Biol Invasions (2012) 14:1665–1682
DOI 10.1007/s10530-012-0179-z

ORIGINAL PAPER

Phylogeography of an invasive land snail: natural range expansion versus anthropogenic dispersal in *Theba pisana pisana*

Carolin Däumer · Carola Greve ·
Rainer Hutterer · Bernhard Misof · Martin Haase

Received: 31 January 2011 / Accepted: 29 January 2012 / Published online: 10 February 2012

Abstract The invasive land snail *Theba pisana pisana* has been widely dispersed through human activities. The Mediterranean and adjacent Atlantic coasts are considered as natural range of this species, which also occurs northwards to the Netherlands, the British Islands and in Ireland. In addition, *T. p. pisana* has been introduced to some Macaronesian Islands as well as to Argentina, Australia, South Africa, and the United States. One important part of understanding biological invasions is the history of the processes including the routes of spread and introduction. Reconstructing the phylogeography based on COI sequences, we asked to which extent it may still be possible to trace natural as opposed to human causes for the current distribution. In addition, we attempted to infer the origins of the apparently recent human-mediated introductions. Tree reconstructions and demographic analyses showed that apart from the origin in NW-Africa, where *T. p. pisana* already underwent considerable differentiation, and an initial spread to the Iberian Peninsula, the phylogeographic signal was largely blurred. All available evidence indicated that primarily human activities rather than natural causes have shaped and still shape the current distribution. Already the spread through the Mediterranean basin may be anthropogenic and even the origins of our samples from South Africa and Australia could not unambiguously be traced. One clade was geographically particularly diverse suggesting that different lineages differ in their adaptive and invasive potential.

Keywords Human-mediated dispersal · Long-distance dispersal · Phylogeography · Range expansion · *Theba*

Introduction

The increasing mobility of humans in particular during the last centuries has—inadvertently or not—transplanted a large number of animal and plant species from their native habitats to areas which they would otherwise never have reached. One important part of understanding biological invasions is the history of the processes including the routes of spread and introduction (Estoup and Guillemaud 2010).

Non-marine gastropods are often considered excellent model organisms for biogeographic questions due

C. Däumer · M. Haase (✉)
Vogelwarte, Zoologisches Institut und Museum,
Universität Greifswald, Soldmannstraße 23,
17489 Greifswald, Germany
e-mail: martin.haase@uni-greifswald.de

Present Address:
C. Däumer
Institute for Medical Informatics and Statistics,
Christian-Albrechts-University, 24105 Kiel, Germany

C. Greve · R. Hutterer · B. Misof
Zoologisches Forschungsmuseum Alexander Koenig,
Adenauerallee 160, 53113 Bonn, Germany

to their low potential for active dispersal, thus conserving information about their origin and distribution better and longer than vagile species (e.g. Davison 2002; Haase et al. 2003, 2007; Haase and Misof 2009; Pfenninger et al. 2009; Sauer and Hausdorf 2009; Cameron et al. 2010). In order to cover larger distances and to overcome dispersal barriers such as open water, snails depend on vectors. Several patterns of distribution including spectacular ones such as the occurrence of the genus *Balea* on the Tristan da Cunha islands (Gittenberger et al. 2006) can only be explained by long distance transportation through birds (e.g. Darwin 1859; Vagvolgyi 1975; Haase et al. 2010a, b). Animals may get caught in the plumage, stick to the feet, or survive passage through the gut (Malone 1965; Rees 1965; Cadée 1988; Green and Figuerola 2005). Accidental or deliberate human-mediated long distance transportation of non-marine gastropods is documented for a large number of species (e.g. Ponder 1988; Robinson 1999; Cowie 2001; Hayes et al. 2008) and many of them are considered threats to the local fauna (e.g. Civeyrel and Simberloff 1996; Kerans et al. 2005), to human health (Pointier et al. 2005; Majoros et al. 2008) or agricultural pests including the land snail *Theba pisana pisana* (Müller 1774) (e.g. Henderson 1996; Barker 2002).

Theba p. pisana, which is highly polymorphic with respect to shell color and banding pattern, is the only representative of the genus with a wide range. It has a circum-Mediterranean distribution and occurs along the Atlantic coast from Morocco northwards to the Netherlands including some localities on the British Islands and Ireland. It also occurs on some Atlantic islands including Gran Canaria, Tenerife and Madeira. Biogeographic considerations and a phylogenetic analysis indicate Morocco as place of origin (Sacchi 1955; Gittenberger and Ripken 1987; Greve et al. 2010). The species prefers the sparse coastal vegetation and experiences a single reproductive season after 1 or 2 years of development, depending on the climatic conditions of a particular area (Heller 1982; Cowie 1984; Baker 1991; Sacchi 1995). Relatively recently, probably during the 19th and 20th centuries, *T. p. pisana* has been introduced to the USA, South Africa, Australia, and Argentina, where it has gained its legacy as a pest species (Chace 1915; Connolly 1916; Dürr 1946; Pomeroy and Laws 1967; Odendaal et al. 2008; Rumi et al. 2010), although it is known to be harmful also in plantations along the Mediterranean coasts (e.g. Castillejo et al. 1996). In the USA, the species was presumably eradicated but reappeared and now "enjoys" high priority with quarantine (Cowie et al. 2009 and literature therein). Introductions to the Netherlands (van Benthem Jutting 1964; van Dalsum 1964) and the Macaronesian Islands (Gittenberger and Ripken 1987; Cook et al. 1993) are recent as well. However, considering that the Mediterranean area has been one of the cultural cradles of mankind including extensive trade relations established by the Phoenicians already more than 3,000 years ago (Sommer 2005), and that the distribution of *T. p. pisana* almost perfectly matches the extent of the Roman Empire at its maximum extent under Trajan during the early 2nd century (Wells 1995), the spread of this species may have already much earlier been influenced by human activities.

The goal of the present paper is the reconstruction of the phylogeography of *T. p. pisana*. In particular, we asked to which extent it may still be possible to trace natural as opposed to human causes for the current distribution. We also attempted to infer the origins of the apparently recent human-mediated introductions.

Materials and methods

Sampling and laboratory methods

A total of 63 samples from 17 countries comprising 343 specimens were collected and conserved in 99.8% ethanol largely between 2007 and 2009 or provided by museum collections (Table 1, Fig. 1). A part of this material, viz. 227 snails, has already been sequenced for Greve et al. (2010) (GenBank accession numbers of unique haplotypes: HM034556-HM034563, HM034570- HM034603). From another 116 individuals (HQ864649-HQ864687), DNA was extracted from foot muscle tissue using QIAGEN's DNeasy Blood and Tissue kit following the manufacturer's protocol. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) was amplified by polymerase chain reaction (PCR) using the primer pair LCO-1490 [5′-GGTCAACAAATCATAAAGATATTGG-3′ (Folmer et al. 1994)] and C1-N-2191 [5′-CCCGGTAAAATTAAAATATAAACTTC-3′ (Simon et al. 1994)]. Amplifications were carried out in a total volume of 25 µl

Table 1 Sampling localities. Locality data and acronyms

Country	Region	Acronym: specimen (N), (sub)species (if not *T. p. pisana*)	Locality	Coordinates	Collector(s)	Date
Australia	Perth	AUS: 1–5 (5)	Bold Park, City Beach	S 31°57′06″, E 115°45′21″	M.S. Johnson	Oct 2010
Cyprus		CYL1: 1–12 (12)	Limassol	N 34°35′45′’, E 33°00′02′’	V. Sternkopf	10 Nov 2007
		CYL2: 1–5, 7–11, 13–15 (13)	Limassol	N 34°38′52′’, E 32°59′41′’	V. Sternkopf	10 Nov 2007
France	Brittany	FRB1: 1–3 (3)	Bassin d’Epuration, Planguenouel	N 48°32′10″, W 02°35′24″	G. Poppe	16 Jun 2009
		FRB2: 1–3 (3)	Palué, Pleslin-Trigavou	N 48°31′10″, W 02°06′33″	C. Greve	11 Sep 2009
		FRB3: 1–3 (3)	Pointe de la Torche, Penmarc′h	N 47°50′15″, W 04°21′18″	D.G. Robinson	14 Sep 2008
		FRB4: 1–3 (3)	Quiberon	N 47°28′48″, W 03°08′10″	K. Groh	21 Jun 2009
	Corsica	FRC: 1–3 (3)	Corte	N 42°18′29″, E 09°08′53″	C. Regnier	Jun 2009
		FRF: 1–10 (10)	Cap Ferret	N 44°37′48″, W 01°15′00′’	F. Eppler	03 Sep 2007
		FRM: 1–15, 17–30 (29)	Montpellier	N 43°36′43″, E 03°52′56″	R. Hutterer	07 May 2006
		FRS: 1 (1)	Banyuls-sur-Mer	N 42°29′06″, E 03°07′48″	K. Händeler	05 May 2009
Germany		DEB: 1 (1)	Bonn-Kessenich	N 50°42′03′’, E 07°07′21″	R. Hutterer	02 Dec 2009
Great Britten	Wales	GBW: 1 (1)	Caerhays, Cornish Coast	N 52°33′00″, W 04°06′00″	E. Bailes	Dec 2007
Greece	Chios	GRC: 2, 3 (2)	Karfa	N 38°19′10′’, E 26°09′10′’	K. Vardinoyannis	13 Mar 2009
	Crete	GRK: 1–9 (9)	Lofos Semeli, S Heraklion	N 35°11′00″, E 25°09′00″	K. Vardinoyannis	22 Apr 2008
		GRS: 1–2 (2)	Safonidi Island	N 36°53′10″, E 26°55′17′’	Triantis	30 Mar 2005
Israel		ILN: 1–6 (6)	Kibbutz Netzer Sereni	N 31°55′25′’, E 34°49′24′’	H. Mienis	26 Nov 2007
Italy	Sardinia	ITT: 1–9 (9)	Tharros, Sinis peninsula	N 39°52′45″, E 08°26′15″	G. Peters	Jul 2008
Libya		LYM: 1 (1)	Tripoli	N 32°52′30″, E 13°10′30″	Mus. Leiden	10 Apr 1961
		LYZ: 1 (1)	Zuwarah	N 32°54′54″, E 12°08′00″	Mus. Leiden	11 Apr 1961
Malta		MTG: 1–3 (3)	Ganjsielem, Gozo	N 36°01′03″, E 14°16′57″	Steinmaier	2009
		MTS: 1–6 (6)	San Gwann	N 35°54′27″, E 14°28′27″	P. Schembri	05 Dec 2007
Morocco		MAA: 1–3, 7–10, 12–14, 16, 18–20, 22–23, 25, 28–30 (20)	Rabat, Agdal	N 33°59′39″, W 06°51′10″	R. Hutterer	Mar 2007
		MAB: 1, 12–18, 20–21, 27–32, 34, 37–40, 60–61 (23)	Bouknadel	N 34°11′06″, W 06°42′20″	R. Hutterer	Mar 2007
		MAD: 1–3, 5 (4)	Rabat, Ruines de Chellah	N 34°00′25″, W 06°49′16″	R. Hutterer	Mar 2007
		MAE: 1 (1), *T. p. ampullacea*	Diabat, near Essaouira	N 31°29′09″, W 09°46′27″	J. Eiwanger	Oct 2007
		MAM: 1–3 (3), *T. p. cantinensis*	Meddouza, Dar Caid Zerhouni	N 32°33′33″, W 09°16′57″	R. Hutterer, C. Greve	13 Mar 2008
		MAQ: 1–3 (3)	Abdel Hachs	N 35°13′57″, W 03°36′09″	J. Eiwanger	03 Apr 2005

