

PATENCY OF APERTURAL BARRIERS IN CLAUSILIIDS WITH DIFFERENT REPRODUCTIVE STRATEGIES

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ABSTRACT: We adopted X-ray microtomography images and the specially designed algorithm that mimics the movement of spherical object in the shell channel to compare apertural barriers of two closely related clausiliid species with well documented reproductive strategies. For oviparous *Laciniaria plicata*, the patency of the shell channel was 0.60 mm (SD 0.09) at the ultimate whorl; 18.0% in relation to shell width. For viviparous *Alinda biplicata*, the patency of the shell channel was 1.24 mm (SD 0.06) at the ultimate whorl; 31.8% in relation to shell width. In the studied species, the patency of the shell channel differs significantly at the ultimate whorl, while at the penultimate whorl it is in both cases close to 43%. The technique applied in this study can be useful for analysing apertural patency in any gastropod species that develops a complex protective shell armature.

KEY WORDS: shell armature, Gastropoda, land snails, viviparity, X-ray microcomputed tomography

INTRODUCTION

Door-snails (Clausiliidae) have developed a unique system of apertural barriers in the ultimate shell whorl (NORDSIECK 2007). It includes several folds and a flexible plate (clausilium) which together almost entirely block the entrance to the shell whenever snail retracts its body. When a snail is active the clausilium is pushed towards the columella, opening the shell channel. Such armature possibly evolved as an anti-predatory adaptation. The development of apertural barriers makes the aperture area decrease to a greater or lesser extent, and as a consequence it may be disadvantageous for the delivery of neonates (POKRYSZKO 1997).

The apertural barriers constitute a three-dimensional system which occludes the shell channel but is not visible from outside. The complexity of this system precludes biometrical studies in two-dimensional space. Recently, X-ray tomography has been

adopted to analyse and quantify the internal shell armature in door-snails in relation to their reproductive modes (SULIKOWSKA-DROZD et al. 2014, WALCZAK et al. 2015).

The aim of this study is to compare apertural barriers of *Laciniaria plicata* (Draparnaud, 1801) and *Alinda biplicata* (Montagu, 1803), closely related clausiliids (subfamily Baleinae) of similar shell sizes, wide distribution in Europe and rather similar habitat preferences (NORDSIECK 2008). They differ in reproductive strategies, as according to MALTZ & SULIKOWSKA-DROZD (2008, 2012), the former species lays partly calcified eggs, while the latter delivers neonates with rigid, calcareous shells. Considering the difference between reproductive modes of *L. plicata* and *A. biplicata*, we focused on the diameter of the shell channel between apertural barriers available for the passage of eggs or embryos in both species.

