Microstructural details in shells of the gastropod genera *Carychiella* and *Carychium* of the Middle Miocene

ADRIENNE JOCHUM, THOMAS A. NEUBAUER AND MATHIAS HARZHAUSER

Microstructural details are revealed via scanning electron microscopy (SEM) in two carychiid species from the early Middle Miocene of Styria, SE Austria. The protoconchs of the shells of *Carychiella eumicrum* (Bourguignat 1857) and *Carychium gibbum* (Sandberger 1875) show different types of microstructure on the embryonic shell during ontogeny. Total, superficial punctate structure on the shell of *Carychiella eumicrum* contrasts with the protoconch–teleoconch demarcation (p/t boundary) observed on the protoconch of *Carychium gibbum*. Both species exhibit aragonitic microstructure. Diagenetic effects, prismatic, homogeneous and crossed lamellar microstructures are detectible in both species. Rheomorphic folding and dense pitting within the columella of *Carychiella eumicrum* suggest a structure–function relationship for tensile strength and bulk weight reduction in carychiid snails. We hypothesize that total superficial pitting on the shell of *C. eumicum*, seen here for the first time in the Carychiidae, suggests paedomorphosis as a life-history strategy to palaeoecological conditions of the Rein Basin during the early Middle Miocene.

Carychiella, Carychium, middle Miocene, mollusc assemblage, Rein Basin (Styria Austria), shell structure.

Adrienne Jochum [adrienne.jochum@gmail.com], Naturhistorisches Museum der Burgergemeinde Bern, Bernastr. 15, Bern CH-3005, Switzerland; Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, Bern CH-3012, Switzerland; Thomas A. Neubauer [thomas.neubauer@nhm-wien.ac.at], and Mathias Harzhauser [mathias.harzhauser@nhm-wien.ac.at], Department of Geology and Paleontology, Natural History Museum Vienna, Burgring 7, Vienna A-1010, Austria; manuscript received on 29/04/2014; manuscript accepted on 11/03/2015.

The Carychiidae represent a fascinating taxon of diehard, terrestrial gastropods. Although it is assumed they have a sketchy 330- to 300-million-year-old fossil record (Tracy et al. 1993; Bandel 1994), they are well represented in the rich mollusc assemblages of the Neogene of Europe (Boettger 1870; Wenz 1923; Strauch 1977; Meijer 1986; Prisyazhnyuk & Stowrzewicz 1995; Stowrzewicz 1999; Harzhauser & Kowalke 2002; Bernor et al. 2004; Binder 2004; Harzhauser & Binder 2004; Harzhauser & Piller 2004; Frank 2006; Salvador 2015; Harzhauser et al. 2014a). As the Carychiidae are amongst the earliest gastropod groups to have completed the transition from a marine/estuarine habitat to a terrestrial mode of life, they provide a model for studying the adaptations and ecology characterizing the radiation of terrestrial pulmonates (Barker 2001). The purpose of this study was to examine and report discernible microstructures found in carychiid fossils of this well-documented mollusc assemblage of the early Middle Miocene. In this study using SEM, we describe shell microstructures preserved in the two known carychiid species of the Rein Basin, Styria, SE Austria (Harzhauser et al. 2014a). This investigation is part of an ongoing search for ecological information and potential new characters for phylogenetic analysis in congruence with recent molecular analyses of extant species (Weigand & Jochum 2010; Weigand et al. 2011, 2012a,b, 2013) and fossil Carychiidae.

*Carychium* Müller 1773, frequently dominates mollusc assemblages and often characterizes communities, such as the ‘Carychium beds’ of the Vienna Basin (Jirícek & Seněš 1974; Bernor et al. 2004). Carychiidae are considered facies indicators for moist lakeshore habitats during the warm temperate to marginally tropical early Middle Miocene Badenian stage (Lueger 1981; Harzhauser & Kowalke 2002). Extant *Carychium* inhabit shady, moist, interstitial layers of leaf, grass and wood litter of mesic environments in riparian zones, damp meadows, mountain forests and tropical woodlands. The Carychiidae show a Holarctic distribution (Pilsbry 1948; Morton 1955) and today comprise two genera: *Carychium* (epigean) and *Zospeum* (troglobitic).

