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Final thesis

**Dispersal, Molecular Phylogeny and Phylogeography of Land Snails with  
Focus on the Family Clausillidae**

**Disperse, molekulární fylogenetika a fylogeografie suchozemských plžů se  
zaměřením na čeled' Clausiliidae**

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## **Abstract**

The active dispersal abilities of land snails are considered to be very poor. In general, most long-distance dispersal happens passively by water, erosion, winds and other organisms, including humans. Climbing reflex of xerophilous snails plays an important role in passive dispersal. Dispersal of a certain species is closely related to gene flow and patterns of dispersal over the time can be reconstructed by using several genetic markers, both nuclear and mitochondrial. In this thesis I've tried to give a summary of molecular approaches so far used on land gastropods and summarized the general trends in the study of phylogeography of European land snail biota. In Europe, distribution of animal and plant species has been greatly influenced by Quaternary climatic oscillations in Pleistocene, when the species survived in southern and montane refugia during the ice ages. From these they later colonized more northern parts of the European continent. Because our further research should be focused on the phylogeography of genus *Cochlodina* (Clausiliidae), I have given a brief introduction to the family and the genus and some studies that have been carried out on them.

**Key words:** dispersal, phylogeny, phylogeography, land snail, Gastropoda, Clausiliidae, *Cochlodina*

## Abstrakt

Schopnosti aktivního šíření jsou u suchozemských plžů považovány za velice malé. Většina rozšiřování na dálku se děje pasivně vodou, erozí, větrem a jinými organizmy, včetně člověka. Šplhací reflex xerofilních plžů hraje významnou roli v pasivní disperzi. Disperze určitého druhu je úzce spojena s tokem genů. Možné cesty rozšiřování druhů v minulosti můžou být zrekonstruovány pomocí použití různých genů, jak mitochondriálních, tak i jaderných. V této práci jsem se pokusila shrnout obecné trendy ve studiu fylogeografie evropské fauny suchozemských plžů. Distribuce živočišných a rostlinných druhů v Evropě byla velice ovlivněna kvartérními klimatickými oscilacemi v pleistocénu, kdy druhy přežívaly ledové doby v jižních a horských refugiích. Z nich posléze kolonizovaly severnější části evropského kontinentu. Protože náš další výzkum by měl být zaměřen na fylogeografii rodu *Cochlodina* (Clausiliidae), představila jsem čeled' i tento rod a uvedla některé studie, které byly na této čeledi provedeny.

**Klíčová slova:** šíření, fylogeneze, fylogeografie, Gastropoda, Clausiliidae, *Cochlodina*

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# 1. Introduction

Land snails are generally considered to be poor dispersers. However, there have been significant historical movements of European land fauna, including land snails, owing to climatic changes. This coursework is divided into three main sections. In the first one, I concentrated on different ways of dispersal of land snails, both active and passive, by which they could have travelled long distances in spite of their low mobility. I have also outlined the relationship between dispersal of a species and gene flow among its populations.

In the second part, I have given a brief introduction to materials, methods and conclusions of some of the earlier phylogenetic and phylogeographic studies carried out on land snails.

Because our future research will be focused on land snails of the genus *Cochlodina* (Clausiliidae), I have given an introduction to the family, its unique morphological features and research outcomes in the third part.

## 2. Species Distribution, Vagility and Dispersal of Land Snails

### 2.1. Distribution

A range of a certain species is formed by several processes such as speciation, extinction, transformation and selection. (Gaston 1998). Abiotic and biotic changes of the environment that the species inhabits are also significant factors determining the species range. Speciation adds new ranges, while extinction acts in opposition and removes ranges. Due to these processes ranges expand or contract. Responses to environmental changes and species dispersal abilities influence species-range distribution greatly (Gaston & He 2002).

## 2.2. Gene Flow and Dispersal

Animals move through complex landscapes and dispersal rates can vary significantly between habitat types (Baur & Baur 1995). Taxa with low vagility and poor dispersal abilities, such as land snails, tend to have patchy populations and form isolates, and as such they can have higher speciation rates (Gaston 1998). Gene flow between populations is ruled by dispersal abilities of organisms and range discontinuity can be a barrier to gene flow (Pfenninger 2002). Species are „reproductively isolated, meaning that they do not freely interbreed under natural conditions“, which means there should be no gene flow between species (Slatkin 1987). In the same study, Slatkin also stated that the geographic range of species is determined by a „series of historical accidents“. The range of a species will extend until it reaches a barrier. However, barriers can change or disappear over time. Their identification and the estimation of their significance in species distribution is a subject of historical biogeography. Gene flow can act as a constraining force in evolution. Because of gene flow there will be very little or no differentiation throughout the species range and this can prevent the splitting of the population into two species. From a different point of view, gene flow can be a creative force and promote genetic evolution, but this theory is less developed than the former one (Slatkin 1987). It is thought that rare, but not the rarest species have the highest rates of speciation (Chown 1997 in Gaston 1998). The general opinion is that more widespread taxa undergo speciation with higher probability (Rosenzweig 1978 in Gaston 1998), but on the other hand can go around the barriers more easily, which counters the previous statement. Large species ranges are often gained by high dispersal abilities of organisms forming the population, which consequently means that the gene flow is well maintained even in the large range and thus inhibits speciation (Mayr 1963). Gene flow is generally thought to go against speciation, the movement of gametes, individuals and whole populations inhibits differentiation. Genetic differentiation is a product of processes such as mutations, genetic drift (which happens owing to finite population size) and natural selection (which favours adaptations to environmental conditions). Rates of gene flow are different for different species (Slatkin 1987).

### **2.2.1. Active Dispersal**

Dispersal, by definition, is a distance covered by a snail during the period of its daily activity (Baur 1991 in Popov et al. 2004). Land snails are considered to have a very low vagility and poor dispersal abilities (Aubry et al. 2006). Several experiments have been carried out to find out rates of dispersal of land snails, which turn out to be different for different species, e.g. 20m range expansion per year in *Theba pisana* with distances of 0.1-1.1m moved per day (Baker 1988) , 1.1-2.6m per month in *Arianta arbustorum* with daily dispersal between 23 and 71 cm (Baur 1986) and *Xeropicta derbentina* individuals were found 38m from the original release point within a period of six months (Aubry et al. 2006). This seems quite a lot compared to *Pomatias elegans* with a dispersal distance of only  $15.95 \pm 12.35$  cm per 42 days (Pfenninger 2002) , *Trochoidea geyeri*, which only moves an average distance of 3 meters during its whole annual life cycle (Pfenninger et al. 1996) and *Arianta arbustorum* with yearly dispersal of one meter (Schilthuizen & Lombaerts 1994). It also seems that the type of habitat influences dispersal of the species significantly (Baur & Baur 1995). The rates of active dispersal differ seasonally, depending on meteorological characters as well as changes in the state of vegetation and physiological state of snails during the year (Baur 1986). From environmental factors influencing active dispersal, temperature and humidity seem to be of crucial importance – active dispersal is most common during wet periods, while during hot and dry seasons snails are more often dispersed passively (Aubry et al. 2006)

