# Introduction

How beetles cope with the environment and competition provoked scientists for generations. Ultimately, studies on ecological and evolutional strategies become a very broad topic with various impacts on our lives and it is important to see that general understanding of direct and indirect interactions in nature is highly important for applied and theoretical fields of science (Begon *et al.*, 2006; Galante & Angeles, 2008).

Beetles are very diverse order with some 300,000 – 400,000 described species (Stork, 2009), so in this thesis I narrowed my focus down only to some selected species of necrophagous beetles of Middle Europe. I would like to provide a new view at how the ecological and evolutional strategies could be used for answering pressing issues in forensic entomology and nature protection.

My selected species came from two particular groups of beetles, family Silphidae and subfamily Cholevinae, more specifically, necrophagous Middle European species of family Silphidae and one particular species of subfamily Cholevinae (Leiodidae), *Sciodrepoides* *watsoni* (Spence, 1813). All those taxa are connected by the fact that they develop and feed on carrions, which is also very interesting ecological and evolutional strategy itself (Szymczakowski, 1961; Šustek, 1981).

Only one species in group I selected, *Necrodes* *littoralis* (Linnaeus, 1758), was earlier recognized as potential bio-indicator species for forensic entomology (Matuszewski, 2011; Fratczak & Matuszewski, 2014). This disinterest of forensic entomology in beetles is global phenomenon (Midgley *et al.*, 2010). Beetles were for a long time perceived as not as good as flies (Diptera), because they usually arrived latter at the scene, but this image is breaking.

The first crack in this picture was made when it was proven that African silphid, *Thanatophilus micans* (Fabricius, 1794), is able to locate and start to breed on corpse in the first 24 hours (Midgley & Villet, 2009a). Although, beetles in the Middle Europe are not that fast, probably due to lower temperatures (Šuláková 2014, pers. comm.), but there are other benefits they could offer.

First of all, beetles tend to have longer development than flies (Midgley & Villet, 2009a; Richards *et al.*, 2009; Velásquez & Viloria, 2009; Ridgeway *et al.*, 2014); therefore they stay on the body for longer period and we can use them as evidence not only in early stages of decomposition. Second of all, they also do not need to form a maggot ball and individual rearing is less difficult (Midgley *et al.*, 2010), so they are easier to handle in laboratory conditions. But we think that the biggest advantage is possibility to cross validate the estimates between species and groups like flies and mites. This is important mainly in times when one of these groups or species could have been affected by external factors (restricted access to body, too high or low temperature, etc.) and give biased estimate.

Only few beetle species were studied specifically for further use in forensic entomology, so in this thesis I would like to look closely on developmental biology of *S. watsoni* and propose its thermal summation model, which will enable to estimate the post mortem interval. Further I would like to propose new character for instar determination and also new methodology for estimating size-based larval characters (section **B**).

Some species burying beetles that we studied could not only be useful bio-indicators. They are also listed in the Red List of endangered species (Růžička, 2005), but their protection is lacking behind of other groups and nothing happened except the statement that they are indeed endangered.

In comparison, American burying beetle (*Nicrophorus* *americanus* (Oliver, 1790)) (Silphidae) was recognized as federally endangered in 1989. In 1991, two years after, the recovery plan was prepared and this plan is still running (Jurzenski *et al.*, 2014). What is maybe even more important than the recovery plan is a fact, that this unfortunate fait put this beetle in a spot light of a scientific world. On the Web of Science you can find 38 articles for a topic query: "Nicrophorus americanus", but nothing on "Nicrophorus antennatus" or "Nicrophorus vestigator" (accessed 27.8.2015).

Because we are few decades behind on this task, it is necessary to address very basic questions, which will allow us to proceed further. One of those, which I will cover in this thesis, is current geographical distribution (section **C**) and some ecological requirements of endangered species of burying beetles (section **A**).

# Literature review

## Necrophagy

In every natural or semi-natural habitat we can found three types of organism – producers, consumers and decompositors. Function of the whole ecosystem is affected by their direct and indirect interactions and it is balancing itself. Producers are organisms that transform an inorganic compounds and energy to organic compounds; consumers are heterotrophic organisms directly feeding on producers or other organisms (Galante & Angeles, 2008), and they can be labeled as predators, parasites or herbivores. These organisms are influencing the rate in which their resources are created. Therefore they harm the production of their food source. Decompositors are very different in that aspect and the production of their food is beyond their control and they depend on misfortune of others (illness, age or injury) (Begon *et al.*, 2006).