Understanding the Carychiidae has as much to do with carychiid evolutionary history, ontogeny, biochemistry and palaeoecology as with their reciprocal influences in the constructional morphology of the remarkably thin and translucent carychiid shell. Luchtel et al. (1997) emphasized that gastropod shell...
structure varies from species to species, and within each species, distinctive details are specifically configured for general shape, pigment, mineral organization and other features. Kano et al. (2008) observed that the smaller the snail’s body size, the more simplified anatomical structures become and that a more specialized ecology is required to sustain it. Within the Ellobioidea, the degree of inner shell resorption varies within the different sub-families, whereby shell sculpture is considered most informative at lower taxonomic levels and varies between genera (Martins 2007). To comprehend the Carychiidae and their role as baseline taxa in molluscan assemblages, it is essential to recognize and compare the peculiarities of shell structure in both extant and fossil taxa. Although advanced assessment techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), nanocomputer tomography (NanoCT), transmission electron microscopy (TEM) and atomic force microscopy (AFM) have given many authors insight into the complex structure of calcareous mollusc shells (Berman et al. 1993; Hedegaard & Wenk 1998; Chateigner et al. 2000; Hess et al. 2008; De Paula & Silveira 2009; Frýda et al. 2009; Furuhashi et al. 2009; Guzmán et al. 2009; Vendrasco et al. 2010; Rodriguez-Navarro et al. 2012; Hickman 2013), very few studies (Jochum 2011; Medaković & Popović 2012; Jochum et al. 2013) have been conducted at the microstructural level on terrestrial ellobioid taxa. It is well known that environmental influences play a pivotal role in shell mineralization and thus, influence shell form, development and repair (McMahon & Whitehead 1987; De Paula & Silveira 2009; Medaković & Popović 2012; Hickman 2013). As the Carychiidae inhabit semi-subterranean, subterranean and extreme subterranean habitats, some existing 980 m below the surface (Weigand et al. 2013), their shells harbour a potential reservoir of valuable information.

Mollusc shells are polycrystalline complexes of calcium carbonate, diverse proteins rich in acidic amino acids and glycoproteins (Lowenstam & Weiner 1989; Berman et al. 1993; Hedegaard & Wenk 1998). Gastropod and bivalve shells demonstrate a comparable range of shell mineralogy and microstructure (Carter et al. 1998), whereby gastropod shells comprise an outer organic periostracal sheet and an inner carbonate layer (Saleuddin & Petit 1983; Slapnik & Medaković 2007). Their rigidity and durability (texture) are governed by hierarchically organized layered carbonates (Hickman 2013) in the form of calcite, aragonite and vaterite (rarest form) such that the fractions of minerals, grain morphology and aggregation and crystal orientation comprise a microstructure identifiable and characteristic for specific structure at the genus to family levels (Hedegaard & Wenk 1998; Fuchigami & Sasaki 2005; Medaković & Popović 2012). Although knowledge of the structure and composition of the organic components constituting the biominerals within the crystalline composites has grown considerably, that of their fossilization process is still sparse (Guzmán et al. 2009). Moreover, the specificity of structural organization reflects structure-function relationships, which have recently become increasingly useful in designing models for material sciences (Ehrlich et al. 2011).

Our knowledge of mineralogy in the Carychiidae is limited to three studies (Medaković et al. 1999; Slapnik & Medaković 2007; Medaković & Popović 2012), whereby X-ray powder diffraction (XRD) was used on Zospeum shells to investigate mineral composition and phase fractions. A high concentration of aragonite and a small fraction of calcite in the shells were detected. As calcite is a better insulator than aragonite, these authors attributed the calcite fraction to adaptation to the fluctuating temperatures in certain cave habitats. As very little is known about ellobioid, let alone extant or fossil carychiid microstructure, our investigation presents new information about the microstructure of the Carychiidae.

Carychiid taxonomy and systematics
As fossil and extant carychiid gastropods are known to be highly morphologically variable (Watson &Verdcourt 1953; Strauch 1977; Burch & Van Devender 1980; Stworzewicz 1999; Weigand & Jochum 2010; Weigand et al. 2012a,b; Weigand et al. 2013), they have been subject to frequent taxonomic re-evaluation. Consequently, the plethora of forms has systematically been shuffled and reshuffled into genera and sub-genera, based on the degree of character expression in shell size, shape, form, dentition, whorl number, whorl height and, most specifically, the degree of sinuosity of the columellar lamella (Strauch 1977; Lueger 1981; Prisyazhnuyk & Stworzewicz 1995; Salvador 2015). For the herein treated carychiids, we follow the latest taxonomic and systematic revisions of Miocene freshwater deposits by Harzhauser et al. (2014a,b). Although recent molecular analyses focusing on phylogenetic and phylogeographical studies of extant Carychiidae have answered some profound questions regarding carychiid phylogeny (Weigand & Jochum 2010; Weigand et al. 2011, 2012a,b, 2013), shell microstructural investigations of extant and fossil material are still requisite to support these studies in an integrative,
contemporary taxonomic approach (Jochum 2011; Jochum et al. 2013).

**Faunal composition and palaeoecological setting**

Recently, Harzhauser et al. (2014a) performed a comprehensive survey on all mollusc fauna of the Lake Rein (Fig. 1), including an up-to-date systematic concept, critical synonymy lists, revisions of many taxa and descriptions of several new species. Along with the two species of Carychiidae comprising this present study, this work revealed a moderately diverse assemblage including 47 gastropod species (12 freshwater, 35 terrestrial) and one bivalve.

During the early Middle Miocene, the basin harboured a geologically short-lived wetland with ephemeral ponds, altogether referred to as Lake Rein. More precise information on the palaeoenvironment was recently inferred from the mollusc fauna (Harzhauser et al. 2014a). These authors emphasize that the freshwater molluscs comprise a large number of pulmonate gastropods, indicating a shallow and stagnant water body with densely vegetated banks and limited riverine influx. Moist woodland habitats fringing Lake Rein are indicated by most of the terrestrial taxa. The herein discussed carychiids, *Carychiella eumicrum* (Bourguignat 1857) and *Carychium gibbum* (Sandberger 1875), most likely settled the permanently moist to inundated habitats near the lakeshore.

