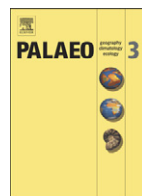




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Phenotypic evolution in a fossil gastropod species lineage: Evidence for adaptive radiation?

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ABSTRACT

Detecting speciation in the fossil record is a particularly challenging matter. Palaeontologists are usually confronted with poor preservation and limited knowledge on the palaeoenvironment. Even in the contrary case of adequate preservation and information, the linkage of pattern to process is often obscured by insufficient temporal resolution. Consequently, reliable documentations of speciation in fossils with discussions on underlying mechanisms are rare. Here we present a well-resolved pattern of morphological evolution in a fossil species lineage of the gastropod *Melanopsis* in the long-lived Lake Pannon. These developments are related to environmental changes, documented by isotope and stratigraphical data. After a long period of stasis, the ancestral species experiences a phenotypic change expressed as shift and expansion of the morphospace. The appearance of several new phenotypes along with changes in the environment is interpreted as adaptive radiation. Lake-level high stands affect distribution and availability of habitats and, as a result of varied functional demands on shell geometry, the distribution of phenotypes. The ongoing divergence of the morphospace into two branches argues for increasing reproductive isolation, consistent with the model of ecological speciation. In the latest phase, however, progressively unstable conditions restrict availability of niches, allowing survival of one branch only.

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1. Introduction

Whenever traditional taxonomy meets high morphological variability, a whole host of new species, subspecies, and phenotypes is frequently described, often based on minor morphologic deviations. In such a case, the morphologic species concept is unsatisfactory and an evolutionary point of view will allow more comprehensive conclusions. In the fossil record, this inevitably leads to complications, as natural populations cannot be precisely captured in respect to coeval generations (e.g. Hunt, 2010). Also, soft-part anatomy is not preserved, precluding molecular analyses. However, the fossil record is the only chance to study morphological change and speciation over long time intervals (Benton and Pearson, 2001; Allmon and Smith, 2011). Long-lived lakes are virtually predetermined for these studies, because of their duration and relative stability, being therefore often called ‘islands of evolution’ (Glaubrecht, 2011). Many studies have proven this fact repeatedly, including the papers on the impressive morphological developments in the Middle Miocene Lake Steinheim planorbids (Hilgendorf, 1867; Gottschick, 1920; Mensink, 1984; Gorthner and Meier-Brook, 1985; Nützel and Bandel, 1993), the Neogene Aegean freshwater gastropods (Willmann, 1981, 1985; Rust,

1997), or the Recent Lake Tanganyika gastropods (e.g., West et al., 1991; Michel et al., 1992; West and Cohen, 1994).

The present study employs data from Lake Pannon, a prime example of a long-lived lake in the fossil record (Fig. 1). It existed from the Late Miocene to Early Pliocene (~11.6–5.8 Ma) and had a surface area of approximately 290,000 km² during its maximum extent (Magyar et al., 1999; Harzhauser et al., 2004). Numerous gastropod groups experienced outstanding patterns of morphological evolution, including several lineages among the genus *Melanopsis* (e.g. Papp, 1953; Geary, 1990; Harzhauser and Mandic, 2008). This taxon is widely known for its highly variable shell morphology, in extant as well as fossil species (Glaubrecht, 1993). For this reason, its species lineages in Lake Pannon were recognized early (Papp, 1953).

Lately, these developments became the focus of evolutionary studies including first morphometric approaches (Geary, 1990, 1992; Geary et al., 2002). All of the performed analyses, however, treat only a restrictive set of individuals of geographically isolated populations and contain stratigraphic errors, making the resulting conclusions doubtful. Moreover, the authors act on the *a priori* assumptions of the distinctiveness of species within a lineage. To retain objectivity, we refrained from any predefined species/subspecies delimitations, many of which were proposed in earlier studies, and refer to the different morphologies as phenotypes (definition after Schluter, 2000).

The aim of this paper is to document and discuss the phenotypic evolution in a single evolutionary lineage by modern morphometric

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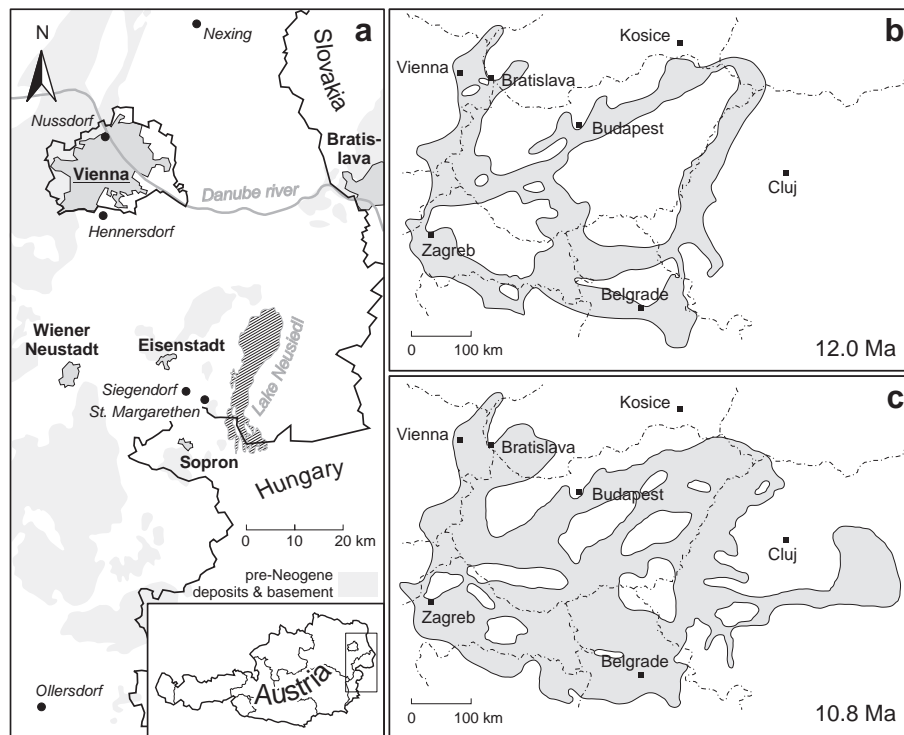


Fig 1. (a) Geographic setting of the studied localities in the Vienna Basin (Nexing, Nussdorf, Hengersdorf), the adjacent Eisenstadt–Sopron Basin (St. Margarethen, Siegendorf) and the Styrian Basin (Ollersdorf) (modified after Kroh, 2005). (b, c) Palaeolimnological maps of Lake Pannon during its initial phase (b) and its largest extent (c) (from Magyar et al., 1999).

analysis. The investigated geological interval of c. 1.6 Ma provides an excellent opportunity to study alternating modes of evolution in the fossil record, including stasis, the proposed adaptive radiation, and the final extinction of one of the emerging branches. The integration of a comprehensive set of palaeoenvironmental data taken from the literature provides the necessary link between the observed phenotypic evolution and underlying parameters, potentially allowing evaluation of the taxonomic status of the phenotypes.

2. Material

Subject of study was the species lineage of *Melanopsis impressa* Krauss, 1852 (Fig. 2). For the morphometric analysis a set of 448 ideally preserved individuals could be considered (Table 1, Fig. 1; stratigraphic ages after Papp, 1951, 1953; Harzhauser et al., 2004). For each locality/bed preservation was uniform over all melanopsid phenotypes. This precludes potential corruption of the analysis by a preservational bias. Additionally, a few reference specimens of the ancestor species *M. impressa* were included from the locality Nexing (Table 1, Fig. 1). Particularly the integration of stratigraphically successive layers from a single section (i.e. St. Margarethen) proved to be especially important for the interpretation of the results. It ruled out that different geographical origins of the samples account for the varying ecological conditions, which are proposed to cause the morphological evolution.

Because the morphometric analysis normalises for size, dimensions were measured separately. Measurements were taken from 752 specimens including all those used for outline analysis (Table 1).

3. Methodology

Morphometric analysis of gastropod shells has certain limitations: The most obvious one is the problem of homology – gastropods have very few homologous points that can be used, e.g., in a landmark

analysis (Johnston et al., 1991; Stone, 1998). In the absence of homologous points analysis of curves (e.g. outlines, which are considered homologous in their entirety; Sampson et al., 1996) is a promising approach. A series of analytical methods has been established over the last decades, depending on the complexity of the curves and whether they are open or closed (e.g. Rohlf, 1990; Sampson et al., 1996; MacLeod, 1999, 2002; Loy et al., 2000; Sheets et al., 2006). A group of analytical methods typically used for closed complex curves is the so-called Fourier analyses (e.g. Kuhl and Giardina, 1982; Davis, 1986; Foote, 1989; Haines and Crampton, 2000; Loy et al., 2000; Sheets et al., 2006; Van Bocxlaer and Schultheiß, 2010). These techniques are based upon the decomposition of a complex function into a combination of simpler trigonometric functions.