### **2.2.2. Passive Dispersal**

In spite of low mobility and not very good active dispersal abilities, many of the land snails become invasive pests (Aubry et al. 2006) or colonize new areas much faster than the speed of their active dispersal would suggest. In fact, the role of active dispersal is considered to be negligible in these cases (Aubry et al. 2006). Even organisms with as low a vagility as snails can be transported long distances by all sorts of transport agents, such as storms, water floods, avalanches and other organisms, including humans. Baur (1986) has also observed that snails, which have fallen down from rock walls and vegetation on steep slopes, have been rolling down the slopes thanks to the globular shape of their shell in the springtime.



### **2.2.2.1. Climbing Reflex**

Many xerophilous land snail species tend to aestivate on vegetation, often in large clusters of individuals. This is called „climbing reflex“ and some authors consider it „one of the main and most efficient features in the process of passive dispersal“ (Aubry et al. 2006).

Aestivation on the above-ground objects helps snails escape from ground heat (Jaremovic & Rollo 1979). The measurements showed that the temperature decrease between the ground and 6cm above the ground was 9.8 °C. This helps the snails reduce the water loss during the seasons with highest temperatures. When the snail is dislodged and falls down from the aestivation site for some reason, it gets to the ground and remaining inactive poses a threat of death by dessication. Fallen-down individuals become active very quickly and immediately search and climb a nearby above-ground object, which might be another piece of vegetation as well as a car, train, animal or human, which can transport them further and act as a passive dispersal vector (Aubry et al. 2006)

### **2.2.2.2. Water**

Rivers and streams can carry away snail individuals and remove them from their original sites. Snails can also be washed down slopes (Pfenninger 2002) or into the rivers and streams by heavy rain and storms, which can very often be fatal (Baur 1986). *Arianta arbustorum* individuals have survived a 10 hour long submersion in water at 18°C (Künkel 1916 in Baur 1986). Similarly, it can happen that an individual is washed into the sea or ocean. Darwin himself tried submerging a hibernating land snail *Helix pomatia* in seawater, where they survived for 20 days (Darwin 1859). Many authors thought that rafting might be the most important way of overseas dispersal, however, nowadays winds are considered to be the main agents (Vagvolgyi 1976).

### **2.2.2.3. Aerial Dispersal**

Minute species are easily carried away by winds, either on their own or with a piece of vegetation on which they dwell. In fact, in minute species, wind is considered the most

important agent in the overseas dispersal (Vagvolgyi 1976). Dispersal by air is more rapid than rafting (Miller 1966) and provides direct transport from one habitat to another without the risk of the snail being harmed on the way (Vagvolgyi 1976). Moreover, dispersal by rafting is non-directional, whereas air currents tend to have a specific direction (MacArthur & Wilson 1967). Minute genera are light enough to be picked up by air currents, and so have the advantage in wind dispersal. Larger species can be dispersed by wind in juvenile stages, but cannot reproduce as soon as they reach the new habitat (Gulick 1932 in Vagvolgyi 1976), which puts them at a disadvantage. It might be owing to these advantages that the minute genera form a much larger part of the island snail fauna than they do on the continent. For rafting size nor weight seem to be significant (Vagvolgyi 1976).

#### **2.2.2.4. Animals**

Animals of different species and phyla can become unintentional vectors for land snail dispersal. Aforementioned long-distance dispersal by birds has already been observed by Charles Darwin (Darwin 1859). Today, as some studies suggest, land snails can be transported by migrating birds as far as 9000 km (Gittenberger et al. 2006). In this case again, the minute genera and smaller species are at advantage, because they can hide in the bird plumage more easily and their weight is not a problem even for the smallest bird species. Soil-dwelling snails encounter burrowing animals, such as oceanic birds (Vagvolgyi 1976). However, species which burrow deeper into the soil are less likely to be carried away passively by biological vectors (Pfenninger 2002). Biggs (1968 in Vagvolgyi 1976) discovered live landsnails of genus *Succinea* in a crop of a dead pigeon several hours after its death, but whether a snail can survive a passage through whole digestive system unharmed is still unknown.

#### **2.2.2.5. Humans**

Humans can be accidental as well as intentional vectors of land snails. Molluscs in general have always been a part of human diet and it is thought that people took snails with them as they moved from one place to another, which was the case of *Helix aspersa*, which were bred by Romans (Aubry et al. 2006, Rogers 1908) as a food source. This might have significantly

facilitated the snail's dispersal. Walkers can also become unintentional carriers of snails when walking in the areas where many snails aestivate and dislodging them from vegetation. With development of traffic, more ways of transport became possible not only for humans, but also for snails (see climbing reflex). These have been noticed on large vehicles, such as retrograde military cargos (Aubry et al. 2006).

### **2.2.2.6. Erosion**

Soil erosion can transport ground-dwelling species or individuals hibernating in the soil. Similarly, avalanches can carry away a part of the soil together with the soil fauna, including hibernating snails, which have been found live on melting avalanches. This way, they have been observed to be moved 350-550m down the hill (Baur 1986).

## **3. Phylogeographic and Phylogenetic Studies on the Class Gastropoda**

Each taxon's unique role in the ecosystem is influenced by the geographic origin of the taxon as well as their duration as members of the community (Wilson 1961). The main goal of molecular systematics is to „describe, classify, and name the world's taxa on the basis of evolutionary relationships“ ( Eshbaugh 1995 in Lydeard & Lindberg 2003) and to create an evolutionary tree of the given taxon (Lydeard & Lindberg 2003). Phylogeography is a subset of this discipline (Lydeard and Lindberg 2003). By definition by Avise (2000) it „attempts to identify the mechanisms governing the geographic distribution of genealogical lineages (phylogenies) among and within closely related species“, with particular interest in the role of historical processes. Terrestrial mollusks seem to be good material for phylogeographic studies, because they resulted into numerous populations with different levels of geographic and genetic isolation, owing to their low vagility and high persistence. Habitat of each species is structured by both geography and climate, which governs the distribution of organisms in the end (Hugall et al. 2003). In general there are four main sources for reconstructing animal evolution: anatomy, fossil record, molecular sequences and genomic characters and ideally, all sources of evidence should be used together to give the most objective and accurate results

(Medina & Collins 2003). As the results of some studies suggest, morphology alone is not able to recognize homogeneous groups, especially not in those species where morphological variation is continuous. When comparing morphology and DNA-based taxonomy, the latter is more reliable (Pfenninger et al. 2006). Shell morphology and size variation is both genetically and environmentally induced (Goodfriend 1986), thus study of shell shape alone can not provide reliable information.

