# Abstract

Necrophagous beetles (Coleoptera) are very interesting and diverse ecological group of species with an immense impact on a natural nutritient cycle. Their main food source and breeding ground are carrions of vertebrates, human remains included. This relationship is often used in various ways by forensic entomology, but its potential was not jet fully reached, because our knowledge of biology of these beetles is very much incomplete.

In this thesis I would like to explore geographic distribution, ecological requirements and developmental biology of several Middle European necrophagous beetles as an outcome of their ecological and evolutional strategies. For that I raised three broad research questions. Which factors are determining the local abundance of carrion beetles (Coleoptera: Silphidae)? How the current geographical distribution of open-landscape carrion beetles looks like in the Czech Republic? How is the temperature affecting the development of *Sciodrepoides watsoni* (Spence, 1813)?

We found that soil type can have significant effect on abundance of carrion beetles and they showed prefference for chernozem – *Nicrophorus* *antennatus* (Reitter), *N. germanicus* (Linnaeus), *N. interruptus* (Stephens), *N. sepultor* (Charpentier), *Silpha obscura obscura* (Herbst), *T. sinuatus* (Fabricius) or fluvisol *N. humator* (Gleditsch). These findings support our hypothesis that soil type could be an important factor determining the occurrence of necrophagous European carrion beetles.

To collect novel data of the current geographical distrubution of carrion beetles we used 420 baited pitfall traps and, at 84 localities, we collected 71 234 specimens of 15 silphid species. Among them, three endangered carrion beetle species listed on the Czech Red List of Invertebrates, were found. Two are vulnerable thermophilic species of open landscapes, *Nicrophorus antennatus* (Reitter, 1884) (collected around Louny and Židlochovice) and *Nicrophorus germanicus* (Linnaeus, 1758) (Louny, Zábřeh and Židlochovice). The third is the near threatened species, *Nicrophorus sepultor* Charpentier, 1825 (collected around Louny, Kutná Hora, Zábřeh and Židlochovice), which also prefers open landscapes.

We studied development of common Holarctic beetle *S. watsoni* under five constant temperature regimes in laboratory (15, 18, 21, 25 and 28°C). Parameters of thermal summation models and their standard errors were calculated for each developmental stage (egg, three larval instars and pupae). We also find a new character for larval instar determination (head width) and proposed novel approach for future studies of size-based characters in instar determination.

**Keywords**: Coloptera, Silphidae, Cholevinae, geographical distribution, developmental biology, larval instar determination, forensic entomology, nature protection

# Abstrakt

Nekrofágní brouci (Coleoptera) jsou zajímavou a velmi diversifikovanou ekologickou skupinou, s velkým dopadem na přirozený cyklus živin. Jejich hlavní složkou potravy, a zároveň místem rozmnožování, jsou mršiny obratlovců, člověka nevyjímaje. Tento vztah je často různě využíván ve forenzní entomologii, ale jeho potenciál není zdaleka využit, protože znalost biologie těchto brouků je velmi kusá.

V této disertační práci chci prozkoumat geografické rozšíření, ekologické nároky a vývojovou biologii několika středoevropských druhů nekrofágních brouků, tedy výsledek jejich ekologických a evolučních strategií. Za tímto účelem jsem si položil tři, velmi široké otázky. Které faktory jsou určující pro lokální abundanci mrchožroutovitých brouků (Coleoptera: Silphidae)? Jaké je současné geografické rozšíření mrchožroutů otevřené krajiny v České republice? Jak ovlivňuje teplota vývoj druhu *Sciodrepoides watsoni* (Spence, 1813)?

Zjistili jsme, že půdní typ má statisticky významný vliv na početnost mrchožroutů. Šest druhů preferovalo černozemě – *Nicrophorus* *antennatus* (Reitter), *N. germanicus* (Linnaeus), *N. interruptus* (Stephens), *N. sepultor* (Charpentier), *Silpha obscura obscura* (Herbst), *T. sinuatus* (Fabricius), a jeden fluvizemě – *N. humator* (Gleditsch). Tyto závěry podporují naši hypotézu, že půdní typ by mohl být jedním z určujících faktorů pro výskyt nekrofágních Evropských mrchožroutů.

