Susceptibility of tropical mountain forests to biological invasions from the temperate and subtropical zone, exemplified by *Zonitoides* (Gastropoda: Gastrodontidae)

César Capinha[1,2], Jaap J. Vermeulen[3,5], Maklarin bin Lakim[4], Menno Schilthuizen[5,6,7] and Heike Kappes[5,8*]

Abstract. Colonisation by, and spread of, animal species from the temperate zone are rather uncommon observations in the tropics. The study provides the first reports of two snail species of the genus *Zonitoides* in Sabah, Borneo, namely *Z. arboreus* (Say, 1819) and *Z. nitidus* (O.F. Müller, 1774). The identification was aided using partial sequences of 28S rDNA, and the barcoding sequence of COI. So far, the two *Zonitoides* species were found in locations where the natural forest cover had been disturbed, and only in the montane forest at elevations between 1500 and 2000 m a.s.l.. Niche modeling suggests that both taxa could be widely distributed in the mountains of tropical South America and Africa. *Z. arboreus* finds suitable climates in many places in SE Asia and especially at many conservation areas in Borneo. In contrast, *Z. nitidus* finds only marginal climatic conditions in the same area, and introductions of *Z. nitidus* probably will remain spatially restricted. This prediction, however, needs to be monitored because the latter species can display molluscivory. Our results additionally point to the possibility of *Z. arboreus* being a species complex that, given its potential economic impact, urgently needs a taxonomic revision.

Key words. biological invasion, climatic suitability, invasive species, land use change, long-range dispersal, Mahalanobis distances

INTRODUCTION

The introduction of non-native species is a global problem that is both, one of the drivers and an indicator of global change (Sala et al., 2000). Biotic exchange between regions occurs irrespective of climatic zone, and lists of introduced terrestrial gastropods have been compiled for many countries (e.g., Hausdorf, 2002; Herbert, 2010; Hayes et al., 2012). The process of introduction and the establishment of non-native land snails is usually linked to disturbances of the original vegetation (e.g., Kappes, 2006; Kappes et al., 2009), and the (subsequent) use of alien plants in horticulture (e.g., Cowie et al., 2008). Thus, it can also be expected that the malacofauna of the tropical island of Borneo contains several non-native

[1]Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[2]Zoologisches Forschungsmuseum Alexander Koenig, Museumsmeile Bonn, Adenauerallee 160, 53113 Bonn, Germany

[3]jk.artandscience, Lauwerbes 8, 2318 AT Leiden, The Netherlands

[4]Research and Education Division, Sabah Parks, Gunung Kinabalu Park, Sabah, Malaysia

[5]Naturalis Biodiversity Center, Postbus 9517, 2300 RA Leiden, The Netherlands; Email: heike.kappes@uni-koeln.de (*corresponding author)

[6]Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, 88999 Kota Kinabalu, Sabah, Malaysia

[7]Centre for Ecological and Evolutionary Studies, Rijksuniversiteit Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands

[8]Department of Ecology, Cologne Biocenter, University of Cologne, Zülpicher Str. 47b, 50674 Köln, Germany

species. Indeed, Schilthuizen & Liew (2008) list several (potentially) introduced slugs and semislugs from Sabah, Borneo, namely *Laevicaulis alte* (Férussac, 1821), *Semperula wallacei* (Issel, 1874), *Philippinella möllendorffi* Collinge, 1899, *Parmarion martensi* Simroth, 1893, and *Deroceras* cf. *laeve* (Müller, 1774). Concerning shelled gastropods, the giant African land snail, *Achatina fulica* Bowdich, 1822, was found along forest paths in a recently disturbed forest at Danum Valley (Schilthuizen & Rutjes, 2001). The pantropical subulinid species *Lamellaxis clavulinus* (Potiez & Michaud, 1838) *(*syn. *Allopeas clavulinum*) and *Lamellaxis gracilis* (Hutton, 1834) (syn. *Allopeas gracile*) were recorded from forests in the Tabin and Danum regions (Schilthuizen & Rutjes, 2001; Schilthuizen et al., 2003). Schilthuizen (2011) observed that a pitfall trap baited with lamb meat to attract carrion beetles also lured hundreds of the non-native *Subulina octona* (Bruguière, 1798) in a secondary forest (at Kota Kinabalu). All three subulinids are rather common in Southeast Asia (e.g., Vermeulen & Whitten, 1998 and references therein). Recently, Uchida et al. (2013) studied snails from several natural forests and disturbed places across Sabah, and found several pantropically introduced snail species, namely *Bradybaena similaris* (Férussac, 1821), *Allopeas gracile*, *Subulina octona*, and *Achatina fulica*.

With the exception of *Deroceras* cf. *laeve*, the species mentioned above are typical for the tropical climate zone. The invasibility of the tropics by species that originate from subtropical or even temperate climatic conditions has hardly been assessed. Here, we provide first details on occurrences of two hitherto unreported temperate/subtropical

zone snail species in Borneo. Both species belong to the genus *Zonitoides*. DNA markers were used in this study as an additional trait for species determination, along with morphological assessments. Further, the area that could be colonised under current climate was evaluated using a GIS-analysis of climatic suitability. An involvement of a cooler climate at higher altitudes was also suggested for gastropods on Hawaii (Meyer & Cowie 2010), but our study is the first to illustrate and quantify the susceptibility of tropical mountains to temperate snail invasion in a global context.

