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Genetic and Morphological Variability of the European Genus *Cochlodina* (Mollusca: Gastropoda: Clausiliidae) with Focus on Species *C. laminata* (Montagu, 1803)

Genetická a morfologická variabilita evropského rodu *Cochlodina* (Mollusca: Gastropoda: Clausiliidae) se zaměřením na druh *C. laminata* (Montagu, 1803)

Master thesis

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Prohlašuji, že jsem diplomovou práci vypracovala samostatně pod vedením školitelky RNDr. Lucie Juřičkové, PhD., a že veškerá použitá literatura a další zdroje byly řádně citovány. Svoluji k zapůjčení této práce s tím, že veškeré prameny budou řádně citovány.

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Contents:

Abstrakt:

Tato práce se zaměřuje na genetickou a morfologickou diverzitu vřetenovky hladké (*Cochlodina laminata*). Zatímco většina druhů patřících do rodu *Cochlodina* je typická svými malými areály rozšíření, areál *C. laminata* pokrývá většinu Evropy, vyjma jejích nejteplejších a nejchladnějších částí. Již drive zazněly názory, že tento taxon není pouze jedním druhem, ale že by mohlo jít o komplex několika druhů. Předchozí studie také upozornily na velkou, avšak zatím nepopsanou, diversitu jak na genetické úrovni, tak i v morfologii. Pro studium diversity byly použity dva mitochondriální geny (16S rDNA, COI) a bylo hodnoceno třináct morfologických znaků. Zjistila jsem, že druh *C. laminata* v současném pojetí jediného druhu neodpovídá zjištěné genetické ani morfologické variabilitě. Původní druhy *C. laminata*/C. *dubiosa* vytvářejí společný druhový complex a rovněž klasické pojetí druhu *C. fimbriata* bude třeba podrobněji zhodnotit.Ostatní středoevropské druhy rodu *Cochlodina* jsou validními druhy.

Klíčová slova: suchozemští plži, Clausiliidae, *Cochlodina*, fylogeneze, taxonomie, fylogeografie, 16S rDNA, COI

Abstract:

This thesis focuses on the genetical and morphological diversity of plaited door snail (*Cochlodina laminata*). While small distribution ranges are typical for most species belonging to genus *Cochlodina*, the distribution range of *C. laminata* covers most of the European continent, except for its coolest and warmest parts. It has been previously suggested that this species may in fact be a complex of several species and large genetical as well as morphological diversity - yet undescribed - has been mentioned in previous studies. Sequences of two mitochondrial genes were used (16S rDNA, COI) and thirteen morphological characteritics have been assessed to investigate this diversity. I discovered that the current concept of *C. laminata* as a species is not in accordance with the discovered genetical nor morphological variability. The original species *C. laminata*/*C. dubiosa* form a common species complex and also interpretation of *C. fimbriata* will need to be assessed in more detail in the future. Other Central European species are valid species.

Key words: terrestrial snails, Clausiliidae, *Cochlodina*, phylogeny, taxonomy, phylogeography, 16S rDNA, COI

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1. Introduction

This study is aimed at the diversity of land snails belonging to genus *Cochlodina* (Gastropoda: Clausiliidae), particularly on *C. laminata*.

Most species belonging to genus *Cochlodina* are known to have patchy and disjunct distributions with small distribution ranges. *Cochlodina laminata* appears to be an exception, with a large distribution range covering substantial part of the European continent, except for its coldest and warmest parts (Kerney et al. 1983, Welter-Schultes 2012). Area of this size is unusual for a species of *Cochlodina*, as has been pointed out earlier (Cameron 2008). A study carried out by Tlachač (2008) revealed that *C. laminata* is very variable in both morphological and molecular traits. Interestingly, the morphological variability appears to be higher in the areas of potential Central European glacial refugia, such as the Carpathians, than in the western Europe (L. Juřičková, pers. com). Survival of this species in Carpathian refugia was directly documented by fossil records (Ložek 2006). There appears to be a significant intraspecific radiation lacking any more distinct features for easy determination. The realized relationships within this species are thought to be a product of migratory processes in the evolutionary past of the species (Tlachač 2008). Intermediate forms between *C. laminata* and *C. dubiosa corcontica* that could be a result of interspecific hybridisation were observed on sites where both of these species live conspecifically. Intermediate morphotypes were associated with haplotypes of both *C. laminata* and *C. dubiosa corcontica*, thus indirectly supporting the hybridisation of these two species (Tlachač 2008).

Here, we hypothesise that *C. laminata* is a complex of cryptic species, among which hybridisation is possible. Another hypothesis is that *C. laminata* is a genetically very variable species due to various glacial refugia of particular populations. The most probable explanation is the combination of both of these hypotheses.

Because of the aforementioned characteristics, genus *Cochlodina* can be used as a model species to investigate the existence of northern glacial refugia in Central Europe.

The aims of this study are:

1) to investigate, whether the Central European species of genus *Cochlodina* are valid species

2) to asses the intraspecific genetical and morphological variability of *C. laminata* and find

out whether morphological variability corresponds with variability on genetical level

3) to investigate, whether genetical variability has a geographical pattern

4) to assess which morphological characteristics are variable and which are stable and could be used as easy determination markers

2. Review

2.1 Clausiliidae

Cochlodina is a genus of pulmonate land snail (Stylommatophora), belonging to the family Clausiliidae (door snails). This family is characterized by prolonged, usually sinistrally coiled turret-like shells and a clausilium – an apomorphic structure that is created from the plicae of the inner side of the shell and that is used to close the shell and thus protect the body of the snail from predators and unfavourable environmental conditions (Nordsieck 2007a).

The shells of Clausiliidae are usually coiled sinistrally (Nordsieck 2007a). There are several exceptions to sinistrality, such as genus *Alopia*. Dextrality of the shell coiling appears to be unoriginal state and seems to have occured several times during the evolutionary history of these species (Fehér et al. 2013).

The monophyly of the family has been confirmed by several studies (Nordsieck 2007a, Uit de Weerd & Gittenberger, *in press*). According to Nordsieck (2007a), the family has a European origin and most lineages are assumed to have originated in the Upper Cretaceous, according to the oldest dated fossils. Current centres of distribution of Clausiliidae are in Europe, Eastern Asia and South America (Nordsieck 2007a). The centre of diversity of genus *Cochlodina* is in the morthwestmost Dinaric regions (such as Eastern Friuli and Venezia Giulia, western Slovenia, Istria and northwestern Croatia with Croatian islands) (Nordsieck 2013).

Nordsieck (2007a) divides the family into nine subfamilies, of which *Cochlodina* belongs to Alopiinae.

Clausiliidae are also a family that is often represented in the fossil record (e.g. Ložek 2006). Land snails with hard calcareous shells are subjects to easy fossilisation and therefore can often be found in fossil record. Most of the times, it is rare that a fossilised whole shell is found. Most often, the scientists are only left fragments of shells to work on (L. Juřičková, pers.com.). This means that a piece of a mouth of the shell may be all that is available to determine the species. In this case, if a piece of evidence contains a very stable morphological character, the species can be determined easily, however, if the character is highly variable, then it may lead to misinterpretation of the species and to errors, which may further influence the studies that are being carried out. Therefore, the result of this thesis could be beneficial in determining fossilized shells as well, where dissection or evaluation of anatomy of the snail are impossible.

2.2 Genus *Cochlodina*

Genus *Cochlodina* contains land snails with sinistrally coiled shells, of thin or cylindrically spindle-like shape. Superior, inferior and subcolumellar lamella and clausilium are developed, while lunella is missing (Ložek 1956, Kerney et al. 1983). The shell is smooth, glossy or irregularly grooved, can be sparsely bluntly ribbed, the mouth of the shell is without groove (Ložek 1956).

Land snails of genus *Cochlodina* are forrest snails (Kerney et al. 1983, Norsieck 2007a, Ložek 1956). In their natural habitats, they feed on microscopic fungi, plants and decaying organic material (Nordsieck 2007a).

From the palatal side, the principal plica is the longest, upper and lower palatal plicae are shorter than principal. The shell measures between 22-18mm and is consider to be „larger“ among Clausiliidae (Ložek 1956).

Nordsieck divided genus *Cochlodina* into four subgenera (*Paracochlodina*, *Cochlodinastra*, *Procochlodina* and *Cochlodina*), which he defined by genital characters and not by morphology (Nordsieck 2007a). However, genital characters are impossible to observe in museal collections when the shells are stored and the bodies in them are dried. Genital characters can be used with live individuals, in which the tissues still maintain their original shape. In absolute ethanol-stored samples, the tissues are hard and contracted and this makes them less suitable for dissection. Therefore, my aim was to use the molecular approach to define genetically defined clades of mid-European *Cochlodina* species and try to find morphological characteristics that would correspond with the division into these clades. If these are found, then a series of morphological characteristics can be defined and used for easy determination in the field, without breaking the shell or having to dissect the individual.

While Nordsieck (2013) states the genital characters very clearly, the morphological characters of individual species are less clear and descriptions used for determination often include several options (such as missing or present plicae, which may or may not merge with the palatal callus and similar). He also mentions that species of *Cochlodina* occurring in this region are very similar in morphology and therefore their identification is difficult, but the differences in reproductory system are suitable for identification of individual species (Nordsieck 2013).

The fact that there are no clear characteristics alone sometimes leads us to question the validity of given and presently valid determination characteristics, use and test them. In this study, I compare the usual determining characteristics from various literature sources with my own findings – observations of morphology of the individuals that were obtained for this study.

2.3 *Cochlodina laminata*

Cochlodina laminata (Montagu, 1803), originally described as *Turbo laminatus* Montagu 1803, is a forrest land snail (Kerney et al. 1983, Pilāte and Korotkaja undated) with “ten smooth, glossy, pellucid, reversed spires“ with shell of a “rufous horn-colour“. The body of the living snail usually gives the shell darker colour (Montagu 1803).

The shell is widest in the middle, the aperture is sub-orbicular and slightly compressed on the top, where it joins the body of the shell. The lip of the shell is white and slightly marginated (Montagu 1803). Inside the mouth of the shell, Montagu (1803) describes “three or four prominent ridges“, these probably being the palatal callus, principal fold, upper palatal fold and lower palatal fold as pictured in Nordsieck (2007a) and Kerney et al. (1983) (more characteristics of *C. laminata* from various sources are listed in tables 17a and 17b).

Land snails of this family are long-lived (Tlachač 2008, Heller and Dolev 1994, Stelfox 1969, Piechocki 1982, Wirth et al. 1997, Giokas and Mylonas 2002, Gergely 2010). As they live for a long time, their shell is a subject to corrosion, causing the shells to be “worn and decorticated, becoming of a cinereous colour in streaks and blotches“ (Montagu 1803).

Maltz and Sulikowska-Drozd (2008) considered the species moderately fast-growing, and state that it takes 18-22 weeks from hatching until the formation of the closing apparatus and full development of the lip of the shell under laboratory conditions. Tlachač (2008) observed various rates of growth in a field experiment aimed at *C. dubiosa corcontica* and *C. laminata* individuals, during which the rate of growth varies between 0 and 4 ⅓ whorls per 89 days and suggested that results from laboratory experiments where conditions are kept stable may vary greatly from actual growth rates on the natural sites, where these land snails occur. From his personal observations, he suggested that the snails mature 2-3 years after hatching.

C. laminata is oviparous. The individuals lay eggs in moist places and preffer bark as egg-laying substrate. Reproduction of all studied Clausiliidae was intensified in spring (March-May) and autumn (August-October) and mean incubation time is 10-14 days (Maltz and Sulikowska-Drozd 2008).

There have not been many studies carried out on *C. laminata* in the past. Currently, there is no published study regarding molecular biology of *Cochlodina*, except for an unpublished preliminary study by Hausdorf, which is mentioned by Nordsieck (2013).

Nordsieck (2007b) describes *C. laminata* as being uniform in shell morphology, but describes several forms based on differences in the anatomy of their reproductory system. Ložek (1956) describes *C. laminata* as “changeable, but with stable deviations”.

2.4 Climatic changes and refugia

The phylogeography of any taxon cannot be studied without reference to the impact of the climate changes on its distribution range during the species′s evolutionary history (Rowe et al. 2004).

The climatic oscillations in the past two million years of the Quaternary influenced evolution and distribution ranges of the European taxa. These climatic changes, caused by changes in the Earth′s orbit, resulted in changes that can be seen in the present-day distributions of European biota (Hays 1976 in Taberlet et al. 1998). The repeated global cooling periods and formation of southwards expanding ice sheets over the continent resuted in the species′ ranges being "pushed down" to warmer refugial regions in southern Europe, while following warming periods and ice-melting enabled the species to expand northwards again (Taberlet et al. 1998). It was generally assumed that present-day northern species were related to those inhabiting more southern areas (Hewitt 2004), because of the mechanism mentioned above. The southern European penninsulas of Iberia, Italy and Balkans-Greece and the Caspian/Caucasus region are considered "southern" temperate refugia (Hewitt 2004).

However, views on refugia and survival of species of the last glacial period have changed rapidly over the last few decades. Even termophillic species which were thought to have been restricted to the area of the Mediterranean refugia, have survived in the extra-mediterranean parts of Europe as well (Schmitt and Varga 2012). Species could also expand or move its distribution range in the mountaneous areas, where a change in altitude is accompanied by a change in temperature (Hewitt 2004) and western Palearctic species exhibit highly complex biogeographical patterns (Schmitt and Varga 2012).

Hewitt (2004) mentions that in mountains at about 40° of latitude, montane species could have moved vertically – descending with warming and ascending with cooling of the environment. He also states that Alps have been a major feature that influenced phylogeography of taxa on the European continent, including land snails. Pinceel et al. (2005) suggested the peripheral area of Alps to be a refugium of land slug *Arion fuscus*.

Numerous populations could have survived the cool periods in the mountaneous areas isolated with no gene flow from other population, which resulted in today's genetical and morphological variability of these species. These small refugial areas in montane regions in

Europe occur more to the north from the classic “southern” glacial refugia, and therefore are often referred to as “northern” refugia, “cryptic” refugia or “microrefugia”(Rull 2005). Rull (2005) proposed the term “microrefugium” (pl. "microrefugia”) to be used for these areas and suggest its definition to be “*a small area with local favourable environmental features, in which small populations can survive outside their main distribution area (the macrorefugium), protected from the unfavourable regional environmental conditions”*. Magri (2008) described the northern Pleistocene refugia of *Fagus sylvatica* as “likely to have been a mosaic of sparse stands of small populations in multiple regions”and the post-glacial colonisation of Europe by this species as“diffuse spread from scattered nuclei”. These “northern” or “cryptic” microrefugia have been suggested for several plant (e.g. *Fagus sylvatica* - Magri 2008) and animal (e.g. *Arianta arbustorum* – Haase et al. 2003, *Triturus vulgaris* and *Triturus montandoni* - Babik et al. 2005, Candidula unifasciata – Pfenninger and Posada 2002) species, for review see Schmitt and Varga (2012). In many cases it seems that both southern refugia and microrefugia contributed to the present-day diversity of the species (*Alnus glutinosa* - King & Ferris 1998, *Bombina bombina* and *Bombina variegata* – Hofman et al. 2007).

With the repeated cooling and warming of the climate, extinctions, bottlenecks and founder effects have occurred repeatedly. Some species might have diverged and split into new species, whilst other might still be capable of hybridisation (Hewitt 2004).

As molluscs are a group with a well-preserved fossil record, a lot of information can be obtained about the morphological evolution and can serve as aid in interpreting molecular phylogenetic patterns or estimating divergence times (Lydeard and Lindberg 2003). It is also the fossil record that confirms the survival of *Cochlodina* species in the Carpathian regions during the last glacial period (Ložek 2006), thus the northern refugia are an option.

Of course, these climate changes influenced other continents as well and here too, cryptic refugia were suggested (for example Anderson et al. 2011, Rowe et al. 2004). Each taxon responds to the climatic changes individually and therefore has a unique evolutionary history (Taberlet et al. 1998).

3. Materials and Methods

3.1 Collecting samples and extracting tissue

From 2009 to 2012, samples have been collected by hand. Sampled land snails belonged to seven different species. Geographical positions where the *Cochlodina* species were found are listed individually for each species in “results”.

Several techniques of obtaining the soft tissue sample have been tried out during this study. The first technique was used for live specimen. The snails were left in moist environment (such as a wet piece of tissue paper in a petri dish) until they came out of their shells and then they were placed into a freezer for several minutes and frozen to -80°C. Because of the relatively small size of the snails, the freezing process was very quick. After the necessary period of time, the dishes with frozen snails have been removed from the freezer, left to defrost, and a piece of tissue has been removed for extracting DNA. The rest of the soft tissues, together with the shell, has been stored in 96-100% ethanol. A little hole has been drilled through the shell above the clausilium, in the second whorl of the shell to ensure complete and thorough preservation of the soft tissues of the specimens. While this process makes tissue removal very easy, the remaining tissue in the mouth of the shell covers most of the morphological features necessary for species identification. Removing this tissue afterwards is a time-consuming task. Therefore, I started using a different approach to tissue removal after some time.

The second technique was used mostly for dead ethanol-stored samples and later on for any samples obtained. When a live snail is fixated in ethanol, it retracts into its shell, into the older whorls and past clausilium. Therefore, it was necessary to break the shell into halves and remove a soft tissue from the body. This has not necessarily been the tissue from the foot. This technique requires extreme care when breaking the shell. Damage to the mouth or mouth of the shell can mean difficulties with identifying the species and observing the morphological features afterwards. After the tissue has been removed, the remains of the bodies and all parts of the shells have been stored in 96-100% ethanol as described before. The ethanol was replaced before long-term storage.

All specimens are currently deposited at the Departments of Invertebrate Zoology and Ecology at the Faculty of Natural Sciences of Charles University in Prague.

3.2 DNA extraction and obtaining DNA sequences

Both mitochondrial and nuclear genes are being used for phylogeographic and molecular studies on land gastropods. In this study, I used sequences of two mitochondrial genes - 16S rDNA and COI – which has already proven to be easily sequenceable in the previous studies (e.g. Thomaz et al. 1996, Pfenninger and Posada 2002, Pfenninger et al. 2003, Tlachač 2008). Total genomic DNA was extracted from a piece of foot tissue or other soft tissue of the snail′s body, using DNeasy Blood & Tissue Kit (QIAGEN) and later Genomic DNA Mini Kit (Tissue) (Geneaid). Amplification of chosen genes was performed according to the protocol by Steinke et al. (2004) with minor changes.

The reaction solution for amplification of the 16S gene had total volume of 25 µl and contained pure water, 2 µl of template DNA, 2.5 mM MgCl2, 1x PCR Buffer, 0.1 mM dNTP, primers (0.2 µM of each) and 0.7 U of TAQ polymerase. The names and sequences of used primers are summed up in Table 2.

The reaction solution for the amplification of COI gene had a total volume of 35 µl and contained pure water, 1 µl of template DNA, 3 mM MgCl2, 1x PCR Buffer, 0.17 mM dNTP, primers (0.13 µM of each) and 1 U of TAQ polymerase. The names and sequences of used primers are summed up in Table 1.

Table 1: Names and sequences of primers used for PCR.

Gene	Primer name	Primer sequence	Source
16S	16S-F	5′- GGTCAACAATCATAAAGATATTGG-3′	(Palumbi et al. 1991)
	16S-R	5′ -TAAACTTCAGGGTGACCAAAAAATCA-3′	
COI	COI-F	5′ -CGGCCGCCTGTTTATCAAAAACAT-3′	(Folmer et al. 1994)
	COI-R	5′ -GGAGCTCCGGTTTGAACTCAGATC-3′	

The quality of PCR products has been checked by electrophoresis on 1.5% agarose gel.

Until 2011, the unpurified PCR products were sent to Macrogen, Soul, South Korea and sequenced there. After 2012, the samples have been purified by ethanol precipitation in the laboratory of the Department of Ecology of Charles University in Prague and the sequencing analysis has been performed by the Laboratory of DNA sequencing at Charles University.

3.3 Molecular analysis

Altogether, 239 sequences of 16S and 41 sequences of COI were obtained from sixteen European countries (Austria, Croatia, Czech republic, Denmark, France, Germany, Hungary, Italy, Motenegro, Romania, Serbia, Slovakia, Slovenia, Sweden, Switzerland and Ukraine), including 35 sequences from Tlachač (2008), to which the corresponding shells were not retrieved and therefore not used in the analysis of morphological features. Outgroup sequences of *Albinaria* sp., *Charpentieria itala* and *Isabellaria saxicola* have been obtained from GenBank.

DNA sequences were checked by eye using BioEdit Sequence Alignment Editor 7.4.0.1. (1999) and aligned in MEGA 5.05 (Tamura et al. 2011).

Areas of alignment with a large number of gaps that were unsuitable for further analysis were identified by Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) and excluded from the alignment. A concatenate alignment was created from the sequence of both genes and later used for the construction of the tree by Bayesian analysis.

jModelTest 1.0 was used to carry out statistical selection of best-fit models of nucleotide substitution. Phylogenetical tree was constructed, using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2001). Markov Chain Monte Carlo (MCMC) method has been applied for 3 million generations with two paralell runs and tree sampling of every 100th tree. First third of the trees, including the trees sampled in the burn-in phase, was excluded from the analysis.

3.4 Morphological analysis

Morphological features have been observed under stereo microscope Olympus SZX9. I did not perform dissections and observe anatomical features, even though according to Nordsieck (2007a) and Brabenec (1967) there seem to be specific differences visible in anatomy. My aim was to find out whether these species can be easily recognizable on the morphological level in cases when the dissection is not possible, such as during fieldwork and when working with materials stored in museum collections, or when obtaining material suitable for molecular analysis is not possible and shells are the only material that is available.

Species were determined using the original descriptions of individual species and identification guides. Later, thirteen morphological characteristics have been assessed for each specimen used in this study. These were:

-the colour of the shell
-the shape of the mouth of the shell (figure 1)
-presence/absence of spiral lines on the surface of the shell (figure 2)
-presence of constricted/callous area on superior lamella
-presence of constricted/callous area on inferior lamella
-visibility of principal plica from apertural point of view
-visibility of lower palatal plica from apertural point of view
-the position of beginnings of the principal plica and upper palatal plica
-the mutual position of principal plica and upper palatal plica (parallel/nonparallel)
-visibility of subcolumellar lamella from apertural point of view
-presence and character of the palatal callus
-position of the palatal callus
-extensions of the palatal callus

All terminology used in this thesis is according to Nordsieck (2007a).

Typical as well as unusual forms have been photographed. All photographs have been taken using stereo microscope Olympus SZX12, using programmes QuickPHOTO MICRO 2.3 and Deep Focus 3.1. and edited in Adobe Photoshop 7.0 CE.

Shells were left to dry and in dry state were positioned under the microscope in a Petri dish filled with salt or other loose material. This was performed for photographs of apertural views of the shells. More difficult procedure was necessary for photographs of abapertural views of the shells, as in many cases plicae of the palatal side were not visible when the shell was dry. These shells were positioned on a piece of reusable pressure sensitive adhesive Blu Tack (Bostik) on the bottom of a Petri dish and covered with water. This procedure requires great caution, especially with very young and fragile shells that might be broken by pressure easily. The resulting photographs of shells in water show the plicae of the palatal side well, however, they are not as sharp as the ones of apertural views of dry shells.

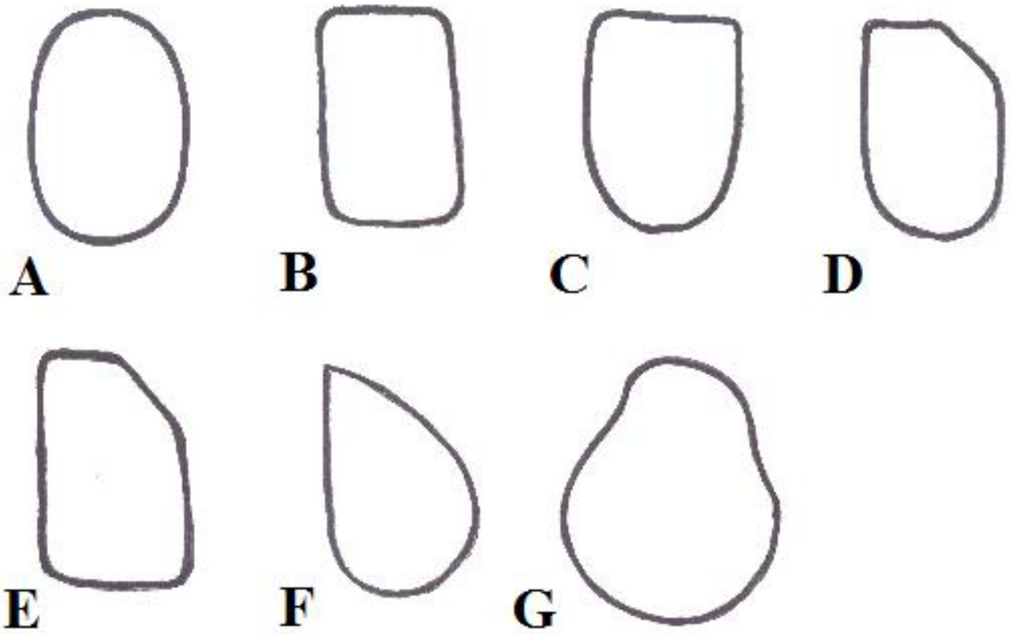

Figure 1: Various shapes of the mouth of the shell from the apertural view that were observed in different species of genus *Cochlodina* (A: oval, B: angular, C: angularly oval, D: bevelled oval, E: bevelled angular, F: drop shaped, G: pear shaped)

Damaged, anomalous or young individuals were excluded from the analysis of morphological features, as the morphology of the former two was influenced by internal or mostly external factors and the young shells lacked the last whorl, in which most of the determination characteristics occur (figure 3).

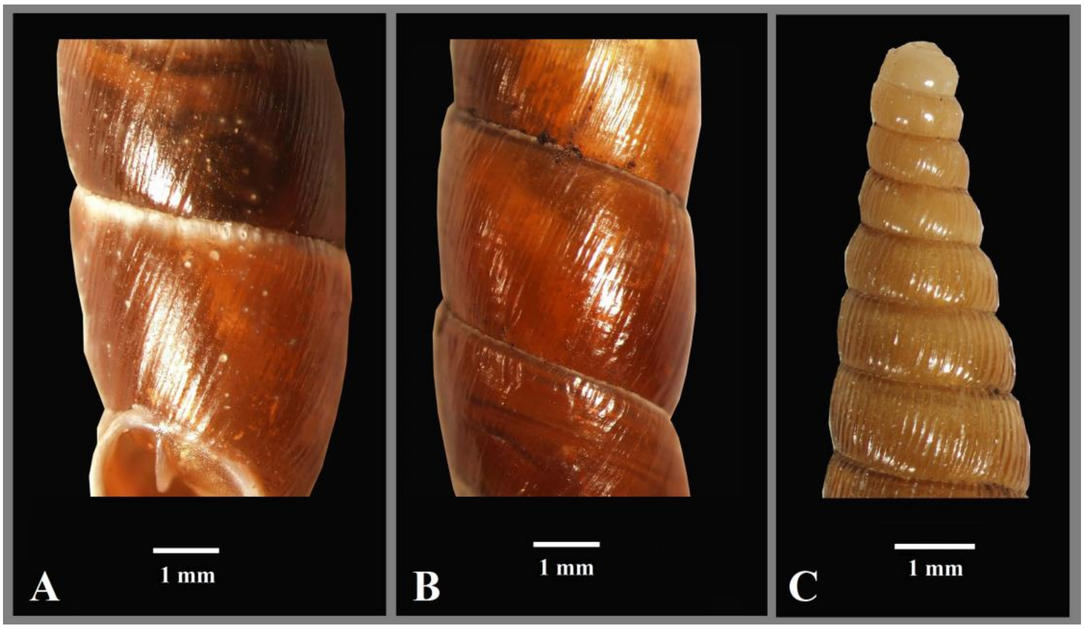

Figure 2: Different types of shell surface (A: smooth without spiral lines (COSIBB), B: smooth with spiral lines (CEHUBU1), C: ribbed (OSKZA1))

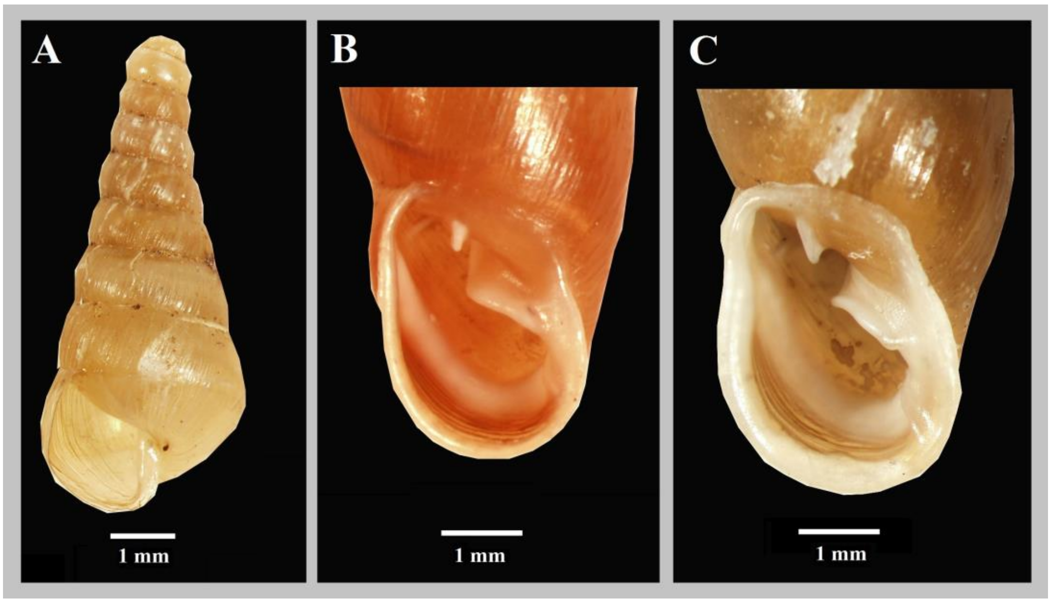

Figure 3: Shells of various ages, shown on three different individuals of *C. fimbriata* . (A: FITPA– the last whorl is not developed yet and thus all morphological characteristics except for colour of the shell and presece of spiral lines of its surface were not assessable; B: FSIVR2 – aperture, palatal callus, lamellae and plicae are formed, the lip of aperture is white, but not wide, C: FSIPL – wide apertural rim, more calcified that in the previous case)

3. Results

4.1 Molecular analysis

Bayesian analysis of the sequences resulted in a tree presented in Figure 4 (full tree) and Figure 5 (compressed tree).

Most of the seven species used in the analysis were included in separate and well supported clades. Paradoxically, the best sampled species (*C. laminata*) with the most individuals sequences had the smallest probability values (0.49). Similar situation occurred with *C. fimbriata* (possessing a probability value of 0.89).

As the existence of these two clades is not very well supported, I divided them both into four smaller and well supported lineages and assessed each of these lineages individually. Detailed images of the resulting tree and list of samples will be given in the following chapter.

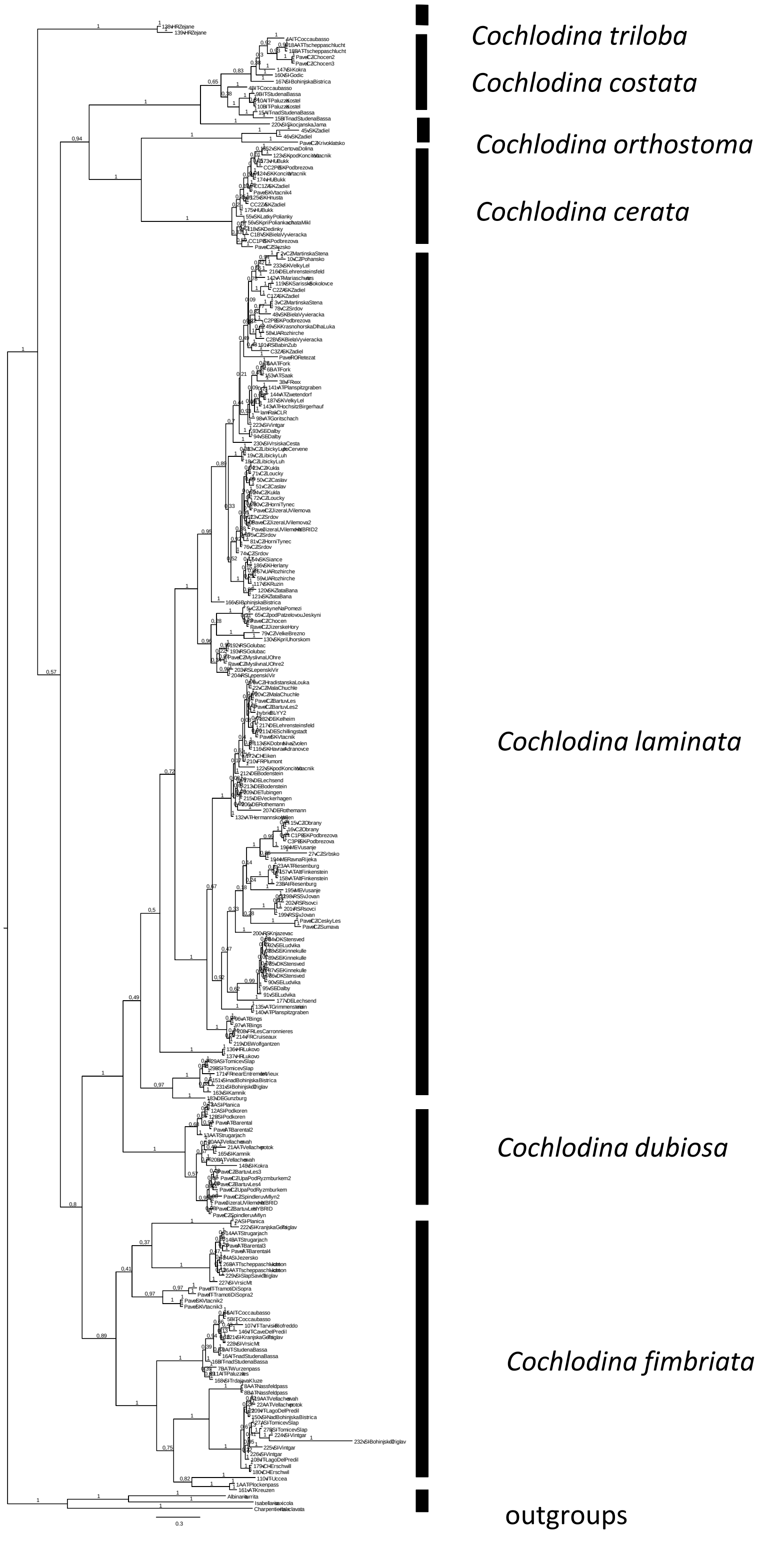

Figure 4: Tree based on the 239 sequences of gene 16S rDNA and 41 sequences of COI gene calculated by Bayesian analysis.

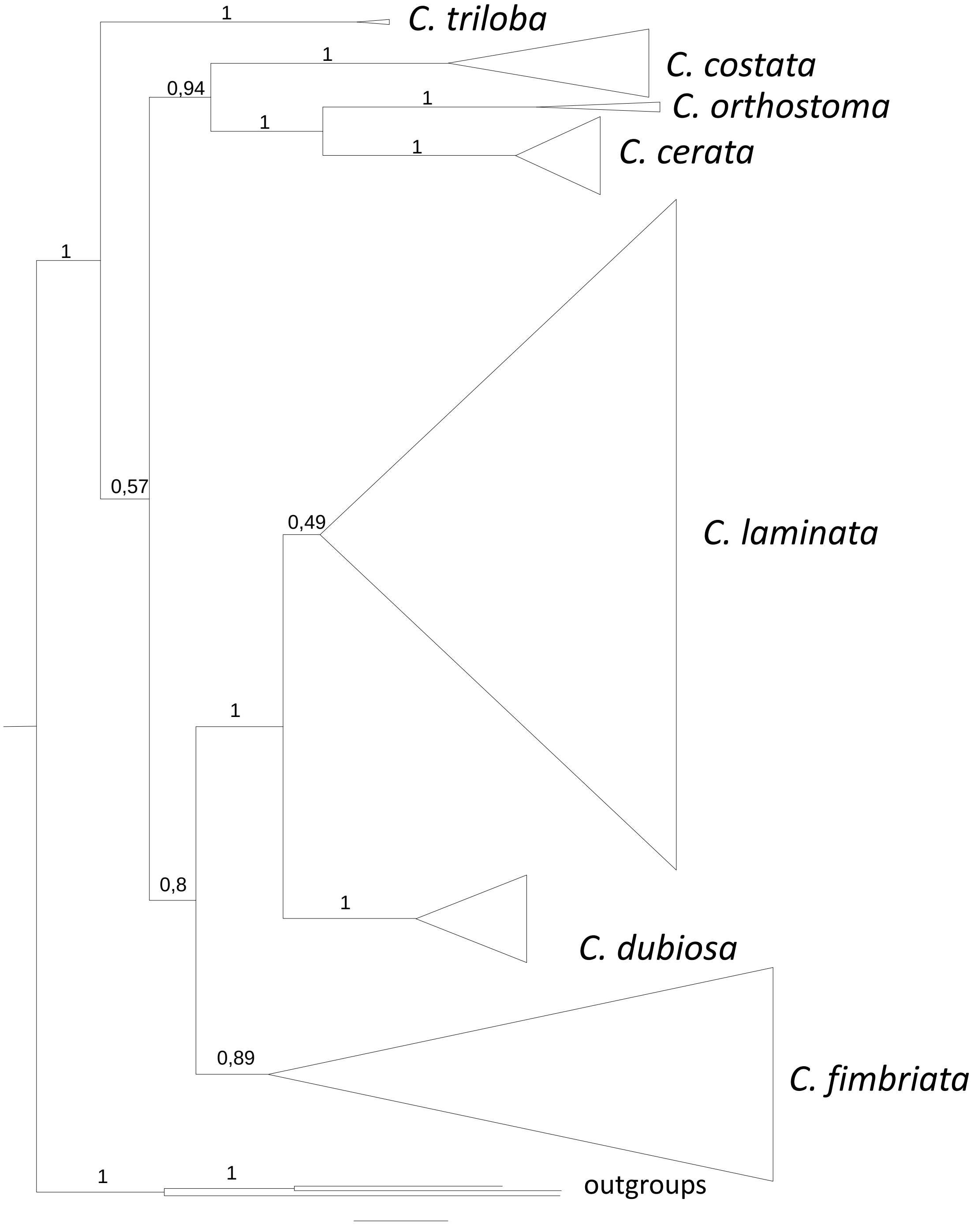

Figure 5: A compressed tree based on the 239 sequences of gene 16S rDNA and 41 sequences of COI gene calculated by Bayesian analysis. Probability values are shown at nodes.

4.2. Morphological analysis of the individual clades defined by molecular analysis

In this chapter, the results obtained from observing the thirteen aforementioned morphological caracteristics will be presented.

Samples were divided into groups representing individual clades from the formerly presented Bayesian tree created from the sequences of 16S and COI mitochondrial genes. Each of these groups were assessed individually

Firstly, individual groups and their morphological characteristics are presented. After that, individuals with damaged shells or shells with clearly anomalous conchological characteristics will be shown.

4.2.1. *Cochlodina triloba*

Only two individuals of *Cochlodina triloba* from the same site (Žejane, Croatia) (shown in table 2) were used in this analysis.

Table 2: List of individuals of *C. triloba* used in this study.

