

## Spatial distribution and seasonal dynamics of non-biting moth flies (Diptera, Psychodidae) in confound conditions of a stable

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**Abstract:** Many small Diptera adopted at some level endophilic life style, including man-made buildings. Stables create a specific type of microhabitat, which provides shady and relatively humid conditions in combination with excess of organic matter. Unlike the parasites (mosquitos, biting midges, etc.), the commensal fauna of stables is poorly studied. Moth flies (Psychodidae) were collected in cow stable located in Šenkvice, SW Slovakia. Special traps (derived from Malaise traps) were installed along the stable internal wall and in three different heights. In total, we recorded 6325 moth flies belonging to 8 species. The flight period lasted from spring to autumn. Seasonal dynamics was strongly influenced by rainfall and mean week temperature, e.g. high temperature in mid-summer caused drop in moth flies captures. The moth flies clearly preferred the ground and moderately preferred the interior of stable.

**Keywords:** drain flies, endophilic, cow stable, temperature, rainfall, traps

### Introduction

Most of the invertebrates are exophilic, living and preferring open, free space, but some prefer enclosed space, such as burrows, cracks or caves (Oosterbroek 2006). Given that burrows and natural cavities are frequently inhabited by mammals (carnivores, small rodents, bats), it is expected that there will be many commensals or parasites adapted to the sheltered conditions (Kinlaw 1999). The “artificial caves” (human constructions, such as stables) are now an integral part of intensive animal husbandry, creating a combination not present in nature before – cows, horses, pigs and even birds living in sheltered conditions. Naturally, many of the endophilic parasites and commensals make use of new resources.

Typical examples of endophilic parasite are mosquitoes, most frequently genus *Anopheles*, which was recorded in stables of

Hungary (Božičić 1985), Netherlands (Ibañez-Justicia & Cianci 2015) and Germany (Kronefeld *et al.* 2012).

The biting midges (Ceratopogonidae) are treated as either endo- or exophilic, as was demonstrated by Bishop (2002) who finds that *Culicoides brevitarsis* Kieffer, 1917 does not entrance closed areas (including stables), and in contrast, the work by Kameke *et al.* (2017) in which results showed that most *Culicoides* species (~60%) can be find inside cow, horse and sheep stables in Germany; these authors linked preference of sheltered conditions to the collecting season: it was conducted in the coldest part of the year (late winter and spring) (Kameke *et al.* 2017). Thus, the exo/endophilic behaviour might be conditional on the weather. Meiswinkel *et al.* (2000) found that African *Culicoides bolitinos* Meiswinkel, 1989 intrudes the stables, while *C. imicola* Kieffer, 1913 does not, so the



Fig. 1. Trap used to collect psychodid samples.

species endophilic habits are different according to species. However, these concepts cannot be extended to Psychodinae since they are not hematophagous. So, the collections inside of buildings depend of species, weather and, in relation this one, the latitude/climate (see Napp *et al.* 2011).

The sandflies (Phlebotominae, Psychodidae) are other candidates for endophilic lifestyle: they are nocturnal, but use as adult diurnal resting sites tree holes, buttress roots, rock crevices, leaf litter, houses, animal shelters and burrows (Alexander 2000). The immature stages of subfamily Phlebotominae develop in soil, rodent burrows, and in any kind of holes/caves (Theodor 1958). Where studied, most species preferred stable and dwelling over natural enclosures, such as caves (Arroub *et al.* 2012, Ramdane & Berchi 2018).

The commensals living in stables are poorly studied. In most cases, specimens are determined to the family level. Dipteran fauna is commonly neglected in ecological research due to extremely complex and demanding method for identification and is mostly referred to and analyzed at the family level. Since this is the most abundant order both by number of species and by abundance, to neglect them may disguise many of the ecological processes and relationships (e.g. Ivković *et al.* 2015). The published results suggest that representatives of several Diptera families are regularly present in stables or buildings, e.g. Drosophilidae,

Milichiidae, Phoridae, Psychodidae, Scatopsidae, Sciaridae, Sepsidae and Sphaeroceridae belong to the most numerous (Papp 1975, Farkas & Papp 1989, Demény 1989, Del Río *et al.* 2014). Similar presence of Diptera families was found in dairy in Sicily. Species of families Drosophilidae, Fannidae, Milichidae, Muscidae, Phoridae, Sphaeroceridae and in small portion also Psychodidae were recorded (Russo *et al.* 2002). From the limited evidence it seems that the moth flies are along others a regular part of stable fauna.