Decomposition process is one of the most important processes for ecosystem functions. Decomposition can be defined as a process of transformation of dead organic matter to molecules or basic elements. In general it is a process of releasing energy contained in the matter and mineralization of nutrients from organic back to inorganic (Galante & Angeles, 2008).

Decomposition can be divided into two inseparable parts, destruction and degradation of organic matter. Destruction is the first phase of the process. It is done by mechanical means and outcome are smaller particles of organic matter (Galante & Angeles, 2008). Degradation is mostly done by bacteria and fungi (Begon *et al.*, 2006), therefore it will not be cover in here. But destruction is often done by saprophagous species of insect, which are the ones i was dealing with.

Saprophagy is general term for group of animals feeding on dead matter of plants and animals (Begon *et al.*, 2006). This term and can be narrowed down to more specific types as is necrophagy. Necrophagous species feeds on dead bodies of other animals. Main advantage of this strategy is that animal tissues are nutrient, especially with nitrogen, and they have very low level of indigestible parts, compared to plant tissues. These qualities make a perfect food source in, but carrions are very rare on spatial and temporal scale (Zimmer, 2008).

Necrophagous species of invertebrates are very common among Diptera and Coleoptera species (Zimmer, 2008). In Europe we can find an obligate necrophage, thus species feeding predominantly on carrions, in several beetle families: Silphidae, Trogidae, Dermestidae, Nitiduliade, Leiodidae (Cholevinae more specifically) and Staphylinidae (Szymczakowski, 1961; Kočárek, 2003; Archer *et al.*, 2006; Dekeirsschieter *et al.*, 2013).

Species composition of necrophagous community on carrion is largely affected by its stage of decomposition (Zanetti *et al.*, 2015). These stages are a bit arbitrary and differ between authors and studies. First time they were defined by (Mégnin, 1894) and then re-described by many others (e.g. (Fuller, 1934; Reed  Jr., 1958; Payne, 1965; Abell *et al.*, 1982) so to avoid confusion I chose to follow the stages described by (Goff, 2009)

1. Fresh - phase between time of dead and the first signs of bloating. In this phase is carrion usually discovered by flies from family Calliphoridae and Sarcophagidae and they will lay eggs on it. (these species prefer to lay their eggs around the openings (natural or other)) (Goff, 2009).

2. Bloated - carrion is starting to decay and gases are causing the bloating. Bacteria and fly larvae activities are rising inner temperature and fluids are leaking from the body and they change pH of surrounding soil to more alkaline. Calliphoridae are strongly attracted to the body in this stage (Goff, 2009).

3. Decay - this stage begins by rupture of the bloated body due to accumulated gases. Fly larvae are predominant group and they occur in big feeding groups that clean soft tissues of the bones. Carrion attracts many necrophages and predators like beetles, ants and wasps (Goff, 2009).

4. Postdecay - only skin, cartilages and bones are remaining. Diptera are no longer predominant. Diversity and predominant taxas are predetermined by humidity of the habitat. In xerophytic and mesophytic habitats Coleoptera starts to dominate, which is associated with increased number of their predators and parasites. Although in wet habitats like swamps or rain forests do Diptera and their predators and parasites predominate over Coleoptera (Goff, 2009).

In the Czech Republic this phase is often linked with presence of family Dermestidae, Trogidae, Cleridae and some Staphylinidae (Kočárek, 2003).

5. Skeletal - this stage can be recognize when only fur and bones remains. This stage does not have any clear ending because even composition of the soil fauna, which was changed during the second stage, remains noticeable after months and years. This phase is not linked with occurrence of any specific taxa (Goff, 2009).

Carrion is rather ephemeral and unpredictable food source therefore species have to be able to locate it quickly and at the right moment (Dekeirsschieter *et al.*, 2011). This is crucial especially in severe competition of other necrophagous species. To accurately locate their food source, without wasting time on random search, burying beetles use chemosensory organs on their antennae, which are sensitive to multiple volatile organic compounds emitted by decomposing carrion, like methanethiol, methyl thiolacetate, dimethyl sulphid, dimethyl disulphide and dimethyl trisulphid (Kalinová *et al.*, 2009; Podskalská *et al.*, 2009). There is even some evidence that insect can detect chemical changes and thus their appearance in particular time is not coincidence (Archer & Elgar, 2003b; von Hoermann *et al.*, 2011).

