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SMALL WATER BODIES

Weak altitudinal pattern of overall chironomid richness is a result of contrasting trends of subfamilies in high-altitude ponds

Ladislav Hamerlík · Marek Svitok · Milan Novikmec · Marta Veselská · Peter Bitušík

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Abstract The decline of species richness with altitude is one of the most obvious patterns in ecology and results from the combination of ecological and evolutionary mechanisms. In harsh high-altitude environments, the effect of altitude usually overrules other environmental variables related to biodiversity. Studies using species richness along altitude gradients in high altitude are relatively numerous for lakes, but not for ponds. However, due to their special features, such as small size, high isolation and regional variability, ponds have been proved to be different systems compared to lakes. In high-altitude waterbodies, species of the family Chironomidae often dominate in benthic invertebrate communities and thus serve as an ideal model to study aquatic community changes along an altitude gradient. However, due to the time-consuming processing and expertise needed to identify the species, chironomids are often excluded from regular surveys. In the present study, we sampled 66 Tatra ponds over a 1100-m altitude gradient for benthic invertebrates, with special attention to chironomids. Out of the total 122 taxa collected, Chironomidae constituted the richest group with 58 taxa, being present in all the study ponds. The most diverse pond supported 13 chironomid taxa, and mean diversity was 6 taxa/pond. While total invertebrate richness decreased with altitude, chironomid richness showed only a weak negative response to altitude. The proportion of total chironomid diversity made up of Tanypodinae and Chironominae subfamilies decreased with altitude, while the opposite trend was recorded for the proportion of Diamesinae and Orthocladiinae.

Keywords Non-biting midges · Biodiversity · Phylogeny · Altitudinal gradient · Tatra Mountains · Central Europe

Introduction

The decrease of species richness with altitude is one of the general patterns in ecology (e.g. Rahbek, 1995). It is obvious, however, that this pattern is not a result of altitude *per se* and altitude is just a surrogate for multiple factors that directly influence species richness (Rahbek, 2005). The most important drivers affecting biota that is globally associated with increasing altitude are the decline in air temperature and the reduction of land area (Körner, 2007). Changes of

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L. Hamerlík (✉) · P. Bitušík
Department of Biology and Ecology, Matej Bel University, Tajovského 40, 97401 Banská Bystrica, Slovakia
e-mail: ladislav.hamerlik@umb.sk

M. Svitok · M. Novikmec · M. Veselská
Department of Biology and General Ecology, Technical University in Zvolen, T. G. Masaryka 24, 96053 Zvolen, Slovakia

climatic and geographic properties are reflected by other environmental properties, such as water and air temperature, duration of the ice-free season, precipitation, catchment area and vegetation cover settings. Moreover, due to age and isolation of mountain areas, the ecological and evolutionary consequences of increasing altitude for biota are slower and shorter speciation, greater extinction, environmental filtering and limiting similarity (Graham et al., 2014) generally favouring species richness in lower altitude.

Due to enhanced climatic variation over short spatial scales together with simple ecosystems and the presence of organisms well adapted to the local conditions, mountain areas offer ideal conditions for exploring evolutionary adaptations and biodiversity trends over short spatial distances (Körner, 2007; Graham et al., 2014). In mountains, altitude changes rapidly, resulting in dramatic decrease in average air temperature and increased precipitation (Hinden, 2005). Due to the combination of short growing season, and limited energy and nutrient resources, aquatic ecosystems at high elevations experience extremely harsh conditions (Füreder et al., 2006), which require special adaptations from organisms (e.g. Lencioni, 2004). Surveys of species richness along an altitude gradient in high-altitude lakes in some European mountain ranges (Catalan et al., 2009; Fjellheim et al., 2009), the Alps (Füreder et al., 2006) and the Pyrenees (de Mendosa & Catalan, 2010), showed decreasing or hump-shaped patterns of richness with altitude. Other studies did not find any clear relationship between taxa richness and altitude (e.g. Boggero et al., 2006; Martinez-Sanz et al., 2012). The reason for this discrepancy is probably that the gradients selected included environmental changes that reflect local or regional peculiarities not generally associated with altitude (Körner, 2007).

Compared to high-altitude lakes, high-altitude ponds have been proved to be different systems (Catalan et al., 2009; Hamerlík et al., 2014). Due to their smaller size and catchment area (Novikmec et al., 2016) ponds represent isolated and highly heterogeneous systems that are more sensitive to environmental changes than lakes (e.g. Hinden, 2005; Novikmec et al., 2013). Therefore, it is likely that their response, either biological or environmental, to an altitudinal gradient is also different compared to lakes. Indeed, the few existing studies focusing on species richness of ponds in relation to altitude are quite contradictory: some report a sharp decline of species richness of various taxonomic groups (Oertli et al., 2000; Hinden et al., 2005; Ilg & Oertli, 2014), while other studies failed to find a significant relationship between altitude and richness in mountain ponds (Martinez-Sanz et al., 2012).

Even though altitude is a key factor, it is not the only variable affecting biodiversity in mountainous areas. Waterbody size, often correlating with depth and volume, thermal regime, stability and habitat complexity, can also influence species composition and diversity (Magnuson et al., 1998). It has been demonstrated that pond size can have a positive effect on biodiversity for some organisms in lowland ponds (Oertli et al., 2002), though with increasing altitude, pond size loses its effect (e.g. Hinden et al., 2005; Hamerlík et al., 2014). This could be because altitude and its interrelated variables have a greater effect over other variables influencing biodiversity (Hinden et al., 2005) and because of the simplicity and uniformity of littoral microhabitats of alpine ponds (Hamerlík et al., 2014).

Chironomidae (Diptera) is a species rich family whose diversity is usually among the highest of aquatic invertebrate groups in many water bodies. Due to their exceptional tolerance to extreme environmental variables, Chironomidae frequently occur in high abundance in mountain areas (Ferrington, 2008 and references therein), as a consequence of which they provide an ideal model for studying biodiversity patterns along altitude gradients. However, due to the time-consuming processing and expertise needed to identify the species, chironomids are often excluded from regular surveys as well as from pond diversity studies in high elevations (Oertli et al., 2000; Hinden et al., 2005; Ilg & Oertli, 2014). It has also been noted that studies of bioidiversity patterns in alpine ponds should include taxonomic groups with high species richness e.g. chironomids (Hinden et al., 2005) to confirm the validity of previously identified altitude-related biodiversity patterns.