Table 1 continued

Country	Region	Acronym: specimen (N), (sub)species (if not *T. p. pisana*)	Locality	Coordinates	Collector(s)	Date
	Rif	MAR1: 1, 3–4 (3)	Afso, Province Nador	N 34°49′45″, W 03°11′11″	R. Hutterer	Apr 2009
		MAR2: 1–3 (3)	Aklim, E river Moulouya	N 34°55′34″, W 02°26′15″	R. Hutterer	05 Apr 2009
		MAR3: 1–3 (3)	Asilah	N 35°28′27″, W 6°01′40″	R. Hutterer	28 Feb 2009
		MAR4: 1–4 (4)	Nador, near Kariet Arkmane	N 35°05′42″, W 02°50′42″	R. Hutterer	16 Mar 2008
		MAR5: 1–3 (3)	Zaio, near Ras El Ma	N 34°56′42″, W 02°45′18″	R. Hutterer	18 Mar 2008
		MAS: 1 (1)	Oum el Aioun, near Safi	N 31°42′36″, W 09°39′	R. Hutterer, C. Greve	18 Mar 2008
		MAS: 2–3 (2), *T. p. ampullacea*	Oum el Aioun, near Safi	N 31°42′36″, W 09°39′	R. Hutterer, C. Greve	18 Mar 2008
		MAT: 1 (1)	Cap Spartel, Tanger	N 35°47′24″, W 05°55′40″	J. Linstädter	12 Dec 2009
Netherlands		NLD: 1–6, 9–11, 15 (10)	Domburg	N 51°33′27″, E 03°28′30″	C. Greve	01 Dec 2007
Portugal	Madeira	PTL: 3 (1)	Lagoa, Algarve	N 37°06′03″, W 08°27′38″	Rourke	26 Dec 1997
		PTM1: 1–3 (3)	Machico, E marina Quinta do Lorde	N 32°43′03″, W 16°46′03″	D.G. Robinson	20 Dec 2006
		PTM2: 1–3 (3)	Porto Santo, Pico do Facho, 347 m	N 33°04′60″, W 16°18′60″	J. Astrin	26 Mar 2008
South Africa	Western Cape	ZAW1: 1 (1)	Gans Bay	S 34°35′15″, E19°20′42″	Natal Museum	13 Nov 2005
		ZAW2: 1 (1)	Mossel Bay	S 34°10′30″, E22°08′06″	Natal Museum	25 Mar 2005
		ZAW3: 1 (1)	Paternoster	S 32°48′33″, E 17°53′51″	Natal Museum	05 Feb 2005
		ZAC: 1–7 (7)				
Spain	Andalusia	ESA1: 1 (1)	Embalse de Almodóvar, Cortijo la Luna	N 36°44′15″, W03°41′06″	T. Wagner	24 Aug 2007
		ESA2: 1–8, 10 (9)	Conil de la Frontera	N 36°16′39″, W06°05′33″	T. Wagner	23 Aug 2007
		ESA3: 1–3 (3)	Los Barrios, El Tiradero	N 36°10′04″, W 05°34′50″	T. Wagner	24 Aug 2007
		ESA4: 1–6 (6)	Malaga, Alcazaba de Malaga	N 36°43′24″, W 04°24′40″	R. Hutterer	28 Oct 2007
		ESA5: 1 (1), *T. andalusica*	Sanlucar de Barrameda	N 36°46′12″, W06°22′12″	Mus. Leiden	Apr 1988
		ESB: 1–3 (3)	Caiòn, Playa Barrahan	N 43°19′09″, W 08°36′09″	F. Eppler	14 Sep 2007
		ESC: 1–3 (3)	Cedeira	N 43°39′41’’, W 08°03′20’’	F. Eppler	10 Sep 2007
	Gran Canaria	ESG1: 1–8 (8)	Tafira, Clinica Bandama	N 28°03′27″, W 15°27′39″	R. Hutterer	Aug 1993
		ESG2: 1–20 (20)	Las Palmas de Gran Canaria, Tamaraceite-Campus	N 28°05′46″, W 15°28′05″	R. Hutterer, C. Greve, I Bischoff	05 Oct 2007

Table 1 continued

Country	Region	Acronym: specimen (N), (sub)species (if not *T. p. pisana*)	Locality	Coordinates	Collector(s)	Date
		ESG3: 1–12 (12)	Las Palmas de Gran Canaria	N 28°05′46″, W 15°28′05″	R. Hutterer, C. Greve, I Bischoff	05 Oct 2007
		ESG4: 1–3 (3)	Telde, Jinamar	N 28°01′50″, W 15°25′21″	R. Hutterer	08 Oct 2007
		ESG5: 2–8; ESG 6: 1–24 (31)	Telde, Las Piletillas	N 27°57′21″, W 15°25′22″	R. Hutterer, C. Greve, I. Bischoff	06 Oct 2007
		ESG7: 1–10 (10)	Vega de San Mateo, ca. 700 m	N 28°00′36″, W 15°31′57″	R. Hutterer, C. Greve, I. Bischoff	07 Oct 2007
Tunesia	Djerba	TND1: 1–9 (9)	Midoun	N 33°48′18″, E 10°59′06″	K. Uhlenbruch	13 Feb 2008
		TND2: 1–2 (2)	El May	N 33°48′57″, E 10°52′35″	Mus. Leiden	Nov 1982
Turkey		TRI: 1–3 (3)	Dalyan, Iztuzu	N 36°50′06″, E 28°39′00″	H. Meinig	Jun 2009
USA		USC: 2–3 (2)	Chula Vista, California	N 32°37′58″, W 116°59′48″	Marques	28 Nov 2007
		USS 1, 3 (2)	Saint Paul, Minnesota, intercepted from Iran	N 44°57′15″, W 93°04′12″	S. Rourke	15 May 2003

consisting of 16 μl de-ionized water, 20–100 ng template DNA, 2.5 μl Buffer (Bioline), 2.0 μl 50 mM MgCl$_2$, 1 μl of each primer (10 pm), 0.5 μl dNTP (each 2.5 mM), 1 μl BSA (1%) and 0.1 units of Taq polymerase (Bioline). The PCR conditions were as follows: an initial denaturation step at 95°C for 3 min, followed by 15 cycles of touchdown PCR (94°C for 0:35 min, 55°C–40°C annealing for 1:30 min and 72°C extension for 1:30 min) followed by 25 cycles (95°C for 0:35 min, 40°C for 1:30 min and 72°C for 1:30 min) and a final extension step at 72°C for 10 min. PCR products were purified using ExoSAP-IT. Subsequent cycle sequencing was performed using the BigDyeTerminator v3.1 kit of Applied Biosystems (ABI). The cycle sequencing products were purified with magnetic beads (Agencourt CleanSEQ, Beckman Coulter) and directly sequenced in both directions on an ABI 3130xl Genetic Analyzer.

Sequence analyses

Sequences were edited in BioEdit 7.0.5 (Hall 1999) and aligned using MAFFT (Katoh and Toh 2008). The final alignment had a length of 610 bp. The X^2 test implemented in PAUP* 4b10 (Swofford 2003) conducted with exclusion of constant sites as proxies of invariant sites (Lockhart et al. 1995) suggested homogeneity of base frequencies ($X^2 = 75.648$, $p = 1.000$). Saturation of substitutions was controlled for by the test of Xia et al. (2003) and by visually inspecting a plot of transitions and transversions against corrected distances (F84). Both tests were conducted in Dambe (Xia and Xie 2001) and rejected saturation. Analyses of sequence polymorphism were carried out using DnaSP 5.10.0.1 (Librado and Rozas 2009) and Arlequin 3.11 (Excoffier et al. 2005).

