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Camouflaged invasion of Lake Malawi by an Oriental gastropod

MARTIN J. GENNER,*†** ELLINOR MICHEL,‡ DIRK ERPENBECK,* NICOLE DE VOOGD,* FRANS WITTE§ and JEAN-PIERRE POINTIER¶

Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, PO Box 94766, 1090 GT Amsterdam, the Netherlands, †Department of Biological Sciences, University of Hull, Hull, HU6 7RX, UK, ‡Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK, §Institute Biology Leiden, University of Leiden, PO Box 9516, 2300 RA Leiden, the Netherlands, ¶Centre de Biologie et Ecologie Tropicale et Méditerranéenne, EPHE, UMR 5555 CNRS, Avenue de Villeneuve, 66860 Perpignan cedex, France

Abstract

In this study we report the first animal invasion, to our knowledge, into Lake Malawi. The colonizer is a non-native morph of the gastropod *Melanoides tuberculata* that differs substantially in external shell characters from co-occurring indigenous forms. However, because the species possesses extensive within-Africa geographical variation in shell morphology, it was unclear whether the invasion was range expansion of a native African morph, or a colonization from elsewhere. Mitochondrial DNA sequences indicate a south-east Asian origin for the invader, suggesting that shell variation found among indigenous allopatric populations camouflaged an intercontinental invasion.

Keywords: cryptic invasion, mitochondrial DNA, parallel evolution, phylogeny, thiaridae

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Introduction

Introductions of nonindigenous species can result in substantial economic damage and extirpation of native faunas (Rahel 2002). Introduced taxa may be morphologically indistinguishable from local populations or 'cryptic' and only identifiable on the basis of distinct genotypes (Geller *et al*. 1997). However, it is also possible that introduced taxa may be easily distinguished from indigenous populations on external appearance, but their true origins are concealed because their morphology appears to be within the bounds of regional allopatric variation. Hence, between-region introductions may go unrecognized, or be misidentified as within-region range changes. If so, the scale of introductions could be higher than currently recognized. Here we report evidence for one such case where an intercontinental invasion was camouflaged not by morphological similarity with sympatric native taxa but rather by morphological variation among indigenous allopatric populations.

Correspondence: M. J. Genner. **Present address: Department of Biological Sciences, University of Hull, Hull. HU6 7RX. UK. Fax: + 44 (0) 1482 465458; E-mail: mjgenner@hotmail.com

The effects of invasions are often greatest in 'island' ecosystems, such as isolated freshwaters (Davis 2003). A location that may be vulnerable is Lake Malawi in East Africa, an ancient lake with species-rich flocks of endemic fish and molluscs (Michel 1994). One of the molluscs native to the lake is *Melanoides tuberculata* (Müller) (Brown 1994). This taxon is common to freshwaters within its native distributional range that covers much of tropical Africa, Asia and Oceania. It is now also present in much of the tropical and subtropical New World as a consequence of introductions that started during the last century (Madsen & Frandsen 1989). The species exhibits considerable geographical polymorphism in shell ornamentation, but within sites discrete lineages or 'morphs' of *M. tuberculata* can be separated on shell characters, such as coloration and ornamentation, because of predominantly parthenogenetic reproduction resulting in negligible intrapopulation variability in these traits (Samadi *et al*. 1999). Our results suggest that Lake Malawi has been recently colonized by an additional non-native morph of *M. tuberculata*. Mitochondrial DNA (mtDNA) sequence data identified the origins of this invader that were initially unclear because of high morphological variation among allopatric native populations.

Materials and methods

Sampling and shell description

Collections of *Melanoides tuberculata* were made within the Lake Malawi Basin between July 1999 and September 2002. Morphs were assigned three-letter codes following Pointier (2001). Where morphs were present at more than one location, populations were assigned site identification numbers (Fig. 1). Two morphs were present in the Lake Malawi Basin; one (morph LMI — 'Lake Malawi Introduced', named in retrospect) was only found at localities in the southern section of Lake Malawi and Lake Malombe, the other was widespread (morph LMN — 'Lake Malawi Native'; Fig. 2a). Outside the Basin, a single morph was present at each of the 10 sampling sites, except for the Lower Selatar Reservoir, Singapore, where two were found (Fig. 1). Morphs were compared using the categorical morphological character scoring system of Facon *et al*. (2003), and using PRIMER-5 (Primer-E Ltd), a normalized Euclidean distance matrix of shell characters was constructed. Multi-dimensional scaling was then used to create a two-dimensional ordination illustrating overall shell-character similarity. This scoring system is robust for delimiting genetic lineages of *M. tuberculata*. Representatives of morphs identified using this method possess high similarity of microsatellite DNA alleles and mtDNA sequences, indicating heritability of characters (Samadi *et al*. 1999; Facon *et al*. 2003). In contrast, delimitation of *M. tuberculata* lineages using morphometric methods to quantify shell shape alone has been proven to be unreliable because of substantial between-lineage overlap in the continuous quantitative measures (Samadi *et al*. 2000).

