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SUBFOSSIL CHIRONOMID ASSEMBLAGES IN ALPINE LAKES OF THE PARÂNG AND THE FĂGĂRAŞ MOUNTAINS (ROMANIA, SOUTH CARPATHIANS)

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A PARENG (PARÂNG) ÉS A FOGARAS (FĂGĂRAŞ) MAGASHEGYI TAVAINAK SZUBFOSSZILIS ÁRVASZÚNYOG EGYÜTTESEI (ROMÁNIA, DÉLI-KÁRPÁTOK)

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ABSTRACT: The Parâng and the Făgăraş mountains are the highest mountain ranges of the South Carpathians. Many alpine lakes are located in the valleys among the hills. The non-biting midge (Diptera, Chironomidae) fauna of the lakes are poorly known and only sporadic information is available about the environmental parameters that influence their distribution. Our aim was to study the recent chironomid fauna of fifteen lakes (nine from Parâng and six from Făgăraş) and assess the environmental factors influencing the distribution of the assemblages. Water and surface sediment samples were collected in the summer of 2012 and 2013. The surface sediment samples were obtained from the deepest part of the lakes. Two cubic centimeters of sediment samples were processed to get the chironomid head capsules. The examined sediments contained 13 to 81 well-preserved head capsules. The most remains and taxa were found in lakes of the Parâng, tribe *Tanytarsini*, genus *Procladius* and *Psectrocladius sordidellus*-type were common in these lakes. Chironomid fauna of the Făgăraş was poor in remains, among them taxa of tribe *Tanytarsini* and genus *Pseudodiamesa* were specific for these lakes. According to the results of the multivariate statistical analysis (CCA, LDA), composition of the chironomid assemblages were different in the two mountains. Distribution of the chironomid assemblages was defined significantly by the maximum water depth and iron(III)-oxide concentration of the sediment of the lakes.

Key words: Chironomidae, subfossil remains, South Carpathians, surface sediment, CCA

KIVONAT: A Déli-Kárpátok legmagasabb hegységei a Parâng és a Făgăraş. Sok gleccsertó található a hegycsúcsok által körbezárt völgyekben. A terület árvaszúnyog (Diptera, Chironomidae) faunájáról és az együttesek összetételét meghatározó környezeti paraméterekről kevés ismerettel rendelkezünk. Jelen tanulmányban célunk volt 15 magashegyi tó (9 tó a Parâng- és 6 tó a Făgăraş-hegységekből) recens árvaszúnyog faunájának vizsgálata és az együttesek eloszlását meghatározó környezeti tényezők feltárása. A víz- és üledékminták vételére 2012 és 2013 nyarán került sor. A felszíni üledékmintákat a tavak legmélyebb pontjáról gyűjtöttük be. Két köbcentiméternyi üledékminták kerültek feldolgozásra, melyekből 13–81 árvaszúnyog fejkapszula került elő. A legtöbb maradvány és taxon a Parâng-hegység tavaiból került elő. A Făgăraş-hegységben található tavak maradványokban szegénynek bizonyultak, itt főleg a *Tanytarsini* tribusz és a *Pseudodiamesa* génusz képviselői fordultak elő. A statisztikai elemzések (CCA, LDA) alapján az árvaszúnyog-együttesek összetétele különbözött a két hegységben. A tavak vízmélysége és az üledék vas(III)-oxid tartalma volt szignifikáns hatással az árvaszúnyog-együttesek eloszlására.

Kulcsszavak: árvaszúnyog, szubfosszilis maradványok, Déli-Kárpátok, felszíni üledék, CCA

Introduction

Among macroinvertebrates, the larval remains of the family Chironomidae (Diptera) are most abundant in lake sediments because the chitinous head capsules are normally well preserved, and thus have proven to be especially useful in paleoenvironmental studies (WALKER 2001). Chironomid (non-biting midge) larvae can be found in a wide range of biotopes, and play an important role in life of lakes due to the large number of species and specimens (ARMITAGE et al. 1995).