Phylogenetic relationships were inferred using maximum parsimony (MP), bio-neighbor joining (NJ), maximum likelihood (ML), and Bayesian analyses (BA). MP and NJ were conducted in PAUP*. For MP, heuristic searches used TBR-branch swapping in 1,000 replicates. Starting trees were constructed by stepwise addition with random addition sequence. For NJ, TPM1uf+Γ+I was determined as best-fitting model with jModeltest (Posada 2008) and for BA, HKY+Γ+I using MrModeltest (Nylander 2004), applying the Akaike Information Criterion corrected for small sample sizes in both cases. BA was conducted with MrBayes (Huelsenbeck and Ronquist

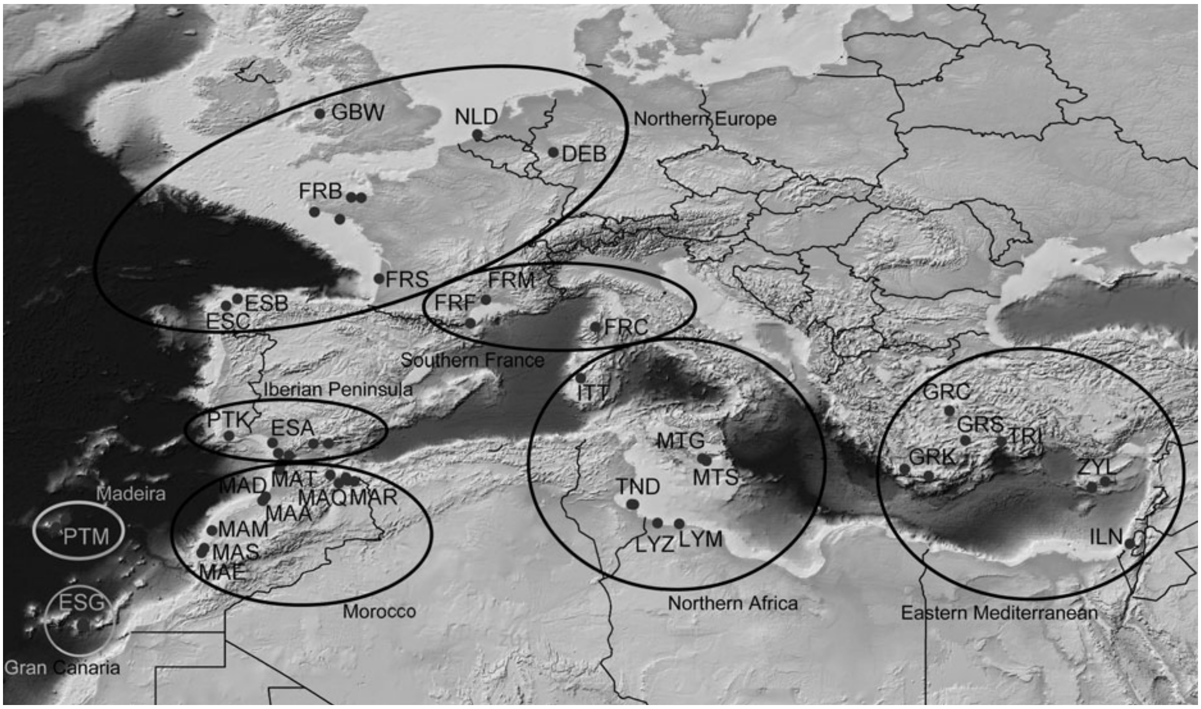

Fig. 1 Map showing sampling localities and definitions of regions. For abbreviations see Table 1

2001). Every 100th tree was sampled over 6 Mio generations with a burnin of 15% and otherwise default settings. Convergence of runs and parameters was controlled by the diagnostics implemented in MrBayes and in Tracer 1.5 (Rambaut and Drummond 2009). ML was performed by GARLI (Zwickl 2006). This program uses a genetic algorithm estimating the tree and substitution model parameters simultaneously. Robustness of MP, NJ, and ML reconstructions was assessed by bootstrapping with 100, 1,000, and again 1,000 replicates, respectively. *Theba p. cantinensis* (HM034567-HM034569), *T. p. ampullacea* (HM034564-HM034566), both confined to Morocco, and the closely related *T. andalusica* (HM034555) from southern Spain served as outgroup taxa (see Greve et al. 2010). Since intraspecific relationships are often better displayed as networks due to the potential presence of ancestral sequences, we conducted a statistical parsimony analysis using TCS (Clement et al. 2000).

In order to compare rates of evolution a Bayesian relative rates test was performed based on the last 1,000 trees sampled during one of the parallel Bayesian tree searches. For each of these trees the distance from the most recent common ancestor to each terminal taxon was estimated with the help of the software Cadence (Wilcox et al. 2004). Means and 95%-confidence intervals for each lineage were calculated and plotted using a program written by CD in R (R Development Core Team 2010).

Phylogeographic analyses

For population genetic analyses, localities were united to eight regions based on the assumption of isolation by distance as most natural model of genetic differentiation (see e.g. Arter 1990; Arnaud et al. 2003 and references therein; Fig. 1).The number of individuals with identical haplotypes was restricted to five for each locality. This design aimed at compensating for the uneven sampling design both in terms of geography and number of specimens per sample. Individuals from the USA, South Africa and Australia were excluded in order to avoid bias. Thus, a total of 275 individuals remained for the following analyses.

Genetic differentiation of regions was expressed by F_{ST}-values calculated in Arlequin. Linearized F_{ST}-values [$F_{ST}/(1 - F_{ST})$, Slatkin 1995] were used to display the differentiation by non-metric multidimensional scaling (NMDS) performed in PAST 2.0

(Hammer et al. 2001). Using Mantel-tests, again conducted in Arlequin, correlation of geographic and genetic distances was investigated. These tests were conducted based on individuals and individual localities with genetic distances obtained in PAUP* and geographic distances (as the crow flies) in ArcGis 9.2 (ESRI, Redlands, California) as well as on regions using linearized F_{ST}-values and distances between centers of gravity and smallest distances between regions, respectively. Coalescence-based methods not assuming mutation-drift equilibrium and symmetric gene flow possibly modeling differentiation and gene flow better than F-statistics could not be implemented successfully, probably because of the high degree of sequence differentiation exhibited by *T. p. pisana*. To test for neutral evolution, respectively demographic expansion, Tajima's D (Tajima 1989), Fu and Li's F* and D* (Fu and Li 1993) as well as Fu's F (Fu 1997) were calculated using DnaSP. Based on these results, the demographic history was analyzed using Bayesian skyline plots (Drummond et al. 2005) generated in BEAST 1.6.0 (Drummond and Rambaut 2007) and Tracer based on the HKY+Γ+I substitution model. We assumed a log normal uncorrelated relaxed clock and constant population size coalescent model. The skyline plots were created for the entire dataset as well as for each region and according population and group sizes varied from 4 and 3 to 8 and 7, respectively. Markov chains ran for at least 20 Mio generations until effective sample sizes of parameter estimates were >200. We did not use the dates suggested in Greve et al. (2010) for calibration, because AFLP data (Greve et al. unpublished) suggest major modifications to the sequence-based phylogeny of the genus. Sudden spatial expansion was controlled for by mismatch distributions using Arlequin based on the model developed by Excoffier (2004). Plots were made in an R routine written by CD. In contrast, the assumptions for testing sudden demographic expansion by mismatch distributions (see Marjoram and Donnelly 1994) were not met and therefore we dispensed with these tests.

Results

There were 146 unique haplotypes among the total of 343 sequenced individuals. Identical haplotypes were mostly collected at the same or geographically close localities. Overall haplotype diversity was high with 0.961 and that of regions at least 0.890 except for Gran Canaria and Madeira, the latter represented only by a small sample, though (Table 2). Likewise, overall nucleotide diversity was high with 0.058, but more heterogeneous among regions with a maximum of 0.081 in Morocco (Table 2). Among the 610 bp, 195 were variable and of these 171 parsimony informative. Tree reconstructions based on different methods gave very similar results with slight topological differences only within clades indicating unambiguous phylogenetic signal. The major clades showed identical relationships and were largely well supported (Fig. 2). Three haplotypes from five morphologically unambiguous *T. p. pisana* individuals, MAR2_3, MAR3_1-3, and USS_3, fell among the outgroup taxa *T. p. ampullacea* and *T. andalusica*. The position of MAR3_1 may well be due to long-branch attraction, although it is geographically consistent among several divergent, basal lineages from Morocco. The basal-most true

Table 2 Genetic diversity and neutrality tests (Tajima 1989; Fu and Li 1993; Fu 1997)

	N	H	π	Tajima's D	Fu and Li's D*	Fu and Li's F*	Fu's F
Total	275	0.961	0.058				
Eastern Mediter.	44	0.948	0.031	−0.014	1.084	0.819	2.097
Gran Canaria	43	0.732	0.011	−0.618	0.010	−0.247	−0.367
Iberian Peninsula	20	0.968	0.044	1.368	0.383	0.792	−1.205
Madeira	6	0.733	0.013	–	–	–	–
Morocco	68	0.986	0.081	0.166	0.368	0.343	−0.729
Northern Africa	31	0.933	0.020	−1.894*	−2.936 *	−3.061*	0.388
Northern Europe	38	0.913	0.033	−0.244	1.074	0.727	4.270
Southern France	25	0.890	0.012	−1.798	−3.281**	−3.303**	2.087

