Original Research

# Distribution and Environmental Determinants of Chironomids (Diptera, Chironomidae) in *Sphagnum* Microhabitats

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#### Abstract

Our study investigates the role of *Sphagnum* mosses as habitat for chironomid larvae with regard to peatbog type (raised bog, transitional bog, fen). Samples were collected within Roztocze National Park (eastern Poland) from May to October 2012 with two monthly intervals. Studied peatbog types differ significantly in terms of density and domination structure of *Sphagnum*-associated chironomids. The results of CCA analysis indicated that pH, N-NO<sub>3</sub>, and chlorophyll-a are significant for chironomid distribution between peatbog types. Moreover, chironomid assemblages were affected by environmental factors specific for a given peatbog type. Temperature and chlorophyll-a determined the chironomid assemblage in raised bogs. Water level, temperature, and total phosphorous were significant for chironomid structure in transitional bogs, whereas conductivity affected chironomids associated with *Sphagnum* in fens. *Sphagnum* mosses in transitional bogs were the preferred habitat for larval chironomids in this study. In transitional bogs chironomid density was the highest and typical, macrophyte-associated taxa, larvae of *Psectrocladius sordidellus* gr. (Zetterstedt, 1838), *Cricotopus sylvestris* gr. (Fabricius, 1794), and *Glyptotendipes* sp., were dominant. In raised bogs and fens, pelophilous taxa, larvae of *Chironomus* sp., and *Procladius* sp. showed higher abundances.

**Keywords:** chironomidae larvae, *Sphagnum*, peatbogs

## Introduction

Larvae of chironomid midges are ubiquitous components of epiphytic invertebrates in wetlands and lake ecosystems [1, 2]. Many taxa are primary consumers of

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algae and detritus and are food for other components of food webs (invertebrates, fish, waterfowl); they form a crucial link between energetic inputs and higher trophic levels [3-5]. Live macrophytes serve mostly as substrate, whereas plant detritus serves as both habitat and food source [6, 7]. Efficient colonization capacity, great feeding plasticity, and flexibility to occupy different substrates allow chironomids

to live in a wide range of habitats [8, 9]. As reported by many researchers the structure of plant associated chironomids is regulated by many environmental factors, such as availability of food resources (periphytic algae), habitat complexity (spatial distribution and architecture of macrophytes), nutrients, salinity, oxygen, and pH [10-15].

Despite their reported importance, the ecology of chironomids associated with peatbog vegetation is not sufficiently described. In temperate regions, the most notable plants are mosses of the genus *Sphagnum*, which form the bulk of living and dead biomass in peatbogs [16]. Due to their high morphological complexity (arrangements of shoots, branches, and leaves) and specific microtopography (mosaic of peaty hummocks, shallow wet hollows, and flatted lawns) [17, 18], *Sphagnum*-dominated peatlands create the diversity of microhabitats which may colonize many group of organisms. The majority of recent studies on the role of *Sphagnum* as substratum for invertebrates have focused on testate amoebae and ciliates [19-22]. The *Sphagnum*-chironomid relationship has not been investigated.

In this study we analyzed the structure of chironomids and their distribution in *Sphagnum* microhabitats in the Roztocze National Park (eastern Poland; 50°37.663′663 N, 23°03.147′E). Within the area, peatbog ecosystems representing a wide environmental gradient, from nutrient-poor ombrotrophic raised bogs, to mesotrophic transitional bogs, to minerotrophic, nutrient-rich fens. It has been hypothesized that:

- 1) The role of *Sphagnum* as microhabitat for larval chironomids is determined by the type of peatbog.
- Water level, pH, nutrients and conductivity, and variables discriminating the type of peatbog have a primary influence on chironomid spatial distribution.

- The structure of chironomids associated with Sphagnum microhabitats on a peatbog type is affected by environmental conditions specific for a given peat- bog.
  - The primary objectives of our paper are:
- 1) To determine the structure and abundance of chironomids on *Sphagnum* mosses in raised bogs, transitional bogs, and fens.
- 2) To recognize environmental variables significant for chironomid spatial distribution between peatbog types.
- 3) To describe the determinants of chironomid structure within a peatbog type.