MATERIAL AND METHODS

Origin of the snail specimens. A population of blackish snails was found at a shallow ditchlike brook below a restaurant in the area of Mount Kinabalu Park headquarters, Mount Kinabalu Park, Sabah, Borneo, MY (ca. 1550 m a.s.l., ca. 6.00629° N, 116.54332° E), coll. H. Kappes, 11 September 2012. Shells were about 5–6 mm in diameter, had about 4.5 convex whorls with irregular radial stripes, a discontinuous but almost roundish unlipped aperture, and an umbilicus that measured about 1/5 of the shell diameter. Both the habitus and the wet habitat immediately gave the impression of (subadults from) the European species *Zonitoides nitidus* (O.F. Müller, 1774). The two specimens Sabah Park collection numbers SP 13543 and SP 13544 are from this site.

A juvenile snail was found at the roadside of Gunung Alab substation, Crocker Range, Sabah, Borneo, MY (ca. 1900 m a.s.l., ca. 5.82156° N, 116.34157° E), coll. H. Kappes, 23 September 2012. The shell was yellowish-brown and had some irregular radial stripes, a discontinuous but almost roundish unlipped aperture, and a narrow umbilicus. Because the shell was not fully grown, it was impossible to assign it to a species using traditional shell morphological methods. It seemed not to fit any other gastropod family from that area. However, it somehow resembled a pale *Zonitoides*. This specimen has the Sabah Park collection number SP 13548.

These two cases were compared to reference samples. To this end, specimens of the wide-spread European wetland snail *Zonitoides nitidus* were collected from a ditch at the Einsteinweg, Leiden, The Netherlands (ca. 52.16585°N, 4.46315°E), coll. H. Kappes, 28 October 2012. The two specimens have been deposited in the collection of Naturalis in Leiden, NL, under collection numbers RMNH. MOL.328276 (*Zoni*-NL1) and RMNH.MOL.328277 (*Zoni*-NL2).

Zonitoides arboreus (Say, 1816), a species of North American origin, has been introduced to numerous greenhouses in Europe (Kerney et al., 1983; Horsák et al., 2004). The specimen *Zoar*-Hortus originated from a greenhouse of the botanical garden (Hortus Botanicus) in Leiden, The Netherlands (ca. 52.15673° N, 4.48320° E), coll. H. Kappes, 14 January 2012. The individual also is deposited in the Naturalis collection (collection number RMNH. MOL.328280).

Genetic identification. DNA was extracted from sections of the foot after dissolving the tissue with Proteinase K at 56°C using the DNeasy Blood & Tissue kit, following the standard protocol for tissue extraction. We considered both a nuclear ribosomal gene, 28S rDNA, and a mitochondrial protein coding gene, Cytochrome *c* oxidase subunit I (COI). A partial sequence of the COI gene was amplified using the standard primers L1490 and H2198 (Folmer et al., 1994). The 25 μl reaction volume was composed as follows: ddH$_2$O 17.25 μl, 10× Reaction Buffer 2.5 μl, MgCl$_2$ (25 mM, Qiagen) 1.5 μl, L1490 1 μl, H2198 1 μl, dNTP (2.5 mM) 0.5 μl, Taq Polymerase (5u μl^{-1}, Qiagen) 0.25 μl, plus 1 μl of template DNA. PCR amplifications were run with 1 cycle at 94°C for 1 min, followed by 38 cycles of denaturation for 30 s at 94°C, annealing for 45 s at 50°C, and extension for 60 s at 72°C, with a final extension at 72°C for 7 min. The partial sequence of the COI gene that could be reconstructed from forward and backward amplification spanned over 683 bp.

The fragment of the 28S rDNA was amplified using the primers D23F and D6R (Park & Ó Foighil, 2000). Each 25 μl reaction volume was composed as follows: ddH$_2$O 11.75 μl, 10× Reaction Buffer (contains 15 mM MgCl$_2$) 2.5 μl, MgCl$_2$ (25 mM, Qiagen) 1.5 μl, D23F 1 μl, D6R 1 μl, dNTP (2.5 mM each) 0.5 μl, Taq Polymerase (5 u μl^{-1}, Qiagen) 0.25 μl, Q-Solution (5×, Qiagen) 5.5 μl, plus 1 μl of template DNA. PCR amplifications were run with 1 cycle at 94°C for 5 min, followed by 30 cycles of denaturation for 15 s at 94°C, annealing for 30 s at 55°C, and extension for 40 s at 72°C, with a final extension at 72°C for 7 min. The amplified (readable) partial sequence of the 28S rDNA was 769–771 bp long.

Sequencing was performed in both directions by Macrogen Europe. Sequences of an additional specimen of *Zonitoides arboreus* were made available by T.-S. Liew (Universiti Malaysia Sabah). The specimen was collected at Mesilau, Kinabalu Park, and sequenced at Universiti Malaysia Sabah by T.-S. Liew with the same COI primers (558 bp).

Sequences were deposited in GenBank under accession numbers KF147206–KF147212 (COI) and KF147213–KF147218 (28S). We compared our results on the COI sequence using public sequences from BOLD (SSEIA4371-13, RBNII049-13, RBNII047-13, RBNII045-13, RBNII043-13, RBNII027-13, RBNII020-13, RBNII019-13, RBNII018-13). All these sequences were kindly supplied from the Biodiversity Institute of Ontario and labelled as *Z. arboreus* (last checked: 3 May 2014).