H haplotype diversity; *N* number of haplotypes; π nucleotide diversity

* $p < 0.05$; ** $p < 0.01$

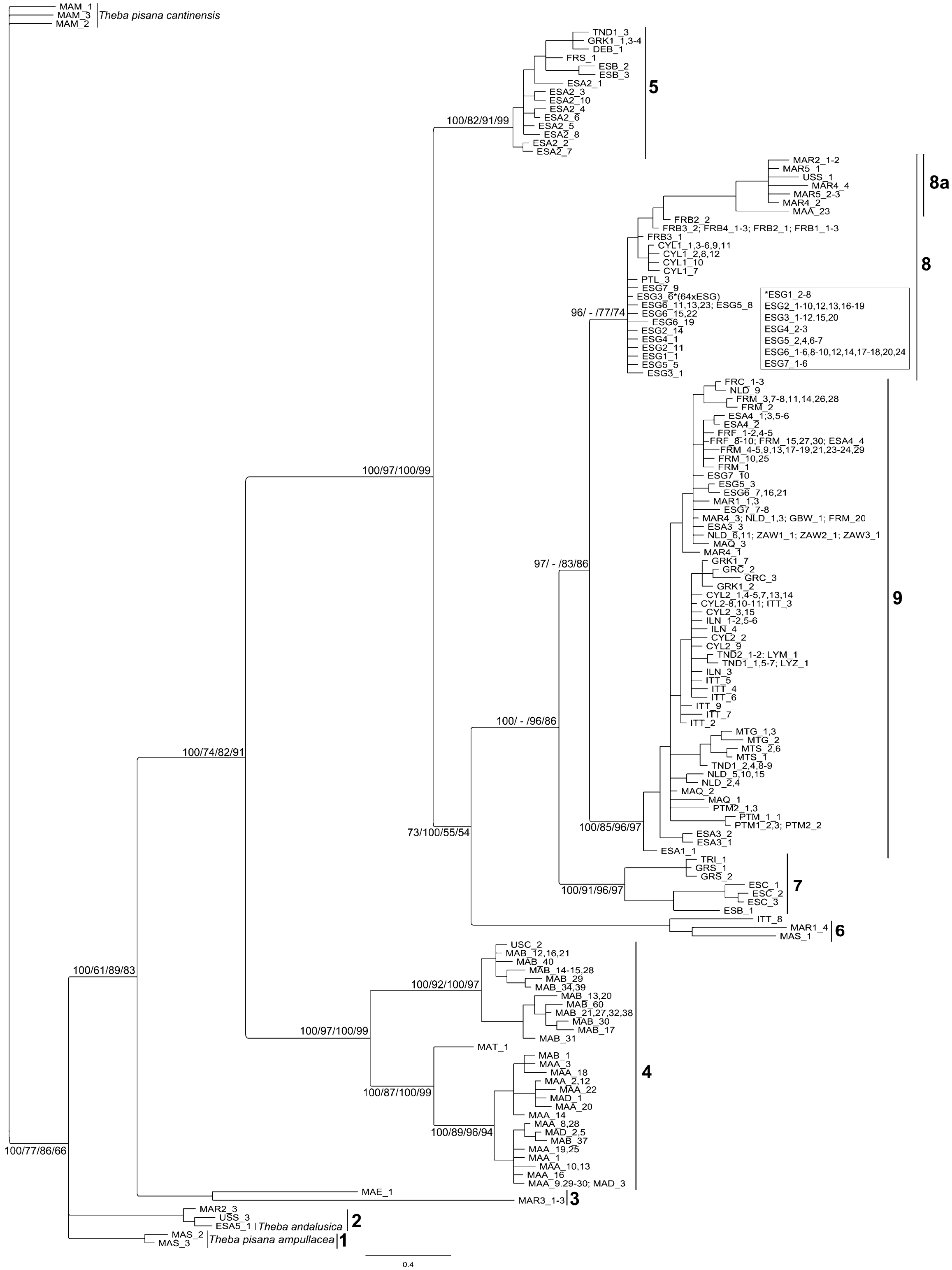

Fig. 2 50% majority rule consensus tree from Bayesian analysis with support values for major clades from all four analyses (BI, ML, MP, NJ). Clades are numbered for easier reference in text. For abbreviations see Table 1

ingroup-clade (clade 4) consisted of snails collected near Rabat in NW-Morocco and two individuals from California. Continuing in a ladder-like fashion a clade (5) composed of individuals collected in Andalusia, NW-Spain, southern France, Tunisia, Greece and the specimen from a market in Bonn (Germany) was followed by a small, well defined group of haplotypes from Morocco and the Italian island of Sardinia (clade 6). Snails from NW-Spain and the Eastern Mediterranean were sister group of the remaining two clades 8 and 9. Clade 8 consisted of snails from Gran Canaria, Portugal, Cyprus, Brittany (France) and a Moroccan subclade (8a) including again an individual from the United States. In the NJ and MP analyses, this Moroccan subclade was sistergroup to the remaining sequences. The largest, least structured clade, No 9, was composed of haplotypes from all eight defined regions. The snails displaced to South Africa had haplotypes identical to individuals collected in the Netherlands and on Corsica (France), respectively. The five specimens from W-Australia shared a single haplotype with two Dutch and three South African specimens.

The statistical parsimony network analysis reflected the tree topology and resulted in a total of 22 individual networks based on the parsimony connection limit of 95%, 11 of which were individual haplotypes including four belonging to the outgroup (not shown). In order to unite all haplotypes in a single network, the connection limit had to be raised to 66 mutational steps. Only the two most derived clades contained a total of three loops indicating a low degree of ambiguity of evolutionary pathways.

Relative rates of sequence evolution were surprisingly heterogeneous indicating that a strict molecular clock did not hold (Fig. 3). In general, basal lineages mainly from Morocco and Southern Spain evolved slower than younger ones. The highest substitution rates were again found among Moroccan haplotypes, viz. among representatives from the foothills of the Rif-Mountains. Interestingly, both the slowest (MAR2-3) and one of the fastest (MAR2-1) lineages occurred at the very same locality. These snails had practically identical shell banding patterns. Their uncorrected genetic distance was 12.3%.

Genetic variation within regions (56.77%) was higher than between regions (43.33%). All F_{ST}-based pairwise comparisons except Iberian Peninsula versus Madeira were highly significant as well (Table 3). Relationships among regions are represented by non-metric multidimensional scaling in Fig. 4. Distortions were moderate as indicated by a stress value of 0.177. A superimposed minimum spanning tree connected Morocco with the Iberian Peninsula and Northern Europe with the Eastern Mediterranean, Southern France, Gran Canaria and Madeira. The distortions in the two dimensional representation of genetic distances suggest that alternative configurations and connections of the regions are not much worse. Therefore, we connected the regions also based on the second lowest pairwise F_{ST}-values in addition to the minimum spanning tree (Fig. 5) assuming that both networks together possibly represent the main dispersal routes more comprehensively.

In accordance with these representations, all Mantel-tests conducted did not reject the null-hypothesis of no

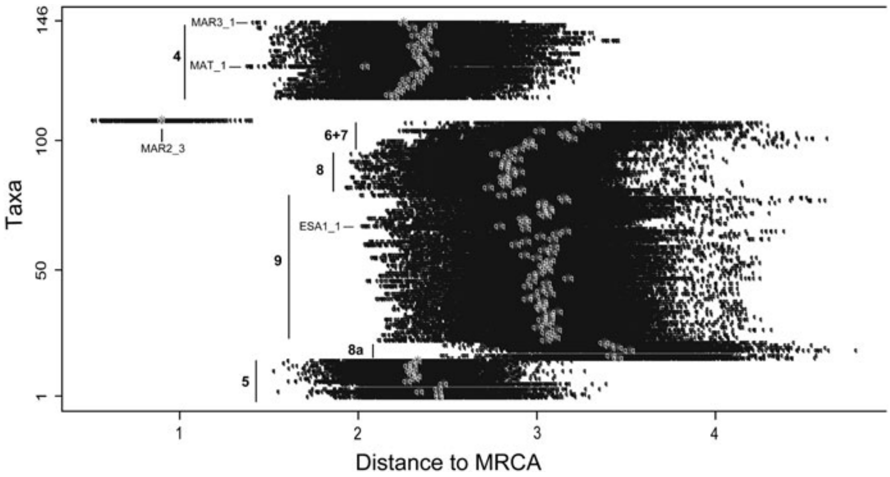

Fig. 3 Bayesian relative rates test based on last 1,000 trees sampled in one of the MrBayes runs. Order of haplotypes according to order in alignment; gaps indicate alignment positions of taxa not considered in this test (see Appendix). *Black* q's represent distance to most recent common ancestor (MRCA) in each particular tree; grey stars enclosed by *light grey* q's denote means and 95% CI. Numbers indicate clades (see Fig. 2)