**Material and methods**

The shell dimensions of *Carychiella eumicrum* range from a height of 0.95 to 1.1 mm and a width of 0.45 to 0.5 mm. The larger *Carychium gibbum* shows a height ranging from 1.40 to 1.45 mm and a width of 0.75 to 0.85 mm (Harzhauser et al. 2014a).

Two microscopically well-preserved specimens of *Carychiella eumicrum* (Bourguignat 1857) and six specimens with the exposed columella of *Carychium gibbum* (Sandberger 1875) were selected and mounted on 13-mm aluminium pin stubs and coated with gold–palladium in a sputter coater. Shell microstructures were examined on the shell surfaces, interfaces and fractured sections of the shells using the JEOL JSM-6610LV (Germany) scanning electron microscope (SEM) at the Natural History Museum Vienna (NHMW, Austria). In this study, we describe the preserved microstructures according to the nomenclature established by Grégoire (1972), Carter & Clark (1985) and Carter (1990). These descriptions were recently modified by Génio et al. (2012) (Table 1).

We interpret carychiid shell microstructures and discernible textures using SEM images collated from the vast pool of literature spanning geological time-scales. We use the following abbreviations to designate structures: Hom, homogeneous structure; Lam, laminar (tablet) structure; Le, lamellar structure; Li,
Table 1. Modified definitions by Génio et al. (2012) of shell microstructure from Carter (1990).

<table>
<thead>
<tr>
<th>Microstructure</th>
<th>Description</th>
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<tbody>
<tr>
<td>Simple prismatic (SP)</td>
<td>First-order prisms that show low-to-moderate length–width ratios: individual first-order prisms lack fan-like arrangements of their second-order structural subunits; the boundaries between adjacent simple prisms are generally well defined</td>
</tr>
<tr>
<td>Nacreous</td>
<td>Polygonal to round aragonitic tablets arranged in broadly continuous, regular, mutually parallel laminae, by definition, this structure is always aragonitic</td>
</tr>
<tr>
<td>Homogeneous (Hom)</td>
<td>More or less equidimensional, irregularly shaped crystallites or crystal morphotypes lacking clear first-order structural arrangement except for possible accretion banding</td>
</tr>
<tr>
<td>Crossed lamellar (CL)</td>
<td>First-order lamellae of thin mutually parallel laths or rods, with two non-horizontal dip directions of their elongate sub-units in adjacent lamellae. The structure is co-marginal, if the first-order lamellae are parallel to the shell margin, radial if perpendicular</td>
</tr>
<tr>
<td>Complex crossed lamellar (CCL)</td>
<td>Crossed structure with three or more non-vertical dip directions of other elongate structural units. It differs from crossed lamellar in that the largest sub-units are not concentrically elongate first-order lamellae but are much less regular and more variable in form</td>
</tr>
<tr>
<td>Intersected cross-platy</td>
<td>A crossed structure with only two predominant dip directions in which the first-order lamellae consist of short, rhomboidal platy crystallites</td>
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laminated structure; Gr, granular structure; and SP, simple prismatic structure.

The studied material is housed at the Natural History Museum Vienna, Austria, under the collection number NHMW 2012/0154.

Results

Descriptions of shell microstructures

*Carychiella eumicrum* (Bourguignat 1857) (Figs 2A–F, 3A–C, 5A, B): the outer layer of the shell shows homogeneous structure (Fig. 2F) consisting of granular and shoal-like formations. Shallow semi-elliptical pits are regularly distributed over the entire shell surface. These pits show a generally granular, homogeneous structure embedded by larger, aragonitic, rhomboidal laminate (RLi) tablets described by Carter (1990, p. 611) as ‘rods, laths, blades or tablets’ (Fig. 2E, F). Faint transverse rheomorphic striations intersperse the cavities and follow the curvature of the shell.

The region of dense calcium carbonate (Fig. 3A, B), constituting the columella at the second abapical columellar fold, demonstrates an inner and outer layer. The outer layer (Fig. 3B) is composed of simple prismatic laminar shell microstructure. At the homogeneous, horizontal interface between the two layers (Fig. 3B), individual crystals show prismatic rhomboidal and tabular form. The curvature of the inner layer shows strongly diagenetically altered crossed lamellar structure abutting a region of diagenetically altered simple prismatic microstructure. The surrounding structure (Fig. 3B, right side) is prismatic, but the exact underlying morphology is difficult to ascertain.

The ventral surface (Fig. 3A) of the columella encompassing the second abapical columellar fold is densely punctate. A pattern of regular pits (Fig. 3C) is located on the planar surface emanating from the interface of the outer, columellar shell layer.

By close inspection of the pitted rheomorphic folds, still smaller equidistantly arranged, distinct pinprick-like pores symmetrically pock a series of non-joining segments of a lightly discernible track-like pattern of horizontal meshwork. These non-fibrillar (at least with SEM) ‘caterpillar feet-like’ tracks are co-marginally concentrated on the mid-outermost section of the shell fracture. They demonstrate an irregular pattern. Although no reference to these tracks in other molluscs is found in the literature, they do not resemble growth lines or microstructural details described by previous authors. We speculate this pattern is due to a Middle Miocene bacterium, fungus, parasite or commensal interloper. From a constructional perspective, the irregular positioning of these peculiar microstructures suggests no function–structure relationship.