The South Carpathians consist of three main mountain ranges: Retezat, Parâng and Făgăraş mountains. Although many paleoecological investigations were carried out in the area of Retezat (BRAUN et al. 2012, BUCZKÓ et al. 2012, 2013., KORPONAI et al. 2011, MAGYARI et al. 2012, 2013, TÓTH et al. 2012), midge fauna and environment of Parâng and Făgăraş mountains are poorly known. The Parâng and the Făgăraş mountains are the highest mountain ranges of the South Carpathians. Many alpine lakes are located in the valleys among the hills. Approaching these lakes is difficult, so this area is impacted by a negligible anthropogenic disturbance, although the sheep grazing is common near a few lakes. The intensive grazing may also cause the disappearance of some rare alpine species (BAUR et al. 2007).

Aim of our study was to explore the distribution of the recent chironomid fauna of fifteen lakes and assess the environmental factors influencing the distribution of the chironomid assemblages.

Materials and methods

Water and surface sediment samples were collected in the summer of 2012 and 2013 at 15 sites (Figure 1, Table 1) in the Parâng and the Făgăraş mountains. The surface sediment samples were obtained from the deepest part of the lakes using gravity corer. Surface samples were sliced off to get the undisturbed top layer of 0-2cm. The surface sediment samples (topmost 0-2 cm) represent the current fauna (e. g. BROOK and BIRKS 2004), so characterizing the recent conditions. Water samples were obtained from the lake surface. The samples were stored cool until processing

A wide range of chemical and physical variables were measured in the field (using by Hand-held Water Quality Meter WQC-24) and in the laboratory (Table 2).

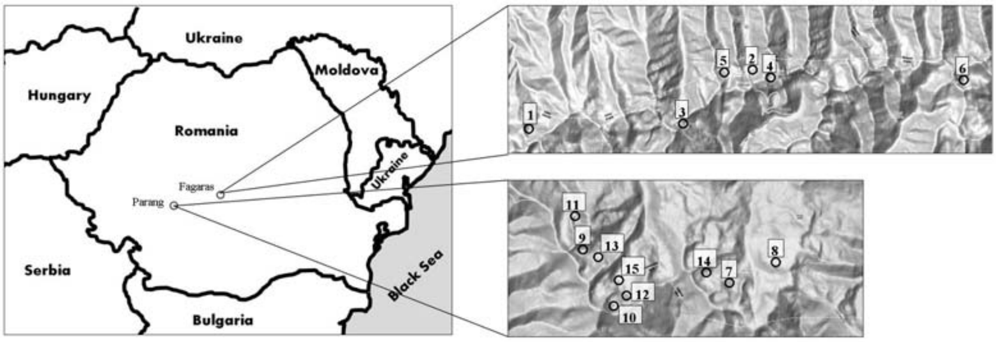

Figure 1. Sampling sites in the Parâng and the Făgăraş mountains (for codes see Table 1).

Table 1. Sampling sites in the Parâng and the Făgăraş mountains with the sampling dates, geo-coordinates and altitude.

Code	Sampling sites	Date	Latitude (N)	Longitude (E)	Altitude (m)
1	Avrig (Făgăraş)	07.08.12	45°34'42,92"	24°28'54,40"	2007
2	Bâlea (Făgăraş)	08.08.12	45°36'12,22"	24°36'59,94"	2038
3	Căltun (Făgăraş)	11.08.12	45°34'54,36"	24°34'21,84"	2135
4	Capra (Făgăraş)	10.08.12	45°36'02,76"	24°37'38,22"	2249
5	Doamnei (Făgăraş)	09.08.12	45°36'18,66"	24°36'00,72"	1890
6	Valea Rea (Făgăraş)	13.07.13	45°35'59,60"	24°44'37,60"	2160
7	Câlcescu (Parâng)	14.08.12	45°21'01,50"	23°36'44,04"	1934
8	Cărbunele (Parâng)	13.08.12	45°21'29,40"	23°38'18,60"	2054
9	Cârja (Parâng)	17.08.12	45°21'49,79"	23°31'51,13"	2129
10	Mândra (Parâng)	15.08.12	45°20'31,98"	23°32'51,12"	2140
11	Mija (Parâng)	16.08.12	45°22'32,28"	23°31'33,54"	1988
12	Rosiile (Parâng)	15.08.12	45°20'41,28"	23°33'15,84"	1978
13	Verde (Parâng)	18.08.12	45°21'36,54"	23°32'18,78"	2030
14	Zănoaga Mare (Parâng)	14.08.12	45°21'15,24"	23°35'55,38"	2018
15	Zănoaga Stanei (Parâng)	15.08.12	45°21'04,68"	23°33'01,26"	1909