Table 3 Genetic differentiation between regions

	Eastern Mediter.	Gran Canaria	Iberian Peninsula	Madeira	Morocco	Northern Africa	Northern Europe	Southern France
Eastern Mediter.	44	***	***	***	***	***	***	***
Gran Canaria	0.326***	43	***	***	***	***	***	***
Iberian Peninsula	0.183***	0.465***	20	ns	***	***	***	***
Madeira	0.278***	0.705***	0.322**	6	**	**	***	***
Morocco	0.432***	0.506***	0.357***	0.429***	68	***	***	***
Northern Africa	0.120***	0.582***	0.285***	0.350***	0.463***	31	***	***
Northern Europe	0.068***	0.257***	0.173***	0.268**	0.428***	0.186***	38	***
Southern France	0.248***	0.660***	0.321***	0.521***	0.477***	0.271***	0.197***	25

Pairwise F_{ST}-values with significance levels from resampling test below diagonal; significance levels from exact test above diagonal; diagonal gives number of haplotypes of each region (max 5 identical per site)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

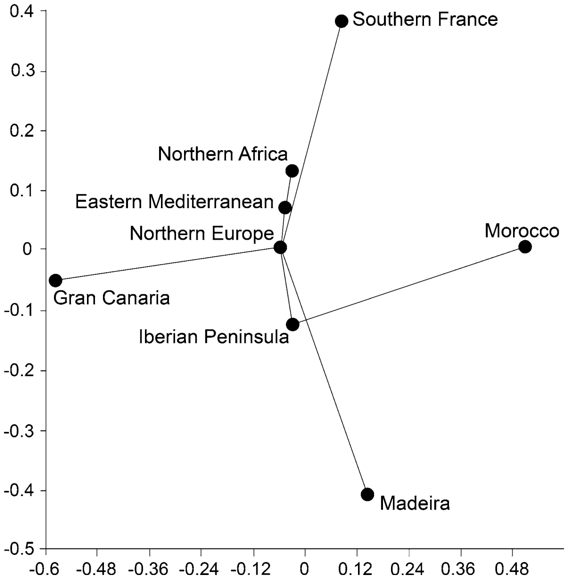

Fig. 4 Nonmetric multidimensional scaling of regions based on linearized F_{ST}-values ($F_{ST}/(1 - F_{ST})$) (Table 3) with superimposed minimum spanning tree

correlation between genetic and geographic distances between regions ($p > 0.5$ in all cases). Therefore, genetic isolation of regions by geographic distance could be ruled out. This result could already be expected considering the composition of the major clades.

Across the entire alignment, neutral evolution of haplotypes could not be rejected. All test statistics were negative, but non-significant (Table 2). These results also suggested demographic stability in the recent past. Narrowing the focus onto single regions, the picture became more heterogeneous with positive as well as negative values for the four test statistics including significant ones suggesting recent demographic population growth in North Africa and Southern France (Table 2).

The previous findings, however, did not concur with the Bayesian skyline analyses modeling the demographic development over all regions. The resulting plot indicated a long period of stable population size throughout the first 75% of the evolutionary life time of *T. p. pisana*. Then the subspecies experienced a slow increase in population size with a peak at about 2% of its life time before present followed by a sharp decline until today. Skyline plots for individual regions basically showed the same shape (Fig. 6).

Mismatch distributions across all regions and for each region separately (not feasible for Gran Canaria and Madeira) could not be distinguished from distributions modeling spatial expansion (Fig. 7).

Discussion

In line with previous findings for several species of land snails (e.g. Thomaz et al. 1996; Pinceel et al. 2005; Haase and Misof 2009) mitochondrial diversity and divergence within *T. p. pisana* were very high. Greve et al. (2010) suggested assigning separate subspecies status to the basal Moroccan clades. However, the sympatry and morphological identity of the extremely divergent individuals MAR2_1 and MAR2_3 indicated unrestricted gene flow. Similarly, the morphologically well defined (sub)species

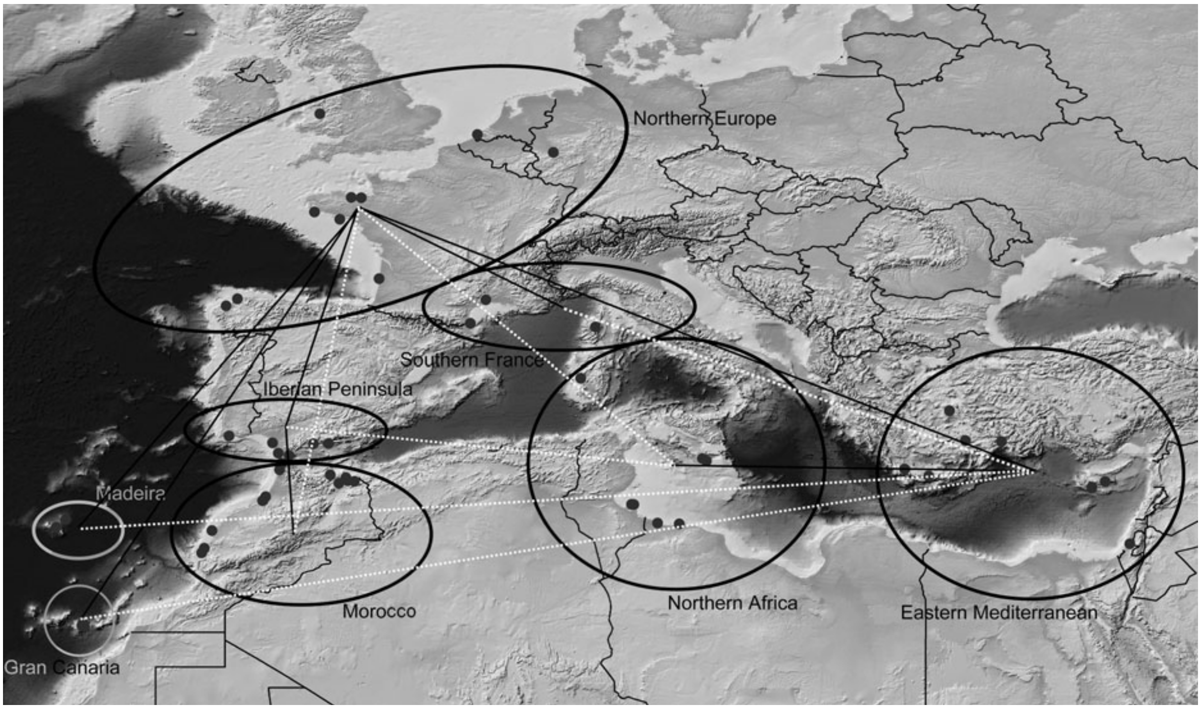

Fig. 5 Hypothesized major routes of dispersal deduced from minimum spanning tree from Fig. 4 plotted on map (*black lines*) and connections based on second lowest F_{ST}-values associated with each region (*white lines*)

T. andalusica and *T. p. ampullacea* could not be unambiguously delimited based on mitochondrial data. Preliminary AFLP data (Greve et al. unpublished) confirm this pattern of incongruence between morphological and genetic data difficult to handle taxonomically. Therefore, the extreme divergence and diversity were probably primarily due to the hermaphroditic nature of *T. p. pisana* (see Haase et al. 2003; Davison 2006). The deep clade structure was most likely a consequence of lineage sorting (Hoelzer et al. 1998), although we cannot exclude that our sampling missed other still extant lineages. The likewise high heterogeneity of substitution rates is so far unparalleled among land snails. Interestingly, older lineages seemed to evolve slower than younger ones. We do not believe that this is an artifact due to saturation of substitutions, because visual inspection of plots of transitions and transversions against genetic distance as well as the test of Xia et al. (2003; data not shown) indicated that saturation was negligible. Similar observations have recently been made for birds (Nabholz et al. 2011).

The tree reconstructions confirmed the origin of *T. p. pisana* in NW-Africa (Greve et al. 2010) and suggested a fairly long history of local differentiation, also expressed by the highest gene diversity among the eight regions defined, before extending the range onto the Iberian Peninsula. Plate tectonic, i.e. vicariance events in the Western Mediterranean did obviously not play a role for the present distribution of *T. p. pisana* as shown or assumed for other land snails (Pfenninger et al. 2009; Guillier and Madec 2010) with at least partly overlapping distribution. The spread across its current circum-Mediterranean range has occurred only in the younger past. However, a pattern of isolation by distance or a more linear stepping-stone arrangement of populations or regions one would expect for a naturally dispersing land snail (Arter 1990; Arnaud et al. 2003 and literature cited therein) could not be detected. In contrast to the test statistics for neutrality, the Bayesian skyline plot indicated a demographic expansion after about 75% of the lifetime of *T. p. pisana*, probably in the late Pleistocene considering substitution rate estimates for land snails in the absence of suitable calibration points (see Guillier and Madec 2010 and references therein). The most recent drop of effective population size probably masked the foregoing increase for the statistical tests for