Moth flies are known for a wide range of larval habitats, often semiaquatic (dendrotelmata, phytotelmata, kitchen waste, etc.) or more terrestrial. Psychodids also develop in cow dung (Papp 1993) and can use stagnant cattle manure left in stables (Papp 1975). Here we present the study of moth flies (Psychodidae) found in a stable. We aim to provide information about species composition, seasonal dynamics, impact of environmental factors and spatial distribution of moth flies within stables.

## Material and methods

### *Study site*

The field experiments were conducted at the cooperative farm PD Šenkvice located in the village of Šenkvice, in central Slovakia, Pezinok District, Bratislava region. The cattle farm is situated in close vicinity to the village (48°18'10" N, 17°21'34" E, altitude 177–178 m above sea level) in the foothills of the Little Carpathian Mountains.

### *Material collection*

The farm where this study took place consists of several stables arranged in parallel in a southeastern (entrance) to northwestern (exit) direction. One stable was selected to accomplish the material collection. Collecting of the material was conducted by trap

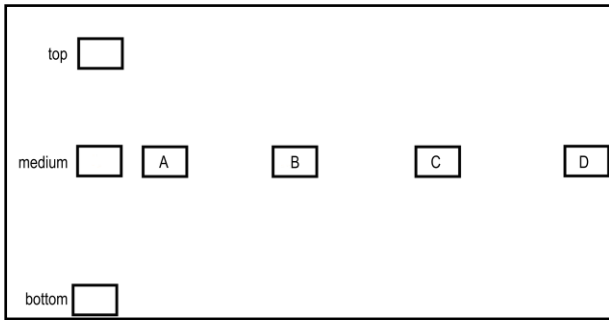


Fig. 2. Arrangement of the traps on the wall.

designed for this purpose. Essentially it was derived from a Malaise trap (Fig. 1). The traps (total number of traps 7) were arranged as follows: four traps were positioned along the stable at heights of 1.30 m, three traps were positioned in three different heights (at the bottom, 1.30 m and 2.10 m) close to the entrance of the stable (Fig. 2). The samples were taken every week from April 2016 to April 2017. Climatic data (mean week temperature and weekly precipitation) were obtained from the Slovak Hydrometeorological Institute and originated from a meteorological station Kráľová pri Senci, situated approximately 10 km from the farm.

### Identification

Identification and classification are in accordance to Vaillant (1971-1983), Ježek (1977, 1983, 1990, 1997), Oboňa & Ježek (2014).

### Data analysis

Data were analyzed in R, version 3.0.2 (R Core Team 2013) and lattice (Sarkar 2008) were used for the analysis and as graphical tools. The following environmental variables were used as explainables: mean temperature in the collecting week, precipitation in the collecting week and precipitation during one to nine weeks preceding the data collecting week. Prior to analysis, the explaining variables were checked for collinearity by pairs plot. Further analysis followed general protocol described in detail by Zuur *et al.* (2009).

As our data are counts and there are sources of correlation (the data can be seen as repeated measures of the same objects in time), we used generalized estimation equations, which allows for Poisson error as well as correlation in the residuals. The analysis was performed in package Geepack (Yan 2002). Once the optimal variance structure was selected (Poisson error, autocorrelation in the residuals), the optimal fixed structure was found by backward selection, that is, non-significant terms were dropped (one each time) and compared against the original model by likelihood-ratio test. Once all of the terms were significant, the backward selection was stopped.

Counts of specimens of all psychodids, counts of species and counts of *Tinearia alternata* (Say, 1824) were evaluated. We were interested, whether the sex influences behaviour of the species. For *Logima satchelli* (Quate, 1955), only females were available and the effect of sex could not be evaluated.

## Results

A total of 6 325 Psychodid moth flies belonging to 8 species were obtained by all traps during the sampling period. The most abundant taxon was *Logima satchelli* with 3 220 captured specimens, followed by *Tinearia alternata* with 3 009 specimens. The two most numerous species accounted for almost 98% of all specimens. An overview of other taxa is given in Tab. 1. and Fig. 3.