Sediment samples of two cubic centimeters were processed to get the chironomid head capsules. The standard preparation technique (e.g. BROOKS 1997, WALKER 2001, 2006) begins with the deflocculating the sample in 10% potassium hydroxide (KOH) on a hot plate at 80 °C for 15–20 minutes. The deflocculated sample is then washed with destilled water on a 92 μm sieve to eliminate clay and other fine sediment components. Eventually the chironomid remains must be hand-picked from the sediment in a Bogorov counting tray (GANNON 1971) at 30× magnification. The picked head capsules should be dehydrated in absolute ethanol before preparating onto microscope slides by using Euparal®. Remains were identified using the keys by BROOKS et al. (2007) and WIEDERHOLM (1983). As only chironomid head capsules were examined, identification to genus and species-group level was possible.

The assemblage dataset was $\log_{10}(x+1)$ transformed before the analyses. The environmental variables were $\log_{10}(x)$ transformed exepting LOI%, which was arcsin(x/100) transformed. Detrended correspondence analysis (DCA) was used to explore patterns in distribution of the taxa within spatial dimension and to choose between linear- or unimodal-based methods in further numerical analyses by estimating the lenghts of compositional gradients (DCA axes 1 and 2). DCA is an indirect ordination method that summarizes the variation in species assemblages along the DCA axes (TER BRAAK and ŠMILAUER 2002). The lengths of compositional gradients were long: 4.151 standard deviation (SD) units for axes 1, 1.8–2.3 SD units for axes 2–4, so the unimodal-based method (CCA) was applied in further analyses (JONGMAN et al. 1995, LEPŠ and ŠMILAUER 2003). Canonical correspondence analysis (CCA) was used to explore the relationship between chironomid assemblages and environmental variables. CCA is a direct gradient procedure that can be used to identify environmental variables that are strongly related to the species assemblages (TER BRAAK 2003). Rare taxa were down-weighted in the analysis. The statistical significance of each variable was tested with a random Monte Carlo permutation test (499 unrestricted permutations), and environmental variables were considered significant if the permutation test value (p) was under 0.05. The statistical analysis (DCA, CCA) were performed using the program CANOCO 4.55 (TER BRAAK and ŠMILAUER 2002). Finally, linear discriminant analysis (LDA) was carried out to classify the lakes of the two mountain ranges. LDA is a method to find a linear combination of features characterizing or separating two or more classes, which provide the best discrimination between the groups (PODANI 1997). Two sample t-tests and ANOVA were carried out to examine significance of the separated groups. LDA, t-tests and ANOVA were performed using 'R 2.14.0' software (R DEVELOPMENT CORE TEAM 2011).

Table 2. Parameters measured in the field and laboratory.

Properties	Specific variables
Water chemistry	Major anions and cations: Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} CO_3^{2-}, HCO_3^- , total phosphorus and nitrogen (TP, TN), pH, alkalinity and conductivity
Water properties	Water transparency (Secchi depth), chlorophyll-a, dissolved oxygen (DO), water temperature
Physical properties	Water depth, longitude, latitude, altitude
Sedimentary properties	Loss on ignition (LOI), sediment chemistry

Results and discussion

Altogether 583 chironomid remains were identified from the sediment samples (Table 3) belonging to 30 taxa (3 Tanypodinae, 3 Diamesinae, 1 Prodiamesinae, 14 Orthocladiinae, 9 Chironominae). Numbers of head capsules in an unit sediment were very low in lakes of Făgăraş, with exception of Lake Doamnei. Tribe *Tanytarsini* and genus *Pseudodiamesa* were specific for these lakes (Table 3). The remarkable number of chironomids in Lake Doamnei could be due to altitude of the lake. All other lakes in Făgăraş are situated above 2000 m, while Lake Doamnei is located 1890 m above sea level. Inverse relationship was found between altitude and nutrient content (especially in cases of TP and TN) in the Swiss Alps (MÜLLER et al 1998), so higher chironomid abundance could be explained by more sources of nutrients. The most remains and taxa were found in lakes of the Parâng, tribe *Tanytarsini*, genus *Procladius* and *Psectrocladius sordidellus*-type were common in these lakes (Table 3). *Chironomus anthracinus*-type, a common taxon in eutrophic lakes (BROOKS et al. 2007), occurred in Lake Carbunele. The presence of this taxon in an alpine lake might be caused by the disturbing effect of sheep grazing.