The strongly diagenetic deteriorated outer layer of the ventral side of the columella is comprised of homogeneous structure, whereby a nacreous ‘aragonitic laminar structure consisting of polygonal to rounded tablets arranged in a regularly formed, parallel sheet’ (Carter & Clark 1985, p. 55) can be discerned. Laminate laths and tablets loosely flank the perimeters of this structure.

*Carychium gibbum* (Sandberger 1875) (Fig. 4A): The surface structure of *C. gibbum* reveals a different morphology than *Carychiella eumicrum*. The protoconch and, in a few cases, the early teleoconch (Fig. 4B) are marked by the regular pattern of characteristic carychiid punctuation (Jochum 2011; Jochum et al. 2013). In contrast to *Carychiella eumicrum*, the second protoconch whorl bears the clear line indicating the demarcation zone of larval torsion and the reorganization of shell secretion between the protoconch and teleoconch (the p/t
This significant feature was observed only in one specimen of *Carychiella eumicrum* from this assemblage. Revealed is a plane of tabular aragonite prism comprised of vertical crystalline sub-units constituting the simple prismatic laminar shell microstructure on the inner marginal side of the shell (Fig. 4C, E, F). Below this layer is a stratum of granular and diagenetic homogenous structure surrounds the pit.
homogeneous structure marked by a bifurcated ribbon of potential accretion banding (Fig. 4C). As this banding oddly bifurcates and is oblique to the shell growth surfaces, it could possibly be organic material attached to the fossil shell surface. Slightly discernible, co-marginal crossed lamellar microstructure of thin, mutually aligned laths or lamellae are arranged parallel to the point of bifurcation of the aforementioned banding. At the endpoint of the accretion banding ribbon is an isolated, superimposed ropey structure of mineral sheets built up of laths (Fig. 4C, D). These laths show a regularity of crystal thickness in an arrangement of successive sheets of obliquely aligned crossed lamellar structure surrounded by homogeneous structure and diagenetic rubble (Fig. 4D).

A second fractured shell of *C. gibbum* provided a clearer and different perspective of microstructures at approximately the same region along the edge of the shell (Fig. 4E–F). This specimen shows sheets of simple prismatic structure merging into microstructure described by Hedegaard (1997, fig. 6) as ‘first order lamellae perceived as irregular aggregates of intergrown pieces of simple crossed lamellar structure’. Cliffs of simple prismatic structure border a zone of either crossed lamellar structure or an interface of continuation of the simple prismatic layer with variable diagenetic alteration below (Fig. 4E). The crossed lamellar structure shows different orientation of the cleavage planes (Fig. 4E).

Simple prismatic structure is indicated by a plane of tabular crystal aragonite (Fig. 4F). *C. gibbum* here presents Hedegaard’s (1997) aforementioned complex of crossed lamellar structure at the inner shell margin. Additionally, transverse jagged sheets of cross-lamellar structure (Fig. 4F) superficially resemble those described by Chateigner *et al.* (ICP, fig. 7b, 2000) for intersected cross-platy structure. A star-like complex of acicular bodies is embedded in a mass of diagenetic rubble (Fig. 4F). It is not clear whether this formation is due to intrinsic prismatic arrangement, whether the bodies are themselves artefacts or whether these structures comprise a constellation of randomly assembled prisms amassed in diagenetic rubble.

A regular pattern of wedge-shaped planes of aragonitic structure (Fig. 5A–D) is present in different densities on the columellar lamellae of both species. This structure has been consistently observed on the columellar lamellae in extant Carychiidae and likely aids in traction during mobility (Jochum 2011; Jochum *et al.* 2013).
Discussion

Protoconch morphology and structure

Strauch (1977) reported that one of five known species of the genus Carychiella is usually found in European fossil biotopes inhabited concurrently by one to three Carychium or Saraphia species. Our investigation here agrees with this finding in that Carychiella eumicrum was found inhabiting the same biotope with the larger Carychium gibbum. Moreover, although no direct phylogenetic relationship was determined within the few fossil forms
representing Carychiella in his investigations, Strauch (1977) hypothesized that Carychiella most plausibly originated from a common ancestor stemming from the Oligocene (33.9–23 Mya).

Although comparable morphological analyses of Carychiella eumicrum (Bourguignat 1857) (Fig. 2A) are documented by Strauch (1977, pl. 14, fig. 13) and Prisyazhnyuk & Stworzewicz (1995) from Opole (Poland), Stworzewicz (1999, fig. 5–6) from Belchatów (Poland) and Tuchórice (Slovak Republic), and Salvador (2015, fig. 1) from Sandelzhausen, Mainburg (Germany) (2013, fig. 1), the specimens examined here and presented by Harzhauser et al. (2014a, 2014b), pl. 6, fig. 5) differ significantly in that the entire shell is densely punctate beyond the protoconch (Fig. 2B, C). This total pitting of the shell is a structural feature new to both fossil and recent Carychiidae.