A thorough understanding of the patterns of altitudinal diversity gradients is of key importance for predicting the response of biodiversity to environmental changes especially in mountain areas, where climate warming and glacier retreat are most pronounced. Because of the inconsistencies in species richness patterns seen in alpine ponds, we wanted to test whether the proven diversity pattern seen in lakes is also applicable to ponds. Therefore, in the present study, we studied chironomid assemblages of 66 high-altitude ponds spread over a 1100-m altitudinal gradient in the Tatra Mountains to (1) investigate the altitudinal range of particular species/taxa, (2) to describe changes in the overall invertebrate and chironomid richness in relation to the altitudinal gradient, (3) to analyse the altitudinal change in richness in chironomid ecological groups and subfamilies and (4) to compare these patterns with other gradients, such as those related to pond size and depth.

Material and methods

Study area

The present study took place in the Tatra Mountains, situated in Central Europe, at the border between Slovakia and Poland (the West Carpathians; 49°10′N, 20°10′E; Fig. 1). In the Tatra Mountains, precipitation can reach up to 2000 mm year^{-1} (Chomitz & Šamaj, 1974); above 2000 m, the precipitation remains as snow and can last for 9 months, typically from October to June. The corresponding temperature gradient is a reduction of 0.6°C per 100 m altitude gain (Konček & Orlicz, 1974). The related hydrology, soil and vegetation features of the Tatra Mountains have been described in detail by Kopáček et al. (2006). In the present study, 66 ponds with an area of < 2 ha located evenly along an altitudinal range from 1,089 to 2,201 m were surveyed (Table 1). All ponds were situated on granitic bedrock and had glacial origin. Due to the acidic base rock of the Tatra Mountains, some of the ponds are naturally dystrophic and others oligotrophic, including some which were formerly affected by acidification but have now regained their buffering capacity (Kopáček et al., 2002). In our dataset, oligotrophic and dystrophic ponds are represented. All ponds are fishless and macrophyte vegetation is entirely absent. All the ponds are rain and melt water-fed and have no glacial influence.

Overall, altitude is a proxy variable of temperature regime (Novikmec et al., 2013) and physico-chemical characteristics of ponds in the Tatra Mountains (Fig. 2). However, as apparent from the PCA on environmental data, morphological features of studied ponds (area, depth and volume) were virtually unrelated to altitude. We removed pond volume from the dataset due to strong correlation with area (r = 0.80) and depth (r = 0.73) and used these morphological gradients in the analyses for comparison with altitudinal patterns.

Field methods

Benthic invertebrates, including chironomids, were sampled every September in the years 2000–2013 in the Tatra Mountains ponds as a part of a larger study (see Novikmec et al., 2015). Benthic samples were taken from the littoral zone or from the whole area of pond (where possible) by the kicking technique (Frost, 1971) using a D-shaped hand net. Ponds were sampled more or less regularly throughout the study period. In all ponds, the total amount of sampling effort was 3 min and the sampling time was allocated proportionally to the coverage of identified substrate types. The material collected was preserved with 4% formaldehyde and stored in plastic bottles. In the laboratory, organisms were hand sorted, slide mounted and

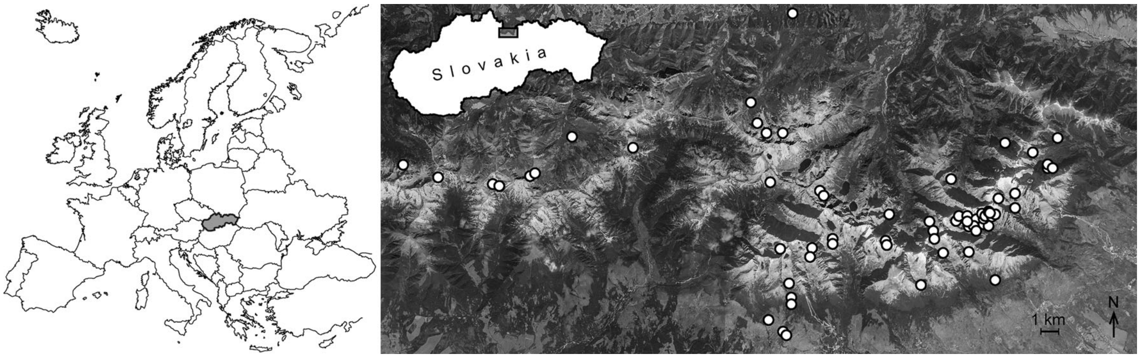

Fig. 1 Map depicting the location of the study region and individual ponds in the Tatra Mountains

Table 1 Summary of environmental characteristics of studied ponds

Environmental characteristics	Mean	Min	Max	SD
Altitude (m)[a,b]	1,838	1,089	2,201	241.9
Morphometric parameters[a,b]				
Surface area (ha)	0.361	0.002	1.889	0.477
Maximum depth (m)	2.3	0.01	8.0	1.8
Volume (m^3)	12,260	7	88,740	19,306
Physico-chemical parameters[c]				
pH	6.13	4.74	7.40	0.78
Conductivity (25°C; µS cm^{-1})	13.7	3.9	40.7	7.3
DOC (mg l^{-1})	1.54	0.06	9.87	2.26
P (µg l^{-1})	7.13	1.50	50.40	9.12

Based on data recorded in the field or from [a] Gregor & Pacl (2005) and [b] Kopáček et al. (2006). [c] Data from Kopáček (unpubl.)