Sequence distances (DNA and protein) between taxa and a phylogenetic reconstruction based on the COI gene were calculated in MEGA 6.0 (Tamura et al., 2013). We used a member of the Oxychilidae (*Oxychilus draparnaudi*, JX911306) as outgroup closely related to the Gastrodontidae, and the Basommatophoran *Carychium mexicanum* (HQ171529) to root the tree. Because the available sequences were of unequal length and we wanted to retain as much information as possible, elimination of positions with alignment gaps and missing data was done

only in pairwise comparisons (pairwise deletion option) to allow analysis across the maximal 683 positions. The tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The percentage of replicate trees in which the associated taxa clustered together were calculated using a bootstrap test with 500 replicates (Felsenstein, 1985).

Niche modeling. Data compilation: Predictions of climatic suitability should be based on distribution data from all regions where the species is found, otherwise, the risk of underestimating suitable conditions is increased (see below). Accordingly, for each species, we compiled native and worldwide invasive occurrence records from a variety of online databases and primary literature (see Appendix). Species occurrences consisting only of locality names or symbols in maps were georeferenced in Google Earth (available from "www.google.com/earth/index.html") and ArcGIS (Esri, Redlands, CA), respectively. Using a coarse resolution such as 20 km allowed more localities to be included, but simultaneously lead to an averaging of local climates which in turn resulted in an unlogic suitability of e.g., the Sahara Desert for *Z. arboreus* and a strikingly low suitability of the Great Lakes Region for *Z. nitidus* despite of many records from there (Appendix). Thus, we only retained records with a spatial precision equal or higher to 10 km. In total we collected 3154 occurrence records for *Z. arboreus* and 6534 for *Z. nitidus* from different geographic areas and climatic regions (Maps in Appendix).

In addition to species occurrences, we also collected six worldwide spatially explicit climatic variables that are considered to have an important role on the physiology, activity and reproduction of terrestrial gastropods (e.g., Prior, 1985; Cook, 2001; Sulikowska-Drozd et al., 2013): annual mean temperature, maximum temperature of warmest month, minimum temperature of coldest month, annual precipitation, precipitation of the driest quarter and precipitation of the wettest quarter. These variables were collected from the WorldClim database (accessible at "www.worldclim.org") at a spatial resolution of 5 arc minutes ($\approx$ 9.3 km at the equator).

Predictions of climatic suitability: The existence of suitable climatic conditions is a pre-requisite for non-native species to establish in new areas. In fact, given adequate propagule pressure, climate seems to be the only factor impeding the success of many invasive species (e.g., Aragón et al., 2010; Rice & Silverman, 2013). Because of this high importance, many researchers have attempted to estimate climatic suitability based on the statistical analyses of the climatic conditions occupied by species, assuming niche conservatism (Soberón & Nakamura, 2009). However, since species ranges are also shaped by interactions between dispersal constraints versus probabilities of long-distance dispersal events, along with biological characteristics and biotic interactions that modify colonisation probabilities, this correlative approach may not express the full range of suitable climates (e.g., Capinha et al., 2014). Using the entire range of occupied regions, i.e., native and invaded ranges, simultaneously is a necessary step toward more reliable estimates (Broennimann & Guisan, 2008). Nonetheless, it still does not guarantee that the full suite of suitable climates is captured.

We here used Mahalanobis distances for predicting climatic suitability to our species. Mahalanobis distances is a similarity metric that differ from the simple Euclidean distances because it is scale-independent and corrects for the correlation among variables (Farber & Kadmon, 2003). This method is a popular alternative to more sophisticated group discriminative techniques, such as MaxEnt or GARP, particularly because it only requires information about the climatic conditions occupied by the species, i.e., a profile technique. Group discrimination techniques, on the other hand, also require information about the climatic conditions in which the species is absent or, alternatively, the climatic conditions that were accessible to it (i.e., 'pseudo-absences' or 'background data'; Soberón & Nakamura, 2009). Such information was not available for any of our two species. Moreover, some authors also suggest that profile techniques should be preferred over group discrimination techniques when predictions of climatic suitability are made for invaders because species' absences may be due to non-climatic factors (Soberón & Nakamura, 2009; Jiménez-Valverde et al., 2011).

We used a GIS application by Jenness et al. (2012) to calculate the Mahalanobis distance of each worldwide climatic combination (as represented by the grid cells of the six climate variables) to the mean of the climatic conditions occupied by each species. Because Mahalanobis distances are continuous, the distinction between suitable and unsuitable climate requires the use of a distance threshold. If one assumes that all species' occurrence are representative of viable populations, this threshold can be easily set as the maximum Mahalanobis distance in which the species is found. However, because some occurrence records may suffer from geographical or taxonomic identification errors or represent sink-populations, more conservative thresholds are also often considered. We here applied three progressively conservative thresholds that are commonly applied for delimiting suitable climates. These corresponded to the Mahalanobis distance bounding 100%, 95% and 90% of the species occurrences. (e.g., Baselga et al., 2012; Capinha et al., 2013).

RESULTS

Identity of the specimens from Sabah. Barcoding matched the identifications using shell characteristics, suggesting that shell characteristics can be used to assign other specimens to the species as well. The individuals from Sabah were *Zonitoides nitidus* (SP13543, SP13544) and *Zonitoides arboreus* s.l. (SP 13548) (Table 1). Despite the huge geographic distance, the genetic distances were only marginally larger between the Dutch individuals and the Sabah individuals than within the two locations (Table 1). Please also note that there are two lineages of *Z. nitidus* from

Table 1. Genetic distances for the partial sequences of COI (683 pb, 558 bp for Borneensis sample) and 28S rDNA (769 bp), examplified using the own *Zonitoides* specimens only. The partial sequence of the 28S rDNA amplified by the primer pair D23F and D6R was not available for the specimen from Mesilau.