Another prerequisite for success among completion is ability to fly, because it allows to cover more ground while searching (Ikeda *et al.*, 2007, 2013). Chemical analysis of food composition in guts of some carrion beetles shown that only true necrophages posses functional wings while closely related wingless species, which were previously considered as necrophagous, are in fact predators (Ikeda *et al.*, 2007).

## Thermal summation models and others

Body temperature of insect is dependent on the ambient temperature and they have very limited options how to control it (muscle contractions, behavioral responses) (Denlinger & Lee, 2010; Chapman, 2012). Also their development rate is dependent on the temperature, and because of that even their seasonal pattern and phenology is governed by temperature (Schwartz, 1998). The effect of temperature on developmental rate can be model as curve where the rate increase with increasing temperature until optimum is reached (Tmax) and then it decreases rapidly (Lardeux *et al.*, 2008; Voss *et al.*, 2010). Around the extreme values, the mortality increases and could prevent development altogether (Régnière *et al.*, 2012).

Developmental rate characteristics were historically considered as species specific trait, but this is not supported by current research. Geographically distant populations of the same species differ as they probably adapt to their environment (Kipyatkov & Lopatina, 2002, 2010). Interestingly, those characteristics could differ even between methodologies. When one group was reared in constant temperature and the other one in fluctuating, the group in fluctuating will develop faster. This is called Kaufmann effect (Ikemoto & Egami, 2013).

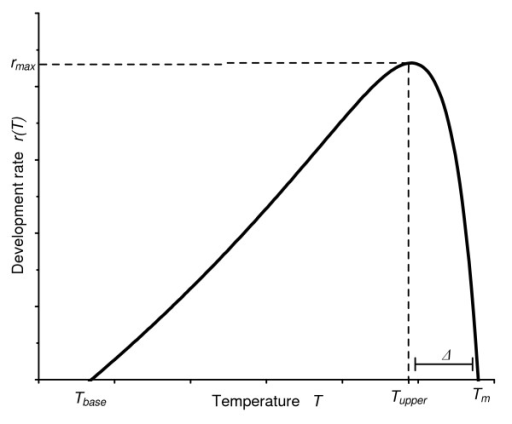


Fig. 1: Relationship between developmental rate and temperature (Lardeux *et al.*, 2008).

There are three approaches to modeling development of poikilotherms. The first is based on assumption that development of immature stages is linear (Higley *et al.*, 1986). This model is very simple and easy to establish, because only few measurements over optimal range of temperatures are needed (Kipyatkov & Lopatina, 2010). Its mathematical formulation is following 1/D = — (t/k) + (1/k)T where D is duration of development, t is lower developmental threshold, k is sum of effective temperatures and T is ambient temperature and is called linear degree-day model or thermal summation model (Briere *et al.*, 1999; Richards & Villet, 2008). This model had some flaws and especially it was disproportionate for upper and lower parts, which distorted and exaggerated lower temperature range and also resulted in lower slope of regression. In reaction to these issues, new formula was offered by (Ikemoto & Takai, 2000): (DT) = k + tD. This formula corrected some problems of the first one and also gives estimation of the variance directly in contrast to the first method where it needed to be recalculated separately. This method is widely accepted and was used in number of studies across different fields of research (Brewer & Hoff, 2002; Chong & Oetting, 2006; Villet *et al.*, 2006; Richards & Villet, 2008; Midgley & Villet, 2009a; Velásquez & Viloria, 2009; Kay, 2010; Ridgeway *et al.*, 2014).

The second and third approach is nonlinear. They both try to model the entire range of temperatures including the extremes based fitting data, but third also tries to incorporate biophysical and biochemical constants to explain processes behind the development (Sharpe & DeMichele, 1977; Jarošík *et al.*, 2002). These models allow estimating the optimal temperature and go beyond linear parts of development (Briere *et al.*, 1999). Problem with many of them is that their parameters have no biological meaning and the fitting process reflects not only true trend, but also biases of data (Jarošík *et al.*, 2002).