Superficial semi-ellipsoidal pitting (Fig. 2D) has been consistently observed in the protoconch of extant Carychiidae (Jochum 2011; Jochum et al. 2013) as well as in the protoconch of Carychium gibbum here (Harzhauser et al. 2014a,b) (Fig. 3A). Stworzewicz (1999) observed pitting on the protoconch of Carychium rhenanum (Strauch 1977) although Strauch (1977) did not describe this feature in this species nor in any other in his extensive analysis of Miocene carychiids. Moreover, Stworzewicz (1999) equally did not report similar finds on the shells of Carychiella eumicrum material she examined from Belchatów, Opole or Tuchórice. On the other hand, and contrary to these observations, it is notable that Harzhauser et al. (2014b) recently found punctate microstructure on the protoconch and early teleoconch of shells of C. eumicrum from Tuchórice.

The pronounced, globular protoconch (Fig. 2C) of Carychiella eumicrum (Harzhauser et al. 2014a,b; pl. 6, figs 5, 6), although not uncommon to lesser degrees for extant Carychiidae, is indicative of ontogenetic development derived from a large egg containing a large yolk supply (Lima & Lutz 1990). Doll (1982) and Bulman (1990) confirm that the egg capsule of extant Carychium tridentatum (Risso 1826) is
‘gigantic’ compared to the adult, encompassing about one-fifth the maximal length of the adult shell (Doll 1982). This observation holds equally well for the relationship of protoconch to adult shell length in Carychiella eumicrum here (Fig. 2A). Together, these two structural features are striking in that they fail to consistently demonstrate the usual protoconch–teleoconch boundary (the p/t boundary), indicating when hatching occurred during the heterotectonic constructional processes of shell secretion (Hickman 2013). This line is associated with larval reorientation and the accompanying ontogenetic shift in shell morphology during metamorphosis. The p/t boundary, however, is well marked on the shells of Carychium gibbum (Fig. 3A–B). A likely similar constructional transition was reported on the shells for Carychiella puissegurii (Truc 1972) of the Early Pliocene from Celleneuve (Hérault), France (Strauch 1977). Our observations suggest a partial developmental and/or transitory stage for the genus Carychiella of the Lake Rein.

Shell microstructure

Microstructure in molluscs is characterized by the morphology of crystal units and their mode of orientation and layering arrangement. The most common extant shell microstructures (spherulitic prismatic, crossed lamellar, nacre and foliated calcite) are known since the Middle Cambrian (Runnegar & Jell 1976). Carter (1979) detected microstructural similarities in brachiopods and mollusc shells, suggesting a similarity in biomineralization processes which may have derived from protoconch–teleoconch processes which may have derived from the Middle Cambrian of Australia (Vendrasco et al. 2010; pl. 1, fig. 10). These tablets within the pits of C. eumicrum are rhomboidal- and blade shaped (Fig. 2E, F), suggesting similarity with the nacre tablet forms known in extant bivalves (Taylor et al. 1969; fig. 10, Pinctada margaritifera; Wada 1972; fig. 8, Pinna attenuata) and from fossils described by Vendrasco et al. (2010, text-fig. 4E–J) dating from the Middle Cambrian. Additionally, the moderately advancing homogeneous wave of structure initiating the depth of each pit (Fig. 2E) suggests a merging of tabular structure as described by Vendrasco et al. (2010, fig. 4H–J).

The microstructure comprising the outer surface of the columnella (Fig. 3A, B) of Carychiella eumicrum consists of simple prismatic structure. The interior, crossed lamellar structures show the ‘plate-like elongated aspect’ of lamellae described for Conus omaria by Rodriguez-Navarro et al. (2012, fig. 1E, F).

The regular punctate microstructure observed at the lower ventral face of the Carychiella eumicrum columnella (3A, C), just below the second apical columnellar fold, is reminiscent of similar superficial poch-like structures found on shells of brachiopods and diverse molluscs. Warén & Gofas (1996, fig. 5a) reported external pitting in the monoplacophoran Veleropilina reticulata. Vendrasco et al. (2010) described a pattern of regular pitting structure on the interior surface of the shell in Figurina specimens (pl. 4, fig. 8) and in specimens of Corystos thorntoniensis (Pl. 5, figs. 7–8) from the Middle Cambrian. These authors attributed this punctate morphology to either an accelerated rate of organic matrix degradation in relation to the dissolution of shell structure or to shell diagenesis. For Carychiella here, we hypothesize with Jochum (2011) that a function–structure relationship, similar to that carried by trabecular bone in vertebrate skeletons, is an intrinsic element of design. The vast network of pores most plausibly serves to underscore shell strength while reducing shell weight during the unwieldy, semi-rotary maneuvering of the carychiid snail in action. In addition, the densely pitted microstructure most likely enhanced tensile strength in the columnella of Carychiella eumicrum during the tractional exertion of the columellar muscle on the columnella. Moreover, it can be assumed that Carychiidae from the Middle Miocene maneuvered in the same characteristic, semi-rotational jerking movements observed in extant species when they circumvent obstacles or enter tight crevices in wood or rocks.

