18	4.21	0.87
Clade B	1.68	8.91	4.32	4.29	0.91
Last whorl height					
Morph A	5.91	19.55	12.48	12.47	2.44
Morph B	5.77	20.37	12.60	12.78	2.35
Morph C	6.81	15.83	11.99	11.92	1.87
Morph B+C	5.77	20.37	12.50	12.65	2.29
Timor	6.53	17.78	12.35	12.48	2.22
Clade A	5.91	17.81	12.25	12.38	2.29
Clade B	5.77	20.37	12.69	12.81	2.33
Last three whorls height					
Morph A	7.93	26.43	16.84	17.04	3.29
Morph B	7.73	28.74	16.93	17.13	3.28
Morph C	9.20	21.34	15.97	15.95	2.49
Morph B+C	7.73	28.74	16.77	16.85	3.19
Timor	9.46	23.89	16.56	16.40	3.12
Clade A	7.93	26.22	16.49	16.54	3.22
Clade B	7.73	28.74	17.01	17.13	3.17
H/W					
Morph A	1.77	2.95	2.31	2.29	0.25
Morph B	1.22	3.13	2.37	2.38	0.22
Morph C	1.50	3.05	2.41	2.41	0.24
Morph B+C	1.22	3.13	2.38	2.39	0.22
Timor	1.92	2.87	2.41	2.41	0.18
Clade A	1.50	3.13	2.39	2.41	0.22
Clade B	1.22	2.93	2.34	2.35	0.23
Last three whorls/width					
Morph A	1.27	2.54	2.05	2.04	0.13
Morph B	1.22	2.53	2.03	2.03	0.15
Morph C	1.39	2.65	2.02	2.03	0.16
Morph B+C	1.22	2.65	2.03	2.03	0.15
Timor	1.66	2.28	2.03	2.04	0.13
Clade A	1.27	2.65	2.05	2.06	0.16
Clade B	1.22	2.38	2.02	2.01	0.13

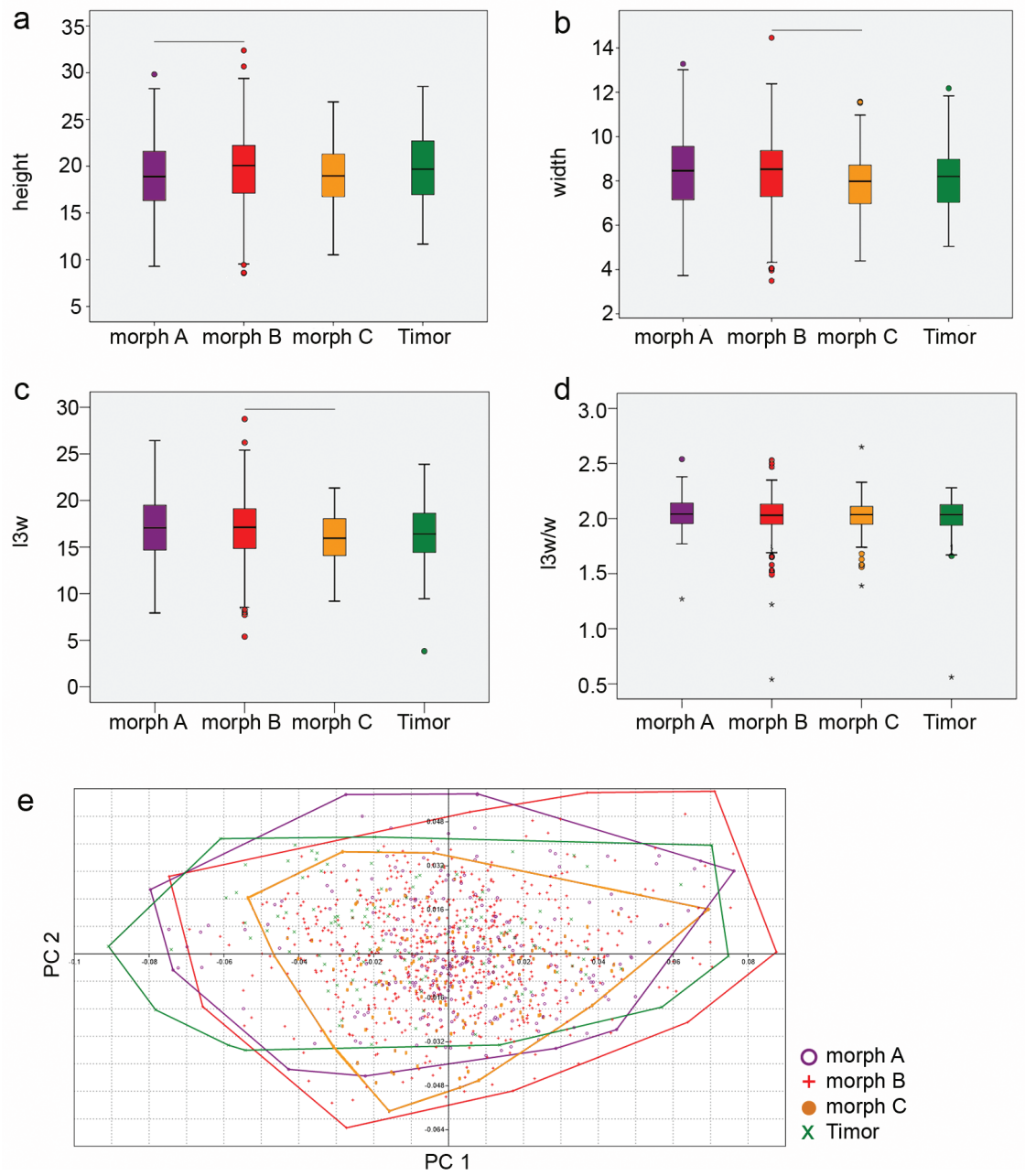

Figure 6. Results of biometric (**a–d**) and geometric morphometrics study (**e**), for four different morphs (A,B,C,Timor) of *Tarebia granifera* (Lamarck, 1816). Boxplots of (**a**) shell height, (**b**) shell width, (**c**) height of the last three whorls and (**d**) index of height of last three whorls agaianst shell width. Significant differences between groups are indicated by bars above the boxplots (**e**) Relative variance in shell shape along PC1 and PC2. Colour corresponding planes indicate the spread of each morph in the data set.

