

Synthesising the trait information of European Chironomidae (Insecta: Diptera): Towards a new database



Sónia R.Q. Serra^{a,*}, Fernando Cobo^{b,c}, Manuel A.S. Graça^a, Sylvain Dolédec^d, Maria João Feio^a

^a MARE – Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Largo Marquês de Pombal, 3004-517 Coimbra, Portugal

^b Department of Zoology and Physical Anthropology, Faculty of Biology, University of Santiago de Compostela, 15782 Santiago de Compostela A Coruña, Spain

^c Estación de Hidrobiología 'Encoro do con', Castroagudín, 36617 Vilagarcía de Arousa, Pontevedra, Spain

^d University Lyon 1, UMR 5023 LEHNA, Biodiversité des Ecosystèmes Lotiques, Bât Forel, 69622 Villeurbanne Cedex, France

ARTICLE INFO

Article history:

Received 30 April 2015

Received in revised form 28 July 2015

Accepted 17 September 2015

Available online 6 November 2015

Keywords:

Chironomidae

Fuzzy coding

Bioassessment

Eltonian traits

Grinnelian traits

ABSTRACT

Chironomidae are among the most conspicuous and ecological diverse group of freshwater invertebrates. They may dominate unimpacted communities in abundance and biomass, accounting for more than 50% of macroinvertebrate species in standing and flowing waters. In deep zones of eutrophic lakes and highly human-impacted streams, they are often the only family of aquatic insects remaining. In bioassessment programmes, Chironomids are often identified at the family and subfamily levels, due to difficulties in the taxonomic identification of larvae resulting from a high intrinsic morphological similarity. This may potentially result in bias as, similarly to Ephemeroptera, Trichoptera or Plecoptera, *Chironomidae* species, which are replaced along natural and human-impacted gradients due to differences in their ecological requirements. Recently, multiple trait-based approaches have been proposed to complement taxonomic-based assessment of streams and rivers using macroinvertebrates. However, the lack of specific trait information for Chironomidae prevents their use in the functional assessment of communities. Therefore, here, we aimed to: (1) develop a trait database for European Chironomidae genera that can be used in future bioassessment and ecological studies; (2) evaluate, by multivariate analyses, whether our new database provides additional information on Chironomidae compared to the trait information provided in the commonly used European trait database (Tachet et al., 2010); and (3) determine whether the new information on Eltonian traits (proxy to biological traits) translates the most accepted phylogenetic relationships among Chironomidae subfamilies. We gathered information on 744 species and 178 genera, for 37 traits covering 186 trait categories, and found substantial differences between our database and the commonly used European trait database. In addition, available information on traits was not always in agreement with phylogenetic relationships among subfamilies. Orthocladiinae and Chironominae which are considered sister groups in evolutionary terms actually showed confident trait relatedness based on Eltonian traits tree while the remaining relationships between subfamilies are questionable. In addition, different traits can occur in closely related taxa depending on the environmental drivers operating on their habitats. Our study reveals that the usually accepted redundancy within the Chironomidae family and subfamilies must be a product of averaging the information from finer taxonomic resolution added to the substantial lack of information for this insect group.

© 2015 Elsevier Ltd. All rights reserved.

* Corresponding author. Tel.: +351 916635553.

E-mail addresses: sonia.rqs@gmail.com (S.R.Q. Serra), fernando.cobo@usc.es (F. Cobo), mgraca@ci.uc.pt (M.A.S. Graça), sylvain.doledec@univ-lyon1.fr (S. Dolédec), mjf@ci.uc.pt (M.J. Feio).

1. Introduction

Chironomidae is the most widely distributed dipteran family; its larvae have colonised terrestrial habitats, as well as marine habitats, and fresh waters. The family can tolerate a wide range of environmental conditions, and some taxa can be found in extreme environments including ice-cold glacial trickles, hot springs, and rather unusual environments such as sub-desert steppes, aquatic hygropetric habitats and leaf axis of plants or rot-hole of trees (Armitage et al., 1995; Cobo and Blasco-Zumeta, 2001; Vallenduuk and Pillot, 2007; Pillot, 2009, 2013).

Chironomidae richness worldwide is estimated at 20,000 species, but the lack of adequate description and identification difficulties at finer taxonomic resolution such as genus or species suggest that this number is underestimated (Armitage et al., 1995; Coffman and Ferrington, 1996).

In fresh waters, the Chironomidae family can account for ~50% of the macroinvertebrate community (Armitage et al., 1995; Coffman and Ferrington, 1996), it is particularly abundant in reservoirs, lakes and in lowland rivers and urban streams, and may be the only insect remaining in highly human-impacted water bodies (Coffman and Ferrington, 1996; Raunio et al., 2011; Andersen et al., 2013). Chironomidae play a key role in organic matter processing by consuming fine particles of organic matter and transferring energy and nutrients to upper trophic levels since they represent prey for an array of organisms, including other invertebrates, fish and birds. They thus have a great influence over productivity and population dynamics of top consumers. Finally, Chironomidae assemblages change along the river continuum similarly to EPT taxa (Ephemeroptera, Plecoptera and Trichoptera) (e.g. Prat et al., 1983; Cobo and González, 1990, 1991; Lindegaard and Brodersen, 1995; Puntí et al., 2009) and according to the lake typology (Saether, 1979; Brodersen and Lindegaard, 1999; Mousavi, 2002).

Historically, Chironomidae family played an important role in lake and running water classification based on its trophic level and saprobity, which reflected the production and decomposition of organic material (Saether, 1979). Fossil chironomid assemblages also provided insights on past environmental conditions (Walker, 2001; Brooks, 2006) whereas abnormalities in body parts, mostly mouthpart deformities, have been used as indicator of contaminant effects in both water and sediments (Rosenberg, 1992). Therefore, the bioassessment potential of Chironomidae is great, being of particular importance in environments where other invertebrate groups are not present. This family includes taxa tolerant to different water salinity, pH, depth, temperature, organic carbon, nutrients and oxygen concentration (e.g. Laville and Vinçon, 1991; Schmidt et al., 2010; Servia et al., 2004) among other environmental variables. Some Chironomidae occur in good quality waters (e.g. *Rheopelopia* spp., *Conchapelopia pallidula*, *Orthocladus thienemanni* and *Zavrelimyia melanura*; Vallenduuk and Pillot, 2007; Marziali et al., 2010; Pillot, 2013), whereas others are rather tolerant to high organic contamination and high trophic degrees (e.g. *Chironomus riparius*, *Rheocricotopus fuscipes* and *Rheocricotopus chalybeatus*; Brodersen and Quinlan, 2006; Marziali et al., 2010; Prat et al., 2013) or low levels of dissolved oxygen (e.g. *Procladius* sp. and *Eukiefferiella claripennis*; Bazzanti and Seminara, 1987; Marziali et al., 2010). Despite the wide range of responses to the environmental gradients, the bioassessment of running waters generally use a coarse taxonomic resolution for depicting Chironomidae assemblages (Rosenberg, 1992; Coffman, 1995; Hawkins and Norris, 2000) because of the difficulties associated with the morphological identification of larvae beyond family and subfamily.