## Estimation of the post mortem interval (PMI)

Estimation of post mortem interval (PMI) is one of the most common usages of insect in forensic entomology. There are several ways how the PMI can be estimated. The most basic way how to estimate PMI is succession pattern. This method is based on knowledge of chronological changes in species composition on carrion (Matuszewski *et al.*, 2010, 2011). Other method is isomegalen diagram. These diagrams are visualization of larval growth for various constant temperatures (Reiter, 1984). These estimates are quick, but they can be misleading when specimen is killed improperly or it experienced some issues during the development (Amendt *et al.*, 2007; Midgley & Villet, 2009b). Another methods is pre-appearance interval, which is estimating the time of arrival at the corpse based on ambient temperature (Matuszewski, 2011; Matuszewski & Szafałowicz, 2013). There is very little experience with this type, but it could be nice complementary information for thermal summation models.

Thermal summation models are considered as the most sophisticated available models used for PMI estimation (Amendt *et al.*, 2011). They are estimating PMI based on calculation of time from colonization of cadaver by necrophagous species till the moment of its discovery (Amendt *et al.*, 2007). This estimate can be done only for species with known relationship between rate of development and temperature as was described in previous part. Additional and very obvious condition is that it should be possible to correctly identify the species and distinguish between larval instars.

Identification of adult specimens is often not a problem and there are many keys for identification of adult specimens (Szymczakowski, 1961; Šustek, 1981), but determination of eggs and larvae is much more challenging especially for beetles. Morphological descriptions of larvae are unknown for many species and especially older descriptions suffer with many issues. Only one instar was described; description was inadequate; figures are not accurate and features are simplified or imagined; determination of larvae was based on association with adults not by confirmed parent-offspring relationship (Jan Růžička, pers. comm.). This problem can be solved by modern morphological description or re-description of larvae as (Kilian & Mądra, 2015) did in case of *Sciodrepoides watsoni* (Spence, 1813) or applying molecular methods like DNA bar-coding for species identification (Schilthuizen *et al.*, 2011).

Another challenge is correct larval instar determination and this problem is closely connected to the previous one, but goes beyond that. Often species does not possess any morphological features that could be used for instar determination (Velásquez & Viloria, 2010; Fratczak & Matuszewski, 2014). In these papers authors tried to estimate the stage of development based on the size of selected characters without prior knowledge of the true stage of the specimen. This approach is from our point of view a little bit problematic, because those measured characters are correlated, therefore bigger larvae could be misidentified as higher instar than they really are. This bias would probably not affect the obtained mean values, but it would give distorted picture about variation.

## Beetles in forensic entomology

Because utility of beetles in forensic entomology was overlooked for a long time (Midgley *et al.*, 2010), there is not many species of beetles with known and statistically robust thermal summation models. Recent studies were focused on carrion beetles (Silphidae). Namely *Thanatophilus micans* (Fabricius, 1794) (Ridgeway *et al.*, 2014), *T. mutilatus* (Castelneau, 1840) (Ridgeway *et al.*, 2014) and *Oxelytrum discicolle* (Brullé, 1840) (Velásquez & Viloria, 2009). But also other PMI related features were studied in this group such as pre-appearance interval of *N. littoralis* and some other silphids (Matuszewski, 2011; Matuszewski & Szafałowicz, 2013)

These carrion beetles colonize corpses in earlier stages of decomposition often along with flies. Larder beetles (Dermestidae) on the other hand arrive very late (Goff, 2009). There are developmental data for several larder beetles (Amos, 1968; Coombs, 1978, 1979; Jacob & Fleming, 1980; Richardson & Goff, 2001) and some notes about their rearing and breeding (Roche & Smith, 1974; Woodcock *et al.*, 2013), but the more statistically robust models are needed (Midgley *et al.*, 2010).

As far as my target group is concerned, Nicropohorinae have very limited utilization in forensic entomology, because they do not breed on larger carrions and they only act as predators of flies (Sikes, 2008). Silphidae and Cholevinae on the other hand can be very useful, because they are breeding on bigger carcasses and even on human remains (Sikes, 2005; Schilthuizen *et al.*, 2011; Ridgeway *et al.*, 2014).

## Silphidae

***Taxonomy***

Family Silphidae (Coleoptera: Staphylinoidea) currently contains 186 described species in two monophyletic subfamilies, Nicrophorinae and Silphinae (Sikes, 2008; Grebennikov & Newton, 2012).

Silphidae are closely related with family Staphylinidae and there is an ongoing scientific discussion if they should be placed inside of Staphylinidae. Main difference between Silphidae and Staphylinidae is that in Silphidae are 3 or 4 visible terga on abdomen instead of 2 as in Staphylinidae. Many similarities were discovered between these two families, namely shortening of elytra and presence of oceli (Šustek, 1981), but molecular taxonomy did not show any sign that they should be merged into Staphylinidae (Dobler & Müller, 2000; Grebennikov & Newton, 2012; Ikeda *et al.*, 2013).