***Cochlodina triloba* (Boettger, 1878)**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
THRZ1 **THRZ2**	Croatia	Žejane	45° 27,045' N, 14° 10,816' E	648m	01-9-12	F.Šťáhlavský, J. Plíšková, J. Kotrbová

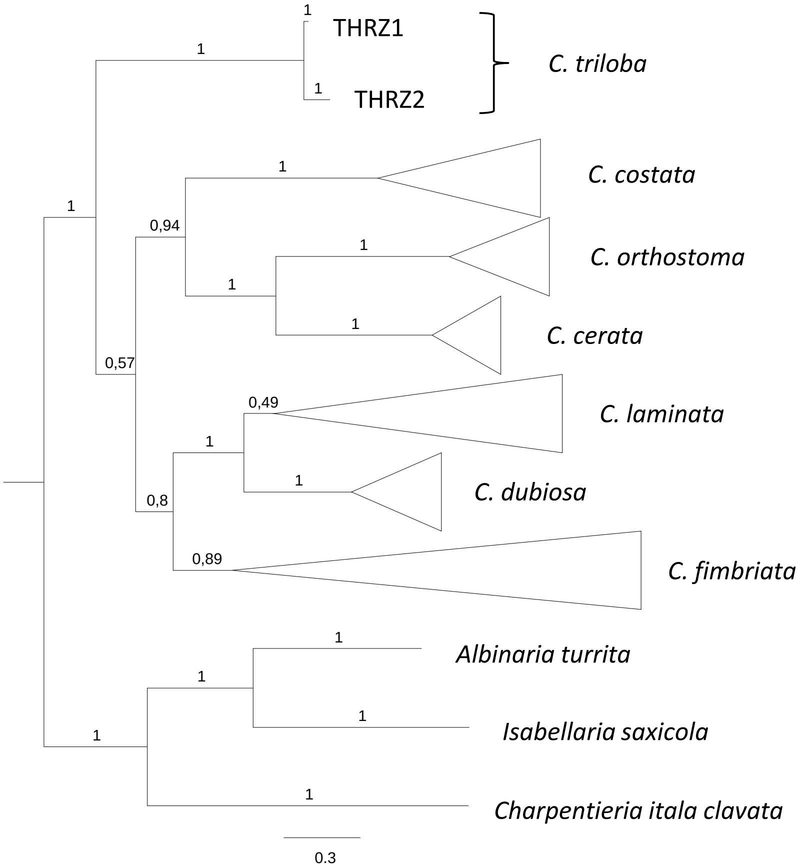

Figure 6: The position of *C. triloba* individuals in the tree created by Bayesian analysis.

Figure 7: Distribution of *C. triloba* individuals analyzed in this study.

These two individuals were very similar in most of their traits, except for the presence of spiral lines, position of the palatal callus and the extensions of palatal callus, as shown in graphs in figures 8a and 8b. Apertural views of both shells are presented in figure 9.

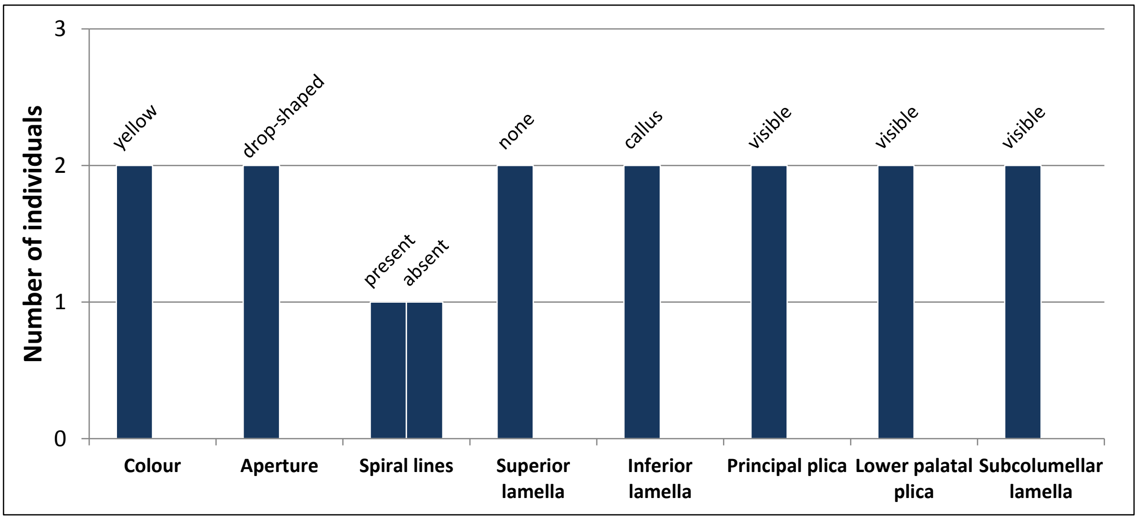

Figure 8a: Morphological characteristics on the surface of the shell and those visible on apertural view of the shell (sup.lam. = superior lamella; inf. lam. = inferior lamella)

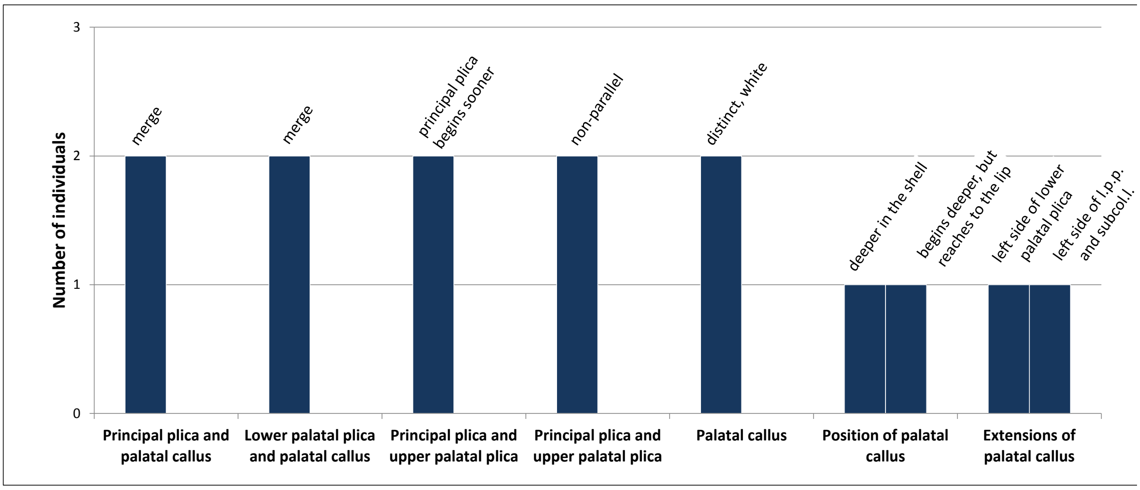

Figure 8b: Interactions and positions of plicae and lamellae in the mouth of the shell. (l.p.p. = lower palatal plica; subcol. lam. = subcolumellar lamella)

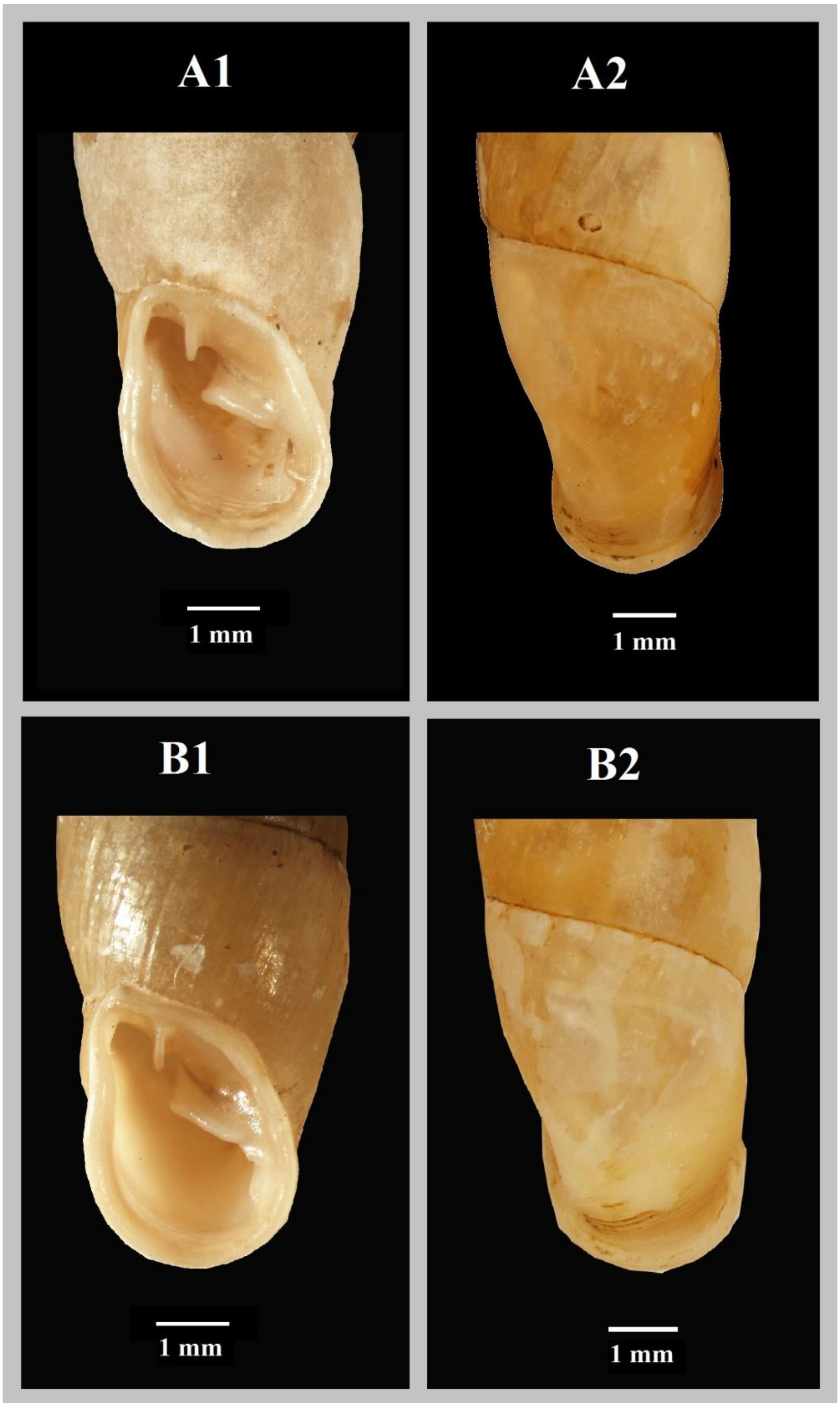

Figure 9: Apertural and abapertural views of *C. triloba* individuals analyzed in this study (A: THRZ1, B: THRZ2)

4.2.2. *Cochlodina costata*

Sequences of fifteen individuals of *Cochlodina costata* were used in the analysis (Table 3, Figure 10). Two of these sequences came from a former study by Tlachač (2008) to which the corresponding shells of snails were not available and therefore only thirteen newly collected individuals were asessed in the analysis of morphological characteristics (Figures 12a and 12b).

Table 3: List of individuals of *C. costata* used in the analysis. Specimens from Tlachač (2008) are listed in *italics*.

***Cochlodina costata* (Pfeiffer, 1828)**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
COITCO1 COITCO2	Italy	Coccaubasso	46°31'55.20" N, 13°38'17.16" E	687m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
COATTS1 COATTS2	Austria	Tscheppaschlucht	46°31'34.89" N, 14°19'24.26" E	455m	09-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
COCZCH1 COCZCH2	*Czech republic*	*Choceň*	*50°00'08.00'' N, 16°14 00.00'' E*	*314m*	*21-8-07*	*P. Tlachač*
COSIKO	Slovenia	Kokra	46°18'12.24" N, 14°28'50.04" E	512m	08-9-12	F.Šťáhlavský, J. Plíšková, J. Kotrbová
COSIGO	Slovenia	Godic	46°15'50.82" N, 14°36'13.20" E	440m	07-9-12	F.Šťáhlavský, J. Plíšková, J. Kotrbová
COSIBB	Slovenia	Bohinjska Bistrica	46°16'45.95" N, 13°57'45.34" E	552m	29-8-12	F.Šťáhlavský, J. Plíšková, J. Kotrbová
COITSB	Italy	Studena Bassa	46°31'24.60" N, 13°15'39.708" E	702m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
COITPA1 COITPA2	Italy	Paluzza	46°31'47.28" N, 13°01'00.84" E	605m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
COITOS1 COITOS2	Italy	over Studena Bassa	46°32'07.08" N, 13°12'28.80" E	1059m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
COSISJ	Slovenia	Škocjanska jama	45°39'48.00" N, 13°59'23.00" E	409m	07-9-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová

The colour of the shell and the shape of aperture seem to be quite variable in this species. Similarly, there were callous and constricted areas on superior lamella of some specimens. However, there were no such morphological shapes on the inferior lamella (figure 12a).

Lower palatal plica, upper palatal plica and subcolumellar lamella were visible from the apertural view of the shell in all cases (figure 12a).

There was an anomaly observed on one of the specimen (COSIKO) which had two plicae in place of lower palatal plica, hence the mutual position of principal plica and upper palatal plica was not assessable. Also the position of the beginning of upper palatal plica was not clear, as it was not possible to distinguish the true upper palatal plica. This individual was excluded from the analysis of the aforementioned two characteristics, but used in assessment of all other characteristics.

Figure 10: Map of collecting sites of the *C. costata* individuals.

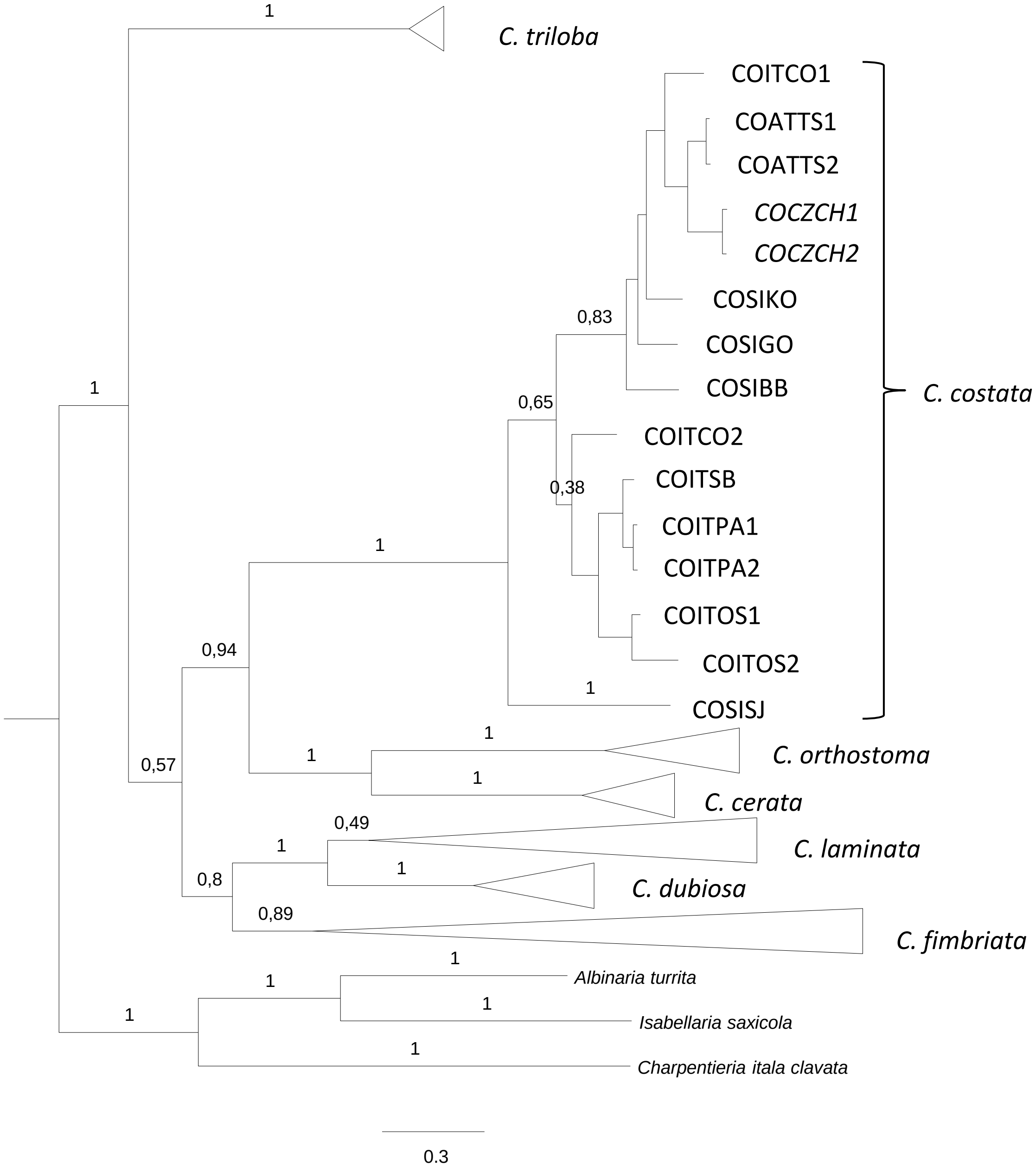

Figure 11: The position of *C. costata* individuals in the tree created by Bayesian analysis.

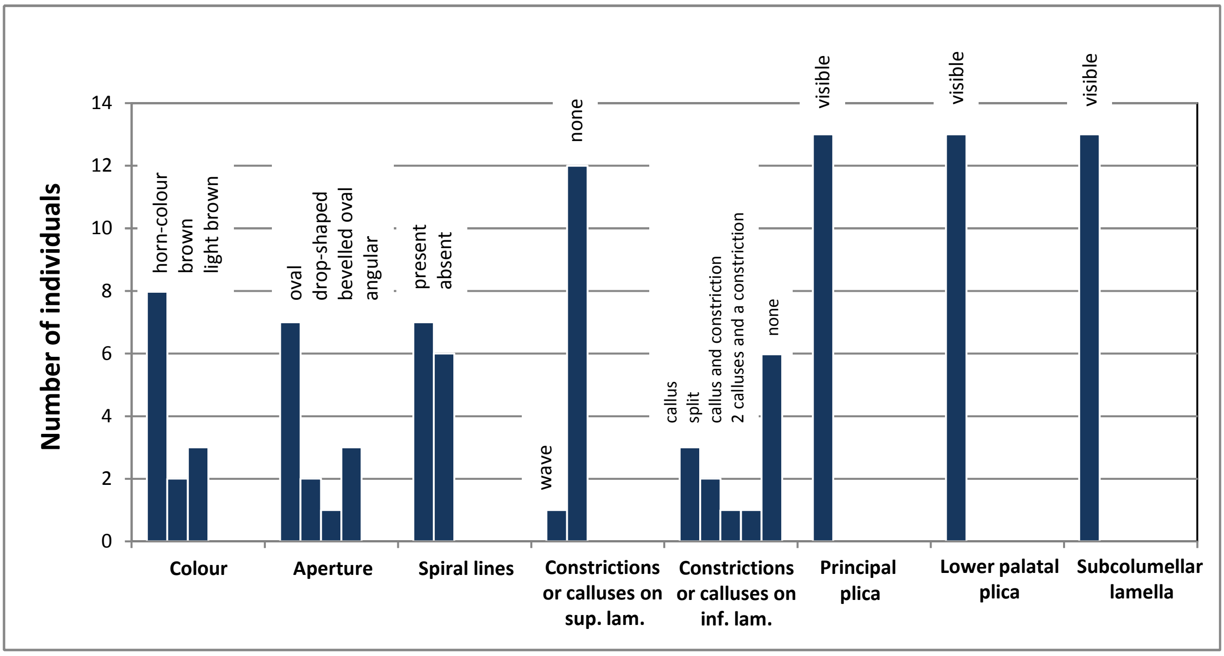

Figure 12a: Morphological characteristics on the surface of the shell and those visible on the apertural view of the shell (sup.lam = superior lamella; inf. lam. = inferior lamella)

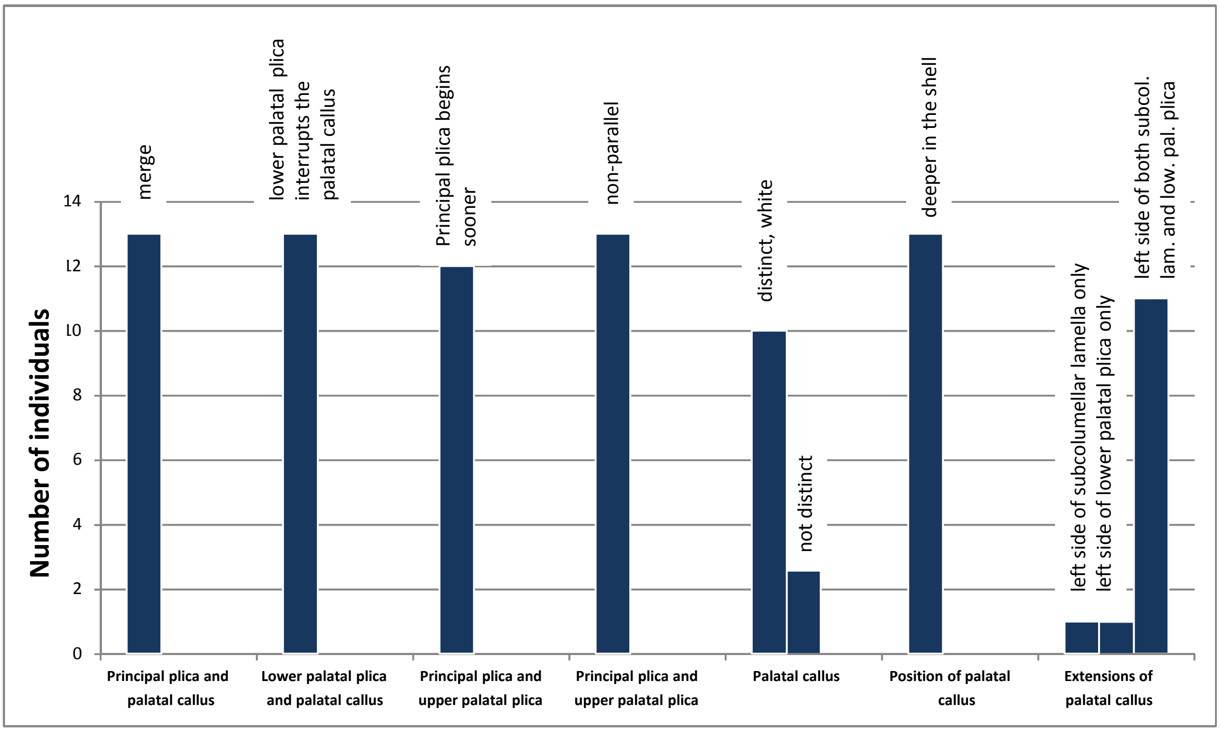

Figure 12b: Interactions and positions of plicae and lamellae in the mouth of the shell (low. pal. plica – lower palatal plica; subcol. lam.- subcolumellar lamella)

While the upper palatal plica merges with the palatal callus, the lower palatal plica interrupts it. The palatal callus is very thin or non-existant around it and the lower palatal fold usually ends on the level of the lower edge of the palatal callus (figure 12b).

This is probably the most prominent morphological feature, already described in detail by Ložek (1956). It can be the first of the traits used to distinguish *C. costata* from other species of this genus. Most of the times, especially then the palatal callus is well-developed and white in colour, this characteristic can also be observed by a naked eye without any means of magnification. This unique nature of the palatal callus and lower palatal fold was observed in all *C. costata* specimen used in this analysis. In all cases the lower palatal fold interrupted the palatal callus.

In all cases, the palatal callus formed an extension or two going up parallel to the lower palatal plica, subcolumellar lamella or both of these structures. Ložek (1956) calls these extensions "untrue plicae" and mentions that its edge is connected to the outer side of upper palatal plica. No such arrangement of plicae was observed in this study. The intensity of the palatal callus has been different in different specimens, however, the lower palatal fold was always placed this way.

Similarly, the position of the palatal callus was always the same – occuring deeper in the mouth of the shell.

Some of the individuals of *C. costata* are pictured in Figure 13.

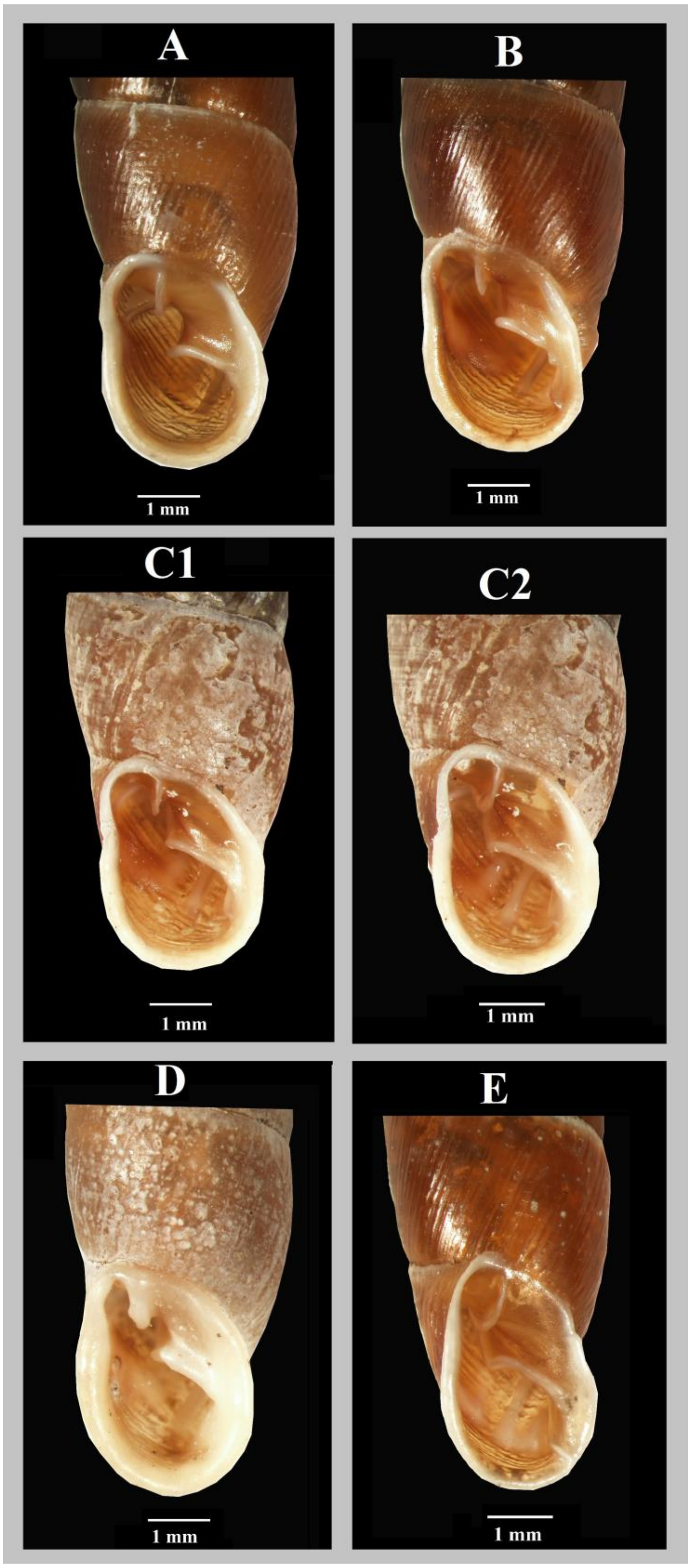

Figure 13: Apertural views of some *C. costata* individuals analyzed in this study.

A: COITPA1; B: COITOS1; C: COITSB; D: COITCO2, E: COSIBB

4.2.3. *Cochlodina orthostoma*

Only two specimen of *C. orthostoma* from one locality in Zádiel gorge, Eastern Slovakia, were obtained for this study. Both of these specimen formed a well separated and well supported clade together with a sequence obtained from study by Tlachač (2008).

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Table 4: List of *C. orthostoma* individuals used in this study. Individual collected by Tlachač (2008) is listed in *italics*.

***Cochlodina orthostoma* (Menke, 1830)**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
OSKZA1 **OSKZA2**	Slovakia	Zádiel gorge	48°37'11.36"N, 20°49'55.24"E	373m	15-6-10	V. Szalontayová, L. Juřičková, P. Synek
OCZKR	*Czech republic*	*Křivoklátsko*	*49°57'11.00'' N, 13°45'00.00'' E*	*281m*	*25-8-07*	*P. Tlachač*

Figure 14: Map of collection sites of the *C. orthostoma* individuals. Individual obtained by Tlachač (2008) is listed in *italics*.

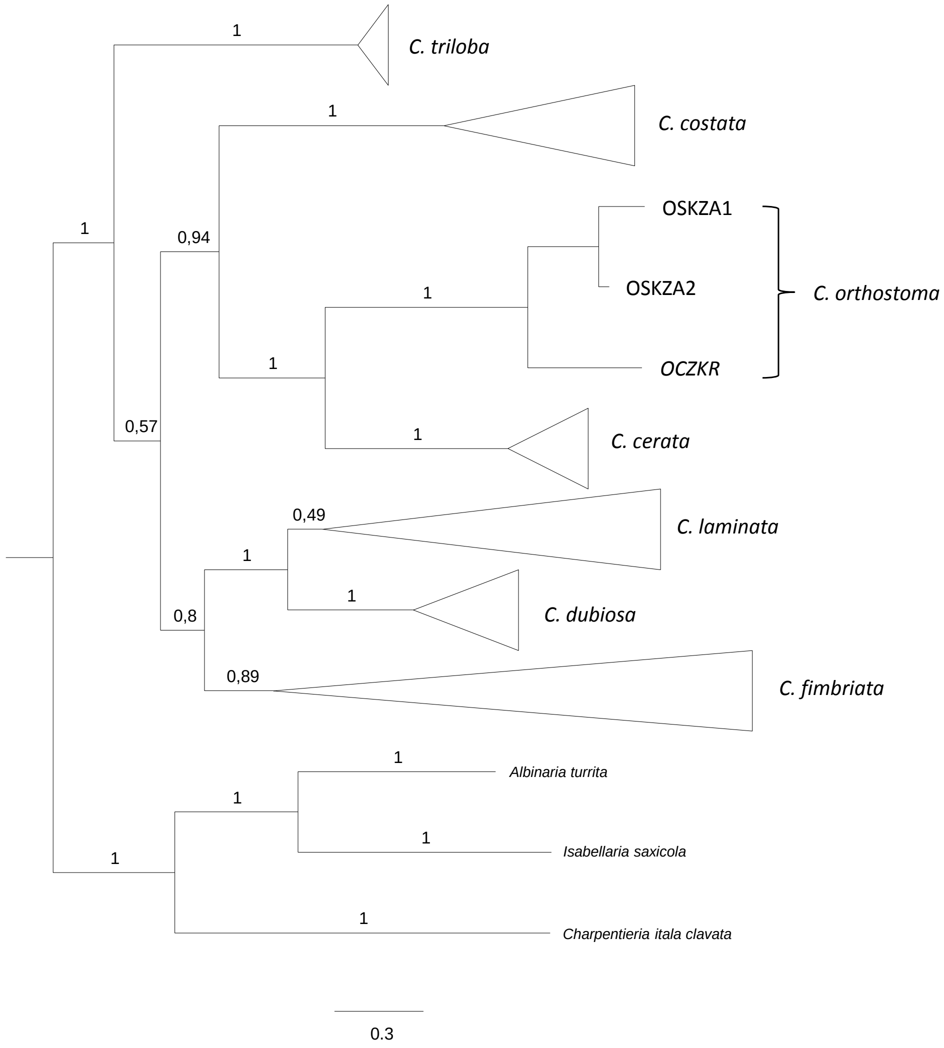


Figure 15: The position of *C. orthostoma* individuals in the tree created by Bayesian analysis.

In spite of the fact that both of these individuals came from the very same sampling site, they differed in one morphological feature (figure 16b).

These individuals were uniform in colour, both being light brown. Ložek (1956) mentions that insufficiently coloured individuals are fairly common. In this very little set of individuals, I did not observe any.

Surface of both individuals was ribbed, as pictured in figure 2 (C).

There were no calluses or constrictions on superior nor inferior lamella (figure 16a).

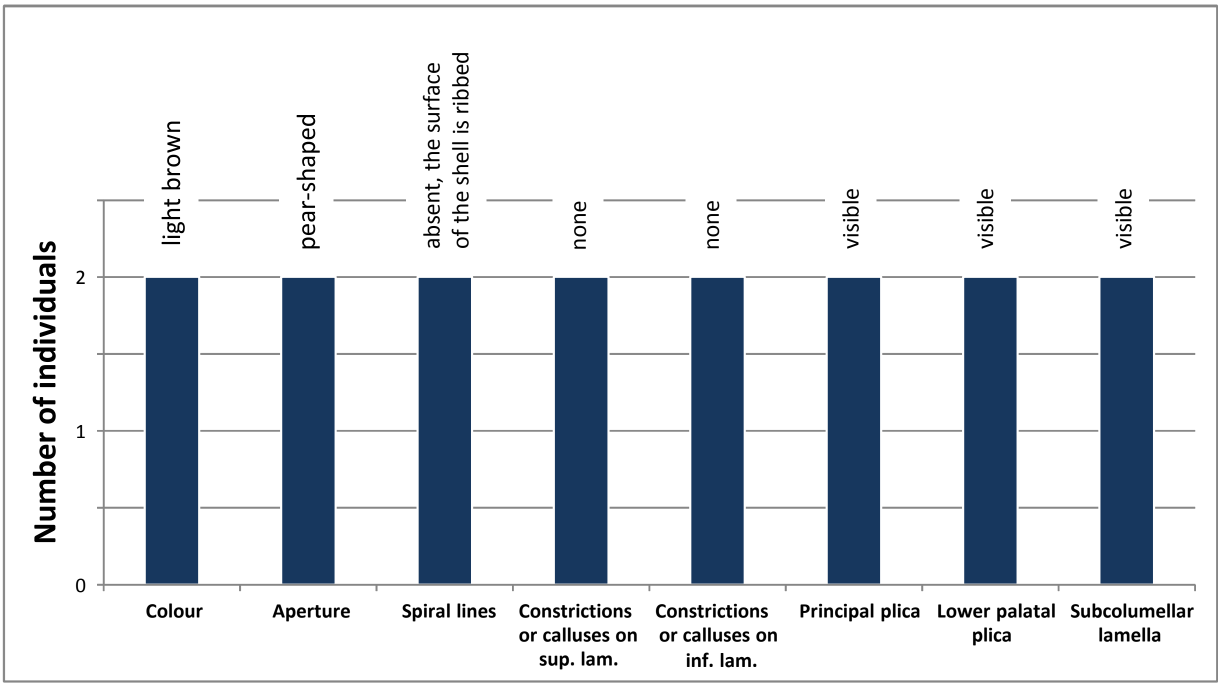


Figure 16a: Morphological characteristics on the surface of the shell and those visible on the apertural view of the shell – sup.lam – superior lamella; inf. lam.-inferior lamella

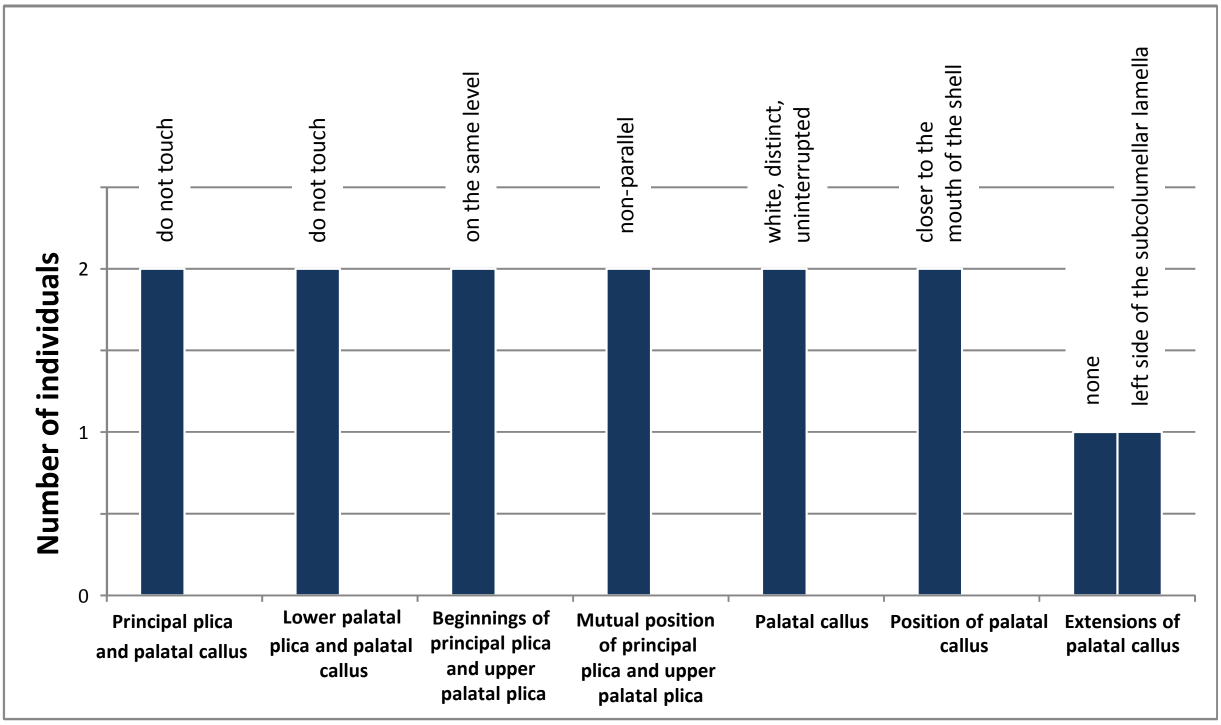


Figure 16b: Interactions and mutual positions of plicae and lamellae in the mouth of the shell.

Principal plica, lower palatal plica and subcolumellar lamella were visible from apertural point of view (figure 16a). None of these interfered with palatal callus.Beginnings of principal plica and upper palatal plica were on the same level and these plicae were non-parallel.Palatal callus was developed, white, occurring closer to the lip of the shell (figure 16b).

The only difference among these individuals was in the way that palatal callus extended upwards, along subcolumellar lamella. Both of the shells were unbroken and showing no apparent sign of a former injury that could cause the shell characteristics to change, therefore I presume that the difference in presence of this extension is natural.