### **3.1. Material for Phylogenetic and Phylogeographical Studies**

Individuals are collected and after that stored either in a frozen form, usually at  $-80^{\circ}\text{C}$  (Schilthuizen et al. 1995; Dépraz et al. 2008), or in 96% ethanol (Fehér et al. 2009). In some studies both frozen and ethanol-preserved tissue samples were used (Uit de Weerd et al. 2004). A piece of tissue from foot (Bargues and Mas-Coma 1997; van Moorsel et al. 2000; Thomaz et al. 1996) or both foot and head (Fehér et al. 2009) is used for further analysis. In some studies, authors used whole individuals which were crushed with their shells (Pfenninger & Posada 2002).

Nowadays the most common of molecular phylogeny methods includes a polymerase chain reaction (PCR), which enables us to amplify DNA fragments *in vitro* using primers. The PCR product is then purified and the followed by determination of the nucleotide sequence (Lydeard and Lindberg 2003), alignment and analysis.

### **3.2. Genes**

Both mitochondrial and nuclear genes have been used for phylogenetic and phylogeographic studies. Genes which show very high variability among studied taxa and cannot be aligned across all taxa are usually excluded from analysis (Wade et al. 2001) and another, more conserved region is used instead (Thomaz et al. 1996).

#### **3.2.1. Nuclear Genes**

Internal transcribed spacers (ITS), variable regions of nuclear DNA, can also provide information about evolution at lower taxonomic levels (Schilthuizen et al. 1995).

Nuclear 18S rDNA gene possesses a well conserved 3' end, which is therefore easily recognizable in different species. However, the 5' end of the 5.8S rDNA gene is less conserved, and it is recognized in alignment with more difficulty (Schilthuizen et al. 1995). Wade et al. (2001) used an rRNA gene-cluster which included a part of 5.8S sequence at 3' end, complete ITS-2 region and several hundred nucleotides from the 5' end of the large subunit 28S gene in their study.

### **3.2.2. Mitochondrial Genes**

Mitochondrial DNA is a supercoiled extrachromosomal circle isolated from the nuclear DNA (Boore et al. 2005). It is predominantly inherited maternally (Thomaz et al. 1996; Simison & Boore 2008). From mitochondrial genes 16S (Pfenninger et al. 2007; Thomaz et al. 1996; Pfenninger & Posada 2002), cytochrome oxidase subunit I (COI) (Fehér et al. 2009) or a combination of both these genes or their fragments (Dépraz et al. 2008) have been used in phylogenetic studies. Mitochondrial large subunit ribosomal RNA (18S rRNA) have also been used (Douris et al. 1998). Mitochondrial genes have the advantage of higher substitution rate than nuclear DNA, which proves useful in resolving relationships in closely related taxa (Simison and Boore 2008).

In some land snail taxa, such as genus *Cepaea*, there is an extreme intraspecific variation and polymorphism of mitochondrial genome. Thomaz et al. (1996) explain, that this could be because of four possible overlapping reasons. First of all, mitochondrial evolution in pulmonates might be exceptionally fast, secondly, different morphs might have originated in separate refugia and come back together. The third reason is that natural selection could have acted to preserve the variation and lastly, it might be owing to the population structure that the persistence of ancient haplotypes has been favoured. As many as five different haplotypes were found coexisting within one population. *Helix aspersa* shows similar intraspecific divergence (Thomaz et al. 1996)

#### **3.2.2.1. Complete Mitochondrial Genomes**

Mitochondrial genome appears to be a good model for studying genome evolution for several reasons. First of all, its content is relatively well conserved among metazoans, secondly it is

advantageous for its small size and thirdly, the ongoing processes in it have been well studied (Serb and Lydeard 2003).

So far only a few mollusks' complete mitochondrial genomes were sequenced. There are complete mitochondrial sequences of bivalves available: *Venerupis philippinarum*, *Inversidens japonensis*, *Meretrix petechialis* (Ren et al. 2009), *Argopecten irradians* and *Chlamys farreri* (Ren et al. 2010), one complete sequence of a polyplacophoran *Katharina tunicata* (Boore and Brown 1994) and several cephalopods: *Nautilus macromphalus* (Boore 2006), *Watasenia scintillans*, *Todarodes pacificus*, *Sepioteuthis lessoniana*, *Sepia officinalis* and *Octopus ocellatus* (Akasaki et al. 2006). From Gastropoda, there are only six sequences available, of which only two : *Albinaria coerulea* (Hatzoglou et al. 1995) and *Cepaea nemoralis* (Terrett et al. 1996) are landsnails. The other four gastropods sequenced are, *Pupa strigosa* (Kurabayashi & Ueshima 2000), *Roboastra europaea* (Grande et al. 2002), *Thais clavigera* (Ki et al. 2010) and *Aplysia californica* (Knudsen et al. 2006). Thus, 16 complete mitochondrial sequences of marine mollusks are known and only two terrestrial ones.

Mitochondrial genomes of mollusks range from approximately 10 to 42 kb, which is much smaller than the nuclear genomes. These can reach up to 5,800,000 kb in size (Simison & Boore 2008).

### **3.2.2.2. Mitochondrial Gene Order**

Mitochondrial genomes of Mollusca also differ from the vertebrate genomes notably by high variation in gene order (Lydeard & Lindberg 2003). Normal metazoan mitochondrial genome contains 37 genes: 2 for rRNAs, 22 genes for tRNAs and 13 genes for proteins (Boore & Brown 1994). Mollusks in general have a higher rate of gene rearrangement compared to other phyla (Simison & Boore 2008; Serb & Lydeard 2003). The gene order does not seem to be subject to selection, so „the likelihood of two lineages independently adopting identical states or of a lineage reverting to an earlier state is low“ (Simison & Boore 2008).

