Commensalism is a form of symbiosis whereby one species benefits while the other is unaffected, contrasting with parasitism in which the host species suffers a decline in fitness (Paracer and Ahmadjian 2000). One form of commensalism is symboresy—an association whereby midge larvae live on the body surface of a larger mobile host without apparent benefit or harm to the host but with clear benefits to the midge (Cranston et al. 1983; Bottorff and Knight 1987). In aquatic habitats, symboresy as a life-history strategy is frequently shown by chironomids (see review by Tokeshi 1993; Roque et al. 2004; Henriques-Oliveira and Nessimian 2009). The chironomid *Epoicocladius ephemerae* (Kieffer 1924), a host-specific midge, shares a resource in the form of the body surface of the nymphal stage of the mayfly *Ephemera danica* Muller 1764 with an unrelated commensal, a generalist epibiont, the ciliated protozoan, *Carchesium polypinum* L. 1758.

The aim of the study was to examine the distribution pattern of the two commensals to understand how these two epibions share host body surface avoiding competition by interference. We tested the hypothesis that *Ephemera* body size influenced the pattern of commensal infestation intensity and prevalence.

Materials and Methods

Study Area

The study was conducted in two small lowland rivers (the Vistula drainage basin, central Poland). One site was located in a first order stretch of the Bzura River, which flows through Łódź City (Fig. 1). This site was characterized by large amounts of allochthonous organic matter, especially tree leaves, covering the stream bed over the whole year. The other site was established in a second stream order section of the Mroga River, a tributary of the Bzura River (Fig. 1), which flows through agricultural areas with riparian trees and bushes.

The study streams differed mainly in the composition of inorganic substrate (Grzybkowska et al. 2012). According to Substrate Inorganic Index (SI) (Quinn and Hickey 1990) in the Bzura, sand was the dominant fraction of bottom substrate (SI=0.4 mm), whereas mud was abundant in the Mroga (SI=0.4 mm).
Whereas in the Mroga sand with scattered gravel and pebbles (SI = 4.9 mm) dominated the river bed. Both rivers differed also in the amounts of the two main fractions of benthic particulate organic matter (BPOM), i.e., coarse (CPOM) and fine (FPOM) (Petersen et al. 1989). In the Bzura River, the amount of both fractions was higher than in the Mroga River (∼9,000 and ∼5,000 g m⁻², respectively). According to water quality data obtained from the Voivodship Inspectorate of Environmental Protection in Łódź (2009), total phosphorus concentration for the Bzura River was 0.38 mg P dm⁻³, whereas for the Mroga River 0.24 mg P dm⁻³, and respectively total nitrogen concentration was 9.80 and 6.81 mg N dm⁻³, total suspended solids 18.91 and 27.10 mg dm⁻³, and conductivity 826 and 513 μS cm⁻¹. Despite these substrate and water quality differences, macroinvertebrate density reached a similar level (mean annual density of ∼5,000 inds m⁻²) in each river, with a dominance of Chironomidae and Oligochaeta. These benthic groups constituted 60% of the total benthic abundance in the former and over 70% in the latter river. In both these lotic ecosystems, mayflies were not numerous, and they were represented by three genera: Caenis, Baetis, and Ephemer. The annual mean density of sand-burrowing nymphs of Ephemer danica was <100 inds m⁻² at each station. The nymphs feed by filtering or collecting fine particulate organic detritus from the water column.

Sampling and Analysis
The microhabitats where mayfly larvae were collected were very similar in both rivers (low stress sandy area with FPOM). Samples were taken using kick-nets on nine occasions in 2009–2011, in spring, summer, and autumn. Living E. danica were transported separately to the laboratory and examined under a stereoscopic microscope to estimate the number of commensals on specific body parts. The occurrence of commensals on the hosts was evaluated on the scope to estimate the number of commensals on specific body parts. The positive (i.e., co-occurrence) or negative (avoidance) type of association was determined by the Yule’s Q index \[ Q = \frac{(a \times d - b \times c)}{(a \times d + b \times c))}, \] which is a point correlation coefficient for presence–absence data (Zar 2010).