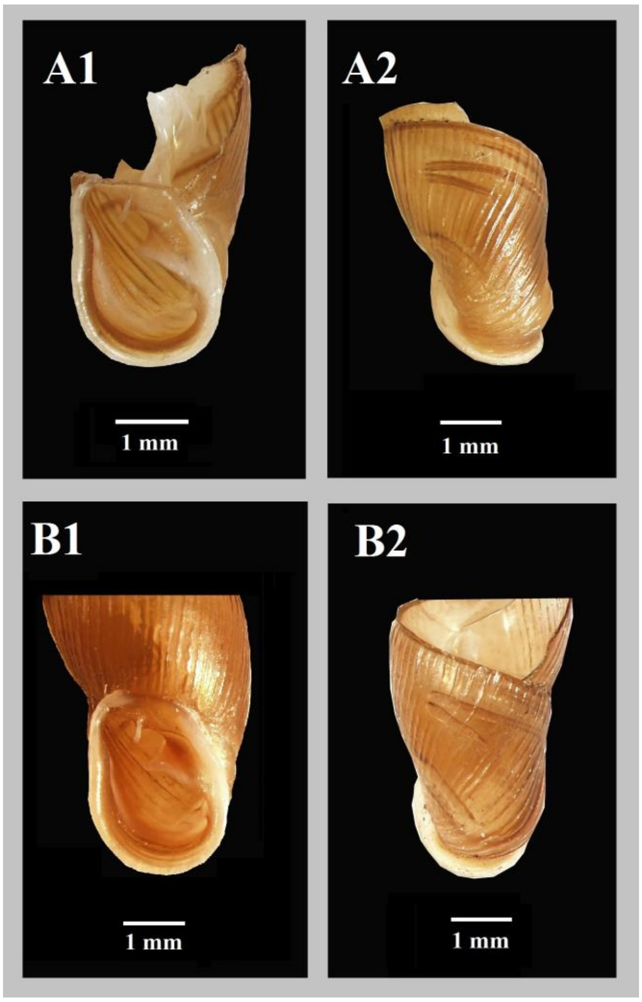


Figure 17: Apertural and abapertural views of *C. orthostoma* individuals analyzed in this study.

4.2.4. Cochlodina cerata

Sequences of seventeen individuals of *C. cerata* have been used in this analysis. Two of these individuals came from a former study by Tlachač (2008) (Table 5) therefore only fifteen individuals were assessed in the analysis of morphological characteristics.

Table 5: List of *C. cerata* individuals used in this study. Individuals collected by Tlachač (2008) are listed in *italics*.

***Cochlodina cerata* (Rossmässler, 1836)**

Code	Country	Locality	Coordinates	Altitude	Date of collecting	Collector(s)
CESKCD	Slovakia	Čertova dolina	48°44'18.20" N, 019°51'26.78" E	715m	01-6-11	I. Černajová
CESKPK	Slovakia	near cottage "Pod Končitou"	48°38'53.87" N, 18°35'18.57" E	504m	01-7-12	V. Szalontayová, A. Hamilton
CEHUBU1 **CEHUBU2** **CEHUBU3**	Hungary	Bükk	47°55'50.66" N, 20°24'59.32" E	148m	23-5-12	D. Říhová
CESKPB1 **CESKPB2**	Slovakia	Podbrezová	48°48'30.47" N, 19°32'19.42" E	503m	15-6-10	V. Szalontayová, L. Juřičková, P. Synek
CESKKO	Slovakia	Končitá	48° 38' 59,60'' N, 18° 35' 10,90'' E	494m	01-7-12	V. Szalontayová, A. Hamilton
CESKZA1 **CESKZA2**	Slovakia	Zádiel gorge	48°37'11.36"N, 20°49'55.24"E	373m	15-6-10	V. Szalontayová, L. Juřičková, P. Synek
CESKVT	*Slovakia*	*Vtáčnik*	*48°38'18.00'' N,* *18°36'47.00'' E*	*787m*	*13-5-08*	*P.Tlachač*
CESKHN	Slovakia	Hnúšťa	48° 34' 34,80'' N, 19° 58' 07,60'' E	368m	01-7-12	V. Szalontayová, A. Hamilton
CESKLP1 **CESKLP2**	Slovakia	Látky-Polianky	48°36'22.74" N, 019°41'43.96" E	917m	01-6-11	Z. Miklušová
CESKDE	Slovakia	Dedinky	48° 51' 48,30'' N, 20° 23' 09,40'' E	793m	01-7-12	V. Szalontayová, A. Hamilton
CESKBV	Slovakia	Biela vyvieračka	48°34'18.39" N, 20°27'59.47" E	231m	16-6-10	V. Szalontayová, L. Juřičková, P. Synek
CECZSL	*Czech republic*	*Slezsko*	*not given (Tlachač 2008)*		*01-7-06*	*Hrabáková*

All of these individuals formed a well supported, compact and monophyletic clade in the tree calculated by Bayesian analysis (Figure 18).

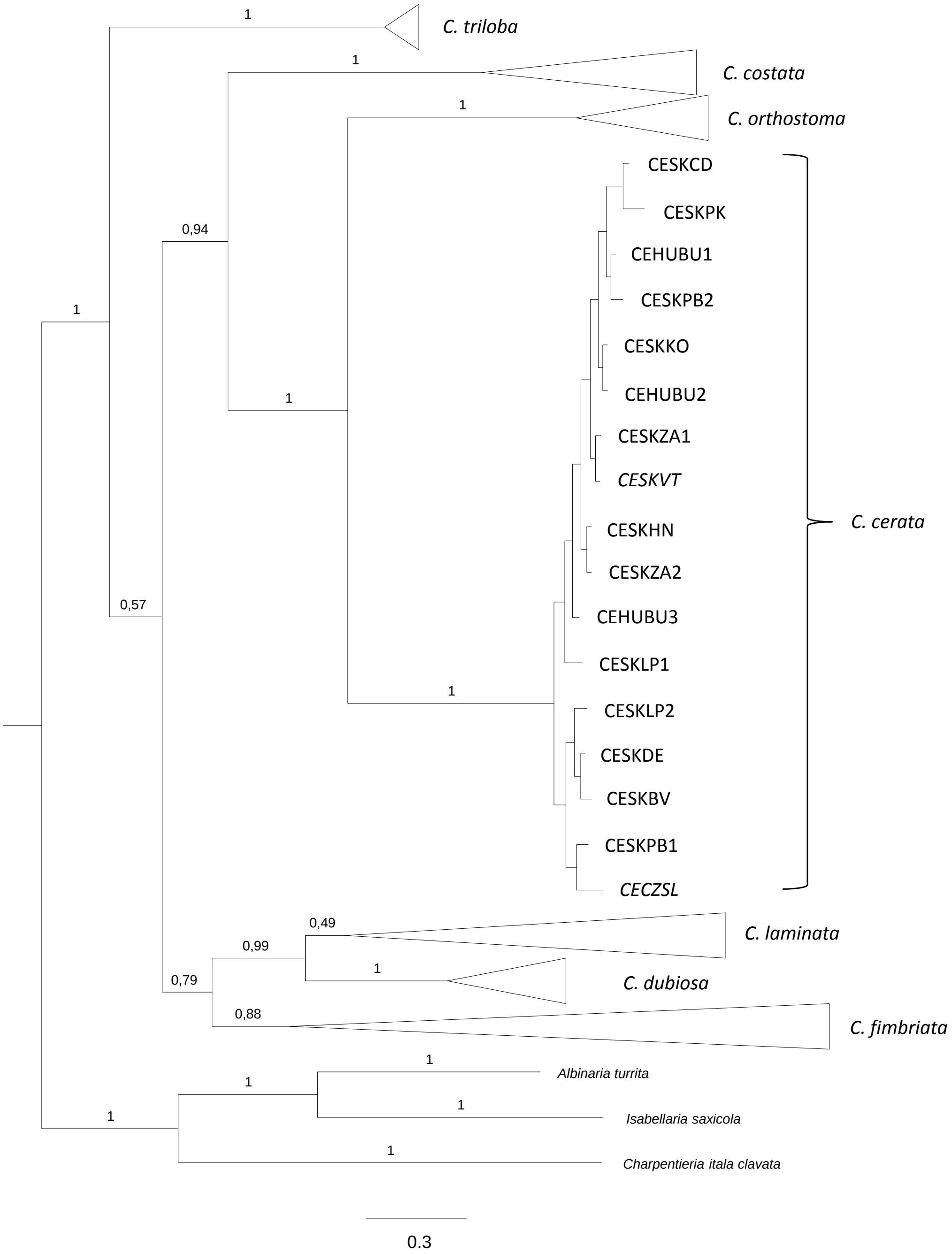


Figure 18: The position of *C. cerata* individuals in the tree created by Bayesian analysis.

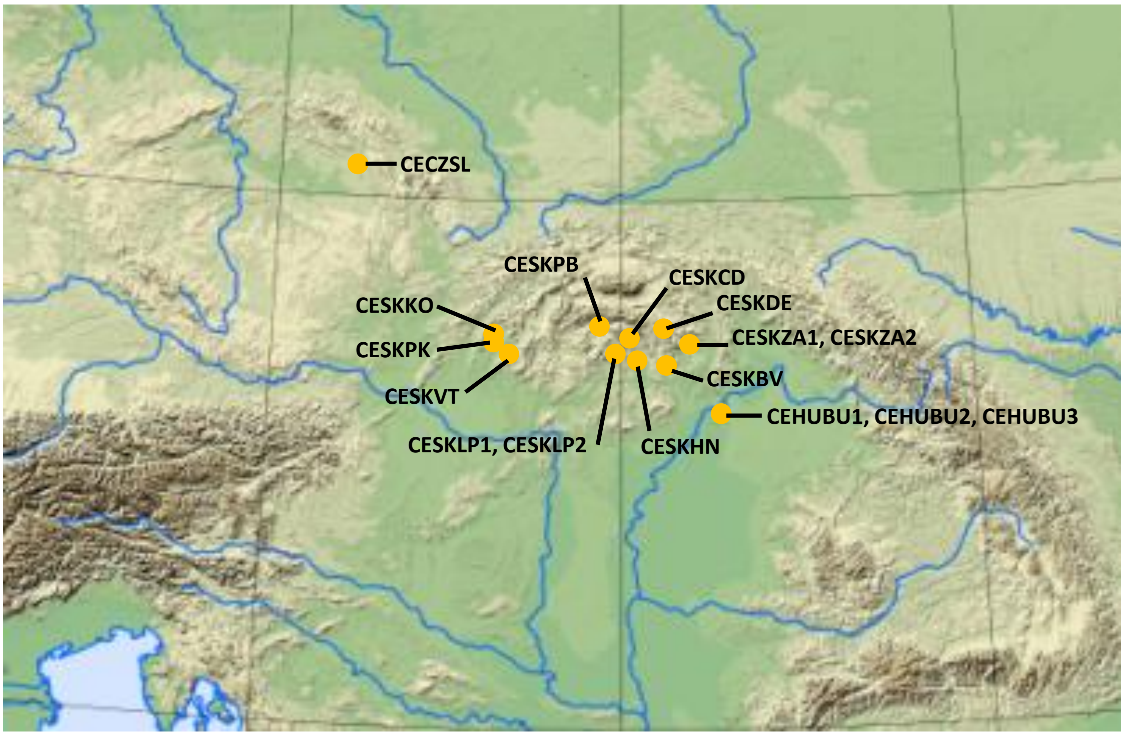


Figure 19: Map of collection sites of the *C. cerata* individuals. Individuals obtained by Tlachač (2008) are shown in *italics*.

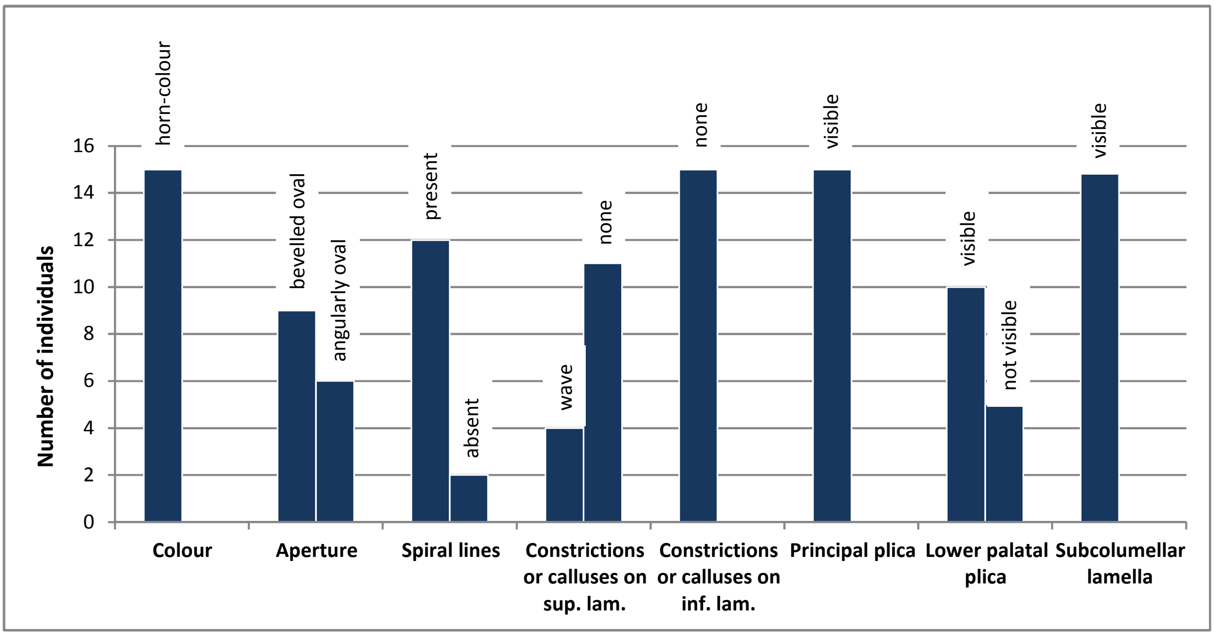


Figure 20a: Interactions and mutual positions of plicae and lamellae in the mouth of the shell (sup. lam. = superior lamella; inf. lam = inferior lamella)

All fifteen individuals were uniform in colour of the shell, which was horn-coloured. Shapes of aperture varied, as did the occurrence of spiral lines on the surface of the shell, presence of constrictions or calluses on superior lamella and surprisingly, visibility of lower palatal plica from the apertural view of the shell. There were no calluses or constrictions present on the inferior lamella and both principal plica and subcolumellar lamella were clearly visible on apertural view of the shell (figure 20a).

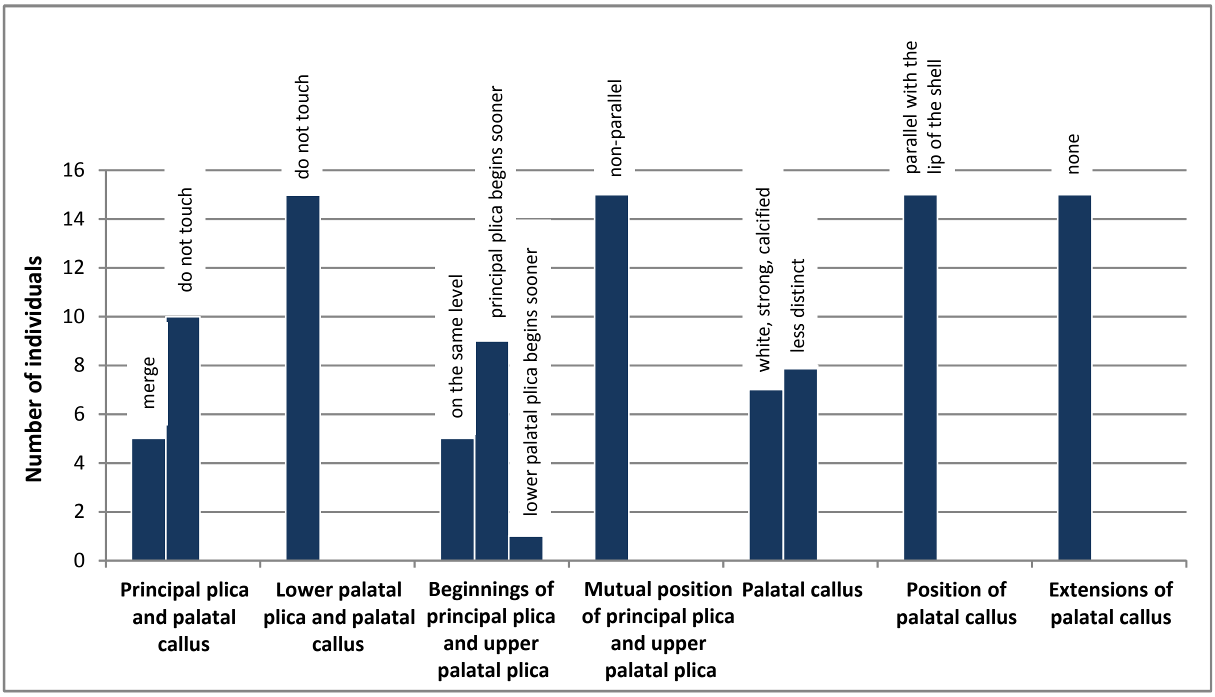


Figure 20b: This graph sums up the morphological characteristics on the surface of the shell and those visible on the apertural view of the shell .

Principal plica, even though always visible on apertural view of the shell, only touched the palatal callus in minority of the cases. Lower palatal plica, always visible, was never long enough to touch the palatal callus or cut through it, as was observed in *C. costata* (figure 20b).

The mutual position of the principal and upper palatal plicae was always non-parallel, however, there was great variability in the positions of the beginnings of these two plicae. In other species mentioned here before, either the beginning of principal plica was positioned more to the left, when viewed from abapertural side or the plicae began on the same level. However, one case was observed here when the beginning of upper palatal plica was positioned more to the left (specimen CESKLP1) (figure 20b), which was not observed in any other specimen used in this study.

Palatal callus was close to the lip of the shell in all cases. It was paralell with the lip, never merging with the lip of the shell itelf (there was always gap between the white lip of the shell and palatal callus). In none of the cases observed did the palatal callus form an extension or untrue plica as it was described in *C. costata* (Ložek 1956).
Photographs of some individuals can be seen in figure 21.

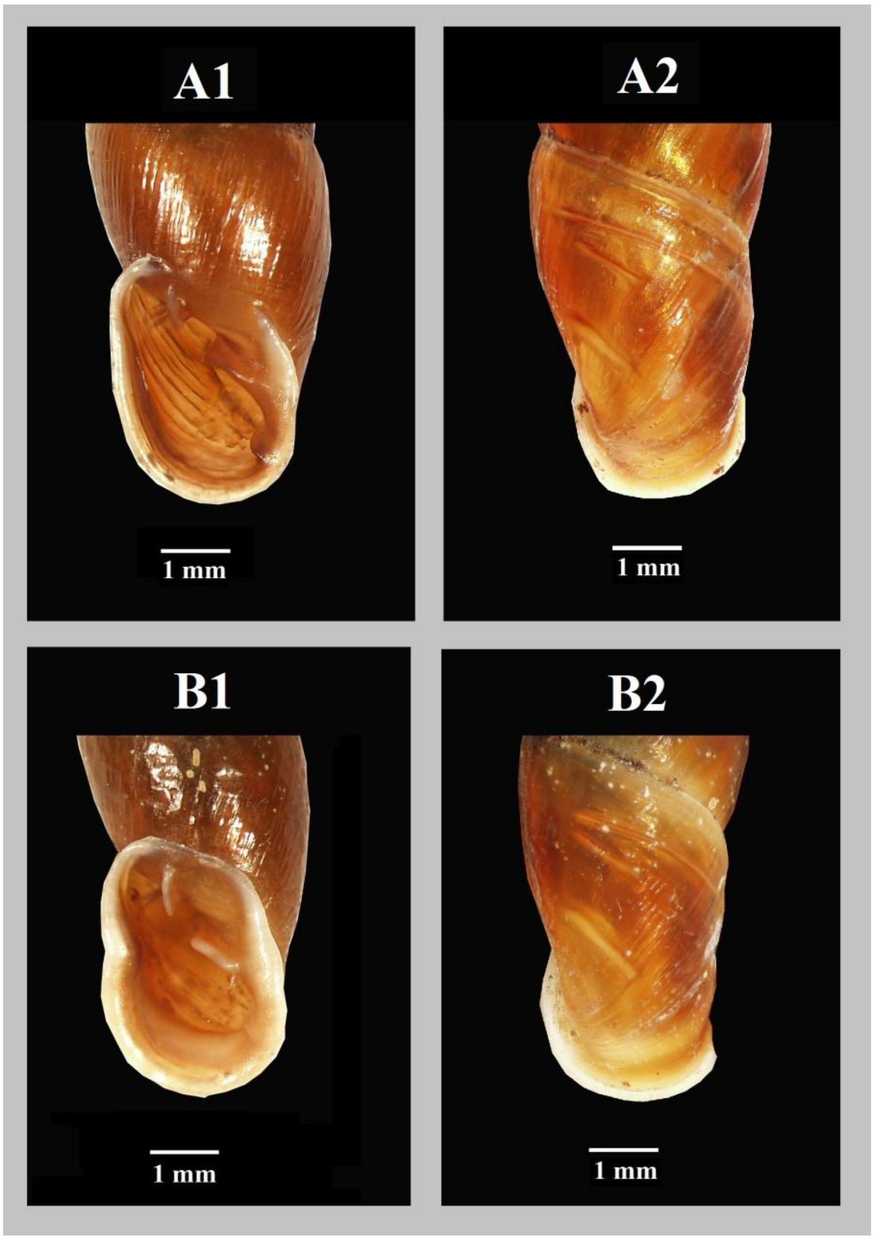


Figure 21: Apertural and abapertural views of some of the *C. cerata* individuals analyzed in this study (A: CESKPK; B: CESKLP2)

4.2.5. *Cochlodina laminata*

Sequences of 140 specimens of *C. laminata* were used for this study. Fifteen of these were from former study by Tlachač (2008). The remaining 125 individuals were used for the following analysis of morphological characteristics.

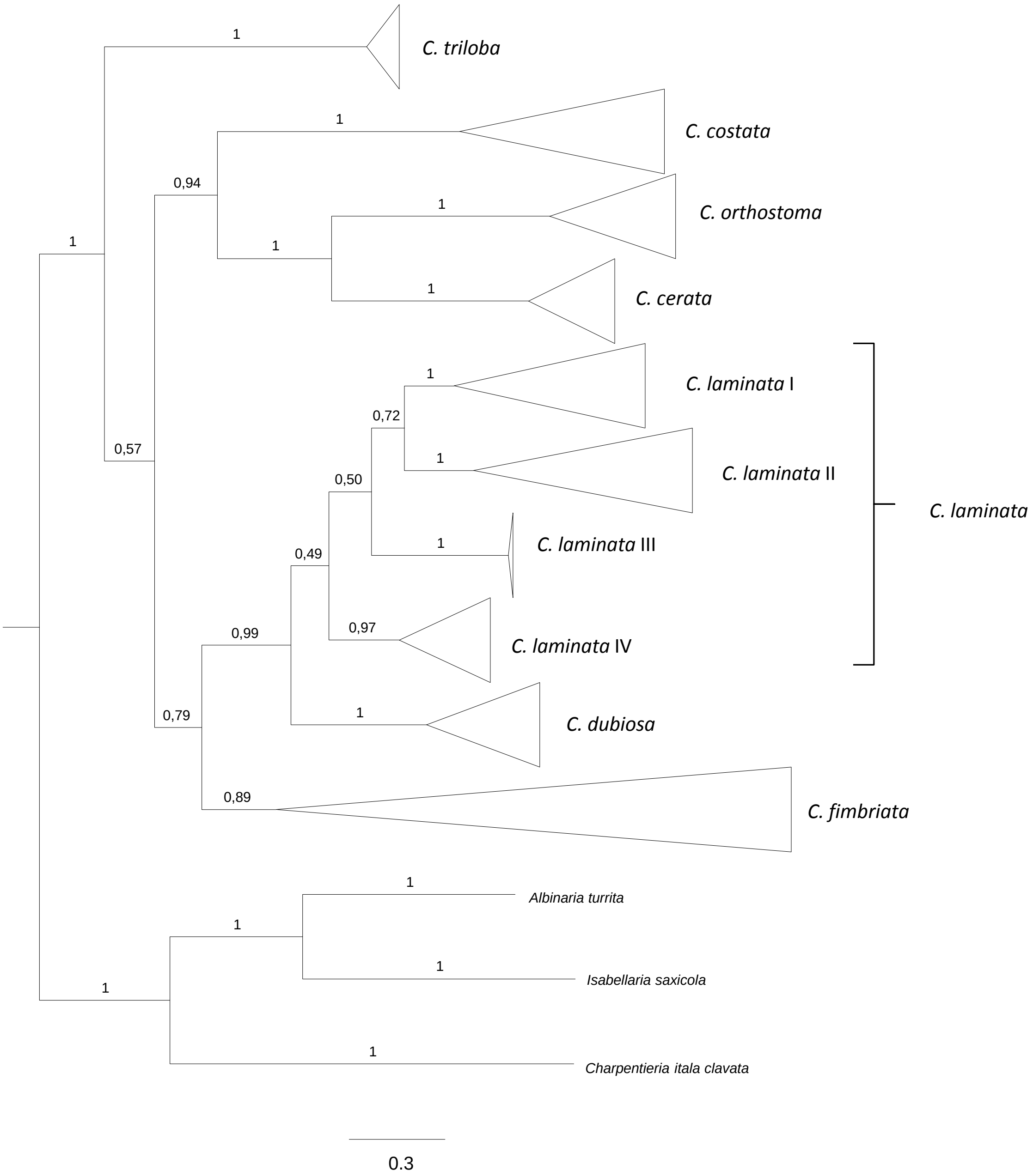
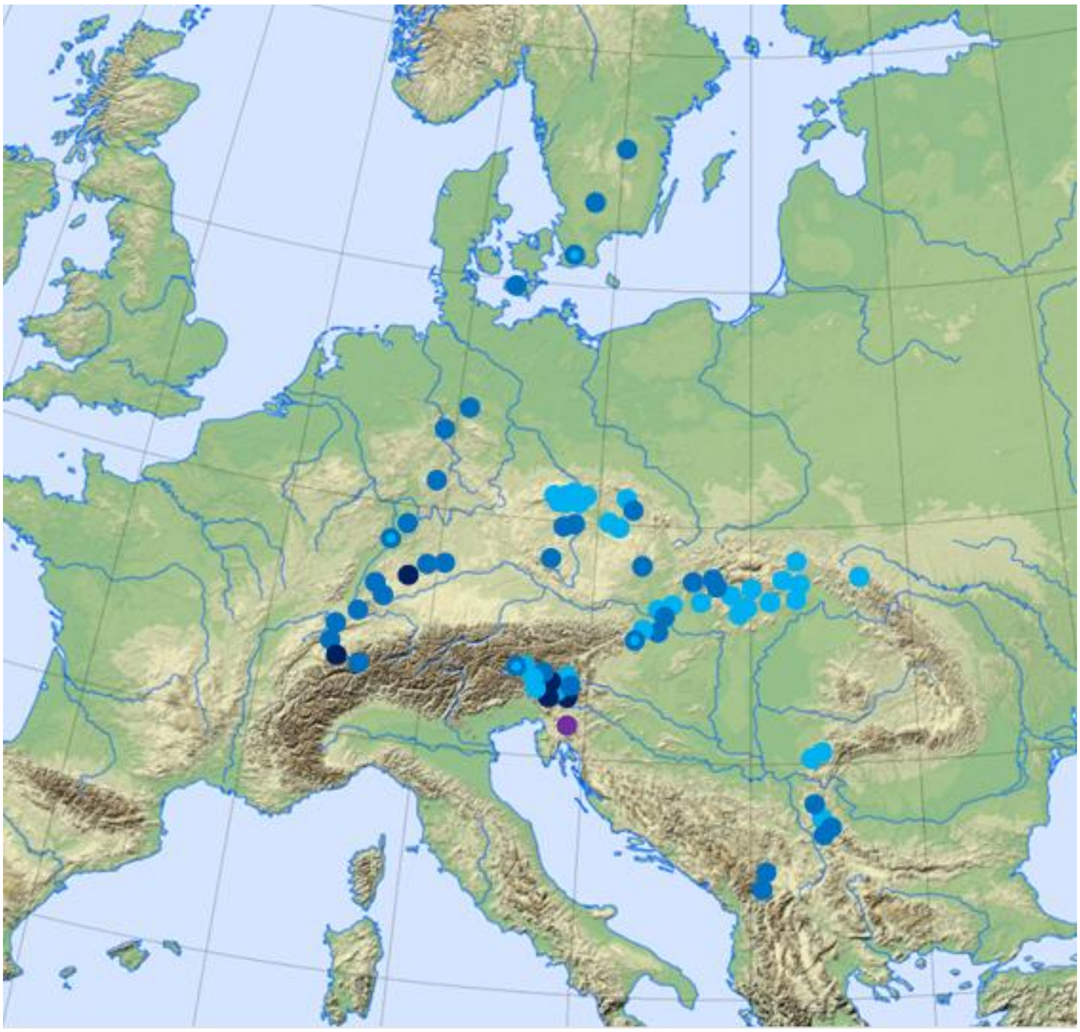


Figure 22: Compressed tree created by Bayesian analysis, showing the position and various lineages of *C. laminata*.

Because the whole clade was not well supported, it was divided into four distinct lineages, each of which possessed very high probability values. Specimens are listed for each lineages individually, in tables 6-9.



● *C. laminata* – lineage I

● *C. laminata* – lineage III

● *C. laminata* – lineage II

● *C. laminata* – lineage IV

◉ sites, where lineages I and II of *C. laminata* occur together

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Figure 23: Map of Europe showing distribution of individual lineages of *C. laminata*.

The geographical distribution of the four lineages of *C. laminata* is shown in figure 23. On most sites from which the material was collected, only one of the lineages occurred. There were only three sites where individuals from lineage I and lineage II occurred simultaneously. These were: Lechsend (Germany), Podbrezová (Slovakia) and Plantspitzgraben (Austria).

Ložek (1956) mentions that *C. laminata* is distributed as far as Crimea, Ukraine, however, I was unable to find *C. laminata* individuals during my field trip in the area, which may be due to very dry conditions at that time.

4.2.5.1. *Cochlodina laminata* -lineage I

Clade I of *C. laminata* consisted of 70 individuals. Nine of these individuals were sequenced by Tlachač (2008) (table 6) and the remaining 61 individuals were newly sequenced for this study and their morphology was assessed.

Table 6: List of individuals of *C. laminata* – lineage I. Individuals obtained by Tlachač (2008) are listed in *italics*, individuals that he described as intermediate forms between *C. laminata* and *C. dubiosa corcontica* are underlined.

Cochlodina laminata **(Montagu, 1803) - lineage I**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
LCZMS1 **LCZMS2**	Czech republic	Martinská Stěna	50°34'51.12" N, 14°27'47.07" E	404m	2009	L. Juřičková, V. Szalontayová
LCZPO	Czech republic	Pohansko	48°44'2.51" N, 16°54'5.09" E	159m	26-9-09	A. Damaška
LSKZO1 **LSKZO2**	Slovakia	Zlatná na Ostrove - -Veľký Leľ	47°45'28,88'' N, 17°56'42,9'' E	112m	2012	J. Škodová
LDELE1	Germany	near Lehrensteinsfeld	49°06'49.32"N, 09°19'28.98" E	288m	27-6-12	L. Juřičková
LATMA	Austria	Mariaschutz water reservoir	47°37'52.92" N, 15°52'24.96" E	1143m	17-9-10	Naturhistorisches Museum, Vienna
LSKSS	Slovakia	Šarišské Sokolovce	49°07'18.80'' N, 21°10'20.50'' E	497m	2012	V. Szalontayová, A. Hamilton
LSKZA1 **LSKZA2**	Slovakia	Zádiel gorge	48°37'11.36"N, 20°49'55.24"E	373m	15-6-10	V. Szalontayová, L. Juřičková, P. Synek
LATMA	Austria	Mariaschutz water reservoir	47°37'52.92" N, 15°52'24.96" E	1143m	17-9-10	Naturhistorisches Museum, Vienna
LCZVB1	Czech republic	Velké Březno	50°39'40.60'' N, 14°10'17.80'' E	454m	22-4-11	L. Juřičková, V. Szalontayová

LSKBV1 **LSKBV2**	Slovakia	Biela Vyvieračka	48°34'18.39" N, 20°27'59.47" E	231m	16-6-10	V. Szalontayová, L. Juřičková, P. Synek
LSKPB2	Slovakia	Podbrezová	48°48'30.47" N, 19°32'19.42" E	503m	15-6-10	V. Szalontayová, L. Juřičková, P. Synek
LSKKD	Slovakia	Krásnohorská Dlhá Lúka	48°37'19.12" N, 20°34'25.73" E	339m	30-3-11	F. Šťáhlavský
LUARH1 **LUARH2** **LUARH3**	Ukraine	Rozhirche	49°07'04.00'' N, 23°40'10.00'' E	361m	29-6-11	V. Szalontayová
LRSBZ	Serbia	Babin Zub	43°22'20.28" N, 22°36'29.01" E	1590m	2012	O. Korábek
LRORE	Romania	Retězat	45°18'13.11"N, 22°58'21.61"E	1022m	01-9-07	F. Šťáhlavský
LATFO1 **LATFO2**	Austria	Förk	46°35'19.68" N, 13°38'21.12" E	700m	10-5-09	L. Juřičková, A. Petrusek, V. Szalontayová
LATSA	Austria	Saak	46°35'31.81" N, 13°37'33.96" E	595m	29-8-12	F.Šťáhlavský, J. Plíšková, J. Kotrbová
LATPG2	Austria	Planspitzgraben	47°35'17.82" N, 14°38'19.62" E	625m	26-4-08	Naturhistorisches Museum, Vienna
LATZW	Austria	Zwetendorf	48°20'33.06" N, 15°55'28.86" E	182m	05-4-10	Naturhistorisches Museum, Vienna
LATHB	Austria	Hochsitz Bürgerhäufl	48°22'39.30" N, 16°11'47.70" E	178m	10-4-08	Naturhistorisches Museum, Vienna
LATST	Austria	Steinfeld	46°45'01.00'' N, 13°15'31.00'' E	584m	04-8-06	L. Juřičková, P. Tlachač
LATGR	Austria	Goritschach	46°33'23.65'' N, 14°24'03.55'' E	452m	2010	F. Šťáhlavský
LSIVI	Slovenia	Vintgar	46°23'36.00'' N, 14°05'07.00'' E	593m	02-9-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
LSEDA1 **LSEDA2**	Sweden	Dalby	55°40'25.62" N, 13°19'46.80"E	67m	09-8-11	L. Juřičková
LSIVC	Slovenia	Vršiška cesta	46°26'22.00'' N, 13°45'24.00'' E	1367m	30-8-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
LCZLL	Czech republic	Libický Luh	50°06'21.52" N, 15°10'18.01" E	198m	2010	D. Říhová
LCZLI1 **LCZLI2**	Czech republic	Libice	50°06'59.43" N, 15°10'37.08" E	197m	18-9-10	D. Říhová
LCZKU1	Czech republic	Kukla	50°37'19.61" N, 14°7'35.25" E	672m	2009	L. Juřičková, V. Szalontayová
LCZLO1 **LCZLO2**	Czech republic	Loučky	50°39'30.50'' N, 14°21'1.70'' E	480m	23-3-11	L. Juřičková, V. Szalontayová
LCZCA1 **LCZCA2**	Czech republic	Čáslav	50°40'7.80'' N, 14°16'44.30'' E	519m	16-4-11	L. Juřičková, V. Szalontayová
LCZHT1 **LCZHT2**	Czech republic	Horní Týnec	50°36'35.00'' N, 14°14'02.00'' E	510m	21-4-11	L. Juřičková, V. Szalontayová
LCZJV1 **LCZJV2** **HCZJV2**	Czech republic	Jizera u Vilémova	50°44'55.00'' N, 15°24'23.00'' E	563m	24-5-08	P. Tlachač
LCZSD2 **LCZSD3** **LCZSD4**	Czech republic	Srdov	50°35'38.00'' N, 14°15'55.00'' E	650m	21-4-11	L. Juřičková, V. Szalontayová
LSKSC	Slovakia	Šiance	48°46'26.16" N, 20°05'24.42" E		2011	I. Černajová
LSKHE	Slovakia	Herľany	48°48'06.59'' N, 21°30'06.73'' E	485m	2011	V. Szalontayová

LSKRU	Slovakia	Ružín	48°52'17,60'' N, 21°06'54,00'' E	300m	2012	V. Szalontayová, A. Hamilton
LSKZB1 **LSKZB2**	Slovakia	Zlatá Baňa	48°56'21.70'' N, 21°26'00.90'' E	672m	2012	V. Szalontayová, A. Hamilton
LSIBB	Slovenia	Bohinjska Bistrica	46°16'45.95'' N, 13°57'45.34'' E	550m	29-8-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová
LCZCP	Czech republic	Cave Na Pomezí	50°14'46,4"N, 17°08'17,6" E	573m	08-9-09	D. Říhová
LCZPC	Czech republic	Patzelova Cave	50°09'46.42"N 16°49'53.44"E	899m	2011	D. Říhová
LCZCH	*Czech republic*	*Choceň*	*50°00'08.00'' N,* *16°14'00.00'' E*	*301m*	*14-5-08*	*P. Tlachač*
LCZJM	*Czech republic*	*Jizerské Mountains*	*not given* *(Tlachač 2008)*	*not given*	*28-9-07*	*L. Juřičková*
LSKUH	Slovakia	Uhorské	48°27'59.50'' N, 19°45'44.70'' E	258m	2012	V. Szalontayová, A. Hamilton
LRSGO1 **LRSGO2**	Serbia	Golubac	44°39'36.90" N, 21°40'37.95" E	85m	2012	O. Korábek
LCZMO1 ***LCZMO2***	*Czech republic*	*Myslivna u Ohře*	*50°23'47.00'' N,* *14°04'49.00'' E*	*174m*	*28-6-07*	*P. Tlachač*
LRSLV1 **LRSLV2**	Serbia	Lepenski Vir	44°33'05.94" N, 022°01'39.00" E	98m	2012	O. Korábek

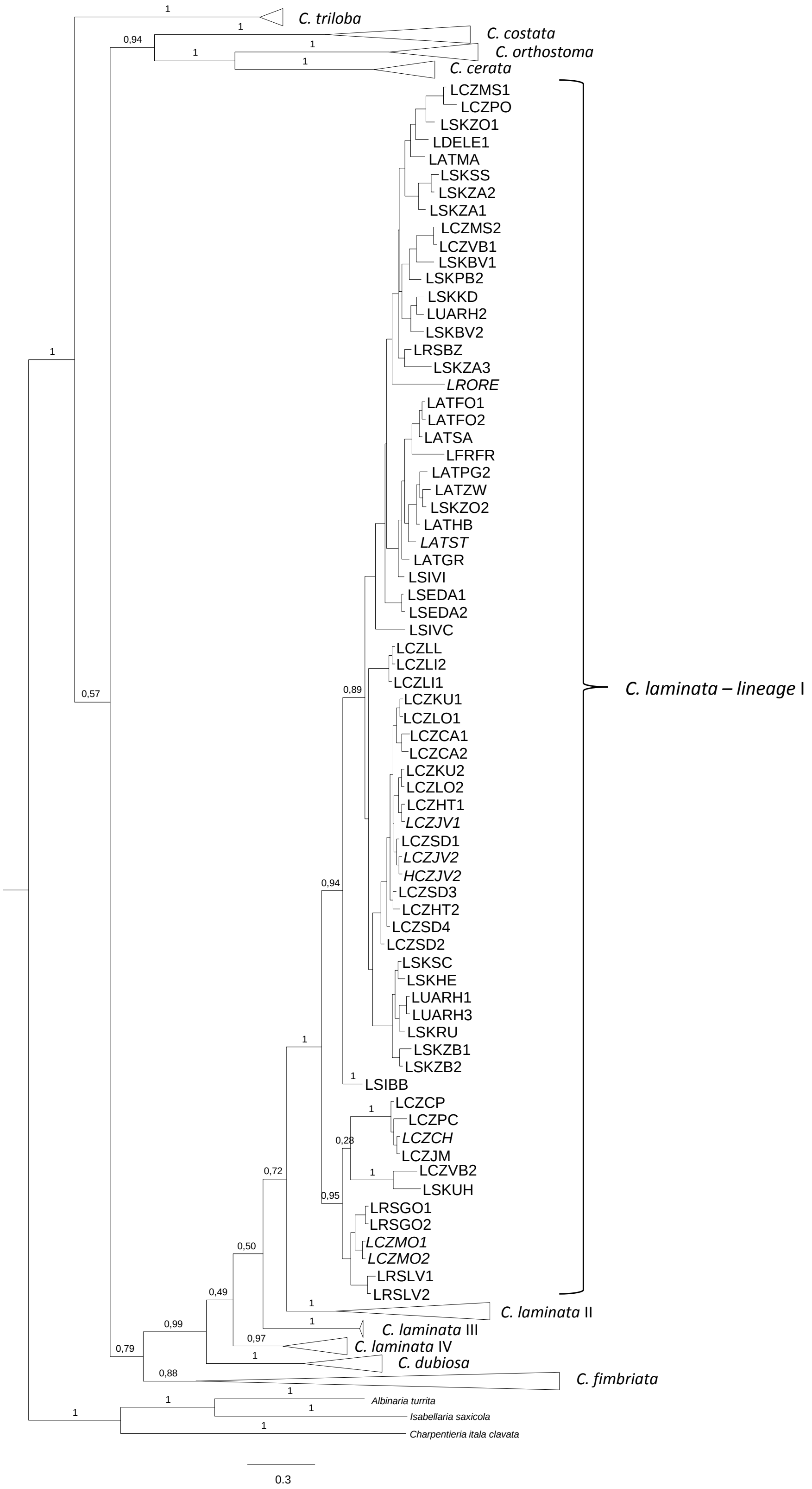

Figure 24: Tree created by Bayesian analysis, showing the position of individuals within the lineage I of *C. laminata*.