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 Figs. 8 and 9 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. We were also unable 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 (Figs 8, 9).

In all examined populations, the number of early and late embryonic stages was above 50%, in most cases even above 75%; see Fig. 10a for the composition of the brood pouch contents according to the three morphs A-C, and see Fig. 10c 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. 9a, b).

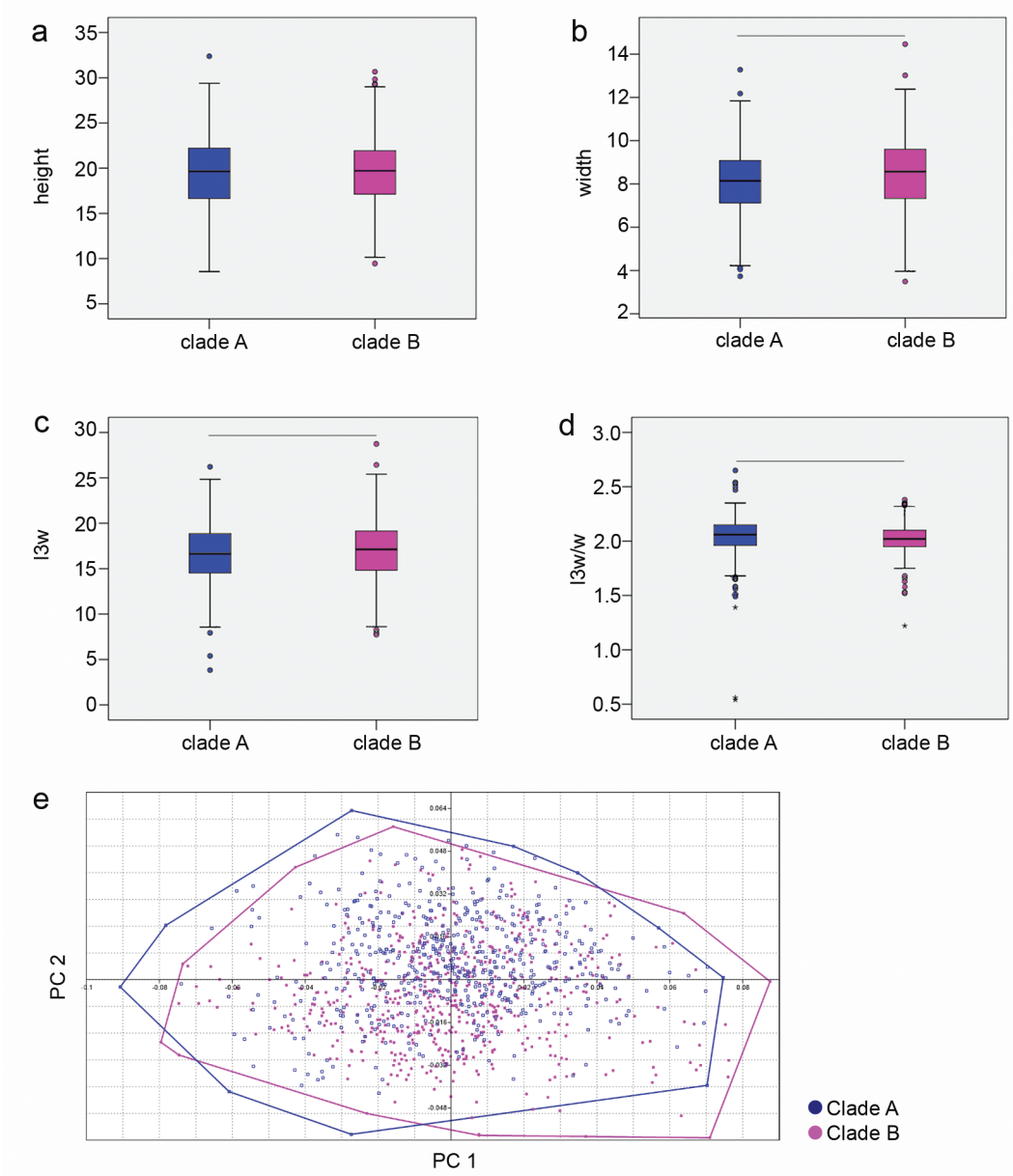

Figure 7. Results of biometric (**a–d**) and geometric morphometrics study (**e**), for the two mitochondiral clades of *Tarebia granifera* (Lamarck, 1816) found in this study. Boxplots of (**a**) shell height, (**b**) shell width, (**c**) height of the last three whorls and (**d**) index of height of last three whorls agaianst shell width. Significant differences between groups are indicated by bars above the boxplots (**e**). Relative variance in shell shape along PC1 and PC2. Colour corresponding planes indicate the spread of each morph in the data set.

When considering the overall distribution of different size classes in the different morphs/geographic clusters or mitochondrial clades, the resulting histograms (Fig. 10a, c) all show essentially the same composition of ontogenetic stages, which suggests the presence of the same reproductive strategy in all investigated groupings. 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 non-gravid vs. gravid specimens was 11:128 (= 8.6%) and that ratio for specimens from Timor Leste was 9:72 (= 12.5%) (Fig. 10b). Considering the two main mitochondrial clades, similar values were observed (Fig. 10d), 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. 11a), clear differences could not be observed. There is, however, one possible exception, i.e. females of *T. granifera* from the Moei River in the Northwest of Thailand, where