The response of chironomid and protozoan commensals to mayfly size was analyzed using Generalized Linear Models (GLM) in the CanoDraw software (Lepš and Smilauer 2003). The abundance of both commensals on mayfly body parts was analyzed with the Friedman test followed by multiple pairwise comparisons using Nemenyi’s procedure as implemented in XLSTAT software package (https://www.xlstat.com/).

Results
Of a total of 389 host individuals, only 28.8% were not affected by commensals, which means that prevalence (f = 95% C.L.) was high (0.712 ± 0.052), whereas the mean intensity of infection was 4.01 (±6.04) commensals on one host. The number of commensals on a single specimen of mayfly varied between 0 and 18 chironomid larvae and from 0 to 46 colonies of ciliates. The maximum number of all commensals on one host was 48 (46 ciliates colonies and 2 chironomids). The comparison of the frequency of commensal chironomid occurrence (f = 95% C.L.) in the two rivers demonstrated that in the Mroga River this index was higher than in the Bzura River (0.505 ± 0.113 and 0.361 ± 0.063, respectively). However, the frequency of the commensal C. polypinum showed the opposite pattern; i.e., this commensal was more frequent in the Bzura River (0.351 ± 0.063) than in the Mroga River (0.119 ± 0.073). Overall, the frequency of both commensal species on one host was low in both rivers; i.e., 0.045 ± 0.028 in the Bzura River and 0.020 ± 0.032 in the Mroga River. Thus, in the Mroga River the commensal chironomid was much more frequent than the ciliate (\[ \chi^2 = 31.82, P < 0.001 \]), but in the Bzura River there was no differences in frequency between E. ephemerae and C. polypinum (\[ \chi^2 = 0.03, P > 0.05 \]).
The abundance of the two commensals also differed significantly among mayfly body parts (the Friedman test $Q = 408.486$, df $= 11$, $P < 0.0001$). Multiple pairwise comparisons (the Nemenyi test) distinguished five homogenous groups difficult to describe, but both commensals were most abundant on the mayfly abdominal part A, tinguished five homogenous groups difficult to describe, but both commensals were 54 times more abundant than chironomids (Fig. 5). The lowest numbers of both commensals were observed on the mayfly cerci and thorax, except on head where ciliates were more frequent (Fig. 3).

The co-occurrence of commensals was not independent with a negative association ($\chi^2 = 50.28$, $P < 0.001$, and $Q = -0.762$). A comparison of their co-occurrence on different mayfly body parts revealed a nonrandom negative association (avoidance) observed only on abdomen parts A and B. For the other parts of the mayfly body, associations tended to be negative, but not significant (Table 1).

In addition, the abundance of E. ephemerae and C. polypinum were affected by host size (Table 2). The optimal mayfly length for chironomids was 28.77 mm (with 26.16 mm as lower and 34.24 mm as upper 95% C.L.). For ciliates, the optimal mayfly size was lower, at 11.63 mm, with 10.61 and 12.38 mm as lower and upper 95% C.L., respectively (Fig. 4).

The abundance of the two commensals also differed significantly among mayfly body parts (the Friedman test $Q = 408.486$, df $= 11$, $P < 0.0001$). Multiple pairwise comparisons (the Nemenyi test) distinguished five homogenous groups difficult to describe, but both commensals were most abundant on the mayfly abdominal part A, and exhibited the lowest abundances on the mayfly cerci and thorax (Fig. 5). The largest differences in numbers between chironomids and ciliate protozoans were noted on the mayfly head and abdomen B, where colonies of ciliate commensals were, respectively, 78 and 54 times more abundant than chironomids (Fig. 5). The lowest numbers of both commensals were observed on the mayfly cerci and thorax. A lack of difference in the numbers of ciliate colonies and chironomids was also noted on mayfly legs, where both commensals reached the intermediate abundances (Fig. 5).