Mean, minimum (Min), maximum (Max) and standard deviations (SD) are displayed

DOC dissolved organic carbon, *P* biologically available phosphorus

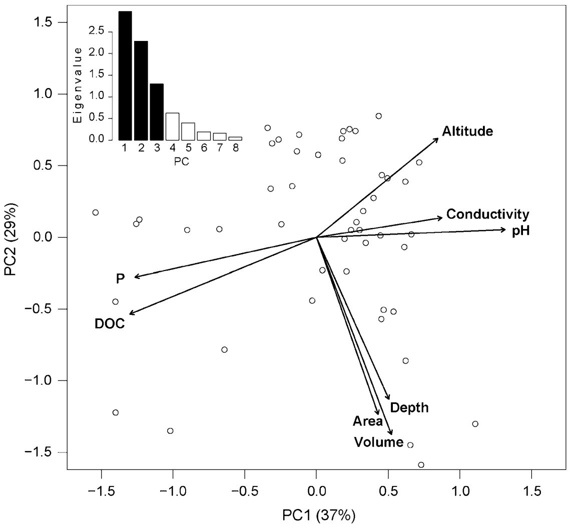

Fig. 2 Principal component analysis (PCA) on standardized environmental characteristics of studied ponds. Variance explained by the first two axes is given in parentheses. *Inset* shows eigenvalues of each principal component. The components explaining non-trivial amount of variance (*black bars*) were determined by broken-stick model. For abbreviations of variable names see Table 1

identified to the lowest possible taxonomic level. Bitušík (2000), Andersen et al. (2013) and Bitušík & Hamerlík (2014) were used as identification literature for chironomid larvae. Pupae and pupal exuviae, if obtained in kick samples, were identified following Wiederholm (1986), and Langton & Visser (2003). Because of the different level of identification reached for larvae (genus, species group, rarely species) and pupae (usually species), pupae were only used in the analyses when they could be allocated to larval level of identification. The prepared samples were added to the permanent slide collection at the Matej Bel University, Banská Bystrica.

Data analysis

Altitudinal distribution of individual chironomid taxa was examined visually (plots) and statistically using generalized linear models (GLM) with logit link function and binomial error distribution (McCullagh & Nelder, 1989). GLMs were also used to assess the effect of the main environmental gradients on richness and taxonomic proportions of benthic invertebrates. Total invertebrate taxonomic richness, total chironomid taxonomic richness, richness of chironomid subfamilies and ecological groups (cold-stenothermal taxa, semi-terrestrial taxa, and feeding groups) were used as response variables in GLMs with log link function and quasi-poisson error. *Diamesa tonsa* (Haliday 1856)/ *cinerella* Meigen 1835/ *vaillanti* Serra-Tosio 1972*, Pseudodiamesa nivosa* (Goetghebuer 1928) and *Pseudokiefferiella parva* (Edwards 1932) were considered as cold-stenothermal taxa (Rossaro, 1991); semi-terrestial taxa are *Chaetocladius piger* group, *Limnophyes* spp., *Metriocnemus hygropetricus* group, *Pseudosmittia* sp. and *Smittia* sp.

(Bitušík, 2000). Moog (1995) and Bitušík & Hamerlík (2003) were used to assign chironomids to feeding groups, such as predators, scrapers and collectors. The proportion (i.e. the ratio of taxa numbers) of chironomids to the total invertebrate richness and proportions of subfamilies and ecological groups to the total chironomid richness were modelled as quasi-binomial variables with logit link function. Major environmental gradients were represented by altitude, pond area (log transformed) and pond depth. Since environmental gradients in field studies are often strongly correlated, as in the present case, we built separate GLMs for each combination of response with gradient. Statistical significance of the relationships in the GLMs were assessed using likelihood ratio tests. The analyses were performed in R (R Core Team, 2015).

Results

Taxonomic composition and altitudinal ranges of particular taxa

In total, 68,965 chironomid larvae and pupae were collected (corresponding to ~80% of all macroinvertebrates recorded) and grouped in 58 taxa/species. Chironomid taxa richness ranged from 1 to 13 taxa, and the mean diversity was 6 taxa/pond. The complete list of macroinvertebrate taxa analysed in the present study is published in Novikmec et al. (2015).

The most common species/taxa were *Heterotrissocladius marcidus* (Walker 1856) (recorded at 47% of all sites), *Paratanytarsus austriacus* (Kieffer 1924) (42%), *Corynoneura scutellata* group, *Micropsectra* spp. (38% each), *Pseudodiamesa nivosa*, *Procladius* (*Holotanypus*) spp., *Tanytarsus* spp. (33% each), *Macropelopia* cf. *nebulosa* (Meigen 1804), *Chironomus* spp. (27%) and *Zalutschia tatrica* (Pagast 1935) (26%).

The recorded taxa showed various elevation ranges, but generally three groups with different patterns could be distinguished (Fig. 3). The first group included taxa covering the full range of altitudinal zones, such as *Paratanytarsus austriacus*, *Chironomus* spp., *Tanytarsus* spp., *Synendotendipes* sp., *Procladius* sp., *Zalutschia tatrica* and *Micropsectra* spp. The second group consists of taxa occurring below ~1,550 m, and the third group of taxa occurring above ~1,550 m representing the timber line in the Tatra Mountains. This classification corresponds well with the results of logistic GLMs for individual taxa (Table 2). Occurrence of 11 chironomid taxa was significantly affected by altitude. Notably, for a 100 metre increase in altitude, we can expect more than 50% increase in the odds of occurrence of *Smittia* sp., *Diamesa* agg., *P. parva*, *C. piger* gr. and *P. nivosa*. In contrast, the presence of *Procladius (H.)* spp., *Synendotendipes* sp. and *A. monilis* agg. was strongly negatively related to altitudinal gradient.

Chironomid richness and environmental gradients

We found a significant effect of altitude on taxa richness in all but two groups (Table 3). While the overall macroinvertebrate richness decreased considerably with altitude, total chironomid richness showed a much weaker response (Fig. 4). Even above 2,000 m a.s.l., the chironomid richness varied considerably from 1 to 11 taxa. However, when we repeated analyses at the subfamily level, clear altitudinal patterns emerged; Tanypodinae and Diamesinae showed strong opposite relationships leading to an overall weak response of the total chironomid richness. When we classified chironomids into ecological groups, both the cold-stenothermal and semi-terrestrial taxa groups showed increased richness along the altitudinal gradient. The taxonomic richness of scrapers also increased with altitude, while predators and collectors decreased significantly with altitude (Table 3). Significant positive species–area relationships were found in total invertebrate richness, total chironomid richness and richness of the subfamilies Tanypodinae and Prodiamesinae (Table 3). Also the proportion of predators showed a significant positive response to pond size. In contrast, the richness of semi-terrestrial taxa decreased with increasing pond size.