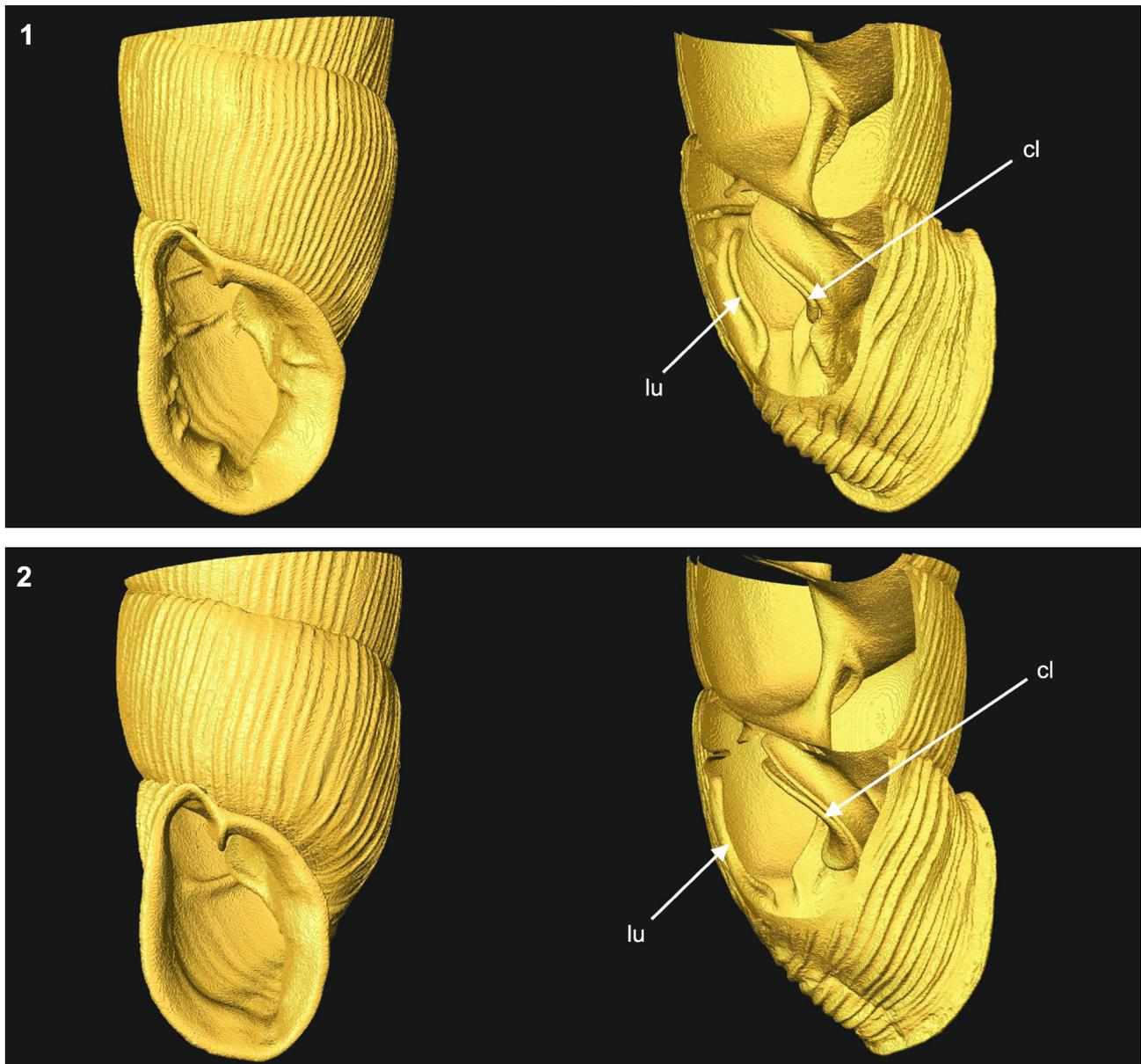


MATERIAL AND METHODS

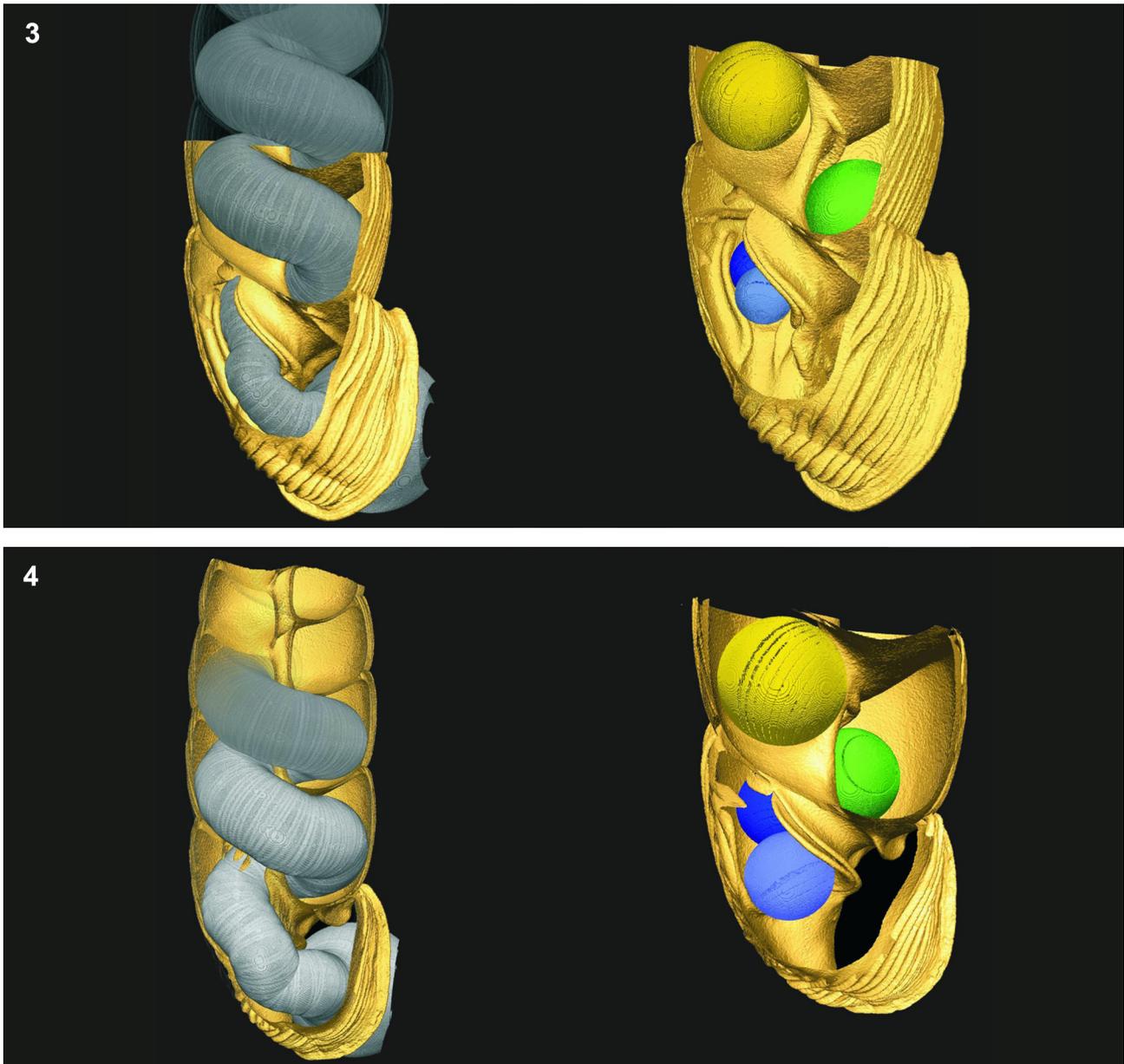
Our material comprised five individuals of *L. plicata* and five of *A. biplicata*. Snails were derived from laboratory culture, which was established with *L. plicata* collected in Wrocław, Lower Silesia, and *A. biplicata* from Łódź, central Poland. The snails were killed in boiling water with their heads outside the shell. This ensured that body soft parts were pressing the clausilium and leaving the shell channel open in the same fashion as during the animal's activity.

Acquiring the three-dimensional data on shell morphology and its analysis followed the methodology described by [SULIKOWSKA-DROZD et al. \(2014\)](#) and [WALCZAK et al. \(2015\)](#). Scanning was performed

by an XMT scanner (GE Sensing and Inspection Technologies, Phoenix/X-ray, Wunstorf, Germany) at the X-ray Microtomography Laboratory at the Institute of Computer Science, University of Silesia (Katowice, Poland). For each shell we obtained a series of about 1,000 axial cross sections of the penultimate and ultimate whorls. After scanning, the reconstructed data set allowed visualizing the internal shell structure and the position of folds and clausilium (Figs 1–2). For measurements of shell interior patency, we adopted the algorithm that mimics the movement of a spherical object (egg or embryonic shell) in the lumen of the parental shell. The pro-



Figs 1–2. X-ray microcomputed tomography images of clausiliid shells: ultimate whorl with aperture and interior of the ultimate whorl showing the passage between apertural barriers (cl – clausilium, lu – lunella): 1 – *Laciniaria plicata*, 2 – *Alinda biplicata*