According to forward selection of CCA analysis, two of the 25 environmental variables proved to be significant ($p < 0.05$) for the distribution of non-biting midge assemblages in the Parâng and the Făgăraş mountains (Figure 2). The iron(III)-oxide concentration of the sediment ($p = 0.002$) and the water depth ($p = 0.026$) of the lakes caused differences between lakes of the Parâng and the Făgăraş mountains. *Pseudodiamesa*, *Heleniella*, *Corynocera oliveri*-type, *Paratanytarsus austriacus*-type were common in lakes of the Făgăraş mountains. Remains of these taxa amounted to 47.6% of the total number of individuals (147) in the lakes of the mountains. On the contrary, frequent occurrence of *Procladius*, *Micropsectra insignilobus*-type and *Psectrocladius sordidellus*-type was typical in lakes of the Parâng mountains. These remains amounted to 39.0% of the total number (436) in the lakes of the Parâng mountains. Large number of occurrence of *Micropsectra radialis*-type and *Tanytarsus lugens*-type was characteristic for both mountains (42% of the total number of remains of the two mountains). *Heterotrissocladius grimshawi*-type, *Micropsectra insignilobus*-type and *Procladius* genus characterized the deeper lakes in the Southern Carpathians.

Taxonomic and environmental separations of the two mountains were confirmed by linear discriminant analysis (Figure 3). Two sample t-tests showed that significant differences occur between lakes of the mountains in case of chironomid assemblages ($df = 6.155$, $t = -11.598$, $p < 0.001$) and also in environmental variables dataset ($df = 8.141$, $t = -9.654$, $p < 0.001$). The group separations were tested by ANOVA, which resulted in the homogeneity of covariance matrices of chironomids and environmental variables.

Our results might be explained by mountains of Southern Carpathians structure: Făgăraş mountains consist of strongly metamorphosed rocks, crystalline slates, conglomerate, dolomite and limestone, while granite and migmatit is the basis of Parâng mountains (SĂNDULESCU and DIMITRESCU 2004). Type of the bedrock can influence water and sediment chemistry variables, which can affect the composition of chironomid assemblages (LENCIONI and ROSSARO 2005, LENCIONI et al. 2011, SZIVÁK et al. 2013).

Depth of the lakes is an important factor, having influence on the distribution and abundance of chironomid taxa. Moreover, most chironomid species show preference for water depth (e. g. HEIRI 2004, LUOTO 2012a, 2012b). The non-biting midge assemblages of South Carpathians consisted of mostly cold stenothermic taxa often occurring in high densities (e.g. *Tanytarsus lugens*-type, *Micropsectra insignilobus*-type, *M. radialis*-type) (Brooks et al. 2007). This alpine community is very sensible to temperature change, thus the global warming will be able to lead to extinction of these cold stenotherm taxa (OERTLI et al. 2008).

In summary, differences could be detected in distribution of non-biting midge assemblages and environment factors between lakes of the two highest mountain of Southern Carpathians. The assemblages of the lakes were separated according to their iron(III)-oxid content of sediment and maximum water depth of the lakes.