Chironomid proportions and environmental gradients

The proportion of total richness comprised by chironomids increased significantly with altitude (Table 4). The most remarkable changes along altitude occurred within the chironomid assemblage structure (Fig. 5). The proportions of total chironomid diversity made up by Tanypodinae and Chironominae subfamilies decreased with altitude, while the opposite trend was recorded for the proportion of Diamesinae and Orthocladiinae. Again, the proportion of cold-

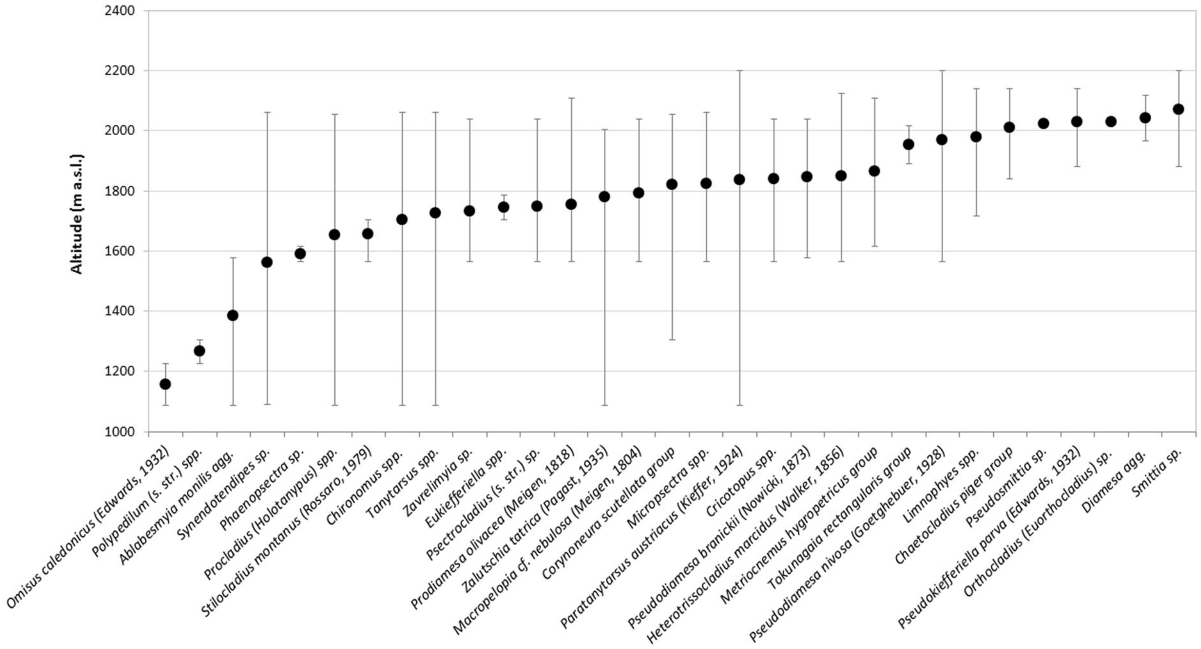

Fig. 3 The altitudinal distribution of particular taxa. Circles represent the mean altitude of occurrence; whiskers show the full range of distribution indicating the lowest and the highest altitude of occurrence. Taxa recorded in only one pond were excluded from the figure. *Diamesa* agg.: *Diamesa tonsa* (Haliday, 1856)/*cinerella* Meigen, 1835/*vaillanti* Serra-Tosio, 1972

stenothermal and semi-terrestrial taxa groups showed a strong positive relationship with altitude. The ratio of scrapers as a proportion of total chironomid diversity increased with altitude, whereas that of predators decreased, and proportion of collectors did not change substantially along the altitude gradient (Table 4).

The proportion of the Tanypodinae and Prodiamesinae taxa significantly increased with the size of ponds. In contrast, the proportion of semi-terrestrial taxa was significantly higher in smaller than larger ponds. The proportion of feeding groups was unaffected by pond size.

The proportion of Orthocladiinae and semi-terrestrial taxa decreased with increasing pond depth.

Discussion

Taxonomic composition and altitudinal ranges of taxa

Taxonomic composition of the surveyed ponds is comparable with other studies on the biota of high-altitude lentic waterbodies in the Tatra Mountains (Bitušík et al., 2006; Krno et al., 2006) along with other alpine areas of Europe (Boggero et al., 2006; Füreder et al., 2006; Catalan et al., 2009).

Many species/taxa that are considered to be cold-stenothermal (Rossaro, 1991; Eggermont & Heiri, 2011) and restricted to high alpine zone (Krno et al., 2006), e.g. *Pseudodiamesa nivosa* or *Paratanytarsus austriacus,* had a surprisingly wide range of occurrence. Larvae of *P. nivosa* are adapted to harsh physical environments, and their occurrence in a water body indicates ultra-oligotrophic status and extremely low water temperatures (Bitušík et al., 2006). Our findings do not necessarily change the interpretation of this species as cold-stenothermal. Instead, they support the idea that constant low temperatures may result from specific, altitude-independent topographical features, such as shading (Novikmec et al., 2013) that create favourable environments for cold-stenothermal taxa at lower altitudes.

It is noteworthy that semi-terrestial taxa, such as *Limnophyes* spp., *Chaetocladius piger* group, *Pseudosmittia* sp. and *Smittia* sp. were characteristic of the uppermost end of the altitudinal gradient (Fig. 3).