Besides the usual taxonomy-based approaches, trait-based approaches are being increasingly used as an alternative to assess stream biological integrity (Dolédéc and Stutzner, 2010). Traits may help to reveal the cause of impairment and give an indirect insight

into which ecosystem functions may be affected by human disturbance (Archambault et al., 2005; Culp et al., 2011; Feio and Dolédéc, 2012). Since traits are indicators of function, community trait composition allows a better understanding of stream functioning (Vieira et al., 2006). However, few researchers have attempted to quantify trait information for Chironomidae, with some of them achieving a trait database at the subfamily and tribe levels (see Tachet et al., 2010 for Europe and Poff et al., 2006 for North America). Few works gathered information at the species or genus level considering a reduced number of traits and/or taxa (see Franquet, 1996 for France; and Vieira et al., 2006 for North America).

Here, we had three objectives. First, we aimed to categorise the European Chironomidae genus characteristics into a set of 21 traits and 110 categories used in Tachet et al. (2010) for all aquatic macroinvertebrates and a set of 16 additional traits specific to Chironomidae. Secondly, we investigated the distribution and variability of trait patterns within Chironomidae subfamilies, using the new trait database. Given the great variability reflected in trait heterogeneity within each Chironomidae subfamily, we expected that trait information gathered at higher or lower level of taxonomic resolution would determine differences in traits patterns gathered within each subfamily. To determine whether our database was actually providing additional assessment information, we contrasted trait patterns given by our database at the genus level with that obtained at the subfamily-level in the trait database of Tachet et al. (2010), which is commonly used in bioassessment studies. Finally, assuming that heritable traits (Eltonian) of organisms could disclose evolutionary processes operating among taxa, Chironomidae subfamily traits relatedness was expected to reflect their phylogenetic distances across subfamilies. Therefore, we compared the subfamily Eltonian trait relatedness with the most accepted Chironomidae phylogeny found in literature (Saether, 2000; Cranston et al., 2010, 2012).

2. Methods

2.1. European freshwater Chironomidae traits

We defined an a priori list of European species and genera. Due to a lack of consensus among European Chironomidae fauna taxa lists and guides (e.g. Illies, 1978; Andersen et al., 2013; Soriano et al., 1997; Cobo et al., 2001; Vallenduuk and Pillot, 2007; Pillot, 2009, 2013) we followed the Fauna Europaea database (<http://www.faunaeur.org/see>, Saether and Spies, 2013) with 194 and 1261 genera and species entries distributed among eight subfamilies: Buchonomyiinae, Chironominae, Diamesinae, Orthoclaadiinae, Podonomyiinae, Prodiamesinae, Tanypodinae, and Telmatogetoninae.

Our database includes the most widespread European Chironomidae species, covering a wide geographic area, different categories of water bodies at different altitudes and latitudes. Lotic and lentic freshwater systems were given equal importance, being mentioned in at least 20% and 19% of total references used, respectively. References covering temporary freshwater systems and hygropetric habitats were also included. Brackish habitat references were also used to support the trait salinity preferences. Other references used did not focus on a specific type of aquatic habitat but addressed ecological, physiological, morphological and/or life history characteristics of specific taxa. Whenever possible, the references for which species were first described in Europe were exploited. Information gathered from publications between 1931 and 2013 (ca. 150), including articles, books and a few PhD theses, were used to describe the species traits.

The initial list was composed of 21 traits and 110 categories of biological, physiological traits and ecological requirements, as used

in Tachet et al. (2010); some traits and categories were adapted given the type of information available for Chironomidae (Table 1). A set of 16 additional traits specific to Chironomidae larvae included respiration (number of tracheas), tube construction, number of eggs per egg mass, flight period, duration of emergence, distance of aquatic and/or aerial dispersion, hibernation stages, length of larval development, oxygen saturation preferences, presence/absence of haemoglobin, migration type, depth preferences, optimal temperature interval for emergence, chlorinity, and general/gross habitat (Table 1). Traits that differed among Chironomidae life stages were gathered for the fourth larval instar (except for number of eggs per egg mass, flight period, and others) and categorised into Grinnellian or Eltonian traits according to the terminology of Devictor et al. (2010) and Mondy and Usseglio-Polatera (2014). Grinnellian traits are related to taxon requirements and performance over a range of environmental conditions considering biotic and/or abiotic resources (e.g. pH, temperature, and food preferences), whereas Eltonian traits focus on the impact of the species on its environment, emphasising their functional role in the ecosystem rather than their response to particular resources (e.g. body size, voltinism, feeding habits).

Following Franquet (1996), the affinity of species or genera to trait category was quantified using the number of references citing this category for a given taxon. The higher the number of references associating a taxon to a trait category, the greater the affinity of that taxon to that particular trait category. Taxa with no available information on a trait were scored 'zero' for all categories, and were treated as missing values, being replaced by the mean of all taxa having information for a given trait category. Trait-affinity scores were further treated as frequency distributions and standardised to sum 1 for a given taxon-trait combination, to give the same weight to each taxon and to each trait in further analyses. This procedure is known as fuzzy coding (Chevenet et al., 1994).

Total number of genera described per trait was estimated to define the best described traits, i.e., with information gathered for more than 50% of the European genera. The genus trait database is provided as Supplementary data with the list of the references used to extract trait information and the list of species used to describe each genus.

2.2. Comparison between the two databases

To determine whether our trait database built at the species and genus levels involved different distributions of taxa (subfamily, tribes) compared to the database of Tachet et al. (2010), we used Fuzzy Correspondence Analysis (FCA) that enables the joint ordination of taxa and trait categories (Chevenet et al., 1994). FCA uses a matrix ($n \times p$) to interpret the relationships between trait categories (p) and resemblances among individual taxa (n). The affinity profile of each trait category among taxa enables the positioning of each trait category at the weighted average of taxa that uses this category. The variance of these positions corresponds to a correlation ratio (i.e. the highest the correlation ratio the highest the separation of taxa across trait categories) and FCA maximises the average correlation ratio across traits when FCA was performed separately on Grinnellian and Eltonian traits. For comparison with the European trait database of Tachet et al. (2010) (hereafter TDB – Tachet DataBase), the fuzzy information of our database at the genus level (hereafter GDB – Genus DataBase) was averaged at the subfamily and tribe levels. Afterwards, these average affinity scores were rescaled so that their sum, for each of these coarser taxonomic groups for a given trait, equals one. Thereby, traits were described at the same scale for all different taxonomic levels of resolution. While the biological information in Tachet et al. (2010) describes only the Podonominae, Tanypodinae, and Orthocladiinae subfamilies and the Chironomini and Tanytarsini tribes, our database included

Table 1

Traits and their categories and codes used in the European Chironomidae database. Eltonian and Grinnellian traits are ordered with (1) traits coded by the first author of this paper, (2) traits adapted from the European database of Tachet et al. (2010) and (3) traits similar to those in Tachet et al. (2010).