Za účelem zjištění geografického rozšíření mrchožroutů, jsme položili 420 vnazených padacích pastí na 84 lokalitách a takto jsme získaly 71 234 kusů od 15 druhů těchto brouků. Mezi nimy byli i tři druhy hrobaříků, kteří jsou na Červeném seznamu bezobratlých. Dva z nich jsou Zranitelné, teplomilné druhy otevřené krajiny *Nicrophorus antennatus* (Reitter, 1884) (nalezen okolo Loun a Židlochovic) a *Nicrophorus germanicus* (Linnaeus, 1758) (Louny, Zábřeh a Židlochovice). Třetí je Téměř ohrožený *Nicrophorus sepultor* Charpentier, 1825 (nalezen okolo Loun, Kutné Hory, Zábřeha a Židlochovic), který taktéž preferuje otevřenou krajinu.

Studium vývoje běžného, Holarktického druhu *S. watsoni* probíhalo v laboratoři za několika konstantních teplot (15, 18, 21, 25 a 28°C). Na základě pozorování délky vývoje jsme vypočítaly parametry termálně sumačního modelu s jejich standardní chybou pro každé stádium vývoje (vajíčko, tři larvální instary a kukla). Zároveň jsme zjistili, že šířka hlavové kapsule je u tohoto druhu dobrým nástrojem pro určení stupně larválního instaru. Popisná statistika tohoto znaku a nová metodika jak studovat velikostně definované znaky je přiložena k práci.

**Klíčová slova**: Coloptera, Silphidae, Cholevinae, geografické rozšíření, vývojová biologie, determinace larválního instaru, forensní entomologie, ochrana přírody

# 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).

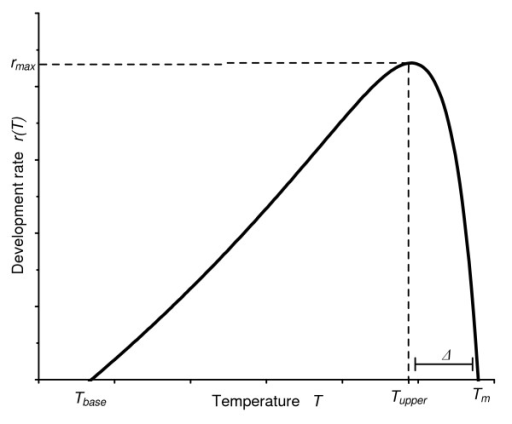


Fig. 1: Relationship between developmental rate and temperature (Lardeux *et al.*, 2008).

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).

# 4. Principal conclusions of the thesis

In this theses I was asking couple of questions about the ecological and evolutional strategies of necrophagous beetles.

During the field work we captured and identified 15 species of carrion beetles. Three of which are currently considered as rare and are on the Czech Red List of Invertebrates as Endangered (N. antennatus and N. germanicus) or Nearly Endangered (N. sepultor) (Růžička, 2005a). The last ecological studies on these species in Europe were done almost 50 years ago when they were probably much more common (Novák, 1966; Petruška, 1968). These species deserve much more attention, because they could play a major role in nature conservation as bio-indicators or umbrella species (see Guarisco, 1997; Holloway & Schnell, 1997; Walker & Hoback, 2007; Creighton et al., 2009; Crawford & Hoagland, 2010; Jurzenski et al., 2014).

The most frequent species was T. sinuatus, whose dominance was overshadowed by N. vespillo and T. rugosus, but only in autumn. All these species seem to be very common in open landscape habitats and our findings confirm the earlier observations of Novák (1962, 1965, 1966) and Petruška (1964).

T. sinuatus and T. rugosus are considered to be co-occurring species without spatially or temporally differentiated niches (Novák, 1966). The higher abundance of T. rugosus in autumn samples could indicate a temporal niche differentiation.

Some of the carrion beetles caught are considered to be forest species (N. humator, N. investigator, N. vespilloides and O. thoracicum) by several authors (e.g. Růžička, 1994; Kočárek & Benko, 1997). Although the traps were not set in forested areas, our observations are in line with their findings, because few of these species were caught in this study (in total 32, 7, 6 and 6 specimens, respectively). The more frequent occurrence of N. humator was probably due to the greater flight activity of this large and common beetle.

CCA analysis revealed that the factors that are significantly associated with the species composition are both soil types (chernozem and fluvisol) and three crops (Zea mays, Hordeum vulgare and Heliantus annus). As depicted in Fig 4 the positions of these factors are roughly orthogonal, with the exception of H. vulgare and H. annus, therefore they are probably unrelated.