Table 2 Summary of logistic GLMs testing the relationships between presence/absence of chironomid taxa and altitude

Test statistics ($\chi^2_{(1)}$), probabilities (p) and relative changes in odds ratios per 100 m (OR) along with bootstrap 95% confidence intervals (95% CI) are displayed. Results significant at α = 5% are highlighted in bold. The table is ordered by odds ratios. Taxa recorded in less than 5% of sites were excluded from the analysis

Taxa	OR (95% CI)	$\chi^2_{(1)}$	p
Smittia sp.	**120 (59,373)**	**10.73**	**0.001**
Diamesa agg.	**78 (36,165)**	**4.75**	**0.029**
Pseudokiefferiella parva	**72 (29,171)**	**6.41**	**0.011**
Chaetocladius piger gr.	**59 (22,203)**	**5.75**	**0.017**
Pseudodiamesa nivosa	**52 (21,100)**	**13.08**	**<0.001**
Limnophyes spp.	**45 (25,90)**	**6.63**	**0.010**
Metriocnemus hygropetricus gr.	6 (−20,56)	0.11	0.737
Heterotrissocladius marcidus	5 (−20,23)	0.22	0.642
Pseudodiamesa branickii	2 (−21,22)	0.04	0.851
Cricotopus spp.	1 (−16,22)	0.01	0.938
Paratanytarsus austriacus	0 (−19,24)	0.00	0.999
Micropsectra spp.	−4 (−23,19)	0.11	0.740
Corynoneura scutellata gr.	−4 (−21,19)	0.12	0.729
Macropelopia cf. nebulosa	−10 (−40,8)	0.84	0.359
Zalutschia tatrica	−13 (−33,4)	1.26	0.262
Prodiamesa olivacea	−14 (−50,11)	0.71	0.399
Psectrocladius (s. str.) sp.	−17 (−45,−1)	1.98	0.159
Zavrelimyia sp.	−23 (−67,−6)	3.76	0.052
Stilocladius montanus	−27 (−54,−15)	1.53	0.216
Tanytarsus spp.	**−30 (−57,−9)**	**6.92**	**0.009**
Chironomus spp.	**−32 (−64,−13)**	**7.46**	**0.006**
Procladius (Holotanypus) spp.	**−61 (−94,−40)**	**20.88**	**<0.001**
Synendotendipes sp.	**−65 (−112,−30)**	**18.70**	**<0.001**
Ablabesmyia monilis agg.	**−78 (−173,−51)**	**12.83**	**<0.001**

Indeed, these taxa are typical for the cold end of the temperature gradient both in rivers (Rossaro, 1991) and lakes (Eggermont & Heiri, 2012). Interestingly, however, their distribution seems to be limited by high altitude rather than by low temperature, since the semi-terrestial taxa were entirely missing from cold ponds of lower altitudes, where other oligo-stenothermal species were common. Because of the specific habitat requirements of semi-terrestrial species, their occurrence in the uppermost ponds might indicate that higher altitude ponds were more prone to water-level fluctuation than those at lower altitudes. Small and shallow waterbodies in general are sensitive to water-level fluctuation (Wantzen et al., 2008). In addition, ponds with rocky substrates and small amounts of fine suspended sediment are more permeable and thus more susceptible to water loss than those surrounded by alpine meadows with well-developed soils and vegetation cover.

Apart from the semi-terrestrial taxa, the rheophilic/rheobiontic *Diamesa tonsa/ cinerella/ vaillanti*, *Orthocladius* (*Euo.*) sp. and *Tokunagaia rectangularis* group were characteristic of the uppermost ponds. Many non-lacustrine species occur in the littoral zone of high-altitude lakes (Bitušík et al., 2006) because low temperature and high oxygen content combined with wave action produces conditions resembling flowing waters and supporting species composition similar to that of streams (Lindegaard, 1992; Armitage et al., 1995).

Taxa preferring warmer temperatures (*Omisus caledonicus* (Edwards, 1932), *Polypedilum* spp. and *Ablabesmyia monilis* agg.) were restricted to the lowest part of the altitude gradient not reaching above 1550 m a.s.l. At the same time, this altitude represented the lower limit for the distribution of many cold-restricted taxa (Fig. 3). In the Tatra Mountains, 1550 m a.s.l. presents an ecological boundary dividing

Table 3 Results of GLMs testing for the effects of altitude, pond area (log transformed) and pond depth on taxonomic richness of total benthic invertebrates (total), total chironomids (Chironomidae), five chironomid subfamilies and five ecological groups

Richness	Altitude				Area				Depth			
	b (95% CI)×100	$F_{1,64}$	p	D	b (95% CI)	$F_{1,34}$	p	D	b (95% CI)	$F_{1,34}$	p	D
Total	**−0.09 (−0.13,−0.05)**	**17.90**	**<0.001**	**22.6**	**0.19 (0.06,0.33)**	**7.62**	**0.008**	**10.70**	0.02 (−0.04,0.08)	0.41	0.526	0.67
Chironomidae	**−0.06 (−0.10,−0.01)**	**5.59**	**0.021**	**7.7**	0.13 (−0.02,0.28)	2.94	0.091	4.25	<0.01 (−0.07,0.07)	<0.01	0.995	<0.01
Tanypodinae	**−0.20 (−0.28,−0.11)**	**19.73**	**<0.001**	**21.4**	**0.39 (0.06,0.75)**	**5.47**	**0.023**	**6.77**	0.04 (−0.11,0.17)	0.26	0.612	0.34
Diamesinae	**0.26 (0.12,0.42)**	**14.51**	**<0.001**	**17.4**	−0.05 (−0.38,0.30)	0.08	0.778	0.11	0.05 (−0.12,0.20)	0.31	0.577	0.46
Prodiamesinae	−0.13 (−0.39,0.19)	0.70	0.406	2.2	**1.63 (0.46,3.17)**	**8.57**	**0.005**	**19.40**	0.03 (−0.45,0.41)	0.02	0.900	0.05
Orthocladiinae	0.03 (−0.04,0.11)	0.64	0.427	0.9	0.14 (−0.08,0.36)	1.53	0.221	2.08	−0.05 (−0.16,0.05)	1.01	0.320	1.48
Chironominae	**−0.16 (−0.21,−0.10)**	**24.21**	**<0.001**	**24.8**	0.01 (−0.22,0.26)	0.01	0.907	0.02	0.03 (−0.09,0.13)	0.20	0.653	0.31
Cold-stenothermal	**0.42 (0.23,0.63)**	**23.68**	**<0.001**	**29.4**	−0.12 (−0.48,0.27)	0.37	0.546	0.53	−0.02 (−0.21,0.16)	0.03	0.862	0.05
Semi-terrestrial	**0.39 (0.20,0.61)**	**18.92**	**<0.001**	**23.7**	**−0.42 (−0.81,−0.01)**	**4.08**	**0.048**	**6.18**	−0.20 (−0.46,0.02)	3.04	0.086	5.12
Predators	**−0.16 (−0.24,−0.08)**	**15.57**	**<0.001**	**17.4**	**0.34 (0.05,0.65)**	**5.19**	**0.026**	**6.39**	0.06 (−0.06,0.18)	0.98	0.326	1.32
Scrapers	**0.21 (0.08,0.36)**	**10.32**	**0.002**	**14.5**	−0.04 (−0.35,0.30)	0.05	0.828	0.07	−0.08 (−0.25,0.07)	1.01	0.319	1.45
Collectors	**−0.07 (−0.12,−0.02)**	**6.55**	**0.013**	**8.3**	0.11 (−0.06,0.27)	1.64	0.205	2.26	< 0.01 (−0.08,0.07)	0.01	0.930	0.01