Figs 3–4. X-ray microcomputed tomography images of clausiliid shells: 3 – *Laciniaria plicata*, 4 – *Alinda biplicata*. The sequence of spheres visible inside the shell was entered in the 3D virtual model of snail as a proxy of the space available for the passage of embryo, i.e. the patency of the shell channel at a given place

cedure worked on a three-dimensional image with voxel size of $4.94 \mu\text{m}$. It allowed us to measure the largest possible sphere that can be entered in the shell channel at a given place (Figs 3–4). The diameter of such a sphere represents shell whorl patency (WALCZAK et al. 2015). To allow the comparison between species we calculated shell whorl patency in

relation to shell width for each individual. Based on X-ray microtomography images we measured also the shell height and width of the studied individuals. The interspecies differences in mean relative shell channel patency were tested at penultimate and ultimate whorls with the unequal variance t-test (RUXTON 2006).

RESULTS

Shell heights of *L. plicata* ranged from 14.25 mm to 15.80 mm (mean 14.73; SD 0.66) and shell width from 3.28 mm to 3.46 mm (mean 3.35; SD 0.09). The diameters of the fitted sphere that can pass be-

tween folds of clausilial apparatus equal 1.43 mm (SD 0.03) at the penultimate whorl and 0.60 mm (SD 0.09) at the ultimate whorl (Figs 5–6). In relation to shell width, these are 42.6% and 18.0%, respectively.

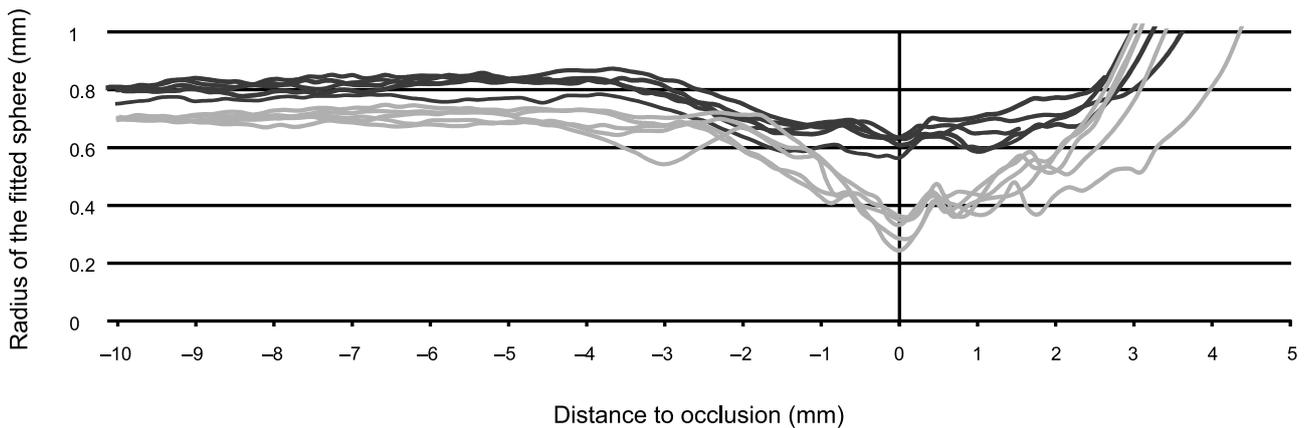


Fig. 5. Shell channel patency estimated by the algorithm: X-axis represents a distance from the occlusion – the narrowest part of the shell channel situated between lunella and bent clausilium in the ultimate whorl, measured along the centre of shell channel; Y-axis refers to the size of the largest possible spherical object that can be entered in the shell channel at a given place as a proxy of its patency. For *Alinda biplicata* – dark grey lines; *Laciniaria plicata* – light grey lines