Species	*Zonitoides nitidus*				*Zonitoides arboreus* s.l.		
Shortcode	NL1	NL2	SP 13543	SP 13544	Hortus	SP 13548	BORNEENSIS
COI partial sequence							
GenBank-No.	KF147209	KF147212	KF147210	KF147211	KF147206	KF147207	KF147208
Zoni-NL2	0.0036	–	0.0036	0.0072	0.1382	0.1382	0.1357
Zoni-SP 13543	0.0036	0.0036	–	0.0036	0.1329	0.1329	0.1305
Zoni-SP 13544	0.0072	0.0072	0.0036	–	0.1329	0.1329	0.1305
Zoar-Hortus	0.1329	0.1382	0.1329	0.1329	–	0.0036	0.0018
Zoar-SP 13548	0.1329	0.1382	0.1329	0.1329	0.0036	–	0.0018
Zoar-BORNEENSIS	0.1305	0.1357	0.1305	0.1305	0.0018	0.0018	–
28S partial sequence							
GenBank-No.	KF147215	KF147218	KF147216	KF147217	KF147213	KF147214	–
Zoni-NL2	0.0000	–	0.0013	0.0013	0.0145	0.0171	–
Zoni-SP 13543	0.0013	0.0013	–	0.0013	0.0160	0.0173	–
Zoni-SP 13544	0.0013	0.0013	0.0013	–	0.0159	0.0172	–
Zoar-Hortus	0.0145	0.0145	0.0160	0.0159	–	0.0026	–
Zoar-SP 13548	0.0171	0.0171	0.0173	0.0172	0.0026	–	–
Zoar-BORNEENSIS	–	–	–	–	–	–	–

Ontario in BOLD, in one of which the Dutch material falls (Figure 1). Also, *Z. arboreus* seems to consist of two main clades (compare Figure 1), one of which is mainly occurring in the temperate zone and often labelled as *Retinella spec.* in BOLD, and one that includes our material and other, usually warm-temperate to (sub-) tropical, and invasive specimens (results from the BLAST procedure in BOLD that includes private data, tree not shown here).

Almost all of the genetic variation within and especially between species was from the third codon position. We did not observe any differentiation in the second codon position, that is, it was strictly conserved. These patterns were also found in the public sequences from BOLD.

The amino acid sequences from the COI barcoding sequence were the same for the *Zonitoides* species, including the nine public COI sequences from BOLD. Only two specimens had slight deviations (Table 2). These two deviations were from mutations in the first codon position, namely a T substitution of A in base 426 in RMNH.MOL.328277 (KF147212), and a T substitution of C in base 477 in SP13544 (KF147211).

More records from Borneo based on empty shells. Because the barcoding indicated that the snails indeed belong to already described non-native taxa, other populations in Sabah showing the same shell features highly likely also belong to these taxa. During studies on the snail fauna of forests in Sabah, performed by two of the authors (M.S., J.V.) and T.-S. Liew (UMS, Kota Kinabalu), only three more observations on *Zonitoides* were made. The three additional observations had been instantly assigned to the two *Zonitoides* species by their collectors. The three observations were geographically close to those of the barcoded specimens:

– *Zonitoides nitidus*, Sabah, West Coast Prov., Kinabalu N.P., Kinabalu Park Headquarters, near Rajah Lodge, about 10 years ago (M.S., personal observation). This observation of living individuals was made only a few hundred meters from the location with the barcoded individuals.

– *Zonitoides arboreus* s.l., Sabah, West Coast Prov., Crocker Range N.P., km 54 road Kota Kinabalu-Tambunan, Gunung Mas, 1750 m a.s.l., disturbed primary montane forest on sandstone/shale soil, near a habitation, coll. J.J. Vermeulen & M. Schilthuizen, 11 April 2002 (collection J.J. Vermeulen, label no. 9770). This is at ca. 5.827°N, 116.337°E and thus a little NW of Gunung Alab, from where the barcoded individual originated.

– *Zonitoides arboreus* s.l., Sabah, West Coast Prov., Kinabalu N.P., access road to Mesilau resort, 2000 m a.s.l., damp roadside with high grass and shrubs, litter in concrete drain, coll. J.J. Vermeulen, 16 July 2005 (collection J.J. Vermeulen, label no. 12733). This record is from the same village as the barcoded individual provided by T.-S. Liew.

Areas of climatic suitability for the two *Zonitoides* species. We thus explored how similar the climatic conditions were in Sabah when compared to those from other occurrences of the two species, and how high the invasion potential of the two non-native species is in the region under study. To this end, we calculated the global distribution of grid cells with a climatic similarity to the conditions from the known distribution of the two species.

Although much of the patterns were similar, *Zonitoides arboreus* could occur in large areas around the globe, whereas the potential distribution of *Z. nitidus* seems to be a more

Table 2. Distances from the translated partial COI gene, examplified using the own *Zonitoides* specimens only. The nine public COI sequences from BOLD translate in the 'general' amino acid sequence. Only two individuals of *Zonitoides nitidus* had an effective change in the amino acid sequence.