Model coefficients (b) along with the 95% confidence intervals (95% CI), results of likelihood ratio tests (F, p) and percentage of deviance in the data explained by the models (D) are displayed. Results significant at α = 5% are highlighted in bold

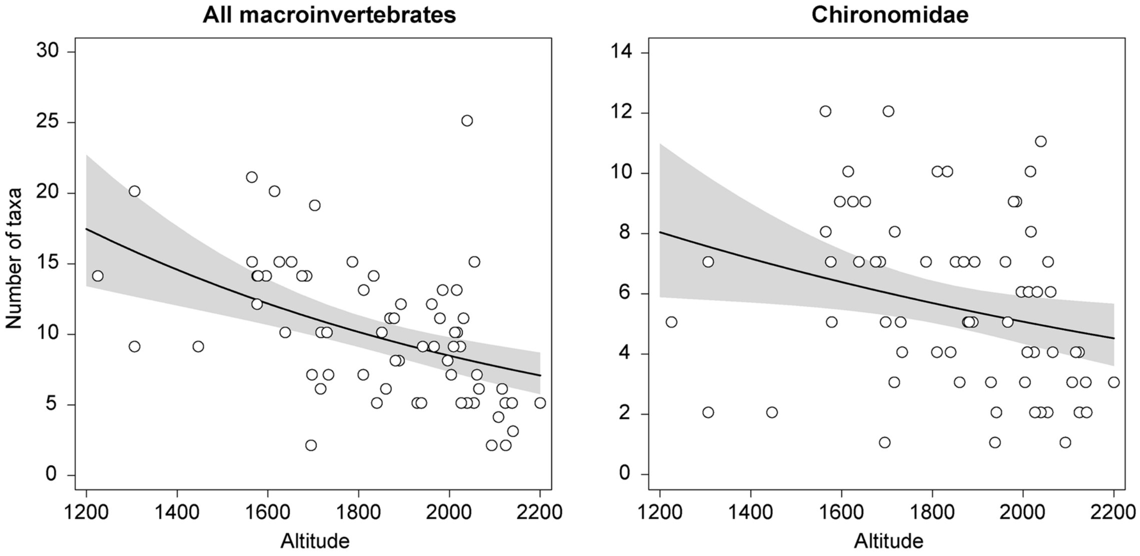

Fig. 4 Altitudinal distribution of the total macroinvertebrate diversity (*left*) and diversity of chironomids (*right*). The *lines* represent GLMs, and the *shaded areas* are the 95% confidence envelopes for these models

the forest and the subalpine zone where associated variables such as temperature, nutrient influx, soil quality and soil quantity drop significantly, while precipitation increased (Vološčuk, 1994). The composition of the chironomid assemblages tend to respond to these environmental variables. For example, Medeiros et al. (2015) found that beside the major temperature gradient, dissolved organic carbon (DOC) and total Kjeldahl nitrogen are important secondary gradients structuring lake chironomid assemblages, especially those located near ecotonal areas. Thus, we focused on these secondary gradients and searched for sudden altitudinal changes in DOC using recursive partitioning by conditional inference (Hothorn et al., 2006). Concentration of DOC in the studied ponds declined significantly ($c_{quad} = 25.0$, $p < 0.001$) around 1577 m a.s.l. (95% bootstrap CI: 1448 – 1717 m a.s.l.) which fits well with the observed changes in chironomid assemblages and treeline position in the Tatra Mountains It appears that changes in food supply (associated with DOC) across the treeline between forest and subalpine zone are important drivers of altitudinal pattern of chironomid diversity. Studies of high-altitude lake systems have identified ecological thresholds at about 1800 m a.s.l. (subalpine-alpine boundary, Bitušík et al., 2006) or at 2000–2200 m a.s.l., where richness, abundance and community composition changed considerably (Catalan et al., 2009; Füreder et al., 2006; de Mendosa & Catalan, 2010). These differences are natural since altitudinal thresholds change along latitudes, and may shift from marine to continental climates.

Diversity patterns along altitudinal gradient

In the Tatra ponds, both the total macroinvertebrate and chironomid richness decreased with altitude; however, the reduction in chironomid richness was much less pronounced. This difference may be a consequence of different evolutionary processes and phylogeny of specific taxonomic groups (Serra et al., 2015). Chironomidae, the dominant group in the high Arctic and alpine regions, have evolved in well-oxygenated cool mountain waterbodies (Brundin, 1966), thus simultaneously developing special adaptations for winter survival and limited summer development (Füreder et al., 2006). Chironomids have weak competition potential compared to other larger-bodied aquatic groups, and may be outcompeted in milder environments (Armitage et al., 1995). However, with increasing altitude, biotic interactions tend to have a smaller affect (Peckarsky, 1983), whereas the abiotic conditions become increasingly more important. This can result in a smaller degree of interspecific competition and in turn a higher diversity of well-adapted species like chironomids.