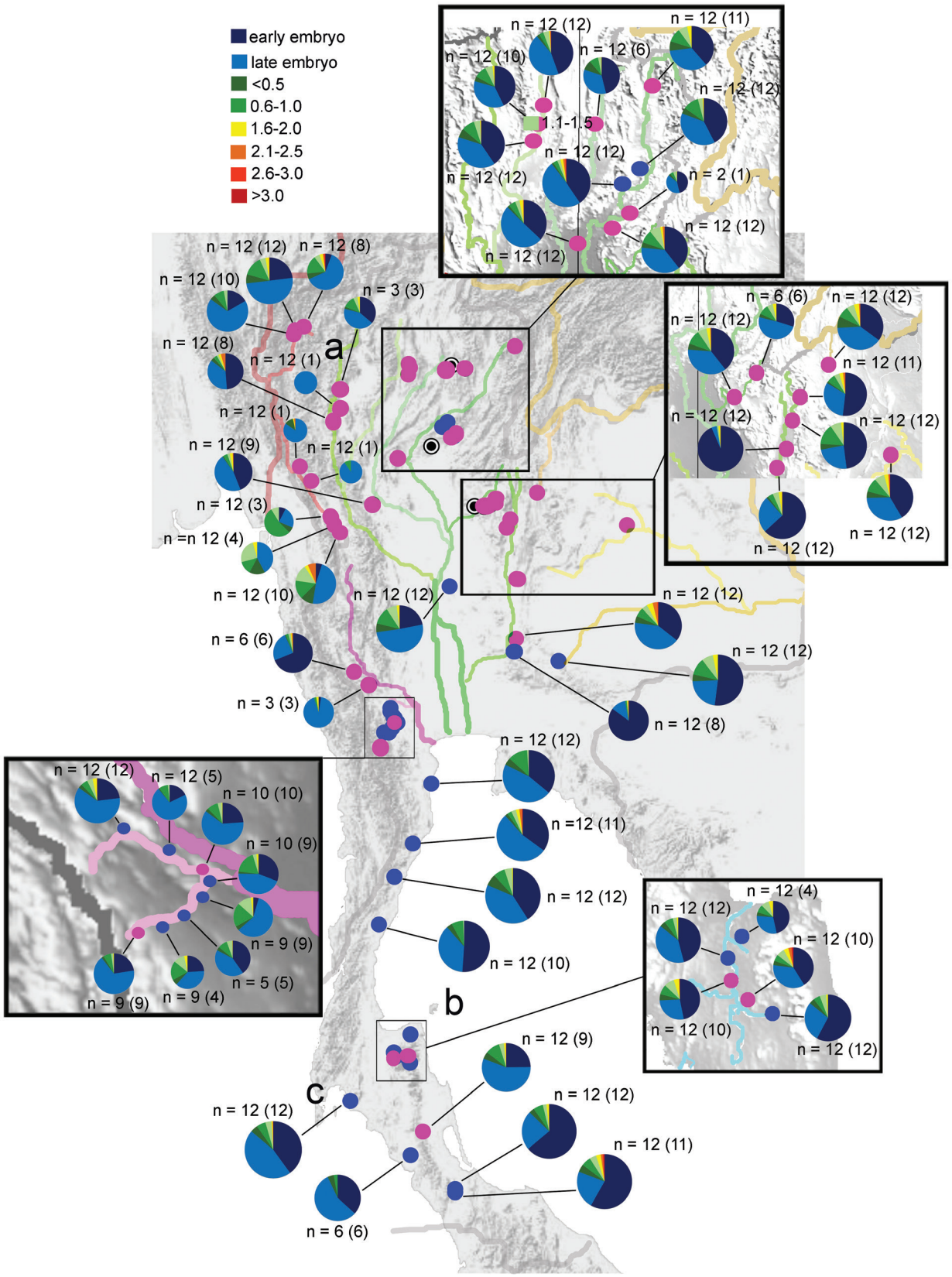

Figure 8. Frequency of ontogenetic stages in the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (morph B) depending on occurrence in Thailand. Blue dots: mitochondrial clade A; pink dots: mitochondrial clade B. Size classes are assigned different colours in the pie charts (see legend) and rivers are coloured 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). The small letters refer to the stations Chiang Mai (**a**), Ko Samui (**b**) and Phuket (**c**) for which meteorological data representing the different climatic regions of Thailand were analysed (see Fig. 12).