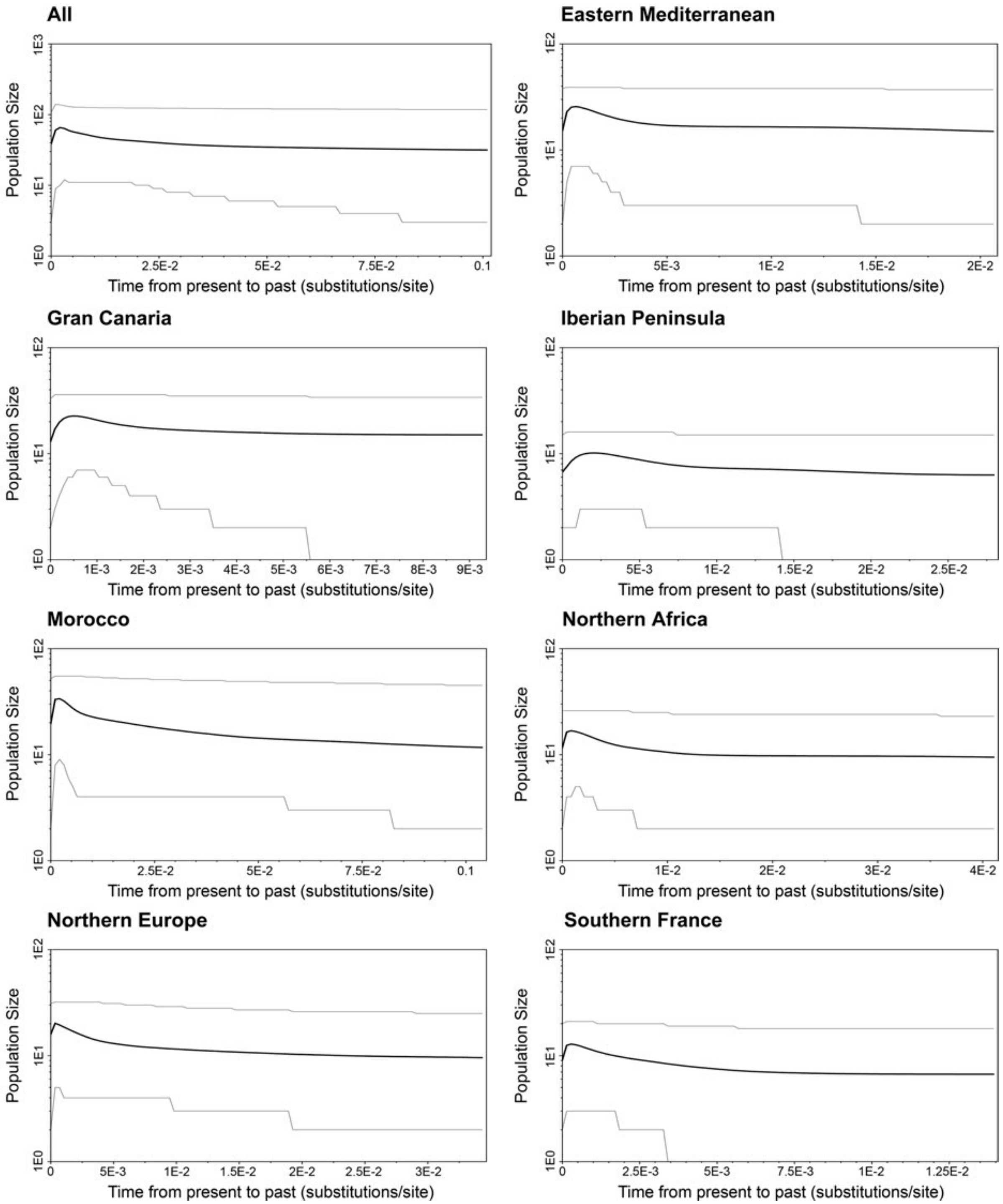

Fig. 6 Bayesian skyline plots depicting mean effective population size (*black line*) through time and 95% highest posterior density interval (*grey lines*)

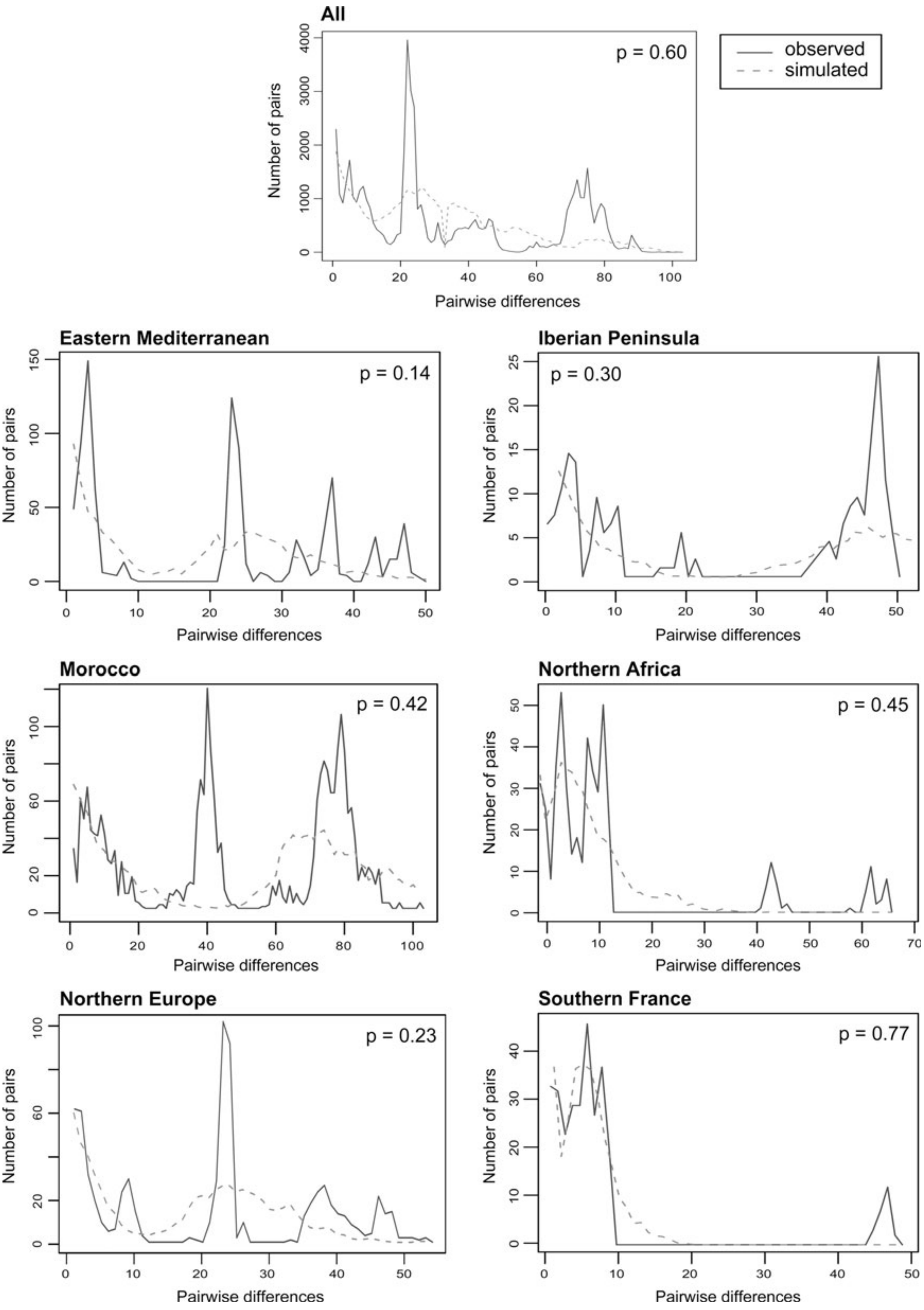

Fig. 7 Comparisons of observed and expected mismatch distributions to test for spatial expansion (Excoffier 2004)

demographic expansion, which largely yielded non-significant *p* values. Expecting the demographic expansion to be linked with spatial expansion, the signal of which was still detectable in mismatch distributions, suggests that *T. p. pisana* started to naturally spread long before the antique civilizations had established. The lack of pattern indicates, however, that long distance dispersal must have played a major role to conquer the Mediterranean coasts. Although birds are known to consume species of *Theba* (Mienis 1971, 1978; Gittenberger and Ripken 1987; Hutterer personal observation), it seems unlikely that they were chiefly responsible for the current distribution. The genetic differentiation among regions suggested important North–South connections paralleling the major flyways (e.g. Berthold 2001). However, "Northern Europe" can hardly be considered a hub for the dispersal of *T. p. pisana*, as Figs. 4 and 5 would suggest, because we know that *T. p. pisana* has been deliberately introduced to northern France, Belgium and the Netherlands only during the last 120 years (Dutertre 1927; Adam and Leloup 1937; van Benthem Jutting 1964; van Dalsum 1964). Only in the British Islands, the species has been present already in the 18th century (Pennant 1777; Turk 1966, 1972). The West-East tracks indicated in Fig. 5, on the other hand, do not coincide with any important flyway, disqualifying birds as long distance vectors again. The lack of any fossil record in Israel has prompted Heller and Tchernov (1978) to assume that *T. p. pisana* has reached the Eastern Mediterranean area only in historical times. However, Holocene records from Malta (Giusti et al. 1995) do suggest an earlier spread towards the East. Recently, Jesse et al. (2011) demonstrated that already the human Neolithic expansion across the Western Mediterranean area has shaped the distribution of another land snail, *Tudorella sulcata*.

The detours a snail invasion can take are illustrated by the sample intercepted by quarantine in St. Paul, Minnesota (USA). This sample was collected on mint plants shipped in a container from Iran, which is not part of the native range, either. Our phylogenetic analyses indicated a Moroccan origin. The two individuals sequenced appeared in very divergent clades suggesting that already the ancestors in Morocco had a turbulent history. Also the Californian sample could be traced to Morocco. The genetic identity of most South African and the Australian specimens with an individual from the Netherlands seems to indicate an unambiguous route of translocation reminiscent of the Netherlands as colonial and trading power. However, this conclusion is misleading since *T. p. pisana* has been introduced to the Netherlands only in the middle of the 20th century (van Benthem Jutting 1964; van Dalsum 1964) when it was already present on the Southern hemisphere (Connolly 1916; Dürr 1946; Pomeroy and Laws 1967). One haplotype was shared by a South African specimen and a sample from Corsica. Interestingly, this haplotype was sister group to another Dutch specimen and both were closely related to snails collected in Montpellier/Southern France. Therefore, Southern France may be a better guess for the origin of both the Dutch—via Northern France and Belgium (Van Benthem Jutting 1964; van Dalsum 1964)—as well as the South African and Australian populations. Our single sample from Western Australia is certainly not representative for the entire continent, where *T. p. pisana* occurs more or less continuously along the entire South coast. Hence, we cannot exclude several independent invasions of Australia. Interestingly, based on allozyme data including samples from southern France, Israel and Wales, Johnson (1988) already assumed a southern French origin of the Australian populations, speculating about a "stop-over" in South Africa.

