An ontogenetic description of *Orbulina suturalis* (Foraminifera) from the Danube Basin (Slovakia)

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Abstract: *Orbulina suturalis* (Brönnimann, 1951) is a planktic foraminifera species that occurred in the Central Paratethys during the Middle Miocene, throughout the Badenian (Langhian, Serravallian) stage. Despite the stratigraphic and paleoecologic significance, the ontogeny of the species remained seldom explored. In this study the inner shell morphology was observed by using a tomographic microscope to identify the ontogenetic stages. The entire shell development of *Orbulina suturalis*, from the monothalamous prolocular stage to the polythalamous terminal stage, was interpreted. Each stage is described by its general morphological features, such as the dimensions of the shell, the shape and number of the chambers, the position of the aperture, the peripheral outline, the texture of the wall and the coiling direction. The constructed ontogenetic model of *Orbulina suturalis* was correlated with the „five stage concept" of *Trilobatus sacculifer* introduced by Brummer et al. (1987).

Key words: *Orbulina suturalis*; tomographic microscope; Badenian; Western Carpathians

1. INTRODUCTION

Orbulina suturalis (Brönnimann, 1951) is an abundant polythalamous planktic foraminifera in Badenian sediments of the Central Paratethys. The first appearance of *Orbulina suturalis* in the Western Carpathians was accepted as a marker of biostratigraphic zonation to define the base of the local CPN 7 or the global M6 Planktic Foraminifera Zone (Cicha et al., 1975; Wade et al., 2011). In spite of their biostratigraphic application, the ontogeny and ecological demands of the species have been a subject of much speculation since their first description by d´Orbigny in 1839.

Ontogenetic investigations of planktic foraminifera are often limited by a lack of preservation of non-adult individuals in fossil records (Gerber et al., 2008; Caromel et al., 2016). Thin-walled juvenile individuals are easily destroyed by taphonomic factors of the environment. In some cases, when younger individuals of genus *Orbulina* are preserved, they may be incorrectly classified (Hemleben et al., 1989). The shell morphology during the earlier development stages could morphologically resemble the adult morphology of different species or genus (Brummer et al., 1986, 1987; Hemleben et al., 1989).

The shell construction of *Orbulina suturalis* is a continuous biological process, characterised by the sequential growth of calcite chambers during the entire life of the specimen (Huang, 1981). In fact, each morphological feature remains an integral part of the growing foraminifera shell, providing a unique possibility to reconstruct the complete ontogeny from the first calcified chamber (proloculus) to the last (terminal) chamber (Huang, 1981). According to the five-stage model of growth in the globigerinids proposed by Brummer et al. (1987), the development of fossils and recent spinose planktic foraminifera is ongoing thoroughout the prolocular, juvenile, neanic, adult and terminal stages.

The aim of the presented investigation was to analyse the general ontogenetic stages of *Orbulina suturalis* by tomographic microscope and to correlate them with the „five stage concept" (*sensu* Brummer et al., 1987) of *Trilobatus sacculifer*. This observation could contribute to the correct taxonomic classification of species from genus *Orbulina*, as well as in identification of evolutionary processes within the phylogenetic lineages of Badenian planktic foraminifera.

2. MATERIALS AND METHODS

Our studied material was obtained from Middle Miocene deposits of the Danube Basin (Slovakia) which presented a part of the Central Paratethys (Fig. 1). The settling of this intermountain basin was affected by several eustatic changes during the Badenian and Sarmatian (Hudáčková & Kováč, 1993; Kováč et al., 2007). A bulk sample was taken from the Modrany-1 borehole (Kováč et al., 2017). To separate foraminiferal tests, the traditional wet sieving method was used (Kováčová & Hudáčková, 2009). A foraminiferal assemblage containing the studied specimen was rich in planktic foraminiferal tests. Age was given as NN5 Zone accordingly to calcareous nannoplankton association from the same bulk sample (Kováč et al., 2017). For the purpose of this paper, 80 specimens of *Orbulina suturalis* were separated and transferred to a Petri dish with water (∅ 15 cm, 2 dcl) due to their tests weight classification. Foraminifera shells filled with pyrite settled on the bottom (72 specimens), while the remaining 8 shells were isolated for further observation. Only one specimen was chosen with an unfilled shell and well-preserved wall for tomographic analyse.