## Methods

## Study Area

Our study was conducted within six peatbog ecosystems representing three types: raised bogs (RB) - Międzyrzeki-RB1, Tory I-RB2, transitional bogs (TB) – Kosobudy-TB1, Kruglik-TB2, and fens (F) - Tory II-F1, Tory III las-F2 situated on the area of Roztocze National Park. The peatbog types differ in the trophic status and the structure of vegetation. In raised bogs, Eriophorum vaginatum (L.), Carex acutiformis Ehrhart., Carex gracilis Curt., Sphagnum angustifolium (C.C.O. Jensen ex Russow), Sphagnum cuspidatum Ehrh. ex Hoffm., and Sphagnum magellanicum Bird dominated. In transitional bogs, Eriophorum vaginatum, Carex acutiformis, Carex gracilis, Sphagnum angustifolium, Sphagnum cuspidatum, Polytrichum sp., and Molinia coerulera have overgrown the largest area. In fens, Phragmites australis (Cav.) Trin ex Steudel, Carex acutiformis, Utricularia sp., and Sphagnum angustifolium were mostly observed.

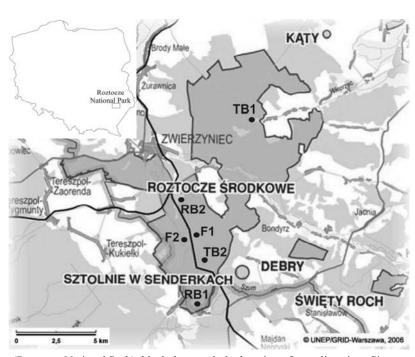


Fig. 1. Map of study area (Roztocze National Park), black dots mark the location of sampling sites. Sites are coded as follows: raised bogs – RB1, RB2; transitional bogs – TB1, TB2; fens – F1, F2. Image source: UNEP/GRID-Warszawa, 2006.

		RB			TB		F			
	May	July	October	May	July	October	May	July	October	
WL (m)	0.11±0.04	0.12±0.03	0.09±0.01	0.16±0.03	0.17±0.02	0.18±0.01	0.72±0.02	0.39±0.01	0.51±0.03	
Temp (°C)	20.2±3.4	21.4±5.3	18.8±0.8	23.4±2.5	8.1±0.1	8.3±0.2	25.5±0.9	7.0±0.5	7.1±0.5	
рН	4.2±0.1	5.2±1.2	4.9±0.9	4.4±0.5	5.1±0.3	5.3±0.4	7.4±0.1	8.1±0.3	8.7±0.2	
Tot. sus (mg·dm <sup>-3</sup> )	10.8±7.3	11.3±7.7	13.0±3.0	95.7±30.3	325.8±189.8	327.4±189.4	28.5±16.4	8.9±6.3	9.3±5.9	
Cond (μS·cm <sup>-1</sup> )	45±9	49±5	54±2	106±31	77±2	86±9	259±13	267±93	270±92	
Dis.oxy (mg·dm <sup>-3</sup> )	6.2±2.5	6.7±1.9	5.4±1.1	7.6±1.9	10.3±1.8	10.9±2.3	6.5±1.6	8.8±2.6	9.1±2.5	
N-NO <sub>3</sub> (mg·dm <sup>-3</sup> )	0.54±0.29	0.59±0.24	0.67±0.02	1.30±0.24	1.34±0.07	1.43±0.17	0.46±0.12	0.81±0.29	0.76±0.25	
P-PO <sub>4</sub> (mg·dm <sup>-3</sup> )	0.077±0.014	0.074±0.01	0.058±0.01	0.453±0.04	0.498±0.48	0.503±0.48	0.115±0.04	0.003±0.001	0.008±0.002	
TP (mg·dm <sup>-3</sup> )	0.098±0.031	0.103±0.026	0.521±0.123	1.006±0.154	0.895±0.163	0.900±0.162	0.159±0.051	0.303±0.241	0.311±0.239	
Chl-a (μg·dm <sup>-3</sup> )	6.1±1.3	6.6±0.9	16.3±1.9	151.7±77.8	321.4±218.5	322.5±219.3	8.3±4.6	18.3±7.1	18.8±7.2	

Table 1. Mean values (±SD) of physical and chemical water parameters of Sphagnum microhabitats in studied peatbogs.