Traits	Categories	Code
Eltonian		
Emergence sea-son	Winter	EMWINT
	Spring	EMSPRI
	Summer	EMSUMM
	Autumn	EMAUTU
Flight period ^a	Winter	FLYWINT
	Spring	FLYSPRI
	Summer	FLYSUMM
	Autumn	FLYAUTU
Emergence duration	Short period (some hours to few days; <15 d.)	EDSHORT
	Long period (several days; >15 d.)	EDWIDE
Number of eggs per egg mass	<100	EGGMAS1
	100–500	EGGMAS2
	500–1000	EGGMAS3
	> 1000	EGGMAS4
Length of larval development (months)	≤1	DEVLARV1
	2	DEVLARV2
	3	DEVLARV3
	4	DEVLARV4
	5	DEVLARV5
	6	DEVLARV6
	7	DEVLARV7
	8	DEVLARV8
	≥9	DEVLARV9
Hibernation phase/instar (overwinter diapause)	Egg	HIBEGG
	1st instar	HIBINST1
	2nd instar	HIBINST2
	3rd instar	HIBINST3
	4th instar	HIBINST4
Distance travelled in aquatic habitat (m)	<10	DISAQU1
	10–100	DISAQU2
	100–1000	DISAQU3
	>1000	DISAQU4
Distance travelled in aerial habitat (m)	<10	DISAER1
	10–100	DISAER2
	100–1000	DISAER3
	>1000	DISAER4
Tube construction	Tube absent	TUBNON
	Tube without shape, unorganised	TUBUNO
	Tube rigid	TUBRIG
Haemoglobin	Present	HBPRES
	Absent	HBNONE
Eltonian adapted from Tachet et al. (2010)		
Respiration (#tracheas)	12 tracheas	TRACH1
	6 tracheas	TRACH2
	3 tracheas	TRACH3
Substrate relation	Free living	FREELV
	Burrower	BURROW
	Miner	MINER
	Fixed (substrate or plants)	FIXED
Potential number of generations per year/Voltinism ^a	1	GENY1
	2	GENY2
	3	GENY3
	>3	GENYM
Resistance forms/habits	Eggs, gemmule, statoblast, shell	RFEGG
	Cocoons	RFCCO
	Resistant stages to desiccation	RFSTA
	Diapause or quiescence	RFDIAP
	None	RFNON
	Deeper penetration in substrate during dryness	RFSUB

Table 1 (Continued)

Traits	Categories	Code
Eltonian taken from Tachet et al. (2010)		
Dispersal	Passive aquatic	AQUPAS
	Active aquatic	AQUACT
	Passive aerial	AERPAS
	Active aerial	AERACT
Feeding habits	Fine sediment eater	DEFEE
	Shredder	SHR
	Scraper, grazer	SCR
	Filter	FFEEDT
	Predator (piercer, cutting or swallowing)	PRED
	Parasite	PARAS
Life cycle duration	≤1 year	LCEQ1
	>1 year	LCMO1
Maximal body size of the 4th instar ^a (mm)	<2.5	SIZE1
	>2.5–5	SIZE2
	>5–10	SIZE3
	>10–20	SIZE4
	>20–40	SIZE5
Reproduction type	Free isolated eggs	FREEGG
	Attached isolated eggs	CEMEGG
	Clutches (cemented or attached)	CEMCLU
	Free clutches	FRECLU
	Endophytic clutches	CLUVEG
	Terrestrial clutches	CLUTER
Type of aquatic stages ^a	Asexual reproduction	ASEXU
	Egg	EGG
	Larva	LARVA
	Pupa	PUPA
	Adult (imago)	IMAGO
Grinnellian		
Chlorinity (g Cl ⁻¹)	<0.3	CHLOR1
	>0.3–1	CHLOR2
	>1–3	CHLOR3
	>3–10	CHLOR4
	>10	CHLOR5
Oxygen saturation preferences ^a	Stable always > 50%	OXSTAB
	Unstable 10–50%	OXUNST
	<5% for few hours	OXLOW
	Rotting summer daily <5%	OXROTT
Depth preferences ^a	Profundal habitat	DPSHALL
	Indifferent and/or medium depth	DPINDIF
	Shallow habitat littoral/sublittoral	DPSHALL
General/gross Habitat ^{a,b}	Lotic	LOTIC
	Lentic	LENTIC
	Creeks, brooks	BROOKS
	Small streams	SSTRM
	Large rivers	LSTRM
	Semi-terrestrial	SEMTER
	Terrestrial	TERRES
Optimal temperature of emergence (°C)	≤6	6OPTEM
	>7–9	7OPTEM9
	>10–12	10OPTEM12
	>13–15	13OPTEM15
	≥16	OPTEM16
Type of migration	Horizontal	MIGHOR
	Vertical	MIGVER
Grinnellian adapted from Tachet et al. (2010)		
Food type ^a	Fine sediment + microorganisms	SEDMIC
	Debris < 1 mm	DEBR1
	Plant debris > 1 mm	DEBR2
	Living microphytes	MICPHY
	Living macrophytes	MACPHY
	Dead animals	DEADAN
	Living microinvertebrates	MICINV
	Living macroinvertebrates	MACINV
Living vertebrates	VERTEB	
Wood	WOOD	

Table 1 (Continued)

Traits	Categories	Code
Temperature preferences	Bacteria	BACTER
	Psychrophilic <15 °C	TPSYCH
	Thermophilic >15 °C	TTHERM
	Eurythermic	TEURYT
pH preferences ^a	Hemistenothermic	THEMIS
	<4	4PH
	>4–5	4PH5
	>5–6	5PH6
	>6–7	6PH7
	>7–8	7PH8
	≥8	PHM8
	Substrate preferences ^a	Stone, boulder, cobble, pebble
Gravel		GRAVEL
Sand		SAND
Silt		SILT
Macrophytes and filamentous algae		MAPFAL
Microphytes		MIPHYT
Twigs, roots		BRANCH
Litter, finer organic matter		LITTER
Mud		ORGMUD
Invertebrates		MINVER
Wood microhabitat		WOODM
Mosses		MOSSES
Trophic status preferences	Oligotrophic	OLIGTR
	Mesotrophic	MESOTR
	Eutrophic	EUTR
	Hypertrophic (Mesohumic)	HYPTR1
	Hypertrophic (Polyhumic)	HYPTR2
Longitudinal distribution along stream channel ^a	Crenon	CRENO
	Epirhithron	EPIRIT
	Metarhithron	METRIT
	Hyporhithron	HYPRIT
	Epipotamon	EPIPOT
	Metapotamon	METPOT
	Estuary	ESTUAR
	Outside river system	OUTFLU
	Kryon (glacial feed habitats)	KRYON
	River channel	CHANNEL
Transversal distribution along stream channel ^a	Banks, connected side-arms	BANKSD
	Ponds, pools, disconnected side-arms	POOLPN
	Marshes, peat-bog	MARSHB
	Temporary waters	TEMPOR
	Lakes	LAKES
	Groundwaters	UNDERG
	Hygropetric	HYGROP
	Artificial water medium (Impoundment reservoirs, ditch, canal, pipeline, sewage filter bed),	ARTIF
	Water surface	WATSUR
	Bottom	BOTTOM
Grinnellian taken from Tachet et al. (2010)		
Saprobity	Xenosaprobic	XENOSAP
	Oligosaprobic	OLIGSAP
	β-Mesosaprobic	BMESSAP
	α-Mesosaprobic	AMESSAP
	Polysaprobic	POLYSAP
Salinity preferences ^a	Fresh water	FRESHW
	Brackish water	BRACKI
Altitudinal preferences ^a (m)	<1000 (lowlands)	ALT1
	>1000–2000 (piedmont)	ALT2
	>2000 (Alpine)	ALT3
Current velocity preferences ^a (cm s ⁻¹)	None	VELO1
	<25	VELO2
	>25–50	VELO3
	>50	VELO4

^a Traits that were described for more than 50% of European genera.