Of these, the Fast Fourier Transform method was applied in the present study for the following reasons: a) the method is highly accurate in capturing details of the outline (Haines and Crampton, 2000; Brusoni and Basso, 2007); b) the method allows smoothing of the outline to reduce potential pixel noise and minor outline irregularities produced by outline tracing programmes (Haines and Crampton, 2000). Such graphical artefacts could severely corrupt the analysis.

Elliptical Fourier Analysis (Kuhl and Giardina, 1982; Crampton, 1995), another frequently applied method for analysis of outlines, in contrast, has some technical limitations and thus was not used in this study. First, it produces computationally not independent coefficients, a fact that compromises following statistical analyses (Haines and Crampton, 2000). Second, it progressively downweights finer elements of shape, lowering the explanatory power of the result (Haines and Crampton, 2000). Another possibility for analysing outlines is the use of semi-landmarks (Bookstein, 1996, 1997; Sheets et al., 2004). This requires a very careful assessment of homology (Van Bocxlaer and Schultheiß, 2010). The melanopsid gastropods studied here expose an extremely broad morphological disparity, thus hampering the definition of sufficient homologous points and outline segments that could be used.

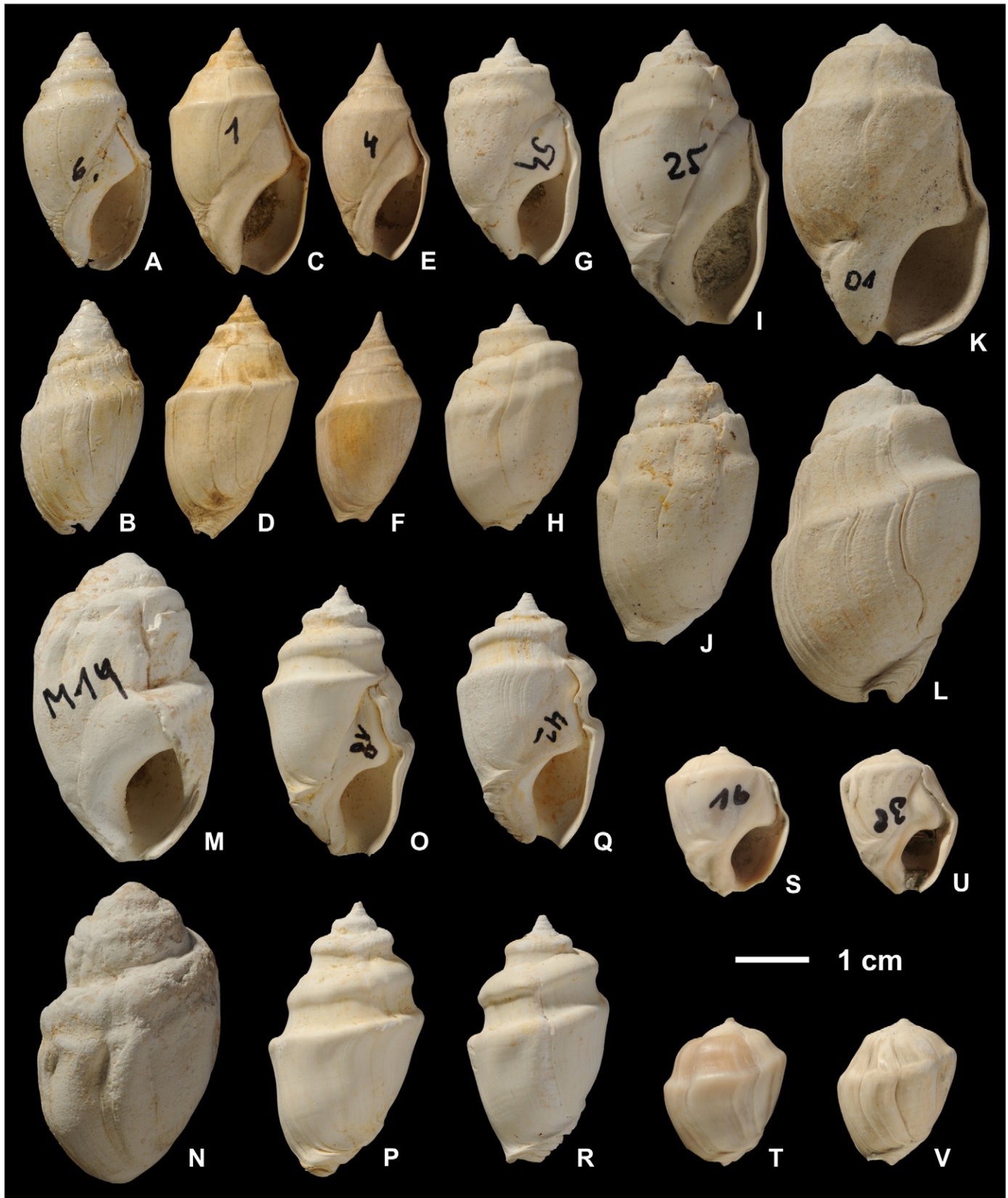


Fig. 2. Images of typical representatives of each phenotype of the *Melanopsis impressa*-species lineage, in ventral and dorsal view. (A, B) *impressa*-phenotype (Nexing). (C–F) *pseudonarazolina*-phenotype (Nussdorf). (G, H) *coaequata*-phenotype (Siegendorf). (I, J) *coaequata*-phenotype (St. Margarethen, bed 12). (K, L) *fossilis*-phenotype (Ollersdorf). (M, N) *rugosa*-phenotype (St. Margarethen, bed 20). (O–R) *handmanniana*-phenotype (Siegendorf). (S–V) *vindobonensis*-phenotype (Hennersdorf). Note that the *rugosa*-phenotype was not used in the morphometric analysis (for details see Section 2).

Table 1
Material used for the morphometric analyses. Note that not all the measured specimens could be used in the Fourier analysis because of insufficient preservation. Abbreviation: NHMW – Natural History Museum Vienna.

Locality	GPS	Age [Ma]	n (size)	n (analysis)	Specimen storage
Nexing	N48° 29' 36", E16° 39' 32"	12.0	27	10	NHMW 2012/0214
Nussdorf	N48° 15' 43", E16° 21' 43"	11.5–11.4	83	82	NHMW 2012/0227
St. Margarethen bed 4	N47° 45' 45", E16° 37' 47"	11.5–11.4	50	20	NHMW 2012/0215
St. Margarethen bed 8	N47° 45' 45", E16° 37' 47"	11.2	45	no data	NHMW 2012/0216
St. Margarethen bed 10	N47° 45' 45", E16° 37' 47"	11.0	100	20	NHMW 2012/0217
St. Margarethen bed 12	N47° 45' 45", E16° 37' 47"	10.8	70	21	NHMW 2012/0218
St. Margarethen bed 20	N47° 45' 45", E16° 37' 47"	10.7	77	no data	NHMW 2012/0219
Ollersdorf	N47° 10' 54", E16° 09' 53"	10.7–10.6	100	100	NHMW 2012/0220
Siegenderf	N47° 46' 44", E16° 34' 55"	10.7–10.6	100	100	NHMW 2012/0221
Henndorf	N48° 06' 16", E16° 21' 11"	10.4	100	95	NHMW 2012/0222

In contrast to these two methods, the Fast Fourier Transform method is expected to provide the most reliable and most detailed picture and consequently has been employed for the analysis of the morphological evolution of the *M. impressa*-lineage here.

Specimens are sampled from single coquina layers to avoid any temporal bias. Only non-fragmented and non-deformed shells were used in the morphometric analysis. Where necessary, objects were cleaned from sediment. To avoid a bias from growth allometry, juvenile specimens were excluded. As many specimens had a slightly damaged aperture, objects were photographed from backside, minimizing the potential impact of a preservational bias. Images were taken overexposed to maximize contrast and create sharp outlines. They were analysed with the software TpsDig 2.12 (Rohlf, 2008), which translated outlines into x,y-coordinates. These were processed with the programme Hangle, which employs the Fast Fourier Transform method for outline analysis (Crampton and Haines, 1996; Haines and Crampton, 2000). Outlines were smoothed 10 times. To reproduce morphological details significantly, 20 harmonics were used. Subsequently, outlines were normalized for starting point (i.e. the apex) with the programme Hmatch (Crampton and Haines, 1996). Principal component analysis (PCA) from a variance–covariance matrix was computed in PAST 2.14 (Hammer et al., 2001). For visualization of outlines in the PCA plots we used CorelTrace 11.633.

Data on the abiotic conditions are provided by comprehensive isotope and stratigraphic studies (Magyar et al., 1999; Harzhauser et al., 2004, 2007). Both the samples used for the morphological analysis as well as the major part of the isotope samples studied by Harzhauser et al. (2007) originate from a geographically small area at the western margin of Lake Pannon. Based on the previously published isotope data a rather detailed model of palaeoenvironmental change is available for the time interval and area studied. This model is used to correlate changes observed in gastropod morphology with palaeoclimate shifts.