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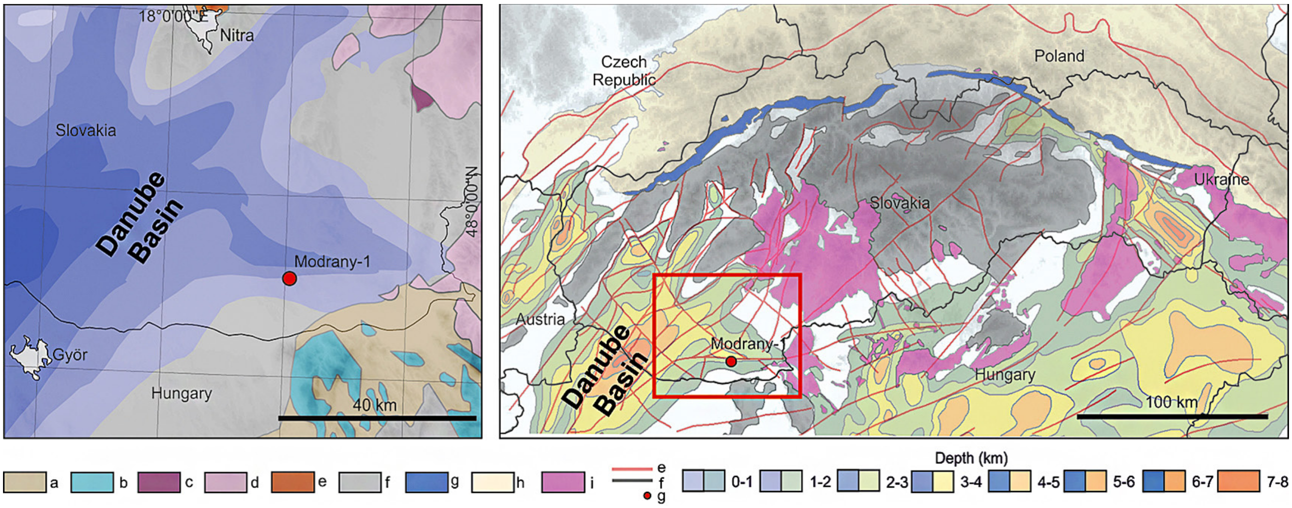

Fig. 1. **(A) Map of the Southeastern Danube Basin; (B) Position of the study area in the Europe and in the Carpathian–Pannonian system.**
Explanatory notes: **(1) Paleogene sediments of the Buda Basin; (2) Bukkikum and the Pelsonia; (3) Silicicum; (4) Miocene volcanic fields; (5) Tatricum; (a) Inner Alpine, Carpathian and Dinaric mountains; (b) Klippen Belt; (c) Foredeep and Flysch belt; (d) Miocene volcanic fields; (e) Faults; (f) State boundaries; (g) Wells. Modified after Horváth et al. (2015) and Hók et al. (2014).**

The chosen specimen was small, about 0.5 mm, preserved as a three-dimensional fossil. Our specimen was studied using X-ray micro-tomography SkyScan 1172. The effective pixel size was ca. 0.56 µm. Tube voltage was set to 40 kV and the current source was 250 µA. No filter was used. Random Movement was set to 5. Data were acquired with an angle slope of 0.120 degree. 180° rotation was used. The acquired data were processed using flat field correction and reconstructed by the supplied software NRecon (Bruker). Photographs and video visualizations were created by Avizo 9.1 software. Presented figures were designed by the graphic program CorelDRAW Graphics Suite X5, and for 3D visualization, Microsoft Paint 3D was used.