^b Refers to the preferential habitat: lentic or lotic, small or large running water bodies, or semi-terrestrial habitats.

additional tribes: Pseudochironomini (Chironominae), Diamesinae, Telmatogetoninae, Buchonomyiinae, and Prodiamesinae.

Finally, to assess the variability in community trait composition explained by the difference between GDB and TDB, we computed between-class variance (with class as type of database; see Dolédec and Chessel, 1987; ter Braak, 1988) and tested its significance against simulated values obtained after 999 permutations of the rows of the trait-composition arrays.

2.3. Chironomidae subfamily trait relatedness

FAC was performed on Eltonian traits of genera averaged at the subfamily level. The resulting FCA coordinates of the 8 subfamilies along the 7 axes ($n - 1$; in which n is the smallest rank of the trait matrix; here, the number of subfamilies) was used to yield the Euclidean distance matrix among subfamilies. Finally, neighbour-joining (Saitou and Nei, 1987; Studier and Keppler, 1988) allowed estimated a tree among subfamilies. Bootstrap procedure was used to assess tree's accuracy and the 'confidence' of each tree bipartition (Efron et al., 1996). This representation was visually compared with the most accepted evolutionary relationships of Chironomidae subfamilies derived from cladistics analysis (Saether, 2000) and molecular analysis (Cranston et al., 2010, 2012).

Statistics and graphical outputs were computed with the ade4 (Thioulouse et al., 1997; Chessel et al., 2004; Dray et al., 2007) and ape libraries (Paradis et al., 2004; Paradis, 2012) implemented in R freeware (R Development Core Team, 2013).

3. Results

3.1. European freshwater Chironomidae trait database

Our final list contained 178 Chironomidae genera and 744 species distributed among 8 subfamilies. Biological information on species and genera was found in the literature for ~59% of the most widespread European species, and 92% of the European genera for 37 traits (Table 2 and see in the information supplied as Supplementary data). From all of the gathered trait information, 11 Grinnellian and 4 Eltonian traits had information for more than 50% of the European Chironomidae genera present in the database (indicated in Table 1). The best described Grinnellian traits were: transversal distribution in streams and general/gross habitat preferences (>90% of genera present in the database). Food types, pH tolerance, salinity preferences, longitudinal distribution along streams, altitude, substrate preferences, current velocity preferences, oxygen and depth preferences were described for 53–74% of the genera. The best described Eltonian traits (for more than 95% of genera) were: maximal size of the fourth larval instar and type of aquatic stages. Potential number of generations per year (voltinism) and flight period were described for 50–53% of genera.

Chironomidae subfamilies with less information were the Buchonomyiinae, Podonominae and Telmatogetoninae. Tanypodinae, Orthocladiinae and Chironominae subfamilies had also

genera with less information such as *Meropelopia* sp. (Tanypodinae), *Lappodiamesa* sp. (Diamesinae), *Stackelbergina* sp. (Orthocladiinae), *Gillotia* sp. and *Neostempellina* sp. (Chironominae). Prodiamesinae were relatively well characterised; only *Monodiamesa* sp. had less information than the other 2 genera included in the subfamily (*Prodiamesa* and *Odontomesa*). A total of 16 European genera of the Diamesinae, Orthocladiinae and Chironominae subfamilies lacked trait information resulting in the absence of entries in the database: *Arctodiamesa*, *Pagastia*, *Arctosmittia*, *Bavarismittia*, *Boreosmittia*, *Corynoneurella*, *Lappokoefferiella*, *Mollerella*, *Neobrillia*, *Prosmittia*, *Tavastia*, *Baeotendipes*, *Carbochironomus*, *Nilomyia*, *Olecryptotendipes*, *Synendotendipes*.

The distribution of trait categories varied greatly across subfamilies for some traits (food types, salinity preferences, longitudinal distribution, Fig. 1a–c), whereas for other traits, all Chironomidae behaved in a very similar way (e.g. altitudinal preferences, pH tolerance, number of generation per year, Fig. 1d–f). Tanypodinae, Orthocladiinae and Chironominae that contained the highest diversity of genera described (ca. 89% of all Chironomidae) covered wide ecological amplitude. Other less diversified subfamilies were associated with specific environments: Buchonomyiinae (*Buchonomyia thienemanni*) were recorded at low altitudes and in lotic habitats, whereas Diamesinae had a higher affinity for upper reaches (e.g. kryon, reaches fed by ice-melt) with higher current velocities and water temperature <15 °C. Telmatogetoninae were well represented in brackish and marine habitats but there is a substantial lack of information about their species traits. Prodiamesinae were generally recorded in sites with a heavy load of organic pollution. Podonominae were mostly represented in ponds and pools, temporary habitats, and marshes and bogs.

On average, most Chironomidae subfamilies have intermediate sizes ranging from 5 to 20 mm. Only Tanypodinae (e.g. *Anatopynia plumipes*) and Chironominae (e.g. *Axarus fungorum*, *Chironomus* spp., *Glyptotendipes* spp.) larvae can achieve a body length of 20 mm. The Buchonomyiinae subfamily (*Buchonomyia thienemanni*) presents 1 generation per year, whereas Prodiamesinae are characterised by at least 2 generations per year, and Chironominae have higher affinities for more than 3 generations per year (Fig. 1).

Projecting the genus trait information against the taxonomic tree showed a great variety of traits within each Chironomidae subfamily, exemplified by two subfamilies and two traits in Fig. 2. For example, Chironominae (Chrn in Fig. 2) and Diamesinae (Dmsn in Fig. 2) had genera with affinities for contrasted trait categories. For example within Chironominae, *Axarus* sp. and *Chironomus* sp. had high affinities for the large size categories (SIZE 4 and 5, Fig. 2) whereas *Kloosia* sp. and *Lauterborniella* sp. had high affinities to small size categories (SIZE 2; Fig. 2). Similarly, for food type, within the Chironominae subfamily *Demeijerea* sp. and *Demicryptochironomus* sp. had high affinities for animal food (MICINV and MACINV; Fig. 2) whereas *Paratendipes* sp. and *Pagastiella* sp. had high affinities for plant debris (DEBR1 and 2; Fig. 2) and live microphytes (MICPHY; Fig. 2). Within the Diamesinae family, despite its lower

Table 2

Genera and species diversity and percentage of genera and species described in the literature used to develop the Chironomidae European trait database.