| | *Zonitoides nitidus* | | | | *Zonitoides arboreus s.l.* | | |
	NL1	NL2	SP 13543	SP 13544	Hortus	SP 13548	BORNEENSIS
NL2	0.0054	–	0.0054	0.0108	0.0054	0.0054	0.0054
SP 13543	0.0000	0.0054	–	0.0054	0.0000	0.0000	0.0000
SP 13544	0.0054	0.0108	0.0054	–	0.0054	0.0054	0.0054
Hortus	0.0000	0.0054	0.0000	0.0054	–	0.0000	0.0000
SP 13548	0.0000	0.0054	0.0000	0.0054	0.0000	–	0.0000
BORNEENSIS	0.0000	0.0054	0.0000	0.0054	0.0000	0.0000	–

restricted to temperate conditions (Fig. 2). Both species display low climate matching in most of the tropical zone. However, they also show the same pattern of potentially suited climatic conditions in the mountainous regions of the tropics (Fig. 2). In tropical South America, the Andes are potentially vulnerable to invasion. In tropical Africa, susceptible areas are the Ethopial Highlands, the Uganda-Tanzanian Highlands with the Rwenzori Mountains, Mount Kenya and Mount Kilimanjaro, and the mountains north of the Congo Basin (Fig. 2).

Zonitoides arboreus finds suitable conditions across much of Southeast Asia (Fig. 3a). On Borneo, suitable conditions include large parts of the Federal State of Sabah with the Crocker Range and the adjacent hillands in SE (Fig. 3a). The species was to be expected, as climatic conditions at Mount Kinabalu and in the Crocker Range are within climatic conditions that envelope 90 % and 95 % of those of its already known distribution (Fig. 4a). Several other conservation areas of Sabah, such as the Trusmadi Forest Reserve, Maliau Basin, and Danum Valley, may be susceptible to invasion by *Z. arboreus*, as are some Indonesian conservation areas, including the Kutai National Park and the Kayan Mentarang National Park (compare Fig. 3a).

In contrast, *Zonitoides nitidus* seemingly finds marginal conditions in Southeast Asia, and environmental conditions that best ($\leq 95\%$) fit those from its known distribution occur, for example, only in a very few grid cells from Peninsular Malaysia, Sumatra, and Borneo (Fig. 3b). In this region, even the outermost 5% of the climatic suitability envelope has an insular occurrence (Fig. 3b). The population at Kinabalu Park Headquarters is within this outermost 5% of the climatic suitability envelope (Fig. 4b). Within the outmost 5%, *Zonitoides nitidus* met sufficiently suitable conditions to persist for at least 10 years, and it probably even spread locally (see above).
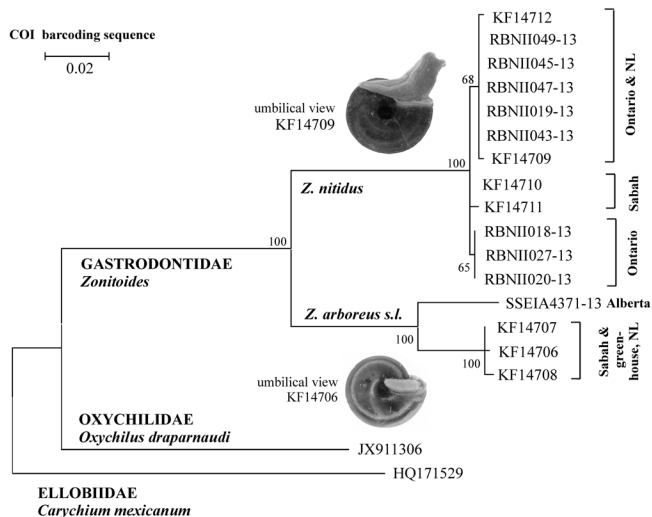

Fig. 1. Estimation of the phylogenetic relationships of the COI barcoding sequence from the *Zonitoides* specimens and two outgroups (labeled with their BOLD or GenBank accession numbers) using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (−2138.3583) is shown. Branch lengths equal genetic distances in terms of the number of base substitutions per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree was constructed in MEGA 6.0. Please note that the shell of (living) *Z. nitidus* is darkly pigmented, and that the extended animal of the depicted *Z. nitidus* started fading.
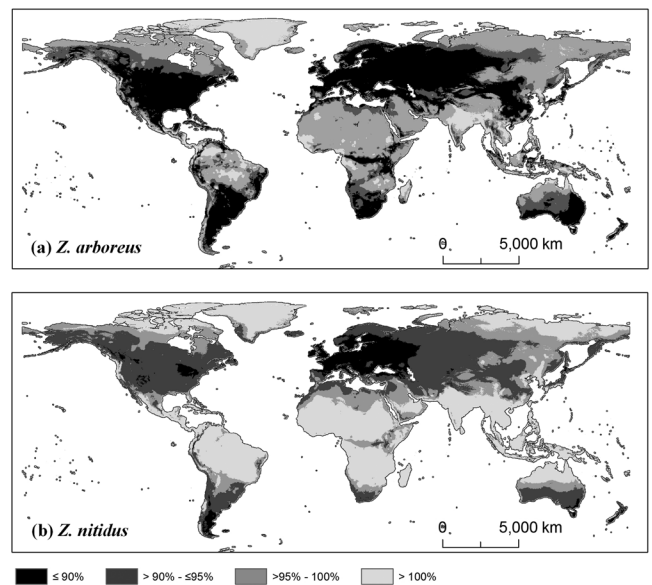

Fig. 2. Global climatic suitability for (a) *Zonitoides arboreus* s.l. and (b) *Zonitoides nitidus* based on Mahalanobis distances from the 10 km grid resolution. The higher the threshold, the more dissimilar are the climatic conditions to those of the majority of known occurrences, and >100 % means that the climatic conditions are dissimilar to those of any other available record.