Examination of museum material

To investigate whether morphs sampled in the Lake Malawi Basin had historically been present within the lake and elsewhere within Africa, we examined *Melanoides* deposited at the Royal Museum for Central Africa, Tervuren, Belgium (RMCA), the Natural History Museum, London, UK [BM(NH)], the Museum National d'Histoire Naturelle, Paris, France (MNHN) and the Geologische Instituut, University of Gent, Belgium (GIG). From Lakes Malawi and Malombe, RMCA houses 264 specimens in 30 lots dating between 1924 and 1972, BM(NH) houses 960 specimens in 51 lots dating between 1877 and 1982, MNHN houses 66 specimens in 26 lots dating between 1885 and 1945 and GIG houses 299 specimens in seven lots dating between 1962 and 1992. Specific collection sites were not available for many of these lots, but importantly BM(NH) houses 824 specimens in 20 lots collected at Cape Maclear between 1972 and 1982, and GIG houses 140 specimens in a single lot collected at Lake Malombe in November 1991.

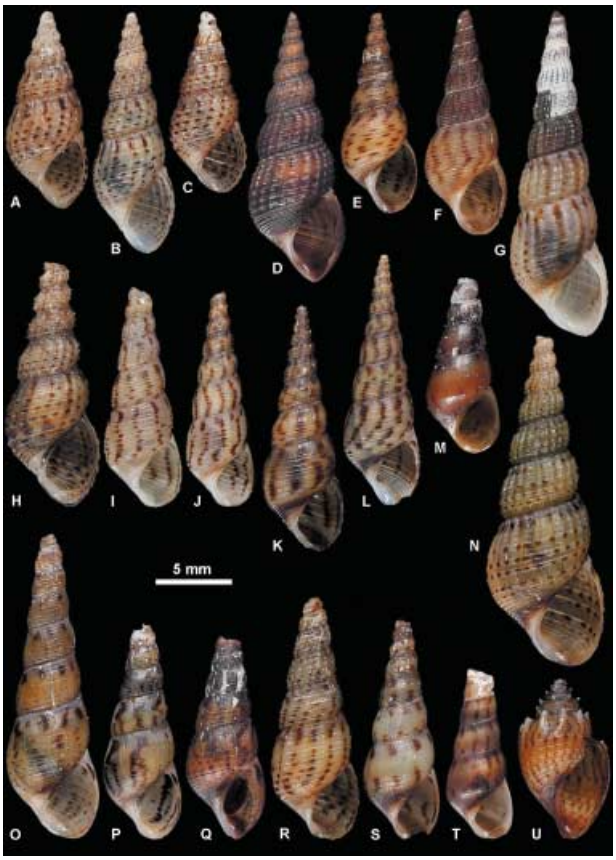

Fig. 1 *Melanoides tuberculata* morphs included in this study, numbers in subscript following the morph names refer to populations. (A) Morph LMN_1, Nkhata Bay, Lake Malawi, collector and date MG 2002. (B) LMN_2, Cape Maclear, Lake Malawi, MG 2002. (C) Morph LMN_3, Mkungula, Lake Malombe, MG 2002. (D) LIS, Lisuli Dam, Chikwawa, Malawi, MG 2002. (E) MAY, Mayotte, Comores Archipelago J-PP 2000. (F) SOM, Eil Spring, Somalia, Tim Fison 1992 via David Brown, BM(NH). (G) ISR, Ilan, Israel. Joseph Heller 1993. (H) VIC, Mwanza Gulf, Lake Victoria, FW 2002. (I) LMI_1, Kambiri Point, Lake Malawi, MG 2002. (J) LMI_2, Cape Maclear, Lake Malawi, MG, 2002. (K) LMI_3, Mkungula, Lake Malombe, MG 2002. (L) LMI_4, Makakola, Lake Malawi, EM 1999. (M) BAN, Pathun