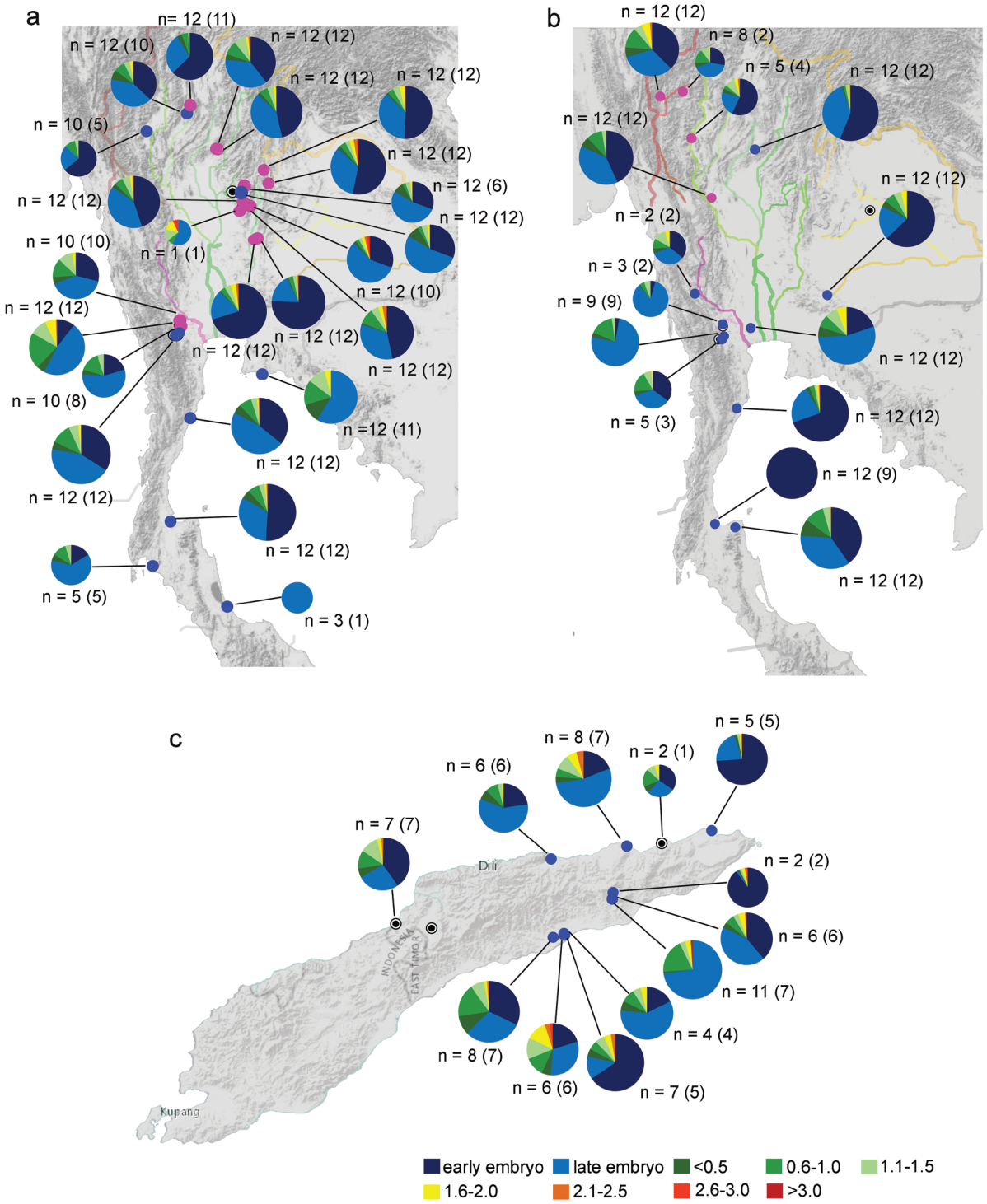

Figure 9. Frequency of ontogenetic stages in the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) depending on occurrence in Thailand and Timor Leste. **a**. Morph A in Thailand; **b**. Morph C in Thailand; **c**. Timor Leste. Blue dots: mitochondrial clade A; pink dots: mitochondrial clade B. Size classes are assigned different colours in the pie charts (see legend) and rivers are coloured 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).

a very low amount of early embryonic stages and less later embryonic stages were found, while there was the largest proportion of larger shelled juveniles. Also, there is 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 exhibits some variation (Fig. 11b), albeit with usually (far) more gravid

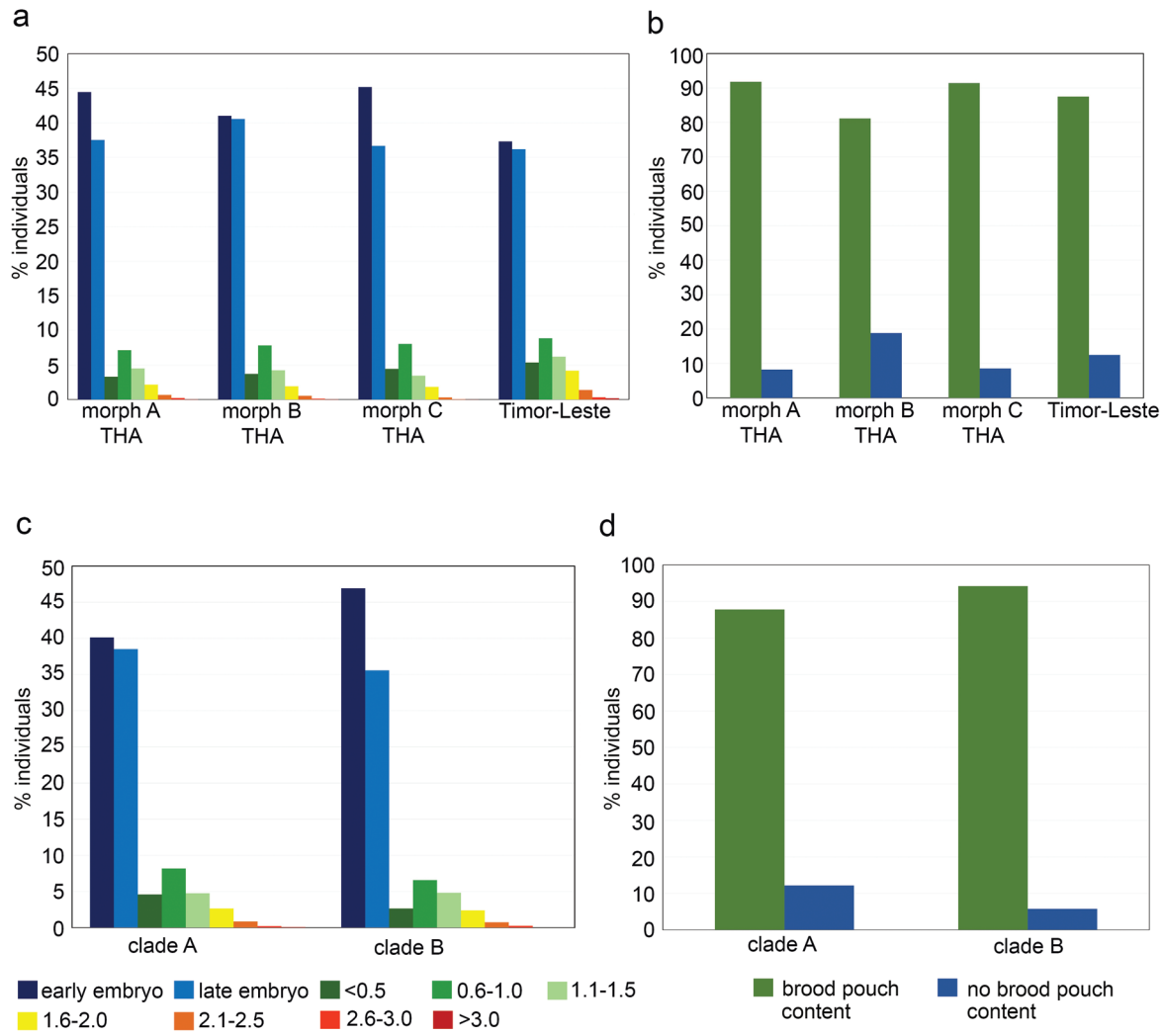

Figure 10. Composition of contents of the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (**a, c**) and proportions of gravid animals, i.e. those with filled brood pouch, versus non-gravid specimens (**b, d**) from Thailand and Timor Leste. **a**. Composition of contents of the brood pouches for morph A, B and C from Thailand (THA) and specimens from Timor Leste (see Figs 1, 8 and 9). **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 (see also Figs 4, 8, 9). **d**. Proportion of gravid vs. non-gravid specimens for mitochondrial clades A and B, respectively. For colour coding, see the inset legends.