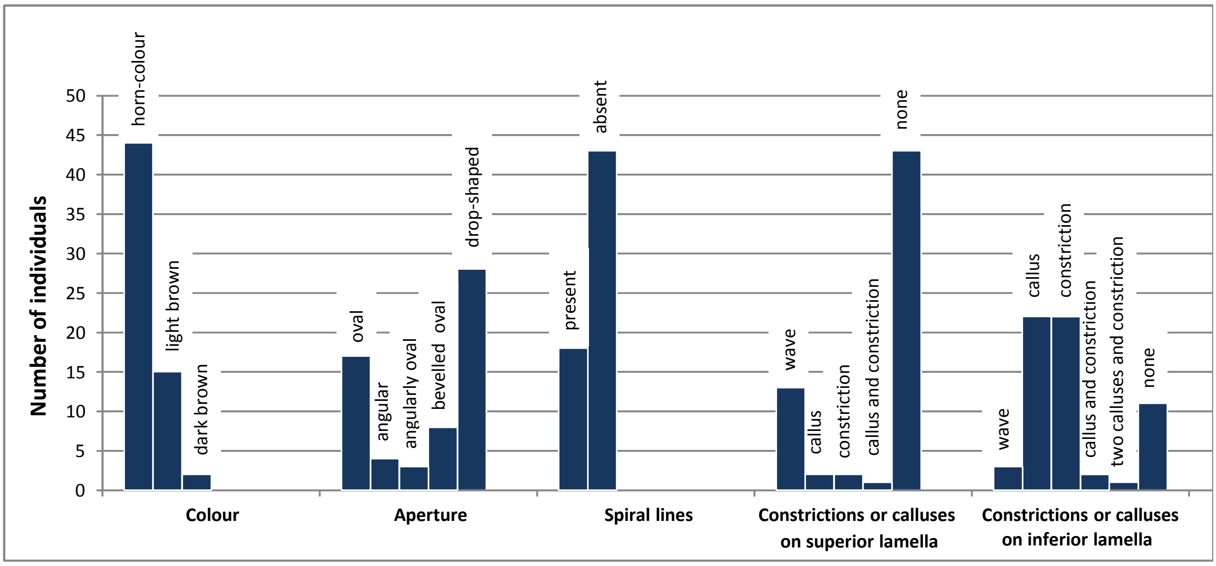

Figure 25a: Some of the morphological characteristics on the surface of the shell and those visible on the apertural view of the shell of *C. laminata* individuals belonging to lineage I.

This group of land snails was very variable in their traits. Colour varied from horn-colour to dark brown. Five different apertural shapes occurred here. Spiral lines were present in less than a half of the specimens and there was great vaiability in forms of superior and inferior lamellae (Figure 25a).

Principal plica was always visible. Lower palatal plica and subcolumellar lamella were visible in majority of the cases. In all cases but one, the principal plica and lower palatal plica both merged with the palatal callus (Figure 25b).

In one individual (LUARH3), plicae of the palatal side did not touch the palatal callus were which seems to be a younger individual and the palatal callus was almost undeveloped here. However, principal plica and lower palatal plica both reached the place where palatal callus usually occurs. Had it been more developed, these plicae would merge with it.

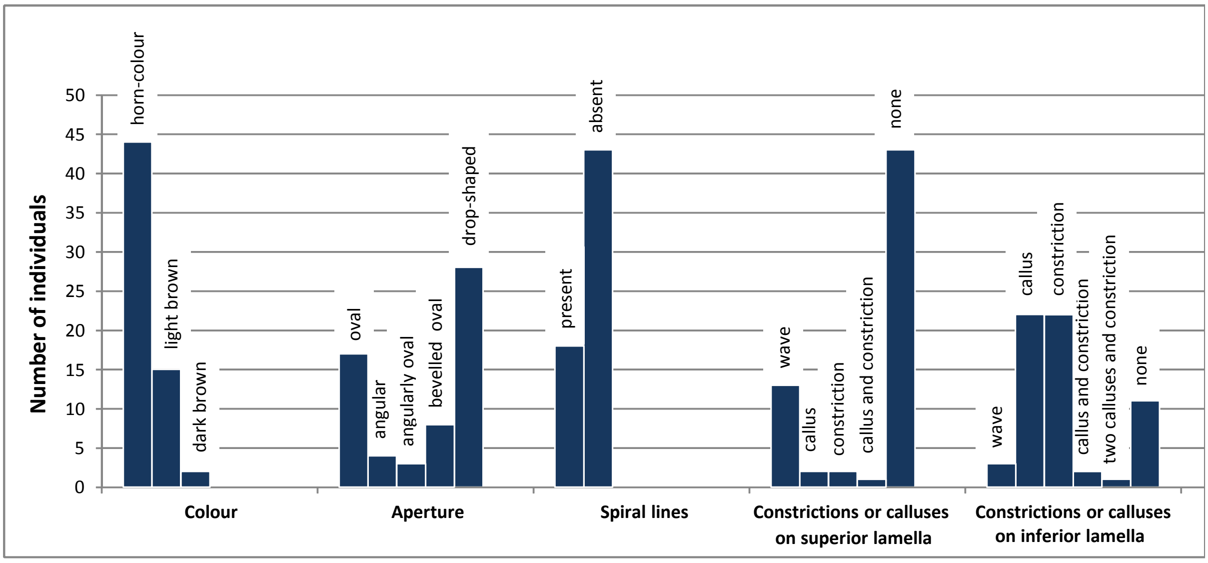

Figure 25b: Some of the morphological characteristics visible on the apertural view of the shell of *C. laminata* individuals belonging to lineage I.

In majority of cases, the beginning of principal plica was placed before the beginning of upper palatal plica (Figure 25c). In two individuals (LCZLL and LCZPC), the position of these two plicae was impossible to assess due to severe corrosion of the shell. These individuals were excluded from the analysis of the positions of the beginnings of the plicae and their mutual position.

Other individuals were excluded from the assessment of the mutual position of principal plica and upper palatal plica for various reasons. There was an extra plica on the palatal side of the individuals LRSBZ and LRSGO2, which made it impossible to state, which of the plicae is the extra one and which is the original upper palatal plica. In individual LATGR, the upper palatal plica had a very short, knob-like form. In this case it was impossible to assess, whether it is parallel or non-parallel to the principal plica.

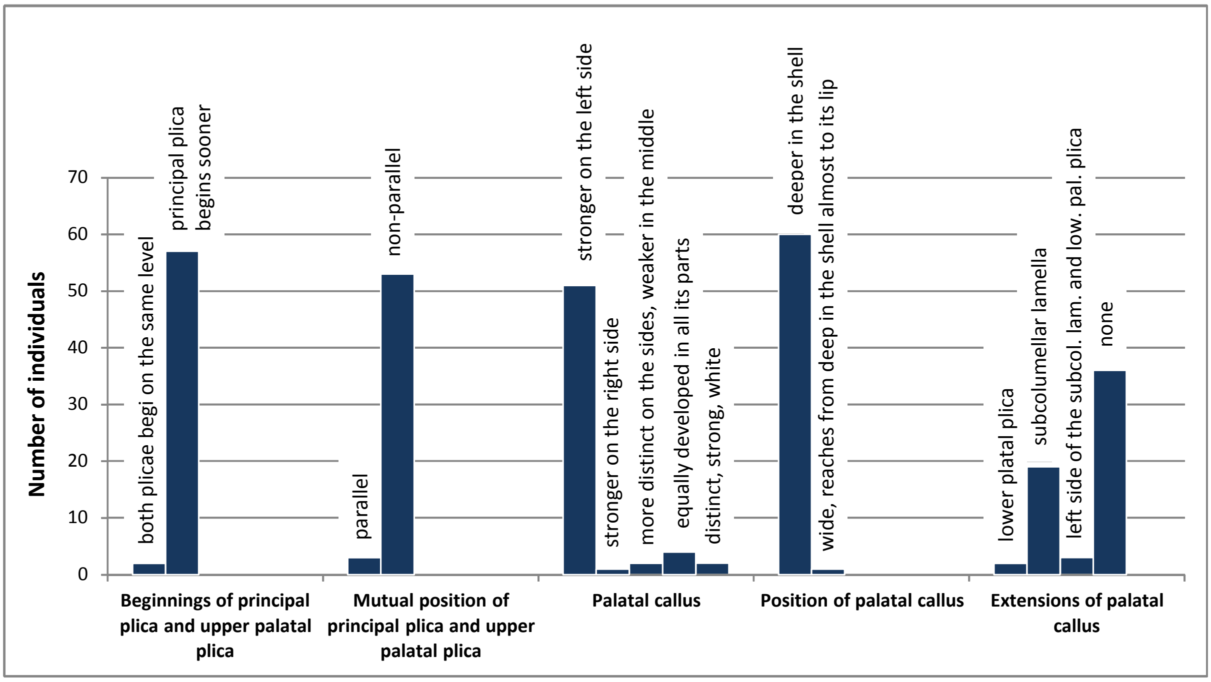

Figure 25c: Some of the morphological characteristics regarding plicae of the palatal side and palatal callus of *C. laminata* individuals belonging to lineage I.

Palatal callus was unexpectedly variable in its form, reaching different strength in various of its parts, however, it was mostly stronger on its left side, as described by Brabenec (1967). However, individuals with palatal callus stronger more developed on the right side or on both sides occurred as well, and most surprisingly, there were a few individuals with a very strong and distinct white palatal callus (LCZSD3 and LATPG2). What is interesting is that there were three more specimens from the same site as LCZSD3 and this kind of palatal callus was not present in any of them. Moreover, this type of palatal callus is in strong discordance with Ložek (1956), who describes it as not having distinct edges .

Photographs of some of the individuals belonging into this lineage are presented in figure 26.

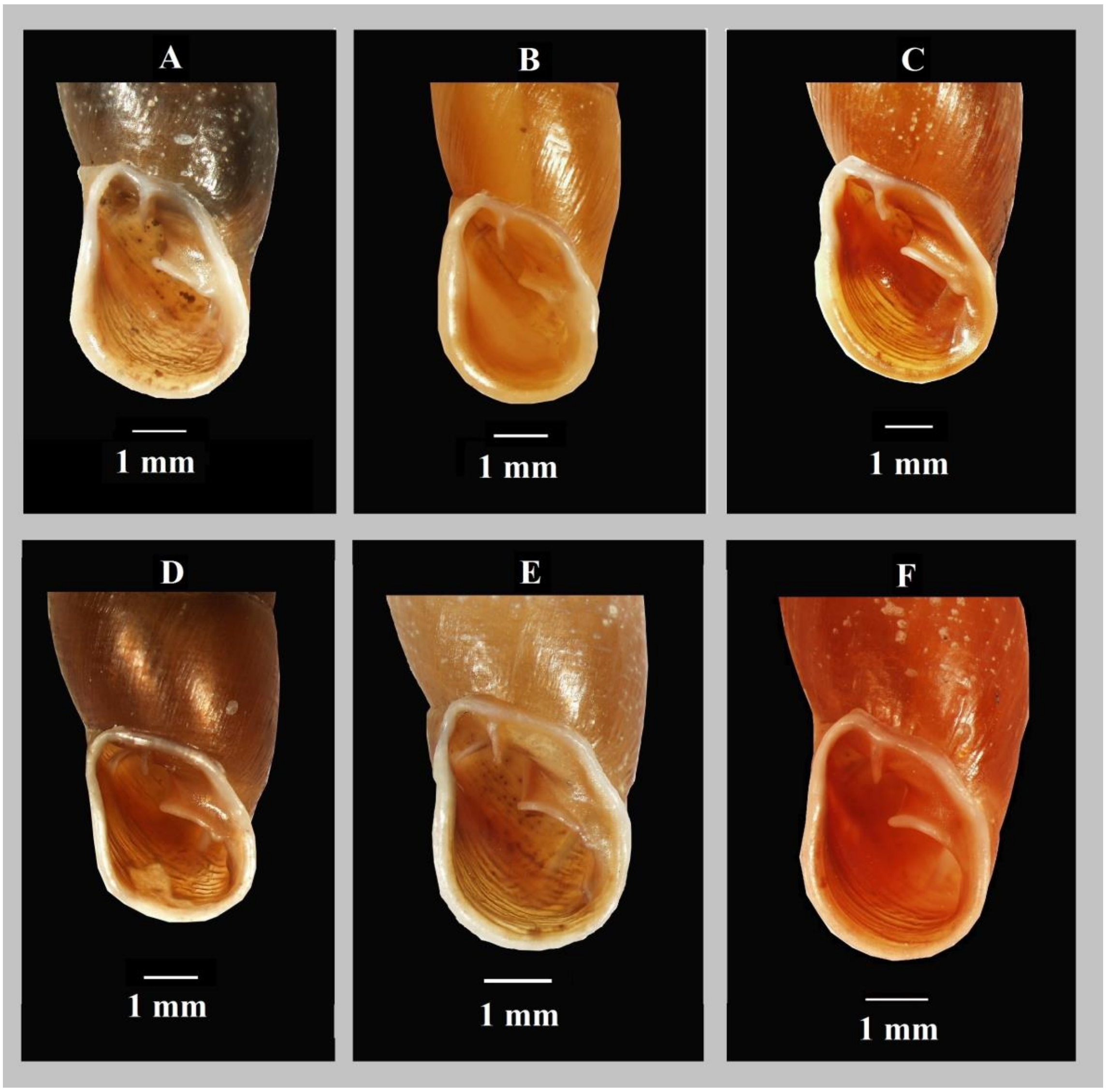

Figure 26: Photographs of apertural views of some of the *C. laminata* I individuals analyzed in this study (A: LATFO2; B: LATPG2; C: LSIBB; D: LRSBZ; E: LDELE1; F: LSKZA1)

4.2.5.2. *Cochlodina laminata* – lineage II

Clade II of *C. laminata* consisted of 60 individuals (table 7). 6 of these individuals were sequenced by Tlachač (2008). Of the remaining 54 individuals that were newly sequenced for this study, 4 were excluded from the analysis of morphological characters. The reason for this exclusion were following. LFRPL LDELC1 and LDELC2 were young individuals apertures of which were not formed yet. Shell of individual LCZSR was highly anomalous due to severe physical injury (Figure 51c). Table 7 lists all individuals belonging to this clade.

Table 7: List of *C. laminata* individuals from lineage II used in this study. Individuals collected by Tlachač (2008) are listed in *italics* and those that he determined as intermediate forms between *C. laminata* and *C. dubiosa corcontica* are underlined.

***Cochlodina laminata* (Montagu, 1803) - lineage II**

Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
LCZHL	Czech republic	Hradišťanská louka	50°30'29.45"N, 13°52'12.03" E	745m	2009	L. Juřičková, V. Szalontayová
LCZMC1 **LCZMC2**	Czech republic	Malá Chuchle	50°01'29.46" N, 14°23'24.31" E	254m	08-10-10	D. Říhová
LCZBL1 ***LCZBL2*** ***HCZBL2***	*Czech republic*	*Bártův Les*	*50°37'31.00'' N, 15°51'02.00'' E*	*679m*	*24-6-07*	*P. Tlachač*
LDEKE	Germany	Kelheim	48°54'54.54" N, 11°51'58.14" E	345m	09-8-11	M. Proćków
LDELE2	Germany	near Lehrensteinsfeld	49°06'49.32"N, 09°19'28.98" E	288m	27-6-12	L. Juřičková
LDESC	Germany	Schillingstadt	49°26'35.64" N, 09°32'27.96" E	392m	27-6-12	L. Juřičková
LSKVT	*Slovakia*	*Vtáčnik*	*48°38'18.00'' N, 18°36'47.00'' E*	*923m*	*13-5-08*	*P. Tlachač*
LSKDN	Slovakia	Dobrá Niva	48°27'59.50'' N, 19°06'15.70'' E	359m	2012	V. Szalontayová, A. Hamilton
LSKHA	Slovakia	Havran near Adranovce	48°33'55.20'' N, 17°52'38.10'' E	402m	2012	V. Szalontayová, A. Hamilton
LCHEI	Switzerland	Eiken	47°31'03.42" N, 07°59'28.08" E	471m	11-8-11	M. Proćków
LFRPL	France	Plumont	47°06'53.40" N, 05°43'52.92" E	263m	26-6-12	L. Juřičková
LSKPK	Slovakia	near cottage "Pod Končitou"	48°38'53.87" N, 18°35'18.57" E	504m	01-7-12	V. Szalontayová, A. Hamilton
LDEBO1 **LDEBO2**	Germany	Bodenstein	52°01'34.80" N, 10°13'15.06" E	248m	30-6-12	L. Juřičková

LDETU	Germany	Tübingen	48°30'35.94" N, 09°01'32.58" E	421m	21-6-12	L. Juřičková
LDEVE	Germany	Veckerhagen	51°29'21.36" N, 09°33'11.16" E	259m	29-6-12	L. Juřičková
LDERO1 **LDERO2**	Germany	Rothemann	50°27'17.82" N, 09°42'29.82" E	418m	29-6-12	L. Juřičková
LATHK	Austria	Hermannskogel	48°16'03.66" N, 16°11'47.70" E	460m	24-3-11	Naturhistorisches Museum, Vienna
LCZOB1 **LCZOB2**	Czech republic	Obřany	49°22'1.20" N, 17°43'59.87" E	685m	28-9-10	L. Juřičková, V. Szalontayová
LSKPB2	Slovakia	Podbrezová	48°48'30.47" N, 19°32'19.42" E	503m	15-6-10	V. Szalontayová, L. Juřičková, P. Synek
LMEVU1	Montenegro	Vusanje	42°29'23.56" N, 19°48'26.60" E	1245m	2012	O. Korábek
LRSSJ1 **LRSSJ2**	Serbia	St. Jovan	42°57'52.20" N, 22°37'14.88" E	534m	2012	O. Korábek
LRSRS1 **LRSRS2**	Serbia	Rsovci	43°10'31.40" N, 22°44'45.45" E	764m	2012	O. Korábek
LCZCL	*Czech republic*	*Český les*	*49°40'51.00'' N, 12°39'59.00'' E*	*814m*	*02-5-08*	*Schläglová*
LCZSU	*Czech republic*	*Šumava*	*49°06'54.00'' N, 13°14'17.00'' E*	*756m*	*17-5-08*	*P. Tlachač*
LRSKN	Serbia	Knjaževac	43°32'18.63" N, 22°17'11.76" E	255m	2012	O. Korábek
LDKSN1 **LDKSN2** **LDKSN3**	Denmark	Stensved	54°58'42.54" N, 12°01'10.50" E	36m	10-8-11	L. Juřičková
LSELU1 **LSELU2** **LSELU3**	Sweden	Ludvika	60°07'08.10" N, 15°07'13.56" E	254m	30-7-11	L. Juřičková
LSEKI1 **LSEKI2** **LSEKI3**	Sweden	Kinnekulle	58°34'08.82" N, 13°26'40.80" E	83m	29-7-11	L. Juřičková
LSEDA3	Sweden	Dalby	55°40'25.62" N, 13°19'46.80"E	67m	09-8-11	L. Juřičková
LDELE1	Germany	near Lehrensteinsfeld	49°06'49.32"N, 09°19'28.98" E	288m	27-6-12	L. Juřičková
LATGS	Austria	Grimmenstein	47°37'56.94" N, 16°07'17.34" E	600m	04-7-10	Naturhistorisches Museum, Vienna
LATPG1	Austria	Planspitzgraben	47°35'17.82" N, 14°38'19.62" E	625m	26-4-08	Naturhistorisches Museum, Vienna
LATBI1 **LATBI2**	Austria	Bings	47°08'42.67'' N, 09°52'00.10'' E	672m	2010	F. Šťáhlavský
LFRLC	France	Les Carronnières	46°01'24.84" N, 05°16'36.06" E	345m	26-6-12	L. Juřičková
LFRCR	France	Cruiseaux	46°29'28.08" N, 05°20'32.04" E	228m	22-6-12	L. Juřičková
LDEWO	Germany	Wolfgantzen	48°01'24.48" N, 07°29'29.70" E	195m	27-6-12	L. Juřičková

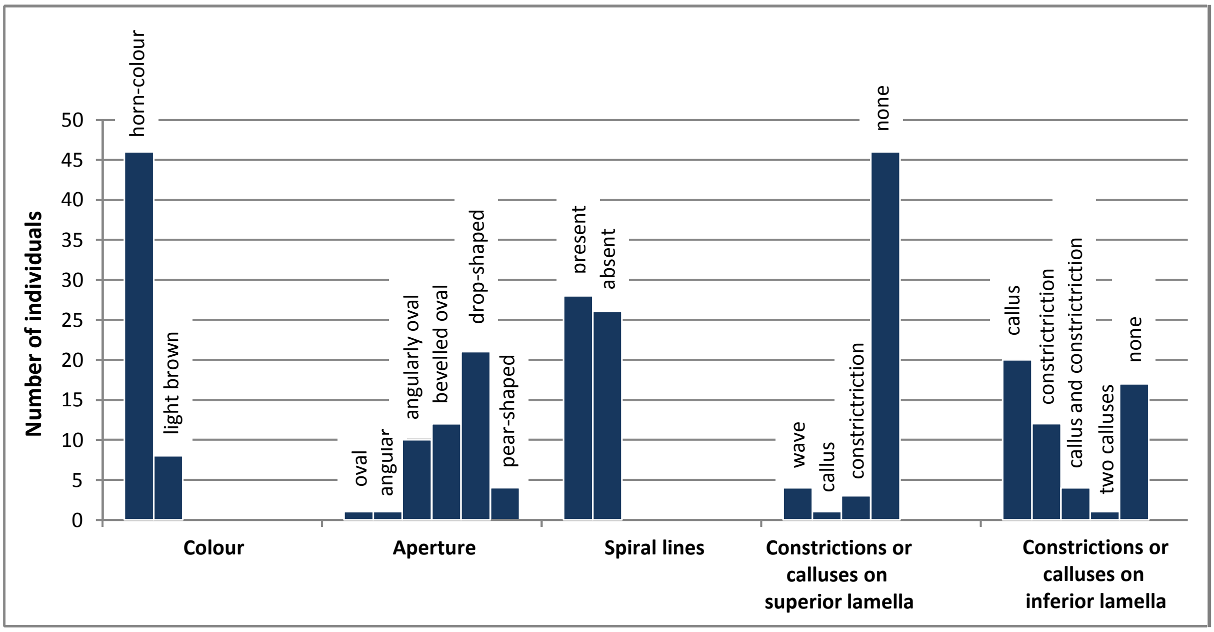

Figure 27a: Some of the morphological characteristics regarding surface of the shell and morphological characteristics visible o apertural view of the shells of *C. laminata* individuals belonging to lineage II.

This group of land snails was very variable in their traits. Colour varied from horn-colour to light brown. Six different apertural shapes occurred here. Spiral lines were present in approximately half of the specimens and there was great vaiability in forms of superior and inferior lamellae (Figure 27a).

Principal plica and lower palatal plica were always visible. Subcolumellar lamella was visible in majority of the cases (Figure 27b).

In all cases but one, the principal plica and lower palatal plica both merged with the palatal callus. In all cases but one (LDEVE) did principal plica touch the palatal callus (Figure 27b).

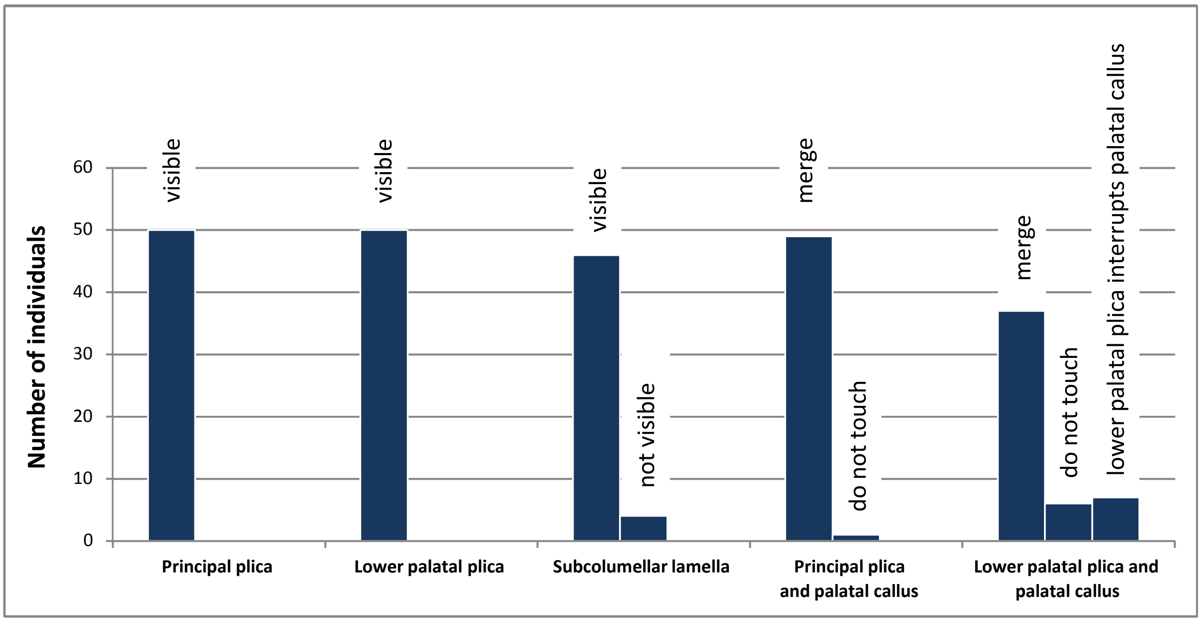

Figure 27b: Some of the morphological characteristics regarding plicae of the palatal side and palatal callus of *C. laminata* individuals belonging to lineage II.

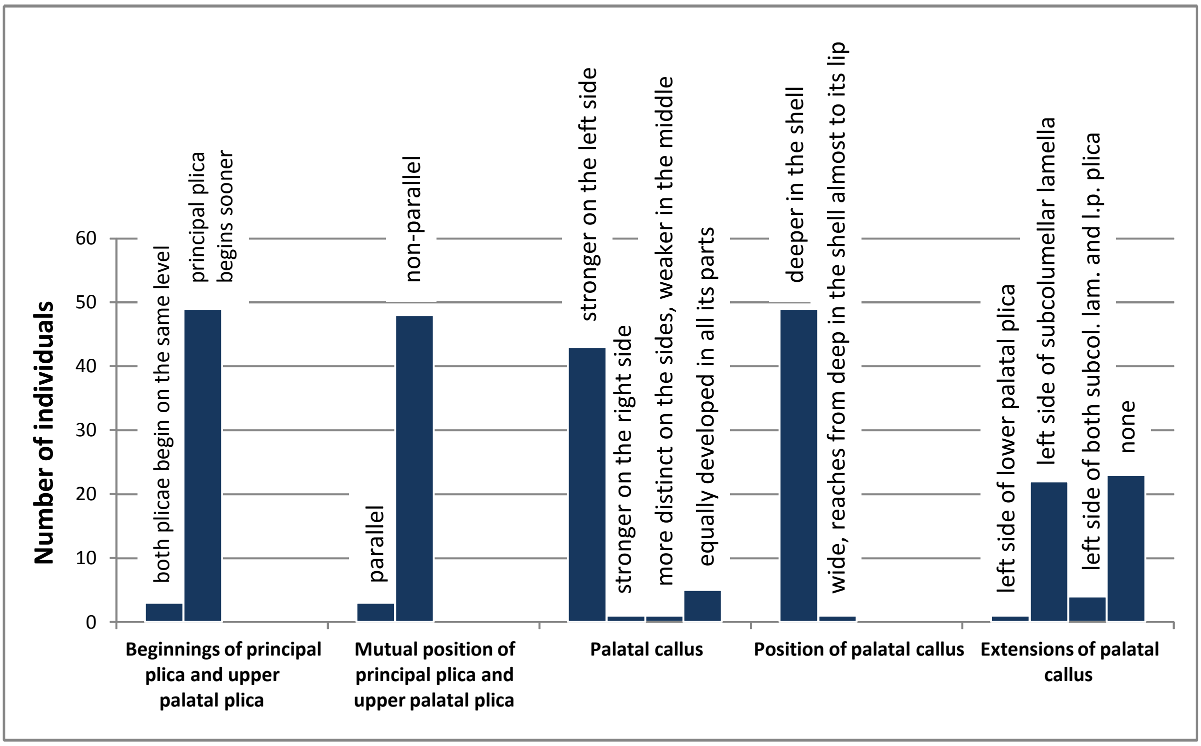

Figure 27c: Some of the morphological characteristics regarding plicae of the palatal side and palatal callus of *C. laminata* individuals belonging to lineage II.

Lower palatal plica merged with palatal callus in most cases. In four cases (LATBI1, LATBI2,LMEVU1, LMEVU2) it did not, because the palatal callus was not present on the right side. However, the lower palatal plica did reach the area where the callus would be, had it been more developed. Had the individuals been older or had the palatal callus been more developed, these individuals would be included in the first category, with majority of individuals from this clade.

In five cases (LDKSN2, LRSKN, LRSSJ2, LRSRS1, LDEBO1 and LCZMC2), lower palatal plica interrupted palatal callus, in a similar way that is typical for *C. costata*.

In majority of cases, the beginning of principal plica was placed to the left of the beginning of upper palatal plica. These two plicae were mostly parallel to each other (Figure 27c).

Palatal callus was differently developed in different individuals, however, it was never white and distinct, as was observed in some of the individuals belonging to lineage I. Wider form of palatal callus was observed in LATPG1. Numerous individuals formed extensions of the palatal callus, extending along the subcolumellar lamella, lower palatal plica or both of these structures (Figure 27c).

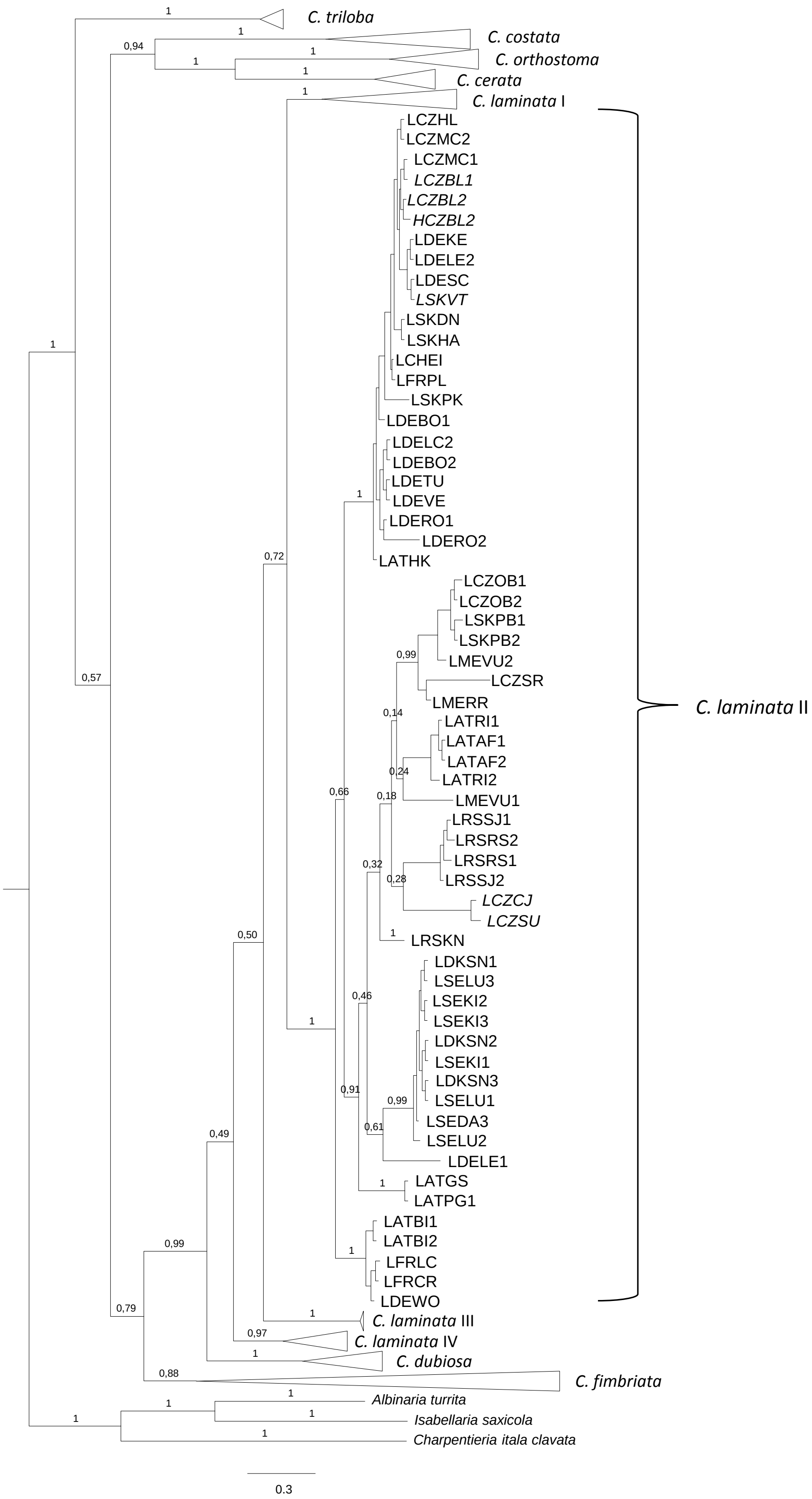

Figure 28: Tree created by Bayesian analysis, showing the position *C. laminata* individuals belonging to lineage II.

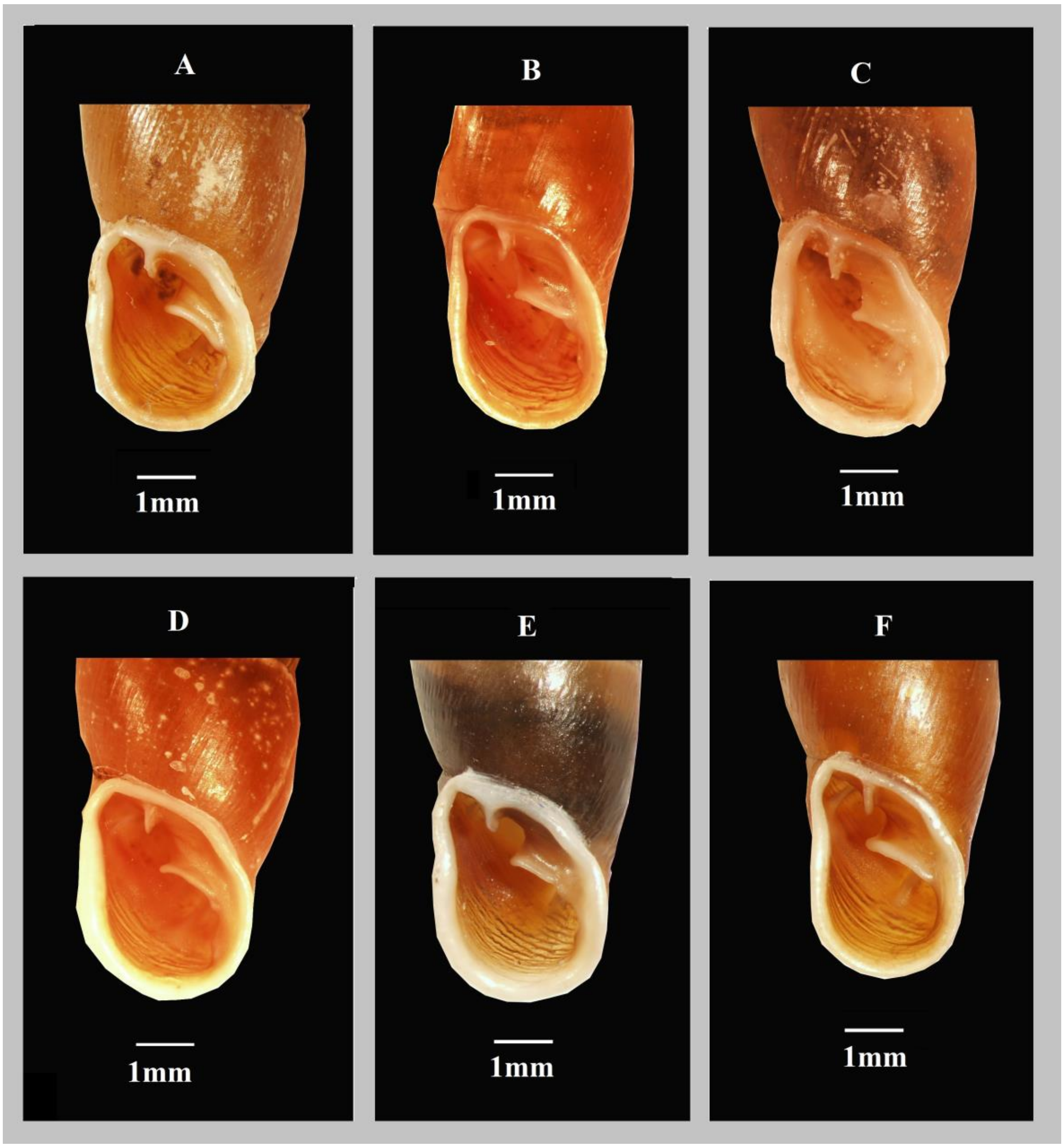

Figure 29: Apertural views of some of the *C. laminata* individuals belonging to lineage II, analyzed in this study.