Tani, Bangkok, Thailand, J-PP 1998. (N) SRI, Kandalama Lake, Dambulla, Sri Lanka, NdV 2003. (O) PAN, Pandan Reservoir, Singapore, MG 2003. (P) LSD, Lower Selatar Drain, Singapore, MG 2003. (Q) USR, Upper Selatar Reservoir, Singapore, MG 2003. (R) LSR, Lower Selatar Reservoir, Singapore, MG 2003. (S) LSS, Lower Selatar Reservoir, Singapore, MG 2003. (T) NAP, Napier Road, Singapore, MG 2003. (U) Outgroup, *Thiara amarula*, Mayotte, Comores Archipelago, J-PP 2000.

Molecular analyses

A 580-base-pair section of mtDNA cytochrome oxidase subunit 1 (CO1) was sequenced from 38 *M. tuberculata* specimens belonging to 20 populations. *Thiara amarula* (L.) from Mayotte was selected as an outgroup. Mitochondrial

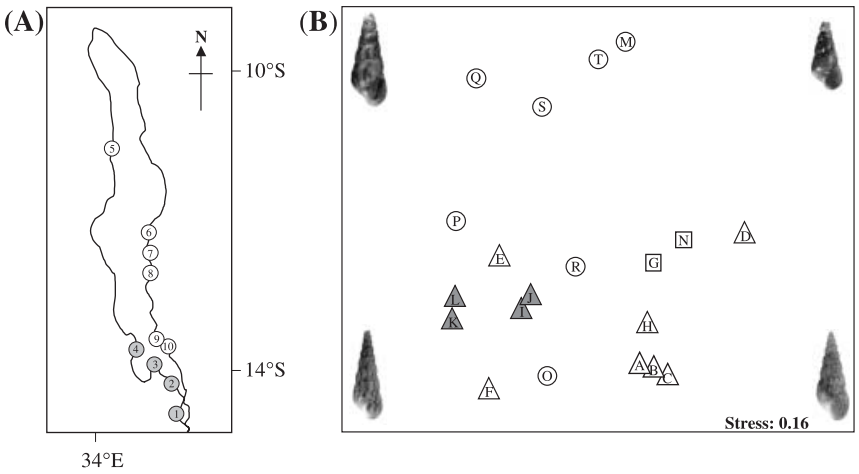

Fig. 2 (A) Distribution of morphs LMI and LMN within the Lake Malawi Basin during 1999–2002. Grey circles represent sites where the both morphs were present, white circles represent areas where LMN was present and LMI was absent. 1 Mkungula, Lake Malombe (14°29′ S, 35°09′ E); 2 Makakola (14°18′ S, 35°08′ E); 3 Chembe, Cape Maclear (14°01′ S, 34°49′ E); 4 Kambiri Point (13°43′ S, 34°38′ E); 5 Nkhata Bay (11°36′ S, 34°18′ E); 6 Nala Point (12°11′ S, 34°42′ E); 7 (12°21′ S, 34°42′ E); 8 Ncolongue (12°48′ S, 34°47′ E); 9 Chinune (13°30′ S, 34°48′ E); 10 Msinji (13°31′ S, 34°52′ E). (B) Multi-dimensional scaling plot illustrating the similarity of shell characters of morphs. Closer symbols indicate more similar morphs. Letters refer to images in Fig. 1, and symbols refer to sampling locations. Circles, southeast Asia; squares, Sri Lanka and Israel; triangles, Africa. Morph LMI from Lake Malawi is shaded in grey. Morphs characterizing the extremes of each axis are illustrated in each corner. Morph LMI is pictured in the lower left, morph LMN in the lower right.