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 are in this respect exceptional because only there we 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 our 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, we have used published meteorological data (e.g. minimum/maximum temperature and precipitation) 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. 8 for these locations). As is evident from Fig. 12, 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. 12a); 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

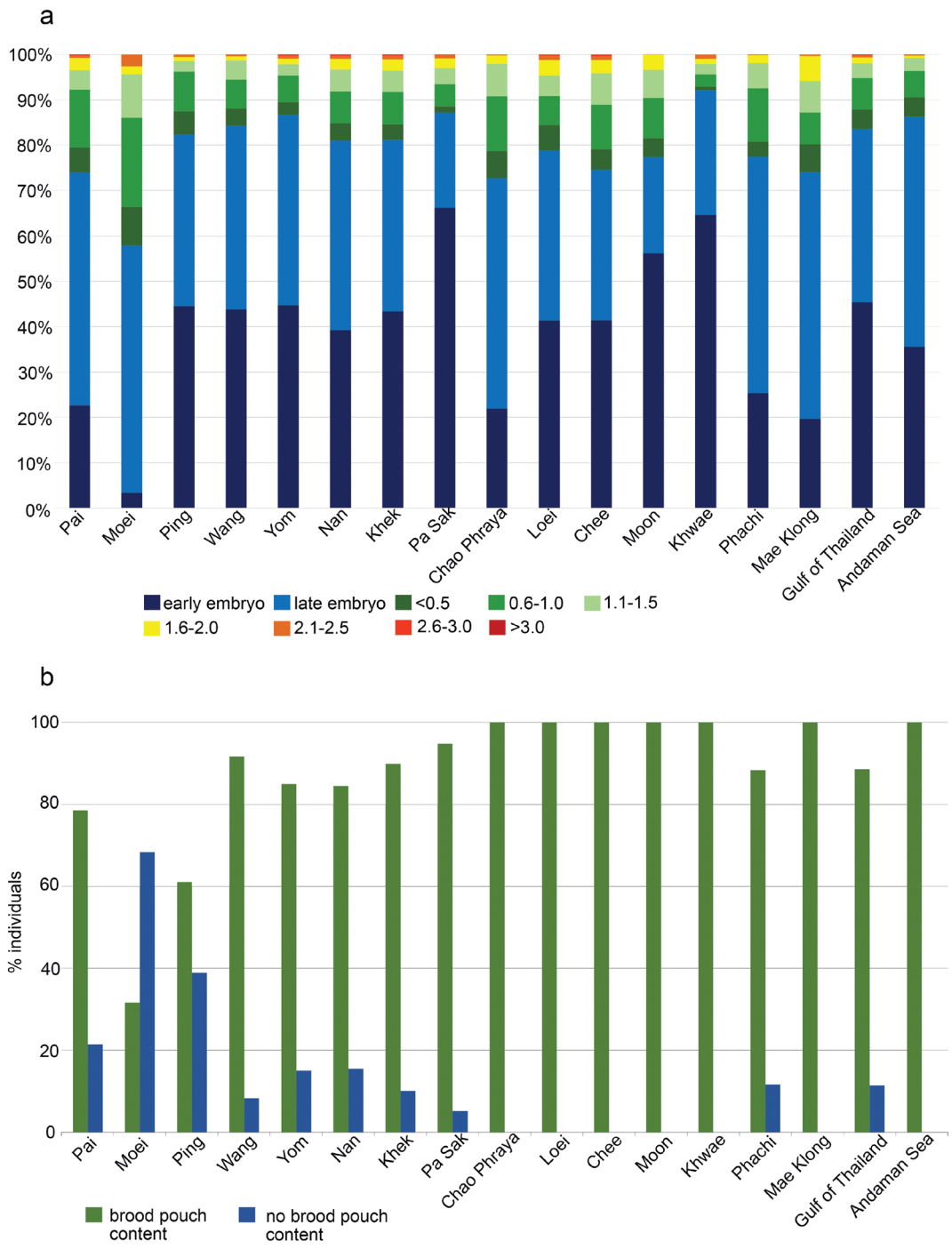

Figure 11. Composition of contents of the subhemocoelic brood pouches of female *Tarebia granifera* (Lamarck, 1816) (**a**) and proportions of gravid animals, i.e. those with brood pouch containing juveniles or other stages, and non-gravid specimens (**b**) from Thailand grouped according to rivers. For colour coding, see the inset legends.

of specimens with brood pouch content were found both during the little (May–June) and great (Oct–Nov.) rainy season; however, we lack sufficient collecting data for the dry season (Fig. 12b). 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. 12c).

Discussion

As evolutionary biologists working with molluscs, we should aim at testing the universality of known and disputed speciation mechanisms, and it is with a clear focus on these mechanisms we should choose our molluscan models to increase their frequency as a source of data in order to decipher the underlying mechanisms of biodiversity.