The association between the abundance of the carrion beetles and these crops is very interesting. We think that it could be due to the microclimatic conditions in fields with these crops. Based on our experience, Z. mays provides a much more humid and cooler environment than H. vulgare and H. annus. Further study is needed to reveal causality.

We were able to show that the abundance of seven of the carrion beetles (N. antennatus, N. germanicus, N. humator, N. interruptus, N. sepultor, Silpha obscura obscura and T. sinuatus) differed significantly in areas with chernozem and fluvisol soils, and therefore soil type is an important factor in determining the occurrence of these carrion beetles. Our findings are supported by the results of the CCA analysis and Wilcoxon rank-sum tests.

This association is assumed by many authors, but only for beetles of the subgenus Nicrophorus (e.g. Pukowski, 1933; Paulian, 1946; Theodorides & Heerdt, 1952; Novák, 1961, 1962). Heretofore, this phenomenon was empirically proven only for North American species (Muths, 1991; Bishop et al., 2002; Looney et al., 2009), where the association of beetles of the subfamily Silphinae with a particular soil was previously reported by Bishop et al. (2002).

All these findings raise an important question as to what mechanisms drive this phenomenon. Muths (1991) has shown that burying beetles are able to distinguish among different types of soil and choose the best substrate for digging in the laboratory. This experiment was conducted at a small scale (an area with a diameter of 1.5 m) and it is reasonable to think that this type of response occurs only during microhabitat selection. Thus, it does not answer our question. Our goal was to determine if the type of soil could be important in habitat selection. Looney et al. (2009) offers three possible explanations. Beetles are either simply more abundant in areas with a particular type of soil, or they preferentially colonize, or they are more competitive in such areas. From our point of view the last option is the most likely for the following reasons.

It is likely that one of the main factors influencing long range habitat selection by carrion beetles is the presence of food (carrion) (Kalinová et al., 2009). The greater abundance of these beetles in areas with particular types of soil (chernozem or fluvisol in our case) could be caused indirectly.

Although burying beetles are good fliers and can cover long distances they mostly choose to stay close to their original locations (e.g. Nicrophorus americanus, which is a relatively large and robust beetle, is capable of flying as far as 7.41 km in a single night, but more typically travels less than 1.6 km/night) (Jurzenski et al., 2011). Limited mobility coupled with adaptation to local conditions could cause the observed spatial structure rather than individual habitat choice (preferential colonization). This is also in line with general local adaptation hypotheses (Alstad, 1998).

The same reasons might account for our finding in the case of T. sinuatus, because it has functional wings (Ikeda et al., 2008), but only a short flight range (Petruška, 1964). Therefore, it has a very similar lifestyle to the burying beetles studied: N. antennatus, N. germanicus, N. humator, N. interruptus, N. investigator, N. sepultor, N. vespillo and N. vespilloides. This hypothesis is supported by the fact that all these species were recorded in areas with both of the different types of soil and often in large numbers, so they are not closely associated with a particular type of soil (see Table 1).

The biology of Silpha obscura obscura is not well known. There is little known about the diet and flight ability of the species in the genus Silpha (Ikeda et al., 2007). There is also a strong possibility that this species is not strictly necrophagous. Its preference for areas with a chernozem soil is surprising in this case, but it could be due the spatial structure of the population, which is driven by an adaptation to chernozem soil.

Loess loams are proposed as the preferred soils of many species of burying beetles (N. antennatus, N. germanicus, N. interruptus, N. vespillo and N. sepultor), but according to Novák (1962) N. vestigator prefers sandy soils. This species unfortunately was not recorded in our study despite the fact that we set traps close to locations where it was often recorded by collectors in the past. It is possible that we missed the population peaks of this rare species. Future studies on this species should focus on earlier months in the year (April–May), when it could be more abundant (Novák, 1962; Šustek, 1981).

Our findings are limited to two types of soil (chernozem and fluvisol) and can be extended only to similar types of soil (e.g. Phaeozem and haplic Luvisol). It is also possible that the abundances of the species studied are different throughout their distribution area, as pointed out by Scott (1998).