### Seasonal dynamics

The flight period of moth flies lasts from April to October. Seasonal captures of the two dominant species were fairly similar except of the beginning of the flight period, when the onset of flight activity is rather abrupt for *L. satchelli* while more gradual for *T. alternata*. Captures of the two dominant

Table 1. The overview table of the psychodid species recorded during the survey.

species	sum	males (m)	females (f)	percentage (%)
<i>Logima satchelli</i> (Quate, 1955)	3220	19	3201	56.8
<i>Tinearia alternata</i> (Say, 1824)	3009	664	2345	41.6
<i>Pneumia plumicornis</i> (Tonnoir, 1922)	45	12	33	0.5
<i>Psychoda cinerea</i> Banks, 1894	38		38	0.6
<i>Logima albipennis</i> (Zetterstedt, 1850)	7		7	0.1
<i>Clogmia albipunctata</i> (Williston, 1893)	3	1	2	>0.1
<i>Logima sigma</i> (Kincaid, 1899)	2		2	>0.1
<i>Trichomyia</i> cf. <i>urbica</i> Haliday in Curtis, 1839	1		1	>0.1

Table 2. Rain, temperature and position of the trap within stable had significant effect on the number of captured psychodids along the stable. RainX: precipitation in weeks preceding the collecting week (X).

	Df	X <sup>2</sup>	P(> Chi )
Trap	3	5.32e+13	< 2e-16 ***
Temp	1	2.20e+01	3.3e-06 ***
l(temp^2)	1	3.00e+00	0.089
l(rain0^2)	1	7.50e+01	< 2e-16 ***
Rain0	1	2.80e+01	1.1e-07 ***
Rain1	1	6.10e+01	6.1e-15 ***
Rain4	1	2.00e+00	0.127
Rain5	1	1.00e+00	0.239
Rain7	1	3.80e+01	6.5e-10 ***
Rain8	1	3.00e+00	0.063
Rain9	1	1.18e+02	< 2e-16 ***
Temp:rain	1	1.90e+01	1.0e-05 ***

Table 3. The number of psychodids captured significantly differs in the three height levels. RainX: precipitation in weeks preceding the collecting week (X).

	Df	X <sup>2</sup>	P(> Chi )
Trap	3	4.8849e+14	< 2.2e-16 ***
Temp	1	5.2410e+03	< 2.2e-16 ***
Rain0	1	6.0000e+00	0.0187216 *
Rain1	1	1.4000e+01	0.0001752 ***
Rain3	1	7.0000e+00	0.0097597 **
Rain5	1	2.4000e+01	1.060e-06 ***
Rain6	1	1.1400e+02	< 2.2e-16 ***
Rain7	1	1.7800e+02	< 2.2e-16 ***
Rain8	1	8.9000e+01	< 2.2e-16 ***
Rain9	1	3.5000e+01	2.705e-09 ***
l(temp^2)	1	7.3000e+01	< 2.2e-16 ***
Temp:rain	1	4.8000e+01	4.956e-12 ***

moth flies reach roughly 3 peaks in May, June and September. The flight activity was depressed during the midsummer (August). Occasionally, few specimens were recorded outside of the main flight season in autumn and winter (Fig. 4).

Table 4. Rain, temperature and position of the trap within stable had significant effect on the number of species along the stable. RainX: precipitation in weeks preceding the collecting week (X).

	Df	X <sup>2</sup>	P(> Chi )
Trap	3	9.03e+08	< 2e-16 ***
Temp	1	1.42e+02	< 2e-16 ***
Rain3	1	3.90e+01	4.8e-10 ***
Rain4	1	8.00e+00	0.00414 **
Rain5	1	6.00e+00	0.01709 *
Rain7	1	1.10e+01	0.00093 ***
l(temp^2)	1	6.00e+00	0.01142 *

Table 5. The number of psychodid species captured significantly differs in the three height levels. RainX: precipitation in weeks preceding the collecting week (X).

	Df	X <sup>2</sup>	P(> Chi )
Trap	3	2.70e+10	< 2e-16 ***
Temp	1	1.59e+02	< 2e-16 ***
Rain3	1	4.00e+00	0.054
Rain5	1	6.00e+00	0.012 *
Rain6	1	3.00e+00	0.092
Rain9	1	3.50e+01	3.2e-09 ***
l(temp^2)	1	5.00e+00	0.031 *

### *Number of specimens in relation to height above ground and along the stable*

GEE approach was used to allow for Poisson error and autocorrelation, as the data is a time series. The factor trap is significant, as well as the air temperature and rain fallen 1 to 9 weeks before the sampling week (Tab. 2).