Table 4 Results of GLMs testing for the effects of altitude, pond area (log transformed) and pond depth on proportion of total taxonomic richness represented by chironomids (Chironomidae) and richness proportions of five chironomid subfamilies and five ecological groups to the total invertebrate chironomid richness

Proportion	Altitude				Area				Depth			
	b (95% CI)×100	$F_{1,64}$	p	D	b (95% CI)	$F_{1,34}$	p	D	b (95% CI)	$F_{1,34}$	p	D
Chironomidae	**0.09 (0.03,0.16)**	**8.00**	**0.006**	**10.4**	−0.15 (−0.35,0.04)	2.30	0.135	3.28	−0.06 (−0.15,0.03)	1.72	0.195	2.55
Tanypodinae	**−0.29 (−0.40,−0.19)**	**30.48**	**<0.001**	**30.0**	**0.42 (0.03,0.85)**	**4.47**	**0.039**	**5.92**	0.04 (−0.13,0.21)	0.26	0.609	0.37
Diamesinae	**0.51 (0.29,0.76)**	**26.78**	**<0.001**	**28.2**	−0.06 (−0.53,0.44)	0.06	0.813	0.09	0.12 (−0.11,0.33)	1.08	0.302	1.68
Prodiamesinae	0.02 (−0.34,0.48)	0.01	0.938	<0.1	**1.55 (0.24,3.38)**	**5.70**	**0.020**	**16.18**	0.10 (−0.44,0.54)	0.15	0.696	0.63
Orthocladiinae	**0.18 (0.09,0.28)**	**15.84**	**0.001**	**16.5**	−0.15 (−0.41,0.12)	1.20	0.278	1.56	**−0.14 (−0.27,−0.01)**	**4.65**	**0.035**	**6.04**
Chironominae	**−0.21 (−0.31,−0.11)**	**18.42**	**0.001**	**19.9**	−0.09 (−0.38,0.21)	0.33	0.568	0.44	0.03 (−0.10,0.16)	0.20	0.655	0.28
Cold-stenothermal	**0.69 (0.41,1.04)**	**31.75**	**<0.001**	**34.9**	−0.07 (−0.61,0.50)	0.06	0.812	0.10	0.08 (−0.18,0.32)	0.42	0.519	0.72
Semi-terrestrial	**0.64 (0.39,0.95)**	**33.92**	**0.001**	**36.4**	**−0.70 (−1.17,−0.24)**	**8.94**	**0.004**	**12.55**	**−0.31 (−0.63,−0.04)**	**5.22**	**0.026**	**8.32**
Predators	**−0.25 (−0.35,−0.15)**	**25.59**	**<0.001**	**25.2**	0.35 (0.00,0.72)	3.90	0.053	4.97	0.08 (−0.07,0.23)	1.16	0.285	1.57
Scrapers	**0.50 (0.31,0.72)**	**33.26**	**<0.001**	**32.0**	−0.08 (−0.52,0.38)	0.12	0.730	0.18	0.02 (−0.20,0.22)	0.02	0.882	0.03
Collectors	−0.04 (−0.13,0.04)	0.98	0.327	1.2	−0.15 (−0.41,0.10)	1.41	0.239	1.82	−0.06 (−0.18,0.05)	1.23	0.272	1.65

Model coefficients (b) along with the 95% confidence intervals (95% CI), results of likelihood ratio tests (F, p) and percentage of deviance in the data explained by the models (D) are displayed. Results significant at α = 5% are highlighted in bold

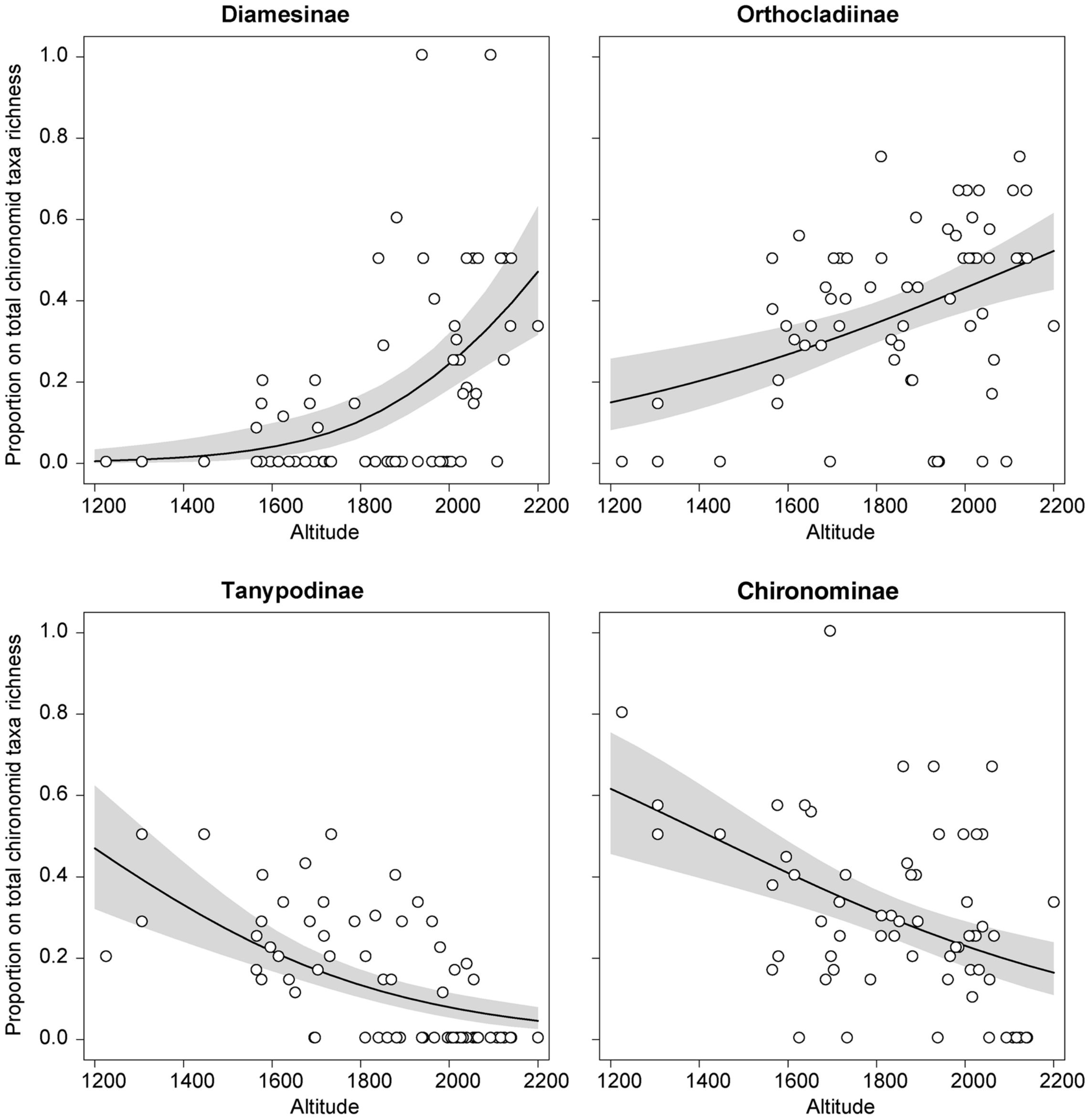

Fig. 5 Altitudinal distribution of the proportion of four chironomid subfamilies (Diamesinae, Orthocladiinae, Tanypodinae and Chironominae) on the total chironomid taxa richness. The lines represent GLMs, and the shaded areas are the 95% confidence envelopes for these models