DISCUSSION

Our study has shown the potential of temperate species to colonise elevated areas in the tropics. Hence, climatic suitability might indeed be the predominant factor in shaping invasion success (e.g., Aragón et al., 2010; Rice & Silverman, 2013). Both invasive taxa, *Z. nitidus* and *Z. arboreus* s.l., seem to be widespread and thus can be considered being good dispersers and colonisers. The autecological requirements of the two species differ for example in soil moisture preferences, as *Z. nitidus* only occurrs in wetlands (Kerney
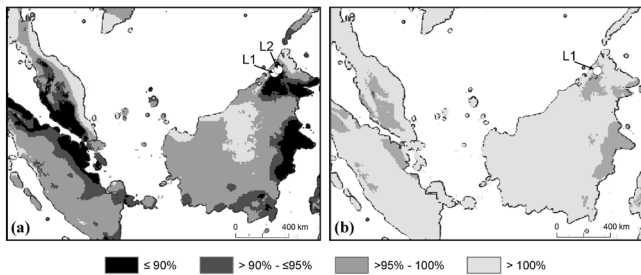
Fig. 3. Climatic suitability for (a) *Zonitoides arboreus* s.l.; and (b) *Zonitoides nitidus* on the Malaysian Peninsula, Sumatra and Borneo, based on Mahalanobis distances. The higher the threshold, the more dissimilar are the climatic conditions to those of the majority of known occurrences. >100% means that the climatic conditions are dissimilar to those of any available record. The new records for the species (white circles) were not included in the calculation of the climatic suitability.
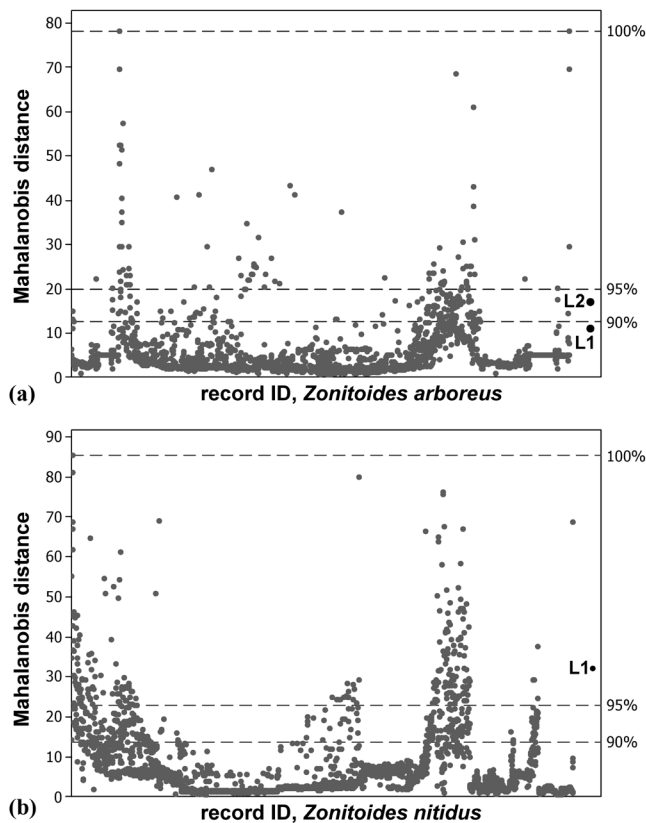
Fig. 4. Position of the new locations (L1 and L2, enumeration for each species separately) in Sabah in relation to the Mahalanobis distances of climatic variables for: (a) *Zonitoides arboreus* s.l., and (b) *Z. nitidus*. Record ID refers to the order of the data entry.

et al., 1983), but the distributional ranges, and areas with potentially suitable macroclimatic conditions, seemingly broadly overlap. Yet, part of the overlap could be an outcome of an unresolved taxonomic issue in *Z. arboreus* (s.l.), as the phylogenetic reconstruction in Fig. 1 suggests, and we will detail it below.

Zonitoides nitidus. *Zonitoides nitidus* is a temperate zone species with a Holarctic distribution (e.g., Kerney et al., 1983) that, however, also has been reported from subtropical areas of North America (e.g., Minton & Perez, 2005). Despite the huge geographic distances covered in our study, the genetic distances showed no clear geographic separation (compare Fig. 1). Indeed, *Zonitoides nitidus* might be a good coloniser, as individuals of the species seem capable of selfing (Jordaens et al., 1998, 2003). Selfing also interferes with the traditional biological species concept that amongst others implies the biparental production fertile offspring (e.g., Mayr, 1942). We tentatively consider all closely related strains from the COI gene as one species. Conseqently, we recognise three taxonomic units belonging to the genus *Zonitoides* in Fig. 1.

In the temperate zone, *Zonitoides nitidus* reproduce during the warm season, that is, from June until the end of October (Kuznik-Kowalska, 2011). The species usually feeds on plant detritus (Frömming, 1962), but it has been acknowledged as a potential biocontrol agent for wetland snails (e.g., Rondelaud, 1975; Moens & Vase, 1986; Rondelaud et al., 2006). In the temperate zone, the timing of predatory behaviour coincides with the egg production period (Rondelaud et al. 2006, and references therein). Its life cycle and foraging behavior under the climate of tropical mountains remains to be studied.

Zonitoides arboreus. Similarly, interactions between *Zonitoides arboreus* and the indigenous components of biodiversity are possible, but need to be scrutinised. Damages to a large variety of plants have been attributed to this species complex (e.g., Bartsch & Quick, 1926; Godan, 1979; Hayes et al., 2012).