### **3.2.3. Combinations of Sequences**

As many authors claim, results obtained from only one gene sequence are not reliable enough to be a reason for proposing fundamental changes in generally accepted system or

nomenclature (Fehér et al. 2009). Therefore there are studies which combine information from two genetic sequences, either mitochondrial (Fehér et al. 2009), nuclear (Schilthuizen et al. 1995) or combined mitochondrial and nuclear (van Moorsel et al. 2000; Pinceel et al. 2005; Uit de Weerd et al. 2004; Uit de Weerd & Gittenberger 2005)

### **3.2.4. Rates of Evolution of Genes**

In general we can say that the rate of evolution of mitochondrial genes is higher than of nuclear genes. Different evolution rates can also be observed among different mitochondrial genes. Example of this can be the evolution of 18S rRNA gene which has a slower evolution compared to that of 28S rRNA and for this reason is used in reconstructing deeper phylogenies (Bargues & Mas-Coma 1997). Mollusks are also a group with a well-preserved fossil record, which can be significant in aiding the interpretation of molecular phylogenetic patterns, estimating divergence times, and also serves as an excellent source of information about morphological evolution (Lydeard & Lindberg 2003).

Rate of evolution of mitochondrial genes is considered to be different in different studies. In arthropods it was estimated to 2.3 % per million years (Masta 2000). However, in land snails, much higher rates were considered. Pfenninger et al. (2005) considered a divergence of 5 % per million years, while in a different study 5.6 % per million years evolution rate was considered (Pfenninger et al 2003).

Broadness of taxon sampling influences the accuracy of obtained results. More samples from more places can lead to different outcomes. Fewer samples could lead to conclusion that the group is paraphyletic, whereas when using broader taxon sampling, the results support monophyly of the same group. This was the case of aquatic genus *Lymnaea* (Remigio 2002).

### **3.2.5. Secondary Gene Structures and Microsatellite Analyses**

Secondary gene structures are sometimes determined in phylogenetic analyses. Several studies used the secondary structure of 18S rRNA gene (Bargues and Mas-Coma 1997; Dutra-Clarke et al. 2001). Some studies (Dépraz et al. 2008) also used microsatellite analyses.

## 4 Quaternary Climate Changes and Phylogeography

In the past two million years during the Quaternary, the evolution of species on the European continent has been greatly influenced by climatic oscillations, which have been caused by changes in the Earth's orbit, consequences of which can be seen in the present-day distributions of European biota (Hays 1976 in Taberlet et al. 1998). This period is typical by repeated global cooling connected with the formation of ice-sheets over the continent. These events have of course influenced the ranges and distribution of species and have led to extinction of some taxa. As the ice sheet expanded, species ranges were pushed to south to warmer regions. With melting of the ice, species moved north again (Taberlet et al. 1998). Thus, the phylogeography of European land snails cannot be studied without reference to climate change impact on its ranges.

### 4.1. Refugia

There are several areas in Europe which could have acted as temperate refugia – the Caspian/Caucasus region and the southern peninsulas of Iberia, Italy and Balkans-Greece (Hewitt 2004). Several phylogeographic studies on vertebrates as well as invertebrates and plants have been carried out, which conclude that the northern parts of the continent have been colonised mostly from Iberic and Balkanic refugia, while the Italian refugia were often isolated, because the Alps prevented the northward expansion of Italian populations. Two refugia, Italy and Balkans, form a clade, which is thought to be because of their close proximity as well as of the fact that there might have been a connection between them across the Adriatic sea when the sea level was lower during the cold periods (Taberlet et al. 1998). It is generally assumed that the present-day northern species are related to the ones inhabiting more southern regions. Similarly, the species movements have occurred in the mountains at about 40° of latitude, where the montane species movement has been vertical, with the species descending with climate cooling and moving to higher altitudes with warming. In Europe the Alps have been the major feature that influenced the phylogeography of the taxa (Hewitt 2004). From a conservation point of view, the southern refugia are of greatest conservation interest, because they support the most of extant genetic variation and their preservation is



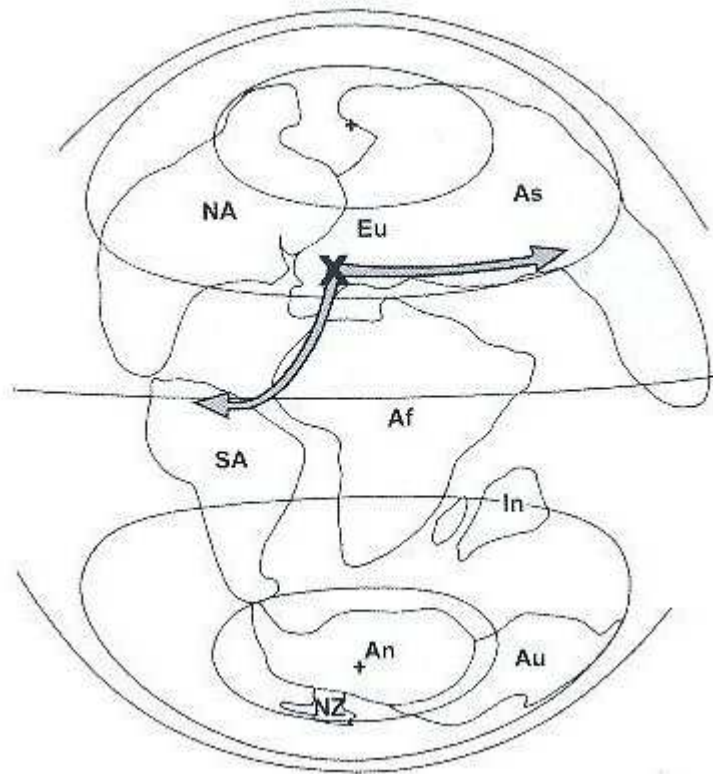
desirable for long-term conservation. Areas such as Italy have many endemic lineages. When a species disappears from the southern warm regions during the warm period, its survival during the cold periods is endangered too (Taberlet et al. 1998). All of these events have shaped the current genetic structure of populations. Extinctions, bottleneck and founder effects have occurred here repeatedly. The use of DNA markers combined with palaeoclimatic reconstruction can aid the reconstruction of the species evolution during this period of time. While some species have diverged and split into new species, others might still be capable of hybridization (Hewitt 2004).

Land snails, having limited dispersal abilities, are ideal organisms to study phylogeographical patterns and evolutionary processes on, as they cannot actively escape the fast-changing ecological conditions, that is why majority of gastropods went extinct (Pfenninger & Posada 2002) and those that remained, survived in suitable refuges, from which they later colonized other areas (Ant 1966 in Pfenninger and Posada 2002). Because of the low dispersal capacity of these animals, there are strong tendencies to preserve genetic variation patterns that arose in the past with no blurring by more recent gene flow (Cruzan & Templeton 2000). In the genus *Candidula*, the colonization of the more northern areas from refuges in southeast France could have occurred through range expansions along the river valleys, which formed several independent corridors for dispersal, probably by larger mammals and grazing routes (Pfenninger & Posada 2002) and led to formation of secondary contact zone, where formerly divided lineages met again (Hewitt 1996).