(A: LCZMC1; B:LSKDN; C: LATHK; D: LCHEI; E: LDEKE; F: LRSRS1)

4.2.5.3. *Cochlodina laminata* – lineage III

Of the all 140 sequenced individuals, only two happened to be included in lineage III (figure 31). Both of these were obtained from the same locality (Lukovo, Croatia) and share most morphological characteristics and are listed in table 8. Morphology of this lineage is very different from that described as typical for *C. laminata*

Table 8: List of *C. laminata* individuals from lineage III used in this study

***Cochlodina laminata* (Montagu, 1803) - lineage III**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
LHRLU1 **LHRLU2**	Croatia	Lukovo	45°12'18.00" N, 14°46'58.86" E	845m	01-9-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová

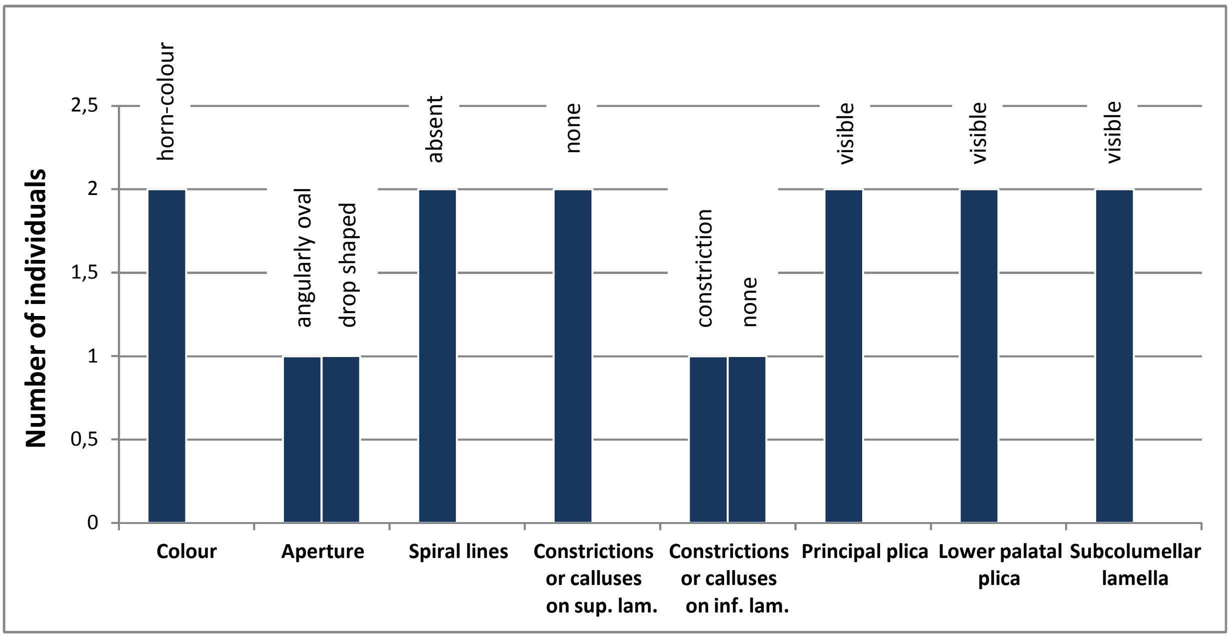

Figure 30a: Some of the morphological characteristics of *C. laminata* individuals belonging to lineage III (sup. lam. = superior lamella; inf. lam. = inferior lamella).

Both individuals were very similar. Their shells were horn-coloured. They did have different shapes of aperture, but this is not surprising, given the apertural shape variability already presented in other species mentioned in this thesis. Both individuals posessed spiral lines. Principal plica, lower palatal plica and subcolumellar lamella were visible on apertural view of the shell (Figure 30a).

There were no constrictions or calluses on superior lamella and one of the individuals (LHRLU1) posessed a constriction on its inferior lamella (Figure 30a).

Principal plica and upper palatal plica were non-parallel in both cases, with the beginning of the principal plica being shifted to the left side compared to the beginning of upper palatal plica when viewed from the abapertural side. Palatal callus was distinct, strong and calcified, white in colour in both individuals. It was developed deeper in the shell and formed an extension along the left side of subcolumellar lamella (Figure 30b).

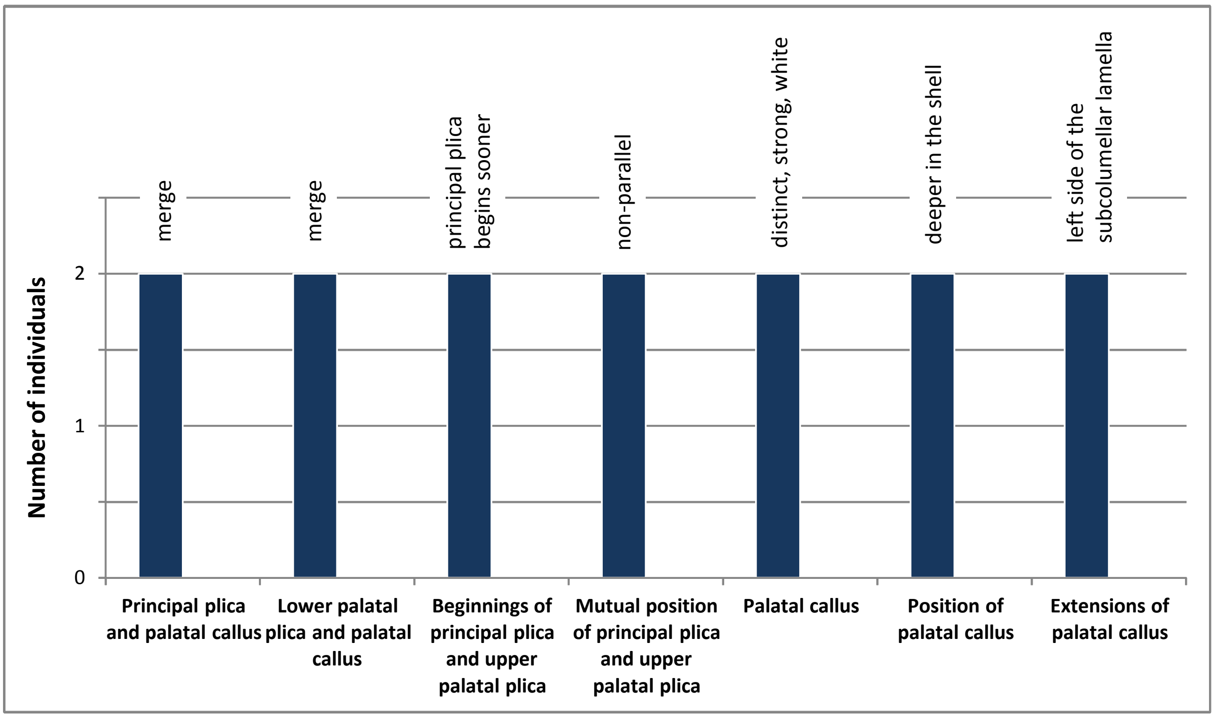

Figure 30b: Some of the morphological characteristics regarding plicae of the palatal side and palatal callus of *C. laminata* – lineage III individuals.

Even though both palatal calluses lie in position which is typical for *C. laminata* (deeper in the shell), they are very strong and similarly as some individuals of lineage I and lineage II, they do not match Ložek's (1956) description of the species. However, they do match almost all morphological features that I regarded as reliable and non-variable for *C. dubiosa* in „Discussion", except for presence of extensions of palatal callus in lineage III and their absence in *C. dubiosa*.

Lower palatal plica of both individuals merged with palatal callus, even though in LHRLU1 this was obvious as the lower palatal plica was very white in colour, while in LHRLU2 the plica was coloured differently and its white part did not reach as far as the palatal callus, giving the impression that these two do not touch. However, on closer observation, the lower palatal plica merges with palatal callus with its brown part.

It is possible that individual LHRLU2 is younger and its lower palatal plica is not as calcified as in LHRLU1 and therefore not as white in colour (as can be seen in figure 32).

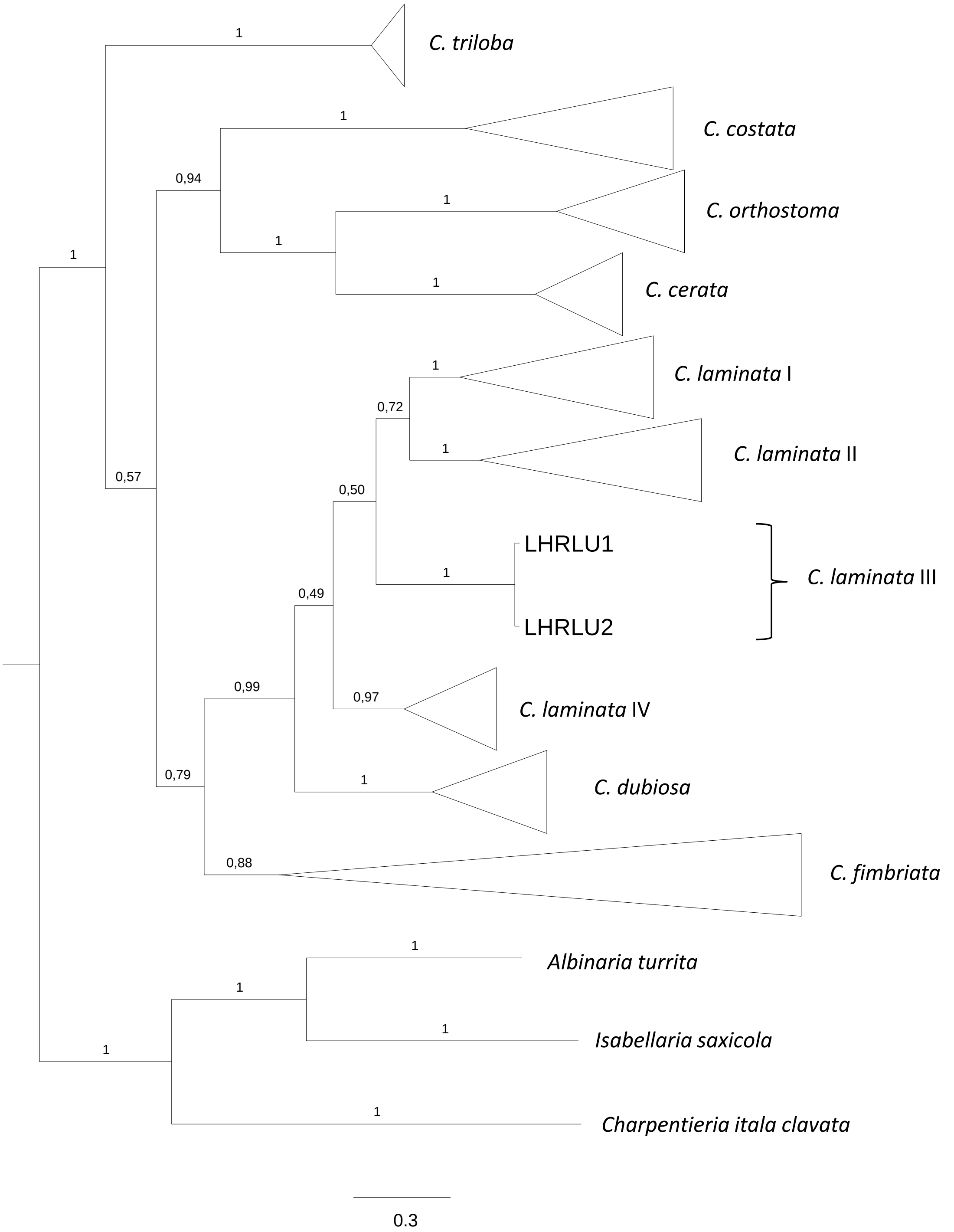

Figure 31: Tree created by Bayesian analysis, showing the position of individuals within lineage III of *C. laminata*.

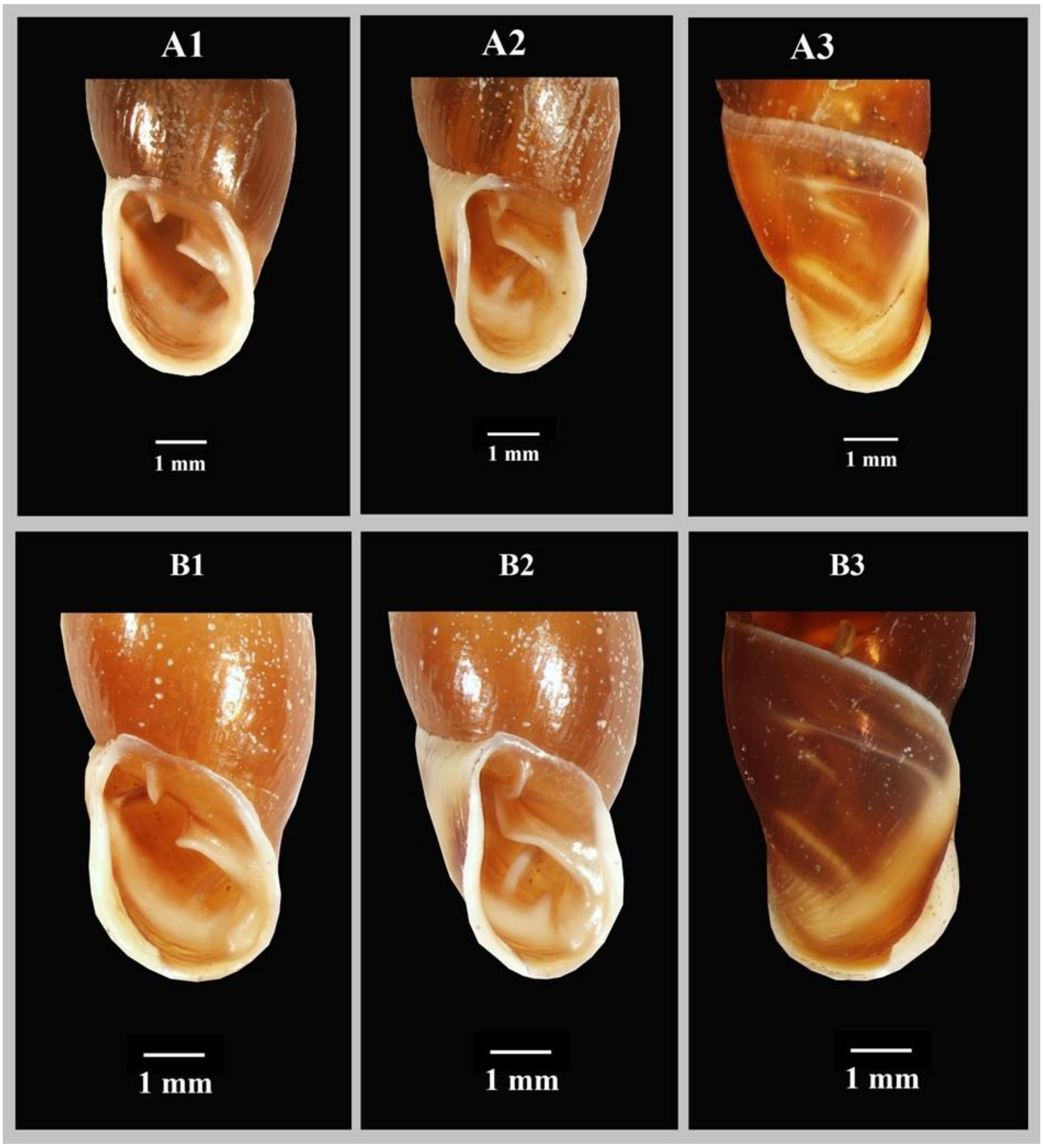

Figure 32: Apertural views of both of the *C. laminata* – lineage III individuals analyzed in this study (A: LHRLU1; B: LHRLU2)

4.2.5.4. *Cochlodina laminata* – lineage IV

There were seven specimen of *C. laminata* in lineage IV (table 9, figure 34). All of them were included in the assessment of morphological characteristics.

Table 9: List of *C. laminata* individuals from lineage IV used in this study.

***Cochlodina laminata* (Montagu, 1803) - lineage IV**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
LSITS1 **LSITS2**	Slovenia	Tomičev Slap	46°23'54.24" N, 14°16'26.76" E	700m	8.5.2009	L. Juřičková, A. Petrusek, V. Szalontayová
LFRLP	France	Le Pont du Lac	45°26'01.20" N, 05°52'31.80" E	764m	13-8-11	M. Proćków
LSIOB	Slovenia	over Bohinjska Bistrica	46°16'33.66" N, 14°00'27.72" E	856m	30-8-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová
LSIBJ	Slovenia	Bohinjsko jezero	46°16'53.00'' N, 13°50'53.00'' E	531m	02-9-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
LSIKA	Slovenia	Kamnik	46°12'03.18" N, 14°36'04.92" E	356m	06-9-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová
LDEGU	Germany	Günzburg	48°27'56.10" N, 10°17'08.70" E	448m	05-8-11	M. Proćków

Colour of the shell ranged from horn-colour to light brown. Shape of aperture was highly variable. Spiral lines were only present in one specimen (LFRLP). There were no constrictions or calluses on superior lamella, while there was a great variety of shapes occurring on inferior lamella (Figure 33a).

Principal plica, lower palatal plica and subcolumellar lamella were all visible on apertural view of the shell (Figure 33a).

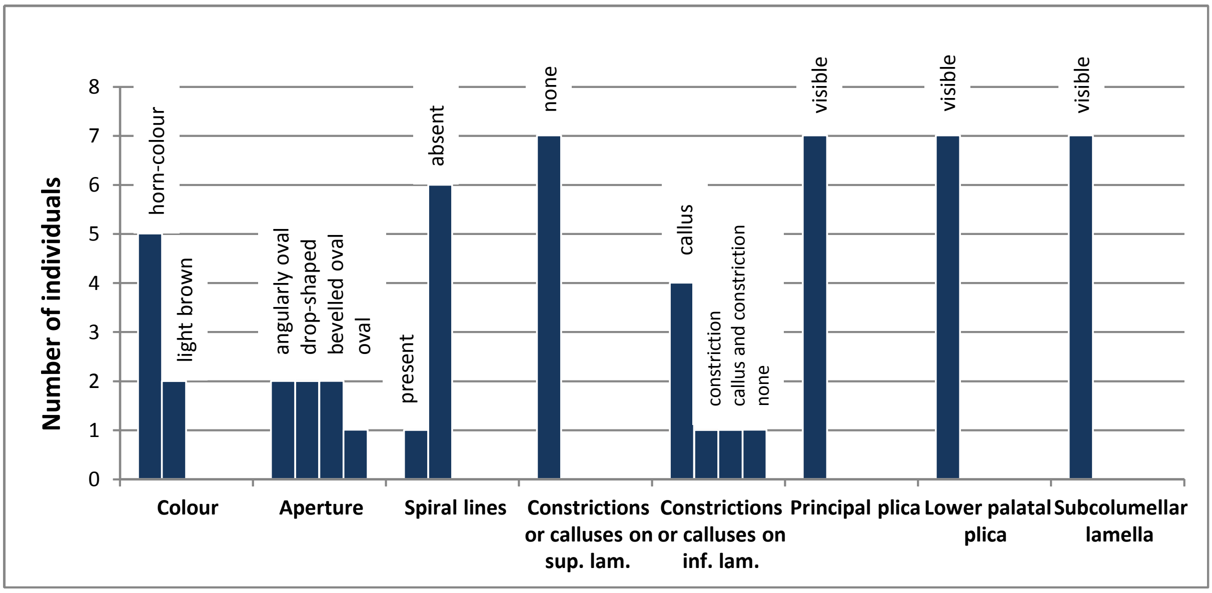

Figure 33a: Some of the morphological characteristics visible on the surface of the shell and apertural side of the shell of *C. laminata* individuals belonging to lineage IV.

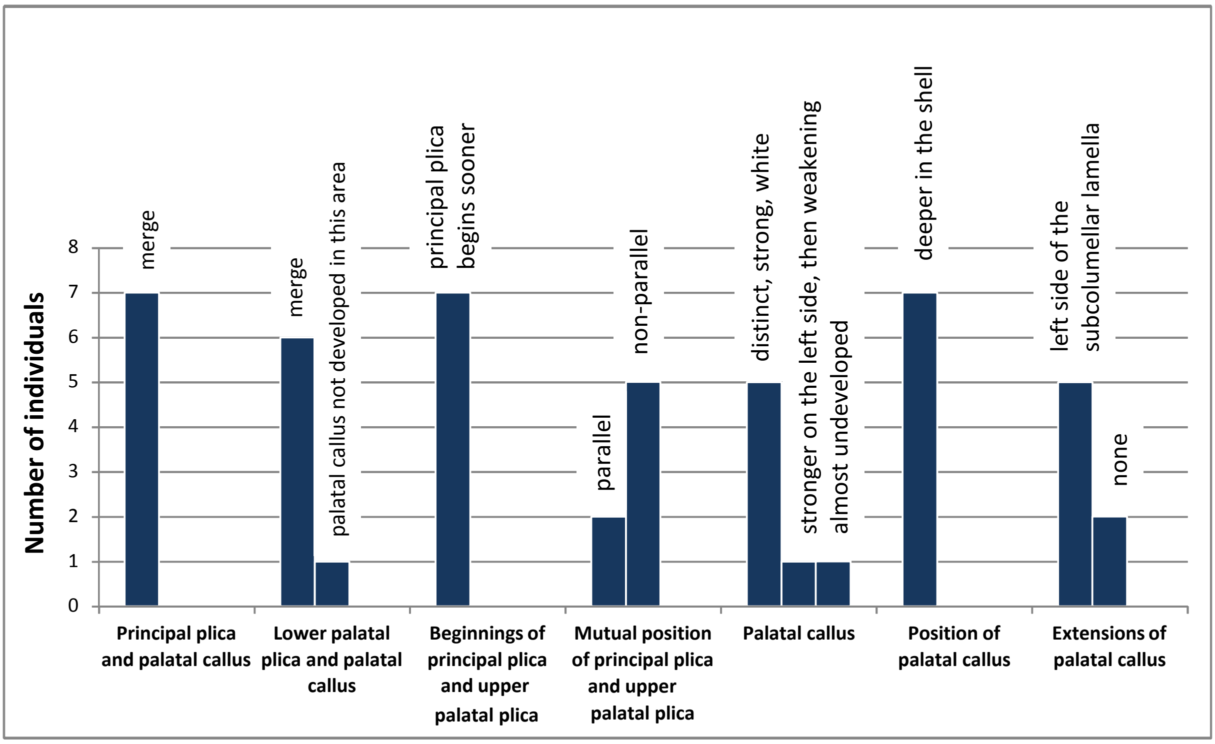

Figure 33b: Some of the morphological characteristics regarding plicae of the palatal side and palatal callus of *C. laminata* individuals belonging to lineage IV.

In all cases, principal plica joined and merged with palatal callus. Lower palatal plica joined palatal callus in most cases, except for one (LDEGU, pictured in figure 35) where palatal callus was very poorly developed and was not visible on the right side even when strong light was shone through the shell. However, lower palatal plica reached as low as it did in other specimens, the only reason that it did not join palatal callus was the absence of the palatal callus in this part of shell (figure 33b).

Most of the specimen in this lineage have a strong, calcified and well visible palatal callus, extending upwards along the left side of subcolumellar lamella, which is in disagreement with all descriptions of this species mentioned in the determining keys and other literature (Ložek 1956, Brabenec 1967).

Five of the specimen formed extensions along the left side of subcolumellar lamella. All these specimen had a very distinct and strongly calcified palatal callus. Two other idividuals (LSIBJ and LDEGU) had poorly developed palatal calluses, which did not form any extensions.

Specimen LSIBJ had a poorly developed palatal callus. This callus did not extend along any of the lamellae and weakened from left to right. Specimen LDEGU had even less developed palatal callus than specimen LSIBJ. The callus here is almost non-existent and is only visible as a darker area on the shell when the light is shone through the s

As the palatal callus is weak on its right side in both of these individuals, it is not surprising, that the lower palatal fold, even though it reaches to the area where the palatal callus would be, does not merge with it, because the palatal callus is not visible in this part of the shell.

None of these two specimen looked juvenile and they both had a well developed and fully grown aperture and so the weakness of the palatal callus is most likely not age-related in this case.

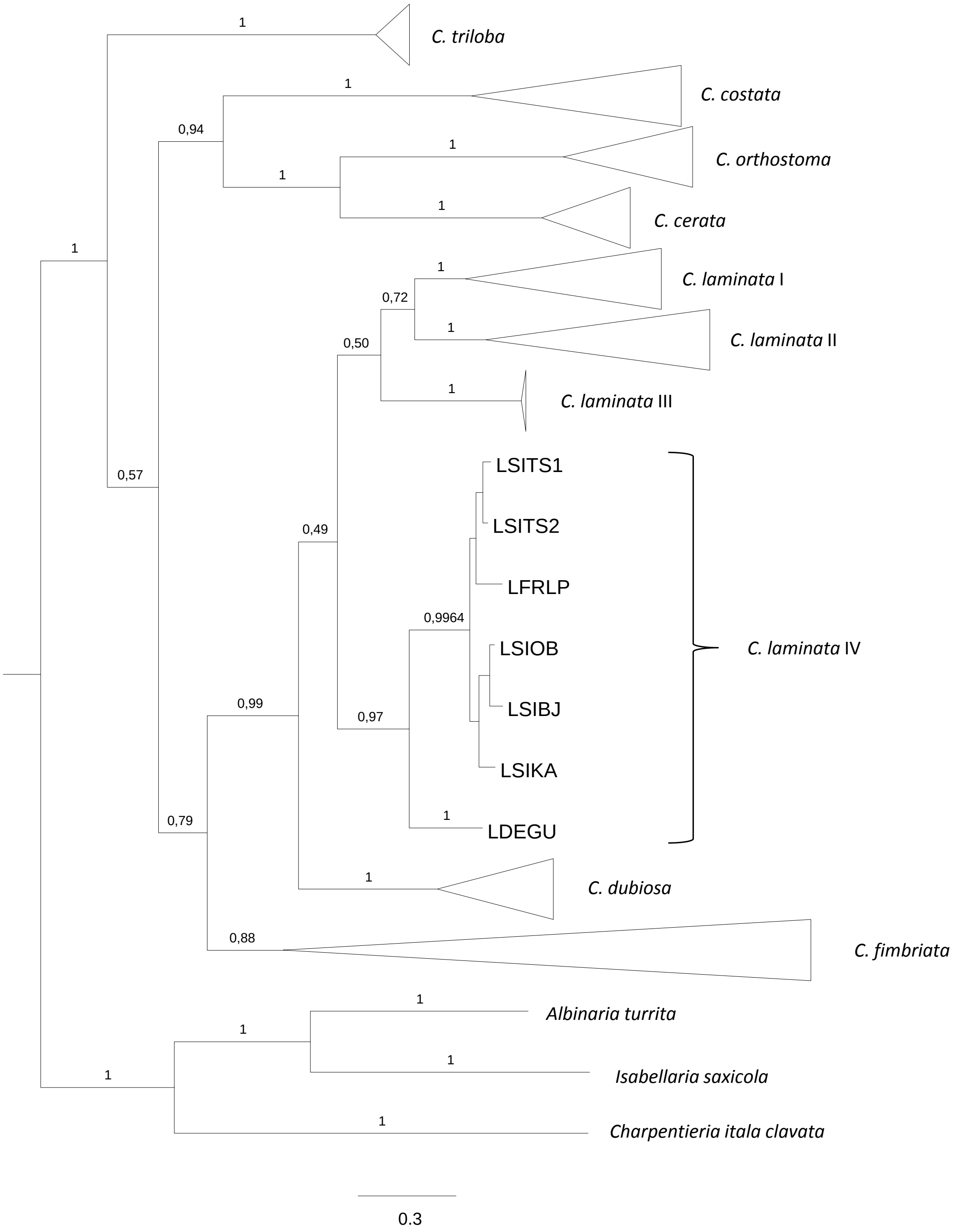

Figure 34: Tree created by Bayesian analysis, showing the position of individuals within the clade of *C. laminata* individuals belonging to lineage IV.

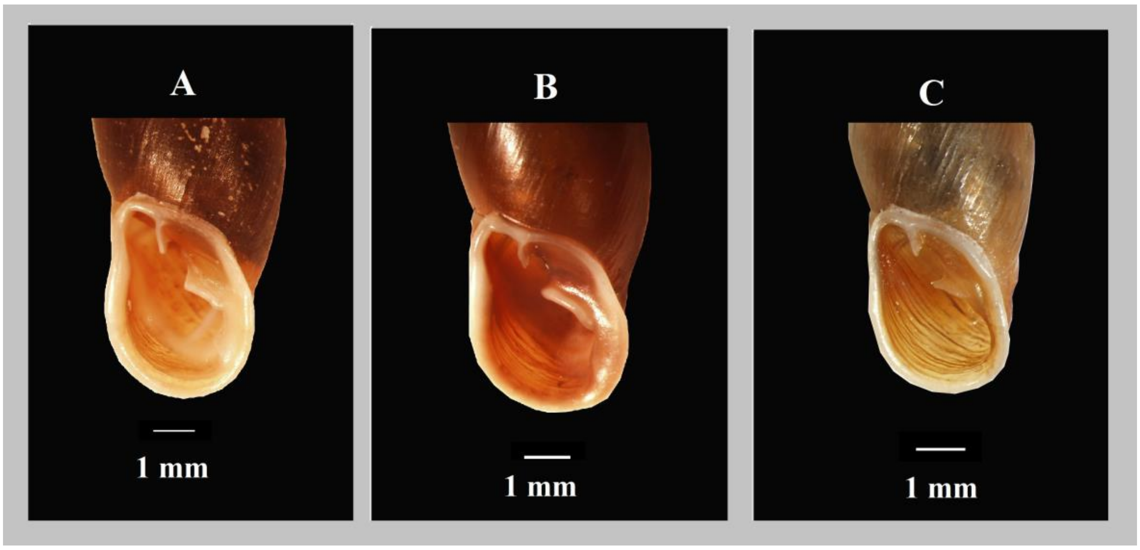

Figure 35: Apertural views of some of the *C. laminata* individuals belonging to lineage IV that were analyzed in this study (A: LSITS2 ; B: LSIKA; C: LDEGU)

4.2.6. *Cochlodina dubiosa*

Sequences of 19 individuals of *C. dubiosa* were used in this analysis. Ten of them were retrieved from Tlachač's study (2008) and nine were newly acquired. These nine individuals were analyzed for their morphological traits.

Table 10 sums up all individuals used. Geographical position of collection sites is shown in map in figure 36.

Table 10: All individuals of *C. dubiosa* used in this study. Individuals listed in *italics* are the ones from study by Tlachač (2008). Those marked with a dot (•) were determined as subspecies *C. dubiosa corcontica*, while underlined codes belong to individuals that were determined as hybrids between *C. laminata* and *C. dubiosa corcontica* by Tlachač (2008).

***Cochlodina dubiosa* (Clessin, 1882)**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
DSIPL	Slovenia	Planica	46°28'50.88" N, 13°43'22.80" E	916m	10-5-09	L. Juřičková, A. Petrusek, V. Szalontayová
DSIPO1 **DSIPO2**	Slovenia	Podkoren	46°30'07.92" N, 13°45'06.84" E	1025m	10-5-09	L. Juřičková, A. Petrusek, V. Szalontayová
DATBA1 ***DATBA2***	*Austria*	*Bärental*	*46°28'57.00"N, 14°10'13.00"E*	*800m*	*31-7-06*	*L. Juřičková, P. Tlachač*
DATST	Austria	Strugarjach	46°29'13.92" N, 14°12'33.12" E	1050m	09-5-09	L. Juřičková, A. Petrusek, V. Szalontayová
DATVL1 **DATVL2**	Austria	Vellacher - slope	46°25'27.84" N, 14°32'25.80" E	1063m	08-5-09	L. Juřičková, A. Petrusek, V. Szalontayová
DATVT	Austria	Vellacher-stream	46°27'02.16" N, 14°34'17.04" E	691m	08-5-09	L. Juřičková, A. Petrusek, V. Szalontayová
DSIKA	Slovenia	Kamnik	46°12'03.18" N, 14°36'04.92" E	356m	06-9-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová
DSIKO	Slovenia	Kokra	46°18'12.24" N, 14°28'50.04" E	512m	08-9-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová
•DCZBL1 ***•DCZBL2*** ***HCZBL1***	*Czech republic*	*Bártův les*	*50°37'31.00'' N, 15°51'02.00'' E*	*679m*	*07-8-06*	*P. Tlachač*
•DCZUP1 ***•DCZUP2***	*Czech republic*	*Úpa pod Rýzmburkem*	*50°25'46.00'' N, 16°02'24.00'' E*	*329m*	*10-5-06*	*P. Tlachač*
•DCZSM1 ***•DCZSM2***	*Czech republic*	*Špindlerův Mlýn*	*50°44'47.00'' N, 15°36'06.00'' E*	*819m*	*08-8-06*	*P. Tlachač*
HCZJV1	*Czech republic*	*Jizera u Vilémova*	*50°44'55.00'' N, 15°24'23.00'' E*	*563m*	*24-5-08*	*P.Tlachač*

Figure 36: Map of Europe showing collection sites of *C. dubiosa* individuals used in this study.

All individuals assessed here were of horn-colour. Shape of aperture, presence of spiral lines and shapes of inferior and superior lamella were variable. On the other hand, principal plica, lower palatal plica and subcolumellar lamella were visible in all cases (Figure 37a).

Principal plica merged with palatal callus in all cases, while lower palatal plica was variable in this characteristic as well. The beginning of principal plica was more to the left compared to the beginning of upper palatal plica in eight cases. In one case (DATVL2) the beginnings of both plicae were on the same level. The mutual position of these two plicae was mostly non-parallel, only in one case (DSIPL) it was parallel (Figure 37b) .

Palatal callus was developed to different extent, from very strong, white and calcified to almost unrecognizable. However, the position of the palatal callus was stable and so was the occurrence of untrue plicae formed by it (Figure 37b).

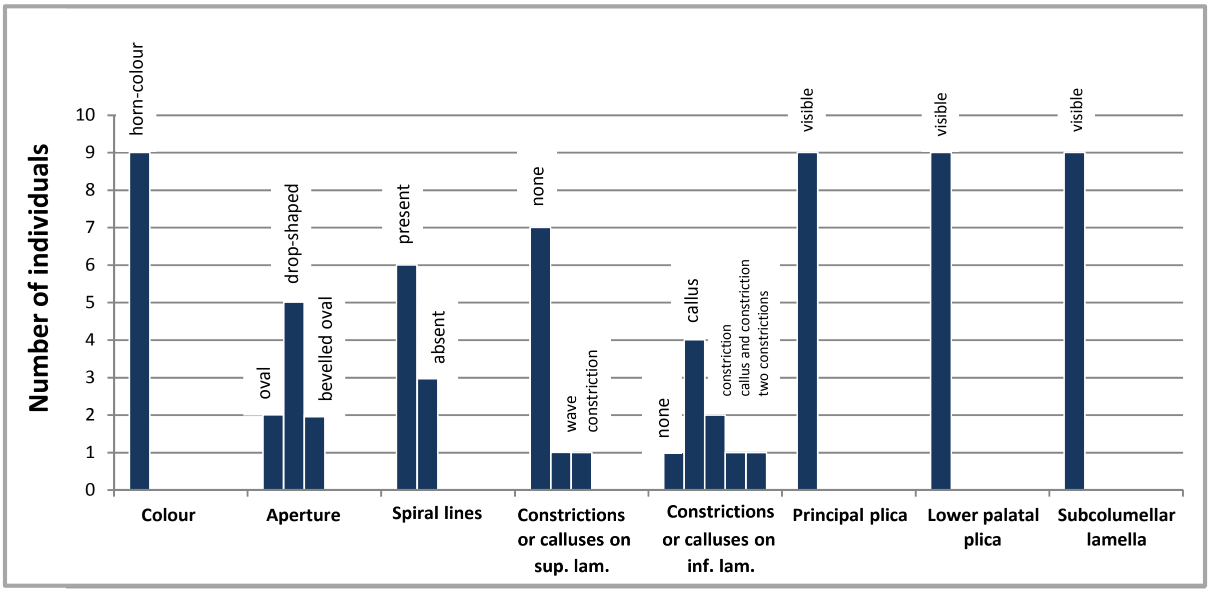

Figure 37a: Some of the morphological characteristics visible on the surface and apertural side of the shells of *C. dubiosa* individuals (sup. lam. = superior lamella; inf. lam. = inferior lamella).

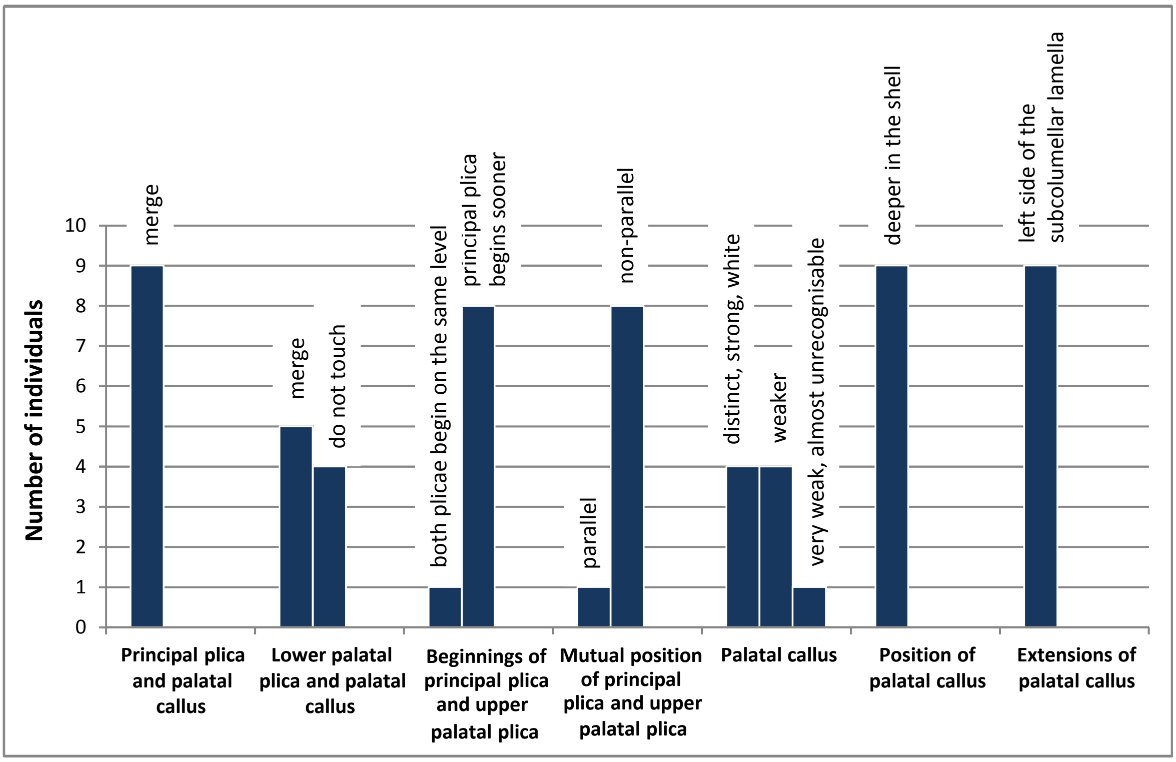

Figure 37b: Some of the morphological characteristics regarding plicae of the palatal side and palatal callus of *C. dubiosa* individuals.