The same approach was applied to number of psychodids captured in three Malaise traps installed in three different heights. As expected, the traps significantly differed in number of captured psychodids (Tab. 3. Fig. 5A).

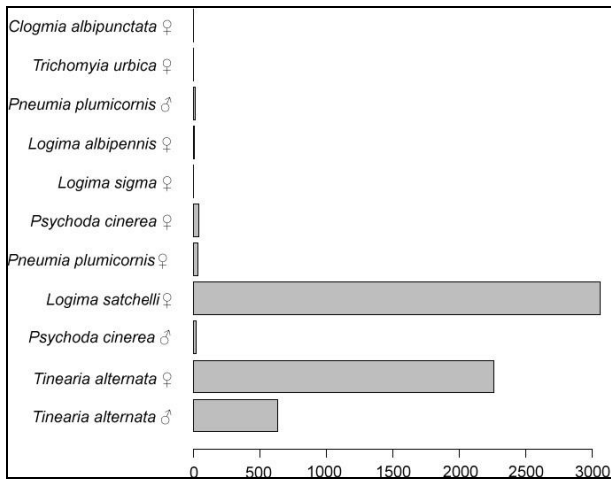


Fig. 3. Barplot of specimens counts per species and sex.

### Number of species in relation to height above ground and along the stable

We also investigated whether there are differences in number of species along the stable or along the heights (Fig. 6). As with the previous data, we used GEE model with Poisson error and autocorrelation. As expected, results of the analysis proved that the number of species is different along the stable, and that the number of species is highest near bottom of the stable (Tabs. 4, 5, Fig. 6).

From the environmental variables, both air temperature and rainfall had significant effect on the number of captured species. Interestingly, only rain fallen three–six and nine weeks before the sampling week had significant effect.

## Discussion

In total, we have identified over six thousand specimens belonging to eight species, of which only two species (*L. satchelli* and *T. alternata*) accounted for almost 98 % of all specimens. From the eight recorded species, only two were previously recorded in stables: *T. alternata* and *C. albipunctata*. Demény (1989) reared two species of sand moths from calf house and cow barns: *T. alternata* (as *Psychoda alternata*, 156 specimens) and undetermined psychodid (as *Psychoda* sp., 132 specimens). *T. alternata* was netted also from cattle

stables (Papp 1993) and reared from samples in poultry houses (Farkas & Papp 1989). *T. alternata* and *C. albipunctata* were captured by light traps in dairy in Sicily (Russo *et al.* 2002). The results of Demény (1989) and Farkas & Papp (1989) thus prove that at least two species of moth flies breed within stables, though one species remains unknown. From the limited available evidence, there are psychodids species that appear to be a regular part of stable fauna, though the species composition is usually reduced to one–two species (Papp 1975, Farkas & Papp 1989, Demény 1989, Papp 1993).

*Psychoda cinerea* Banks, 1894, *Logima albipennis* (Zetterstedt, 1850) and *Pnemia plumicornis* (Tonnoir, 1922) were present throughout the flight season, though in lower numbers. Their regular presence suggests that they also breed within (or close to) the stable, but due to their scarcity they could not be recorded by rearing experiments of Demény (1989) and Farkas & Papp (1989). The biology of *P. cinerea* and *L. albipennis* is consistent with life in stable, as they were previously recorded on excrements, manure, or near toilets (Jung 1956, Ježek 1977, Ježek 1990). The lifestyle of *P. plumicornis* is in strong contrast with this. This conspicuous large species is mostly collected in autumn in slope spring areas, mountain wells or fountains and wet meadows and banks of streams (Ježek 1997).