Our SEM analyses suggest that homogeneous microstructure is prevalent in both carychiid species.
Carter & Clark (1985, p. 63) defined this seemingly amorphous structure as ‘aggregations of more or less equidimensional, irregularly shaped crystallites lacking clear first-order structural arrangement except for possible accretion banding’. Irregularly shaped crystallites are present on the protoconch of Carychiella eumicrum, while a large ribbon of accretion banding is clearly visible on the shell fracture of Carychium gibbum. Although accretion banding has often been observed in extant Carychiidae (Jochum personal observation), it is not necessarily an element of repair but rather an ‘extra’ manifestation of conchiolin fabric. The mechanisms responsible for why and where it appears are not yet clear.

The ‘intersected cross-platy’ variety of crossed lamellar microstructure may be demonstrated by C. gibbum here (Fig. 4F). This form, however, is perplexing in that a microstructural consensus is difficult to reach with SEM here. For example, transverse jagged sheets of crossed lamellar structure resemble those described for ‘intersected cross-platy’ reported by Chateignier et al. (2000, ICP, fig. 7b), Hedegaard (1990), on the other hand, observed this microstructure only in vetigastropods. Chateignier et al. (2000) found it to be identical to the ‘Type II crossed lamellar structure’ reported by Batten (1975). Methods such as TEM or the implementation of a wider sample basis from the same facies of Lake Rein would shed more light on this type of CL microstructure.

Small shell size and paedomorphosis in Carychiella

The minute (max. 1.1 mm shell height), completely punctate shell of Carychiella eumicrum, suggests a paedomorphic life strategy. To understand the significance and singular context of paedomorphism and small body size in C. eumicrum, a cursory discussion of the literature is necessary.

Paedomorphosis involves the heterochronic process by which descendants resemble the juveniles of their ancestors (Bhullar et al. 2012). It is a frequent mechanism of phenotypic change (Gould 1977; McNamara 1997), whereby larvae do not complete metamorphosis and individuals reach sexual maturity while retaining a larval morphology. Paedomorphosis is largely driven by ecological factors (Diz et al. 2012; Bonett et al. 2013). Moreover, paedomorphosis via progenesis promotes early somatic maturation and an abbreviated ontogeny (Bhullar et al. 2012). Although small size alone does not result in paedomorphic morphology, it has factored in discussions about the evolution of paedomorphosis (Bhullar et al. 2012; Diz et al. 2012). For example, if tininess of C. eumicrum (and all Carychiella species) promoted increased survival by wedging into tight crevices to avoid predation or desiccation in extreme environmental situations, then an apparent trade-off may have encompassed their avoidance in reaching the minimum possible size/age for sexual maturation. Thus, in this context, a selective pressure favouring a shift towards paedomorphosis via progenesis is plausible (Diz et al. 2012). In a recent model, Edeline et al. (2013) observed that warming-induced body down-sizing (Bergmann’s rule and the temperature-size rule) was a primary determinant of life-history trends in river fish spanning decades and encompassing 52 species. These authors determined that interspecific competition favoured small body size and that this played an advantageous role in high diversity communities. In the light of Harzhauser et al. (2014a,b) survey depicting the diverse assemblage of 47 gastropod species of the Lake Rein environment, Carychiella eumicrum factors specifically, with the larger member of the genus Carychium, Carychium gibbum as a potential competitor in this context.

Paedomorphosis, on the other hand, has been found to be reversible over relatively long time intervals (i.e. millions of years) in a recent phylogenetic study of spelerpine salamanders, suggesting that metamorphosis and adult traits can re-evolve after being evolutionarily lost (Bonett et al. 2013). Hereby, genes were found to counteract time by assuming alternate functions.

Paedomorphosis also occurs in small-sized, vent-seep bathymodiolin mussels found at organic falls (Génio et al. 2012). The bathymodiolin genus Idas is known from wood and whale-falls dating from the Eocene to Miocene (Amano & Little 2005). In the light of their extreme habitat status, Idas favours a transitory, ephemeral lifestyle much like the extant terrestrial Carychiidae (i.e. Zospeum in caves, Carychium in semi-subterranean habitats). Génio et al. (2012) hypothesize that the small size of Idas may well reflect an adaptation to ephemeral habitats that disappear in a few months or a few years. These authors attribute the evolution of paedomorphic forms to the distinct ecological selective pressures at organic falls such as space limitation, community productivity and ephemeralism. Truncated lifespans, accommodated by a trade-off of resource allocation during early development, would likely favour reproduction rather than growth. In agreement with Ockelmann & Dinesen (2011) and Génio et al. (2012), it is plausible that ‘a short generation time and small population size are preconditions for the possibility of an accelerated evolution’. Specifically,
Carychiella eumicrum likely represents this scenario for Lake Rein.