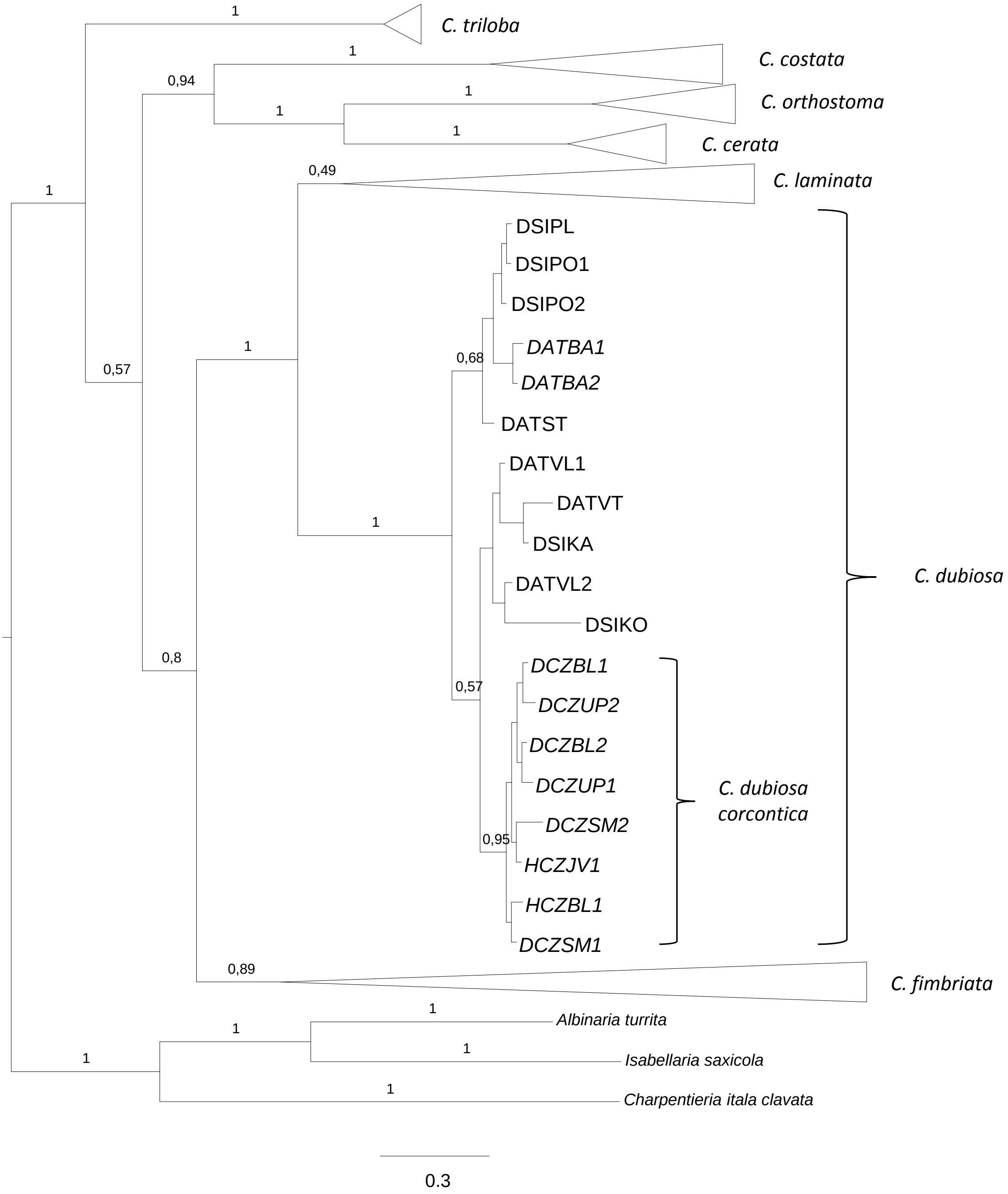

Figure 38: Tree created by Bayesian analysis, showing the position of individuals within the clade of *C. dubiosa*.

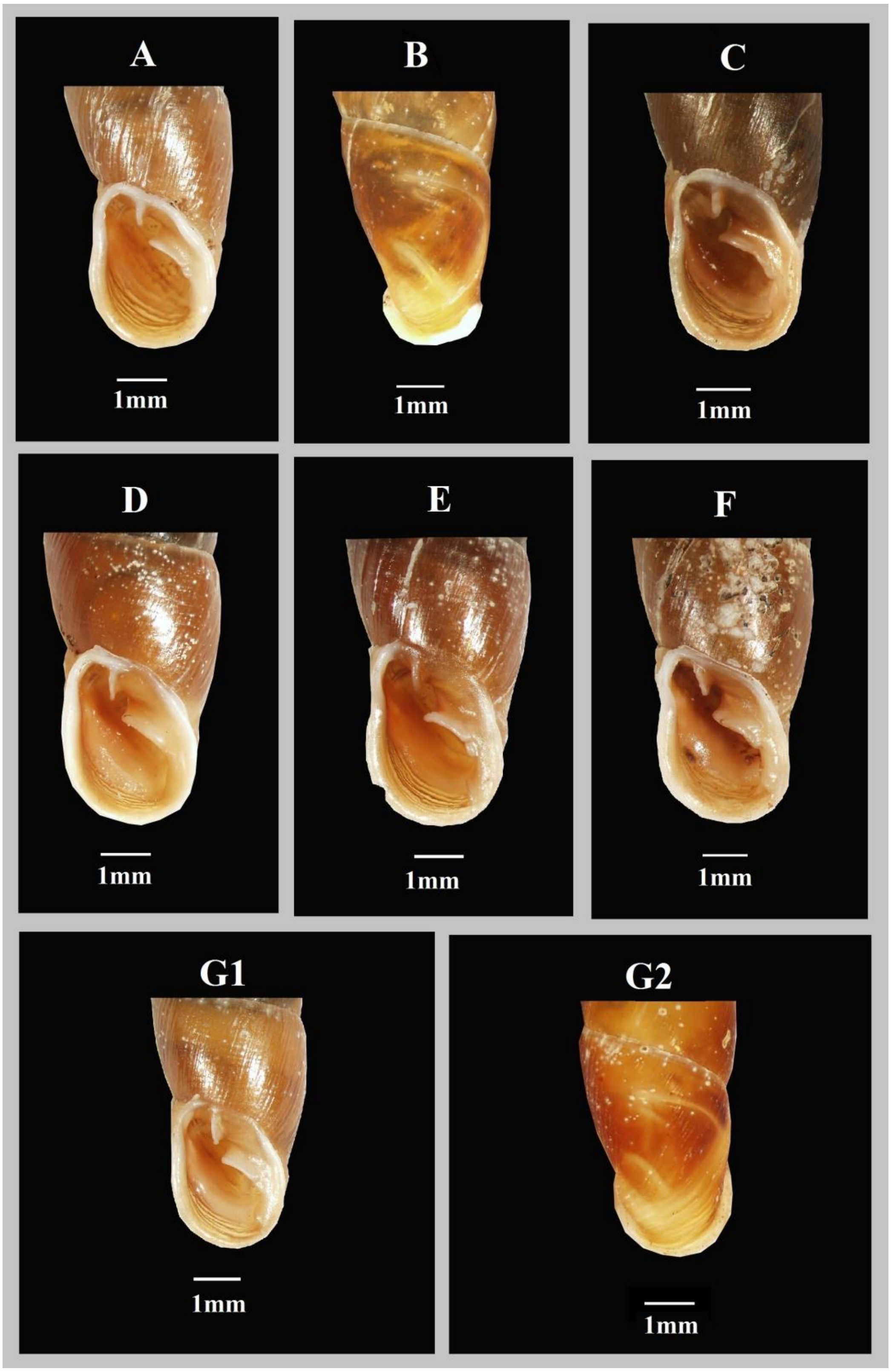

Figure 39: Apertural views of some of the *C. dubiosa* individuals analyzed in this study (A: DATST; B: DSIPL; C: DSIPO1; D: DATVT; E: DSIKO; F: DSIKA; G: DATVL2)

4.2.7. *Cochlodina fimbriata*

Sequences of 45 specimens of *C. fimbriata* were used for this study. Six of these were from former study by Tlachač (2008). The remaining 39 individuals were used for the following analysis of morphological characteristics.

All individuals assessed within this species shared only two characteristics. Lower palatal fold did not touch the palatal callus and subcolumellar lamella was always visible on the apertural side of the shell. However, more similarities are discovered when lineages are treated separately, as in *C. laminata*.

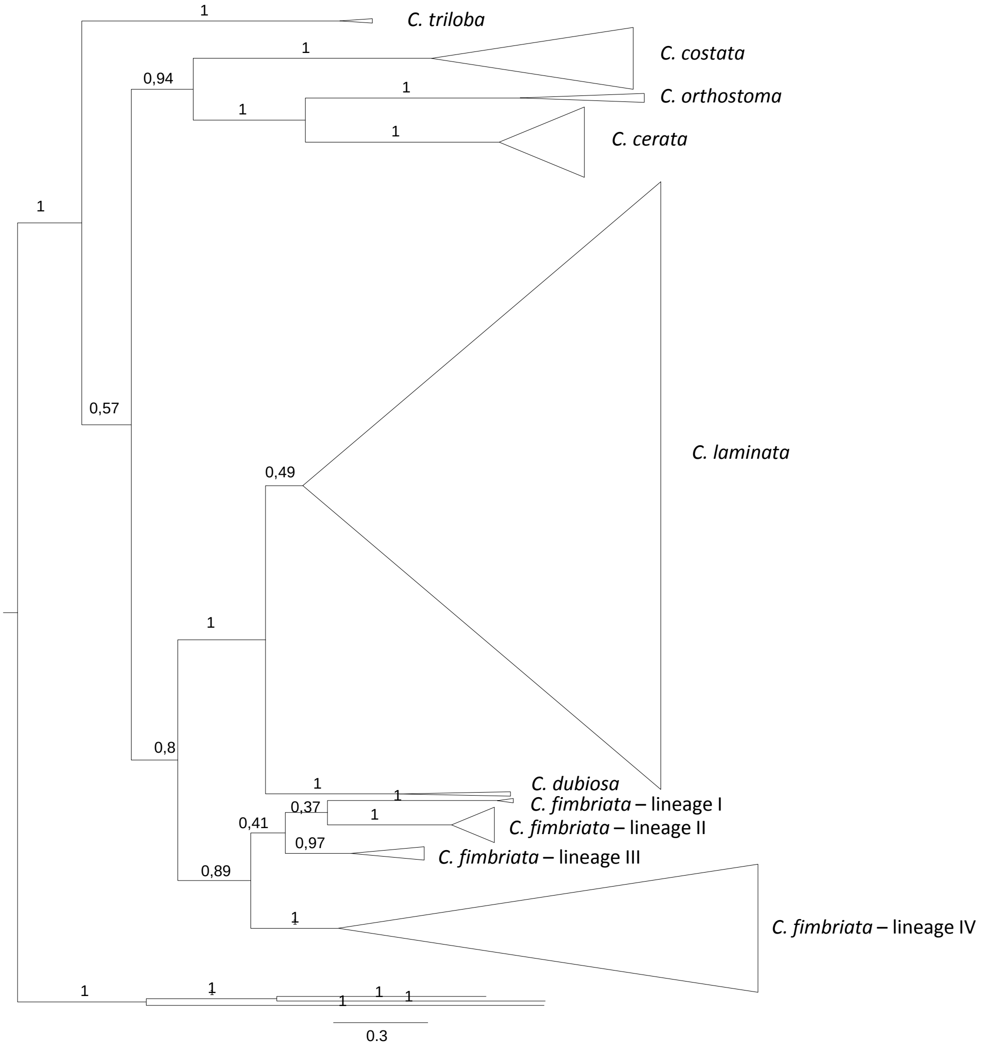

Figure 40: Tree created by Bayesian analysis, showing the position of four lineages within the clade of *C. fimbriata.*

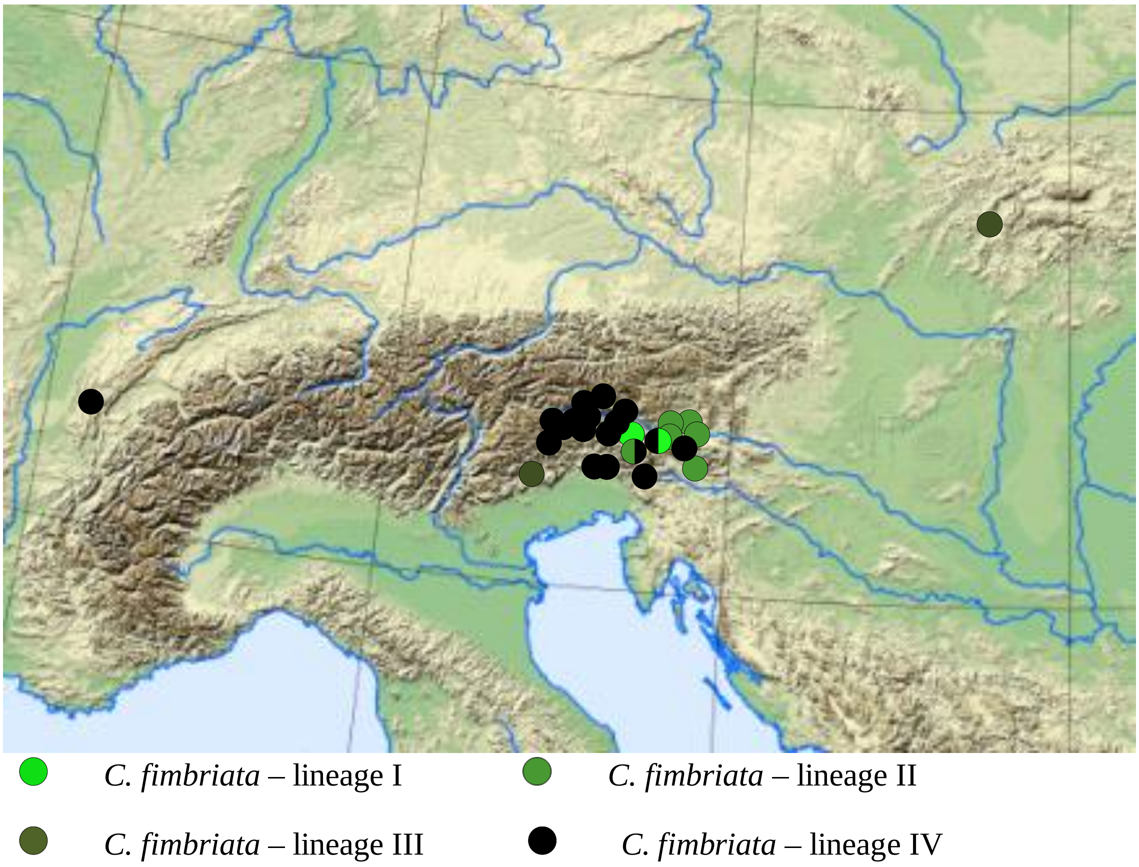

Figure 41: Map showing distribution of the four lineages of *C. fimbriata*, each represented by a different colour. Sites where more than one lineage occurred, are represented by a multicoloured point of the corresponding colours.

Lineage IV included most od individuals of *C.fimbriata* sampled for this study. Individuals FCHER1 and FCHER2 were the ones that occurred most to the west, almost at the western border of the expansion range of this species (according to the map by Kerney et al. 1983). Only four individuals from two sites belonged to lineage III and both were sampled by Tlachač (2008). It was suggested before that *C. fimbriata* occurring in Vtáčnik mountains in Slovakia is a relative of the individuals inhabiting Eastern Alps. Lineage II was distributed more to the east than lineage IV.

In most sampled sites, only one individuals belonging to one lineage were found. Only in two sites (Kranjska Gora mountain and Vršič mountain, both in Slovenia) did two different lineages occur together.

4.2.7.1. *Cochlodina fimbriata* – lineage I

The first lineage consisted of 2 individuals (listed in table 11), both of which were assessed in morphological analysis, result of which are listed below.

Table 11: List of *C. fimbriata* individuals belonging to lineage I that were used in this study.

***Cochlodina fimbriata* (Rossmässler, 1835) - lineage I**						
Code	**Country**	**Locality**	**Coordinates**	**Altitude**	**Date of collecting**	**Collector(s)**
FSIPL	Slovenia	Planica	46°28'50.88" N, 13°43'22.80" E	900m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FSIKG2	Slovenia	Kranjska Gora	46°27'40.00'' N, 13°46'48.00'' E	936m	30-8-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová

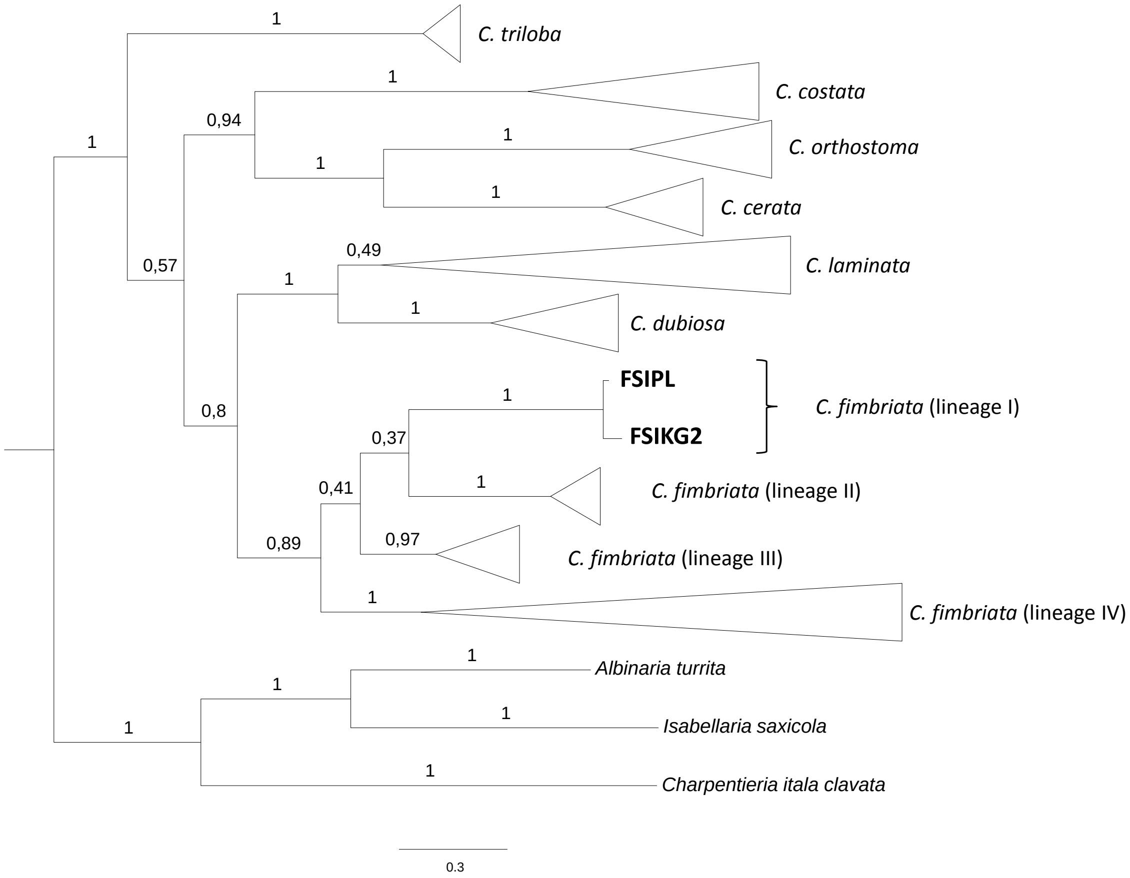

Figure 42: Tree created by Bayesian analysis, showing the position of individuals within the clade of *C. fimbriata* – lineage I.

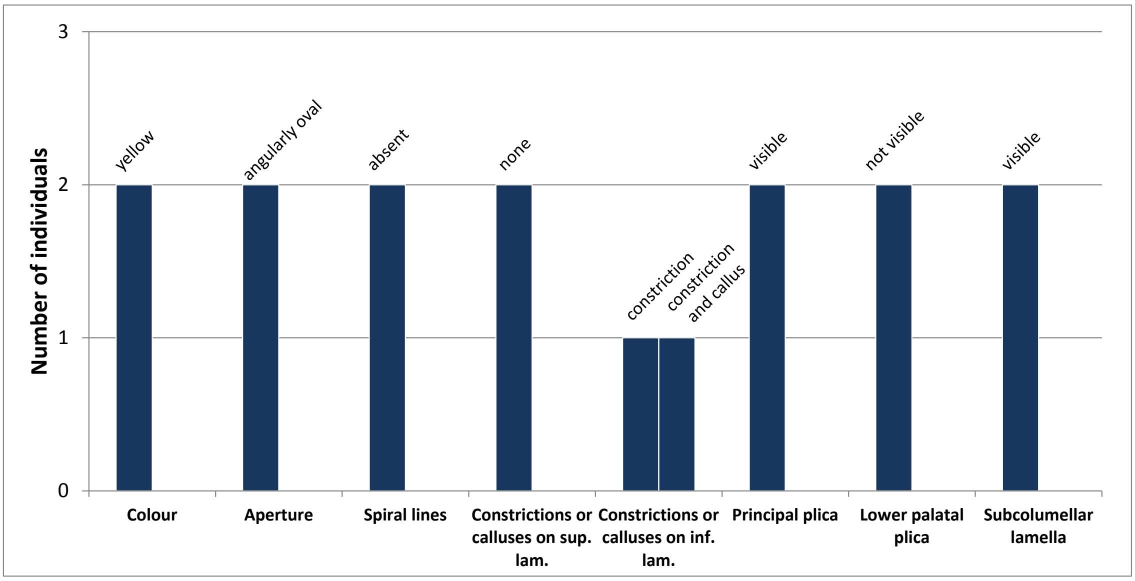

Figure 43a: Some of the morphological characteristics visible on the surface and apertural side of the shells of *C. fimbriata* I individuals (sup. lam. = superior lamella; inf. lam. = inferior lamella).

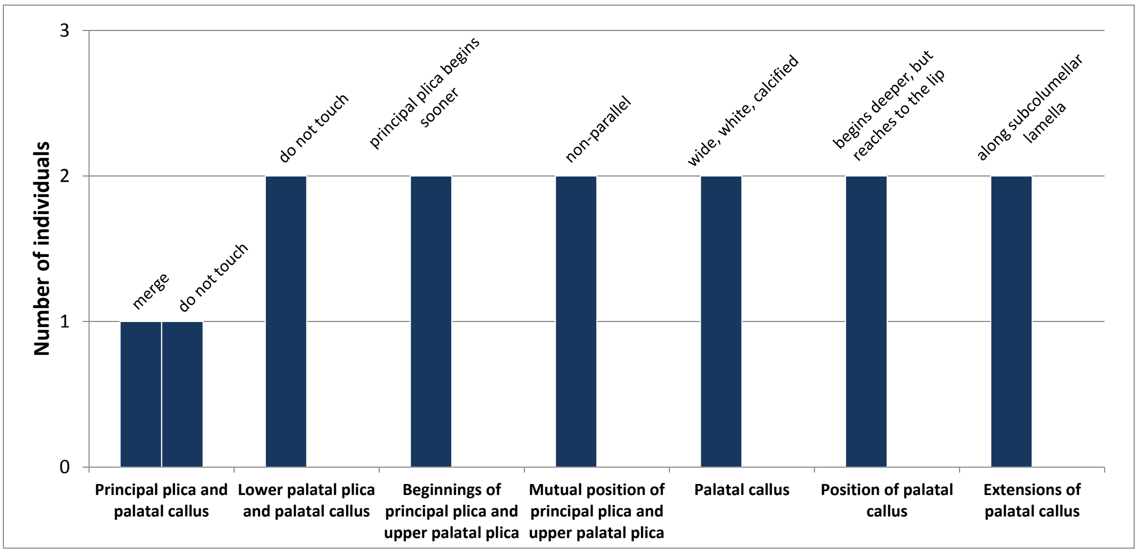

Figure 43b: Morphological characteristics of the palatal side of the shell of individuals belonging to *C. fimbriata* – lineage I.

These two individuals were identical in most of morphological characters investigated, except for the shapes on inferior lamella (individual FSIPL had a constriction on inferior lamella, while individual FSIKG2 had a callus and a constriction on this lamella) and the mutual position of principal plica and palatal callus (while these two touched and merged in FSIPL, they did not touch in FSIKG2. However, both of these shells seem to be of similar age, so the difference is most likely not age-related). Apertural views of these individuals are shown in figure 44.

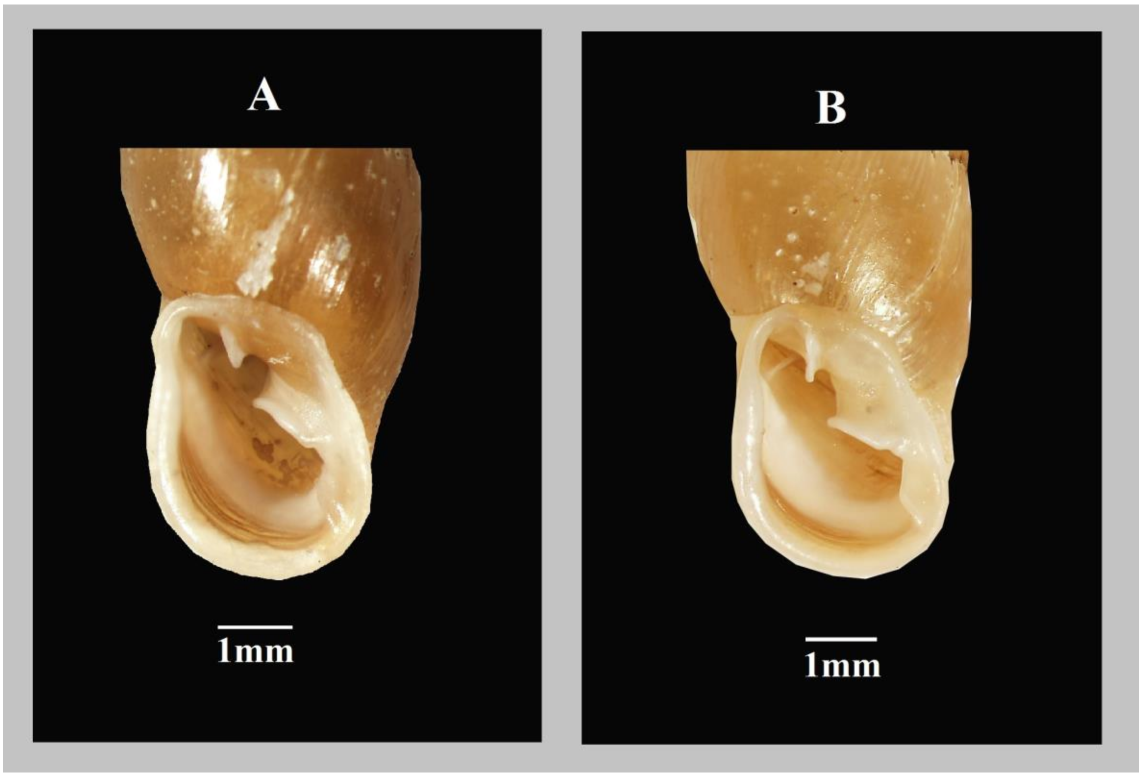

Figure 44: Apertural views of the *C. fimbriata* - lineage I individuals analyzed in this study (A: FSIPL; B: FSIKG2)

4.2.7.2. Cochlodina fimbriata – lineage II

Lineage II contained seven individuals from five different sites in two different countries, all of which are listed in the following table (Table 12). Two of these individuals (FATBA and FATBL) were sampled by Tlachač (2008). Morphology of these individuals was not assessed in this study.

Table 12: List of *C. fimbriata* individuals belonging to lineage III. Samples collected by Tlachač (2008) are listed in *italics*.

Cochlodina fimbriata **(Rossmäller, 1835) - lineage II**						
FATST1 **FATST2**	Austria	Strugarjach	46°29'13.92" N, 14°12'33.12" E	1030m	09-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FSIJE	Slovenia	Jezersko	46°23'02.04" N, 14°28'22.44" E	720m	08-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FATTS1 **FATTS2**	Austria	Tscheppaschlucht	46°31'58.23" N, 14°19'13.29"E	453m	09-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FATBA	*Austria*	*Bärental*	*46°28'57.00''N, 14°10'13.00'' E*	*800m*	*31-7-06*	*L. Juřičková, P. Tlachač*
FATBL	*Austria*	*Bärental*	*46°26'57.00''N, 14°09'45.00'' E*	*1249m*	*01-8-06*	*L. Juřičková, P. Tlachač*
FSISS	Slovenia	Slap Savica	46°17'21.00''N, 13°48'07.00''E	661m	02-9-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
FSIVR1	Slovenia	Vršič Mt.	46°26'07.00'' N, 13°44'38.00'' E	1650m	30-8-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová

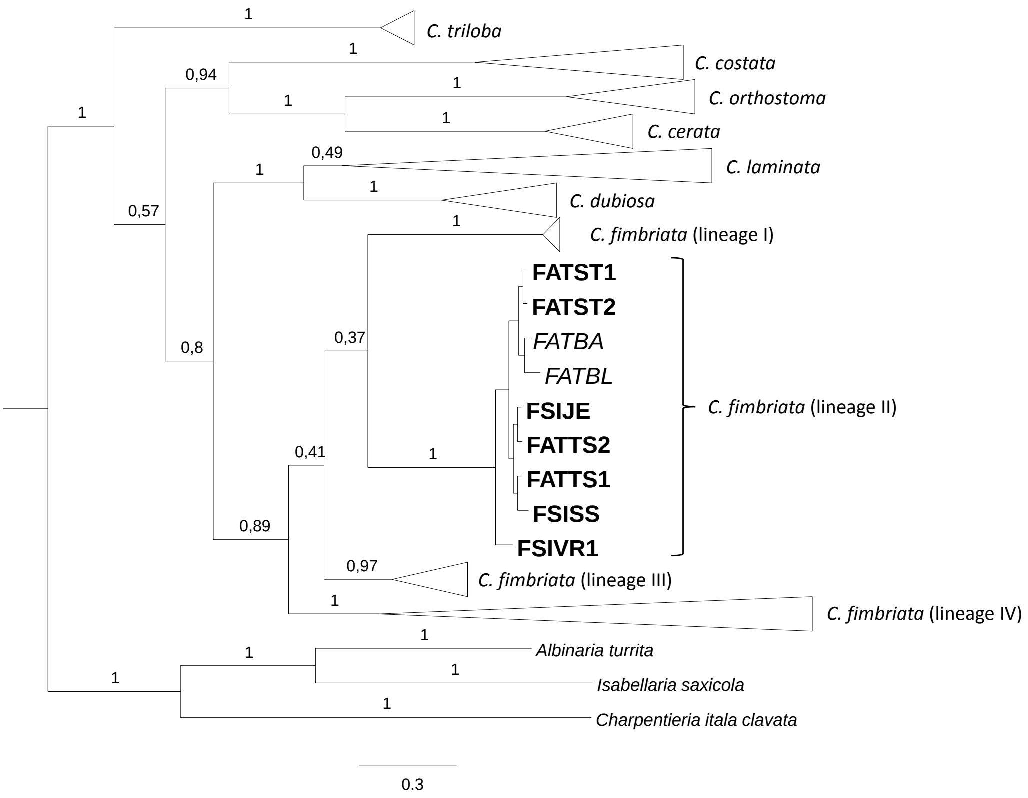

Figure 45: Tree created by Bayesian analysis, showing the position of individuals within the clade of *C. fimbriata* – lineage II.

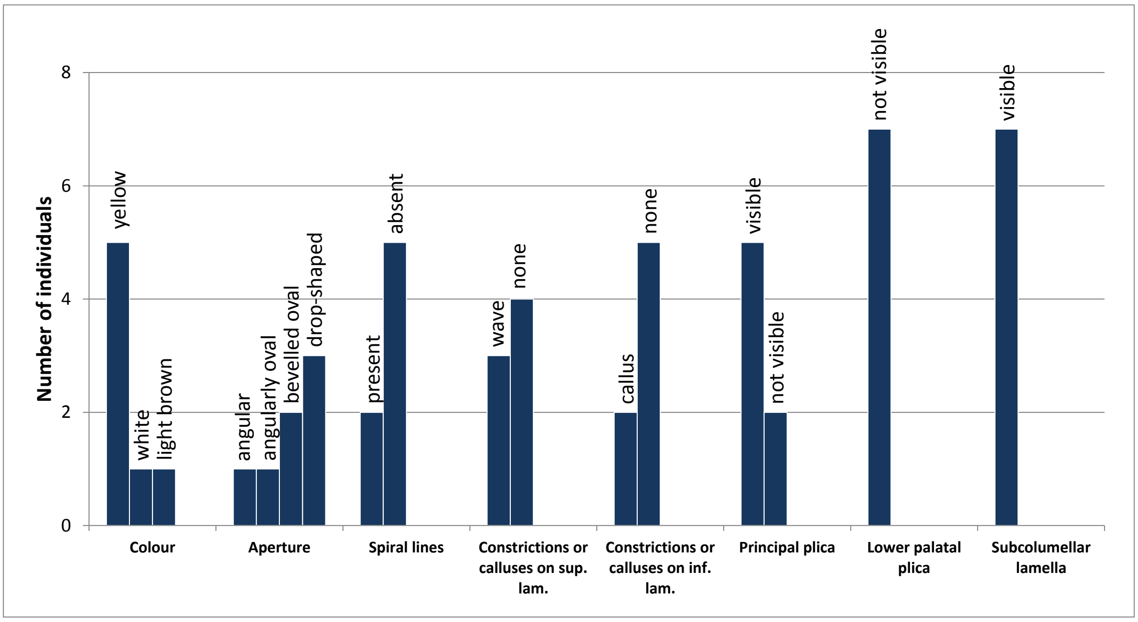

Figure 46a: Some of the morphological characteristics visible on the surface and apertural side of the shells of *C. fimbriata* individuals belonging to lineage II.

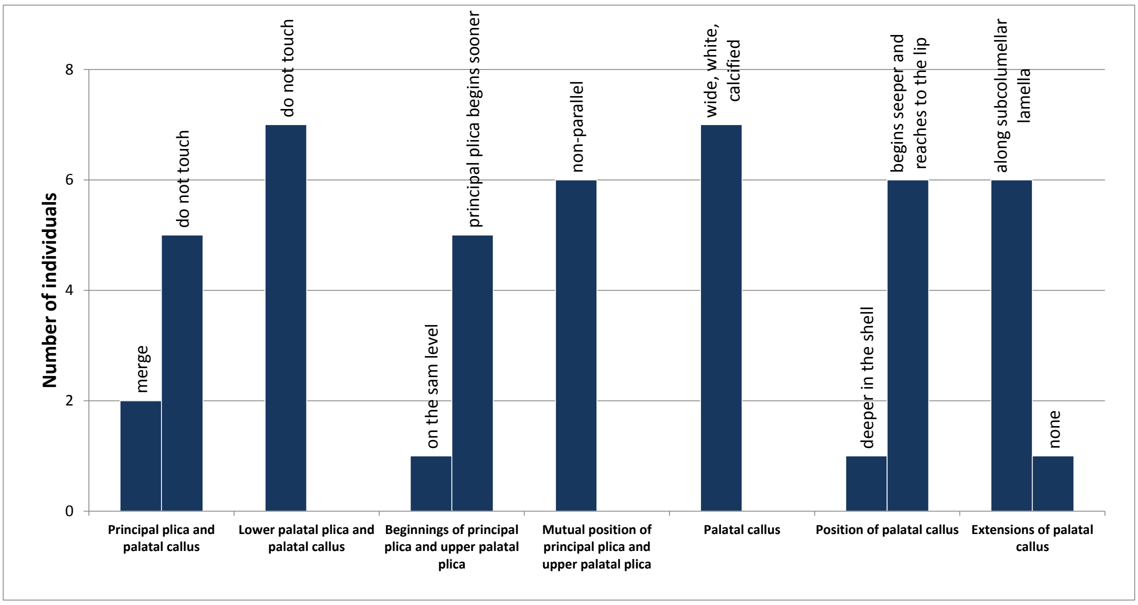

Figure 46b : Interactions and positions of plicae and lamellae in the mouth of the shell of individuals of *C. fimbriata* belonging to lineage II.

Principal plica only merged with the palatal callus in two individuals (FATTS1 a FATTS2), both from the same locality (figure 46b).

Palatal callus was very wide in all individuals except for one (FATTS2). The callus started deeper in the shell and reached almost to the lip of the shell and formed an untrue plica extending upwards along subcolumellar lamella. In FATTS2, the palatal callus was white and distinct, but not as wide as in other individuals from this lineage and the untrue plica extending from it was also missing. When the two individuals from this site (FATTS1 and FATTS2) are compared, FATTS1 seems younger, which would suggest that the character of palatal callus and presence of untrue plica are not age-related characters (figure 46b).

Principal and upper palatal plica were always non-parallel, with the beginning of principal plica being positioned to the left from the beginning of upper palatal plica in all cases except for one (FITTS2) in which both plicae began on the same level (figure 46b).

The mutual position of the principal and upper palatal plica was not assessed in specimen FSIVR, which had an extra plica on the palatal side of the shell and thus it was not possible to see which plica was the aberrant extra one.

Photographs of chosen individuals from this lineage can be viewed in figure 47.

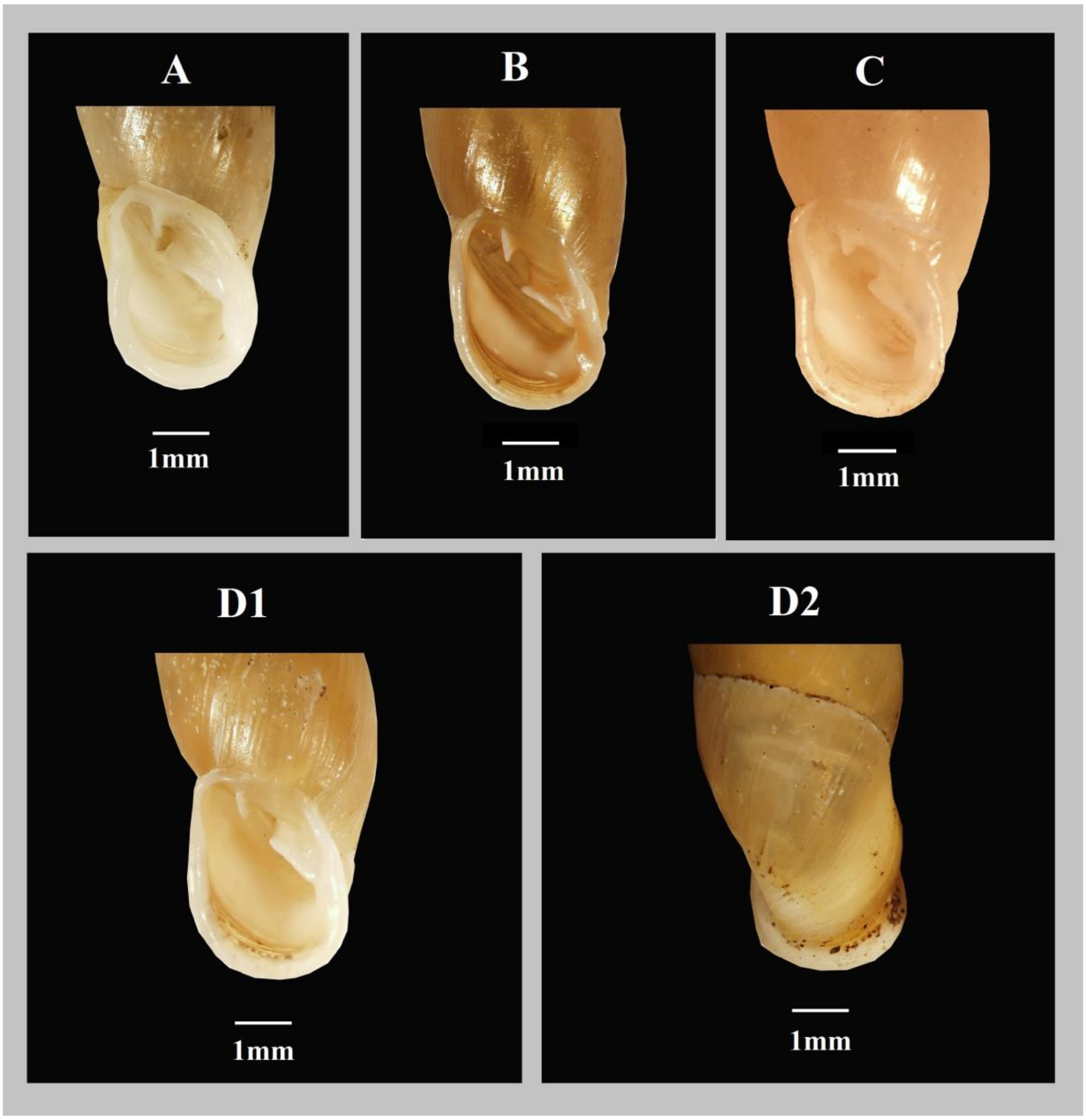

Figure 47: Apertural views of some of the *C. fimbriata* – lineage II individuals analyzed in this study (A: FATST2; B: FSIVR1; C: FSISS; D: FSIJE).