## **5 Clausiliidae**

### **5.1. Introduction**

Clausiliidae are a family of pulmonate land snails (Stylomatophora) with main centres of distribution in Europe, East Asia and South America. The family is thought to have an European origin, (Fig. 1), with most lineages having originated in the Upper Cretaceous. Woods and rocky areas are habitats that they mostly inhabit, and microscopic fungi, plants and decaying organic matter are foods they most likely feed on. The oldest fossils date back to the Upper Cretaceous.



**Figure 1:** History of the distribution of Clausiliidae in Upper Cretaceous. X – centre of origin, arrows show the main directions of dispersal (Nordsieck, 2007).

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There are 156 described extinct and 1278 extant species with 50 other considered, but undescribed. Extant species have been divided into 9 subfamilies (Phaedusinae, Serrulininae, Laminiferinae, Garnieriinae, Neniidae, Alopiinae, Mentissoideinae, Clausiliinae and Baleinae) by Nordsieck (2007) according to shell and genital characters. The monophyly of the family has been confirmed by several studies and is also proven by clausilial apparatus – a distinct autapomorphic feature, which is formed from the interior wall-folds and can be found in the two lowermost whorls of the turret-shaped clausiliid shell. The formation of the clausillium, the most important part of clausilial apparatus, is carried out by mantle epithelium and it has been concluded that the clausilium itself originated from a lamella between inferior and subcolumellar lamella. Sinistrality of their shell is another autapomorphy (Nordsieck, 2007).

## 5.2. Studies Carried out on Family Clausiliidae

The number of molecular, phylogenetic and phylogeographic studies carried out on the land snails belonging to this family is not very numerous. Without doubt, genus *Albinaria* is the most studied from among Clausiliidae. It was subject to several studies (Schilthuizen et al. 1995) and full mitochondrial genome of species *Albinaria coerulea* has been sequenced (Hatzoglou et al. 1995). From mitochondrial DNA it has also been inferred *Albinaria* is a monophyletic genus (Douris et al. 1998) However, while certain nominal taxa were confirmed to be monophyletic, other seemed to be polyphyletic (Giokas 2000). Several years later, the study which used combined analysis of nuclear and mitochondrial genes showed that *Albinaria* is not monophyletic and some of the species should be grouped together with genus *Cristataria* (Uit de Weerd & Gittenberger 2005).

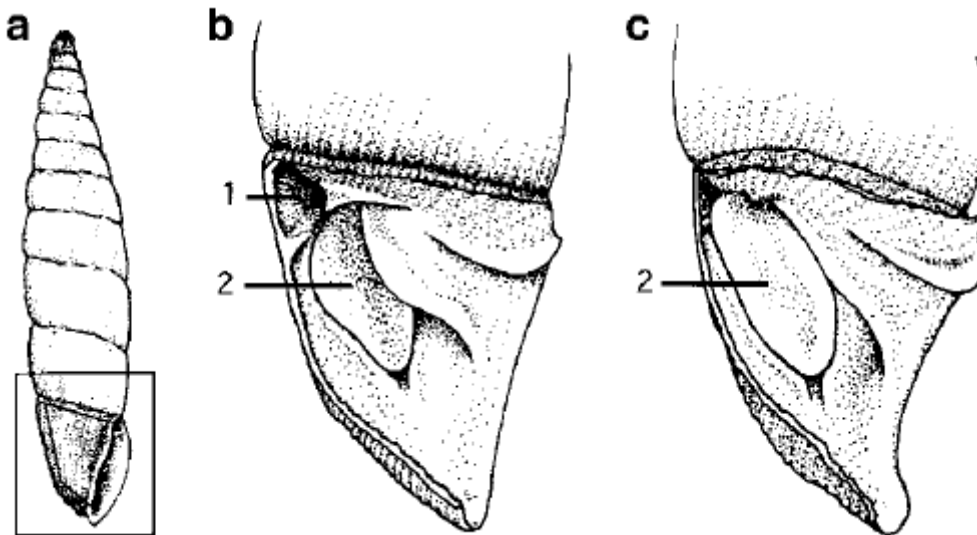
Another genus, *Isabellaria*, is thought to be a polyphyletic entity. It was proved in several studies by the nuclear sequence for ITS 1 (Schilthuizen et al. 1995) and both ITS1 and ITS2 (van Moorsel et al. 2000). It has also been proposed that current species which have been found to be placed within other species, should not have a status of species, because as Giokas (2000) stated, a species should be monophyletic.

## 5.3. Clausilial apparatus

Clausilial apparatus, being characteristic for the family, has been a subject of several studies. There are two types of clausilial apparatus – the normal (N) type and Graciliaria (G) type (Fig. 2). These structures seem to be highly homoplasious and have probably evolved repeatedly several times (van Moorsel et al. 2000; Schilthuizen et al. 1995).

The N type clausilial apparatus possesses a bypass canal, which enables contact between the outer and inner environment of the shell. The G type clausilial apparatus lacks this canal and enables the shell to close completely and is less common (van Moorsel et al. 2000).

N type is thought to be plesiomorphous (Nordsieck 1982 in van Moorsel et al. 2000). The change of N into G type has occurred several times in the evolutionary history of the family (van Moorsel et al. 2000; Uit de Weerd et al. 2004). The adaptive significance of the G type is not well explained. One of the possibilities is that it provides better protection against predation, e.g. by *Drilus* beetle larvae (Schilthuizen et al. 1994 in van Moorsel et al. 2000).



**Figure 2:** a - side-view of a clausiliid shell, b – normal-type clausillial apparatus, c – Graciliaria type clausillial apparatus; 1 – bypass canal, 2 - clausilium (van Moorsel et al. 2000)

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Transformations of N type to G, type involve loss of two lamellae and have been found more often than vice versa. However, these transformations are not irreversible. In addition to this it has been assumed that the type of clausillial apparatus is not a good diagnostic character in genus definition because of the N type's plesiomorphism and recurrent evolution of the G type in several lineages. Parallel evolution can occur in different lineages owing to common selection pressure. One of the factors enabling this is the limited dispersal ability of land snails, which prevents advantageous traits from spreading rapidly and gives opportunity for parallel evolution (Uit de Weerd et al. 2004).