4. Results

Both the morphometric analysis and the size measurements indicate massive changes in the morphology of the melanopsid lineage through time (Figs. 3–5). During the first four time slices (12.0–11.2 Ma) only small and slender specimens occur, traditionally separated as two subspecies *M. impressa impressa* Krauss, 1852 and *M. impressa pseudonarzolina* Papp, 1953 (Fig. 2). Still, within the entire period no net change in the morphology is recorded, reflecting morphological stasis. The clusters show low variability and thus occupy a restricted region of the available morphospace. The plots show that individuals from both ‘subspecies’ cannot be separated, neither from the outline analysis (no separation along any principal component) nor from the measurements.

The first distinct morphological shift happens within the following 200,000 yr (11.2–11.0 Ma). This involves not only a movement but a beginning extension of the clusters, resulting from a much greater

variety of forms and size ranges. Both *impressa* and *pseudonarzolina* are entirely replaced by newly occurring phenotypes. Depending on the degree of shouldering, globosity and size, earlier taxonomic descriptions assigned these specimens to up to four species/subspecies/forms (*M. fossilis* (Gmelin, 1791), *M. coaequata* Handmann, 1887, *M. handmanniana* Fischer, 1996a, and *M. vindobonensis* Fuchs, 1870; Fig. 2; see also Papp, 1953; Fischer, 1996b). The trend towards an expanding size range continues over the next 200,000 yr, when few specimens of even larger and more highly shouldered phenotypes appear (*M. handmanniana* Fischer, 1996a and *M. rugosa* Handmann, 1887; 10.8 Ma). The *rugosa*-phenotype is not covered by the morphometric analysis, as the criterion for its taxonomic separation is the presence of strong ribs, which is not captured by the outline analysis. This phenotype appears during the interval 10.8–10.7 Ma, whereas it becomes common only in late stages.

The beginning divergence of phenotypes culminates in a maximum within the interval 10.7–10.6 Ma. In the PCA, this is shown as expansive cloud with a bimodal distribution. The broad and highly shouldered *handmanniana*-phenotype is the most common, with individuals varying greatly regarding apical angle and outline complexity. On the other side of the morphological spectrum is the small and globular *vindobonensis*, which, in contrast, shows reduced variability and occupies a dense zone in the morphospace. Between the two extremes, a great variety of morphologies (*fossilis*, *coaequata*) still exist.

Finally, again within 200,000 yr (10.6–10.4 Ma), this impressive morphological diversity collapses completely. Only a single phenotype (*vindobonensis*) is present, forming a dense cluster in PCA (Fig. 3). Moreover, the mean and range of its dimensions are strongly reduced (Fig. 4).

5. Discussion

Although analytically totally independent, both the raw measurements and the morphometric analysis identified the most distinct morphological changes in the very same intervals. Three steps in the morphological evolution of the melanopsid lineage can be observed, including 1) stasis over 800,000 yr (i.e. zero net change of the morphology), 2) a subsequent distinct shift of the occupied morphospace along with an initial expansion, followed by a divergence towards two different phenotypes, and 3) the extinction of one of the emerging branches together with all intermediate forms.

The first and most crucial question for further discussion is whether the observed changes are any response to environmental change or not. Therefore, we will assess potential functional importance of the morphological changes in respect to changing environmental parameters. Moreover, we are concerned with a varying rate of evolution: Why is there morphological stasis over such a long period, followed by rapid changes? And finally, why does only a single branch survive in the latest interval, showing a quite restricted morphological spectrum?

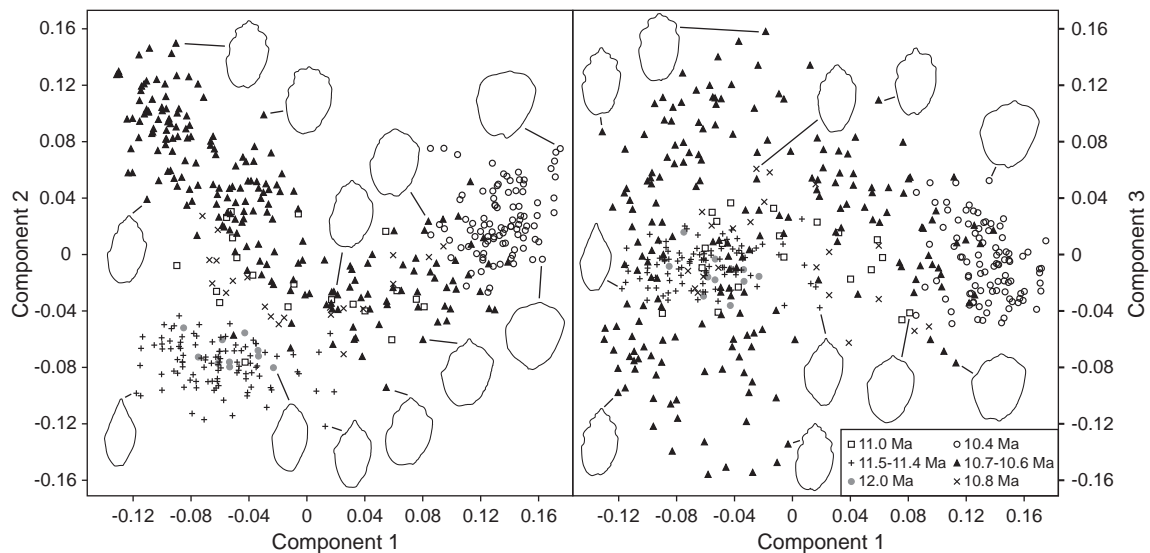


Fig. 3. Principal component analysis (PCA) of Fourier coefficients reflecting morphological differences in shell outlines of the *Melanopsis impressa*-species lineage. The first three components (out of 38) accounted for 32.43%, 15.59% and 10.35% of the total variance. To visualize shifts in the morphologic spectrum over time, several typical specimens of each time slice are illustrated.

5.1. Link to palaeoenvironmental data

Recent studies on isotope and geophysical data and regional stratigraphy present a possible link of the observed morphological shifts to changes in the palaeoenvironment (Magyar et al., 1999; Harzhauser et al., 2004, 2007; Kern et al., 2012). Particularly the studies on stable isotopes are a comprehensive source of information, as the major part of these data also comes from mollusc shells from the Vienna Basin. All these investigations consider the Vienna Basin and its satellite basins in early stages of Lake Pannon as fluvio-deltaic setting with low lake level due to arid conditions (Fig. 1). This is supported by shells of terrestrial gastropods in surrounding settings, which show conchological aridity adaptations (Lueger, 1978). This interval comprises the slender shells of the *pseudonarzolina*-phenotype as common fossils. The subsequent initial phase of morphologic evolution with the appearance of several phenotypes (11.2–11.0 Ma; Figs. 3, 4) parallels a trend of increasing humidity with enhanced precipitation and lake-level high stand. Data from palaeoclimate reconstructions of the Middle to Late Miocene indicate massive increases in average annual temperature and precipitation all over Central and Southwest Europe during this period (Böhme et al., 2008). This trend continues during the interval of morphospace divergence (10.7–10.6 Ma), when Lake Pannon reached its maximum extent (Fig. 1; Harzhauser et al., 2004).

In the latest interval studied herein (10.4 Ma) the situation changes dramatically. Periodic increases of river discharge and accompanying lake level changes, stronger seasonality with drier summer months, lake stratification during winter months and eutrophic conditions due to high productivity and algal blooms generated progressively unstable environmental conditions (Daxner-Höck, 2004; Harzhauser et al., 2007). While many sublittoral mollusc species flourish during this time, the shallow water *Melanopsis* species are successively displaced. The only melanopsids found belong to the small-sized *vindobonensis*-phenotype. This phase coincides with the 'Vallesian Crisis' in mammalian assemblages (Agustí and Moyà-Solà, 1990).

5.2. Fitting a model

The link of morphological shifts and climate change is crucial. Surely, random genetic drift (e.g. Lande, 1976; Raup, 1977; Bookstein, 1987;

Roopnarine et al., 1999; Hunt, 2006, 2007) may have operated to some extent on the studied traits, but the link to environmental data clearly signals the strong influence of environmental parameters on the morphological evolution of the melanopsid species lineage. Moreover, Estes and Arnold (2007) showed that genetic drift does not account for substantial evolutionary change during long geological time, as it is the case in this study.

In the following, we will elaborate the three abovementioned steps in the evolutionary history of the *M. impressa*-lineage, and try to reveal possible links to certain ecological conditions and environmental events. For this we employ the concept of the adaptive landscape (e.g. Wright, 1932; Simpson, 1944; Lande, 1976; Estes and Arnold, 2007; McGhee, 2007; Gavrillets, 2010). When set into environmental context, shifting regions in the morphospace can be related to shifting adaptive optima on the landscape: populations tracing their 'ideal morphologies' in respect to environmental conditions (Lande, 1986; Arnold et al., 2001). As the principal components result from the highly sensitive Fourier coefficients, it is unrealistic to parallel a single component with a specific feature of the shell outline. Nevertheless, excursions in the plot reflect real variations of morphological characters in a multidimensional space. The first two principal components – showing the most variability in the data space – can be used as axes of the adaptive landscape.

