# General 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 Central Europe. I would like to provide a new insight 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 (carrion beetles) and subfamily Cholevinae of the family Leiodidae (small carrion beetles). More specifically, we focus on necrophagous, Central 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 the 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 carrion beetle, *Thanatophilus micans* (Fabricius, 1794), is able to locate and start to breed on corpse in the first 24 hours (Midgley & Villet, 2009). Although beetles in the Middle Europe are not that fast, probably due to lower temperatures (H. Šuláková 2014, pers. comm.), but there are other benefits for forensic entomology they could offer.

First of all, beetles tend to have longer development than flies (Midgley & Villet, 2009; 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 estimation.

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 *Sciodrepoides watsoni* and find the thermal summation model for this species, which will enable to estimate the post mortem interval. Further, I would like to find a new character for instar determination and propose a new methodology for estimaton of size-based larval characters (section **B**).

Some species of 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 U. S. A. 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 currently 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 of necrophagous carrion beetles (section **C**) and what are the ecological requirements of the European carrion beetle (section **A**).

European necrophagous species are overlooked, but they deserve much more attention, because they play major role in nutrient cycling, and information about their ecology and evolution can have great impact on applied fields of science like forensic entomology and nature protection (Lomolino *et al.*, 1995; Lomolino & Creighton, 1996; Ratcliffe, 1996; Begon *et al.*, 2006; Looney *et al.*, 2009; Crawford & Hoagland, 2010; Midgley *et al.*, 2010; Jurzenski *et al.*, 2014; Houston *et al.*, 2015).

During the field work on article **A** and **C**, we captured and identified 15 species of Central European carrion beetles. Three of which are currently considered as rare and are on the Czech Red List of Invertebrates as Endangered (*Nicrophorus antennatus* and *N. germanicus*) or Nearly Endangered (*N. sepultor*) (Růžička, 2005). The last ecological studies of these species in Europe were done almost 50 years ago when they were probably much more common (Novák, 1966; Petruška, 1968).

The most frequently observed species was *Thanatophilus 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; Petruška, 1964). Both species of genus *Thanatophilus* are therefore fairly common in the Czech Republic and they visit regularly carcasses of large animals.

*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.

All these traits could be used in forensic entomology, but larval identification, instar determination and thermal summation models are not established for them yet. In contrast, successful case report of estimation of PMI based on closely related African species of genus *Thanatophilus* (*T. mutilatus* and *T. micans*) was published last year (Ridgeway *et al.*, 2014). These species were only recently studied and proposed thermal summation models made them available for use in forensic entomology (Midgley & Villet, 2009; Ridgeway *et al.*, 2014). *T. sinuatus* and *T. rugosus* are showing similar qualities and options for future research looks very promising.

We were able to show in the article **A** 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 (article **A**).

CCA analysis in article **A** also revealed that the factors that are significantly associated with the species composition are both soil types (chernozem and fluvisol) and also three crops (*Zea mays*, *Hordeum vulgare* and *Heliantus annus*). The association between the abundance of the carrion beetles and these crops is very interesting and we did not expect such a relationship. We think that it could be due to the microclimatic conditions in fields with these crops. Based on our experience, *Z. mays* provides much more humid and cooler environment than *H. vulgare* and *H. annus*. Further study is needed to reveal causality.

Association between abundance of Nicrophorinae and soil characteristics was assumed by many authors (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).

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). It is also possible that the abundances of those species are different throughout their distribution area, as pointed out by (Scott, 1998).

Among other species, which were more abundant on cernozem, we also identified three endangered species (*N. antennatus*, *N. germanicus* and *N. sepultor*). Our finding can help in actions towards the conservation of these species, which is currently not a topical issue in the Czech Republic and Europe generally, but 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*) (Anderson, 1982; Lomolino *et al.*, 1995, 1999; Lomolino & Creighton, 1996; Bedick *et al.*, 1999, 2004; Szalanski *et al.*, 2000; Sikes & Raithel, 2002; Bishop *et al.*, 2002; Walker & Hoback, 2007; Crawford & Hoagland, 2010; Jurzenski *et al.*, 2011, 2014; Black, 2012; Hall *et al.*, 2015; Houston *et al.*, 2015).

The article **B** provides parameter estimates of thermal summation model of *Sciodrepoides watsoni* together with new character for instar determination. *S. watsoni* is very common necrophagous beetle (Růžička, 1994, 2000; GBIF, 2015), which occurs across Holarctic region (Peck & Cook, 2002; Perreau, 2004). This species was offered as with some utility for forensic entomology by (Schilthuizen *et al.*, 2011). This view was recently challenged by (Kilian & Mądra, 2015), but they unfortunately did not show any evidence to support their claim that this species is irrelevant for forensic entomology.

The mean development time decreased with increasing temperature (article **B**, 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.

During the breeding we observed very high levels of mortality in later stages of development (third larval instar and pupae). It is more likely that separation from other larvae and adults was the reason for this phenomenon. (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 (Peck, 1975). 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.

Methodology of measuring the size of the instars of *S. watsoni* 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 my 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.

I found an overlap in the head width between of all instars. This is especially true for the first and second instar, but measuring more, probably correlated, characters would not solve the problem. The 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 in the third instar. 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).

The article **B** 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, 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.

Altogether, these tree articles aim to contribute to growth of our knowledge of necrophagous beetles and I hope that I bring some of them to attention of profesional public.

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