DNA large subunit (LSU) rDNA sequences indicate that the species is part of a well-supported sister clade to *Melanoides* (Lydeard *et al*. 2002). DNA was isolated from ethanol-preserved foot tissue of two individuals per *M. tuberculata* population using the phenol/chloroform method described in Erpenbeck *et al*. (2002), with the exception of samples from Somalia and Sri Lanka (one isolation from each). For most taxa primers LCO-1490 and HCO-2198 were used (Folmer *et al*. 1994). For *T. amarula* a different reverse primer was developed (5′-RAARTTWCGRTCHGTT-3′), where R = A or G, W = A or T and H = A or C or T. Polymerase chain reaction (PCR) was performed in 25-μL reactions including 1 μL genomic DNA, 2.5 μL 10× PCR buffer, 2.5 μL dNTPs (1 mM); 1 μL each primer (10 mM stock), 1 μL $MgCl_2$ (25 mM stock), 0.5 units Super*Taq* (Promega), 1 μL bovine serum albumin (20 μg/mL) and 14.9 μL double-distilled water. PCR conditions were as follows: 1 min at 95 °C; then 34 cycles of 95 °C for 30 s, 43 °C for 30 s and 72 °C for 1 min, followed by 72 °C for 5 min. PCR products were purified and sequenced in both directions using the cloning and sequencing procedure in Erpenbeck *et al*. (2002), or direct sequencing of cleaned PCR product with ABI sequencers and BigDye™ Terminator cycle sequencing kits.

Phylogenetic analyses

Sequences were aligned in DAMBE (Xia & Xie 2001). Phylogenetic trees were reconstructed using maximum likelihood, maximum parsimony and minimum evolution techniques in PAUP* 4.0b10 (Swofford 2002), and Bayesian inference techniques using MRBAYES 3.04 (Huelsenbeck & Ronquist 2001). Prior to analyses, MODELTEST 3.06 (Posada & Crandall 1998) and MRMODELTEST 1.1b (http://www.ebc.uu.se/systzoo/staff/nylander.html) were used to determine the best-fitting model of sequence evolution, the HKY + Γ model was selected. Maximum likelihood analyses were performed using heuristic searches, TBR branch swapping and bootstrap support was calculated from 100 permutations. Maximum parsimony was undertaken with branch and bound searching, TBR branch swapping and branch support was calculated as the bootstrap percentage of 1000 replications. Minimum evolution branch support was calculated from 1000 permutations. Bayesian inference analyses were performed with the HKY + Γ model using four Markov chains, 5 000 000 generations with a burn-in of 10%. The significance of alternative phylogenetic hypotheses was tested using topology-dependent PTP tests (parsimony, 1000 permutations) in PAUP*, and a global molecular clock was tested for using likelihood-ratio statistics. The sequences generated have GenBank Accession codes AY575971–AY575998.

Results

Melanoides tuberculata morph LMI had several shell characters distinguishing it from LMN, the other morph present in Lake Malawi: (i) a more acutely conical shell than LMN; (ii) a columnar band, this trait was absent in LMN; (iii) shallow spiral grooves, these are more pronounced in LMN and; (iv) no axial ribs; these are elevated in LMN

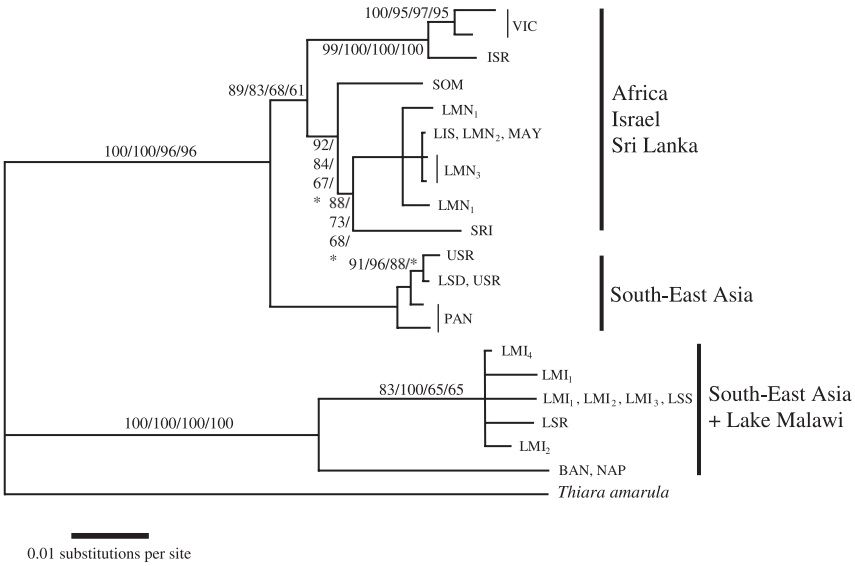

Fig. 3 Phylogenetic tree based on partial CO1 sequences of *Melanoides tuberculata* morphs with the outgroup *Thiara amarula*. Tree constructed using the neighbour-joining method on uncorrected *P*-distances. Numbers on branches represent Bayesian posterior probabilities, followed by maximum parsimony, maximum likelihood and minimum evolution bootstrap percentage support, respectively. Only values greater than 50% are shown, * indicates support less than 50% in minimum evolution analysis. For codes see legend to Fig. 1.