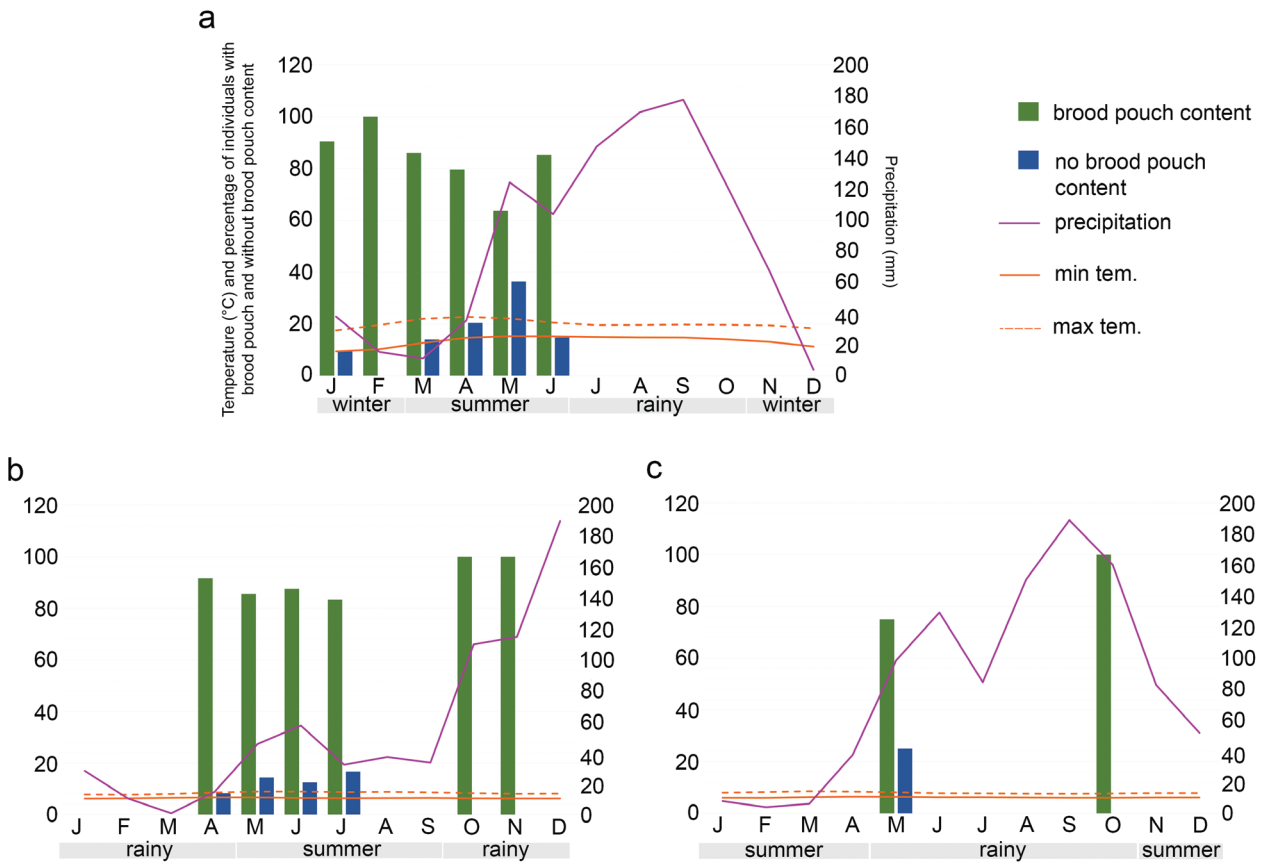

Figure 12. Proportions of gravid vs. non-gravid specimens of *Tarebia granifera* (Lamarck, 1816) collected in different months within a given year, plotted on climate charts for localities that are representative for different climatic regimes in Thailand. (**a**) Chiang Mai for inland locations; (**b**) Ko Samui for the Gulf of Thailand; (**c**) Phuket for the Andaman Sea (see also Fig. 8). For colour coding, see the inset legend.

The combination of molecular genetics and phenotypic analyses in concert with information on the geographical occurrence and additional data, e.g. on biological properties such as reproductive strategies, provides a powerful tool for the study of species differentiation, or diversification indicating speciation. It allows truly biological species to be distinguished, not only as perceivable taxonomic or even genetic units, but also as natural entities of evolutionary significance; if we want to make here the careful distinction between a species taxon (with identifying characteristics) and species entity (as a group of coevolving populations); see for the theoretical background of applications of species concepts in freshwater molluscs Glaubrecht (2004, 2009, 2010, 2011). Thus, within this framework of species as natural entities in space and time an identifiable species taxon can serve as a hypothesis of a species entity.

In freshwater gastropods high levels of morphological disparity and taxonomic diversity are frequently correlated, but often only because traditionally disparity was equated with diversity, as has been exemplified for limnic Cerithioidea, such as e.g. the Mediterranean melanopsids (Glaubrecht 1993, 1996, 2004), the Southeast Asian pachychilids as well as Australian thiarids; see Glaubrecht (2004, 2009, 2010, 2011) and Glaubrecht et al. (2009) for review and additional references.

As has been discussed by the latter author with focus on freshwater gastropods, the widely adopted typological practice during the 19th and way into the 20th century of naming allopatric populations, in isolated fashion and often based on single specimens only, as if representing putatively distinct (morpho-) species, has led to a plethora of species and subspecies names. 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 (e.g. Rensch 1929, 1934, Dillon 2000, Glaubrecht 1993, 1996, 2004, 2009, 2010, 2011). However, even the most pronounced of these conchological features, such as e.g. shell size, shape and sculpture as well as colour, hardly allow for definite species identifications. In limnic gastropods the shells are notoriously phenotypically plastic and variable environmental conditions can produce substantial modifications. Consequently, even marked differences in shell shape, size and sculpture do not necessarily indicate the presence of more than one species. Nevertheless, the former typological perception as to conchological variabil-

ity in molluscs in general and freshwater gastropods in particular, has resulted in long lists of synonyms, causing a considerable amount of taxonomic redundancy in many cases, and, consequently, resulted in an unwanted inflation of biodiversity, as is now also evident from our studies on Thiaridae from Southeast Asia,

In the course of the systematic revision of these thiarids, based on an evolutionary systematic approach (see Glaubrecht 2010) combining morphological and molecular genetic data, not only the number of species in general can be reduced. These investigations also provide the basis for an evaluation of phenotypical (i.e. conchological) plasticity in these gastropods, to be distinguished from genotypical diversity as yet another indication of a differentiation process within and among populations that reflect incipient or other stages of speciation. Here phenotypical plasticity is understood as the ability to express different phenotypes depending on the biotic or abiotic environment within one species (Agrawal 2001), in contrast to a truly speciational process.

However, an assessment of the significance of distinct phenotypic traits is in general lacking, as is an understanding of the genetic basis of phenotypical variation in particular for gastropods. For the limnic pomatiopsid *Oncomelania hupensis*, 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, suggesting that a relatively simple underlying genetic mechanisms (likely controlled by a few genes) might be responsible for gastropod shell traits. In a natural experimental situation in *Oncomelania* from the Miao River in the Yangtze floodplain in China, Davis et al. (1999) found that ribbing is indeed genetically controlled by a single gene with multiple alleles and suggested this to be an adaptation for dealing with annual flooding and survival by water transport. However, understanding the mechanisms that generate phenotypic variation such as shell sculpture and shape and being evolutionary relevant (i.e. inherited and selected with an adaptive value) still remains a fundamental challenge for contemporary evolutionary biology.

Owing to the earlier typological approach that resulted in the traditional overestimation of taxonomical diversity due to conchological disparity, but also in context of the genetically apparently closely related but morphologically highly distinct thiarids found across the distributional ranges throughout Southeast Asia and Australasia, we have to ask whether we are indeed dealing with actually many diverse species as separate evolutionary entities rather than only few, though highly polymorphic species with maybe several sympatric morphs exhibiting different ecophenotypical adaptations in shell response to the many variable environments where thiarids are usually to be found.