4.2.7.3. *Cochlodina fimbriata* – lineage III

Lineage III contained four individuals, sequences of which were taken from Tlachač's study (2008). As the shells of these snails are not available any more, morphology of this group was not assessed. The following table (table 13) contains information about the samples and collection sites of these snails.

Table 13: List of individuals of *C. fimbriata* belonging to lineage III.

Cochlodina fimbriata **(Rossmäller, 1835) - lineage III**						
FITTS1 *FITTS2*	*Italy*	*Tramoti di Sopra*	*46°21'10.00" N, 12°46'59.00" E*	*1228m*	*02-8-06*	*L. Juřičková, P. Tlachač*
FSKVT1 *FSKVT2*	*Slovakia*	*Vtáčnik*	*48°38'18.00"N, 18°36'47.00" E*	*787m*	*13-5-08*	*P. Tlachač*

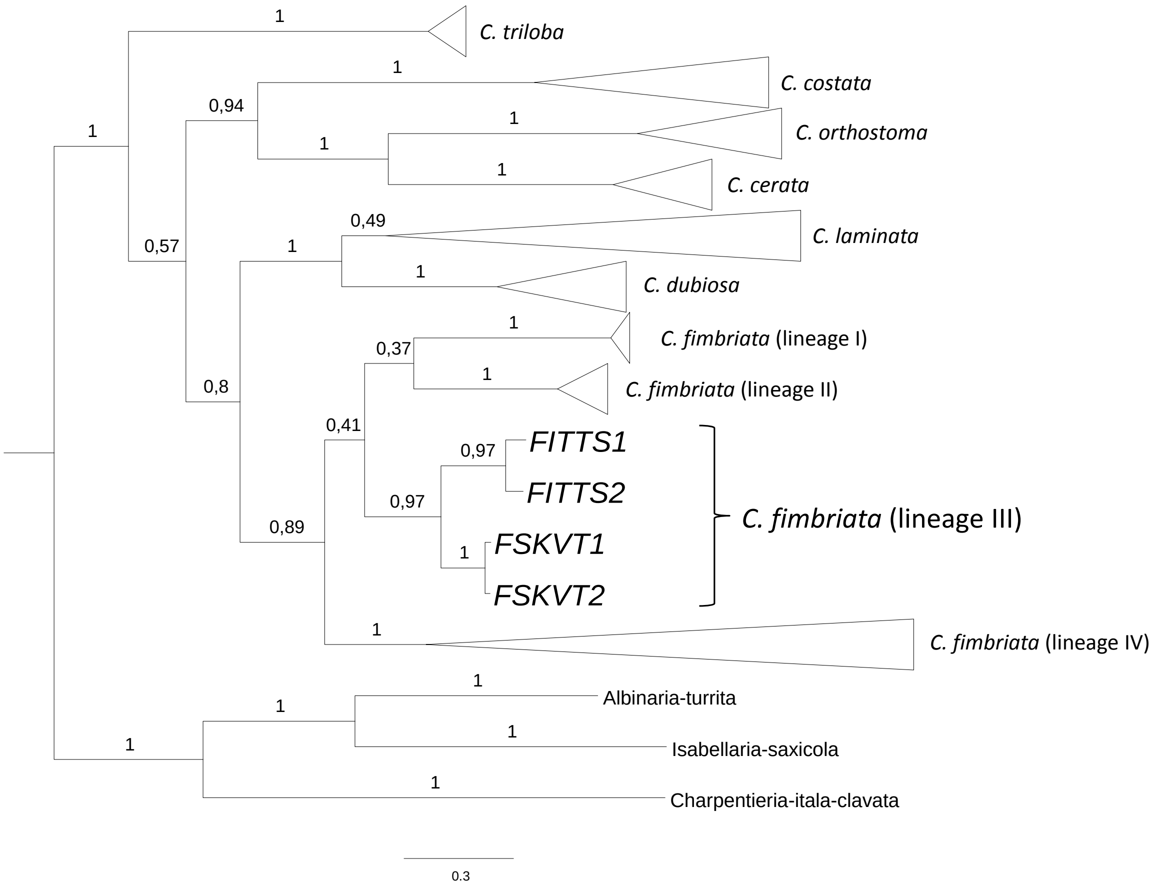

Figure 48: Tree created by Bayesian analysis, showing the position of individuals within the clade of *C. fimbriata*, lineage III.

4.2.7.4. *Cochlodina fimbriata* – lineage IV

The fourth lineage consisted of 30 individuals, all of which were assessed in morphological analysis. All of them are listed in the following table (Table 14).

Table 14: List of individuals of *C. fimbriata* belonging to lineage IV.

Cochlodina fimbriata **(Rossmäller, 1835) - lineage IV**						
FITCB1 **FITCB2**	Italy	Coccaubasso	46°31'55.20" N, 13°38'17.16" E	670m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FITTR	Italy	Tarvisio - Riofreddo	46°27'52.18''N, 13°34'22.45''E	866m	14-10-10	F. Šťáhlavský, M. Peprný
FITCP	Italy	Cave del Predil	46°27'52.66'' N, 13°34'22.95'' E	873m	30-8-12	F. Šťáhlavský, M. Peprný
FSIKG1	Slovenia	Kranjska Gora	46°27'40.00'' N, 13°46'48.00'' E	936m	30-8-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
FSIVR2	Slovenia	Vršič Mt.	46°26'07.00'' N, 13°44'38.00'' E	1650m	30-8-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
FITSB	Italy	Studena Bassa	46°31'24.60" N, 13°15'39.70" E	660m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FITOS1 **FITOS2**	Italy	over Studena Bassa	46°32'07.08" N, 13°12'28.80" E	1050m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FATWU	Austria	Wurzenpass	46°32'36.96" N, 13°18'04.68" E	780m	09-9-09	L. Juřičková, V. Szalontayová, A. Petrusek
FITPA	Italy	Paluzza	46°31'47.28" N, 13°01'00.84" E	650m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FSITK	Slovenia	Trdajava Kluže	46°21'43.86" N, 13°15'22.44" E	709m	30-8-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová
FATNA1 **FATNA2**	Austria	Nassfeldpass	46°36'14.04" N, 13°17'15.72" E	720m	10-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FATVL	Austria	Vellacher-slope	46°25'27.84" N, 14°32'25.80" E	1100m	08-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FATVT	Austria	Vellacher-stream	46°27'02.16" N, 14°34'17.04" E	650m	08-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FITLP1 **FITLP2**	Italy	Lago del Predil	46°24'54.51'' N, 13°33'08.84'' E	978m	14-10-10	F. Šťáhlavský, M. Peprný
FSIBB	Slovenia	over Bohinjska Bistrica	46°16'33.66" N, 14°00'27.72" E	856m	30-8-12	F. Šťáhlavský, J. Plíšková, J. Kotrbová
FSITS1 **FITTS2**	Slovenia	Tomičev Slap	46°23'54.24" N, 14°16'26.76" E	700m	08-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FSIVI1 **FSIVI2** **FSIVI3**	Slovenia	Vintgar	46°23'36.00'' N, 14°05'07.00'' E	857m	02-9-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
FSIBJ	Slovenia	Bohinjsko jezero	46°16'53.00'' N, 13°50'53.00'' E	531m	02-9-12	P. Dolejš, V. Hula, P. Just, O. Košulič, J. Niedobová
FCHER1 **FCHER2**	Switzerland	Erschwil	47°22'02.28" N, 07°33'18.30" E	459m	13-8-11	M. Proćków
FITUC	Italy	Uccea	46°19'40.00'' N, 13°22'06.86'' E	963m	14-10-10	F. Šťáhlavský, M. Peprný
FATPL	Austria	Plöckenpass	46°37'21.72" N, 12°56'48.48" E	1060m	11-5-09	L. Juřičková, V. Szalontayová, A. Petrusek
FATKR	Austria	Kreuzen	46°39'41.44" N, 13°33'49.81" E	934m	29-8-12	F. Šťáhlavský

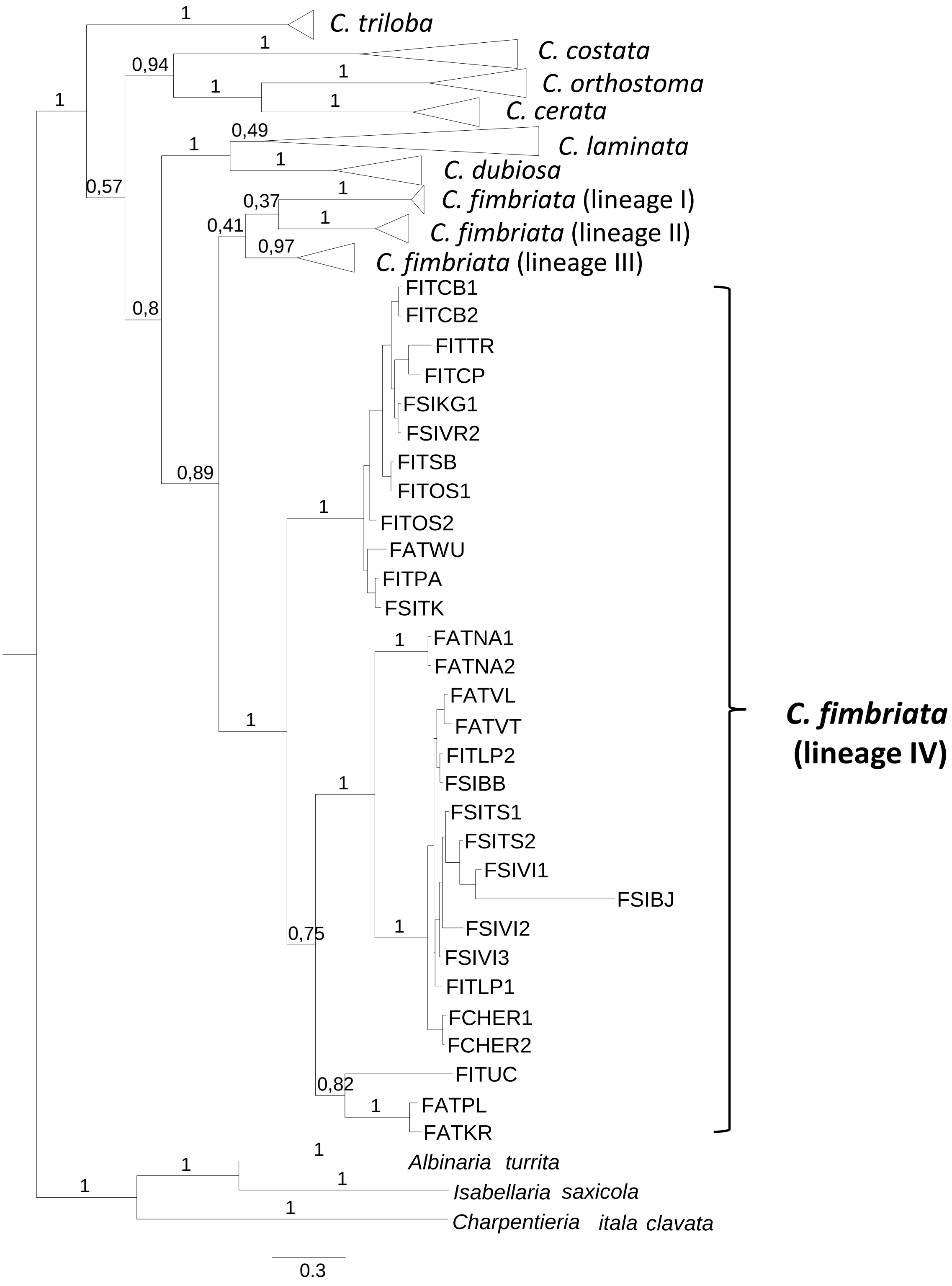

Figure 49: Tree created by Bayesian analysis, showing the position of individuals within *Cochlodina fimbriata* – lineage IV.

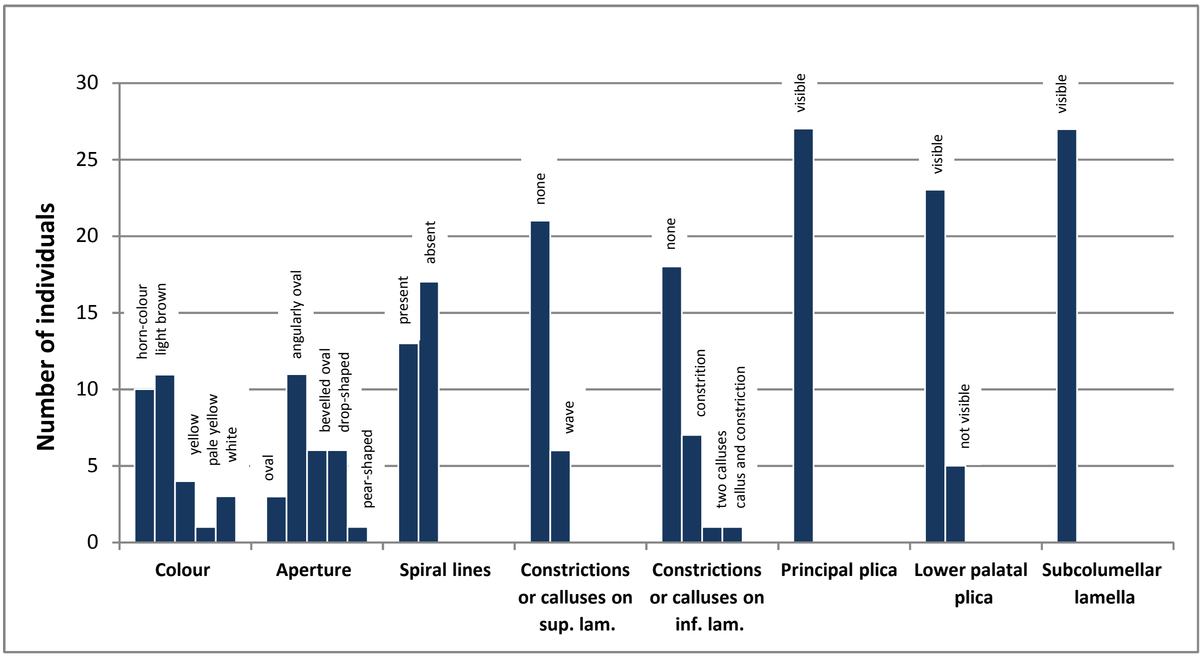

Figure 50a: Some of the morphological characteristics visible on the surface and apertural side of the shells of *C. fimbriata* individuals belonging to lineage IV.
(sup. lam. = superior lamella; inf. lam. = inferior lamella).

Three of the individuals (FATKR, FATNA2 and FITPA) were juveniles, whose apertures were not developed yet and these were excluded from the analysis of every morphological feature except for the colour of the shell and presence of spiral lines.

C. fimbriata IV individuals were very variable in colour. Within these 30 individuals, five types of shape of aperture occurred. Individuals belonging to this lineage were also variable in presence of spiral lines, presence of calluses and constrictions on superior and inferior lamella and visibility of lower palatal plica. On the other hand, principal plica and subcolumellar lamella were always visible (Figure 50a).

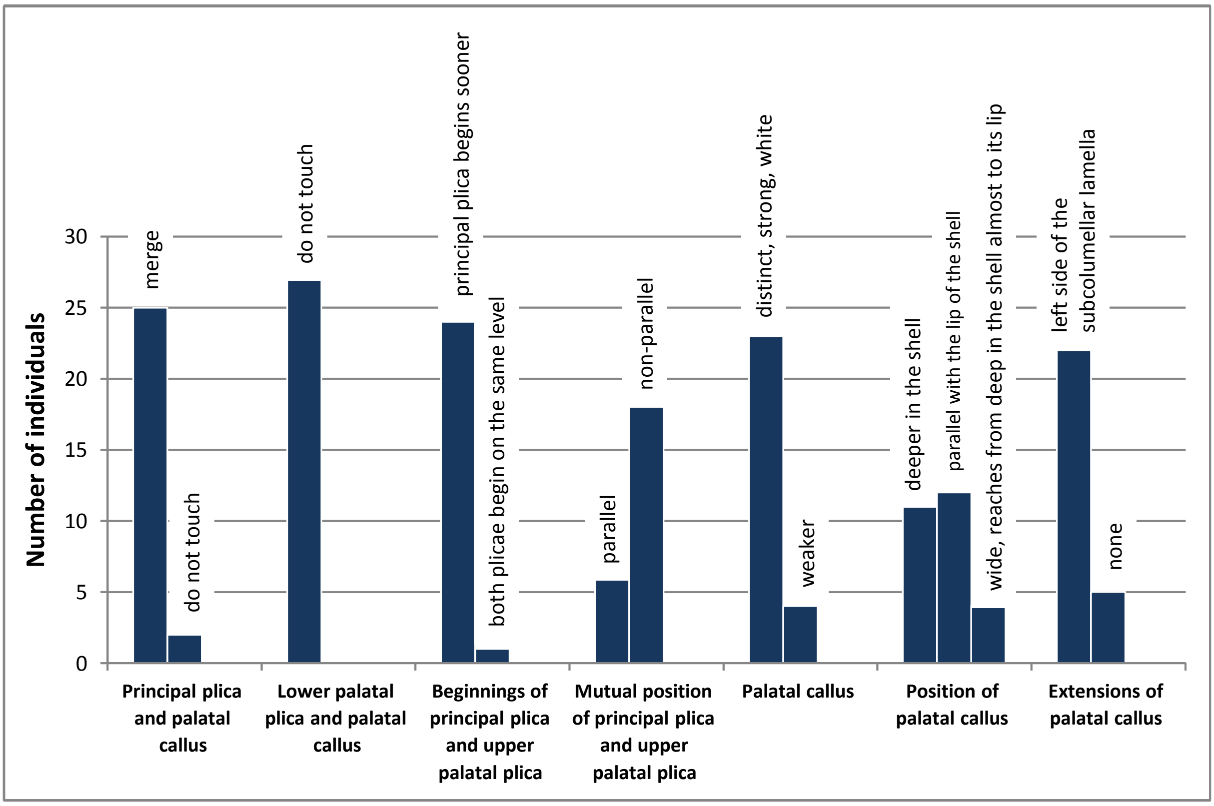

Figure 50b: Some of the morphological characteristics regarding plicae of the palatal side and palatal callus of *C. fimbriata* IV individuals.

In three individuals the mutual position of the principal and upper palatal plica was not assessed. In two cases (FSIVR1 and FSIVI2) this was because of an extra plica on the palatal side and in one case (FATWU) it was due to severe corrosion of the shell surface, which caused the plicae of the palatal side to be invisible.

Principal plica merged with palatal callus in most cases, while lower palatal plica never interacted with the callus. Palatal callus was developed to different extent and in different parts of the shell. In most cases it formed extensions along the left side of subcolumellar lamella, while in some it did not (Figure 50b).

Individual FATWU was excluded from analysis of principal and upper palatal plica, because they were not visible from palatal side of the shell because of corrosion. The mutual position of the principal and upper palatal plica was also not assessed in specimen FSIVR, which had an extra plica on the palatal side of the shell.

Some of the individuals belonging to this lineage are pictured in figure 51.

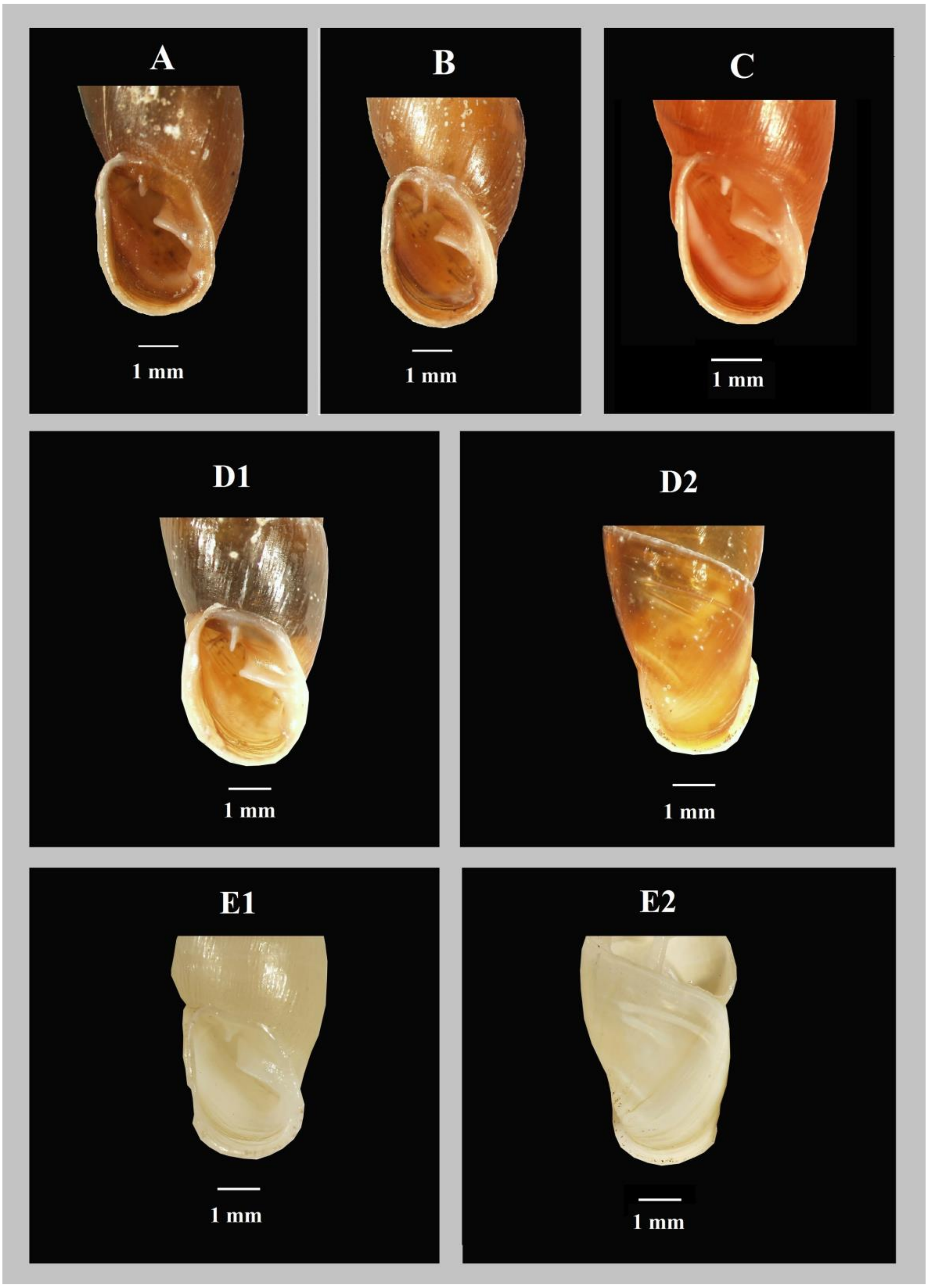

Figure 51: Apertural views of some of the *C. fimbriata* individuals from lineage IV analyzed in this study (A: FATNA1; B: FITSB; C: FSIVR2, D: FITCB1; E: FITLP1)

4.2.8. Damaged shells

I encoutered several damaged individuals during the process of working on this study, where it was clear that the shell was fractured and that the bad shape of it was not a growth or genetical anomaly. The most representative individuals are shown in figure 52. Several shells were corroded (Figure 52 – C: LDEBO1). Corrosion of the shell is caused by external factors, especially by acidity of the environment. This type of damage is closely related to the age of the specimen – the longer the animal lives, the longer it is exposed to unfavourable conditions of the environment and the more corroded it gets. This type of damage does not change the shape or quality of most of the morphological characteristics that I assessed here, but it makes certain characteristics invisible, especially the plicae of the palatal side and their mutual positions cannot be seen through the shell, even when strong light is shone through the cleaned shell.

In certain cases, a crack on the last whorl of the shell can interfere with the plicae or the scar on the shell can resemble a plica itself (figure 52 – D: CEHUBU3) or can cause the apertural shape to be distorted (figure 52 – B: CESKLP2). In all of the cases where damage occurs, extra attention is needed to distinguish original morphological features and those created by damages. In case LCZSR (figure 52 – A), the inferior and superior lamella have been duplicated, possibly as a result of reparation of the shell after a severe injury.

In case FATST1 (figure 52 – E) there is an obvious repared crack visible, in one of the younger whorls of the shell. This crack, even though it did not influence morphologicl characters on either side of the mouth of the shell, did distort overall shape of the shell, especially its width.

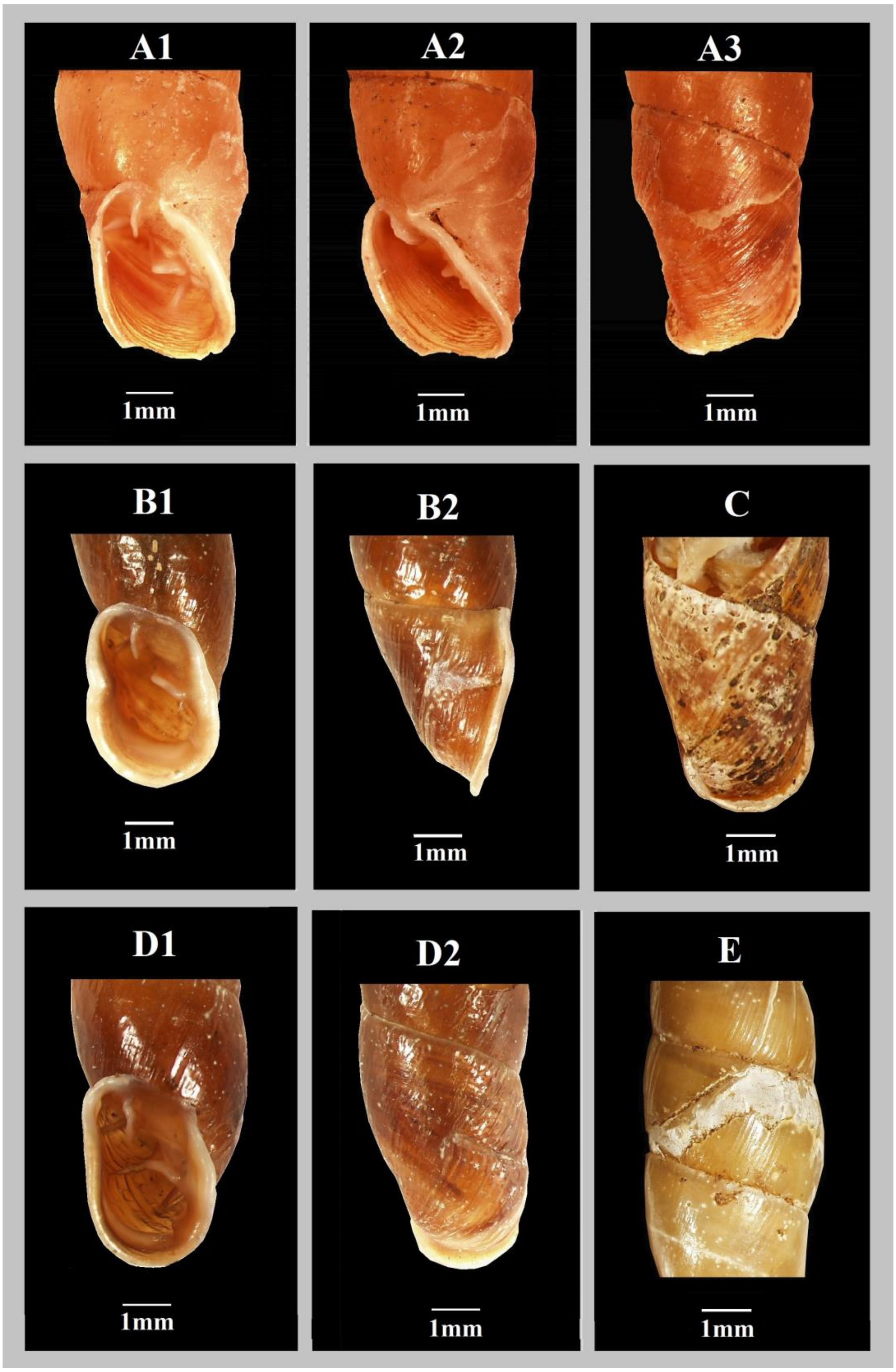

Figure 52: Some of the damaged shells that were present in this study (A: LCZSR; B: CESKLP2; C:LDEBO1; D: CEHUBU3; E: FATST1)

4.2.9. Abnormalities

Apart from previously mentioned abnormalities that were most likely caused by an injury to the shell (figure 52), there were also such anomalies, where a damage was not clearly visible. Some of these less usual forms are showed in figure 53.

There were many individuals with an extra plicae of the palatal side, two of which are shown in figure 53 (A and B). Some individuals had a very short and knob-like upper palatal plica.

One shell of *C. dubiosa* individual (DATVL) was depleted along its axis, in a similar way that Mitov et al. (2003) observed on a shell of *Laciniaria plicata*. It is showed in figure 53 (C, on the left) in comparison with a non-deviantly coiled individual of the same species (DATVT – figure 53 – C, on the right side), that was found very close to it. Even though there is no visible sign of an injury that could cause this shape anomaly, Mitov et al. (2003) suggest that it could be caused by an injury of columella.

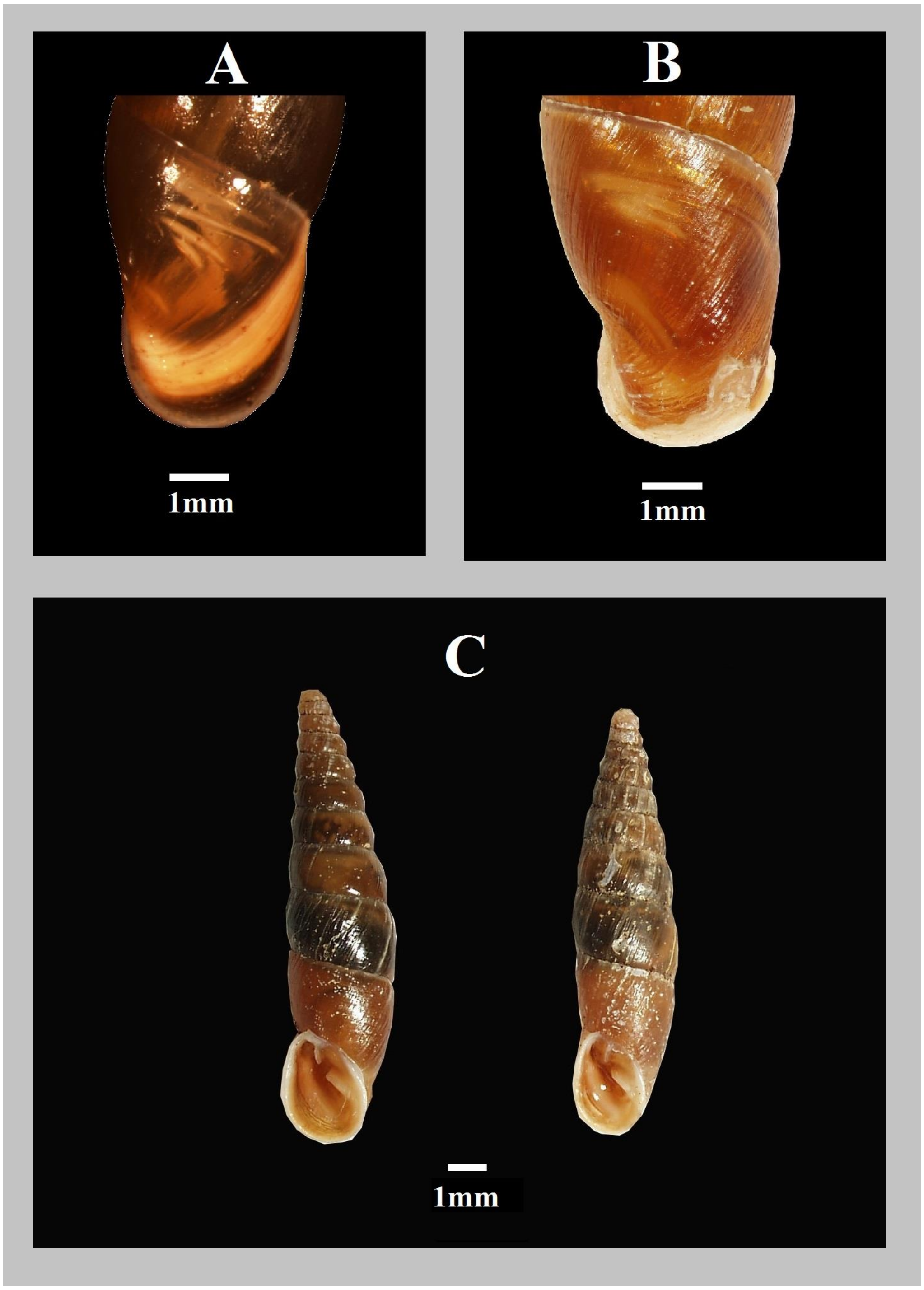

Figure 53: Some of the observed abnormalities, such as an extra plica on the palatal side (A: FSIVR; B: LRSBZ) and a distorted shape of the shell (C: DATVL, left) in comparison with a shell of normal shape (C: DATVT, right)

5. Discussion

Morphological characteristics of seven species of genus *Cochlodina* were investigated. Some species showed out to be more stable in their morphology or at least shared certain stable morphological characteristics thanks to which they could be easily identified , while other (*C. fimbriata* and *C. laminata*) were so morphologically and genetically diverse, that defining them according to their morphological characteristics is very difficult. Moreover, both *C. laminata* and *C. fimbriata* gained poor support in the tree computed by Bayesian analysis and therefore their existence as monophyletic clades is questionable. This was also the reason, why these taxons were divided into several well-supported lineages and each of these lineages was assessed individually.

In many of these clades, the high diversity with no pattern could be caused by insufficient sampling of these species.

The following chapter (5.1.) offers a comparison of the morphological traits of individual lineages with those presented either in original description of the species or commonly used determination literature. Commonly used atlas by Kerney et al. (1983), new atlas of land snails by Welter-Schultes (2012) and Czech determination key by Ložek (1956) were the literature used, as these represent the literature most often used for species determination.

5.1. Evaluation of morphological characteristics

This chapter includes a comparison of commonly used morphological characteristics mentioned in literature and result of the study presented in "Results" section of this thesis.

Certain morphological characteristics showed to be more variable than others. All of the characteristics are summed up for each species separately in a table that compares information about morphological traits given in available literature with the data gained in this thesis and presented in „Results". In *C. laminata* and *C. fimbriata,* each lineage is analyzed separately and an overall characteristic of the whole clade is given as well.

5.1.1. ***Cochlodina triloba***

As only two individuals were obtained in this study, and both were obtained by accident, and as *C. triloba* was not a focus-species, making conclusions about reliability of the morphology of this species is beyond the scope of this study. However, the followig table (Table 15) offers a comparison of morphological characters mentioned in literature and observed in this study.

Presence of spiral lines on the surface of the shell, shape of aperture and width of palatal callus seem variable. However, the latter one and a few other characters can be influenced by aging of the shell. As the shell ages, the palatal callus could be getting wider and more distinct, reaching closer towards the lip of the shell.

This species was only represented in the study by two individuals from one site and analysis of these two individuals is insufficient to draw any conclusions about its morphology, however, I propose that these four morphological characters that could be considered as stable and reliable for future determination:

- palatal callus is very strong
- both principal plica and lower palatal plica are visible from apertural side of the shell and merge with palatal callus
- one or two untrue plicae emerge from the palatal callus and run parallel to the lower palatal fold or subcolumellar lamella
- surface of the shell is smooth (with or without spiral lines)

Table 15:

Cochlodina triloba (Boettger, 1878)		
	Morphological characteristics from literature	**Congruence with the results of this study**
Colour of the shell	•yellowishly horn-coloured (Welter-Schultes 2012; Boettger 1878)	•OK
	• striated (Welter-Schultes 2012; Boettger 1878)	•no
	• heavily striped (Boettger 1878)	•no
Surface of the shell	-	•smooth, growth lines present, with or without spiral lines ✸
Shape of aperture	-	•drop-shaped ⊙
Palatal callus	•strong and white (Welter-Schultes 2012)	•OK
	•well developed, translucent, white (Boettger 1878)	•begins deeper in the shell, sometimes reaches to the lip ❖
		•untrue plicae reach out of the callus along the left side of subcolumellar lamella and sometimes along left side of lower palatal plica as well ❖
Superior lamella	-	•no calluses or constrictions ⊙
Inferior lamella	-	•callus ⊙
Subcolumellar lamella	•emerging as weak nodule (Welter-Schultes 2012)	•OK
Principal plica	-	•begins sooner than upper palatal plica⊙
	•the two lower strong palatal plicae connected with the palatal callus (Boettger 1878)	•visible, merges with palatal callus
Lower palatal plica		•visible, merges with palatal callus
	-	
Upper palatal plica	-	•non-parallel with principal plica ⊙

✸	this symbol suggests that this morphological character is variable
⊙	even though individuals of this species were uniform in this morphological characteristic, evidence from analysis of other species could suggest that this uniformity is an artefact of insufficient sampling and this characteristic could also be highly variable
❖	this morhological characteristic could be age-related

5.1.2. *Cochlodina costata*

Table 16 offers a comparison of morphological characters mentioned in literature and observed in this study.

Palatal callus has a unique character, as mentioned by Ložek (1956), however, the number of untrue plicae emerging from it varied. This could potentially be age-related, as the strength of the callus and plicae could develop with time.

What is important is that all individuals had the unique arrangement of palatal callus described in detail by Ložek (1956). This type of palatal callus interrupted by lower palatal plica was not observed in any other species included in this thesis and therefore I propose that it is the main morphological characteristic to be used when determining species in the field.

- The palatal callus was mostly distinct and this unique arrangement can be visible by naked eye without any means of magnification. The plica interrupts the palatal callus, which disappears around the plica, either completely, or at least in the upper half of the intersection of the two.