This study has revealed more about the distribution and ecology of European carrion beetles, especially the preference of three endangered species (N. antennatus, N. germanicus and N. sepultor) for areas with chernozem soils (see also Jakubec & Růžička, 2012). Our finding that the preferences of some species of carrion beetles are possibly determined by an adaptation to particular types of soil is crucial for the effective conservation of these species, which is currently not a topical issue in the Czech Republic and Europe generally. These beetles are charismatic and could become a widely accepted flagship species for stakeholders and policymakers, as indicated by the public interest in and the vast number of studies on the American burying beetle (N. americanus) (e.g., Anderson, 1982b; Lomolino et al., 1995; Amaral et al., 1997; Crawford & Hoagland, 2010).

This study presents distribution data for beetles of the family Silphidae, collected during ecological studies in the vicinities of Louny, Kutná Hora, Zábřeh and Židlochovice (Fig. 1). These areas are warm lowlands with two different soil-types (loess and fluvisols).

Beetles were collected using 420 pitfall traps with a 1:1 water: ethylene glycol solution, and baited with ripe cheese and fish.

Traps were placed in lines of five on fields at least 50 m from the ecotone and 20 m from each other. Traps were left for two weeks, and only in spring 2008 was this extended to three weeks because of cold weather. Traps were placed in 84 localities. In total, 71 234 individuals of 15 species were collected and determined.

Specimens were determined using keys by Šustek (1981). Doubtful specimens were examined and confirmed by Jan Růžička. All material is housed in the collection of Jan Růžička, except samples from Kutná Hora, which are in the collection of Pavel Jakubec.

The list of localities is divided by place and year of collection. It includes serial number, name of closest habitation, faunistic map code, GPS coordinates of the centre of the trap line and scientific name of the crop-plant surrounding the trap.

Detailed comments are given below on three endangered species, listed in the local Red List of Invertebrates (Růžička 2005b):

(1) *Nicrophorus* *antennatus* (Reitter, 1884) is a Palaearctic species, widely distributed throughout Europe (except Scandinavia and Great Britain), in Asia it is known from Turkey up to Kashmir and north-western China (Růžička & Schneider 2004). In the Czech Republic, the species is known only from several older records from Bohemia and Moravia (J. Růžička, unpubl.). Novák (1961, 1965) reported abundance of this species in the 1950/60s in northern Moravia near Náklo (6368) and Drahanovice (6468), but only rarely near Chválkovice (6369), all in the vicinity of Olomouc. Recently, abundant occurrence was registered in Bohemia, near Žabovřesky nad Ohří (5550) (J. Růžička, unpubl.). In the Czech Republic, *N. antennatus* is evaluated as vulnerable (VU) in the local Red list (Růžička 2005b). Novák (1962) reported abundant occurrence of *N. antennatus* in open landscape, linked with loess soils. We only recorded it in two of the studied regions (Louny and Židlochovice) (Table 1).

(2) *Nicrophorus germanicus* (Linnaeus, 1758) is a western Palaearctic species, distributed from Europe through Turkey, southern Russia and Iran up to Turkmenistan (Růžička & Schneider 2004). In the Czech Republic, abundant older records are known from Bohemia and Moravia (J. Růžička, unpubl.). Novák (1961, 1962, 1965) reported this species as abundant in 1950/60s in northern Moravia near Náklo (6368), Chválkovice (6369) and Drahanovice (6468), and less abundant near Lhota nad Moravou (6368), all in the vicinity of Olomouc. Recently it has only been rarely reported, but we are aware of at least two additional localities with recent abundant occurrence – Žabovřesky nad Ohří (5550) in Bohemia and near Kyjov (7068) in Moravia. In both cases, the habitats consist mostly of meadows (J. Růžička, unpubl.). In the Czech Republic, like the previous species, *N. germanicus* is listed as vulnerable (VU) in the local Red list (Růžička 2005b). Novák (1962) reported *N. germanicus* as abundant in open landscape, on warmer localities with loessy soils. Here, we recorded the species as abundant in two regions (Louny, Židlochovice), with only a single record around Zábřeh (Table 1).