5.2.1. Step 1 – Stasis

During the interval 12.0–11.2 Ma no net change in shape and size is recorded, reflecting morphological stasis (Figs. 3–5). This is nothing unusual. Indeed, many authors favour the theory that morphological stasis persists through most of the time (e.g. Hansen and Martins, 1996; Hansen, 1997; Arnold et al., 2001). Hunt (2007) showed that in almost 50% of fossil sequences stasis is the prevalent evolutionary pattern. After Lande (1986) long-term morphological stasis requires the influence of stabilizing selection, precluding any morphological change from mutation and/or genetic drift. A main factor for stabilizing selection is thought to be individual habitat choice. This concept properly applies here. Environmental data showed that the ecological conditions during that time were largely constant (Harzhauser et al., 2007). Habitat availability and distribution did not change substantially, reflecting an about constant adaptive optimum (or narrow 'adaptive zones' after Simpson, 1944; Estes and Arnold, 2007). Stabilizing selection operated on the investigated traits to keep the

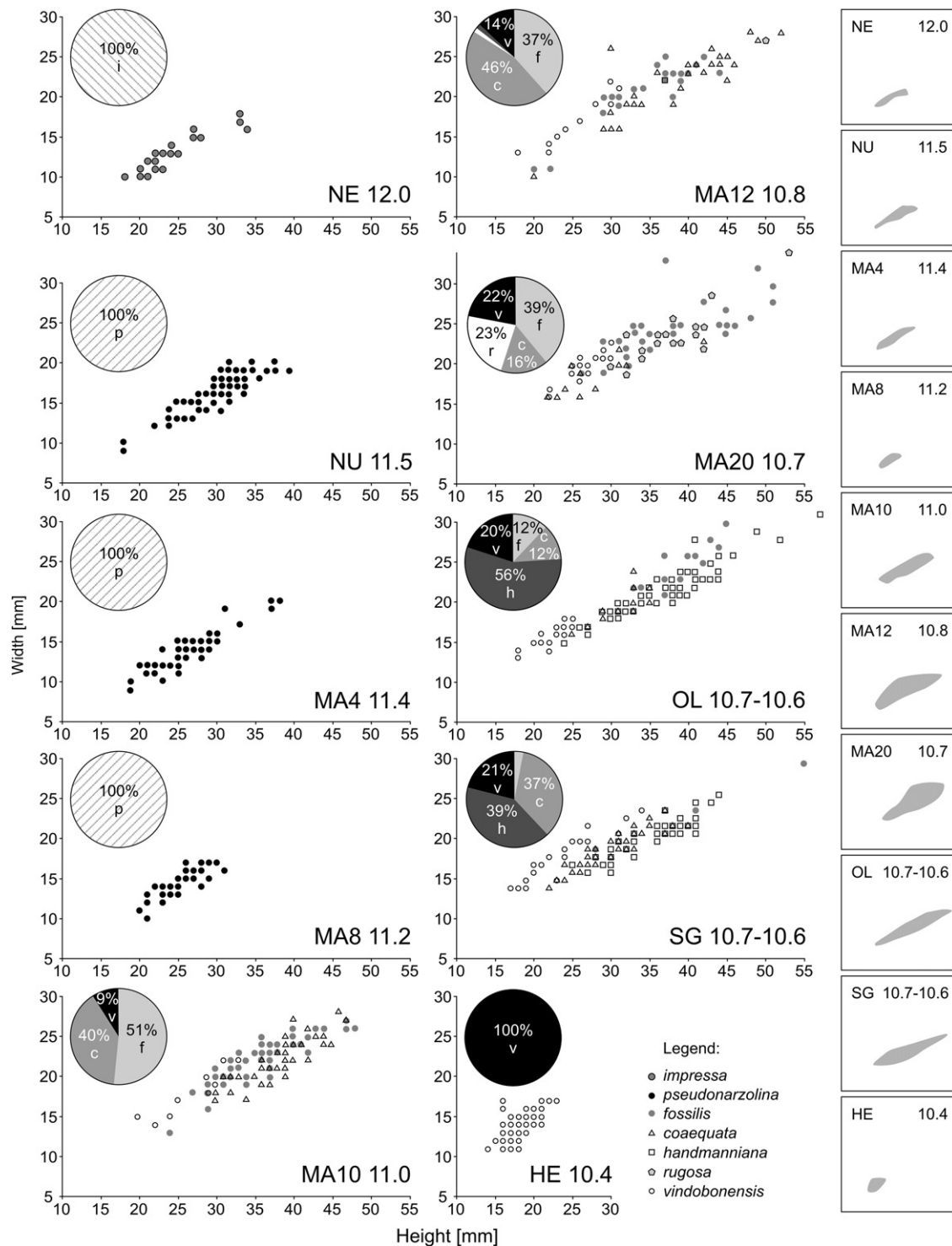


Fig. 4. Size change of the *Melanopsis impressa*-species lineage over time. On the right-hand side size ranges are lumped across phenotypes to visualize the most dramatic shifts. Abbreviations: NE – Nexing, NU – Nussdorf, MA – St. Margarethen (with numbers of horizons), OL – Ollersdorf, SG – Siegendorf, HE – Hengersdorf; c – *coaequata*, f – *fossilis*, h – *handmanniana*, i – *impressa*, p – *pseudonarzolina*, r – *rugosa*, v – *vindobonensis*. Numbers next to locality abbreviations refer to their geological age (in million years).

population close to the peak (Hunt et al., 2008). Consequently, the taxonomic separation of the 'subspecies' *pseudonarzolina* from *impressa*, introduced for stratigraphical differences, has to be rejected.

5.2.2. Step 2 – The adaptive radiation

In respect to the simultaneously changing environmental factors, the subsequent morphological shift with the first occurrence of several new phenotypes, followed by a massive morphological divergence,

is considered to reflect adaptive radiation, i.e. morphologic diversity as a result of rapidly multiplying lineages (e.g. Schluter, 2000; Hendry et al., 2007; Losos and Mahler, 2010). In terms of the adaptive landscape this is best explained by the model of a displaced adaptive optimum (Lande, 1986; Estes and Arnold, 2007; Hunt et al., 2008). In this model, the average phenotype is considered to follow an optimum, which has rapidly moved to a new position. In case of an adaptive radiation, descendant species occupy different adaptive peaks,

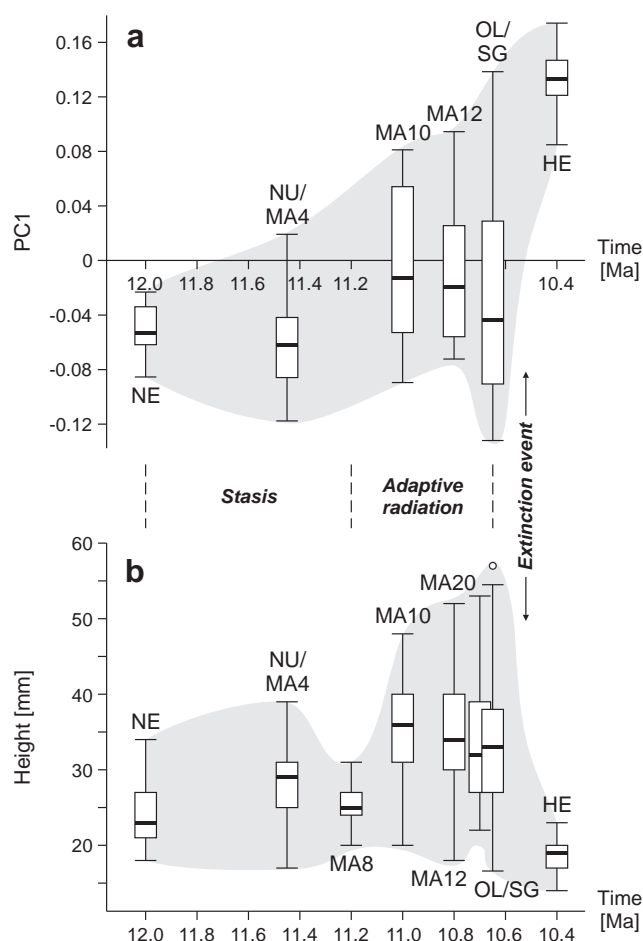


Fig. 5. Box plots depicting changing morphological ranges as described through Fourier analysis (PC1) (a) and shell height (b). The latter plot displays all samples, including those not treated by the PCA (for details see Section 2). White boxes indicate the range from 25th to 75th percentile, black horizontal lines inside boxes represent the median. Minimal and maximal values are given by horizontal lines outside boxes; the circle is the only outlier (Hammer and Harper, 2006). Grey areas indicate the total morphological ranges, interpolated between studied time slices. Direct interpretation of the PC1 should be avoided because it is computed from dimensionless Fourier coefficients; only the variable range and relative vertical position are of importance here. The onset of the adaptive radiation is signalled by conspicuous increase of the morphological variability, in both plots shown by the shifted medians and expanded percentile boxes. The extinction event is reflected by a strong decrease of morphological variability. Likewise, during the interval of stasis variation is comparatively small. Note that for the full interpretation of the results the consideration of all variables is necessary and that the pattern exhibited by single characters as illustrated above may slightly deviate from the general trend.

corresponding to different ecological niches (Schluter, 2000; Arnold et al., 2001). On a global scale the landscape becomes complex with multiple peaks, while on a local scale the individual phenotype/species might simply ascend a (moving) local adaptive optimum (Arnold et al., 2001). The model also accounts for divergence over geologically long time scales. As we are here confronted with c. 200,000 yr for the initial divergence, several shifts of the optimum (presumably in same 'ecological direction') can be expected (Estes and Arnold, 2007).