Three species accounted for only five specimens: *Logima sigma* (Kincaid, 1899), *Trichomyia urbica* Haliday in Curtis, 1839 and *Clogmia albipunctata* (Williston, 1893). Thus, we assume that they do not breed within stables and were present more or less accidentally, what is in accordance with their biology. The larvae of *T. urbica* are xylophagous and occur in shaded slope spring areas and some other habitats with decaying organic matter (Ježek & Omelková 2012). *C. albipunctata* is expansive species that colonizes mainly urban habitats, where it can

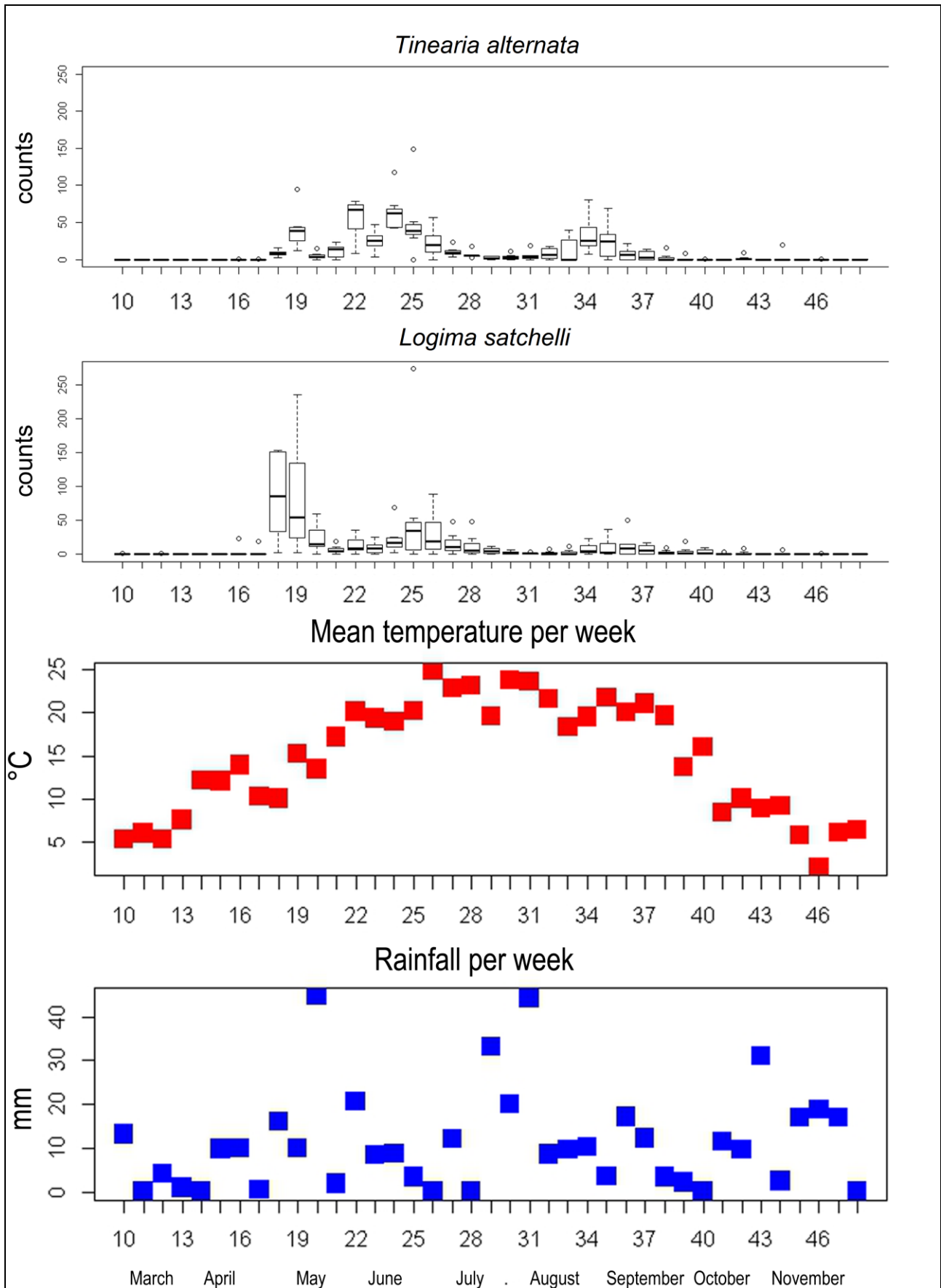


Fig. 4. Seasonal dynamics of *Tinearia alternata* and *Logima satchelli*, mean temperature per week and rainfall per week.