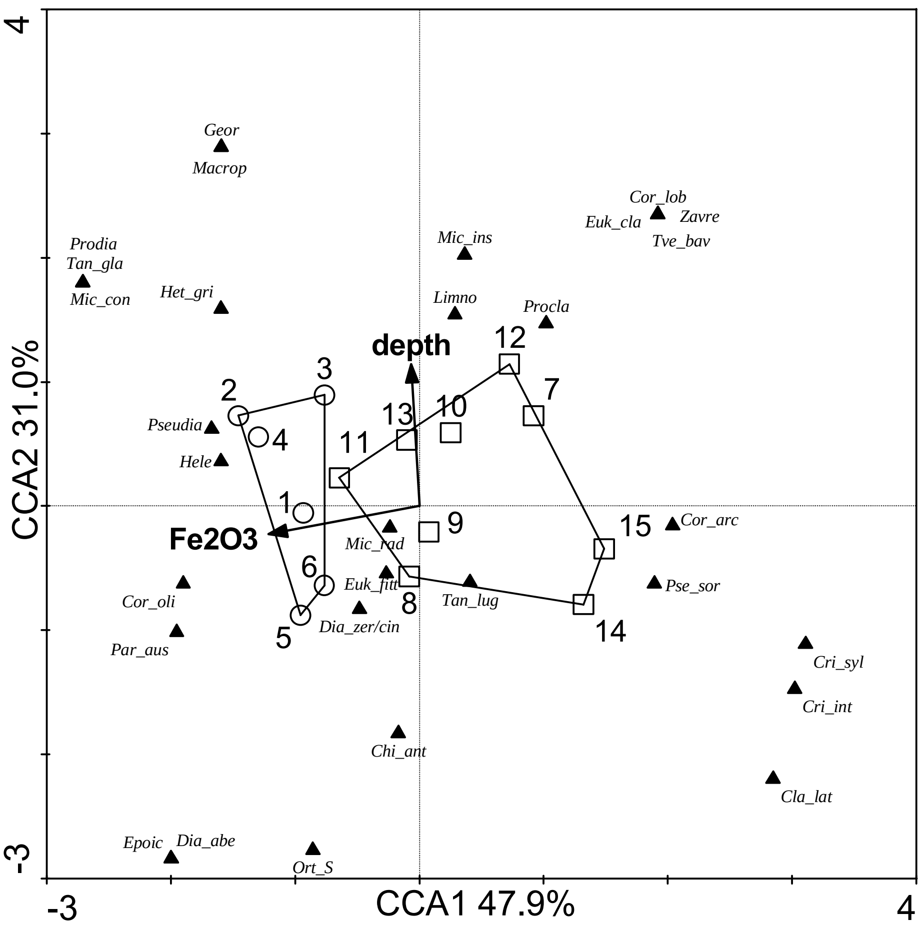

Figure 2. Canonical correspondence analysis (CCA) of chironomid taxa, sampling sites and environmental parameters in the Parâng and the Făgăraş mountains. Different symbols indicate the sampling sites and the taxa: ○ – sampling sites of the Făgăraş mountains, □ – sampling sites of the Parâng mountains, ▲ – taxa. Environmental parameter codes: Fe2O3 – iron(III)-oxide concentration, depth – maximum water depth of the lake. For sampling sites codes see Table 1, for taxa codes see Table 3.