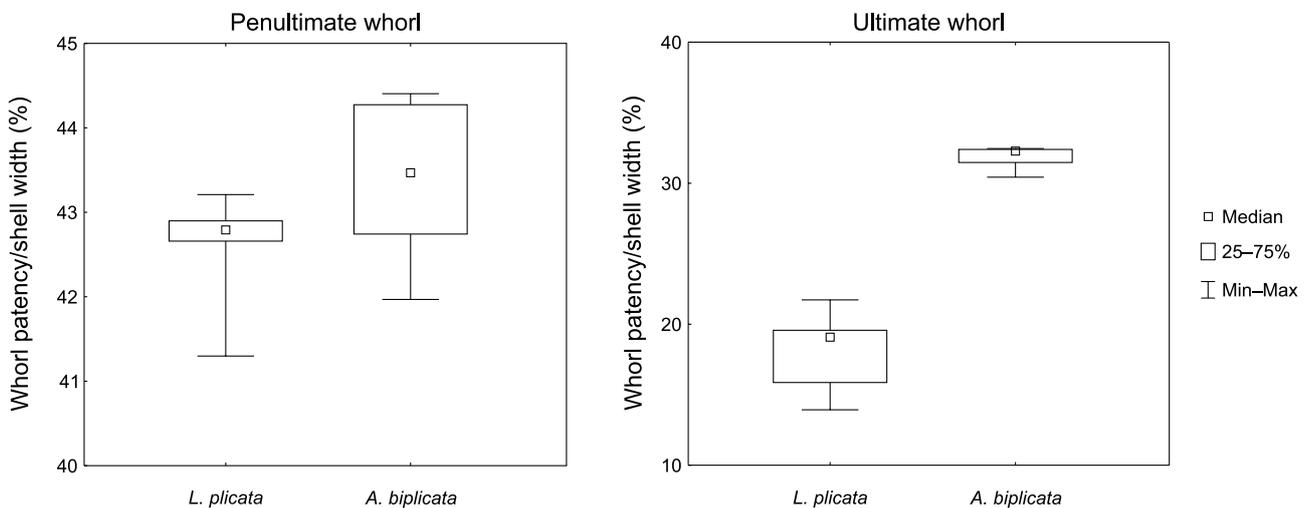


Fig. 6. Shell channel patency estimated by the algorithm in relation to shell width at penultimate and ultimate whorls

Shell heights of *A. biplicata* ranged from 15.03 mm to 16.08 mm (mean 15.57; SD 0.46) and shell width from 3.76 mm to 4.01 mm (mean 3.90; SD 0.11). The patency of the penultimate whorl reaches 1.69 mm (SD 0.07); in relation to shell width thus 43.4%. The patency of the ultimate whorl equals 1.24 mm (SD 0.06), which is on average 31.8% in relation to shell width (Figs 5–6).

There is close similarity in the relative width of the shell channel of the two species at the penultimate whorl (unequal variances *t*-test: 0.201; $p > 0.05$; two-tailed test). The difference became significant at the ultimate whorl: the apertural barriers in ultimate whorl are more constraining in *L. plicata* than in *A. biplicata* (unequal variances *t*-test: 0.000; $p < 0.05$; one-tailed test).

DISCUSSION

We have shown that, in spite of some intraspecific variation in shell internal armature, the patency of the shell channel at the ultimate whorl is significantly greater in viviparous *A. biplicata*, than in egg-laying *L. plicata*. The wider passage through apertural barriers in former species seems to be an adaptation that enables the release of intrauterine embryos with inflexible, calcareous shells. These two species show substantial variation in shell morphology across their

geographical ranges (NORDSIECK 2008), which might affect shell channel patency. Such variation remains to be assessed; it was beyond the scope of our study.

Until now, the data on shell channel patency has been published for only a few species of clausiliids (SULIKOWSKA-DROZD et al. 2014). Besides *L. plicata*, very tight occlusions in the ultimate whorl also occur in *Balea stabilis* and *Vestia ranojevici*, egg-laying species, where only 15–18% of shell width is available for the



passage of eggs. Low patency of the shell, as a mechanical constrain, precludes the successful delivery of shelled embryo.

Acquiring a viviparous reproductive strategy, as well as predation by small arthropods entering the shell through the aperture, are recognized selective forces that might shape shell morphology in clausiliids (MOORSEL *et al.* 2000, SULIKOWSKA-DROZD *et al.* 2014). The technique applied in this study can be

useful for analysing apertural patency in any gastropod species that develops a complex protective shell armature.

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