Taxa richness and proportion of chironomid subfamilies changed with altitude, though two opposite trends were seen: whereas the proportion of Diamesinae and Orthocladiinae taxa making up the total chironomid richness increased, both the proportion and richness of Tanypodinae and Chironominae decreased considerably. The pattern is known for mountain lakes (Boggero et al., 2006; Füreder et al., 2006; Hamerlík & Bitušík, 2009) as well as for flowing waters (e.g. Lindegaard & Brodersen, 1995; Khamis et al., 2014), and this trend follows the general food, temperature and oxygen preferences of the subfamilies. The majority of the Diamesinae and Orthocladiinae species are cold adapted and usually feed on periphyton covering firm substrates. Moreover, many of them are oxy-conformers (Brodersen et al., 2004) i.e. they are not able to regulate oxygen uptake, thus are restricted to waters with a high oxygen content.

Conversely, Chironominae, designated as collectors feeding on detritus, together with the Tanypodinae, a subfamily that are predators, both have adaptations to cope with warmer water and lower oxygen levels (Brundin, 1966). In addition, most groups of the Tanypodinae subfamily are primary lentic (Fittkau, 1962) and not very well adapted to the cold alpine zone.

Broad generalizations and assumptions that all species of a subfamily share the same feeding habit mask the real feeding diversity of chironomids and must be used with caution (Serra et al., 2015). Thus we analysed the response of the richness of particular feeding groups using species resolution. There was a very strong response to the number of predators (negative) and scrapers (positive) to increasing altitudinal change, while collectors showed a weak negative or no response when analysing their proportion. Changing sediment structure and decreasing proportions of fine organic sediment are most likely to be responsible for the changes in the proportion of the feeding groups. Ormerod et al. (1994) also indicated that bankside and catchment vegetation can affect the trophic structure of high-altitude streams, mainly due to the high ratio of terrestrial–aquatic interface, a phenomenon which may also apply to ponds. At lower altitudes, shading by trees and shrubs prevents photosynthetic algae from growing on pond substrates; therefore this can be a disadvantage to scrapers which feed on algae. On the other hand, collectors can benefit from the organic enrichment. With increasing altitude, the quantity and coverage of catchment vegetation decreases and so does the habitat variability of ponds, thereby decreasing the proportion of feeding groups.

Species–area relationships

Waterbody size is known to be a key driver for species richness. Larger water bodies tend to support more species as a result of higher colonization potential and lower extinction rates (MacArthur & Wilson, 1967) combined with increased habitat diversity in larger biotopes (Catalan et al., 2009). In the ponds of the Tatra Mountains, both chironomid and total invertebrate richness showed a weak positive response to pond size. In previous pond surveys, the species–area relationship was not found (Hinden et al., 2005; Martinez-Sanz et al., 2012; Hamerlík et al., 2014) or only applied to selected taxonomic groups (Wissinger et al., 1999; Oertli et al., 2002). It has been shown that local diversity patterns in high-altitude lentic waterbodies changed significantly at an area of 2 ha, and below this limit, a species–area relationship was not detected (Hamerlík et al., 2014). The likely reason was significantly lower habitat diversity and higher isolation of smaller waterbodies compared with those above 2 ha. All our study ponds had an area below 2 ha, and thus species richness showed a weak response to pond size.

The proportion of semi-terrestrial taxa was negatively correlated with pond size, while there was a positive correlation between richness and/or proportion of Tanypodinae, predators and collectors. Since smaller ponds have a greater ratio of terrestrial–aquatic interface, there is greater opportunity to support semi-terrestrial taxa compared to larger ponds. In addition, small ponds present ideal settings for semi-terrestrial species as they are more likely to be ephemeral compared to large ponds. Conversely, subfamilies that are less adapted to drought (e.g. Tanypodinae) with longer development time should be more common in large ponds.

In general, because the effect of altitude and related factors are predominant in high-altitude systems, the influence of other variables (e.g. area and depth) is often hidden. To identify the importance of these factors for biodiversity, a study with a reduced altitude gradient would be needed (Hinden et al., 2005).

Conclusions

(1) Cold-stenothermal species showed a large altitudinal range. These species were not only found at the high altitudes, but also at lower-altitude ponds which were a suitable habitat probably due to local topographical conditions, such as shading. Consequently, future warming will not necessarily cause general upward movement of cold-stenothermal species, since they can survive also at lower altitudes, if conditions are appropriate. Therefore, from the point of view of conservation, it is important to identify those ponds/lakes at lower altitudes that can serve as refuges for cold-stenothermal species.

(2) Chironomid diversity declined much less with altitude than total macroinvertebrate diversity. Phylogenetic reasons are likely behind this pattern. Chironomids have evolved in cold mountain environments, and thus many phylogenetically primitive taxa are well adapted to these conditions. This was especially obvious when the richness of subfamilies with different ecological and evolutional backgrounds was analysed. Consequently, knowledge of the phylogeny of particular taxa could help to predict their responses to changing temperature and thus may be important for nature conservation.

(3) Chironomid diversity increased moderately with pond size. Although this trend has been recorded previously by other researchers, the effect of altitude over pond size may be an important driver in controlling biodiversity.

Our aim with the present study was to point out that patterns of species richness, even in a taxonomically consistent family (in our case Chironomidae), is the result of the interactions within the taxonomic group and that responses at family level can hide considerable changes when higher (e.g. subfamily, tribe) taxonomic resolution is used for analysis.

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