	Genus diversity	Genus described	Genus described (%)	Species diversity	Species described	Species described (%)
Buchonomyiinae	1	1	100	1	1	100
Chironominae	64	59	92	474	300	64
Diamesinae	11	9	82	69	47	68
Orthocladiinae	78	69	88	588	304	52
Podonominae	5	5	100	9	3	33
Prodiamesinae	3	3	100	10	7	70
Tanypodinae	30	30	100	106	81	76
Telmatogetoninae	2	2	100	4	1	25
Total	194	178		1261	744	

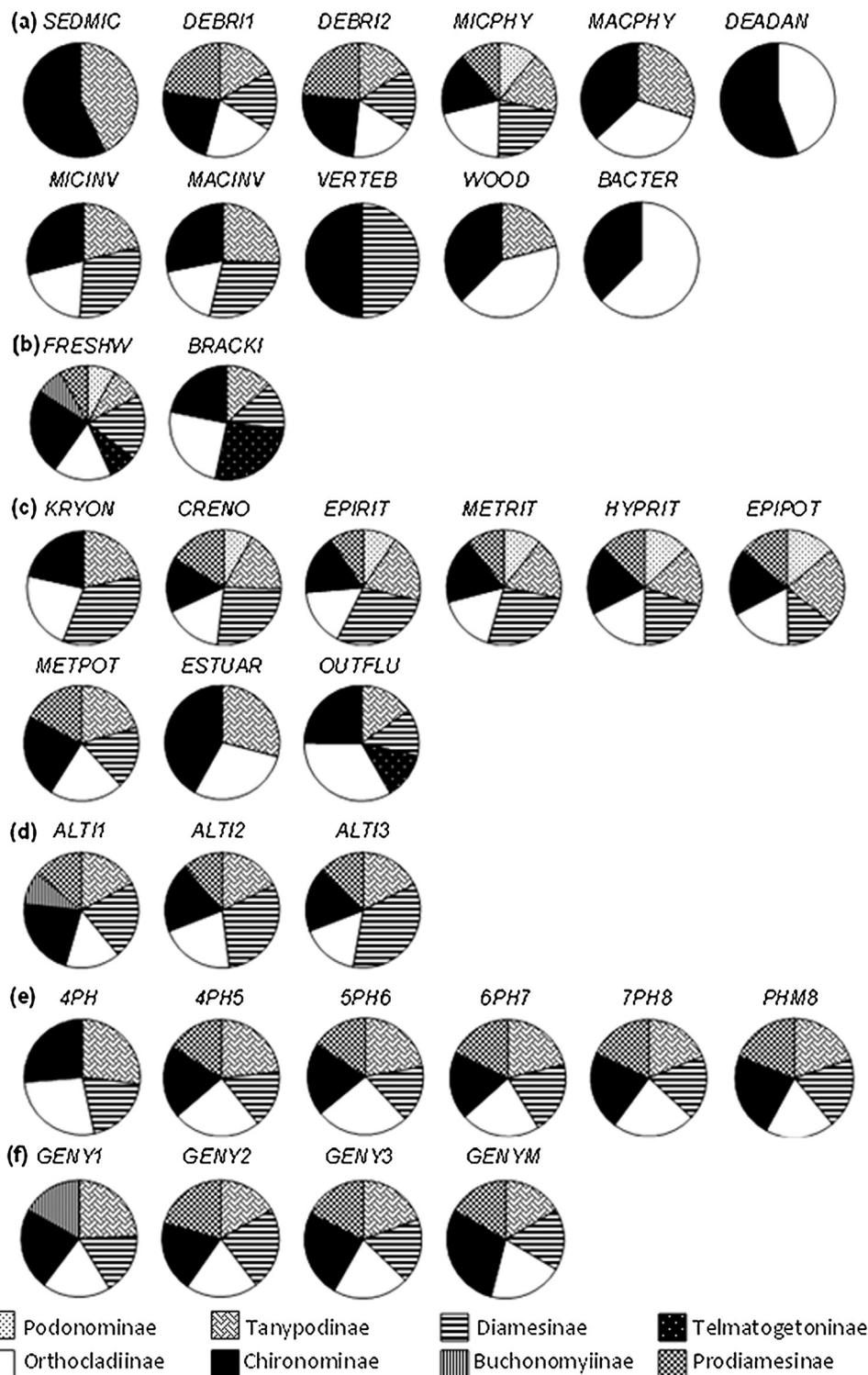


Fig. 1. Proportions of subfamilies for selected traits and respective categories (see Table 1 for acronyms) with (a) food type, (b) salinity preferences, (c) longitudinal distribution, (d) altitudinal preferences (e) pH tolerance, and (f) number of generations per year.

species richness in comparison to other subfamilies, affinities could also vary within the same trait. For instance, the *Diamesa* sp. larvae showed affinities from small to large size categories (SIZE 2 to SIZE 4; Fig. 2), whereas *Protanypus* sp. showed larger sizes (SIZE 4; Fig. 2). Considering food types, *Diamesa* sp. generally consume living microphytes such as diatoms (MICPHY; Fig. 2) whereas *Potthastia* sp. feed on detrital particles (DEBRI1 and 2; Fig. 2).

Diamesinae subfamily also includes genera with a wider spectrum of food preferences (*Protanypus* sp., *Boreoheptagyia* sp.). Similarly, Chironominae subfamily includes opportunistic genera able to feed on almost any food item (e.g. *Chironomus* sp. and *Glyptotendipes* sp.) and genera that live in woody microhabitats and introduce wood in their diets, being true wood miners with the ability to digest wood fibres (e.g. *Stenochironomus* sp.; WOOD).