Discussion
Influence of Environmental Parameters on Hosts and Commensals
Besides temperature, the main important factor controlling the distribution of riverine macrobenthic assemblages is flow regime. Both factors to a large degree regulate the availability of food (quality and quantity of food sources, including BPOM). Another important parameter is the granulometry of the mineral substrate (Minshall and Robinson 1998). Gravel and pebbles are usually colonized by a greater number of macroinvertebrates than smaller inorganic particles (sand) because the former habitat offers surfaces for attachment on which it is easy to forage or construct larval cases, and develops a biofilm (perilithon). It can also provide various refuges from predators enabling numerous macroinvertebrates to co-exist (Rabeni and Minshall 1977; Grzybkowska and Witczak 1990; Bournaud et al. 1998; Heino et al. 2004; Szczekowska-Majchrzak et al. 2010; Grzybkowska and Glowacki 2011). This pattern was observed in the Mroga River, where higher abundances of certain taxa (e.g., Gammaridae, Sphaeridae, and Trichoptera), were observed in comparison to the Bzura River exhibiting a more uniform bed. This pattern also refers to chironomid diversity (Grzybkowska 1995; Grzybkowska et al. 2012). Such trend was a consequence of higher flow variability in the Mroga River, with more events removing and/or redepositing substrate, then creating a gravel-pebble river bed.

Only one of the chironomid taxa living in the Bzura and Mroga Rivers, in the genus Epoicocladius, represents the epoictic mode (also termed commensalistic or symphoretic), living on mayflies (Ephemeroptera). This orthoclad species is widespread, frequently collected from nymphs of the genus Ephemerida in a large number of rivers located in European countries (Fittkau and Reiss 1978; Svensson 1986; Tokesi 1986, 1988; Soldán 1988), and in non-European countries (Winterbourn 2004; Callisto et al. 2006). These associations between orthoclads and mayfly nymphs were also found in Poland (Kłukowska 2002; Kurzawski et al. 2009), in similar reaches to those described by Soldán (1988): unpolluted or weekly polluted fluvial ecosystems up to ~5 m wide, with well aerated and relatively warm water. Note, that some of these authors extend this relationship to other chironomid species (genus Synorthocladius, Sahin and Arslan 1999).

The higher density of chironomid commensals detected in the Bzura River in comparison to the Mroga River seems to be the effect of a higher abundance of the host, which may promote the success of commensals. In addition, aquatic phoretic relationships were relatively common in the Bzura River, probably due in part to the prevalence of sandy lotic habitats, which favor this mode of life. This was in agreement with White et al. (1980) that found an increasing rate of chironomid phoresy on aquatic insects when the percentage of rocks and other coarse substrate types decreased. Despite this, we did not find an ontogenetic microhabitat shift of E. danica from pebbles (young larvae) to sandy areas (older individuals), as shown by Hanquet et al. (2004). In the investigated rivers, mayfly larvae were collected in a low hydraulic stress area, similarly to Møbes-Hansen and Waringer (1998).

Fig. 2. The frequency of occurrence of Epoicocladius ephemerae and Carchesium polypinum on the mayfly body.

Fig. 3. Attachment position of Epoicocladius ephemerae and Carchesium polypinum on the host body parts, ranging from the anterior to posterior of the mayfly body.
Two Commensals on Mayfly Hosts

Interactions between organisms play a key role in the functioning and evolution of the biosphere (Combes 2001). Dodds (1997) and Pennuto et al. (2002) suggested that amensalism and commensalism should dominate large-scale interaction webs, whereas hydrobiologists often focus on competition, predation and parasitism. In the literature, however, there are examples of multidirectional interactions and varying strengths of relationships between chironomids and other aquatic organisms (Roque et al. 2004). A well-recognized commensal association is that between the chironomid *Epoicocladius ephemerae* and the mayfly *E. danica*. Small chironomid larvae (Orthocladiinae) were found living on the gills, appendages and segments of nymphs of *E. danica*. The immature stages, larvae and pupae of this commensal species were described by Sulc and Zavrel (1924), including a description of an imago by Kieffer (after Henson 1957). After some redescriptions, this species is now known as *Epoicocladius ephemerae* (Kieffer 1924).