3. RESULTS

On the studied shell of *Orbulina suturalis*, we managed to identify five ontogenetic stages: prolocular, juvenile, neanic, adult and terminal („five-stage model", *sensu* Brummer et al., 1987).

The shell dimension of the studied *Orbulina suturalis* is 400 µm (average dimension was 390 µm) and it is made up of 12 chambers arranged in ± 2.5 trochospirally (dextrally) coiling whorls (Fig. 2A–E). The coiling of the first whorl is lower in comparison to the second whorl. The size of the chambers increases continuously from the deuteroconch to the terminal chamber. The number of chambers per whorl gradually decreases. The wall porosity tends to be higher during the adult and terminal stages than observed in the earlier phases of development.

The first stage of ontogeny (~ 17 µm), the prolocular stage, is marked by the establishment of the oldest chamber, which is the proloculus or protoconch (Figs. 2A–D and 3A,B). Tomographic analyses have enabled to determine this chamber in the centre of the first trochospiral whorl. Proloculus has a globular shape with the exception of a short and flattened area where the second chamber (deuteroconch) is added (Fig. 2B). The wall texture during this stage is sparsely perforated and the character and position of the primary aperture is unidentifiable.

The second stage of the imaged specimen, the juvenile stage, is composed of six juvenile chambers (Figs. 2A–D and 3A,B). The juvenile shell dimension is ~ 95 µm. Proloculus, together with the juvenile chambers, are arranged in ± 1.25 trochospirally, dextrally, coiling whorls (Fig. 2B). The coiling of the first whorl is more acute (~ 25°) than the subsequent whorls. The juvenile stage is initiated by the growth of the second chamber (> 17 µm) which is the deuteroconch (Figs. 2B and 3A). The proloculus, together with the deuteroconch, forms the first polythalamous morphotype during the life cycle of *Orbulina suturalis*. The shape of the juvenile chambers is globular, apart from flattened areas, where the consecutive chambers are created (Fig. 2B). The size of the chambers gradually increases from the deuteroconch. The suture is depressed and the chamber walls are rounded (Fig. 2B). The primary aperture is open, ovoid and umbilical (Fig. 3A). The peripheral outline of the aperture could be secondarily modified with an imperforated lip. The juvenile wall texture is sparsely perforated with rounded and small pores. Spine bases are detectable between the pores. The juvenile morphology of *Orbulina suturalis* is reminiscent of the morphology of genus *Turborotalita* (Figs. 2A and 3A). We managed to identify the juvenile stage according to the size of the shell, suture, chamber's perforation and number and aperture position.

The third stage of the studied specimen, the neanic stage, is made up of three globular neanic chambers (Figs. 2C,D and 3A,B). The size of the neanic shell reaches ~ 150 µm. Neanic chambers are situated on the second trochospiral whorl (Fig. 3A,B). Their shape is more inflated than the previously built chambers. The neanic shell is characterized by a lobate peripheral outline, a depressed suture and thicker shell walls. The wall texture is spinose and the entire shell becomes densely perforated with larger pores. The primary aperture remains open and umbilical (Fig. 3A). In general, the neanic stage of *Orbulina suturalis* has a globigerinoides-like morphology (Fig. 3A,B). This stage was documented based on coiling variations, the chambers' shape and their dimensions.

The fourth stage of the sample, the adult stage, is composed of one globular and inflated adult chamber (Figs. 2D and 3A,B). This chamber indicates the end of the trochospiral coiling. Due to its large size (120 μm), it occupies the first half of the last trochospiral whorl. The adult chamber has rounded wall instead of a flattened range, where the neanic chambers are localised. The peripheral outline is lobate and the suture is depressed (Fig. 3A,B). The wall texture is densely perforated with large pores. Spine bases appear among the pores. The primary aperture is open and umbilical with a high arch. The total length of the adult individual is ~ 250 μm (Fig. 3A). The adult shell is made up of 11 chambers arranged in 2.5 trochospirally (dextrally) coiling whorls (Figs. 2D and 3A,B). *Orbulina suturalis* during this stage is morphologically similar to *Globigerinoides sacculifer* (Fig. 3A). This stage was determined according to the wall texture, coiling direction, shell size and primary aperture.