Shell morphology

In the present study, we examined phenotypically distinguishable shell morphs of yet another thiarid from Thailand, traditionally assigned to *Tarebia granifera*, in reference to samples from Timor Leste as known type locality of the nominal species, using biometry and geometric morphometrics in combination with phylogeographical analyses of molecular genetics and reproductive strategy.

We found *Tarebia* to be 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. Some of these have even been formally named as distinct species (albeit from other regions in Asia), 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, the name *lineata* (as well as *lateritia*, traditionally used for Philippine forms) have frequently been applied to morphs and/or populations in the Oriental region. Subba Rao (1989) discussed that *T. lineata* was often synonymised with *T. granifera,* or treated as its variety (e.g. Benthem-Jutting 1959), although it is readily distinguished from the latter by the presence of the very distinct spiral ridges. 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.

Applying a drainage-based phylogeographical as well as a biometrical approach, we were unable to find for the populations in Thailand any correlation of the morphs distinguished in this study based on discernable shell features as well as overall "Gestaltwahrnehmung" with any criteria deducible from our observations given above, neither with geographical occurrence or preferred habitat and substrate nor with the molecular genetic substructuring detected (see below). So, all available evidence points at the coexistence of different morphologies or disparate phenotypes in *Tarebia granifera* in this part of mainland Southeast Asia. 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 discussed further below.

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 (e.g. Bocxlaer and Schultheiß 2010, Maaß and Glaubrecht 2012). Geometric analyses are used in addition to traditional morphometrics in order to compare in detail different populations and relationships among variable groupings (e.g. Rohlf and Marcus 1993, Sheets et al. 2006).

Although there are some differences in the biometric parameters and in the geometric morphometrics of Thai

Tarebia, it is generally impossible to delimit distinct entities (in the sense of being at least indicative of the existence as biological species) based on these features, as all of them largely overlap (Figs 6, 7). 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.

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 (e.g. Abbott 1952, Brandt 1974). Also Bradstreet and Rogowsky (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. 6d). Isnaningsih et al. (2017) found the shape of *T. granifera* from the Indonesian 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 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. 6e). 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* (e.g. Facon et al. 2003, Genner et al. 2004, Sorensen et al. 2005, Yousif et al. 2009).

Phylogenetic analyses

In contrast to shell morphology (morphs A–C, or *lineata* vs. *granifera* phenotypes), we found based on molecular genetics strong indication as to the distinction of at least two natural entities within *Tarebia* in Thailand. As our analyses revealed, there is a most pronounced separation of two distinct mtDNA clades in this taxon, marked on the one hand by long branches in the resulting phylogenetic tree connecting these two clades, and on the other hand by very shorter branches within each of them (Figs 4, 5).

Therefore, our analyses would potentially allow for a more narrow species delimitiation 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 (see Fig 4, insert). However, we propose that the latter reflects rather the occurrences in higher mountainous regions in the north than in the south of Thailand than a truly differential habitual preference. 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. Thus, it should be the privilege of a more comprehensive and in-depth analysis of the biogeographical situation based on an ongoing molecular genetic study (Glaubrecht unpubl. data).

Historical biogeography

While we found representatives of clade A in the northern tributaries of rivers such as the Chao Phraya and Mae Klong that run into the Gulf of Thailand, with only few others occurring at some localities in the south of Thailand (Figs 4, 8, 9a,b), those in clade B were found in the Salween River and the headwaters of Ping, Wang, Yom and Nan River. 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 (Figs 1, 4).

However, although being more frequent in the northern provinces, some representatives of clade B also occur in more southern locations, such as in the provinces 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 flows in drainage systems. The results of the median-joining haplotypes network and bGMYC analysis (Fig. 5) reveal that clade A and B exhibit many steps separating these two groups, and have low probabilities of conspecificity between clade A and B (p=0–0.05).

As we found in our molecular analyses this major split of clade A and B in Thai *Tarebia* to be as old as most likely c. 5.32 million years ago (Fig. 5d), it is worthwhile to look for a possible biogeographic explanation of the above distribution. 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 Bruyn et al. (2005) pointed out, historical biogeography while providing crucial insights into the relationship between biological diversity and earth history, as a consequence has its limitations. However, patterns of intraspecific molecular variation may show unambiguous evidence for such historical divides, and can be used to test competing biogeographic hypotheses, such as e.g. the dispersal-vicariance debate, see e.g.

Glaubrecht (2000) and Glaubrecht and Rintelen (2003) for limnic gastropods).

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. It is interpreted as the result of Neogene marine transgressions that breached this isthmus in two locations for prolonged periods of time, i.e. more than 1 million year duration, as was shown e.g. by phylogeographic analyses of a freshwater decapod crustacean, the giant freshwater prawn *Macrobrachium rosenbergii* (cf. Bruyn et al. 2005). In his review 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 and, thus, of mainland Southeast Asia. It is believed to be of the same scale as, e.g., the 'Wallace's Line', albeit it remains less well-known and less well-studied, with its location and cause being still enigmatic. 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 in Pleistocene palaeohabitats. They found in bulbuls, as we suggest in analogy here for thiarid gastropods, Pliocene Indochinese-Sundaic lineage divergence, for which they hypothesized that it coincides with strong vegetational changes on the Peninsula shaping two phytogeographical 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.

While the separation of *Tarebia* and *Thiara* hint at a Late Miocene splitting event (anticipated to have occurred somewhere in the Indo-Malayan insular region of the Sunda and Sahul shelfs), our molecular and distributional data on *T. granifera* (Figs 4, 5d) 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; see Bruyn et al (2005) and literature therein for further details as to the relevant geological data and discussion.