After Schluter (2000) adaptive radiation incorporates three main processes. Related to properties of the adaptive landscape, these are 1) phenotypic differentiation (populations following a peak, which moves as a result of changes in the environment), 2) phenotypic divergence (subpopulations approaching different peaks, as a result of divergent selection from competition or ecological opportunity) and 3) the establishment of reproductive isolation, i.e. ecological speciation. For

discussion whether adaptive radiation necessarily involves (ecological) speciation see Schluter (2000, 2001) and Losos and Mahler (2010).

Each of these steps is documented in the presented data. The initial phenotypic differentiation is mirrored in the morphological shift in the interval 11.2–11.0 Ma. This goes along with a first expansion of the morphospace with the appearance of several new phenotypes (*fossilis*, *coaequata*, *vindobonensis*), i.e. phenotypic divergence. However, there is still large morphologic overlap between these forms, regarding both size and shape traits. Over the following 200,000 yr two new phenotypes emerge (*handmanniana*, *rugosa*), which account for minor changes in the average morphology. In summary, at 10.8 Ma five phenotypes are synchronously present within a limited geographic area (Figs. 1, 4). The most impressive phenotypic divergence happens within the interval 10.7–10.6 Ma. In the PCA, this is expressed as a bimodal distribution towards a large and highly shouldered *handmanniana*- and a small, globular *vindobonensis*-phenotype. Although both phenotypes occur already earlier, they become distinctly more abundant during this period and massively extend the initial divergence. This strong phenotypic bifurcation is considered to reflect the last process, i.e. ecological speciation (Schluter, 2000).

5.2.2.1. Potential trigger mechanisms. This claim implies diverging natural selection leading to reproductive isolation, as a result of ecological specialization of certain phenotypes to certain environments. This raises the question, what promoted the evolution of larger and shouldered/globular phenotypes?

The development of sculptured and/or shouldered shells is not unusual in *Melanopsis*. In Lake Pannon several independent lineages show recurrent patterns of morphological evolution. This includes the lineage of *M. bouei* Férussac, 1823 (Geary, 1990), coevally occurring with the *M. impressa*-lineage, and the Pontian (late Tortonian) lineage of *M. caryota* (Stoliczka, 1862) (Geary et al., 2002). Both species show changes in average shell shape and either a loss of sculpture (*M. bouei*) or an increase of shouldering (*M. caryota*) comparable to the here documented case. For *M. caryota* Geary et al. (2002) discuss potential agents of selection, considering a predator–prey model, or a response to changing water chemistry or hydrodynamic conditions, as equally likely explanations.

Comparable developments are common throughout the history of this genus, and also among the recent populations (e.g. Glaubrecht, 1993, 1996; Bandel, 2000; Heller and Sivan, 2002). Studies on *Melanopsis* species from the Pleistocene Jordan Valley showed a distinct correlation between morphology and water energy (Heller and Sivan, 2002). Smooth and slender forms are considered to represent agitated environments, hence to dwell within or in the proximity of rivers. In contrast, sculptured shells reflect non-turbulent habitats. Likewise, the smooth and slender *impressa*- and *pseudonarzolina*-phenotypes of Lake Pannon dwelled in fluvio-deltaic settings, exposed to elevated water energy. Parallel to the appearance of broader and shouldered phenotypes (*fossilis*, *coaequata*, *vindobonensis*) a massive increase in lake level is recorded, representing low-energy open-lake conditions (Fig. 1; Harzhauser et al., 2007). This trend increases during the broad phenotypic divergence (Figs. 3, 5). Comparable correlations are found in the extant species of the gastropod *Lavigeria* in Lake Tanganyika. Michel et al. (1992) showed that the divisions between morphospecies coincide with habitat barriers (see also Michel, 1994). Likewise, the workgroup around Johannesson (2009, and references therein) presented an evolutionary model from the marine gastropod *Littorina saxatilis*. Two ecotypes arise from divergent selection, resulting from adaptation to different substrates and predation intensity. Similarly, we consider lake-level changes, which strongly affect substrate type and exposure to water energy, to be the trigger of the proposed divergent selection. The changing ecological conditions might have promoted invasion of new adaptive zones with underutilized niches, i.e. ecological opportunity, which is considered the most likely case for speciation (Schluter, 2000; Seehausen, 2006; Losos and Mahler, 2010).

Though it is a common trigger mechanism in gastropod evolution (e.g. Vermeij and Covich, 1978; West et al., 1991; Martens et al., 1994; West and Cohen, 1994; Johannesson, 2001), the frequently cited predator–prey co-evolution as explanation model for the evolution of such morphologies seems unsuitable in the present case. Although larger and highly shouldered shells provide better constructional stability against shell-crushing predators, the evidence for such interactions is poor (Geary et al., 2002). Moreover, different phenotypes with varied constructional stability coexist in the same environment over hundred thousands of years across several localities throughout the entire lake (Geary, 1990, 1992).

5.2.2.2. Limitations. Still, we cannot definitely assure from this kind of palaeontological data that the observed pattern is consistent with adaptive radiation (e.g. Allmon and Smith, 2011), nor can we proof the exact mode of speciation. Palaeontological data will rarely allow a precise reconstruction of the involved processes, e.g. adaptive radiation (e.g. Schluter, 2000; Rintelen et al., 2004; Rintelen and Glaubrecht, 2005; Pinto et al., 2008; Losos and Mahler, 2010) versus non-adaptive radiation (e.g. Rundell and Price, 2009; Wilke et al., 2010), ecological speciation (e.g. Schluter, 1996, 2000, 2009; Dieckmann and Doebeli, 1999; Doebeli and Dieckmann, 2003; Coyne and Orr, 2004; Rundell and Nosil, 2005; Hendry et al., 2007; Hendry, 2009) versus non-ecological speciation (e.g. McKinnon et al., 2004; Rundell and Price, 2009; Schluter, 2009), or a possible influence of hybridization (e.g. Mallet, 2008, 2009; Sadedin et al., 2009). This is still a highly discussed topic in evolutionary biology and usually rests upon studies on molecular data, tests/models for mating preferences (e.g. Gavrillets and Vose, 2009; Sadedin et al., 2009) and behavioural aspects like resource competition – features that can be rarely tested or inferred from fossils (e.g. Hunt, 2010; Louys et al., 2012). Nevertheless, the correlation between the phenotypic changes and the environmental alterations strongly argues for ecologically driven natural selection. Consequently, the strong phenotypic divergence is thought to reflect reproductive isolation. The argument for directional selection is corroborated by the fact that both shape and size traits show massive changes within the interval of the proposed adaptive radiation. Hunt (2007) suggested body size to be a more common subject to directional selection than shape traits.

Finally, the stratigraphic resolution is not precise enough to identify where and when the initial divergence happened, regarding exact location and time, hence gradual vs. punctuated change (e.g. Eldredge and Gould, 1972; Gould and Eldredge, 1977; Hunt, 2008). Unfortunately, there is no possibility to increase stratigraphic resolution, because no fossils are preserved between the studied intervals. Nevertheless, as all samples are collected from proximate localities at the western lake margin, they are suggested to represent populations that thrived under similar conditions. The phenotypic differentiation and divergence is known to occur within a single locality (St. Margarethen, this study) – excluding a spatial bias – and coevally across several localities in the Vienna Basin, the Eisenstadt–Sopron Basin (this study) and even throughout the entire Lake Pannon (Geary, 1990, 1992; Geary et al., 2002). As the environmental changes, proposed to cause the morphological evolution, affected the whole lake (Harzhauser et al., 2007; Böhme et al., 2008), we can at least exclude a bias of our results from immigration events (e.g. Van Bocxlaer et al., 2008).

Limited dispersal by virtue of narrow ecological preferences or the mode of reproduction in *Melanopsis* can also be excluded. Recent *Melanopsis praemorsa* (Linnaeus, 1758) is a generalist, living in a great variety of habitats. These include rivers, ponds, springs, shallow lakes with inundated marshes, mud and gravel shores of estuaries, irrigation canals, and oases. It tolerates high temperatures and brackish conditions (Brown, 1994; Mouahid et al., 1996; Plaziat and Yousif, 2005; Bandel et al., 2007). This species feeds variably on algae, detritus and carrion (Glaubrecht, 1996). *M. praemorsa* is a dioecious, oviparous species with direct development of the larvae into the adult

animal; no planktonic stage occurs (Mouahid et al., 1996). This might result in limited dispersal possibilities over large geographical distances within a short time. However, this is expected to play a minor role for the geological time scales dealt with here.