The last stage of development, the terminal stage, is connected with the construction of the last Trilobatus, the so-called terminal chamber (Fig. 2E). It is unequivocally the largest chamber of the shell (~ 400 μm). The terminal chamber has rounded calcite wall of thickness being more than 5 μm. The terminal chamber envelopes the older chambers (prolocular, juvenile, neanic and adult) situated on the trochospiral whorls (Suppl. 1). The wall texture during this stage is densely perforated with large pores (Fig. 2E). Numerous spine bases are visible among the pores. The terminal stage is marked by the formation of 30 sutural apertures along the peripheral outline of the neanic and adult and real chambers. Morphological features constructed throughout the stages older than the terminal are weakly visible from the spiral side of the terminal shell. This stage was recognised according to the globular terminal chamber (Fig. 2E) and its sutural apertures.

Morphological descriptions of the individual stages were correlated with the ontogenetic concept for spinose species („five stage concept") derived from *Trilobatus sacculifer* by Brummer et al. (1987) (Tab. 1).

4. DISCUSSION

We identified five ontogenetic stages in our studied sample (prolocular, juvenile, neanic, adult and terminal). The shell construction from the monothalamous protoconch to the polythalamous terminal stage fits the „five stage concept" documented by Brummer et al. (1987). Development was interpreted by tracing

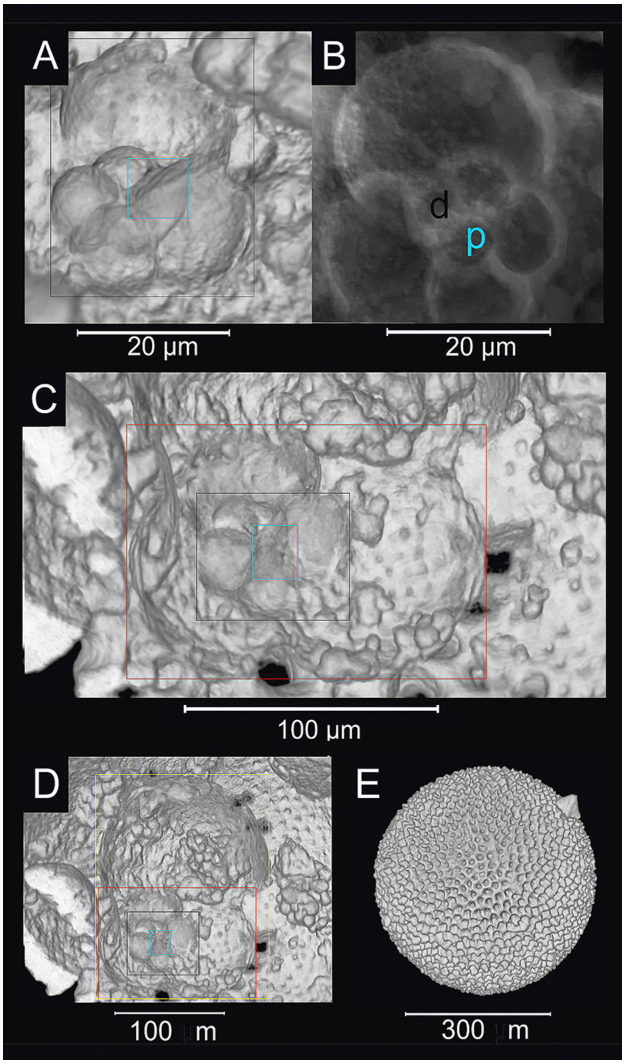

Fig. 2. Ontogenetic stages of *Orbulina suturalis* (A-E). (A) Prolocular and juvenile stages; (B) Position of the proloculus and deuteroconch inside the juvenile shell; (C) Neanic stage; (D) Adult stage; (E) Terminal stage; (p) Proloculus; (d) Deuteroconch; (blue square) Prolocular stage; (black square) Juvenile stage; (red square) Neanic stage; (yellow square) Adult stage.