# Field Sampling

Chironomids associated with Sphagnum angustifolium were collected from May to October 2012 with two monthly intervals. Three sampling sites were randomly selected at each type of peatbog. At each site and sampling date, three replicate samples were taken. Chironomid samples were collected using a perspex cylindrical apparatus (diameter 13 cm, length 32 cm, wall thickness 0.5 cm), with openings covered by net of mesh size 250 µm. The open cylinder was placed inside the Sphagnum bed and the plants were gently inserted into the cylinder using a small floristic fork. The sampler was closed and raised in a horizontal position to the surface. The water from the cylinder was emptied, the sampler was opened, and the plant material was transferred into the plastic bag. At the laboratory, the larvae of chironomids were rinsed from Sphagnum plants (each sample separately) and preserved in 4% formaldehyde solution, counted, and identified. The nomenclature of larval chironomids followed Chernovsky [23] and Wiederholm [24]. The density was calculated per 100 g of dry weight (DW) of plant. The Sphagnum plants collected for analysis were dried at 105°C for 24 h and then weighed to obtain the DW.

Water samples for chemical analysis were taken in three replicates at the same sites and dates as chironomids. *In situ* were recorded water level, temperature, pH, conductivity, and dissolved oxygen by means of YSI 556 MPS electrode. Concentrations of nitrate nitrogen (N-NO<sub>3</sub>), total phosphorous (TP) and dissolved orthophosphates (P-PO<sub>4</sub>) were determined at the laboratory using spectrophotometric methods [25]. The concentration of chlorophyll-*a* was analyzed by spectrophotometric method following a 24-h extraction with 90% acetone in the dark [26].

## Data Analysis

The influence of peatbog type and season on chironomids abundance and species composition were verified

using two-way analysis of variance ANOVA. Kruskal-Wallis test was used to compare significant (P<0.05) differences between means. The analysis was performed by means of Statistica 7.0 Software. Detrended correspondence analysis (DCA) was used to measure the gradient of variability indicated by chironomids [27]. Because the length of the gradient was >4 standard deviations, canonical correspondence analysis (CCA) was used to explore the relationships between the abundance of chironomid species and environmental variables in different types of peatbogs [28]. Automatic forward selection of environmental variables and Monte Carlo permutation test (999 permutations) was used to determine the most important variables. Significant variables (P<0.05) were marked as bold arrows on the diagrams. The ordination analyses were performed by means of CANOCO 4.5 for Windows.

# Results

## **Environmental Variables**

Environmental conditions of *S. angustifolum* habitats were highly variable among studied peatbog types (Table 1). The water level fluctuated from 0.09 m (October, RB) to 0.71 m (May, F). Nutrients showed the highest concentration in TB; N-NO<sub>3</sub> (1.30-1.43 mg·dm³), TP (0.895-1.006 mg·dm³), and P-PO<sub>4</sub> (0.453-0.503 mg·dm³) and the lowest, dependent on the season in RB or F. Chlorophyll-*a* concentration varied between 6.1 μg·dm³ (May, RB) and 322.5 μg·dm³ (October, TB). pH in RB and TB was low (typical for humic waters) and oscillated between 4.2 and 5.3; in F, pH was visibly higher and amounted from 7.4 to 8.7.

# Structure of Chironomid Assemblages

The abundance of chironomids was significantly affected by peatbog type (ANOVA, F = 7.28, P = 0.006) and sea-

	RB			ТВ			F		
	May	July	October	May	July	October	May	July	October
Ablabesmyia phatta (Eggert, 1863)	2±1.1						1±0.3		
Procladius sp.		1±0.7				4±1.0	5±2.1	3±1.6	
Corynoneura lacustris Edwards, 1924							2±0.9		5±2.1
Corynoneura lobata Edwards, 1924		4±1.7							
Cricotopus sylvestris gr. (Fabricius, 1794)				8±2.3					
Psectrocladius sp.				1±1.4					
Psectrocladius dilatatus gr. (v.d. Wulp, 1859)			1±0.9						
Psectrocladius sordidellus gr. (Zetterstedt, 1838)		1±1.6		11±4.1	32±4.9	21±8.3			
Chironomus sp.	1±0.2		3±2.7		7±2.7	6±2.5	1±0.5	2±0.7	5±2.7
Einfeldia sp.					4±2.2	4±1.9			
Endochironomus albipennis (Meigen, 1830)		1±1.6							
Endochironomus impar (Walker, 1856)					7±1.9				
Stictochironomus histrio (Fabricius, 1794)					4±1.7				
Glyptotendipes sp.				9±2.8					
Paratendipes albimanus (Meigen, 1818)	1±0.3				9±3.5				
Phaenopsectra flavipes (Meigen, 1818)			1±1.4					16±4.8	
Polypedilum sordens (v.d. Wulp, 1874)				2±0.7					
Polypedilum nubeculosum gr.(Meigen, 1804)				1±1.2				1±1.1	
Total abundance	4±0.8	7±1.8	5±1.8	32±4.6	63±10.4	35±8.4	9±1.9	22±6.7	10±2.2

Table 2. Species composition and mean densities (ind. 100g DW-1, ±SD) of Sphagnum-associated chironomids in studied peatbogs.

son (ANOVA, F = 1.78, P = 0.047). The highest densities of *Sphagnum*-associated chironomids were noted in TB (32-63 ind.·100 g DW<sup>-1</sup>) and the lowest in RB (4-7 ind.·100 g DW<sup>-1</sup>) (Table 2). Regardless of peatbog type, the lowest densities were observed in May and the highest in July.