As this study revealed the only known carychiid to morphologically suggest paedomorphosis, further investigation of other fossil assemblages containing Carychiella species may reveal more occurrences from the Neogene. The question remains open whether the observed microstructural aspects and protoconch form of C. eumicrum are indicatory of a potential transitory stage of C. eumicrum forms known from Belchatów, Opole, Tuchorice and Sandelhausen. In this context, it is interesting that Strauch (1977) observed C. eumicrum finds frequently associated with other, larger Carychiella species at different geological periods: Miocene (eumicrum, crossei) and Pliocene (marinae, puissegurii). Strauch (1977) also observed apparent constructional transitions on the shells of C. puissegurii (Truc 1972) of the Early Pliocene from Celleneuve. A contemporary investigation emphasizing morphological and anatomical studies of Carychiella’s proposed closest extant relative, Carychium sibiricum (Westerlund 1897) (Strauch 1977; Stworzewicz 1999), could well reveal valuable, ecologically contextual information in this smallest of extant epigean Carychiidae.

Shell morphology reflects ecology

Constructional shifts in shell morphology, such as the p/t boundary observed in Carychium gibbum, often correlate with ecological transitions during ontogeny (Jackson et al. 2007; Hickman 2013). In addition, as similar shell ornamentation in the Carychiidae may reflect similar shifts in gene expression profiles (Grégoire 1972), shell structure may show similar gene regulatory transitions in response to ecological and functional requirements (Jackson et al. 2007; Hickman 2013).

Mechanisms influencing fossil and extant terrestrial gastropod shell structure are manifold. Less apparent but none the less influential for shell structure are those involving cross-ecosystem interactions such as species-specific coupling of plants with soil in extant and paleobiotic communities. Plant species influence the soil biota, including carychiid snails, via changes in the quality and quantity of organic material entering the substrate (Bardgett & Wardle 2010). Ions used for biomineralization are derived from the diet or directly from the environment (Weiner 2008), whereby the onset of shell mineralization may be correlated to feeding requirements (Eyster & Morse 1984). As extant carychiid species demonstrate a marked affinity towards specific plant communities (Morton 1955; Ložek 1957), and plant communities and species preferentially select for decomposer communities that most effectively mineralize their own litter (Wardle 2002; Ayres et al. 2006, 2009; Vivanco & Austin 2008; Strickland et al. 2009), it is fundamental to consider this relationship in the light of direct biochemical influences exerted in the individual mineralization of shells as well as in the subsequent taphonomic processes of both Carychiella eumicrum and Carychium gibbum facies.

Conclusions

The presented data provide additional evidence that shell microstructures are similar (i.e. within similar morphotypes) throughout diverse molluscan groups through geological time (Runnegar 1985; Vendrasco et al. 2010). This study reveals instances of consistency with extant Carychiidae such as pitting on the protoconch and wedge-shaped planes of aragonitic structure on the columella in fossils of two carychiid genera from the Middle Miocene. The Carychiidae of the Lake Rein show similar microstructures and textures in the same parts of the shell of different genera. Our SEM images show the most common crystallographical aragonitic microstructural units according to Carter (1990). These structures include homogeneous, simple prismatic and crossed lamellar structure. However, we conclude that although SEM is effective in detecting these structure types, it is not sufficient enough to reveal finer structural relationships such as crystallographical texture in fossil carychiid shells. Also, as mineralogical diagenetic alteration is highly variable within a single layer of fossil shell structure, mineralogy and chemical composition (Barskov et al. 1997; Guzmán et al. 2009), more carychiid shells would need to be investigated for palaeoecological reconstructions based on these structures for the Lake Rein. As the organic matrix of the periostracum provides a key source of ontogenetic and ecological information setting Carychiella eumicrum of Lake Rein apart, future research using X-ray diffraction of shell tissue and TEM investigations of the periostracal layer would greatly augment these studies. Carychiella eumicrum specifically represents a highly context-dependent dynamic for which ontological, biochemical, genetic and ecological interactions at many different levels contributed to producing either: (1) an ecophenotype capable of exploiting a potentially novel and extreme habitat; or (2) it represents an autapomorphic condition (i.e. character state derived via natural selection) in ancient carychiid phylogeny.

As protoconch pitting has been consistently observed in microstructural investigations of recent Carychiidae, including the troglobitic genus Zospe-
(Jochum 2011; Jochum et al. 2013), it can be assumed that this frequent elloboid microstructural characteristic (Martins 2007) appears more frequently in the fossil record than reported thus far. More importantly, this consistency within the Carychiidae suggests that pitting is a microstructural character demonstrating low phenotypic plasticity and thus reflects evidence of strong genetic control in both extant and fossil species. In addition, as previous investigations (Strauch 1977; Priszyazhnyuk & Stworzewicz 1995; Stworzewicz 1999) predate contemporary technological refinements, the degrees of technological advancement (SEM in this case) as well as the preservation potential of the shell have much, if not all, to do with this discrepancy. Up to now, five records show pitting on the protoconch and early teleoconch of the shells of fossil Carychiidae dating from the Miocene. These include Carychium rhenanum (Strauch 1977) (from Belchatów) and Harzhauser et al.’s (2014b) observations in specimens of Carychiopsis schwageri (Reuss 1868), Carychiopsis priszyazhnyuki (Stworzewicz 1999), Carychiella eumicrum (Bourguignat 1857) (from Tuchörce) and Carychium gibbum (Sandberger 1875) (from Lake Rein). Only one carychid, Carychiella eumicrum (Bourguignat 1857) (from Lake Rein) is known to possess total shell punctation. Two of these records document two genera from the Lake Rein.