The fact that today 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 in Glaubrecht and Köhler (2004). Thus, although being today located north of the Thai-Malay Peninsula in Central Thailand, the Isthmus of Kra and late Miocene/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 biology1

Tarebia snails are all viviparous, i.e. they incubate embryos and later ontogenetic stages in an extra-uterine structure, called the subhemocoelic brood pouch, located at the back of the head in the female's body running alongside and below, but being independent of the pallial organs (e.g. the gonoduct), and formed apparently by an invagination of the genital grove found in other oviparous cerithioidean gastropods (Glaubrecht 1996, 1999, 2006, 2011, Glaubrecht et al. 2009). Based on histology, for *T. granifera* Glaubrecht (1996) described an eu-viviparous strategy, involving matrotrophy (i.e. the nourishment by the female) of the progeny that develop 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*, is in contrast to an ovo-viviparous mode, reported e.g. for *Thiara amarula* and some other Australian thiarids, such as *Stenomelania aspirans* (see Schütt and Glaubrecht 1999, Glaubrecht et al. 2009, Maaß and 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 a clear-cut 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 (see Figs 8, 9, and more details above) that were found to only possess late and/or even early embryonic stages, respectively. This is in contrast to a pronounced correlation as to reproductive biology in Thiaridae from Australia (Glaubrecht et al. 2009), where all ovo-viviparous taxa that release veligers paradoxically exhibit very restricted distributional ranges in the Jardinian biogeographical region only. It is also in contrast to differences in the Thai thiarid *Melanoides jugicostis* (see Dechruksa et al. 2013), that was found to lack viviparous populations at least in some geographical regions and during some time of the year.

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 represen-

tative 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 (see Figs 8, 9, 10, 11a). However, as reported above (see Figs 11b, 12a) some populations in 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. It can be provisionally deduced from these data, that there might be a tendency for *Tarebia* females to be gravid especially during and after the end of the main or great rainy season in the second half of the year (and potentially during the dry season). In contrast to this temporal (spatial) hypothesis, we do not explain the frequency of non-gravid specimens as being indicative of the varying existence of males, as their occurrence would alternatively be regionally specific (in the northwest) and seasonal (little rainy season early in the year), which we doubt.

As in most (if not all) thiarids, *Tarebia* apparently lacks males in most populations, as we failed to find positive evidence for their existence. 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 (Maynard 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* (Jacob 1957, 1958, Berry and Kadri 1974, Ben-Ami and Heller 2005). 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 (Livshits and Fishelson 1983, Heller and Farstey 1990).

It would be tempting to anticipate 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. However, none ad hoc feature such as e.g. shell morphology between male and female could be differentiated in these aphallic Cerithioideans. 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.

Species concepts in parthenogenetic *Tarebia1*

Given the prediction supported here that thiarid gastropods reproduce largely (if not completely) via parthenogenesis, the application in particular of the biological species concept is not made easy in case of thiarids. Morrison (1954) in discussing the enormous shell variability in thiarids in context with parthenogenesis, noted wisely that "wise indeed is the scientist who can tell whether a clone is a species or not, and be right every time, in the case of the Thiaridae". This was shown, for example, for *Melanoides tuberculata* (Jacob 1957, 1958, Berry and Kadri 1974, Facon et al. 2003). Therefore, Stoddart (1985) preferred to apply instead of the biological species concept that of the evolutionary species (ESC) following Wiley (1978, 1981), as in his opinion this concept "stresses the relevance of the process of speciation to species definitions and provides the most appropriate framework for the taxonomy of asexual organisms". Although this statement is debatable for several reasons, admittedly, the biological species concept (BSC) is also not without problems in application to *Tarebia*, as it explicitly uses the reproductive criterion in sexually reproducing organisms. The BSC was introduced by Mayr (1942) and since then widely discussed; see literature survey with references updated and discussion with respect to limnic gastropods e.g. in Glaubrecht (2004, 2009, 2010, 2011, Glaubrecht et al. 2009).

In case of the thiarids it remains to be seen in how far they are actually prone exclusively to parthenogenesis. For example, for populations of *Melanoides tuberculata* in Israel Ben-Ami and Heller (2005) reported sexually as well as asexually reproducing individuals, thus contradicting the general assumption that indeed all thiarids reproduce via apomixis. Apparently, at least in *M. tuberculata* there are both modes realized, securing the exchange of genetic information by sexual reproduction as was shown in earlier allozyme studies (Livshits and Fishelson 1983) and excluding the possibility of gynogenesis, i.e. parthenogenesis with the development of eggs to be induced by contact with sperm, though. Given the fact that we have (albeit indirect) evidence for the presence of males at least in low frequency, as is evident from published records, e.g. on *Thiara amarula* (see Healy and Glaubrecht 2018), as well as unpublished data, we here anticipate at least the occasional sexual reproduction in Thiaridae. As their species either maintain low levels of males in some populations, or by other means switch between asexual and sexual reproduction, there is no objection to not applying species concepts grounded on the reproductive criterion, as explicitly done under the BSC.

Conclusion

In view of the pronounced phenotypic plasticity reported herein for the Thai *Tarebia granifera*, it should be asked, in addition or alternatively to environmental factors, in

how far this conchologically expressed variation is correlated to or even caused by these, at least frequently, parthenogenetically reproducing thiarids. Resulting in monoclonal lineages, populations of morphologically varying freshwater snails with partly or potentially completely parthenogenetic females hitherto have erroneously been treated as species under the traditional typological approach (not only in malacology). However, this simplistic and often non-comprehensive approach has most likely underestimated natural variation and intraspecific disparity by, at the same time, overestimating taxonomic diversity, resulting in taxonomic redundancy as an underrated phenomenon in evolutionary biology.

The development of an accurate and rapid method for the detection of males in aphallic thiarids, in order to evaluate the frequency of parthenogenesis in individual populations and species or higher-level taxa, respectively, remain an essential desideratum in biosystematics research on these snails. In addition, it remains to be analysed thoroughly whether and in how far there is a correlation of partially or completely parthenogenetic populations with parasite infections by digenic trematodes, for example, in the thiarids *Melanoides tuberculata* (see Krailas et al. 2011, 2012, 2014), in *M. jugicostis* (see Dechruksa et al. 2013) and *Tarebia granifera* (Veeravechsukij et al. 2018).

Our preliminary analyses of the brood pouch content in the latter species under study here revealed that infected females tend to have fewer embryos than non-infected specimens, which might be a hint to the influence of parasite load on the reproductive mode of this major intermediate host. Therefore, given the human infection aspects of these trematode-carrying gastropods, our study not only has implication for human health in Thailand. We also hope that with studying trematode infections in the various conchologically disparate and molecular genetically distinct lineages of *Tarebia* we will eventually gain deeper insights into the complex evolutionary interplay of various trematode parasites and their snail hosts mediating infections in the human population.

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