Subfamily Nicrophorinae rather small, with 73 recognized species and according to paleontological records it originated 135 million years ago. It currently contains four genera *Eonecrophorus* Kurosawa, 1985, *Ptomascopus* Kraatz, 1876, and genus *Nicrophorus* Fabricius, 1775, with two subgenera *Necroxenus* Semenov-Tian-Shanskij, 1933 and *Nicrophorus* Fabricius, 1775 (Sikes, 2003; Sikes & Venables, 2013).

Subfamily Silphidae is composed of 14 genera (*Ablattaria* Reitter, 1884, *Aclypea* Reitter, 1885, *Dendroxena* Motschulsky, 1858, *Diamesus* Hope, 1840, *Heterosilpha* Portevin, 1926, *Heterotemna* Wollaston, 1864, *Necrodes* Leach, 1815, *Necrophila* Kirby & Spence, 1828, *Oiceoptoma* Leach, 1815, *Oxelytrum* Gistel, 1848, *Phosphuga* Leach, 1817, *Ptomaphila* Kirby & Spence, 1828, *Silpha* Linnaeus, 1758 and *Thanatophilus* Leach, 1815,) and originated some 165 million years ago (Sikes, 2005, 2008; Sikes & Venables, 2013; Qubaiová *et al.*, 2015).

Many new species were described recently especially from Asia region as result of increased interest of entomologist in this group and also in the region, thus number of species and probably even genera can change in future (Ryabuchin, 1990; Koz’minykh, 1995; Háva *et al.*, 1999; Cho & Kwon, 1999; Růžička *et al.*, 2000; Sikes & Peck, 2000; Sikes *et al.*, 2006; Khachikov, 2011; Ruzicka & Schneider, 2011; Oliva, 2012; Sikes & Mousseau, 2013).

**Geographical distribution**

Distribution of all organisms is outcome of their ecological and evolutional history. The oldest records were found in China and they were at least 165 million years old, therefore the whole group probably occurred first on Laurasia continent in Jurassic period. The appearance of these fossil specimens is very similar to present-day sexton beetles (Nicrophorinae) and they possessed some highly specialized features of their modern cousins as three segmented antennal club covered with sensilla (Sikes, 2008; McHugh & Liebherr, 2009; Cai *et al.*, 2014).

Current distribution of Nicrophorinae is almost exclusively limited to Holarctic region, but some species inhabit mountain regions of Indo-Malaysia islands and South America (Sikes, 2008; Sikes & Mousseau, 2013). This pattern could be explained by competition exclusion of Nicrophorinae in warmer regions by ants and other, more aggressive carrion feeders (Sikes, 2008).

The current distribution of Silphinae compared to Nicrophorinae is covering much bigger area. They inhabit Australia and New Guiney that Nicrophorinae never reached (Peck, 2001). This geographical disparity could be linked with higher taxonomic diversity and longer evolution history of the Silphinae. Other advantage of Silphinae is that they use bigger carcasses for breading, where the size of carcass allows coexistence of multiple necrophagous species. Almost all Nicrophorinae use small carcasses, but they have to usurp the whole body to themselves for successful breading, which is much harder to accomplish in competition of flies, ants and other large necrophagous beetles (Sikes, 2008).

From the Czech Republic was reported presence of 24 species of Silphidae. Nine belongs to subfamily Nicrophorinae and the rest to Silphinae (Růžička, 1993; Háva & Růžička, 1997). Nine of these species are on the Czech Red List. One species is regionally extinct (Thanatophilus dispar (Herbst, 1793)), one species is endangered (*Aclypea souverbii* (Fairmaire, 1848)), five is vulnerable (*Ablattaria* *laevigata* (Fabricius, 1775), *Aclypea* *undata* (O.F. Müller, 1776), *Nicrophorus* *antennatus* (Reitter, 1884), *Nicrophorus* *germanicus* (Linnaeus, 1758) and *Nicrophorus* *vestigator* Herschel, 1807) and two species are near threatened (*Nicrophorus* *sepultor* Charpentier, 1825 and *Silpha* *tyrolensis* Laicharting, 1781) (Růžička, 2005).