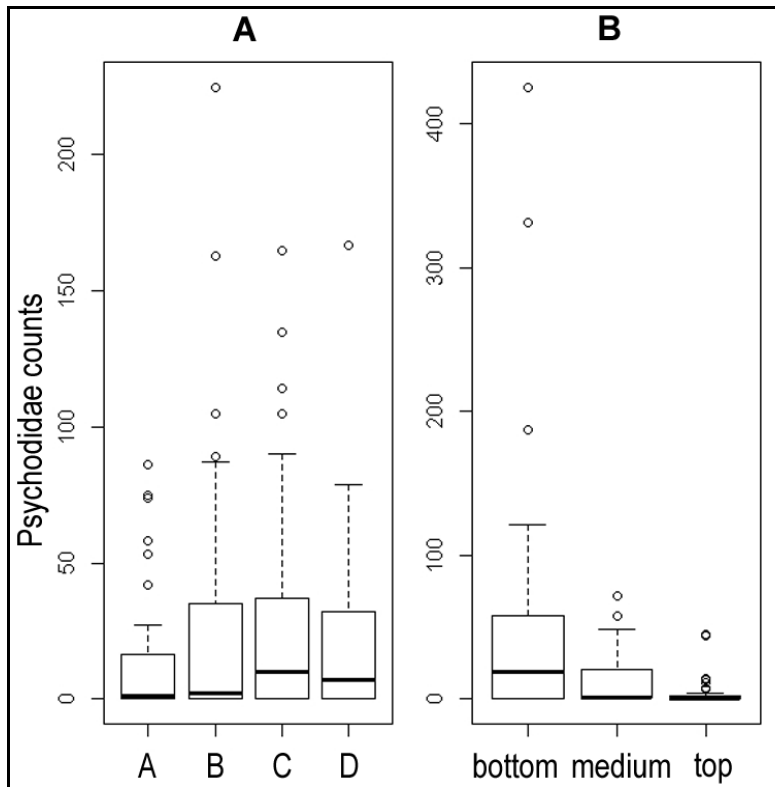


Fig. 5. Observed number of captured moth flies: A) along the stable. B) in three different heights.