(3) *Nicrophorus sepultor* Charpentier, 1825 is a Palaearctic species, widely distributed from Europe to Mongolia, eastern Siberia and north-western China (Růžička & Schneider 2004). In the Czech Republic, individual older and/or recent records are known from Bohemia and Moravia (Vysoký 2007; J. Růžička, unpubl.). Novák (1961, 1962, 1965) reported this species as abundant in 1950/60s in northern Moravia near Náklo (6368), Lhota nad Moravou (6368) and Drahanovice (6468), and less abundant near Chválkovice (6369), all in the vicinity of Olomouc. Recently, only isolated records from the Czech Republic are known (e.g., records from Ústí nad Labem region are summarized by Vysoký (2007)). We know of two localities with recent abundant occurrence of *N. sepultor* in Bohemia – around Žabovřesky nad Ohří (5550) and Kostelec nad Černými lesy (6055); both habitats consist mostly of meadows (J. Růžička, unpubl.). In the Czech Republic, *N. sepultor* is evaluated as near threatened (NT) in the local Red list (Růžička 2005b). Records are mostly from open landscapes, including colder habitats (Vysoký 2007; J. Růžička, unpubl.). Novák (1962) linked its occurrence to habitats with loessy soils. Vysoký (2007) also mentioned records on the margins of forested habitats. Here, we recorded the species relatively abundantly in three regions (Louny, Kutná hora, Židlochovice); two specimens were found at a single locality around Zábřeh (Table 1).

A further two species of carrion beetles, found rarely in open landscapes in central Europe, were not recorded in our study. The first such species is *Thanatophilus dispar* (Herbst, 1793), with a Palaearctic distribution and a preference for tundra biome, with recent occurrence in central Europe in Germany (distribution reviewed by Köhler & Klausnitzer 1998) and Poland (e.g., Aleksandrowicz & Komosiński 2005). This species was historically reported in the Czech Republic from Moravia – with confirmed records near Náklo (6368) (Novák 1966) and Litovel (6268–6368) (Kočárek 1997). In Bohemia, only several old records are known (J. Růžička, unpubl.). There are no recent records of *T. dispar* from the Czech Republic.

The other species not recorded in our study, is *Nicrophorus vestigator* Herschel, 1807. This burying beetle is morphologically very similar to *N. antennatus* (Šustek 1981). Generally, it is regarded as rare in the Czech Republic (Šustek 1981). Novák (1962) reported it as abundant (more than 200 specimens over a two year period of pitfall trapping) in 1950/1960s in northern Moravia near Chválkovice (6369). Single records from Náklo (6368) and Drahanovice (6468) (all in surrounding of Olomouc) from the same period were recorded by Novák (1961, 1965). Novák (1962: 289) discussed a possible preference of *N. vestigator* for habitats with light, sandy and more permeable soil.

In central Europe, field ecosystems can regarded as substitute habitats for species of open landscape carrion beetles. Most of them are primarily steppe or more generally, xerophilous species, more abundant in southern Russia (e.g., Pushkin 2002, Pushkin & Shapovalov 2011).

Tři zjištěné vzácnější druhy hrobaříků (*Nicrophorus* *antennatus*, *N. germanicus* a *N*. *sepultor*) mají společné to, že se jedná o druhy preferující otevřenou krajinu. Zřejmě se primárně jedná o druhy stepní či obecně xerofilní, početnější na jihu Ruska (např. Pushkin 2002, Pushkin & Shapovalov 2011). V podmínkách střední Evropy pro ně kulturní stepi polních ekosystémů mohou představovat náhradní biotopy.

Další dva druhy otevřené krajiny, které jsou vzácné ve střední Evropě, nebyly v naší studii zachyceny. Jedná se o druh *Thanatophilus dispar* (Herbst, 1793), palearktický druh mrchožrouta preferujícího biom tundry, který se ve střední Evropě recentně vyskytuje v Německu (shrnuje Köhler & Klausnitzer 1998) a v Polsku (např. Aleksandrowicz & Komosiński 2005). Tento druh byl historicky hlášen v ČR z Moravy – konkrétní nálezy byly publikovány např. z okolí Nákla (6368) (Novák 1966) a Litovle (6268–6368) (Kočárek 1997). V Čechách známe pouze jednotlivé starší nálezy (J. Růžička, nepubl.). Recentně nebyl na území ČR nalezen.