Zonitoides arboreus was described by Say (1816) from North America without a locus typicus, but Bartsch and Quick (1926) suggest it could have been from the vicinity of Philadelphia where most of Say's new species were collected. Several later described species were soon synonymised with *Zonitoides arboreus*, for which its acknowledged distribution range spans from (sub-) tropical to subarctical conditions (e.g., Binney & Bland, 1869; Mozley, 1937; Gleich & Gilbert, 1976; Minton & Perez, 2005). *Zonitoides arboreus* is known to occur in greenhouses (Karlin, 1956; Kerney et al., 1983; Horsák et al., 2004), with an increasing number of populations recorded from outside greenhouses in Europe (e.g., Dvořák & Kupka, 2007 and references therein). While up to now its establishment in the temperate zone of Europe seems to progress slowly, the taxon has spread widely in warm and tropical climates across the globe (e.g., Herbert, 2010 and references therein).

Yet, our study indicates a well-defined genetic unit from the temperate zone (Alberta) and a genetic unit that is usually found under tropical climatic conditions. If originally

collected at Philadelphia, *Z. arboreus* would be a species from the (warm) temperate zone. In contrast, the synonymised species *Z. ottonis* (Pfeiffer, 1840) was described from Cuba. This taxon was found to live in the tropical areas of Florida as well (Gould, 1851). Gould (1851) indicates that Pfeiffer himself later seemingly considered *Z. arboreus* and *Z. ottonis* as synonyms. Bartsch and Quick (1926) did not find any differences between shells from parts of the eastern coast of the United States but they did not sample in Florida. Gould (1851), who seemingly saw tropical material as well, clearly states that there are shell anatomical differences such as a smaller size and a narrower umbilicus in *Z. ottonis*.

The differences given by Gould (1851) are in line with some discrepancies in species measurements in the standard literature of the two continents. For example, Pilsbry (1939) gives a shell diameter of 6–7 mm for *Z. nitidus* and 5–6 mm for *Z. arboreus* in North America. Kerney et al. (1983) give a shell diameter of 6–7 mm for *Z. nitidus* but only 4.5–5 mm for the greenhouse species *Z. arboreus* in Europe. Here, we want to point out again that our *Z. nitidus* specimens were smaller than expected, but aphallic and/or not fully mature. According to Pilsbry (1939; and the figures therein) the umbilicus fits 5× in the shell diameter for *Z. nitidus* and 4.5–5× in that of *Z. arboreus*. In contrast, the figures in Kerney et al. (1983) suggest it fits about 4.5× in the diameter for *Z. nitidus* and about 5.1× in that of *Z. arboreus*, the relative umbilicus widths thus being reversed between the species. Also, Pilsbry (1939) uses the word 'yellow' to describe the shell of *Z. nitidus*, whereas 'yellowish' is used to describe the shell of *Z. arboreus* in Kerney et al. (1983). All these inconsistencies might have lead to some of the public BOLD sequences from the Great Lake Region being labeled *Z. arboreus* but clustering with specimens from wet habitats in The Netherlands and Sabah, that were blackish-colored and looking like what is typically called *Z. nitidus* in Europe.

Our findings consequently point to the neccessity of a taxonomic revision of the *Zonitoides* species. Only then it will be clear if the tropical strain actually is *Z. ottonis*, and if the few field observations in temperate N Europe are in fact introductions of the true *Z. arboreus*.

Forest disturbance, climate, and invasion potential. In the course of intensive studies on the forest snails of Sabah by two of the authors (M.S., J.V.) and T.-S. Liew (UMS, Kota Kinabalu; pers. comm.), that spanned over several years, *Zonitoides* was never found in undisturbed, remote forests. The additional data from empty shells indicates that the vicinity of the locations where the *Zonitoides* were collected in 2012 seem to have been colonised already for (at least) a decade. This suggests populations are established. All reports of the two introduced *Zonitoides* species from Sabah/Borneo so far were from elevations in between 1500 and 2000 m a.s.l., corroborating the assumption that the climatic conditions have played a role in the colonisation of the almost equatorial region.

Besides of climatic suitability, two processes may have helped the two *Zonitoides* species to establish in Borneo: 1) the creation of new niches by anthropogenic forest disturbances; and 2) the increased probability of passive dispersal with plants from other regions associated with settlements.

Forest disturbances alter the microclimate and nutrient availability (e.g., Vasconcelos & Laurance, 2005; Scharenbroch & Bockheim, 2007). The role of disturbances for the colonisation of forest sites by gastropod species from open habitats and by invasive species has been shown for the temperate and subtropical zone (e.g., Kappes, 2006; Kappes et al., 2009). Both *Zonitoides* species were also found close to anthropogenic disturbances in mountain rainforests of Argentina (Oroño et al., 2007: 24). All sites in Sabah were close to settlements and thus close to gardens. Horticulture in former forests implies transport of cultivated and ornamental plants. This transport is more likely to occur over large distances when an isolated climatic zone, such as in the tropical mountains, is settled. Horticulture has been recognised as one of the main pathways of gastropod species introductions (e.g., Cowie et al., 2008). It thus may be assumed that the settlement in tropical mountainous forests renders these ecosystems vulnerable to biological invasion from the temperate and subtropical zone.