Clade 9 was geographically particularly diverse suggesting that different lineages differ in their adaptive and invasive potential. This has been observed only for few other species (Kelly et al. 2006; Kang et al. 2007; Ciosi et al. 2008). However, as in *T. p. pisana*, experiments comparing the ecological valences of clades are lacking.

In conclusion, apart from the origin in NW-Africa, the territory of today's Morocco, where *T. p. pisana* already underwent considerable differentiation, and an initial spread to the Iberian Peninsula, the phylogeographic signal is largely blurred. All available evidence indicates that primarily human activities rather than natural causes have shaped and still shape the current distribution. A denser sampling design, which to achieve would require a tremendous effort, though, might reveal a few more details. Similarly, non-equilibrium approaches in population genetics, once adapted to divergent lineages such as those found in many species of land snails, will certainly refine the picture. However, although the picture may become more complex, a fundamental change of the general scenario outlined in this study is not to be expected.

Acknowledgments We thank Christel Meibauer for support in the laboratory. Andreas Spillner is gratefully acknowledged for providing access to the computer cluster of the Mathematical Institute in Greifswald and his comments on an earlier version of the manuscript. We are indebted to all collectors listed in Table 1 as well as the curators from the Netherlands Centre for Biodiversity 'Naturalis' in Leiden, Edi Gittenberger, Wim Maassen, and Menno Schilthuizen, and the Natal Museum in Pietermaritzburg, Dai Herbert, for providing specimens. The comments of two anonymous reviewers helped improving our account. Financial support was received from the German Science Foundation DFG (MI 649/7-1, HU 430/2-2) and SYNTHESYS (NL-TAF 3966).

Appendix

See Table 4.

Table 4 Positions of sequences in alignment for identification in Fig. 3 representing the relative rates test

Position	Acronym	Position	Acronym	Position	Acronym	Position	Acronym
1	TND1_3	38	ILN_6	75	MTS_1	112	MAS_3
2	GRK1_4	39	ITT_9	76	PTM2_1	113	MAM_1
3	FRS_1	40	ITT_7	77	PTM1_1	114	MAM_3
4	ESB_2	41	NLD_9	78	PTM1_2	115	MAM_2
5	DEB_1	42	MTG_1	79	FRB3_1	116	MAE_1
6	ESB_3	43	MTG_2	80	CYL1_4	117	USC_2
7	ESA2_2	44	TND1_8	81	CYL1_8	118	MAB_12
8	ESA2_3	45	MTS_6	82	PTL_3	119	MAB_40
9	ESA2_10	46	NLD_15	83	ESG7_9	120	MAB_14
10	ESA2_4	47	GRC_3	84	ESG3_6	121	MAB_29
11	ESA2_5	48	MAR4_3	85	ESG6_11	122	MAB_34
12	ESA2_7	49	ESA3_3	86	ESG6_15	123	MAB_13
13	ESA2_6	50	ILN_4	87	FRB3_2	124	MAB_31
14	ESA2_8	51	CYL2_2	88	FRB2_2	125	MAB_60
15	ESA2_1	52	CYL2_9	89	ESG6_19	126	MAB_38
16	MAR2_1	53	TND2_1	90	ESG2_14	127	MAB_30
17	MAR5_2	54	FRM_10	91	ESG4_1	128	MAB_17
18	USS_1	55	NLD_11	92	ESG2_11	129	MAT_1
19	MAR4_4	56	ITT_2	93	CYL1_10	130	MAB_1
20	MAR5_2	57	GRK1_2	94	ESG1_1	131	MAA_3
21	MAR4_2	58	ILN_3	95	ESG5_1	132	MAA_8
22	MAA_23	59	ESA4_2	96	ESG3_1	133	MAA_19
23	FRC_1	60	MAQ_2	97	CYL1_7	134	MAA_18
24	ESA4_6	61	NLD_2	98	TRI_1	135	MAA_1
25	FRF_5	62	TND1_7	99	GRS_1	136	MAA_13
26	FRF_8	63	ITT_5	100	GRS_2	137	MAA_16
27	FRM_18	64	FRM_14	101	ESC_1	138	MAA_2
28	ESG7_10	65	FRM_2	102	ESC_2	139	MAA_22
29	ESG5_3	66	MAQ_1	103	ESC_3	140	MAD_1
30	ESG6_16	67	ESA1_1	104	ESB_1	141	MAA_29
31	MAR1_1	68	ESA3_2	105	ITT_8	142	MAA_20
32	ESG7_7	69	ESA3_1	106	MAR1_4	143	MAD_2
33	GRK1_7	70	MAR4_1	107	MAS_1	144	MAA_14
34	GRC_2	71	ITT_4	108	MAR2_3	145	MAB_37

Table 4 continued

Position	Acronym	Position	Acronym	Position	Acronym	Position	Acronym
35	CYL2_4	72	MAQ_3	109	USS_3	146	MAR3_1
36	CYL2_8	73	FRM_1	110	ESS_1		
37	CYL2_15	74	ITT_6	111	MAS_2		

For Acronyms see Table 1. Taxa 5, 18, 109–117 were not included in the test resulting in gaps in the figure

References

Adam W, Leloup E (1937) Sur la presence de *Helicella virgata* (Da Costa) et *Theba pisana* (Müller) en Belgique. Bull Mus Royal d'Hist Nat Belg 13:1–8

Arnaud J-F, Madec L, Guiller A, Deunff J (2003) Population genetic structure in a human-disturbed environment: a case study in the land snail *Helix aspersa* (Gastropoda: Pulmonata). Heredity 90:451–458

Arter HE (1990) Spatial relationship and gene flow paths between populations of the alpine snail *Arianta arbustorum* (Pulmonata: Helicidae). Evolution 44:966–980

Baker GH (1991) Production of eggs and young snails by adult *Theba pisana* (Müller) and *Cernuella virgata* (da Costa) (Mollusca: Heliciae) in laboratory cultures and field populations. Aust J Zool 39:673–679

Barker GM (2002) Molluscs as crop pests. CABI Publishing, Wallingford

Berthold P (2001) Bird migration. A general survey, 2nd edn. Oxford University Press, Oxford

Cadée GC (1988) Levende wadslakjes in bergeend faeces. Ned Malac Ver 243:443–444

Cameron RAD, Pokryszko BM, Horsák M (2010) Land snail faunas in Polish forests: patterns of richness and composition in a post-glacial landscape. Malacologia 53:77–134

Castillejo J, Seijas I, Villoch F (1996) Slug and snail pests in Spanish crops and their economical importance. In: Henderson IF (ed) Slug and snail pests in agriculture. British Crop Protection Council, Farnham, pp 327–332

Chace EP (1915) *Helix pisana* Müller in California. Nautilus 29:72

Ciosi M, Miller NJ, Kim KS, Giordano R, Estoup A, Guillemaud T (2008) Invasion of Europe by the western corn rootworm, *Diabrotica virgifera virgifera*: multiple transatlantic introductions with various reductions of genetic diversity. Mol Ecol 17:3614–3627

Civeyrel L, Simberloff D (1996) A tale of two snails: is the cure worse than the disease? Biodiv Cons 5:1231–1252

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9: 1647–1660

Connolly M (1916) Notes on the South African non-marine Mollusca. Ann S Afr Mus 13:187–188

Cook LM, Goodfriend GA, Cameron RDA (1993) Changes in the land snail fauna of eastern Madeira during the Quaternary. Trans R Soc Lond B 339:83–103

Cowie RH (1984) The life cycle and productivity of the land snail *Theba pisana* (Mollusca: Helicidae). J Anim Ecol 53:311–325

Cowie RH (2001) Invertebrate invasions on Pacific islands and the replacement of unique native faunas: a synthesis of the land and freshwater snails. Biol Invasions 3:119–136

Cowie RH, Dillon RT, Robinson DG, Smith JW (2009) Alien non-marine snails and slugs of priority quarantine importance in the United States: a preliminary risk assessment. Am Malac Bull 27:113–132

Darwin C (1859) On the origin of species. Murray, London

Davison A (2002) Land snails as a model to understand the role of history and selection in the origins of biodiversity. Popul Ecol 44:129–136

Davison A (2006) The ovotestis: an underdeveloped organ of evolution. BioEssays 28:642–650

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214

Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. Mol Biol Evol 22: 1185–1192

Dürr HJR (1946) A contribution to the morphology and bionomics of *Theba pisana* (Müller) (Gastropoda: Helicidae). South African Department of Agriculture. Stellenbosch Elsenburg Sci Ser 47:1–34

Dutertre AP (1927) Esquisse biogéographique de l'esuaire de la Canche (Pas-de-Calais). CR Soc Biogéogr 4:51–58

Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? Mol Ecol 19:4113–4130

Excoffier L (2004) Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. Mol Ecol 13:853–864