#### **5.4. Genus *Cochlodina***

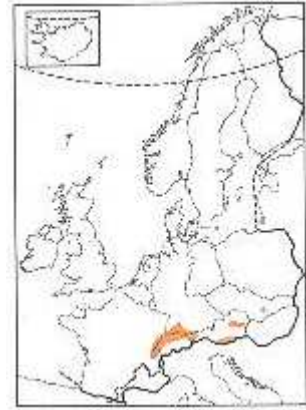
*Cochlodina* (Clausiliidae) is a genus, which consists of species usually with very small distribution areas. However, *Cochlodina laminata* is an exception, with a range covering most of Europe, except its warmest and coolest parts (Fig. 3). This species also exhibits great intraspecific variability, both in genetics and in morphology.



*C. orthostoma*



*C. laminata*



*C. fimbriata*



*C. cerata*



*C. comensis*



*C. costata*



*C. dubiosa*



*C. comensis*

**Figure 3:** distribution of European species from genus *Cochlodina* (Kerney et al. 1983)

Interestingly, the morphological variability is higher in the Carpathian (in the area of potential Central European refugia), then in the western Europe affected by ice shield mostly (L. Juříčková, pers. com.). A previous study on this genus showed that in *C. laminata* there is a significant intraspecific radiation lacking any more distinct features for easy recognition. The realized relationships within this species are thought to be a product of migratory processes in the evolutionary past of the species. *C. laminata* seems to be a complex of cryptic species, among which hybridisation could be possible or genetically very variable species due to various glacial refugia of particular populations. Similarly, intermediate forms between *C. laminata* and *C. dubiosa corcontica* were observed at the sites where both of these species live, which could also be a result of hybridisation. Intermediate morphotypes were associated with haplotypes of both *C. laminata* as well as *C. dubiosa corcontica* haplotypes, which strongly supports hybridisation between these two clausiliids (Tlachač 2008). The aim of our following study is to explain this variability and its causes. This species should be used as a model species for investigation of existence of glacial refugia in Central Europe together with three other land snail species (*Helicodonta obvoluta*, *Faustina faustina* and *Isognomostoma isognomostomos*) and several plant species. The research will be carried out in cooperation with Department of Botany at Charles University in Prague.

## 6 Conclusion

Limited dispersal abilities of land snails make them very suitable model organisms for studying phylogeographical and evolutionary processes. Research of dispersal patterns of these organisms can help us discover the ice age refugia on the European continent. In genus *Cochlodina laminata*, more studies will be needed to explain the high level of variability within this species.

## References

- Akasaki, T., Nikaido, M., Tsuchiya, K., Segawa, S., Hasegawa, M. and Okada, N.** (2006) Extensive mitochondrial gene arrangements in coleoid Cephalopoda and their phylogenetic implications – *Molecular Phylogenetics and Evolution* 38: 648-658
- Ant, H.** (1966) *Die Bedeutung der Eiszeiten für die rezente Verbreitung der europäischen Landgastropoden - Malacologia* 5: 61-62 (sec. cit.)
- Aubry, S., Labaune, C., Magnin, F., Roche, P. and Kiss, L.** (2006) Active and passive dispersal of an invading land snail in Mediterranean France – *Journal of Animal Ecology* 75: 802-813
- Avise, J.C.** (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.
- Baker, G.H.** (1988) Dispersal of *Theba pisana* (Mollusca: Helicidae) – *Journal of Applied Ecology* 25: 889-900
- Bargues, M.D. and Mas-Coma, S.** (1997) Phylogenetic Analysis of Lymnaeid Sails Based on 18S rDNA Sequences – *Molecular Biology and Evolution* 14(5): 569-577
- Baur, A.** (1991) *Effects of Competitive Interactions and Habitat Structure on Life-History Traits and Dispersal in Land Snails, PhD. Thesis, Uppsala* (sec. cit.)
- Baur, B.** (1986) Patterns of dispersion, density and dispersal in alpine populations of the land snail *Arianta arbustorum* (L.) (Helicidae) – *Holarctic Ecology* 9: 117-125
- Baur, B. and Baur, A.** (1995) Habitat-related dispersal in the rock-dwelling land snail *Chondrina clienta*. – *Ecography* 18: 123-130
- Biggs, H.E.J.** (1968) *Succinea putris* (L.) in a pigeon's crop – *The Conchologist's Newsletter* 24:36 (sec. cit)
- Boore, J.L.** (2006) The complete sequence of the mitochondrial genome of *Nautilus macromphalus* (Mollusca: Cephalopoda) – *BMC Genomics* 7: 182
- Boore, J.L. and Brown, W.M.** (1994) Complete DNA Sequence of the Mitochondrial Genome of the Black Chiton, *Katharina tinucata* – *Genetics* 138: 423-443
- Boore, J.L. and Brown, W.M.** (1994) Mitochondrial genomes and the phylogeny of mollusks – *Nautilus* 108: 61-78
- Boore, J.L., Macey, J.R. and Medina, M.** (2005) Sequencing and comparing whole mitochondrial genomes of animals – *Molecular Evolution: Producing the Biochemical Data, Part B. Methods in Enzymology* 395: 311-348 (Ed. by Zimmer, E.A. and

Roalson, E.) Burlington, MA: Elsevier

- Chown, S.L.** (1997) *Speciation and rarity: separating cause from consequence – The biology of rarity: causes and consequences of rare-common differences* (ed. by Kunin, W.E. and Gaston, K.J.) 91-109. London: Chapman & Hall (sec. cit)
- Cruzan, M.B. and Templeton, A.R.** (2000) Paleoeology and coalescence: phylogeographic analysis of hypothesis from the fossil record – *Trends in Ecology and Evolution* 15: 491-496
- Darwin, C.** (1859) *On the Origin of Species* – Murray, London
- Dépraz, A., Cordellier, M., Hausser, J. and Pfenninger, M.** (2008) Postglacial recolonization at snail's pace (*Trochulus villosus*): confronting competing refugia hypotheses using model selection – *Molecular Ecology* 17: 2449-2462
- Douris, V., Giokas, S., Lecanidou, R., Mylonas, M., Rodakis, G.C.** (1998) Phylogenetic analysis of mitochondrial DNA and morphological characters suggest a need for taxonomic re-evaluation within the Alopinae (Gastropoda: Clausiliidae) – *Journal of Molluscan Studies* 64: 81-92
- Dutra-Clarke, A.V.C., Williams, C., Dickstein, R., Kaufer, N. and Spotila, J.R.** (2001) Inferences on the phylogenetic relationships of Succineidae (Mollusca, Pulmonata) based on 18S rRNA gene – *Malacologia* 43 (1-2): 223-236
- Eshbaugh, W.H.** (1995) *Systematics Agenda: An historical perspective – Biodiversity and Conservation* 4: 455-462
- Fehér, Z., Szabó, K., Bozsó, M. and Péntes, Z.** (2009) Phylogeny and phylogeography of the *Lozekia-Kovacsia* species group (Gastropoda: Hygromiidae) – *Journal of Zoological Systematics and Evolutionary research* 47(4): 306-314
- Gaston, K.J. and He, F.** (2002) The distribution of species range size: a stochastic process – *Proceedings of the Royal Society London B* 269: 1079-1086
- Gaston, K.J.** (1996) Species-range-size distributions: patterns, mechanisms and implications – *Trends in Ecology and Evolution* 11: 197-201
- Gaston, K.J.** (1998) Species-range size distributions: products of speciation, extinction and transformation – *Philosophical Transactions of the Royal Society London B* 353: 219-230
- Giokas, S.** (2000) Congruence and conflict in Albinaria (Gastropoda, Clausiliidae) A review of morphological and molecular phylogenetic approaches – *Belgian Journal of Zoology* 130 (Supplement 1): 93-100
- Gittenberger, E., Groenenberg, D.S.J., Kokshoorn, B., and Preece, R.C.** (2006) *Molecular*