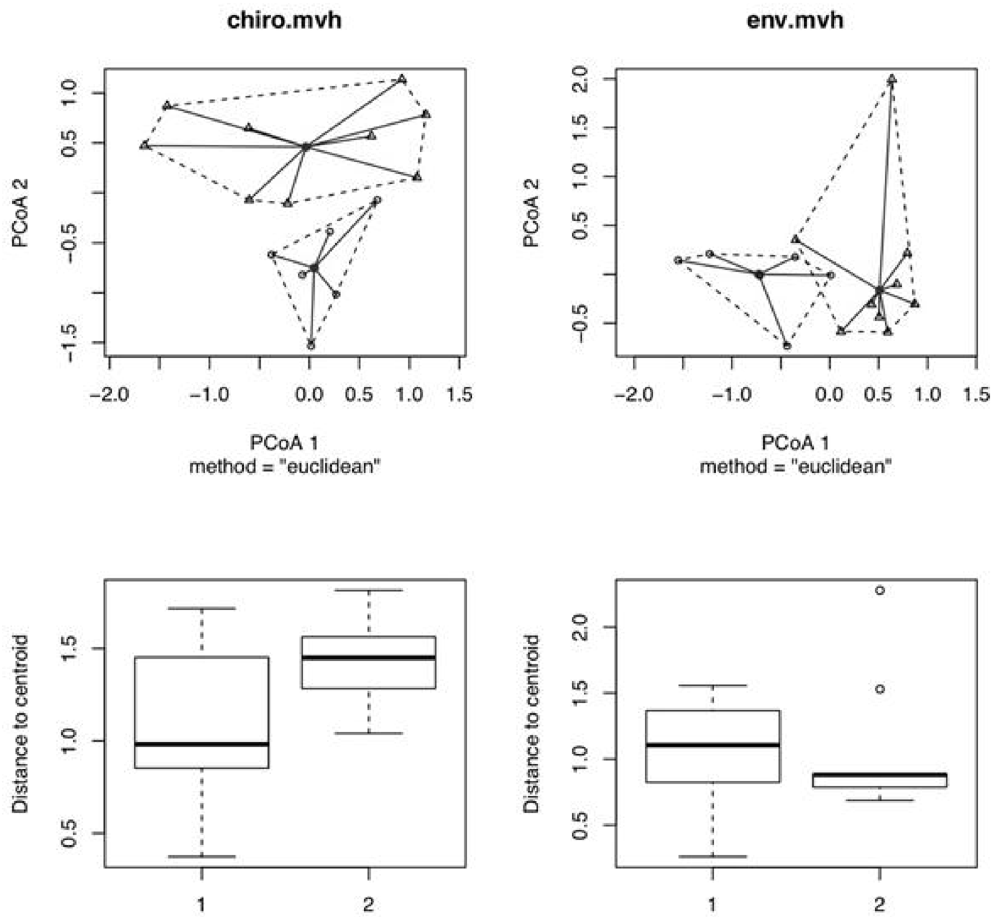

Figure 3. Linear discriminant analysis (LDA) of the chironomid assemblages (chiro.mvh) and the environmental variables (env.mvh). Boxplots illustrate distances to centroids based on Welch two sample t-tests. Different symbols indicate the sampling sites and centroids: ○ – sampling sites of the Făgăraş mountains, ∆ - sampling sites of the Parâng mountains, ● – centroids.

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Table 3. List of Chironomidae taxa found in the Parâng and the Făgăraş mountains, with the codes of taxa and number of remains (for the codes of sampling sites see Table 1).

Taxa	**Code**	**Sampling sites**															∑
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
Tanypodinae																	
Macropelopia	Macrop			1													**1**
Procladius	Procla							3	1		2	6	15			4	**31**
Zavrelimyia	Zavre							2									**2**
Diamesinae																	
Diamesa aberrata-type	Dia_abe					2											**2**
Diamesa zernyi/cinerella-type	Dia_zer/cin					2		1									**3**
Pseudodiamesa	Pseudia	6	1	1	2	3								7			**20**
Prodiamesinae																	
Prodiamesa	Prodia				1												**1**
Orthocladiinae																	
Corynoneura arctica-type	Cor_arc							1			2				3	1	**7**
Corynoneura lobata-type	Cor_lob							1									**1**
Cricotopus intersectus-type	Cri_int														1	7	**8**
Cricotopus sylvestris-type	Cri_syl															2	**2**
Epoicocladius	Epoic					2											**2**
Eukiefferiella claripennis-type	Euk_cla							3									**3**
Eukiefferiella fittkaui-type	Euk_fitt					3		2									**5**
Georthocladius	Geor			1													**1**

Table 2. (continued)

Taxa	**Code**	**Sampling sites**															**∑**
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	
Heleniella	Hele	4										9					**13**
Heterotrissocladius grimshawi-type	Het_gri			6			1										**7**
Limnophyes	Limno							1				1					**2**
Orthocladius type *S*	Ort_S					8									1		**9**
Psectrocladius sordidellus-type	Pse_sor						3	3	1				3		17	32	**59**
Tvetenia bavarica-type	Tve_bav							1									**1**
Chironominae																	
Chironomus anthracinus-type	Chi_ant								4								**4**
Cladopelma lateralis-type	Cla_lat														7	1	**8**
Corynocera oliveri-type	Cor_oli	3	5		1	7	7			2							**25**
Micropsectra contracta-type	Mic_con				3												**3**
Micropsectra insignilobus-type	Mic_ins			1				2		2	37	5	26	11			**84**
Micropsectra radialis-type	Mic_rad	1	2		1	19			17	11	10	1	6		2	1	**71**
Paratanytarsus austriacus-type	Par_aus	8	4			13	5		1								**31**
Tanytarsus glabrescens-type	Tan_gla				3												**3**
Tanytarsus lugens-type	Tan_lug	2	5	3	2		5	21	24	66	25			2	19		**174**
∑		**24**	**17**	**13**	**13**	**59**	**21**	**41**	**48**	**81**	**76**	**22**	**50**	**20**	**50**	**48**	**583**
Number of taxa		**6**	**5**	**6**	**7**	**9**	**5**	**12**	**6**	**4**	**5**	**5**	**4**	**3**	**7**	**7**	**30**