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinf Online 1:47–50

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

Fu YX, Li WH (1993) Statistical tests of neutrality of mutations. Genetics 133:693–709

Gittenberger E, Ripken TEJ (1987) The genus *Theba* (Mollusca: Gastropoda: Helicidae), systematics and distribution. Zool Verh 241:3–59

Gittenberger E, Groenenberg DSJ, Kokshoorn B, Preece RC (2006) Molecular trails from hitch-hiking snails. Nature 439:409

Giusti F, Manganelli G, Schembri PJ (1995) The non-marine molluscs of the Maltese Islands. Mus Reg Sci Nat Torino Monogr 15:1–608

Green AJ, Figuerola J (2005) Recent advances in the study of long-distance dispersal of aquatic invertebrates via birds. Divers Distrib 11:149–156

Greve C, Hutterer R, Groh K, Haase M, Misof B (2010) Evolutionary diversification of the genus *Theba* (Gastropoda: Helicidae) in space and time: a land snail conquering islands and continents. Mol Phylogen Evol 57:572–584

Guillier A, Madec L (2010) Historical biogeography of the land snail *Cornu aspersum*: a new scenario inferred from haplotype distribution in the Western Mediterranean basin. BMC Evol Biol 10:18

Haase M, Misof B (2009) Dynamic gastropods: stable shell polymorphism despite geneflow in the land snail *Arianta arbustorum*. J Zool Syst Evol Res 47:105–114

Haase M, Misof B, Wirth T, Baminger H, Baur B (2003) Mitochondrial differentiation in a polymorphic land snail: evidence for Pleistocene survival within the boundaries of permafrost. J Evol Biol 16:415–428

Haase M, Marshall BA, Hogg I (2007) Disentangling causes of disjunction: the Alpine fault hypothesis of vicariance (New Zealand, South Island) revisited. Biol J Linn Soc 91: 361–374

Haase M, Fontaine B, Gargominy O (2010a) Rissooidean freshwater gastropods from the Vanuatu archipelago. Hydrobiologia 637:53–71

Haase M, Naser MD, Wilke T (2010b) *Ecrobia grimmi* in brackish Lake Sawa (Iraq): indirect evidence for long distance dispersal of hydrobiid gastropods (Caenogastropoda: Rissooidea) by birds. J Moll Stud 76:101–105

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/ NT. Nucl Acids Symp Ser 41:95–98

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological Statistics software package for education and data analysis. Palaeontologia Electronica 4:9

Hayes KA, Joshi RC, Thiengo SC, Cowie RH (2008) Out of South America: multiple origins of non-native apple snails in Asia. Divers Distrib 14:701–712

Heller J (1982) Natural history of *Theba pisana* in Israel (Pulmonata: Helicidae). J Zool Lond 196:475–487

Heller J, Tchernov E (1978) Pleistocene landsnails from the coastal plain of Israel. Israel J Zool 27:1–10

Henderson IF (1996) Slug and snail pests in agriculture. British Crop Protection Council, Farnham

Hoelzer GA, Wallman J, Melnick DJ (1998) The effects of social structure, geographical structure, and population size on the evolution of mitochondrial DNA: II. Molecular clocks and the lineage sorting period. J Mol Evol 47:21–31

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Jesse R, Véla E, Pfenninger M (2011) Phylogeography of a land snail suggests trans-Mediterranean Neolithic transport. PLoS One 6:e20734

Johnson MS (1988) Founder effects and geographic variation in the land snail *Theba pisana*. Heredity 61:133–142

Kang M, Buckley YM, Lowe AJ (2007) Testing the role of genetic factors across multiple independent invasions of the shrub Scotch broom (*Cytisus scoparius*). Mol Ecol 16:4662–4673

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinf 9: 286–298

Kelly DW, Muirhead JR, Heath DD, MacIsaac HJ (2006) Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters. Mol Ecol 15: 3641–3653

Kerans BL, Dybdahl MF, Gangloff MM, Jannot JE (2005) *Potamopyrgus antipodarum*: distribution, density, and effects on native macroinvertebrate assemblages in the greater yellow-stone ecosystem. J N Am Benth Soc 24: 123–138

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Lockhart PJ, Larkum AW, Steel MA, Waddell PJ, Penny D (1995) Evolution of chlorophyll and bacteriochlorophyll: the problem of invariant sites in sequence analysis. Proc Natl Acad Sci USA 93:1930–1934

Majoros G, Fehér Z, Deli T, Földvári G (2008) Establishment of *Biomphalaria tenagophila* snails in Europe. Emerg Infect Dis 14:1812–1813

Malone CR (1965) Killdeer (*Charadrius vociferus* Linnaeus) as a means of dispersal for aquatic gastropods. Ecology 46:551–552

Marjoram P, Donnelly P (1994) Pairwise comparisons of mitochondrial DNA sequences in subdivided populations and implications for early human evolution. Genetics 136:673–683

Mienis H (1971) *Theba pisana* in pellets of an Israelian owl. Basteria 35:73–75

Mienis H (1978) *Theba pisana* in droppings of the stone curlew in Israel. Argamon 6:61–63

Nabholz B, Künstner A, Wang R, Jarvis ED, Ellegren H (2011) Dynamic evolution of base composition: causes and consequences in avian phylogenomics. Mol Biol Evol 28: 2197–2210

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University

Odendaal LJ, Haupt TM, Griffiths CL (2008) The alien invasive land snail *Theba pisana* in the West Coast National Park: Is there cause for concern? Koedoe 80:93–98

Pennant T (1777) British zoology, vol 4. Benjamin White, London

Pfenninger M, Vela E, Jesse R, Arantzazu Elejalde M, Liberto F, Magnin F, Martínez-Ortí A (2009) Temporal speciation pattern in the western Mediterranean genus *Tudorella* P. Fischer, 1885 (Gastropoda, Pomatiidae) supports the Tyrrhenian vicariance hypothesis. Mol Phylogen Evol 54: 427–436

Pinceel J, Jordaens K, Backeljau T (2005) Extreme mtDNA divergences in a terrestrial slug (Gastropoda, Pulmonata, Arionidae): accelerated evolution, allopatric divergence and secondary contact. J Evol Biol 18:1264–1280

Pointier JP, David P, Jarne P (2005) Biological invasions: the case of planorbid snails. J Helminth 79:249–256

Pomeroy DE, Laws HM (1967) The distribution of introduced land snails in South Australia. Rec S Aust Mus 15:483–494

Ponder WF (1988) *Potamopyrgus antipodarum*—a molluscan colonizer of Europe and Australia. J Moll Stud 54:271–285

Posada D (2008) jModelTest: phylogentic model averaging. Mol Biol Evol 25:1253–1256

Rambaut A, Drummond AJ (2009) Tracer v1.5. Available from http://tree.bio.ed.ac.uk/software/tracer/

Rees WJ (1965) The aerial dispersal of Mollusca. Proc Malac Soc Lond 36:269–282

Robinson DG (1999) Alien invasions: the effects of the global economy on non-marine gastropod introductions into the United States. Malacologia 41:413–438

Rumi A, Sánchez J, Ferrando NS (2010) *Theba pisana* (Müller, 1774) (Gastropoda, Helicidae) and other alien land molluscs species in Argentina. Biol Invasions 12:2985–2990

Sacchi CF (1955) Il contributo dei molluschi terrestri alle ipotesi del "Ponte Siciliano". Elementi tirrenici ed orientali nella malacofauna del Maghreb. Arch Zool Ital 40:49–180

Sacchi CF (1995) *Theba pisana* (Mull.) en Afrique du Sud: la revanche des "cadets" (Gastropoda, Pulmonata, Helicinidae). Boll Malacol 31:119–132

Sauer J, Hausdorf B (2009) Sexual selection is involved in speciation in a land snail radiation on Crete. Evolution 63:2535–2546

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139:457–462

Sommer M (2005) Die Phönizier: Handelsherren zwischen Orient und Okzident. Kröner, Stuttgart

Swofford DL (2003) PAUP* 4b10. Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

R Development Core Team (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org

Thomaz D, Guiller A, Clarke B (1996) Extreme divergence of mitochondrial DNA within species of pulmonate land snails. Proc R Soc Lond B 263:363–368

Turk SM (1966) The rediscovery of *Theba pisana* (Müller) in Cornwall. J Conch 26:19–25

Turk SM (1972) A study of the white sand-hill snail. DTNC J 4:138–142

Vagvolgyi J (1975) Body size, aerial dispersal and origin of the Pacific land snail fauna. Syst Zool 24:465–488

Van Benthem Jutting WSS (1964) Aanvullend bericht over *Theba pisana* op Walcheren. Basteria 28:11–12

Van Dalsum J (1964) *Theba pisana* (Muell., 1774) en *Helicella cespitum* (Drap., 1801) in Nederland. Basteria 28:10–11

Wells CM (1995) The Roman Empire, 2nd edn. Harvard University Press, Cambridge

Wilcox TP, García de Léon FJ, Hendrickson DA, Hillis DM (2004) Convergence among cave catfishes: long-branch attraction and a Bayesian relative rates test. Mol Phylogen Evol 31:1101–1113

Xia X, Xie Z (2001) DAMBE: software package for data analysis in molecular biology and evolution. J Hered 92:371–373

Xia X, Xie Z, Salemi M, Chen L, Wang Y (2003) An index of substitution saturation and its application. Mol Phylogen Evol 26:1–7

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Dissertation, University of Texas at Austin