(Fig. 1, Table 1). Examination of museum material revealed that allopatric populations of *M. tuberculata* possessed considerable differences in shell morphology. The majority of African populations had elevated axial ribs similar to LMN, but specimens without distinct ribs and similar coloration to our focal morphs from Somalia (SOM) and Mayotte (MAY) were also in collections from Mauritius, Madagascar, Algeria, Kenya and Namibia. Critically, LMI was absent from all museum collections from the Lake Malawi Basin, including those from Cape Maclear between 1972 and 1982, and Lake Malombe in 1991. All museum specimens of *M. tuberculata* from Lakes Malawi and Malombe, including fossil specimens from deposits near the Upper Shire River ^{14}C-dated to 5800 years before present (D. Van Damme personal communication), had elevated axial ribs and pronounced spiral grooves characteristic of LMN.

Twenty unique mitochondrial haplotypes were present in 38 *M. tuberculata* individuals sequenced. All phylogenetic reconstruction methods resulted in congruent topologies (Fig. 3). *Melanoides tuberculata* from two well-supported mtDNA clades were present within Lake Malawi. Morph LMN belonged to a clade containing all other African populations, and those from Israel (ISR) and Sri Lanka (SRI), whereas LMI belonged to a clade containing the Thai morph (BAN) and three morphs from Singapore (LSR, LSS, NAP). Multiple individuals sequenced from the same morph belonged to the same clades in all cases. Both major lineages contained morphs with a range of shell characters (Figs 1–3). Morphs LMN, LIS and MAY differed strikingly in shell characters, but shared mitochondrial haplotypes. On average there was 13.67% sequence divergence (uncorrected *p*-distances) between individuals from LMN and LMI (range 13.28–14.31%), within these morphs average divergences between individuals were 0.26% for LMN (range 0–0.52%) and 0.36% for LMI (range 0–0.86%). A global clock-like tempo of nucleotide substitution was rejected (likelihood-ratio test: clock enforced LnL = 1915.41, clock not enforced LnL = 1882.71, 19 d.f., $P < 0.001$). Alternative phylogenetic hypotheses that morph LMI was more closely related to other taxa from Africa, than to Asiatic taxa within the clade containing LSR, LSS, NAP and BAN, were significantly rejected by parametric bootstrapping (T-PTP tests, 1000 permutations, $P < 0.001$).

Discussion

An invading taxon

The absence of LMI from historic collections made at Cape Maclear and Lake Malombe, but its current high abundance at these sites (up to 70 individuals/m^2 at a 5-m depth at Cape Maclear; M.J.G. personal observation), suggests that colonization has taken place within the last 22 years. It is possible that the morph may have been elsewhere in the basin prior to this but evidence suggests it is not a long-term resident. The morph evidently has the ability to disperse rapidly, colonize new sites, and become one of the most abundant gastropods present. However, with such rapid dispersal ability, if it were truly indigenous, it would be surprising that it had not colonized the northern or eastern shores during evolutionary history. However, collections made in these areas indicate that LMI was absent, despite suitable habitat being present as indicated by abundant native soft-sediment gastropods. Taken together (i) the distinct shell morphology from other Lake Malawi *Melanoides tuberculata*, (ii) the absence from historic museum collections, and (iii) the limited distribution within the lake are all consistent with recent colonization. On available evidence it is not possible to identify a site or date precisely, or the mechanism of transport to the

Table 1 Shell trait scores of *Melanoides tuberculata* populations; characters were scored using the categorical scoring system of Facon *et al*. (2003)