**Ecology**

Differences between Silphinae and Nicrophorinae are not just in their appearance, but also in their ecology and behavior. Especially striking feature is bi-parental care of Nicrophorinae. This phenomenon attracted a lot of attention of scientific community and is well documented (see (Pukowski, 1933; Špicarová, 1982; Müller *et al.*, 1990; Eggert & M�ller, 1992; Trumbo & Fiore, 1994; Trumbo & Fernandez, 1995; Eggert & Müller, 2011; Steiger *et al.*, 2012; Engel *et al.*, 2014). Adults usually search for fresh carrion in particular part of day, which is often species specific (Kočárek, 2002). If the male finds a carrion without any female on it, they can attracts the female with pheromones (Eggert, 1992). The mating pairs are formed on the carrion and encounter of multiple individuals of the same sex lead to fights. These fights, tends to have some rules and males and females are fighting only individuals of the same sex as they are. The dominant couple will chase away weaker beetles and usurp and bury the carrion. This is sometimes accompanied by nest parasitism when loser females lay their own eggs around the carcass in hope that their larvae might sneak into the winner's nest and develop alongside of their brood (Sikes, 2008).

Majority of Silphidae has functional wings. Ability to fly seems to be crucial for food searching for necrophagous beetles, because as I already mentioned, carrions is very ephemeral food source. This is reflecting reactive nature of necrophagy compared to proactive approach of predators in obtaining the food and according to some authors it can cause a loss of the flight ability of the former group as evolutionary unnecessary trait (Ikeda *et al.*, 2007, 2013).

Flight ability differs between species. Especially bigger species appears to be able to flight over longer distances than the small ones. This was confirmed for *Nicrophorus* *germanicus*, which is the biggest species of Silphidae in the Czech Republic. In experiment this species was recorded to fly over the distance of more than four kilometers during 24 hours (Petruška, 1964). Even bigger sexton beetle (*Nicrophorus* *americanus* (Olivier, 1790)) was recorded as far as 7.41 kilometers from the place of origin after one night**,** but this record was an outlier and 90% of observed beetles did not move further than 1.6 kilometers over the same period of time(Jurzenski *et al.*, 2011). Smaller necrophagous species are less mobile and *Thanatophilus* *sinuatus* Fabricius, 1775 and *T. rugosus* Linnaeus, 1758 distance recorded as they dispersion over period of 24 hours was 395 and 375 m, respectively (Petruška, 1964).

It was found that flight height is probably also positively correlated with body size, and bigger species fly higher than smaller ones (Ohkawara *et al.*, 1998).

Distribution of necrophagous species of silphids over landscape is not homogenous, but as it seems, some species are preferring different types of habitat, which was reported by number of studies (Novák, 1961, 1962; Růžička, 1994; Kočárek, 2003; Looney *et al.*, 2004, 2009).

Many European species seemed to be more abundant in open landscape like *Thanatophilus sinuatus, T. rugosus, Nicrophorus germanicus, N. vespillo* (Linnaeus, 1758), *N. antennatus*, *N sepultor* and *N. vestigator* (Novák, 1962; Růžička, 1994; Kočárek, 2003), but many species like *Oiceoptoma thoracicum* (Linnaeus, 1758), *Nicrophorus vespilloides* Herbst, 1784 and *N. humator* (Gleditsch, 1767) visit open landscape only occasionally and they prefer forest biotopes (Růžička, 1994). Similar pattern was observed between different soil types and some species were observed more often on one particular soil type (Pukowski, 1933; Paulian, 1946; Theodorides & Heerdt, 1952; Novák, 1961, 1962) All these beetles are necrophagous and they are not especially picky about the food source so researchers are very interested in revealing the reason, why they prefer to occupy particular type of habitat or soil, because it could be useful for their protection and it would improve our general understanding of interactions in the nature.

What is the cause of this pattern is still not fully understood, but it was shown for some North American burying beetles, that deeper and loess soils are hosting more abundant communities of these species, but some of them prefer the exact opposite, shallow and rocky soils (Muths, 1991; Bishop *et al.*, 2002; Looney *et al.*, 2009). The exactly same pattern was observed for some European burying beetles. For example *N. antennatus* was observed more often on loessal soils, but *N. vestigator* seems to prefer dry and sandy soils (Novák, 1965). (Looney *et al.*, 2009) offered three possible explanations for this phenomenon. Beetles are either simply more abundant in areas with a particular soil type, or they preferentially colonize, or they are more competitive in such areas.