In total, 18 chironomid taxa were noted on S. angustifolium (Table 2). Species structure of chironomid assemblages was related to peatbog type and season. In RB, the highest abundance revealed *Corynoneura lobata* (July), *Ablabesmyia phatta* (May), and *Chironomus* sp. (October). Larvae of *Psectrocladius sordidellus* gr. prevailed within the chironomid community in TB. Moreover, high abundance showed larvae of *Glyptotendipes* sp. (May), *Cricotopus sylvestris* gr. (May), and *Paratendipes albimanus* (July). In F, larvae of *Procladius* sp. (May), *Phaenopsectra flavipes* (July), *Corynoneura lacustris* (October), and *Chironomus* sp. (October) predominated.

Two chironomids, *Chironomus* sp. and *Procladius* sp., were classified as common taxa; these larvae were noted on *Sphagnum* in all of the studied peatbogs. Moreover, the exclusive chironomid taxa were found for each type of peatbog. Larvae of *C. lobata, Endochironomus albipennis*, and *Psectrocladius dilatatus* gr. were noted only in RB; large group of chironomids, *C. sylvestris* gr., *Einfeldia* sp.,

Endochironomus impar, Stictochironomus histrio, Glyptotendipes sp., and Polypedilum sordens were observed solely in TB and larvae of C. lacustris in F habitat

## **Ordination Analysis**

The results of CCA confirmed the distinction of chironomid assemblages between peatbog types. Samples collected in RB, TB, and F are clearly separated on the CCA biplot (Fig. 2). In the ordination space studied peatbogs are distributed along the gradient of pH, chlorophyll-*a*, and N-NO<sub>3</sub>.

The abundance of *Sphagnum*-associated chironomids within RB, TB, and F was significantly affected (results of Monte Carlo permutation test) by environmental variables specific for a given peatbog. Temperature ( $\lambda = 0.72$ , F = 17.11, P = 0.012) and chlorophyll-a ( $\lambda = 0.10$ , F = 3.87, P = 0.046) showed the significant effect on chironomid abundance in RB. On the CCA biplot, chironomids are separated into three groups (Fig. 3A). Group I (*A. phatta*, *P. sordidellus* gr., *Chironomus* sp., *P. flavipes*) correspond with temperature, conductivity, and N-NO<sub>3</sub>. Group II (*Procladius* sp.) show a relation with chlorophyll-a.

The abundance of taxa in Group III (E. albipennis, P. dilatatus gr., P. albimanus, C. lobata) is related to water level and pH.

Water level ( $\lambda$  = 0.33, F = 3.86, P = 0.042), temperature ( $\lambda$  = 0.07, F = 7.59, P = 0.046) and P-PO<sub>4</sub> ( $\lambda$  = 0.21, F = 4.67, P = 0.028) significantly affected the density of chironomids in TB. On the CCA diagram (Fig. 3B), a large group of chironomid taxa *C. sylvestris* gr., *P. sordidellus* gr., *Chironomus* sp., *E. impar*, *Glyptotendipes* sp., *P. albimanus*, *P. sordens*, *Polypedilum nubeculosum* gr. show the relation to chlorophyll-a, pH, and conductivity; the remaining species are situated separately on the diagram and do not show any visible relation to environmental variables.

In F habitat, conductivity had a significant influence on chironomid assemblage ( $\lambda = 0.59$ , F = 10.94, P = 0.042). On the CCA biplot (Fig. 3C), larvae of *Procladius* sp. and *P. flavipes* are related to dissolved oxygen and conductivity; *Chironomus* sp. corresponds with temperature and P-PO<sub>4</sub>. Larvae of *C. lacustris*, *P. nubeculosum* gr., and *A. phatta* are related to N-NO<sub>3</sub>.