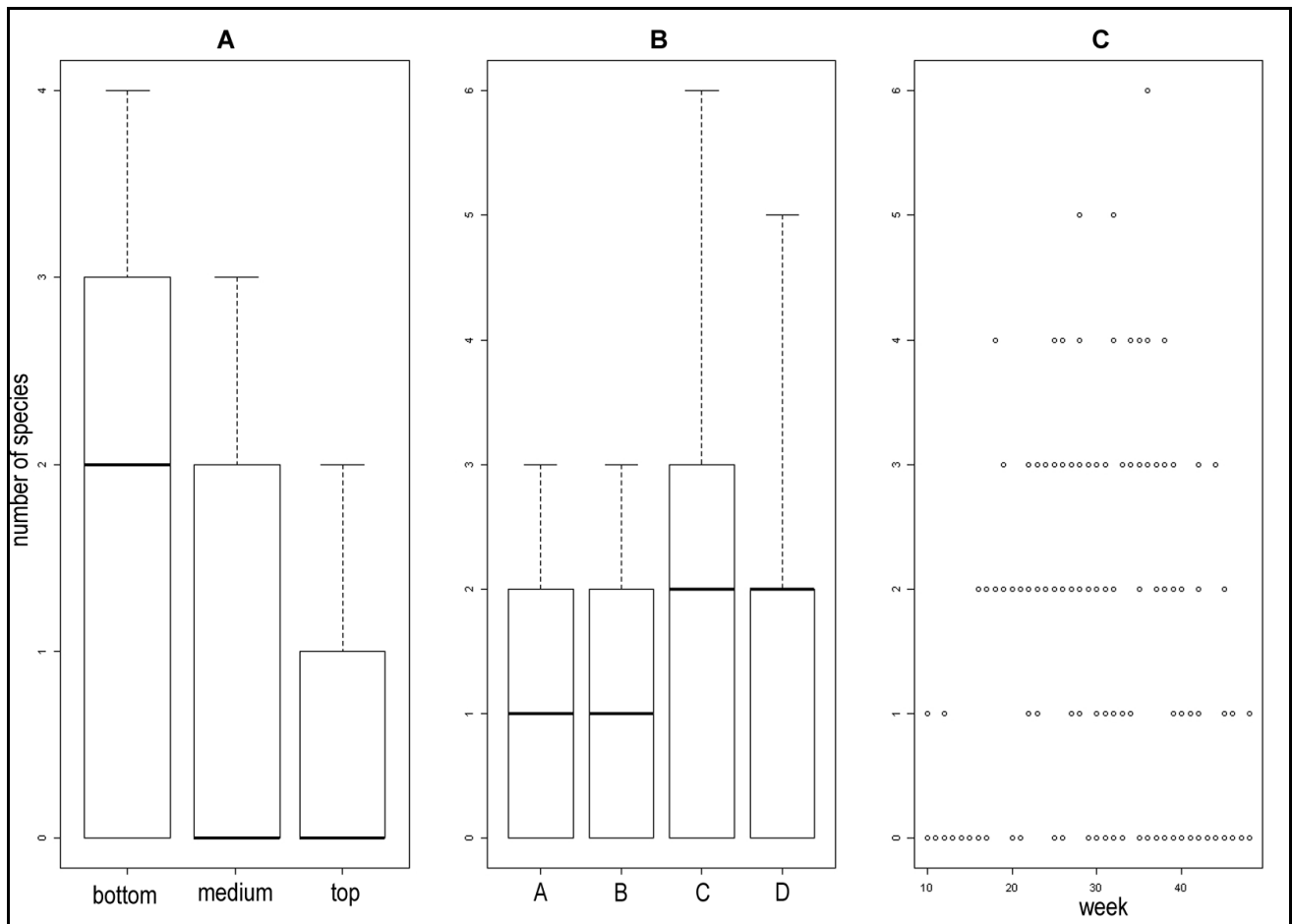


Fig. 6. Number of species recorded per trap: A) in three different heights; B) along the stable; C) throughout the season- each point represents number of species recorded in one of the 7 traps.

find suitable conditions for overwintering (Oboňa *et al.* 2016). The larval breeding site is not known for *L. sigma*. Breeding of *L. albipennis*, *L. satchelli* and *P. cinerea* within stables has yet to be proved.

### Seasonal dynamics and environmental factors

The flight season of moth flies last approximately 6 months (from April to October) and can be divided in three parts: the spring period with high counts and large fluctuations, summer depression and autumn flight period. Ivković *et al.* (2015) hypothesized that a strong effect on assemblage composition of Diptera as well as on diversity, abundance, phenology and feeding guilds composition between sites and that it will have a stronger effect than microhabitat characteristics. According Masteller & Wagner (1984) the tribe Psychodini was most abundant in the sewage-contaminated water. In present study, from the environmental factors, both rainfall and temperature had significant effect on moth flies captures. The start of flight period begins in half of April, when the temperature was for several weeks 10°C or higher. End of the flight season is accompanied by similar conditions, the flight season ends on half of October, when the mean temperature per week decreased to 10°C for two weeks. The mean week temperature thus seems to be limiting for emergence of adult moth flies.

Number of generations are not known for the dominant species, but where known, the life cycle is very short (1-3 weeks) leading to several generations per year. The rain fallen 1-9 weeks before the trapping of adults was consistently highly significant and the predicted and observed numbers of psychodids were very similar in all models. Strong influence of rain also suggests that moth flies only partly breed inside of the stable, as rain can add only little breeding sites within the stable. This suggests that

moth flies use stables predominantly as resting site or in search of sexual partner (in sexual species), due to higher humidity and protection against wind.

### Distribution within stable

The distribution of psychodids moths showed two clear patterns: the number of specimens increased towards the back side of the stable and was maximal near the ground. High density of moth flies close to the ground is a possibly result of their weak flight ability. Alexander (2000) points out that one of the favorite resting site of sand flies is leaf litter. The diurnal resting site is not known for *T. alternata* or *L. satchelli*.

The pattern for number of specimens and number of species was virtually the same. Number of species throughout season shows contrasting pattern to that of number of specimens. The highest number of captured species occurs in June, in the time of relatively low flight activity of moth flies.

### Acknowledgments

We would especially like to thank the reviewers and everyone who helped by providing constructive comments, as well as for improving the manuscript. The work was supported by grant APVV-14-0652 "Pesticide-free control of the house fly and stable fly populations on livestock farms in Slovakia" and VEGA 2/0111/18 „Oribatid mite communities of urban ecosystems”.

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Received: 01.04.2020

Accepted: 02.06.2020

Published online: 09.11.2020