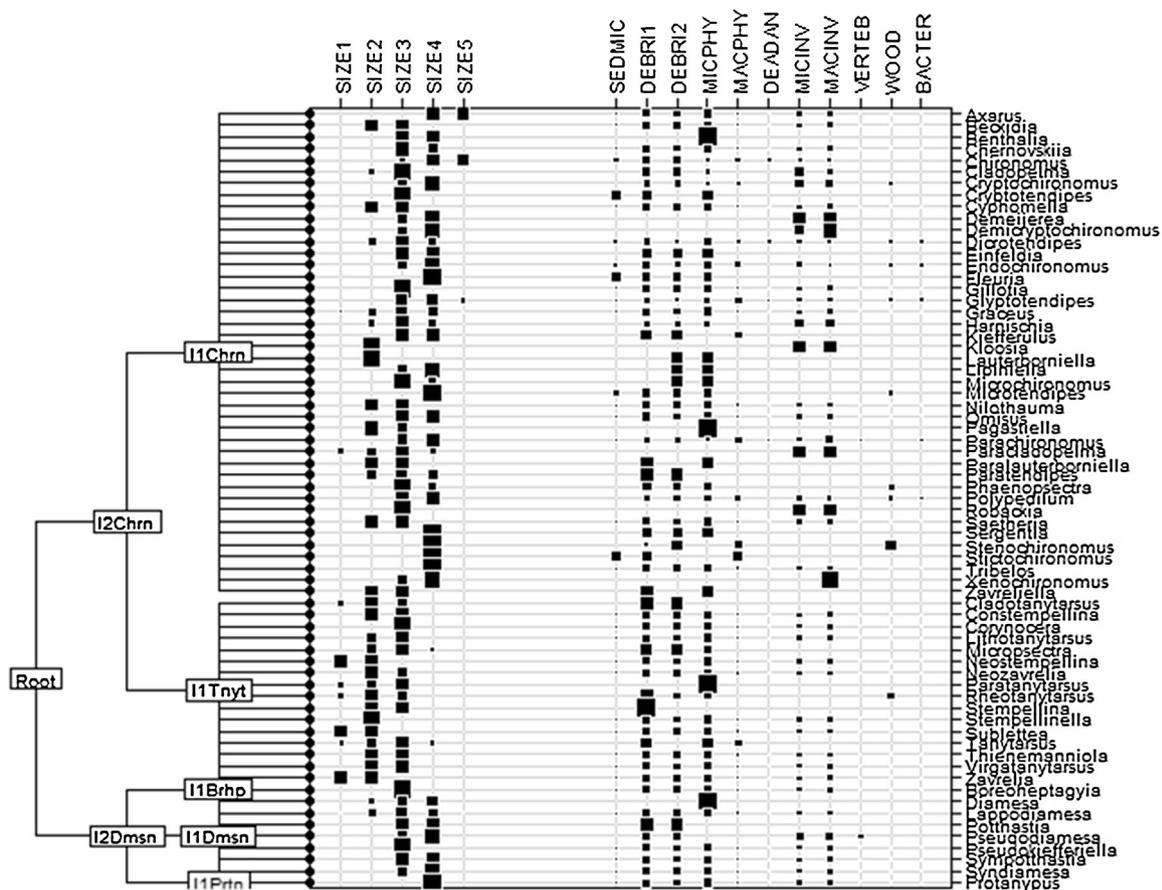


Fig. 2. Representation of two traits (maximal body size of the 4th larval instar and food type) and their categories (see Table 1 for acronyms) for two subfamilies (Chironominae and Diamesinae). The information is presented against a taxonomic tree with two taxonomic levels: I1 (tribe) and I2 (subfamily). Chironominae (I2 Chrn) are represented in the top leaf of the cluster by two tribes (Chironomini, I1 Chrn; and Tanytarsini, I1Tnyt). Diamesinae are represented in the below leaf by three tribes (Boreoheptagyini, I1 Brhp; Diamesini I1 Dmsn; and Protanypodini, I1 Prtn). The size of each square is proportional to the frequency of the corresponding trait category (on top) for a given genus (at right).

3.2. Comparison between the two databases

FCA performed on Grinnellian traits showed low but significant differences between the two databases (15% of variance explained; simulated *p*-value = 0.001; Fig. 3a, Table 3). Transversal distribution along the stream channel, pH, and to a lesser extent food type, were more important contributors for the difference among databases. Substrate preferences and transversal distribution each explained more than 30% of variance over first axis (32% and 40% respectively),

Table 3
Correlation ratios of Grinnellian traits for Chironomidae subfamilies/tribes (from two databases, the new developed in this work and existing in Tachet et al., 2010) on the first-two axes of the fuzzy correspondence analysis and respective eigenvalues.

Variables (Grinnellian traits)	Axis	
	F1	F2
Temperature	0.079	0.131
pH	0.050	0.112
Trophic degree	0.009	0.068
Saprobity	0.091	0.014
Salinity	0.043	0.030
Altitude	0.134	0.007
Longitudinal distribution	0.183	0.052
Transversal distribution	0.398	0.271
Substrate preferences	0.319	0.081
Current velocity	0.059	0.050
Food type	0.135	0.086
Eigenvalues	0.137	0.082

whereas altitude preferences, food type and longitudinal distribution explained 13%, 14% and 18% of the variance, respectively. Transversal distribution also had a high contribution to explain distribution over the second axis (explaining 27% of the variance), whereas temperature and pH preferences were also relevant (13% and 11% variance explained, respectively).

FCA performed on Eltonian traits likewise revealed significant differences between databases (32.7%, simulated *p*-value = 0.002; Fig. 3b, Table 4) to which reproduction type, resistance form and,

Table 4
Correlation ratios of Eltonian traits for Chironomidae subfamilies/tribes (from two databases, the newly developed in this work and the existing one from Tachet et al., 2010), on the first-two axes of the fuzzy correspondence analysis and respective eigenvalues.

Variables (Eltonian traits)	Axis	
	F1	F2
Size (4th instar larva)	0.043	0.066
Life cycle duration	0.334	0.035
Voltinism	0.164	0.428
Aquatic stage	0.019	0.043
Reproduction	0.756	0.084
Dispersal	0.066	0.024
Resistance	0.792	0.017
Feeding habits	0.139	0.099
Respiration	0.004	0.000
Substrate relation	0.158	0.102
Eigenvalues	0.248	0.090

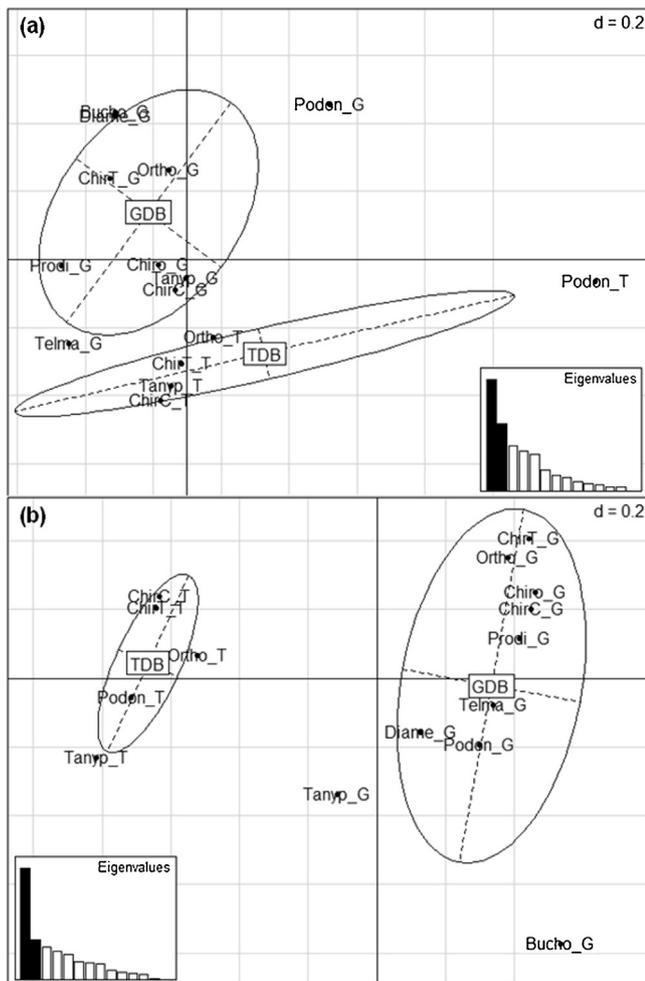


Fig. 3. Fuzzy correspondence analysis (FCA) plot performed on (a) Grinnellian traits and (b) Eltonian traits of the two databases, considering the information existing at the subfamily level and few tribe levels for Chironominae. The information is grouped by database with GDB, the European database developed using the genus information compiled at the subfamilies and tribe levels; and TDB, the database from Tachet et al. (2010) at the subfamily/tribe level. Subfamily or subfamily/tribe level is identified by their 5 first letters (Bucho – Buchonomyiinae; Chiro – Chironominae; ChirC – Chironomini; Diame – Diamesinae; Ortho – Orthoclaadiinae; Podon – Podonominae; Prodi – Prodiamesinae; Tanyp – Tanytopodinae; ChirT – Tanytarsini; Telma – Telmatogetoninae); followed by a .G for the GDB plot, or a .T for the TDB plot. Ellipses include 80% of the points for readability.

to a lesser extent, life cycle duration had the highest contributions. Resistance forms and reproduction type explained 79% and 76% of the variance, respectively, considering the first axis, followed by life cycle duration, which explained 33% of the variation. The variance along axis 2 was explained by voltinism (43% variance explained) and to a lesser extent by the substrate relation, explaining 10% of the variance.