trails from hitch-hiking snails - Nature 436(26): 409

- Goodfriend, G.A.** (1986) Variation in land-snail shell form and size and its causes: a review – Systematic Zoology 35(2): 204-223
- Grande, C., Templado, J., Cervera, L. and Zardoya, R.** (2002) The Complete Mitochondrial Genome of the Nudibranch *Roboastra europaea* (Mollusca: Gastropoda) Supports the Monophyly of Opisthobranchs – Molecular Biology and Evolution 19(10): 1672-1685
- Gulick, A.** (1932) *Biological peculiarities of oceanic islands – The Quarterly review of Biology* 7: 405-427 (sec. cit)
- Harasewych, M.G., Adamkewicz, S.L., Blake, J.A., Saudek, D., Spriggs, T. and Bult, C.J.** (1997) Phylogeny and relationships of pleurotomariid gastropods (Mollusca: Gastropoda): An assessment based on partial 18S rDNA and cytochrome c oxidase I sequences – Molecular Marine Biology and Biotechnology 6(1): 1-20
- Hatzoglou, E., Rodakis, G.C. and Lecanidou, R.** (1995) Complete Sequence and Gene Organization of the Mitochondrial Genome of the Land Snail *Albinaria coerulea* – Genetics 140:1353-1366
- Hays, J.D., Imbrie, J. and Shackleton, N.J.** (1976) *Variations in the Earth's orbit: pacemaker of the ice ages – Science* 194: 1121-1132 (sec. cit)
- Hewitt, G.M.** (2004) Genetic consequences of climatic oscillations in the Quaternary – Philosophical Transactions of the Royal Society London B 359: 183-95
- Hewitt, G.** (1996) Some genetic consequences of ice ages and their role in divergence and speciation – Biological Journal of the Linnean Society 58: 247-276
- Hugall, A., Stanisci, J. and Moritz, C.** (2003) Phylogeography of Terrestrial Gastropods: The case of *Sphaerospira* Lineage and History of Queensland Rainforests – in Molecular Systematics and Phylogeography of Mollusks (ed. by Lydeard, C., and Lindberg, D.R.)
- Jaremovic, R. and Rollo, D.** (1979) Tree climbing by the snail *Cepaea nemoralis* (L.): a possible method for regulating temperature and hydration - Canadian Journal of Zoology 57: 1010-1014
- Kerney, M.P., Cameron, R.A.D. and Jungbluth, J.H.** (1983) Die Landschnecken Nord- und Mitteleuropas: Ein Bestimmungsbuch für Biologen und Naturfreunde – hamburg; Berlin: Parey
- Ki, J., Lee, Y., Jung, S., Horiguchi, T., Cho, H. and Lee, J.** (2010) Mitochondrial genome of *Thais clavigera* (Mollusca: Gastropoda): Affirmation of the conserved, ancestral gene

- pattern within the mollusks – *Molecular phylogenetics and Evolution* 54: 1016-1020
- Knudsen, B., Kohn, A.B., Nahir, B., McFadden, C.S. and Moroz, L.** (2006) Complete DNA sequence of the mitochondrial genome of the sea slug, *Aplysia californica*: Conservation of the gene order in Euthyneura – *Molecular Phylogenetics and Evolution* 38: 459-469
- Künkel, K.** (1916) *Zur Biologie der Landschnecken – Heidelberg (sec. cit)*
- Kurabayashi, A. and Ueshima, R.** (2000) Complete Sequence of the Mitochondrial DNA of the Primitive Opisthobranch Gastropod *Pupa strigosa*: Systematic Implication of the Genome Organization – *Molecular Biology and Evolution* 17(2): 266-277
- Lydeard, C., and Lindberg, D.R.** (2003) Challenges and research Opportunities in Molluscan Molecular phylogenetics – *Molecular systematics and Phylogeography of Mollusks*
- Medina, M. and Collins, A.G.** (2003) The Role of Molecules in Understanding Molluscan Evolution – *Molecular Systematics and Phylogeography of Mollusks* (ed. by Lydeard, C., and Lindberg, D.R.)
- Masta, S.E.** (2000) Phylogeography of the jumping spider *Habronattus pugillis* (Araneae: Salticidae): recent vicariance of sky island populations? – *Evolution* 54(5): 1699-1711
- Mayr, E.** (1963) *Animal Species and Evolution* – Cambridge, MA: Harvard University Press
- van Moorsel, C.H.M., Dijkstra, E.G.M. and Gittenberger, E.** (2000) Molecular Evidence for Repetitive Parallel Evolution of Shell Structure in Clausiliidae (Gastropoda, Pulmonata) – *Molecular Phylogenetics and Evolution* 17(2): 200-208
- Nordsieck, H.** (1982) *Die Evolution des Verschlussapparats der Schliessmundschnecken (Gastropoda: Clausiliidae) – Archiv für Molluskenkunde* 112: 27-43 (sec. cit)
- Nordsieck, H.** (2007) *Worldwide door snails (Clausiliidae), recent and fossil* – ConchBooks, Hackenheim
- Pfenninger, M., Bahl, A. and Streit, B.** (1996) Isolation by distance in a population of a small snail *Trochoidea geyeri*: evidence from direct and indirect methods – *Proceedings of the Royal Society London B* 263: 1211-1217
- Pfenninger, M., Cordellier, M. and Streit, B.** (2006) Comparing the efficacy of morphologic and DNA-based taxonomy in the freshwater gastropod genus *Radix* (Basommatophora, Pulmonata) – *BMC Evolutionary Biology* 6:100
- Pfenninger, M., Nowak, C. and Magnin, F.** (2007) Intraspecific range dynamics and niche evolution in *Candidula* land snail species – *Biological Journal of the Linnean Society* 90: 303-317