#### **Discussion**

Larval chironomids associated with *Sphagnum* microhabitats showed visible spatial variability between peatbog

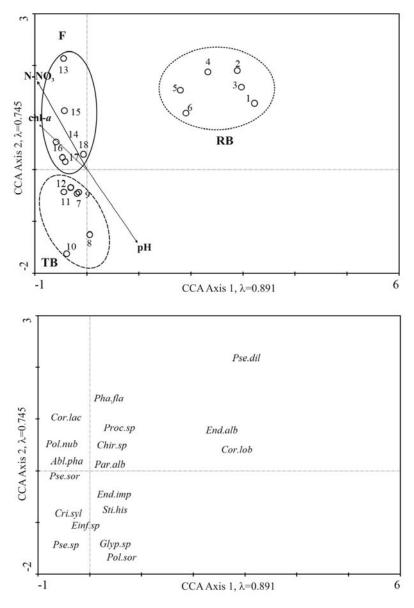


Fig. 2. Scatter plots of canonical correspondence analysis (CCA) showing A) *Sphagnum* samples in studied peatbog habitats and B) chironomid species; 1-16 samples collected in raised bogs; 7-12 samples collected in transitional bogs; 13-18 samples collected in fens. N-NO<sub>3</sub> – nitrate nitrogen; chl-*a* – chlorophyll-*a*.

Taxa codes: Abl.pha – Ablabesmyia phatta; Cor.lac – Corynoneura lacustris; Cor.lob – Corynoneura lobata; Chir.sp – Chironomus sp; Ein.sp – Einfeldia sp; End.alb – Endochironomus albipennis; End.imp – Endochironomus impar; Glyp.sp – Glyptotendipes sp; Par.alb – Paratendipes albimanus; Pha.fla – Phaenopsectra flavipes; Proc.sp – Procladius sp; Pse.sp – Psectrocladius sp; Pse.dil – Psectrocladius dilatatus; Pse.sor – Psectrocladius sordidellus; Pol.nub – Polypedilum nubeculosum; Pol.sor – Polypedilum sordens.

types. In RB and F, the abundance and species richness of chironomids were very low, similar to those observed on *Ceratophyllum demersum* L. in a shallow hypertrophic lake [29]. Densities of chironomids on *Sphagnum* in TB were

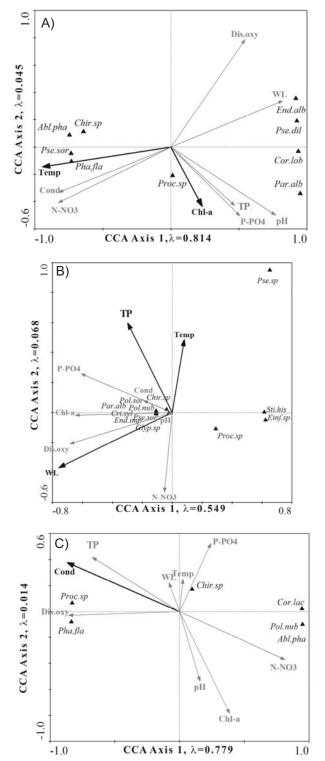


Fig. 3. Biplots of canonical correspondence analysis (CCA) showing chironomid taxa and environmental variables on A) RB; B) TB; C) F.

Chl-a – chlorophyll-a; Cond – conductivity, Dis.oxy – dissolved oxygen; N-NO $_3$  – nitrate nitrogen; P-PO $_4$  – dissolved orthophosphates; TP – total phosphorous; Temp – temperature; WL – water level. Taxa codes are the same as on Fig. 2.

high, close to that on submerged macrophytes (Stratiotes aloides L., Potamogeton lucens L., Myriophyllum spicatum L.) in shallow lakes [30, 31]. Larvae of Psectrocladius sordidellus gr., Cricotopus sylvestris gr., and Glyptotendipes sp., dominant in TB, are typical plant-dwelling chironomids of highly eutrophic and oxbow lakes [32, 33]. In F and RB, pelophilous taxa, Chironomus sp., Procladius sp., and Phaenopsectra flavipes showed high abundances; these chironomids are important components of bottom fauna and are widely assumed to rely on benthic algae and/or associated detritus as food [13, 34].