3.3. Chironomidae subfamily trait relatedness

The neighbour-joining analysis performed on the 20 Eltonian traits revealed the subfamilial trait similarity among Orthoclaadiinae and Chironominae on one hand, and Podonominae with Tanytopodinae on the other hand (Fig. 4). The analysis also showed trait similarity between Diamesinae and Prodiamesinae with Tanytopodinae and Podonominae segregating them from Orthoclaadiinae and Chironominae.

The accuracy of the tree assessed through the bootstrap analysis give confidence to the group formed by Orthoclaadiinae and Chironominae, with 100% of trees showing the same combination. All other nodes and bipartitions do not reveal a strong confidence, with confidence values below 0.44 revealing no trait relatedness signal.

4. Discussion

Most studies in which Chironomidae were used at higher taxonomic resolution than subfamily or tribe are historically associated to lakes, either considering subfossil Chironomidae assemblages for paleolimnological studies or the analysis of communities in deeper zones (Raunio et al., 2011). In running waters, the extensive use of Chironomidae in bioassessment is still a matter of debate. Some authors have suggested that assessments may be more efficient by eliminating Chironomidae from the protocols and by using resources for analysing additional sites (Hawkins and Norris, 2000; Rabeni and Wang, 2001). Some authors fully agreed with the family-level and its ability to detect impairment (Móra et al., 2008), while others have even strongly recommended the use of a finer level of taxonomic resolution for Chironomidae, showing that family level yielded much weaker assemblage–environment relationships, which emphasised the risk of reducing accuracy in bioassessment (King and Richardson, 2002).

Here, we defend the hypothesis that Chironomidae could be appropriate indicators of environmental conditions, as the same taxonomic group includes tolerant (e.g. *Chironomus*) and sensitive (e.g. *Diamesa* spp.) taxa to human impacts (Armitage et al., 1995; King and Richardson, 2002; Lencioni et al., 2012). One main problem for bioassessment purposes arises from the difficulties

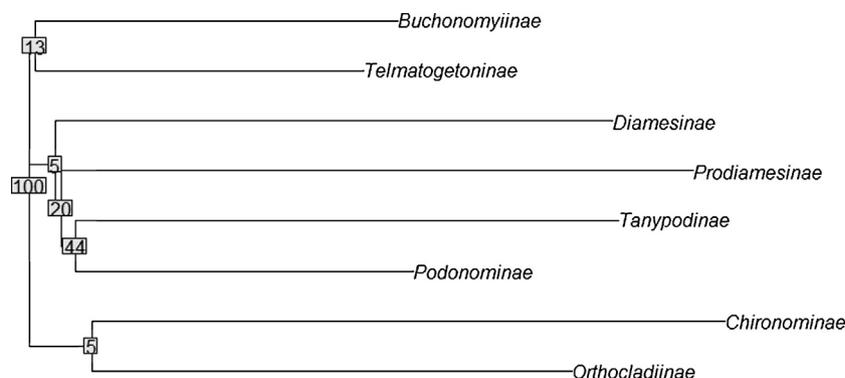


Fig. 4. Trait relatedness tree estimated among Chironomidae subfamilies using neighbour-joining, given the Euclidean distances of their FCA coordinates. Values associated to each node represent the percentage of partitions present in bootstrap trees.

of taxonomic identification and the poor knowledge on traits at the genus or species level, contrary to other groups of freshwater invertebrates (e.g. Poff et al., 2006; Tachet et al., 2010). Aiming to fill this gap, information on 37 Chironomidae traits and 184 trait categories was compiled in this study. The number of species and genera covered by our database (59% and 92% respectively) highlights the great effort that is still needed to understand the behaviour, physiology and ecological tolerances of Chironomidae species. Considering the Eltonian traits only, the development of the database clearly showed the poor information available in the literature as only 4 of this biological type of traits were characterised for more than 50% of the European genera. Our database thus identifies the genera and species to which more attention should be given in future studies due to reduced available information. One of the reasons for the reduced and uneven information on Chironomidae traits is the fact that morphological and physiological studies typically focus on *Chironomus* species (e.g. *C. tentans* and *C. riparius*), because they are easy to keep in the laboratory and use in routine ecotoxicological tests (Ankley et al., 1994; Armitage et al., 1995; Penttinen and Holopainen, 1995).

A total of 16 European genera belonging to Diamesinae, Orthocladiinae and Chironominae subfamilies lacked information (Supplementary data). The inability to characterise these genera may be due to their limited distribution so far (e.g. *Mollerella*, *Neobrillia*) and their small number of species in Europe (e.g. *Baeotendipes noctivagus*, *Nilomyia aculeata*) or by their relatively recent or very recent discover (e.g. *Olecryptotendipes* Zorina 2007; *Arctosmittia* Zelentsov 2006). One advantage of our database is that any additional information available on references not used in the original dataset can be simply added to the information provided in Supplementary data.

Compared to pre-existing information (i.e., from Tachet et al., 2010), the data that we compiled at the genus level resulted in significant differences in the separation of Chironomidae subfamilies. This suggests that differences in specialisation among Chironomidae occur primarily at higher levels of taxonomic resolution (genus and species). Even at the genus level, generalisation should be carefully considered since environmental requirements, life history traits, and sensitivity to anthropogenic pressure may vary considerably within a genus (Rossaro et al., 2006; Lencioni et al., 2007). A high number of species per family in many aquatic environments usually suggests an extensive adaptive radiation by diversification of ancestral species into several ecologically different species by adaptive morphological, physiological and/or behavioural divergence in those environments limiting the utility of the family level

in bioassessment (Hawkins and Norris, 2000; King and Richardson, 2002).

It is common to find autoecological information at the subfamily level mentioning faunistic patterns along environmental gradients (e.g. longitudinal, altitudinal) such as the greater abundance of Diamesinae and Orthocladiinae upstream, giving place to Tanypodinae and Chironominae downstream (Prat et al., 1983; Bitušik et al., 2006; Lencioni et al., 2007). Averaging trait affinities of Chironomidae subfamilies showed that they were distinct from each other considering some traits (e.g. maximal body size, food type); although the great trait diversity within each subfamily suggests that the subfamily level operating in the database of Tachet et al. (2010) is not appropriate. The latter database points out the ecological redundancy in the Chironomidae family and subfamilies, which may be simply due to the averaging operation, that masks the real trait diversity of Chironomids. Such false redundancy was highlighted by others (e.g. Lenat and Resh, 2001) and may compromise the results of studies that attempt to recognise Chironomidae faunistic patterns using a lower taxonomic level.