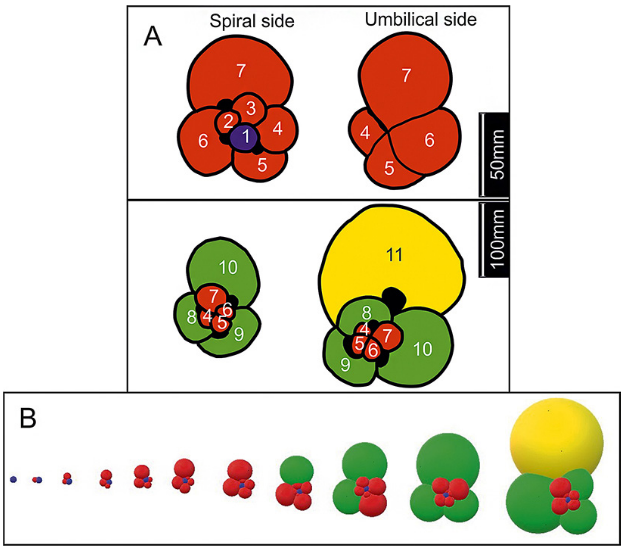

Fig. 3. (A) Position of the apertures and chambers arrangement during the juvenile, neanic and adult stages; (B) Development of the shell from the proloculus to the adult chamber of *Orbulina suturalis*. *Explanatory notes:* (1) Proloculus (blue colour); (2) Deuteroconch (red colour); (3–7) Juvenile chambers (red colour); (8–10) Neanic chambers (green colour); (11) Adult chamber (yellow colour).

Tab. 1. Morphological differences between the ontogenetic stages of species *Orbulina suturalis* (Danube Basin, Slovakia) and *Trilobatus sacculifer* (*sensu* Brummer et al., 1987). *Explanatory notes:* **(SS) Size of the shell; (NoCC) Number of created chambers; (NoW) Number of whorls; (PoPA) Position of the primary aperture.**

	Orbulina suturalis				*Trilobatus sacculifer*			
Ontogenetic stage	SS (µm)	NoCC	NoW	PoPA	SS (µm)	NoCC	NoW	PoPA
Prolocular stage	17	1	-	-	19	1	-	Interiomarginal, marginal
Juvenile stage	95	6	±1.25	Umbilical with lip	90	8-13	±1.5	Umbilical
Neanic stage	150	3	1	Umbilical	210	3-6	0.5-1	Umbilical
Adult stage	250	1	0.5	Umbilical with lip	>210	1-4	-	Umbilical + secondary
Terminal stage	400	1	-	Sutural apertures	350	1-3 + kummerform chamber	-	Umbilical + secondary

the individual stages backwards from the last chamber to the first two-chambered morphotype (proloculus+deuteroconch) (Figs. 2B and 3A). Changes in morphology, such as coiling direction, test diameter, wall texture, and primary aperture have enabled to detect each ontogenetic stage (Figs. 2A–E and 3A,B). Based on the drastic morphological variation and development pattern, the studied specimen belongs to the first group of planktic foraminifera described by Brummer et al. (1986).