- Pfenninger, M. and Posada, D.** (2002) Phylogeographic history of the land snail *Candidula unifasciata* (Helicellinae, Stylommatophora): fragmentation, corridor migration and secondary contact – *Evolution* 56(9): 1776-1788
- Pfenninger, M.** (2002) Relationship between microspatial population genetic structure and habitat heterogeneity in *Pomatias elegans* (O.F. Muller 1774) (Caenogastropoda, Pomatiasidae) – *Biological Journal of the Linnaean Society* 76: 565-575
- Pinceel, J., Jordaens, K. and Backeljau, T.** (2005) Extreme mtDNA divergences in a terrestrial slug (Gastropoda, Pulmonata, Arionidae): accelerated evolution, allopatric divergence and secondary contact – *Journal of Evolutionary Biology* 18(5): 1264-1280
- Ponder, W.F. and Lindberg, D.R.** (2008) Molluscan Evolution and Phylogeny – An Introduction – *Phylogeny and Evolution of the Mollusca* – Regents of the University of California
- Popov, V.N. and Kramarenko, S.S.** (2004) Dispersal of Land Snails of the Genus *Xeropicta Monterosato 1892* (Gastropoda; Pulmonata; Hygromiidae) - *Russian Journal of Ecology* 35(4): 263-266
- Remigio, E.A.** (2002) Molecular phylogenetic relationships in the aquatic snail genus *Lymnaea*, the intermediate host of the causative agent of fascioliasis: insights from broader taxon sampling – *Parasitology Research* 88: 687-696
- Ren, J., Shen, X., Jiang, F. and Liu, B.** (2010) The Mitochondrial Genomes of Two Scallops, *Argopecten irradians* and *Chlamys farrei* (Mollusca: Bivalvia): The Most Highly Rearranged Gene Order in the Family Pectenidae – *Journal of Molecular Evolution* 70: 57-68
- Ren, J.F., Shen, X., Sun, M.A., Jiang, F., Yu, Y., Chi, Z.F. and Liu, B.** (2009) The complete mitochondrial genome of the clam *Meretrix petechialis* (Mollusca: Bivalvia: Veneridae) – *Mitochondrial DNA* 20(4):78-87
- Ricklefs, R.E. and Cox, G.W.** (1972) Taxon cycles in the west Indian avifauna – *The American naturalist* 106(948): 195-219
- Rogers, J.E.** (1908) *The Shell Book* – A popular guide to a knowledge of the families of living mollusks, and an aid to the identification of shells native and foreign – Charles T. Branford co., Publishers; Boston, Massachusetts
- Rosenzweig, M.L.** (1978) *Geographical speciation: on range size and the probability of isolate formation* – *Proceedings of the Washington State University Conference on Biomathematics and Biostatistics* (ed. D. Wollkind): 172-194. Washington University Press (sec. cit)

- Schilthuizen, M.** (2000) Bimodal hybrid zones and the scale of a snail – *TREE* 15(11): 469
- Schilthuizen, M., Gittenberger, E. and Gultyaev A.P.** (1995) Phylogenetic Relationships Inferred from the Sequence and Secondary Structure of ITS1 rRNA in *Albinaria* and Putative *Isabellaria* Species (Gastropoda, Pulmonata, Clausiliidae) – *Molecular Phylogenetics and Evolution* 4(4): 457-462
- Schilthuizen, M., Kemperman, T.C.M., and Gittenberger, E** (1994) *Parasites and predators in Albinaria (Gastropoda Pulmonata: Clausiliidae) – Bios 2: 177-186 (sec. cit)*
- Schilthuizen, M. and Lombaerts, M.** (1994) Population structure and levels of gene flow in the Mediterranean land snail *Albinaria corrugata* (Pulmonata: Clausiliidae) – *Evolution* 48(3): 577-586
- Serb, J.M. and Lydeard, C.** (2003) Complete mtDNA Sequence of the North American Freshwater Mussel, *Lampsilis ornata* (Unionidae): An Examination of the Evolution and Phylogenetic Utility of Mitochondrial Genome Organization in Bivalvia (Mollusca) – *Molecular Biology and Evolution* 20(11): 1854-1866
- Simison, W.B. and Boore, J.L.** (2008) *Molluscan Evolutionary Genomics – Phylogeny and Evolution of the Mollusca* (ed. by Ponder, W.F. and Lindberg, D.R.) – Regents of the University of California
- Slatkin, M.** (1987) Gene Flow and the Geographic Structure of Natural Populations – *Science* 236: 787-792
- Taberlet, P., Fumagalli, L., Wust-Saucy, A.G., Cosson, J.F.** (1998) Comparative phylogeography and postglacial colonization routes in Europe – *Molecular Ecology* 7:453-464
- Terrett, J.A., Miles, S. and Thomas, R.H.** (1996) Complete DNA Sequence of the Mitochondrial Genome of *Cepaea nemoralis* (Gastropoda: Pulmonata) - *Journal of Molecular Evolution* 42: 160-168
- Thomaz, D., Guiller, A. and Clarke, B.** (1996) Extreme divergence of mitochondrial DNA within species of pulmonate land snails – *Proceedings of the Royal Society London B: Biological Sciences* 263: 363-368
- Tlachač, P.** (2008) Taxonomické postavení, biologie a ekologie endemického poddruhu Krkonoš *Cochlodina dubiosa corcontica* (Brabenec, 1967) – Diploma thesis
- Uit de Weerd, D.R. and Gittenberger, E.** (2005) Towards a monophyletic genus *Albinaria* (Gastropoda, Pulmonata): the first molecular study into the phylogenetic position of Eastern *Albinaria* species – *Zoological Journal of the Linnean Society* 143: 531-542
- Uit de Weerd, D.R., Piel, W.H. and Gittenberger, E.** (2004) Widespread polyphyly among

Alopiinae snail genera: when phylogeny mirrors biogeography more closely than morphology – *Molecular Phylogenetics and Evolution* 33: 533-548

**Vagvolgyi, J.** (1976) Body size, aerial dispersal and origin of the Pacific land snail fauna – *Systematic Zoology* 24: 465-488

**Wade, C.M., Mordan, P.B. and Clarke, B.** (2001) A phylogeny of the land snails (Gastropoda: Pulmonata) – *Proceedings of the Royal Society London B: Biological sciences* 268: 413-422