	Background colour			Ornaments				Columellar band		General shape		Sculptures		
Morph	IN	TI	HE	DO	SP	SO	HO	SH	SC	CO	RO	GR	RI	RD
LMN_1	2	1	0	2	2	1	2	1	—	2	3	3	3	3
LMN_2	2	2	0	2	2	1	2	1	—	2	3	3	3	3
LMN_3	2	2	0	2	2	1	2	1	—	2	3	3	3	3
LIS	4	1	1	1	2	1	1	2	3	2	3	3	3	3
MAY	3	1	0	2	1	2	2	3	2	2	3	1	0	—
SOM	3	1	0	3	1	2	1	2	2	2	3	3	0	—
ISR	2	1	0	1	3	1	2	2	2	2	3	2	3	3
VIC	2	2	0	2	2	1	2	1	2	2	3	3	3	3
LMI_1	2	1	0	2	2	2	1	2	2	1	2	2	0	—
LMI_2	2	2	0	2	2	2	1	2	2	1	2	2	0	—
LMI_3	3	2	0	2	2	3	1	3	3	1	3	2	0	—
LMI_4	2	1	0	2	2	3	1	3	3	1	2	2	0	—
BAN	3	3	1	1	3	1	2	2	2	3	2	0	0	—
SRI	2	2	1	2	3	1	2	2	3	2	3	3	3	3
PAN	2	1	0	2	2	2	3	1	—	1	2	1	0	—
LSD	2	1	0	2	1	3	3	2	2	2	2	0	0	—
USR	4	3	0	2	2	1	3	3	3	3	2	1	0	—
LSR	2	2	0	2	2	2	2	2	2	2	2	2	2	2
LSS	2	1	0	1	3	1	3	2	3	2	1	1	0	—
NAP	2	1	1	1	3	1	3	2	3	3	2	0	0	—

— indicates the trait was absent, a value of 0 was substituted for generation of a multi-dimensional scaling plot.
IN, intensity of shell background colour: (1) very pale, (2) pale, (3) medium, (4) dark.
TI, background tint of the shell: (1) yellow to brown, (2) greenish, (3) orange to reddish, (4) white.
HE, heterogeneity of the background colour on the shell whorl: (0) homogeneous, (1) distinctly darker band below the suture.
DO, overall density of colour ornaments on the whole shell, except the zone just below sutures: (0) no ornaments, (1) medium, (2) dense.
SP, ornament type, expressed as proportion of spots to flames: (0) only flames, (1) more flames than spots, (2) more spots than flames, (3) only spots.
SO, size of the ornaments: (1) small spots or narrow flames, (2) medium, (3) large spots or wide flames.
HO, heterogeneity of ornamentation among different parts of the whorl: (1) homogeneous, (2) slightly different ornaments below suture, (3) ornaments below suture very different from the rest of the shell.
SH, presence and sharpness of a dark band on the axial edge of the aperture columelar band: (1) absent, (2) diffuse, (3) sharp.
SC, size of the columellar band, when present: (1) narrow, (2) medium, (3) wide.
CO, conicity of the shell: (1) acute, (2) medium, (3) blunted cone.
RO, roundness of body whorls: (1) flat, (2) slightly rounded, (3) well-rounded.
GR, spiral grooves: (0) absent, (1) shallow grooves, (2) intermediate, (3) very deep grooves.
RI, density and width of axial ribs: (0) none, (1) a few narrow ribs, (2) a few large ribs, (3) many narrow ribs.
RD, elevation of axial ribs when present: (1) shallow, (2) medium, (3) deep.

lake, but it may have arrived with discarded ornamental aquarium contents, as happened with other thiarid gastropods in the Americas (Madsen & Frandsen 1989). Recent spread within the lake may have been aided by internal boat transport; the morph was present in the hulls of small boats that travel between lakeshore settlements (M.J.G. personal observation).

A camouflaged intercontinental invasion

The similarity in shell characters of the colonizing morph to allopatric African *M. tuberculata* populations made it conceivable that the colonization was a within-Africa range extension. However, molecular evidence indicates that other populations from Africa belong to a subclade exclusively containing populations from Africa, Israel and Sri Lanka, suggesting that they are indigenous to that biogeographical region. In contrast the colonizing morph forms part of an otherwise exclusively southeast Asian clade, and representatives of the colonizer were found to share a haplotype with a morph from Singapore, implying that the colonizers of Lake Malawi may have originated from Singapore, an important location for trade in freshwater ornamental fishes and plants.