Lastly, in a second line of consideration, as recent Carychiidae demonstrate a wide spectrum of morphological and intraspecific variability (Winslow 1922; Zimmermann 1925; Bulman 1990; Nekola & Barthel 2002; Weigand et al. 2012a,b), reflecting conditions such as phenotypic plasticity, that is the phenotypic response of an organism to fluctuating environmental conditions (Trussell 1996; Holland & Butlin 2010; Weigand et al. 2013), it can be assumed that fossil Carychiidae, comprising these Neogene assemblages, responded phenotypically in the same manner to paleoenvironmental circumstances. Hence, the multitude of carychid fossil subgenera and the diversity of fossil species assigned to them most likely well reflect the same skewed dynamic. Subsequently, a falsely sufficient and misleading understanding of species comprising these Neogene assemblages is likely. Work discussing the broad spectrum of morphological and intraspecific variability in extant Carychiidae is currently in progress.

Acknowledgements. – We thank Dan Topa for his expertise with the SEM at the NHMW. We also thank Angel Diz, Robert Hershler, Carole Hickman and Silvia de Paula for their expedient help in literature acquisition. Special gratitude goes to Timo Noack for technical help with the image processing. This research received support from the SYNTHESYS Project http://www.syntheses.info/, which is financed by the European Community Research Infrastructure Action under the FP7 ‘Capacities’ Program. It contributes to the FWF-project P 25365-B25 ‘Freshwater systems in the Neogene and Quaternary of Europe: Changes in gastropod biodiversity, provinciality, and faunal gradients’. We are grateful to David Reid and Luciana Genio for their constructive input, the anonymous reviewers and the editor, Peter Doyle, who provided valuable suggestions for improving the quality of the article.

References
Bulman, K. 1990: Shell variability in Carychium tridentatum (Risso, 1826) and its importance for infraspecific taxonomy (Gastropoda, Pulmonata: Ellobiidae). Malakologische
they have a phylogenetic significance? Deep-Sea Research I 64, 86–103.
demic Press, New York, Mollusca.
Guzman, N., Dauphin, Y., Cuij, J.P., Denis, A. & Ortlieb, L. 2009: Diagenetic changes in Concholepas concholepas shells (Gastropoda, Muricidae) in the hyper-arid conditions of Northern Chile – implications for palaeo-environmental recon-
structions. Biogeosciences 6, 197–207.
Harzhauser, M. & Binder, H. 2004: Synopsis of the Late Miocene mollusc fauna of the classical sections Richardhof and Eich-
Harzhauser, M. & Kowalke, T. 2002: Sarmatian (Late Middle Miocene) Gastropod Assemblages of the Central Paratethys. 
Facies 46, 57–82.
Harzhauser, M., Neubauer, T.A., Mandic, O., Zuschin, M. & Corić, S. 2012: A Middle Miocene endemic freshwater mollus
Harzhauser, M., Neubauer, T.A., Gross, M. & Binder, H. 2014a: The early Middle Miocene mollusc fauna of Lake Rein (East-
eren Alps, Austria), Palaeontographica. Abteilung A 302, 1–71.
Harzhauser, M., Neubauer, T.A., Georgopoulou, E. & Harl, J. 2014b: The Early Miocene (Burdigalian) mollusc fauna of the 
Aarhus, Aarhus, Denmark.
64, 133–136.
Hess, M., Beck, F., Ginsler, H., Kano, Y., Kiel, S. & Haszprunar, G. 2008: Microanatomy, shell structure and molecular phylog-
ology of Leptogyra, Xyleptogyra and Leptogyropsis (Gastropoda: Neomphalida: Melanodrymiidae) from sunken wood. Journal of 
Molluscan Studies 74, 383–401.
Hickman, C.S. 2013: Interacting constraints and the problem of similarity in gastropod structure and function. American Mal-
acological Society 31, 155–168.
Hollandier, J. & Butlin, R.K. 2010: The adaptive value of pheno-
typic plasticity in two ecotypes of a marine gastropod. BMC Evolutionary Biology 10, 333.
ecological transitions. BMC Evolutionary Biology 7, 160.
Jirick, R. & Sene, J. 1974: Die Entwicklung des Sarmats in den Becken der Westkarpaten der CSSR. In Papp, A., Marinescu, 
85. Verlag der Slowakischen Akademie der Wissenschaften, Bratislava.
Jochum, A.J. 2011: Evolution and diversity of the troglobitic Carychiidae – A morphological and phylogenetic investigation of 
the terrestrial ephelobidae genera, Carychium and Zospeum. The Malacologist 57, 16–18.
taxonomy (Ellobioidea: Carychiidae) – a new perspective highlights some known and novel morphological characters in 
the shell and radula. Açoreana. World Congress of Malacology, Ponta Delgada, Azores 8, 138–139.
Kano, Y., Chikyu, E. & Warén, A. 2008: Morphological, ecologi-
cal and molecular characterization of the enigmatic planispiral


netic and evolutionary hypotheses. *BMC Evolutionary Biology* 13, 18.