Other morphological characteristics suitable for easy determination are:

- lower palatal plica is visible on apertural side of the shell and distinctly interrupts palatal callus
- principal plica is visible on apertural side of the shell and merges with palatal callus
- subcolumellar lamella is visible on apertural side of the shell
- one or two untrue plicae may emerge from palatal callus
- surface of the shell is smooth (with or without spiral lines)

Table 16:

Cochlodina costata (Pfeiffer, 1828)		
	Morphological characteristics from literature	**Congruence with the results of this study**
Colour of the shell	•reddish to light horn-colour (Ložek 1956)	•OK (from light brown to horn-colour) ✸
	•horny brown, but locally variable (Welter-Schultes 2012)	
Surface of the shell	•weakly striated to ribbed (Welter-Schultes 2012)	•smooth, growth lines present, with or without spiral lines ✸
	•shell usually slightly less smooth than in *C. laminata* and less sculptured that in *C. fimbriata* (Kerney et al. 1983)	
	•irregularly striated (Ložek 1956)	
Shape of aperture	•four-angularly pear-shaped, white mouth (Ložek 1956)	•very variable ✸
	•locally variable (Welter-Schultes 2012)	
Palatal callus	•usually prominent (Welter-Schultes 2012)	•in all except for three cases the callus was well developed ❖
	•palatal callus absent or poorly developed, weaker than in *C. fimbriata* (Kerney et al. 1983)	
	•unusual form, white and distinct in its upper part, then abruptly ends before lower palatal plica and forms an untrue palatal plica, end of which is connected with the outer side of upper palatal plica. There is a part of the callus on the right side of the lower palatal plica, seding up one shorter plica-like extension (Ložek 1956)	•mostly in agreement with Ložek ' s description, except that also cases with only one untrue plica (either along left side of subcolumellar lamella or along left side of lower palatal plica) we observed as well as cases where both of these untrue plicae were developed ✸❖
Superior lamella	•thin and quite high (Ložek 1956)	•either with no shapes at all or with a slight wave at its beginning ⊙✸
Inferior lamella	•strongly bent and very distinct (Ložek 1956)	•very variable ✸
Subcolumellar lamella	•well visible from the apertural view (Ložek 1956)	•visible from apertural view
	•well visible (Welter Schultes 2012)	
Principal plica	•connected with the palatal callus (Ložek 1956)	•visible from apertural view
		•merges with palatal callus
Upper palatal plica	•very short (Ložek 1956)	•non-parallel with principal plica, shorter than principal plica
Lower palatal plica	•breaking through palatal callus (Welter-Schultes 2012)	• OK (interrupts palatal callus, the callus disappears around this plica and then continues behind it towards subcolumellar lamella)
	•visible from the apertural view, long (Ložek 1956)	•visible from apertural view

✸	this symbol suggests that this morphological character is variable
⊙	even though individuals of this species were uniform in this morphological characteristic, evidence from analysis of other species could suggest that this uniformity is an artefact of insufficient sampling and this characteristic could also be highly variable
❖	this morhological characteristic could be age-related

5.1.3. *Cochlodina orthostoma*

Table 17 sums up the congruence and differences between the results of this study and commonly used determination literature.

One feature that makes *C. orthostoma* very easy to distinguish from other species is the **ribbed surface of its shell** – this is probably the most distinct feature of this species, making it very easy to identify, as none of the other six investigated species had a ribbed surface.

Other characters that could be used for identification are:

- palatal callus lies closer to the lip of aperture and runs parallel to it
  plicae of the palatal side do not touch palatal callus
- principal plica and lower palatal plica are visible from apertural side of the shell
- subcolumellar lamella is always visible from apertural side of the shell

It is notable that while both Kerney et al. (1983) and Welter-Schultes (2012) mention that lower palatal plica is not visible in the aperture, both individuals used for this study had a lower palatal plica well visible in the aperture, even though only its small part.

Table 17:

Cochlodina orthostoma (Menke, 1830)		
	Morphological characteristics from literature	**Congruence with the results of this study**
Colour of the shell	•reddish-brown to lightly horn-colour. Individuals with insufficient pigmentation are fairly common (Ložek 1956)	•light brown ⊙
	•translucent, yellowish-brown, often with light green colouring (Kerney et al. 1983)	
Surface of the shell	•shiny (Kerney et al. 1983)	•OK (spiral lines were absent, surface of the shell was always ribbed)
	•regularly bluntly ribbed (Ložek 1956, Kerney et al. 1983) that are relatively strong and clear (Kerney et al. 1983)	
Shape of aperture	•egg-shaped or pear-shaped, with no groove (Ložek 1956)	•OK (pear-shaped) ⊙
Palatal callus	•strong whitish callus, with distinct sharp edges (Ložek 1956)	•OK (white, distinct and uninterrupted)
	•paralell with the lip of the shell (Ložek 1956, Kerney et al. 1983)	•OK
	•not connected with plicae of the palatal side (Kerney et al. 1983)	•OK
	•usually weak (Welter-Schultes 2012, Kerney et al. 1983)	•white and distinct ❖
Superior lamella	•similar to *C. laminata* (Kerney et al. 1983)	•no calluses or constrictions, lamella was smooth ⊙
Inferior lamella	•similar to *C. laminata* (Kerney et al. 1983)	•no calluses or constrictions, lamella was smooth ⊙
	•distinct (Ložek 1956)	
Subcolumellar lamella	•poorly visible from the apertural view (Ložek 1956)	•visible from apertural point of view
Principal plica	•not visible in the aperture (Welter-Schultes 2012)	•visible from apertural point of view
	•almost reaches the palatal callus (Ložek 1956)	•begins on the same level as upper palatal plica
		•non-parallel with upper palatal plica
Upper palatal plica	•a lot shorter than the principal plica (Ložek 1956)	• OK
	-	•begins on the same level as principal plica
	-	•non-parallel with principal plica
Lower palatal plica	•only slightly longer than upper palatal plica (Ložek 1956)	•OK
	•does not touch palatal callus (Welter-Schultes 2012)	•OK
	•not visible in the aperture (Welter-Schultes 2012)	•visible from apertural point of view
	•palatal plicae start deep in the mouth of the shell and therefore are not visible from apertural point of view (Kerney et al. 1983)	

✸	this symbol suggests that this morphological character is variable
⊙	even though individuals of this species were uniform in this morphological characteristic, evidence from analysis of other species could suggest that this uniformity is an artefact of insufficient sampling and this characteristic could also be highly variable
❖	this morphological characteristic could be age-related

5.1.4. *Cochlodina cerata*

Table 18 offers a comparison of morphological characters mentioned in literature and observed in this study.

Several characters were always uniform in this species. Probably the most important one is the nature of palatal callus, therefore it is mentioned as the first of the following:

- palatal callus lies very close to the edge of aperture and is sharply bordered
- principal plica is visible from apertural side of the shell (may or may not touch palatal callus)
- lower palatal plica does not touch palatal callus (may or may not be visible from apertural side of the shell)
- subcolumellar lamella is visible
- surface of the shell is smooth (with or without spiral lines)

Table 18:

Cochlodina cerata (Rossmässler, 1836)		
	Morphological characteristics from literature	**Congruence with the results of this study**
Colour of the shell	•yellowish with reddish to olive brown hue (Welter-Schultes 2012)	•horn-colour ⊙
	•reddish brown to horn-brown (Ložek 1956)	
	•horn-brown to reddish brown (Kerney et al. 1983)	
Surface of the shell	•very distinctly grooved on the upper whorls, almost not grooved or smooth on the lower whorls (Ložek 1956)	•smooth, with spiral lines present or absent ✸
	•matt glossy and finely grained (Kerney et al. 1983)	
Shape of aperture	•apertural margin, often with broad and reddish lip (Welter-Schultes 2012)	•apertural lip in gully grown individuals mostly whitish or white ❖
	•aperture rim whitish (Kerney et al. 1983)	
	•eliptical to rectangular (Kerney et al. 1983)	•angularly oval or bevelled oval ✸
	•eliptical or four-angularly pear-shaped (Ložek 1956)	
Palatal callus	•strongly developed (Kerney et al. 1983), strong and white(Ložek 1956)	•always developed, but to a various degree, but always visible ❖
	•paralell with the lip of the shell, behind which it tightly lies (Ložek 1956, Kerney et al. 1983)	•OK
	• sharply bordered (Ložek 1956)	•OK
Superior lamella	•quite tall (Ložek 1956)	•either with no shapes at all or with a slight wave at its beginning ✸
Inferior lamella	•simple lamella (Ložek 1956)	•no constrictions or calluses on this lamella ✸
Subcolumellar lamella	•subcolumellaris curved and visible in a perpendicular view (Welter-Schultes 2012)	•OK
	•well visible from the apertural view (Ložek 1956)	•OK
Principal plica	•quite short (Ložek 1956)	•approximately twice as long as upper palatal plica
	-	•visible from the apertural view
	-	•merges with palatal callus or does not touch it ✸❖
Upper palatal plica	•significantly shorter than principal plica, approximately the same length as the lower palatal plica (Ložek 1956, Kerney et al. 1983)	•shorter than lower palatal plica
Lower palatal plica	•significantly shorter than principal plica (Ložek 1956, Kerney et al. 1983)	•OK
	• approximately of the same length as upper palatal plica (Ložek 1956, Kerney et al. 1983)	•longer than upper palatal plica
	• not visible from apertural point of view (Kerney et al. 1983)	•visible or not visible from apertural point of view ✸❖
	-	•does not touch palatal callus

✸	this symbol suggests that this morphological character is variable
⊙	even though individuals of this species were uniform in this morphological characteristic, evidence from analysis of other species could suggest that this uniformity is an artefact of insufficient sampling and this characteristic could also be highly variable
❖	this morphological characteristic could be age-related

5.1.5. *Cochlodina laminata*

Tables 19a and 19b offer a comparison of morphological characters mentioned in literature and observed in this study.

C. laminata was the best sampled species in this thesis and the focus species of this thesis as well.
Four genetical lineages defined by the Bayesian analysis were assessed for morphological traits and evaluated separately. The following table sums up the agreement of the data collected in this thesis with information currently available in literature.

Sadly, there are onyl very few characteristics that are common for the whole clade. These are:

- shell colour is of various shades of brown, including light brown, horn-colour and dark chocolate-like brown
- surface of the shell is smooth, with or without spiral lines
- principal plica is always visible on apertural side of the shell

Other characters are mostly in agreement with previously published information, however, in each clade there were exceptions to almost every single characteristic. Especially lineage III differs from traditionally described *C. laminata* tremendously.

One of the most important morphological characteristics for *C. laminata* should be a weak palatal callus, as was mentioned by several authors (see table 19a). However, various types of palatal calluses were observed among the individuals belonging to these four lineages.

There are various posibilities of why the morphological variability of this taxon is so vast. These are discussed in the following chapters.

The overall low support of the clade here referred to as *C. laminata* suggests that this taxon is not a valid species. Similarly to *C. fimbriata*, there were only a few sites where individuals belonging to two lineages inhabited the same site. This would suggest that the individual lineages are reproductively isolated and do not interbreed. If this was true, then each of the lineages could be regarded as species. Lineages I and II could possibly be regarded as *C. laminata* s. str., as they fill most of the traditional criteria for *C. laminata*, while new criteria for lineages III and IV would have to be determined.

However, the rich structures of the lineages I and II (figures 24 and 28) suggest that a more complex mechanism plays role here. Even within a single lineage, the individuals from the same site vary and are placed further away from each other in the resulting tree. There are various reasons that could have influenced the overall diversity of the whole species, which are discussed in chapter 5.3.

Table 19a:

Cochlodina laminata (Montagu, 1803)						
	Morphological characteristics from literature	**Overall congruence with the results of this study**	**Lineage I**	**Lineage II**	**Lineage III**	**Lineage IV**
Colour of the shell	•light horn-colour (Brabenec 1967) •reddish or yellowish (Ložek 1956) •brown to cherry red (Welter-Schultes 2012) •yellowish brown shell (Kerney et al. 1983)	various shades of brown✸	various shades of brown✸	various shades of brown✸	horn-colour⊙	various shades of brown✸
Surface of the shell	•nearly smooth, shiny, pellucid (Welter-Schultes 2012) •pellucid, softly furrowed (Ložek 1956) •shiny and rather translucent (Kerney et al. 1983)	smooth, with or without spiral lines	smooth, with or without spiral lines	smooth, with or without spiral lines	smooth, no spiral lines	smooth, with or without spiral lines
Shape of aperture	•mostly four-angularly pear shaped (Brabenec 1967, Ložek 1956)	variable✸	variable✸	variable✸	variable✸	variable✸
	•aperture rim thickened and white (Kerney et al. 1983)		in older shells only❖	in older shells only❖	in older shells only❖	in older shells only❖
Palatal callus	•very weak, brownish or whitish, receding from the lip of the shell in its lower part, not with distinct edges (Ložek 1956)	variable✸	mostly yes✸	variable✸	distinct, strong and white⊙	from almost undeveloped to very strong ✸
	•very weak, often almost unrecognisable, especially in the lower part (Brabenec 1967)	variable✸	deeper in the shell or closer to lip or very wide and strong✸	variable, very strong forms also present✸		
	•usually weak (Welter-Schultes 2012)		mostly yes✸	no	no	variable✸
	•receding from the lip of the shell in its lower part, not with distinct edges (Ložek 1956)		mostly yes, but sometimes with one or two extensions along either subcolumellar lamella or lower palatal plica or both✸❖	visible on the right side with zero to two extensions along subcolumellar lamella or lower palatal plica✸❖	extension along left side of subcolumellar lamella⊙	zero or one extension✸❖
		variable ✸	deeper in the shell or reaching from depth of the shell almost to the lip ✸	deeper in the shell or reaching from depth of the shell almost to the lip ✸	deeper in the shell⊙	deeper in the shell⊙

Table 19b:

Cochlodina laminata (Montagu, 1803)

	Morphological characteristics from literature	Overall congruence with the results of this study	Lineage I	Lineage II	Lineage III	Lineage IV
Superior lamella	•strongly developed (Kerney et al. 1983)	variable✸	various or no shapes✸	various or no shapes✸	no shapes⊙	no shapes⊙
	•longer (Brabenec 1967)					
	•relatively low (Ložek 1956)					
Inferior lamella	•strongly developed (Kerney et al. 1983)	variable✸	various or no shapes✸	various or no shapes✸	constriction or no shapes ✸	various or no shapes✸
	•ends on the lip usually simply - with no callus or knob (Brabenec 1967)					
	•strong, bent, distinct (Ložek 1956)					
Subcolumellar lamella	•not visible or almost invisible from the apertural view (Ložek 1956)	visible or not visible	visible or not visible	visible or not visible	visible	visible
	•almost invisible from the apertural view (Brabenec 1967)					
Principal plica	•long, parallel with the seam in all its length (Brabenec 1967)	**visible**	**visible**	**visible**	**visible**	**visible**
	•long, usually begins before the upper palatal plica, reaches to the palatal callus (Ložek 1956)	variable✸	merges or does not touch palatal callus,begins before or on the same level of beginning of upper palatal plica	merges or does not touch palatal callus, usually begins before upper palatal plica, but not always	merges with paltal callus	merges with paltal callus, begins before upper palatal plica
Upper palatal plica	• short, not paralell with the seam (Brabenec 1967)	variable✸	mostly non parallel with the seam or principal plica ✸	mostly non-parallel but not always	non-parallel, begins after principal plica	non-parallel to principal plica
Lower palatal plica	•approximateyly twice as long as the upper palatal plica and distinctly connected with the palatal callus, so it is very well visible from the apertural view (Brabenec 1967, Ložek 1956)	variable✸	visible or not visible✸	visible	visible	visible
	•merging with palatal callus or running through it (Nordsieck 2013)	variable✸	merges or does not touch palatal callus or cuts through it and interrupts it like in *C. costata* ✸	merges or does not touch or even interrupt palatal callus - extreme variability✸	merges with palatal callus⊙	merges or does not touch palatal callus, because callus is not developed there
	•reaches the callus and sometimes breaks through (Welter-Schultes 2012)					

✸	this symbol suggests that this morphological character is variable
⊙	even though individuals of this species were uniform in this morphological characteristic, evidence from analysis of other species could suggest that this uniformity is an artefact of insufficient sampling and this characteristic could also be highly variable
❖	this morphological characteristic could be age-related

5.1.6. *Cochlodina dubiosa*

Table 20 offers a comparison of morphological characters mentioned in literature and observed in this study.

The morphological features that were shared by all individuals examined within this study were following:

- principal plica is visible from aprtural side of the shell and merges with palatal callus
- lower palatal plica is visible from aprtural side of the shell
- subcolumellar lamella is visible from aprtural side of the shell
- palatal callus without extensions (untrue plicae), of different strength, lying deeper in the shell
- surface of the shell is smooth (with or without spiral lines)

Similarly as in many other species, while the aforementioned characteristics were stable, other, such as presence and type of shapes on superior and inferior lamella and shape of aperture were variable. Evidence suggests that these characters are extremely variable and so are not suitable for describing new forms or being used as determination characters.

Table 20:

Cochlodina dubiosa (Clessin, 1882)		
	Morphological characteristics from literature	**Congruence with the results of this study**
Colour of the shell	•more distinctly striated than *C. laminata* (Nordsieck 2007b)	•horn-colour ⊙
Surface of the shell	-	•smooth
	-	•with or without spiral lines ✸
Shape of aperture	•narrow, egg-shaped to pear-shaped (Kerney et al. 1983)	•variable (oval, bevelled oval and drop-shaped apertures were observed) ✸
	•pear-shaped (Welter-Schultes 2012)	
Palatal callus	•more or less distinct (Kerney et al. 1983)	•OK (forms from white and very well developed to almost invisible were observed) ✸ or ❖
	•strong (Welter-Schultes 2012)	
	•less diffuse than in C. laminata (Nordsieck 2007	• OK
	•not crossed by basalis (Welter-Schultes 2012)	•OK
Superior lamella	-	•various or no shapes on this lamella were observed ✸
Inferior lamella	-	•various or no shapes on this lamella were observed ✸
Subcolumellar lamella	•reaching out (Kerney et al. 1983)	•OK
Principal plica	•starts not or only a little in front of upper palatal plica (Kerney et al. 1983)	•usually begins sooner than upper palatal plica, but in one case both beginnings of plicae were on the same level ✸
	-	•merges with palatal callus
	-	•visible in the aperture
Upper palatal plica	-	•parallel or non-parallel with principal plica ✸
Lower palatal plica	•ends far away from palatal callus or runs into it (Kerney et al. 1983; Nordsieck 2007b), but never goes through it (Kerney et al. 1983)	•OK ✸❖
	-	•visible from apertural point of view

✸	this symbol suggests that this morphological character is variable
⊙	even though individuals of this species were uniform in this morphological characteristic, evidence from analysis of other species could suggest that this uniformity is an artefact of insufficient sampling and this characteristic could also be highly variable
❖	this morphological characteristic could be age-related

5.1.6.1. Comparison of morphology of *C. laminata* – lineage III, *C. laminata* – lineage IV and *C. dubiosa*

According to the phylogenetical tree obtained from Bayesian analysis, *Cochlodina laminata* and *Cochlodina dubiosa* are closely related species. The clade including both of them is well supported, as well as the clade *C. dubiosa* itself. However, while the individual lineages of *C. laminata* are well supported, the overall clade is not. And even though lineages I and II of *C. laminata* mostly fill the criteria traditionally listed in determination literature, lineage IV and especially lineage III are very different.

Cochlodina dubiosa and *Cochlodina laminata* comprise a well-supported clade throughout which the morphological characteristics are variously distributed.

Therefore I propose that either:

A) none of these two species are valid species and both need to be newly defined

or

B) *C. dubiosa* is a valid species, but *C. laminata* is not. While lineages I and II of C. laminata fit the most criteria for this species, the morphology of lineages III and IV differs and they may be a different morphologically determined species. Lineage currently marked as *C. laminata* could be renamed to *C. laminata* s. str. (including lineages I and II) and lineages III and IV should be described as new species.

In the light of the fact that *C. dubiosa* appears to be relatively stable in morphology (although it has to be mentioned again that there were very few individuals of *C. dubiosa* included in the tudy compared to *C. laminata*), I suggest that option B) is more probable.

Table 21 gives a comparison of these three lineages and sums up variable and non-variable (common) characteristics (marked by *). The character of palatal callus is a questionable shared character (marked by a question mark - ?). It was variably strong within these lineages. However, the level of calcification of the palatal callus could be in relationship with age. Various age of the sampled snail may be a reason of the observed variability in the strength of the palatal callus.

Table 21: Comparison of observed morphological characteristics between two lineages of *C. laminata* and *C. dubiosa*. Shared characteristics are marked with a star (*), while questionable shared characteristics are marked with a question mark (?).

	C. laminata **(III)**	***C. laminata*** **(IV)**	***C. dubiosa***	
Colour of the shell	horn-colour	various shades of brown	horn-colour	
Spiral lines	absent	present or absent	present or absent	
Shape of aperture	variable	variable	variable	
Superior lamella	no shapes	no shapes	various or no shapes	
Inferior lamella	constriction or no shapes	various or no shapes	various or no shapes	
Subcolumellar lamella	visible	visible	visible	*
Principal plica	visible	visible	visible	*
Principal plica and palatal callus	merge	merge	merge	*
Beginnings of principal plica and upper palatal plica	principal plica begins sooner	principal plica begins sooner	both plicae begin on the same level or principal plica begins sooner	
Mutual position of principal plica and upper palatal plica	non-parallel	parallel or non-parallel	parallel or non-parallel	
Lower palatal plica	visible	visible	visible	*
Lower palatal plica and palatal callus	merge	merge or do not touch	merge or do not touch	
Character of palatal callus	strong and distinct	variable	variable	?
Position of palatal callus	deeper in the shell	deeper in the shell	deeper in the shell	*
Extensions of palatal callus	left side of subcolumellar lamella	none or on the left side of subcolumellar lamella	left side of subcolumellar lamella	*

5.1.7. *Cochlodina fimbriata*

Because *C. fimbriata* was divided into four lineages and each of them was assessed individually, they are presented individually also in the following table (table 20). Lineage III is left out, as this contained sequences from Tlachač (2008).

Compared to *C. laminata*, this species was less variable, however, the sampling was not as thorough as in *C. laminata*, so the lower variability may be an artefact of less sufficient sampling.

The whole clade of *C. fimbriata* (lineages I-IV) is more supported than *C. laminata*, however, this could again be an artefact of less samples being included in the analysis. Each of the four lineages is well supported, therefore a further hypothesis could be proposed, that *C. fimbriata* is a complex of cryptic lineages – a very diverse species with rich evolutionary history, which resulted in today's great variability.

As can be seen in the map in figure 41, these lineages rarely occur on the same sites together. Only two cases have been observed. In these two localities, it cannot be said with certainty, whether there is hybridisation possible between the two lineages. If these lineages do not hybridise, each of the four lineages could be a species of its own, therefore the clade that is here refferred to as *C. fimbriata* would be a taxon comprising of several cryptic lineages that could possibly be regarded as species.

Probably most surprising was the fact that the shell can be of various shades of brown or yellow, which is in incongruence with data obtained from literary sources. White individuals occurred as well. Therefore colour is not a good characteristic for determination of the species

The only characters that were common for all three assessed lineages were these:

- subcolumellar lamella is visible
- lower palatal plica does not reach and therefore does not touch or merge with palatal callus
- surface of the shell is smooth (with or without spiral lines)

All other characters were variable.

Table 22:

Cochlodina fimbriata **(Rossmässler, 1835)**

	Morphological characteristics from literature	**Congruence with the results of this study**			
			lineage I	**lineage II**	**lineage IV**
Colour of the shell	•shell similar to *C. laminata* , colouring weaker than on *C. laminata* , often regularly clearly streaked, especially on the first whorls (Kerney et al. 1983) •yellowish red or reddish-yellowish, slightly striped (Rossmässler 1835)	• very variable✸	• yellow⊙	• variable - white, yellow, light brown✸	• variable - white to horn-colour✸
	•lighter than *C. laminata* (Welter-Schultes 2012)	not always✸	• OK⊙	• no✸	• no✸
Surface of the shell	•like *C. laminata* , but with more prominent riblets (Welter-Schultes 2012) •barely ribbed (Rossmässler 1835)	• like *C. laminata* = **smooth** with or without spiral lines✸	• **smooth** without spiral lines✸	• **smooth** spiral lines present or absent✸	• **smooth** spiral lines present or absent✸
Shape of aperture	•egg-shaped to pear shaped (Rossmässler 1835)	• variable✸	angularly oval⊙	very variable✸	very variable✸
Palatal callus	•strong palatal callus present (Welter-Schultes 2012) •white, clear,outstanding (Rossmässler 1835)	• usually strong ❖	• wide, white, calcified, forms an extension along subcolumellar lamella ⊙	• wide, white, calcified, deeper in the shell and either reaches the lip or not, with or without an extension along subcolumellar lamella	• of various strength✸❖ • usually distinct, occurring close to the lip or deeper in the shell, sometimes wide from depth of the shell almost to its lip, with one or no extension along subcolumellar lamella✸
Superior lamella	-	• variable✸	• no shapes⊙	• variable✸	• variable✸
Inferior lamella	-	• variable✸	• variable✸	• variable✸	• variable✸
Subcolumellar lamella	-	**• visible**	**• visible**	**• visible**	**• visible**
Principal plica	-	•visible or not visible	• visible	• visible or not visible ✸	•visible
		•merges with callus or does not touch it-	• merges or does not touch palatal callus ✸	• touches or does not touch palatal callus ✸	• merges with or does not touch palatal callus ✸
		•beginning before or after principal plica-	• begins before upper palatal plica ⊙	• begins before or on the sme level as upper palatal plica ✸	•begins on the same level as principal plica
Upper palatal plica	-	•running parallel or non-parallel to principal plica✸	•non-parallel to principal plica ⊙	•non-parallel to principal plica ⊙	•parallel or non-parallel to principal plica ✸
Lower palatal plica	•in frontal view hardly recognizable (Kerney et al. 1983)	•most of the time not visible✸	• not visible	•not visible	• visible or not visible ✸
	•not associated with palatal callus (Kerney et al. 1983, Welter-Schultes 2012)	**• OK**	**• OK**	**• OK**	**• OK**

✸	this symbol suggests that this morphological character is variable
⊙	even though individuals of this species were uniform in this morphological characteristic, evidence from analysis of other species could suggest that this uniformity is an artefact of insufficient sampling and this characteristic could also be highly variable
❖	this morphological characteristic could be age-related

5.2. Factors influencing morphological analysis

Several complications have been encountered during observing the morphological features on the shells. Here, I list several factors that can influence correct species identification.

5.2.1. Age of the specimen

Most of the morphological characteristics that are used for determining the species within genus *Cochlodina* are placed in the last and youngest whorl of the shell. The lip of the shell grows as the animal ages and after its completion the growth is considered to be complete (Maltz and Sulikowska-Drozd 2008). However, there are no data on whether certain features get more prominent as the animal ages further. Therefore, identification of the juvenile specimen of the genus is almost impossible. Juvenile and young shells are also very susceptible to damage, especially in the area around the uncompleted lip of the shell. Juvenile shells, in which the lip of the shell was not fully developed, were excluded from the analysis of morphological features. However, young and fully grown individuals have been used for analysis, including those individuals whose shells have not yet been fully calcified and some features might be less distinct than in older snails (for example young specimens of *C. laminata* can lack the palatal callus, which is also not very distinct in old shells, completely).

Similarly, principal fold, subcolumellar lamella and other characteristics may not be fully developed yet. In certain cases , the shell lacks the palatal callus completely. On the other hand, sometimes the upper palatal fold seems to be more developed and reaches further out of the shell, while subcolumellar lamella is not visible and the overall shape of the mouth of the shell may not be fully developed yet. Superior and inferior lamella are also not fully calcified yet, however, on many of them constrictions or callous areas are already visible).

5.2.2. Corrosion of the shell

Several original descriptions of species mention the corrosion of the shell (Rossmässler 1835, Montagu 1803). The level of corrosion of the shell is closely related to its age – the longer the animal lives, the longer it is exposed to environmental conditions that damage its shell, and therefore the damage is greater. The fact that these animals are long-lived (Tlachač 2008, Heller and Dolev 1994, Stelfox 1969, Piechocki 1982, Wirth et al. 1997, Giokas and Mylonas 2002), means that corrosion of the shells is very common. Corrosion can also damage surface structures of the shell and therefore structures such as growth lines, ribs and spiral lines, as well as the original colour of the shell, might be unrecognizable. Level of corrosion depends on the environmental conditions and therefore can differ in different environments.

Spiral lines were most easily visible on the darker individuals and when the light was shone on the shell at different angles. The recognition of spiral lines on paler individuals was very difficult, even when the shells not at all corroded.

5.2.3. The position of the clausilium

I encountered one more problem when viewing the plicae from the palatal side. When wet, the plicae are visible from the palatal side when the light is shone through the shell from the side. In certain cases, the clausilium was positioned in the last whorl. When the light is shone through the shell, the clausilium can appear as a vertical straight plica in place of lunulla. While this might lead to certain confusion, a thin sharp object (such as preparation needle or entomological pin) can be used to move the clausilium. If this had been done to a plica, it would stay unmoved.

5.2.4. Injuries

Accidents, in which the shell is broken may cause the shape and dimensions of the shell to be distorted. Such cases have been noticed among clausiliids as well (Mitov et al.2003).

Many injuries were also observed, however, these were mostly not on the mouth of the shell and therefore did not influence determination of the species and observing the morphological characteristics. What they may have influenced, are the dimensions of the shells, however, I did not observe any conspicuously aberrant shell forms or shapes during our study.

5.2.5. Other anomalies

Several abberant forms have been observed during assessing the morphological characteristics of the collected material. Abberant forms and anomalies have been observed in many mollusks, including Clausiliidae. Abnormalities in shell and body shape might result from external and internal factors'influence during the embryonic and post-embryonal development. Certain abnormalities can be of taxonomic importance, when they influence diagnostic characters (Mitov et al. 2003). In certain cases, misdetermination of these individuals may occur.

Albinotic or very pale individuals have been observed in two lineages of *C. fimbriata*. While these individuals had completely white shells, their bodies posessed the usual pigmentation.

This study gives evidence that abnormalities and injuries, or unusual number of plicae are fairly common in *Cochlodina* and should be taken into consideration when determining species.

5.3. Variability of *C. laminata*

This study confirms Tlachač's (2008) finding that *C. laminata* is a very diverse species in genetics and morhology. The same was observed for *C. fimbriata* as well.

Of the seven species researched in this thesis, *C. laminata* was the most variable in its morphological characteristics, which is possibly an artefact of better sampling compared to other species researched here. In general it seems that the more individuals were sampled in a certain species, the more forms of certain morphological characteristic were observed. This is especially true for shapes of aperture and forms on superior and inferior lamella.

In this study, we have discovered four lineages within *Cochlodina laminata*, that could potentially be classified as species. The extreme morphological diversity that was showed in "Results" suggests that *Cochlodina laminata* is a young radiating species, in which morphological diversity does not match the diversity on the genetical level. Even the features that were stable for most of species, such as the position and character of palatal callus, were variable in this species, not only among different lines, but also within them.

As was mentioned in 5.1.5., it is likely that lineages III and IV are separate species and not inner lineages of *C. laminata*. Their different morphology suggests so and so do the high support values of these lineages, while the overall support for the whole clade of *C. laminata* is very low.

However, even in lineages I and II, where most individuals fitted the criteria for *C. laminata* given by Ložek (1956), different forms occurred. It is also likely, that while lineages III and IV do not belong into *C. laminata*, the *C. laminata* s. str. (lineages I and II) is a diverse taxon in itself, influenced by various events in its evolutionary history.

Lineages III and IV seem to be less variable than lineages I and II, however, there is a large difference in the numbers of sampled individuals and therefore the uniformity of these two lineages is very likely an artefact of poor sampling.

5.4. Patterns in distribution of *C. laminata* and possible ways of dispersal

There seems to be no apparent relationship between individual genetical lineages and geographic distribution either, however, a certain weak pattern can be seen.

While lineage I of *C. laminata* was distributed from Eastern Alps to East Europe, lineage II covered most of the Europe, not being restricted to any particular area. This would support the hypothesis that glacial refugia could have been in central Europe (as has been found for many other species, e.g. Magri 2008; Haase et al. 2003; Babik et al. 2005; Pfenninger and Posada 2002), where the genetical diversity is highest, each lineage spreading its own way. The fact that several parts of Europe have been colonised by more than one lineage could mean that long-distance passive dispersal occurred in this species or that lineages were spreading simultaneously.

Isolated occurences of other Clausiliids, such as *Itala ornata* or *Cochlodina commutata* were noted in the Czech Republic, great distances away from their nearest sites of occurrence, which is most probably a result of aerial dispersal by birds (Ložek 2004). Similarly, it seems that *Cochlodina dubiosa corcontica* was also a passive disperser, originating from its Alpine sister taxon *Cochlodina dubiosa* (Tlachač 2008). And lastly, disjunct range of *C. fimbriata* (Kerney et al. 1983) suggests that similar mechanism may play a role in the distribution of this species as well.

There were too few individuals belonging to lineages III and IV to conclude anything about phylogeography from the distribution of these lineages.

The hypothesis that there was a one or a few clear glacial refugia for this species which contributed to current state of morphological and genetical diversity was not confirmed. The question of the origin of the high level of diversity in *C. laminata* therefore remains. However, the relationships within this species realized through the molecular methods that are presented in this thesis suggest that the reason for this incredible level of intraspecific diversity could be the existence of several cryptic glacial refugia, or so called northern glacial refugia. These have been suggested for several other animal (Haase et al. 2003; Babik et al. 2005;Schmitt and Varga 2012) and plant (Magri 2008) species.

Fossil evidence of the species shows that *Cochlodina* did not occur as far south as the traditional southern refugia during the last Glacial (Ložek 2006). However, if there were a few large refugia located more to the north, such as in the Carpathian area, a similar trend as Taberlet et al. (1998) described for the southern refugia could be seen. Diversity would decrease with the increasing distance of dispersal from the given refugium, provided that no lineages go extinct in the refugial areas, these would be richer in diversity that newly colonized ones (Taberlet et al. 1998).

The genetical or morphological variability of *C. laminata* does not appear to follow any pattern – various lineages occurred in various sites, often together. However, some trend can be seen in the distribution of certain lineages – lineage I appears to be of more eastern distribution and lineage II seems to be more western, even though certain overlaps in areas of distribution are visible. This could be explained by occurrence of differened scattered refugia or several events of long-distance passive dispersal, which can occur for small land snail species and have been documented even for Clausiliids (Gittenberger et al. 2006).

Morphological diversity of *C. laminata* decreased towards north of Europe, however, even localities as remote as Scandinavia were colonized by more than one lineage, which is not very congruent with the idea of a single refugium. Moreover, the sampling was not as thorough in the northern parts of Europe as it was in Central Europe and therefore I cannot exclude the possibility that had the sampling been equally in-depth in all parts of distribution range of this species, there would be more sites where several lineages would occur together.

The current distribution of the four lineages would suggest that: A) there were several glacial refugia in different parts of Europe, in which various lineages survived the last glaciation and from which they later spread, colonising different areas and reaching today's overlapping distribution ranges or B) the colonisation by the other lineage – (represented only by a single individual) – can be more recent and has occurred by passive dispersal, which can carry land snails to great distances, as has been shown for other Clausiliids (Gittenberger et al. 2006), even from genus *Cochlodina* (*C. dubiosa corcontica* in Krkonoše Mountains in Czech Republic and *C. fimbriata* in Vtáčnik mountains in Slovakia). It has been recently proven by experiments, that even snails that do not have a structure to close their shells such as clausilium, are able to survive the passage through the digestive systems of birds, possibly only thanks to their small size and mucus (Wada et al. 2012). Thanks to the clausilial apparatus, these snails might be capable of surviving the consumption and all the way throughout the digestive tract of animals that consume them and therefore can transport them for long distances while digesting is in progress. All snails used in these studies are of relatively small size (up to 7 mm for *Hydrobia ulva* in Cadée (2011) and approximately 2.5mm for *Tornatellides boeningi* in Wada et al. (2012)). While *H. ulva* possesses an operculum (Cadée 2011), *T. boeingi* lacks it, but the authors argue that it could be protected from the digestive enzymes by a layer of mucus (Wada et al. 2012). Adult *Cochlodina* specimen are much larger than the snails used in aforementioned studies, therefore they could be crushed within the gizzard of birds, however, there is no reason to predict, that a juvenile *Cochlodina* individual could not be transported long distances this way. Gittenberger et al. (2006) even reported a case of a clausiliid, *Balea perversa*, being transported 9000 km from the European mainland to the Azores and back, most probably by migrating birds. There is no reason why this could not be happening on a smaller scale on the European continent.

Morphological features of *C. laminata* are not stable. It seems that this taxon is a young and currently radiating species, in which the morphological features are not stabilised yet. The complicated relationships within the species are probably a result of several processes, such as surviving the glaciated periods in various northern cryptic refugia, consequently hybridising, possibly with introgression and in land snails of this small size also very likely by long distance passive dispersal.

As snails of genus *Cochlodina* are long-lived species (Heller and Dolev 1994, Stelfox 1969, Piechocki 1982, Wirth et al. 1997, Giokas and Mylonas 2002, Gergely 2010), which climb the trees in their forest habitats, we suggest that this behavior might make them more susceptible to being transportd passively via animal vectors. Snails were also observed on vegetation and leaf litter, therefore they could be transported with this litter by wind. How far they would get in the forest ecosystem is not certain. Heavy rains can result in snails being washed down into rivers and streams and use the river valleys as corridors to colonizing new areas, which is the case of the land snails of the genus *Candidula* (Pfenninger & Posada 2002). All of these ways of dispersal could have contributed to the current distribution of lineages of *Cochlodina*.

6. Conclusion

This study followed Tlachač's study which found that there is a considerable intraspecific diversity in *Cochlodina laminata* in both morphology and on genetical level (Tlachač 2008).

This study shows that most of currently recognized Central European species of genus *Cochlodina* are monophyletic lineages that share morphological characteristics, which make them recognizable from other species of this genus. The stable morphological characters that were most reliable for determination of individual species were given in chapter "Discussion".

Except for *C. laminata* and *C. fimbriata*, there were clear morphological characters for determination of each genetically defined clade. However, other morphological characters often mentioned in literature as important, were variable and therefore should not play a role in determining species.

Individual lineages of *C. laminata* were found in various parts of Europe. Certain geographical pattern was observed. It is very likely that *Cochlodina laminata* is a young radiating taxon, in which various small cryptic glacial refugia have resulted in the observed genetical diversity and later the hybridisation between these lineages has caused the current relationships within the four lineages of the species to be the way they are at present time. Hybridisation, introgression, various glacial refugia and possibly also long-distance passive dispersal are the factors that may have influenced the diversity of the species considerably. I propose that lineages I and II of *C. laminata* are further refferred to as *C. laminata* s str., while lineages III and IV are different species, for which determination characteristics have to be found. This will only be possible of more samples from these lineages are obtained.

Nordsieck (2007b) mentions *C. dubiosa* and *C. inaequalis* to be close species of *C. laminata*. There were no specimens of *C. inaequalis* used in this study, however, the close relationship between *C. laminata* and *C. dubiosa* has been proven to be true on the basis of mitochondrial DNA, similarly as it was proven by Tlachač (2008) and Nordsieck (2007b).

According to my results, *C. laminata* and *C. dubiosa* are closely related species, forming a monophylletic clade together. However, the relationships within this clade have to be further resolved, as the split between *C. laminata* and *C. dubiosa* is not very well supported and as it also contains taxons of uncertain position.

The situation with *Cochlodina fimbriata* may be very similar, however, the sampling of this species was not as thorough as the sampling of *C. laminata* a therefore it cannot be concluded with certainty. However, *C. fimbriata* could be a focus of further investigations.

In the future, hybridisation patterns could be discovered by advanced molecular methods, as well as by obtaining sequences of more genes, both mitochondrial and nuclear, could provide more reliable results in future studies.

Investigation of the formation of determining characters (especially of palatal callus, principal plica and lower palatal plica) could offer more information about how these characteristics develop during ontogeny and therefore add to the knowledge of morphology of these snails.

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