**Temporal distribution**

Temporal distribution of burying beetles of genus *Nicrophorus* is governed by the state in which they overwinter. Some species overwinter as larvae (*Nicrophorus sepultor*, *N. investigator* and *N. interruptus*) and some as adults (*N. antennatus*, *N. vespillo*, *N. vespilloides*, *N. germanicus*, *N. humator* and *N. vestigator*) (Novák, 1962; Šustek, 1981; Růžička, 1994). It is obvious that overwintering as adult give them opportunity to emerge early in the spring and species overwintering as larvae are emerging during late in spring or in summer, depending on the latitude and altitude (Růžička, 1994).

In general, there is difference in number of generations per year between bigger and smaller species. *Nicrophorus germanicus* (body length around 4 cm) has usually one generation per year in the Czech Republic. In comparison, *N. vespillo* (body length around 3 cm), which is sharing the same habitat with the previous, has two generations per year (Novák, 1961). Even smaller silphids like *T. sinuatus* and *T. rugosus* (body size about 0.5 cm) are able to have even three generations per year (Novák, 1966).

**Circadian activity**

Activity of probably all animals is changing on temporal scale. The mechanism behind these rhythms can be triggered by some external (reaction to change of environment) or internal impulse (controlled by circadian or another oscillation) (Sanders *et al.*, 2002). It was found that predominant type of activity (nocturnal, diurnal) differs between ecological groups. Diurnal activity is common for predators and herbivores, probably because they are using sight for food searching, but saprophagous and necrophagous beetles use chemo-receptors to do so therefore they prefer low light conditions (crepuscular and nocturnal activity) (Lewis & Taylor, 1965).

(Špicarová, 1974) shown in the laboratory conditions that young adults of *N. germanicus* are leaving soil predominantly right after sundown. This was in contrast to her laboratory observations of *N. vespillo* (Špicarová, 1972), which left the soil predominantly right after noon. Field observations did not support asserted theory of diurnal activity of *N. vespillo* (Kočárek, 2001), but they found that *T. sinuatus, T. rugosus* and *O. thoracicum* are almost exclusively diurnal. Repeated experiment confirmed that these species have diurnal activity in Central Europe (Kočárek, 2002).

**Intraspecific and interspecific competition**

Carrion is very good source of nutrients and the competition associated with such a source is very harsh. Nicrophorinae and Silphinae differ in their competitive strategies. Nicrophorinae prefer smaller corpses (<100 g) and Silphinae prefer bigger carrions (>300 g), which limits competition between these two groups (Sikes, 2008).

Nicrophorinae avoid an interspecific competition by burying carrion underground, which limits ability of other species to locate it. (Springett, 1968) and others (Anderson, 1982; Ratcliffe, 1996; Sikes, 2008) offer an theory that Silphidae and especially Nicrophorinae have symbiotic relationship with mites from genus *Poecilochirus* G. Canestrini & Canestrini, 1882 (Mesostigmata: Parasitidae), which are often hitchhiking on carrion beetles, and they are feeding on fly eggs. Therefore they eliminate them from competition before they have a chance to escape or hide.

Beetles from subfamily Silphinae have to fight their competition directly, because they cannot hide it from other competitors, but because they use much bigger carrions for breading, they have enough time and resources to finish their development (Sikes, 2005).

Competition between species of subfamily Nicrophorinae is also very intense and each breading pair has to occupy their own, small corpse. In these fights are bigger species of Nicrophorinae in clear advantage (Safryn & Scott, 2000). Such a situation would have to result in competitive exclusion and ultimately extinction of smaller species, but we are not witnessing such situation. Most probable reason for such a peaceful coexistence of several species is their temporal and spatial separation, because fights would result in wasting resources of everybody involved (Anderson, 1982). Intraspecific competition, as I already described, is very physical and bigger individuals tend to win over small ones, but several cases of communal breeding were reported (Trumbo & Fiore, 1994; Trumbo, 1995; Scott *et al.*, 2007).

## Leiodidae: Cholevinae

**Taxonomy and geographical distribution**

Subfamily Cholevinae is monophyletic and currently placed inside of family Leiodidae and it is one of its largest groups with approximately 1 605 species (Newton, 1998, 2005; Perreau, 2004) The place of origin of the entire group was probably on south part of Gondwana continent (today's Antarctica) in Triassic period (250 – 200 MYA). Choleviane spread from there to all continents, but they went extinct on the Antarctica itself (Giachino *et al.*, 1998).