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APPENDIX

Sources of species occurrence data

Online databases:

Analyse Educatie en Marien Oecologisch Onderzoek, (ANEMOON; http://www.anemoon.org; Accessed on 8 April 2013)

Atlas of Living Australia (http://www.ala.org.au; Accessed on 8 April 2013)

Electronic Atlas of the Wildlife of British Columbia (e-FAUNA BC; http://www.geog.ubc.ca/biodiversity/efauna/index.shtml; Accessed on 8 April 2013);

Inventaire National du Patrimoine Naturel, (INPN; http://inpn.mnhn.fr; Accessed on 9 April 2013)

Global Biodiversity Information Facility (GBIF; http://data.gbif.org/; Accessed on 5 April 2013)

Malacology Collection at the Academy of Natural Sciences of Philadelphia (http://clade.ansp.org/malacology/collections/index.html; Accessed on 8 April 2013)

National Biodiversity Network's Gateway (NBN; http://data.nbn.org.uk/; Accessed on 8 April 2013)

SysTax – a Database System for Systematics and Taxonomy (http://www.biologie.uni-ulm.de/systax/; Accessed on 8 April 2013)

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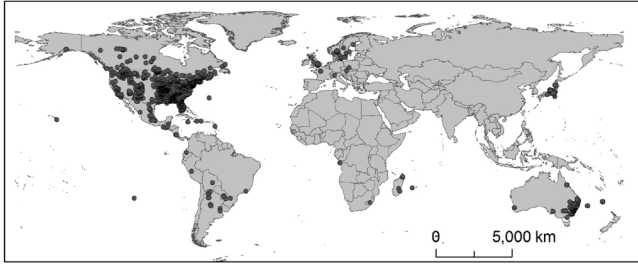
Zonitoides arboreus

Fig. A1. Map of the occurrences of *Zonitoides arboreus s.l.* used for the calculation of climatic suitability for the 10 km grid resolution.
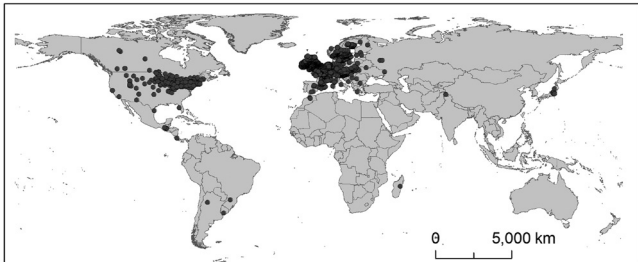
Zonitoides nitidus

Fig. A2. Map of the occurrences of *Zonitoides nitidus* used for the calculation of climatic suitability for the 10 km grid resolution.
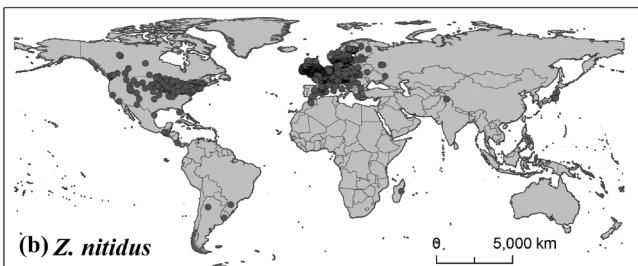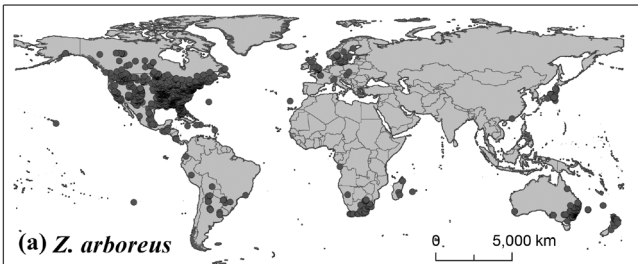

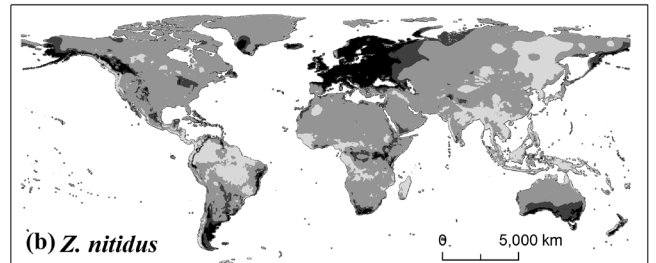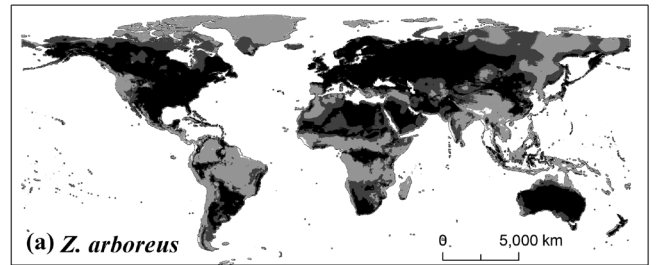Fig. A3. Map of the occurrences of *Zonitoides arboreus* s.l. and *Zonitoides nitidus* used for the calculation of climatic suitability for the 20 km grid resolution.

Fig. A4. Global climatic suitability for: (a) *Zonitoides arboreus s.l.*; and (b) *Zonitoides nitidus* based on Mahalanobis distances using the 20 km grid resolution. The higher the threshold, the more dissimilar are the climatic conditions to those of the majority of known occurrences, and >100% means that the climatic conditions are dissimilar to those of any other available record. Please note that the deserts of Africa, Arabia and Australia are unlikely places for a snail that inhabits (temperate) forests in its native range (*Z. arboreus s.l.*), and that the Great Lakes Region that seems well inhabited by *Z. nitidus* does not fully match its climate, what can only be explained from effects of averaging local climates at a larger grid scale.