The results of CCA indicate the significant role of N-NO<sub>3</sub>, chlorophyll-a, and pH as factors determining the distinction of chironomids between peatbog types. The role of N-NO<sub>3</sub> is important in ombrotrophic RB, whose nutrient balance depends totally on atmospheric input. Sphagnum is a genus of plants typical of ombrogenous mires. Opposite to submerged macrophytes in lakes, which are able to uptake nutrients from bottom sediments or directly from the water column [35, 36], Sphagnum plants accumulate nutrients through direct influx of atmospheric inputs into living cells and thus may regulate the hydrochemical conditions at the bog surface [37, 38]. Deposition of atmospheric nitrogen regulates N availability on the surface and interstitial water of Sphagnum mosses microhabitats, and may affect the decomposition rate and nutritional value of dead organic matter and thus may limit the presence of chironomids feeding on detritus and algae [39, 40]. In our study, larval chironomids in RB were the least abundant and prevailed by pelophilous taxa; in the studied ombrotrophic habitat, the concentration of N-NO3 was the lowest in comparison to other studied peatbog types. Chlorophyll-a, as an indicator of algae biomass, is regarded as a determinant of potential food for grazing invertebrates. Availability of food resources is often pointed to as the major regulator of chironomid distribution and abundance [5, 41]. In our study, the role of algae (food source) seems to be important for Sphagnum-associated chironomids in TB; in these ecosystems, the dominant chironomids (Glyptotendipes sp., P. sordidellus gr., C. sylvestris gr.) are commonly reported algae grazers [30, 41]. pH is a factor that may affect macrophytechironomid relations on wetlands. Studies of Woodcock et al. [1] on chironomids in marine wetlands suggest that competitive dominance of a few taxa was responsible for lower richness in low pH wetlands, whereas competition in high pH wetlands is weaker and enables the coexistence of more chironomid taxa. In the present study, we obtained the opposite results, as chironomids show the highest richness in TB at low values of pH (4.5-5.0).

Chironomids associated with *Sphagnum* mosses showed seasonal variability. The role of season is usually related to temperature and this factor was recognized as significant for chironomid abundance in RB and TB. Temperature affects chironomid phenology (the length of larval stage, emergence time) and growth rate. The studies on development times of floodplain chironomids provided by Tronstad et al. [42] have demonstrated higher growth and shorter development times of chironomids grown at optimum temperatures (summer) and with high-quality food.

Moreover, temperature may regulate grazing pressure of chironomids on lower trophic levels (algae, protozoa). These interactions are extremely important during the summer period. Increase of temperature positively influences the mechanism of "top-down" regulation [43].

Water level and total phosphorous were indicated as determinants of chironomid abundance in TB. The role of phosphorous should be considered in terms of the trophic status of studied ecosystems. The concentrations of TP and P-PO<sub>4</sub> in TB are similar to those noted in shallow eutrophic lakes, where the production of algae (potential food source for larval chironomids) is usually limited by external and internal P loadings [44]. Water level may affect chironomid abundance indirectly through its effect on Sphagnum growth and reproduction [45]. It was reported that species such as Sphagnum magellanicum and Sphagnum rubellum showed higher biomass production on high water levels and temperature [46]. Thus, changes in water table depth may alter changes in the species composition of Sphagnum mosses and, consequently, may diminish/expand the area for chironomid colonization.

Chironomid associated with *Sphagnum* in F habitat was structured by conductivity. This may indicate the effect of salinity on chironomid distribution. In general, chironomids are often one of the most abundant taxa of inland waters with moderate to high salinities [47]. As reported by Cartier et al. [48] and Cañedo-Argüelles and Rieradevall [10], salinity determines species structure, distribution, and the emergence of chironomid species. In the present study, we did not find a clear relationship between conductivity and distribution of larval chironomids between peatbog types. The highest density and species richness of chironomids were observed on *Sphagnum* in TB, where values of conductivity oscillated between 70 and 100 μS·cm<sup>-1</sup>.

# **Conclusions**

The results of the study showed clear responses of Sphagnum-associated chironomids to the changes of habitat conditions (peatbog type). It can be assumed that the structure of chironomids is habitat specific. In this study, the highest abundance and species richness of chironomids were observed in TB, which suggests that larvae of Psectrocladius sordidellus gr., Glyptotendipes sp., Cricotopus sylvestris gr, and Paratendipes albimanus, dominants on Sphagnum mosses, prefer habitats of high concentration of nutrients and chlorophyll-a (biomass of algae). These observations indicate great similarity to macrophyte-chironomid relationships in shallow-lake ecosystems, where P and N as well food resources (periphytic algae) are major determinants of chironomid assemblages. Despite clear differences of environmental conditions, Sphagnum microhabitats in RB and F were less favourable for larval chironomids. The structure of chironomid larvae on both peatbog types was similar, wherein the abundances of pelophilous larvae of Chironomus sp. and Procladius sp. were higher in F habitat.

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