5.2.3. Step 3 – Extinction

In the latest stage included in this study, only very small individuals of the *vindobonensis*-phenotype are present. Environmental data indicate a strong turnover during this period, with large-scale, astronomically-tuned climatic changes (Kern et al., 2012) and strong seasonal fluctuations of lake level, productivity and river discharges (Harzhauser et al., 2007). Martens et al. (1994) concluded that seasonality has strong effect on the biology of long-lived lake animals. This is particularly true for the influence of changing nutrient input. Possibly, the reduced amount and availability of nutrients drove the larger phenotypes of the herbivorous snails to extinction. Moreover, the lake level changes might have strongly influenced the exposure of the snails to increasing water energy and wave action. Probably, the small, globular *vindobonensis*-phenotype was better adapted to these new environmental conditions. Recent biometric analyses revealed that compact shells with low spires have a higher postural stability on the substrate than those with higher spires (Noshita et al., 2012). Of course, this is exceedingly influenced with increasing water energy. Also Heller and Sivan (2002) relate stout shells of *Melanopsis* from the Pleistocene of the Jordan Valley to highly turbulent waters. Consequently, the unstable conditions during the Vallesian Crisis with at least partially higher water energy may have suppressed the large and shouldered phenotypes.

6. Conclusions

In summary, our results demonstrate an evolutionary sequence involving morphological stasis, a shift of the occupied morphospace followed by phenotypic divergence, and finally an extinction of one of the branches. The morphological changes could be reliably linked to palaeoenvironmental data. These suggest about constant conditions for the interval of stasis, promoting the action of stabilizing selection on the studied traits. The following shifting and expanding morphospace parallels an increase in lake level, which resulted in an overall change in the available habitat types. Investigations on the functional importance of shell characteristics (e.g. Heller and Sivan, 2002; Noshita et al., 2012) suggest, amongst others, declining water energy to trigger the evolution of more globular and shouldered phenotypes. This strong correlation between environmental parameters and appearance of several new phenotypes is interpreted as an adaptive radiation (e.g. Schluter, 2000; Losos and Mahler, 2010). The subsequent impressive divergence towards two different optima is considered to reflect adaptation to new habitats. The slowly evolving discrepancies of ecological requirements indicate the action of divergent natural selection. This finally resulted in two separate phenotypes, which are interpreted as distinct species, consistent with the model of ecological speciation (e.g. Schluter, 2001, 2009; Rundell and Nosil, 2005). In the latest investigated stage a massive environmental turnover with strong seasonally fluctuating conditions reduced available niche space and wiped out the majority of formerly existing phenotypes/species. The only surviving species comprised the small and globular *M. vindobonensis*.

Concluding, the employed methods allowed detailed insights into different modes and rates of evolution. The Fast Fourier transform method proved particularly capable of describing the morphospace accurately. Future research should focus increasingly on the application of such comprehensive analyses. This will essentially enlarge our toolbox of methods to reveal morphological changes and relate them to evolutionary mechanisms in the fossil record.