The prolocular stage of growth is formed by the proloculus in both *Trilobatus sacculifer* and *Orbulina suturalis*. Isolated proloculi in the studied bulk sample (Modrany-1 borehole) have not been found. This fact supports the idea that the proloculus has weakly or non-calcified walls (Brummer et al., 1986, 1987). The most important morphological differences were observed during the second, juvenile stage of ontogeny (Tab. 1). In the studied specimen, this stage was marked by the creation of six juvenile chambers arranged in ±1.25 trochospirally coiling whorls (Figs. 2A,B and 3A,B), while in *Trilobatus sacculifer*, eight to thirteen chambers are created, which are situated in ± 1.5 trochospiral whorls (Brummer et al., 1987) (Tab. 1). The aperture of the juvenile shell in both species is umbilical, however, the umbilicus of our studied sample is secondarily modified by an imperforated umbilical lip. One notable morphological difference between the neanic stages was documented in the diameter of the shell. The longer diameter of the studied specimen during this stage is 150 µm (Fig. 2C), while *Trilobatus sacculifer* reaches more than 200 µm in the neanic stage (Tab. 1). The neanic stage in *Orbulina suturalis* is made up of three (Figs. 2C and 3A,B) while in *Trilobatus sacculifer* of 3–6 chambers (Tab. 1). The number of neanic chambers probably depends on the amount of juvenile chambers. According to Bé et al. (1985), as more juvenile chambers are present, less neanic chambers are necessary to reach the size of the adult stage. During the adult stage of the imaged *Orbulina suturalis*, the penultimate, adult, chamber formation was observed (Figs. 2D and 3A,B). Among species of *Trilobatus sacculifer*, Brummer et al. (1987) documented the growth of 1 to 4 adult chambers (Tab. 1). The wall texture, the shell size, the umbilical aperture, the shape of the chambers and the chambers' arrangement of an adult *Orbulina suturalis* are morphologically reminiscent of the adult and terminal stages of *Trilobatus sacculifer* (Figs. 2D and 3A,B). Major morphological changes are marked by the terminal stage. This stage of the studied specimen commences with the growth of the last, terminal chamber, which envelopes the older chambers (Fig. 2E and Suppl. 1), and by the transition of the umbilical aperture into 30 sutural apertures, located on the spiral side of the shell. Brummer et al. (1987) described 1 to 3 terminal chambers among species *Trilobatus sacculifer*, as well as the growth of the kummerform chamber (Tab. 1), which is not typical for genus *Orbulina*.

Throughout the development of *Orbulina suturalis* and *Trilobatus sacculifer*, major changes of the primary aperture are recognised. After the prolocular stage, growth of an umbilical aperture begins, which persists until the end of the adult stage (Fig. 3B). The most significant size variations of the aperture are documented between the neanic and the adult stages, when the umbilicus starts to shift towards its adult position (Brummer et al., 1986, 1987). According to Brummer et al. (1987), these aperture modifications are presumably generated due to trophic behavioural changes. Juvenile individuals with a small aperture usually feed on microplankton, while adult individuals with a massive umbilicus are largely carnivorous in diet (Hemleben et al., 1989) (Fig. 3A). Among species of genus *Orbulina*, the umbilicus is transformed into several sutural apertures after the adult stage.

5. CONCLUSION

Orbulina suturalis is among the most abundant planktic foraminifera in Badenian sediments of the Western Carpathians. This study confirmed the five-stage model of growth of spinose planktic foraminifera, documented by Brummer et al. (1987). In the studiedshell of *Orbulina suturalis*, the prolocular, juvenile, neanic, adult and terminal stages have been shown by using a tomographic microscope SkyScan 1172.

Throughout the visualised ontogenetic stages, major morphological changes were described. Chambers' number and size, coiling direction, wall texture and even aperture modifications have been visible during calcite shell development.

Transitions, such as in the position of the apertures, could be interpreted as a result of changes in trophic demands, which can differ between the ontogenetic stages.

The results confirm the disadvantages of recent classification of planktic foraminifera, which is based on the morphology of terminal stages. With the application of the current classification, ontogenetic stages of *Orbulina suturalis*, could be incorrectly classified into several species. *Orbulina suturalis* during the juvenile stage is morphologically similar to the genus *Turborotalia*, the neanic stage has a globigerinoides-like morphology and the adult stage is reminiscent of the morphology of species *Trilobatus sacculifer*. This investigation contributes to making a more effective classification of pre-adult Badenian (Langhian, Serravallian) planktic foraminifera.

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Supplement 1: Outer and inner test morphology of the studied *Orbulina suturalis. Explanatory notes:* Presented video visualization shows the position of prolocular, juvenile, neanic and adult stages inside the terminal chamber.