Biodiversity hot spot for Cholevinae is Mediterranean thanks to cave dwelling tribe Leptodirini, with high rate of endemism (Newton, 1998; Zoila, 1998).

In the Czech Republic was found 48 species in 11 genera (*Nemadus* C.G. Thomson, 1867, *Eocatops* Peyerimhoff, 1924, *Anemadus* Reitter, 1884, *Nargus* C.G. Thomson, 1867, *Choleva* Latreille, 1796, *Attaephilus* Motschulsky, 1869, *Dreposcia* Jeannel, 1922, *Sciodrepoides* Hatch, 1933, *Catops* Paykull, 1798 and *Cholevinus* Reitter, 1901 (Růžička & Vávra, 1993; Vávra & Růžička, 1993; Perreau, 2004).

Majority of these species are saprophagous generalist, which feeds on decaying biological material or they could be sporophagous or mycophagous (Jeannel, 1936; Peck, 1990, 1998; Růžička & Vávra, 1993; Betz *et al.*, 2003). Some species were often observed on carrions (Szymczakowski, 1961) and it was proposed that they could be used as bio-indicators in forensic entomology (Archer & Elgar, 2003a; Schilthuizen *et al.*, 2011).

The most promising species in this regard are the ones with autumn and winter activity like Catops nigricans (Spence, 1813), Catops tristis (Panzer, 1793), Nargus wilkini (Spence, 1813) Choleva agilis (Illiger 1798) and Choleva elongata (Paykull, 1798) (Topp, 1994, 2003). These species can occur on carrions in temperatures, which are too low for usual forensic bio-indicators – flies (Diptera).

Other promising species are the ones with very wide geographical distribution like *Sciodrepoides watsoni* (Spence, 1913), which occurs across the Holarctic region (Peck & Cook, 2002).

**Ecology**

Cholevinae of the Central Europe prefer to be in climatically stabile environment like leaf litter, ant colonies, caves or burrows and dens of small mammals (Szymczakowski, 1961). It is very interesting that some species are developing during the cold season (sometimes called cold season species), which is season usually avoided by other species (Topp, 1994). These species try to avoid temperature higher than 20°C and they aestivate during summer heats (Topp, 1990). Optimal temperature for their breeding is from 5 to 10°C (Topp, 2003). Some of these species were listed by (Topp, 1994), but this list is far from complete.

Some cold season species were probably uncovered by (Růžička, 1994) when he studied seasonal dynamics of Cholevinae around Velký Blaník in the Czech Republic. He discovered four groups. First group had a one activity peak in spring (*Catops subfuscus subfuscus* Kellner, 1846, *Sciodrepoides fumatus* (Spence, 1815) and *S. alpestris* Jeannel, 1934. Second group peaked during the summer (*S. watsoni* (Spence, 1813) and *C. coracinus* Kellner, 1846). Third group peaked in autumn (*C. grandicollis* Erichson, 1837, *C. kirbyi* (Spence, 1815) and *C. morio* (Fabricius, 1792)). Fourth group had bimodal activity and peaked in spring and autumn (*C. picipes* (Fabricius, 1792), *C. tristis* (Panzer, 1794), *C. fuliginosus* Erichson, 1837 and *Ptomaphagus sericatus* (Chaudoir, 1845)).

It is very likely that autumn group is the one, which contains also the cold season species. This theory could be proved by simple lab breeding experiment or by field observations. The reason for existence of these separate groups is unknown and (Peck & Anderson, 1985) hypothesized that competition is probably not the answer, unlike for Silphidae.

As I already mentioned, Cholevinae are often associated with ant colonies or burrows of mammals. Species living with ants seems to be specialized on specific host, but this was not observed for species living with mammals (Szymczakowski, 1961; Peck & Cook, 2007).

Some species lives in caves and especially Mediterranean is filled with species truly adapted for the life in dark (Peck, 1998), but species occurring in the Czech Republic do not posses any features of true troglobionts, like prolonged legs and antennae, loss of sight or other developmental adaptation (Szymczakowski, 1961).

In the Czech Republic we can found species almost exclusively associated with open landscape (*Catops fuliginosus*, *C. grandicollis*, *C. morio* and *Ptomaphagus sericatus*) or forest species (C*. subfuscus subfuscus, C. tristis, C. coracinus, C. picipes, S. fumatus* and *S. alpestris*). Some species are not specialized and they occur almost regardless the habitat as *C. kibyi* or *Sciodrepoides watsoni* (Růžička, 1994).