Mayflies also host ciliated protozoans. Empirical and mathematical models have shown that two species cannot coexist on the same limiting resource if they use it in the same way (Griffin and Silliman 2011). As illustrated by our data, the occurrence of the two commensal species was not random and showed a negative association. These species competed for both parts of the host abdomen, one with gills and the other without gills. Gill movements may disturb the attachment of protozoans to the host body, whereas they do not appear to affect chironomids. The relationship between these two commensal species may also lead to resource partitioning in another way: ciliated protozoans primarily colonized small larval mayflies and chironomids typically larger individuals. As suggested by Tokeshi (1988), apostome ciliates, epizoic on other macroinvertebrates (crustaceans), are sensitive to change in hormones or other chemical substances during growth or hardening and thickening of the cuticle of older hosts, which become unsuitable for protozoan colonization. Whether these responses occurred in the present study will require further analyses.

These co-existing commensal species did not compete for food resources because their diet did not overlap. *E. ephemerae* larvae are scarpers (owing to the special morphology of the labium), relying on feeding opportunities afforded by a constant supply of detritus or algae accumulating on the host's body. Chironomid larvae are able to attach to the host owing to strong bristles at the end of the abdomen and posterior prolegs, which have hooked bristles as well as spines. In contrast, protozoan ciliates filter bacteria suspended in the water column. Thus, the most effective parts of the host for their attachment are the head and abdomen, especially close to the anus (Tokeshi 1995; M. Grzybkowska unpublished data). We did not find anybody fragments of both ciliates and hosts in the few larval chironomid guts analyzed.

There are four factors that are believed to favor commensalism by chironomid species: (1) a constant supply of food accumulating on the mayfly host’s body, (2) an increased mobility of the commensal when in association with its host, (3) better protection from disturbance, especially in running waters, and (4) reducing the size

![Fig. 4. The abundance of *Epoicocladius ephemerae* and *Carchesium polypinum* as a function of host size.](image)

Table 1: Chi-squared analysis of the co-occurrence of two commensal species on mayfly body parts

| Commensal   | Carchesium polypinum | Epoicocladius ephemerae |
|-------------|----------------------|-------------------------|
| Mayfly      | Body area            | Head                    |
|             |                      | Thorax                  |
|             |                      | Legs                    |
|             |                      | Abdomen A               |
|             |                      | Abdomen B               |
|             |                      | Cerci                   |

|                | *P > 0.05*   | *P < 0.05* | **P < 0.01** |
|----------------|-------------|------------|--------------|
| Head           | 0.018       | 0.012      | 0.018        |
| Thorax         | 0.064       | 0.045      | 0.021        |
| Legs           | 0.193       | 0.081      | 0.094        |
| Abdomen A      | 0.125       | 0.066      | 0.133        |
| Abdomen B      | 0.166       | 0.107      | 0.137        |
| Cerci          | 0.075       | 0.049      | 0.076        |

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*Abbreviations: C20 = Carchesium polypinum, E20 = Epoicocladius ephemerae.*

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*(Table 1 continued on next page)*
spectrum of predators by adhering to larger hosts (Tokeshi 1986, 1988; Soldán 1988).

In addition to chironomid larval stages, eggs and pupae may also colonize the body of a host; e.g., Corydatus (Megaloptera) and Corynoneura (Chironomidae; Callisto et al. 2006) can occur under the gills and between the first and fourth abdominal segments. Predation by their host is probably limited because large megalopterans tend to prey on insects much larger than chironomids and thus the size of the megalopteran used as a host by chironomids seems to diminish the risk of becoming prey. However, exceptions do occur. There are costs and benefits associated with symbiose (Pennuto et al. 2006). In species that do not compete for food, competition for space may be particularly important for internal parasites (Jackson et al. 1988; Soldán 1988).

The 2 × 2 continential table analysis (\(\chi^2\) test) was used to test species independence and the values of the Yule Q index are presented to illustrate the type of association, i.e., avoidance (negative) or co-occurrence (positive).

- \(P > 0.05\),
- \(P < 0.05\),
- \(P < 0.01\).

Fig. 5. The abundance (average and ±95% C.L.) of Epoicocladius ephemerae and Carchesium polypinum on mayfly body parts. Letters above error bars denote significantly different groups as determined by post hoc pair-wise comparisons; values with the same letter did not differ significantly.

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