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References

- Agustí, J., Moyà-Solà, S., 1990. Mammal extinctions in the Vallesian (Upper Miocene). In: Kauffmann, E.G., Walliser, O.H. (Eds.), *Lecture Notes in Earth Sciences*, 30: Extinction Events in Earth History. Springer, Berlin, pp. 425–432.
- Allmon, W.D., Smith, U.E., 2011. What, if anything, can we learn from the fossil record about speciation in marine gastropods? Biological and geological considerations. *American Malacological Bulletin* 29, 247–276.
- Arnold, S.J., Pfrender, M.E., Jones, A.G., 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* 112–113, 9–32.
- Bandel, K., 2000. Speciation among the Melanopsidae (Caenogastropoda). Special emphasis to the Melanopsidae of the Pannonian Lake at Pontian time (Late Miocene) and the Pleistocene and Recent of Jordan. *Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg* 84, 131–208.
- Bandel, K., Sivan, N., Heller, J., 2007. *Melanopsis* from Al-Qarn, Jordan Valley (Gastropoda: Cerithioidea). *Paläontologische Zeitschrift* 81, 304–315.
- Benton, M.J., Pearson, P.N., 2001. Speciation in the fossil record. *Trends in Ecology & Evolution* 16, 405–411.
- Böhme, M., Ilg, A., Winkhofer, M., 2008. Late Miocene “washhouse” climate in Europe. *Earth and Planetary Science Letters* 275, 393–401.
- Bookstein, F.L., 1987. Random walk and the existence of evolutionary rates. *Paleobiology* 13, 446–464.
- Bookstein, F.L., 1996. Biometrics, biomathematics and the morphometric synthesis. *Bulletin of Mathematical Biology* 58, 313–365.
- Bookstein, F.L., 1997. Landmark methods for forms without landmarks: localizing group differences in outline shape. *Medical Image Analysis* 1, 225–243.
- Brown, D.S., 1994. *Freshwater Snails of Africa and their Medical Importance*. Taylor & Francis, London.
- Brusoni, F., Basso, D., 2007. Canonical morphometry versus statistical treatment of outlines through Fourier shape analysis: an empirical comparison. *Bollettino Malacologico* 43, 131–138.
- Coyne, J.A., Orr, H.A., 2004. *Speciation*. Sinauer Associates, Sunderland, Massachusetts.
- Crampton, J.S., 1995. Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. *Lethaia* 28, 179–186.
- Crampton, J.S., Haines, A.J., 1996. Users' manual for programs Hangle, Hmatch and Hcurve for the Fourier shape analysis of two-dimensional outlines. Institute of Geological and Nuclear Sciences, Science Report 96, 1–28.
- Davis, J.C., 1986. *Statistics and Data Analysis in Geology*. Wiley and Sons, New York.
- Daxner-Höck, G., 2004. Flying squirrels from the Upper Miocene of Austria. *Annalen des Naturhistorischen Museums in Wien* 106A, 387–423.
- Dieckmann, U., Doebeli, M., 1999. On the origin of species by sympatric speciation. *Nature* 400, 354–357.
- Doebeli, M., Dieckmann, U., 2003. Speciation along environmental gradients. *Nature* 421, 259–264.
- Eldredge, N., Gould, S.J., 1972. Punctuated equilibria: an alternative to phyletic gradualism. In: Schopf, T.J.M. (Ed.), *Models in Paleobiology*. Freeman Cooper, San Francisco, pp. 82–115.
- Estes, S., Arnold, S.J., 2007. Resolving the paradox of stasis: models with stabilizing selection explain evolutionary divergence on all timescales. *The American Naturalist* 169, 227–244.
- Férussac, A.E.J.P.J.F.d'A. de, 1823. Monographie des espèces vivantes et fossiles du genre melanopside, *Melanopsis*, et observations géologiques à leur sujet. *Mémoires de la Société d'Histoire Naturelle de Paris* 1, 132–164.
- Fischer, W., 1996a. Beiträge zur Kenntnis der rezenten und fossilen Melanopsidea V. *Melanopsis handmanniana* nov. nomen und *Melanopsis fossilis* (Gmelin 1790) (Gastropoda, Prosobranchia). *Club Conchylia Informationen* 28 (1/2), 25–38.
- Fischer, W., 1996b. Beiträge zur Kenntnis der rezenten und fossilen Melanopsidae VI. Über *Melanopsis coaequata coaequata* Handmann 1887 und *Melanopsis coaequata rugosa* Handmann 1887 sowie ein Nachtrag zu Teil V. (Gastropoda, Prosobranchia). *Club Conchylia Informationen* 28 (3/4), 19–26.
- Foot, M., 1989. Perimeter-based Fourier analysis: a new morphometric method applied to the trilobite cranium. *Journal of Paleontology* 63, 880–885.
- Fuchs, T., 1870. Beiträge zur Kenntniss fossiler Binnenfaunen. Die Fauna der Congerischichten von Radmanest im Banate. *Jahrbuch der Kaiserlich Königlichen Geologischen Reichsanstalt* 20, 343–364.
- Gavrillets, S., 2010. High-dimensional fitness landscapes and the origins of biodiversity. In: Pigliucci, M., Muller, G. (Eds.), *Toward an Extended Evolutionary Synthesis*. MIT Press, Cambridge, MA, pp. 45–80.
- Gavrillets, S., Vose, A., 2009. Dynamic patterns of adaptive radiation: evolution of mating preferences. In: Butlin, R.K., Bridle, J., Schluter, D. (Eds.), *Speciation and Patterns of Diversity*. Cambridge University Press, Cambridge, pp. 102–126.
- Geary, D.H., 1990. Patterns of evolutionary tempo and mode in the radiation of *Melanopsis* (Gastropoda: Melanopsidae). *Paleobiology* 16, 492–511.
- Geary, D.H., 1992. An unusual pattern of divergence between two fossil gastropods: ecophenotypy, dimorphism, or hybridization. *Paleobiology* 18, 93–109.
- Geary, D.H., Staley, A.W., Müller, P., Magyar, I., 2002. Iterative changes in Lake Pannon *Melanopsis* reflect a recurrent theme in gastropod morphological evolution. *Paleobiology* 28, 208–221.
- Glaubrecht, M., 1993. Mapping the diversity: geographical distribution of the freshwater snail *Melanopsis* (Gastropoda: Cerithioidea: Melanopsidae) with focus on its systematics in the Mediterranean Basin. *Mitteilungen aus dem Hamburger Zoologischen Museum und Institut* 90, 41–97.
- Glaubrecht, M., 1996. Evolutionsökologie und Systematik am Beispiel von Süß- und Brackwasserschnecken (Mollusca: Caenogastropoda: Cerithioidea): Ontogenese-Strategien. *Paläontologische Befunde und Zoogeographie*, Backhuys, Leiden.
- Glaubrecht, M., 2011. Toward solving Darwin's “mystery”: speciation and radiation in freshwater gastropods. *American Malacological Bulletin* 29, 187–216.
- Gmelin, J.F., 1791. *Caroli a Linné, systema naturae. Tomus I. Pars VI. Beer, Lipsiae*.
- Görthner, A., Meier-Brook, C., 1985. The Steinheim Basin as a paleoancient lake. In: Bayer, U., Seilacher, A. (Eds.), *Sedimentary and Evolutionary Cycles*. Springer Verlag, Berlin, pp. 256–296.
- Gottschick, F., 1920. Die Umbildung der Süßwasserschnecken des Tertiärbeckens von Steinheim a. A. unter dem Einflusse heißer Quellen. *Jenaische Zeitschrift für Naturwissenschaft* 56, 155–216.
- Gould, S.J., Eldredge, N., 1977. Punctuated equilibria: tempo and mode of evolution reconsidered. *Paleobiology* 3, 115–151.
- Haines, A.J., Crampton, J.S., 2000. Improvements to the method of Fourier shape analysis as applied in morphometric studies. *Palaeontology* 43, 765–783.
- Hammer, Ø., Harper, D.A.T., 2006. *Paleontological Data Analysis*. Blackwell Publishing, Oxford.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4, 1–9.
- Handmann, R., 1887. Die fossile Conchylienfauna von Leobersdorf im Tertiärbecken von Wien. *Aschendorff, Münster*.
- Hansen, T.F., 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51, 1341–1351.
- Hansen, T.F., Martins, E.P., 1996. Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution* 50, 1404–1417.
- Harzhauser, M., Mandic, O., 2008. Neogene lake systems of Central and South-Eastern Europe: faunal diversity, gradients and interrelations. *Palaeogeography, Palaeoclimatology, Palaeoecology* 260, 417–434.
- Harzhauser, M., Daxner-Höck, G., Piller, W.E., 2004. An integrated stratigraphy of the Pannonian (Late Miocene) in the Vienna Basin. *Austrian Journal of Earth Sciences* 95 (96), 6–19.
- Harzhauser, M., Latal, C., Piller, W.E., 2007. The stable isotope archive of Lake Pannon as a mirror of Late Miocene climate change. *Palaeogeography, Palaeoclimatology, Palaeoecology* 249, 335–350.
- Heller, J., Sivan, N., 2002. *Melanopsis* from the Pleistocene site of 'Ubeidiya, Jordan Valley: direct evidence of early hybridization (Gastropoda: Cerithioidea). *Biological Journal of the Linnean Society* 75, 39–57.
- Hendry, A.P., 2009. Ecological speciation! Or the lack thereof? *Canadian Journal of Fisheries and Aquatic Sciences* 66, 1383–1398.
- Hendry, A.P., Nosil, P., Rieseberg, L.H., 2007. The speed of ecological speciation. *Functional Ecology* 21, 455–464.
- Hilgendorf, F., 1867. Über *Planorbis multiformis* im Steinheimer Süßwasserkalk. *Monatsberichte der Königlich-Preussischen Akademie der Wissenschaften zu Berlin* 1866, 474–504.
- Hunt, G., 2006. Fitting and comparing models of phyletic evolution: random walks and beyond. *Paleobiology* 32, 578–601.
- Hunt, G., 2007. The relative importance of directional change, random walks, and stasis in the evolution of fossil lineages. *PNAS* 104, 18404–18408.
- Hunt, G., 2008. Gradual or pulsed evolution: when should punctuational explanations be preferred? *Paleobiology* 34, 360–377.
- Hunt, G., 2010. Evolution in fossil lineages: paleontology and *The Origin of Species*. *The American Naturalist* 176, S61–S76.
- Hunt, G., Bell, M.A., Travis, M.P., 2008. Evolution towards a new adaptive optimum: phenotypic evolution in a fossil stickleback lineage. *Evolution* 62, 700–710.
- Johannesson, K., 2001. Parallel speciation: a key to sympatric divergence. *Trends in Ecology & Evolution* 16, 148–153.
- Johannesson, K., 2009. Inverting the null-hypothesis of speciation: a marine snail perspective. *Evolutionary Ecology* 23, 5–16.
- Johnston, M.R., Tabachnick, R.E., Bookstein, F.L., 1991. Landmark-based morphometrics of spiral acrometry growth. *Paleobiology* 17, 19–36.
- Kern, A.K., Harzhauser, M., Piller, W.E., Mandic, O., Soliman, A., 2012. Strong evidence for the influence of solar cycles on a Late Miocene lake system revealed by biotic and abiotic proxies. *Palaeogeography, Palaeoclimatology, Palaeoecology* 329–330, 124–136.
- Krauss, F., 1852. Die Mollusken der Tertiärformation von Kirchberg an der Iller. *Jahreshefte des Vereins für Vaterländische Naturkunde in Württemberg* 8, 136–157.