Druhým druhem, který se nám nepodařilo zachytit, je *Nicrophorus vestigator* Herschel, 1807. Tento hrobařík je morfologicky velmi podobný druhu *N. antennatus* (Šustek 1981). Celkově je u nás hodnocen jako řídce se vyskytující (Šustek 1981). Hojné nálezy (za dva roky více než 200 ex.) z Chválkovic (6369) uvádí z přelomu 50. a 60. let Novák (1962). Jednotlivé nálezy z okolí Nákla (6368) a Drahanovic (6468) v Olomouckém okrese z téže doby zmiňuje Novák (1961, 1965). Novák (1962: 289) diskutuje také možnou vazbu tohoto druhu na lehké, písčité a propustnější půdy.

We did not obtain any larvae from the 28°C treatment probably because adults did not oviposit in this temperature or egg mortality was too high. The second claim is little bit more likely from our point of view, because we did not find any eggs. But as we mentioned in the methodology section, eggs of *S. watsoni* are tiny and we could simply overlook them during our controls in the Petri dish's substrate even under the binocular microscope.

Mortality of our specimens in the breeding experiment was very high over the all treatments especially in the later stages (L3 and pupae). This was in a sharp contrast with what we saw in the observation study. The whole colony in the observation study prospered and even increased in the number of adult over time. Only difference between these two was that we did not separate individuals and we also did not have to handle the larvae for photo documentation.

We did not observed any hostility between specimens in the observation study or signs of cannibalism between individuals as reported by (Kilian & Mądra, 2015), but it is possible that we missed it, because estimated number of individuals in the box was close to one hundred.

We think that photographing process was not so intrusive to be responsible for such high mortality rates thus it is more likely that separation from other larvae and adults was the reason for that. (Peck, 1975) mentioned that Ptomaphagus hirtus (Tellkampf, 1844) (Leiodidae: Cholevinae: Ptomaphagini) needed soil from the cave of its origin to successfully complete the development. Soil bacteria probably played some part in this process, because specimens did not develop on autoclaved soil. It is possible that adults feeding along with larvae could provide such bacteria in our case. Another explanation could be that feeding of multiple individuals is much more effective or improves the quality of the food source.

We had to change our methodology of egg extraction for the second year due to the fact that eggs could be easily overlooked in the substrate and beetles refused to lay their eggs in offered wet cotton balls or small pieces of paper. To prevent bias in recorded time we introduced dish rotation methodology and adults stayed in the same dish only one day and then they were moved to another. Those used dishes were then regularly searched for emerging larvae. The main issue with this approach (dish rotation) is that we could not measure the eggs mortality, because we could not count the original number of eggs.

The mean development time decreased with increasing temperature (Fig. 6), except for L2 and L3 instars in the 25°C treatment. This might indicate that between 21°C and 25°C should be an optimal temperature for the development of these two stages. Optimal temperatures for lower stages are probably even higher. This agrees with findings of (Engler, 1981), who reported *S. watsoni* as warm season species in contrast to some species of Choleva and Catops that prefers to breed during the winter season and their optimal temperatures for development were below 16°C.

As you can see in Table 1, we had low number repeats for L3 and pupae. This was caused by high mortality rates of both instars. Measuring development time for pupae was even more challenging and we had difficulties to measure it precise due to the fact that they did not pupate close to the wall of Petri dish. Therefore we had to search for them. This effort was sometimes unsuccessful and some specimens surprised us after time when they appeared as adults, because they were missing and presumed dead.

Our methodology of measuring the size of the instars was based on continual observation of individuals from egg until pupation. This approached differs from other studies with similar goals (Velásquez & Viloria, 2010; Fratczak & Matuszewski, 2014), where 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.

As you can see on Fig. 8 and Table 3, all instars have some overlap in the head widths. This is especially true for the first and second instar. It would not do us any good to measure more characters, because they are correlated, but we offer different solution. First instar larva has only primary setae on its body, but after molt to the second instar a secondary set of setae will emerge and they are present unchanged also on the third instar larvae. Thus chaetotaxy can be used for the discrimination of the first and second instar larvae. For additional differential diagnosis of those morphological characters, see (Kilian & Mądra, 2015).

We established developmental parameters for *Sciodrepoides watsoni* together with the new and reliable character for instar determination. This species is so far the smallest necrophagous beetle with known thermal summation model. The developmental characteristics provided in this study will help to estimate the PMImin in cases where it was not possible before. The instar determination is the integral part of the PMImin estimation, because without accurate determination it we could not reach the right conclusion. We strongly encourage other authors to adopt our methodology for establishing the size-based instar characteristics, because it provides correct picture about its variability.