The scale of molecular divergence provides further evidence of the independent origins of the *M. tuberculata* morphs in Lake Malawi. Although assumptions of a global molecular clock were not supported, if we apply approximate gastropod CO1 divergence rates of 0.67–2.4% per million years (Hellberg & Vacquier 1999; Marko 2002), sequence differentiation between LMI and LMN would translate to divergence 20.4–5.7 million years ago. Although these dates should be interpreted with caution, they are notably before the earliest known freshwater faunas in the Basin that date between 4.0 and 2.3 million years (Van Damme & Pickford 2003). Such high molecular divergence raises the possibility that comprehensive character analysis of internal morphology may yield between-lineage differences, and provide evidence of taxonomic inconsistencies.

Mitochondrial DNA differentiation between southeast Asian taxa and those indigenous to Africa has also been found in *M. tuberculata* phylogenetic reconstructions by Facon *et al*. (2003) who sequenced the 16S and 12S rRNA regions. These authors found that the Israel morph (ISR) belonged to the clade containing African–Arabian populations (Kenya, Seychelles, Madagascar, Morocco, Oman), and a population introduced into Martinique. The Thailand morph (BAN) was also studied by Facon *et al*. (2003) and belonged to a clade of populations from Chiang-Mai (Thailand), Indonesia and Vietnam, and others introduced into the New World. However, Facon *et al*. (2003) also identified populations from Congo and Nigeria that share a haplotype with the BAN. Hence, it is possible that populations in the immediate mitochondrial sister clade to the Lake Malawi colonizers are in West Africa. However, as Facon *et al*. (2003) discuss, given extensive invasive properties of the BAN lineage, and evidence that these West Africa populations share mtDNA haplotypes with New World invaders, it is probable that they are not indigenous to West Africa, but rather that they have southeast Asian ancestry and have recently colonized West Africa. This explanation is supported by absence of all morphs of *M. tuberculata* from historical Congo Basin collections (Brown 1994).

Consequences for Lake Malawi gastropods

Lake Malawi contains notable molluscan diversity. Of 28 recorded species 16 are endemic (Brown 1994). The colonizing morph is sympatric with many of these, including endemic species of *Melanoides*, *Bulinus*, *Gabbiella* and *Lanistes* (M.J.G. personal observation), and is present in Lake Malawi National Park. Following introductions of *M. tuberculata* into the New World, dispersal and population increases have been rapid, and competitive exclusion of native taxa has taken place. This capacity makes the species effective for biological control of schistosome harbouring gastropods (Pointier 2001). Thus, competitive interactions between the invading morph and the indigenous fauna may occur within Lake Malawi. Another possible consequence is introgression between introduced and native taxa. Despite a propensity for parthenogenetic reproduction, hybrids of introduced *M. tuberculata* have been recorded from the Caribbean (Samadi *et al*. 1999; Facon *et al*. 2003). Control of the Lake Malawi invader is unlikely to be successful given high reproductive potential; rapid spread to littoral habitats throughout the Basin appears likely.

Cryptic invaders

Our evidence suggests that a cryptic intercontinental invasion was camouflaged by high morphological variation among native populations of a polymorphic species. This result has wider significance for our understanding of biological invasions. Many taxa, such as macroalgae, molluscs and crustaceans, exhibit intraspecific morphological variability within natural species distributions. As such, invasions of taxa with morphology that falls within the bounds of regional allopatric variation of native species could go unnoticed, or be ascribed to within-region range changes, even if invaders are morphologically distinct from sympatric natives. The scale of between-region introductions may be higher than appreciated.

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Martin Genner recently completed a Marie-Curie Fellowship at the University of Amsterdam (UvA) and is now pursuing interests in African cichlid fish evolution at the University of Hull. Ellinor Michel, now based at The Natural History Museum in London, focuses her research the speciation and biogeography of African gastropods. Dirk Erpenbeck recently completed a PhD on sponge phylogenetics at UvA, while Nicole de Voogd, also of UvA, is nearing completion of a PhD on marine sponge ecology and taxonomy. Frans Witte of the University of Leiden works primarily on the fish and fisheries of Lake Victoria while Jean-Pierre Pointier of CNRS in Perpignon has a long-standing research programme on the ecology of invading gastropods.