- Kroh, A., 2005. *Catalogus Fossilium Austriae*, Band 2: Echinoidea neogenica. Verlag der Österreichischen Akademie der Wissenschaften, Wien.
- Kuhl, F.P., Giardina, C.R., 1982. Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing* 18, 236–258.
- Lande, R., 1976. Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30, 314–334.
- Lande, R., 1986. The dynamics of peak shifts and the pattern of morphological evolution. *Paleobiology* 12, 343–354.
- Linnaeus, C., 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis locis*. Tomus 1. Laurentius Salvius, Holmiae.
- Losos, J.B., Mahler, D.L., 2010. Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. In: Bell, M.A., Futuyma, D., Eanes, W., Levinton, J. (Eds.), *Evolution since Darwin: the first 150 years*. Sinauer, Sunderland, Massachusetts, pp. 381–420.
- Louys, J., Wilkinson, D.M., Bishop, L.C., 2012. Ecology needs a paleontological perspective. In: Louys, J. (Ed.), *Paleontology in Ecology and Conservation*. Springer, Berlin, Heidelberg, pp. 23–38.
- Loy, A., Busilacchi, S., Costa, C., Ferlin, L., Cataudella, S., 2000. Comparing geometric morphometrics and outline fitting methods to monitor fish shape variability of *Diplodus puntazzo* (Teleostea: Sparidae). *Aquacultural Engineering* 21, 271–283.
- Lueger, J.P., 1978. Klimaentwicklung im Pannon und Pont des Wiener Beckens aufgrund von Landschneckenfaunen. *Anzeiger der Österreichischen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Klasse* 6, 137–149.
- MacLeod, N., 1999. Generalizing and extending the eigenshape method of shape space visualization and analysis. *Paleobiology* 25, 107–138.
- MacLeod, N., 2002. Geometric morphometrics and geological shape-classification systems. *Earth-Science Reviews* 59, 27–47.
- Magyar, I., Geary, D.H., Müller, P., 1999. Paleogeographic evolution of the Late Miocene Lake Pannon in Central Europe. *Palaeogeography, Palaeoclimatology, Palaeoecology* 147, 151–167.
- Mallet, J., 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society B* 363, 2971–2986.
- Mallet, J., 2009. Rapid speciation, hybridization and adaptive radiation in the *Heliconius melpomene* group. In: Butlin, R.K., Bridle, J., Schluter, D. (Eds.), *Speciation and Patterns of Diversity*. Cambridge University Press, Cambridge, pp. 177–194.
- Martens, K., Coulter, G., Goddeeris, B., 1994. Speciation in Ancient Lakes – 40 years after Brooks. In: Martens, K., Goddeeris, B., Coulter, G. (Eds.), *Speciation in Ancient Lakes: Archiv für Hydrobiologie - Beiheft: Ergebnisse der Limnologie*, 44, pp. 75–96.
- McGhee, G.R., 2007. *The Geometry of Evolution. Adaptive Landscapes and Theoretical Morphospaces*. Cambridge University Press, New York.
- McKinnon, J.S., Mori, S., Blackman, B.K., David, L., Kingsley, D.M., Jamieson, L., Chou, J., Schluter, D., 2004. Evidence for ecology's role in speciation. *Nature* 429, 294–298.
- Mensink, H., 1984. Die Entwicklung der Gastropoden im miozänen See des Steinheimer Beckens. *Palaeontographica Abteilung A* 183, 1–63.
- Michel, E., 1994. Why snails radiate: a review of gastropod evolution in long-lived lakes, both recent and fossil. In: Martens, K., Goddeeris, B., Coulter, G. (Eds.), *Speciation in Ancient Lakes: Archiv für Hydrobiologie - Beiheft: Ergebnisse der Limnologie*, 44, pp. 285–317.
- Michel, A.E., Cohen, A.S., West, K., Johnston, M.R., Kat, P.W., 1992. Large African Lakes as natural laboratories for evolution: examples from the endemic gastropod fauna of Lake Tanganyika. *Mitteilungen - Internationale Vereinigung für Theoretische und Angewandte Limnologie* 23, 85–99.
- Mouahid, A., Idaghdour, M., Ghamizi, M., Moné, H., 1996. Observation of spawn in *Melanopsis praemorsa* (Prosobranchia: Melanopsidae). *Journal of Molluscan Studies* 62, 398–402.
- Noshita, K., Asami, T., Ubukata, T., 2012. Functional constraints on coiling geometry and aperture inclination in gastropods. *Paleobiology* 38, 322–334.
- Nützel, A., Bandel, K., 1993. Studies on the side-branch planorbids (Mollusca, Gastropoda) of the Miocene crater lake of Steinheim am Albuch (southern Germany). *Scripta Geologica, Special Issue* 2, 313–357.
- Papp, A., 1951. Das Pannon des Wiener Beckens. *Mitteilungen der Geologischen Gesellschaft in Wien* 39–41, 99–193.
- Papp, A., 1953. Die Molluskenfauna des Pannon des Wiener Beckens. *Mitteilungen der Geologischen Gesellschaft in Wien* 44, 85–222.
- Pinto, G., Mahler, D.L., Harmon, L.J., Losos, J.B., 2008. Testing the island effect in adaptive radiation: rates and patterns of morphological diversification in Caribbean and mainland *Anolis* lizards. *Proceedings of the Royal Society B* 275, 2749–2757.
- Plaziat, J.-C., Younis, W.R., 2005. The modern environments of molluscs in southern Mesopotamia, Iraq: a guide to paleogeographical reconstructions of Quaternary fluvial, palustrine and marine deposits. *Carnets de Géologie/Notebooks on Geology (Article 2005/01 (CG2005_A01))*.
- Raup, D.M., 1977. Stochastic models in evolutionary palaeontology. In: Hallam, A. (Ed.), *Patterns of evolution as illustrated by the fossil record. Developments in Palaeontology and Stratigraphy*, 5. Elsevier, Amsterdam, pp. 59–78.
- Rintelen, T.v., Glaubrecht, M., 2005. Anatomy of an adaptive radiation: a unique reproductive strategy in the endemic freshwater gastropod *Tylomelania* (Cerithioidea: Pachychilidae) on Sulawesi, Indonesia and its biogeographic implications. *Biological Journal of the Linnean Society* 85, 513–542.
- Rintelen, T.v., Wilson, A.B., Meyer, A., Glaubrecht, M., 2004. Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. *Proceedings of the Royal Society B* 271, 2541–2549.
- Rohlf, F.J., 2008. tpsDig, digitize landmarks and outlines, version 2.12. Department of Ecology and Evolution, State University of New York at Stony Brook. Available from <http://life.bio.sunysb.edu/morph>.
- Rohlf, F.J., 1990. Fitting curves to outlines. In: Rohlf, F.J., Bookstein, F.L. (Eds.), *Proceedings of the Morphometrics Workshop*. University of Michigan Museum of Zoology, Special Publication, 2, pp. 167–177.
- Roopnarine, P.D., Byars, G., Fitzgerald, P., 1999. Anagenetic evolution, stratophenetic patterns, and random walk models. *Paleobiology* 25, 41–57.
- Rundell, R.J., Price, T.D., 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology & Evolution* 24, 394–399.
- Rundle, H.D., Nosil, P., 2005. Ecological speciation. *Ecology Letters* 8, 336–352.
- Rust, J., 1997. Evolution, Systematik, Paläoökologie und stratigraphischer Nutzen neogener Süß- und Brackwasser-Gastropoden im Nord-Ägäis-Raum. *Palaeontographica Abteilung A* 243, 37–180.
- Sadein, S., Hollander, J., Panova, M., Johannesson, K., Gavrilets, S., 2009. Case studies and mathematical models of ecological speciation. 3: ecotype formation in a Swedish snail. *Molecular Ecology* 18, 4006–4023.
- Sampson, P.D., Bookstein, F.L., Sheehan, F.H., Bolson, E.L., 1996. Eigenshape analysis of left ventricular outlines from contrast ventriculograms. In: Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P., Slice, D.E. (Eds.), *Advances in Morphometrics: NATO ASI Series A: Life Sciences*, 284, pp. 211–233.
- Schluter, D., 1996. Ecological causes of adaptive radiation. *The American Naturalist* 148, S40–S64.
- Schluter, D., 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford, New York.
- Schluter, D., 2001. Ecology and the origin of species. *Trends in Ecology & Evolution* 16, 372–380.
- Schluter, D., 2009. Evidence for ecological speciation and its alternative. *Science* 323, 737–741.
- Seehausen, O., 2006. African cichlid fish: a model system in adaptive radiation research. *Proceedings of the Royal Society B* 273, 1987–1998.
- Sheets, H.D., Kim, K., Mitchell, C.E., 2004. A combined landmark and outline-based approach to ontogenetic shape change in the Ordovician trilobite *Triarthrus becki*. In: Elewa, A.M.T. (Ed.), *Morphometrics: Applications in Biology and Paleobiology*. Springer-Verlag, Berlin, pp. 67–82.
- Sheets, H.D., Covino, K.M., Panasiewicz, J.M., Morris, S.R., 2006. Comparison of geometric morphometric outline methods in the discrimination of age-related differences in feather shape. *Frontiers in Zoology* 3, 15.
- Simpson, G.G., 1944. *Tempo and Mode in Evolution*. Columbia Univ. Press, New York.
- Stoliczka, F., 1862. Beitrag zur Kenntnis der Molluskenfauna der Cerithien- und Inzersdorfer Schichten des ungarischen Tertiärbeckens. *Verhandlungen der Kaiserlich Königlich Geologischen Reichsanstalt* 529–538.
- Stone, J.R., 1998. Landmark-based thin-plate spline relative warp analysis of gastropod shells. *Systematic Biology* 47, 254–263.
- Van Bocxlaer, B., Schultheis, R., 2010. Comparison of morphometric techniques for shapes with few homologous landmarks based on machine-learning approaches to biological discrimination. *Paleobiology* 36, 497–515.
- Van Bocxlaer, B., Van Damme, D., Feibel, C.S., 2008. Gradual versus punctuated equilibrium evolution in the Turkana Basin molluscs: evolutionary events or biological invasions? *Evolution* 62, 511–520.
- Vermeij, G.J., Covich, A.P., 1978. Coevolution of freshwater gastropods and their predators. *The American Naturalist* 112, 833–843.
- West, K., Cohen, A., 1994. Predator–prey coevolution as a model for the unusual morphologies of the crabs and gastropods of Lake Tanganyika. In: Martens, K., Goddeeris, B., Coulter, G. (Eds.), *Speciation in Ancient Lakes: Archiv für Hydrobiologie - Beiheft: Ergebnisse der Limnologie*, 44, pp. 267–283.
- West, K., Cohen, A., Baron, M., 1991. Morphology and behavior of crabs and gastropods from Lake Tanganyika, Africa: implications for lacustrine predator–prey coevolution. *Evolution* 45, 589–607.
- Wilke, T., Benke, M., Brändle, M., Albrecht, C., Bichain, J.-M., 2010. The neglected side of the coin: non-adaptive radiations in spring snails (*Bythinella* spp.). In: Glaubrecht, M. (Ed.), *Evolution in action. Case Studies in Adaptive Radiation, Speciation, and the Origin of Biodiversity*. Springer, Heidelberg, pp. 551–578.
- Willmann, R., 1981. Evolution, Systematik und stratigraphische Bedeutung der neogenen Süßwassergastropoden von Rhodos und Kos/Ägäis. *Palaeontographica Abteilung A* 174, 10–235.
- Willmann, R., 1985. Responses of the Plio-Pleistocene freshwater gastropods of Kos (Greece, Aegean Sea) to environmental changes. In: Bayer, U., Seilacher, A. (Eds.), *Sedimentary and Evolutionary Cycles*. Springer-Verlag, Berlin, pp. 295–321.
- Wright, S., 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. *Proceedings of the Sixth International Congress of Genetics* 1, 356–366.