The differences between the subfamily/tribe trait patterns gathered at low and high levels of taxonomic resolution (TDB and GDB respectively) were clear for pH tolerance, transversal distribution in the river channel, reproduction types, resistance forms, and, to a lesser extent, food types and life-cycle duration. Food type is often considered a key factor in the distribution of Chironomidae species along with temperature. Additionally, flow regime and pH also have indirect influence on their distribution by regulating food availability, quantity and quality (Lencioni et al., 2007; Vallenduuk and Pillot, 2007). Consequently, differences in these traits can compromise multiple trait-based assessments.

A given set of traits in the organisms of the same species result from the process of evolution and adaptation to specific environmental conditions. Thus, it is generally accepted that there is a link between taxa phylogenetic relatedness and the traits they possess (Kraft et al., 2007). Usseglio-Polatera et al. (2000) recognised that traits related to morphology, physiology and life history (Eltonian traits) appeared to be more constrained by phylogeny than traits related to behaviour and habitat preferences (Grinnellian traits). Therefore, we expected that the most recently diverged subfamilies would tend to share more Eltonian trait categories among their taxa than subfamilies that diverged a long time ago from the Chironomidae common ancestor. The tree estimated by neighbour-joining revealed a small trait distance between Chironominae and Orthocladiinae subfamilies, which is in agreement with cladistics (Saether, 2000; Fig. 5a) and molecular phylogeny studies (Cranston

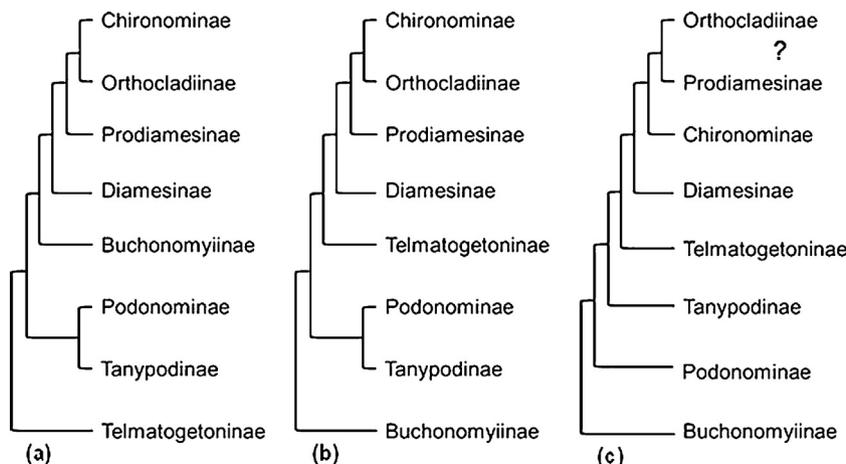


Fig. 5. Subfamily relationships among Chironomidae subfamilies given by: (a) cladistic analyses using parsimony of morphological characters of adults, pupae and larvae (Saether, 2000) and (b) and (c) molecular phylogenies by Cranston et al. (2010, 2012), respectively.

et al., 2010, 2012; Fig. 5b and c). In fact, Orthocladiinae retain some ancestral traits (e.g. respiration type given by the number of tracheas), which slightly differentiates the two subfamilies, whereas the presence of other much more recent traits (e.g. presence of haemoglobin) bring together the two subfamilies.

The fact that Eltonian trait patterns do not necessarily reflect phylogenetic relationships of the subfamilies means that environmental drivers are operating differently in species leading to higher functional diversity, exposing the labile nature of traits. Several authors argued that more closely related taxa may not be ecologically similar since the same ecological function can evolve through different pathways depending on the environmental drivers operating in the habitats, often called trait lability through evolutionary time (Webb et al., 2002; Poff et al., 2006; Cavender-Bares et al., 2009). According to Poff et al. (2006), multiple trait-based approaches should precisely take advantage of the selection of traits relatively unconstrained by phylogeny (i.e. more evolutionary labile), with low statistical and phylogenetic correlations, and more responsive to local selection, such as voltinism, which tell more about the drivers and environmental filters that operate in the systems than about the history of the taxa.

5. Conclusions

Among lacustrine macroinvertebrates, Chironomidae have been well studied and pointed as a powerful paleo-environmental indicator when using preserved subfossil assemblages collected from lake sediments. The value of Chironomidae as an indicator is not only associated to its wide distribution or community composition, but also to potential morphological responses to changes in environmental conditions such as exposure to contaminants. Despite its demonstrated importance and ecological role, in many freshwater studies (e.g. springs, streams, littoral of lakes) Chironomidae are still disregarded or neglected with their identification kept at family/subfamily levels. This has been limiting a more extensive use of Chironomidae in biomonitoring and the knowledge about autecology of taxa therein. Our study shows that Chironomids are indeed a quite diverse group with different ecological requirements and characteristics, and if used at the genus or species level, they have the potential to improve the signals provided by ecological assessment tools, either in taxonomic-based structural assessments or in indirect functional assessments using multiple-traits-based approaches. To further prove these insights, tests comparing both types of assessments based on sub-family level and genus/species level are needed. Our database, which is the first comprehensive European database for Chironomidae traits at the genus level to the best of our knowledge, can be used for that purpose, as well as in ecological studies on functional patterns of freshwater systems, especially those including habitats that are traditionally considered less diverse.

Acknowledgments

This work was made possible by funding of the Portuguese Foundation for Science and Technology (FCT) through a PhD scholarship (SFRH/BD/80188/2011), the cotutelage between the University of Coimbra and the University of Lyon 1, and the cooperation between the MARE, University of Coimbra, Portugal, and the LEHNA – Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés, University of Lyon, France. The research partly benefited from the European Community's Seventh Framework Programme, Grant Agreement No. 603629-ENV-2013-6.2.1-Globaqua.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2015.09.028>.

References

- Andersen, T., Cranston, P.S., Epler, J.H. (Eds.), 2013. *Chironomidae of the Holarctic Region. Keys and diagnoses – Larvae*. Insect Systematics and Evolution. Lund, Sweden, Supplement 66, pp. 1–571 (formerly Entomologica Scandinavica Supplement).
- Ankley, G.T., Benoit, D.A., Balogh, J.C., Reynoldson, T.B., Day, K.E., Hoke, R.A., 1994. Evaluation of potential confounding factors in sediment toxicity tests with three freshwater benthic invertebrate. *Environ. Toxicol. Chem.* 13, 627–635. <http://dx.doi.org/10.1002/etc.5620130412>.
- Archambault, V., Usseglio-Polatera, P., Bossche, J., 2005. Functional differences among benthic macroinvertebrates communities in reference streams of same order in a given biogeographic area. *Hydrobiologia* 551, 171–182. <http://dx.doi.org/10.1007/s10750-005-4459-9>.
- Armitage, P., Cranston, P.S., Pinder, L.C.V. (Eds.), 1995. *The Chironomidae. The Biology and Ecology of Non-biting Midges*. Chapman & Hall, London, UK.
- Bazzanti, M., Seminara, M., 1987. Profundal macrobenthos structure as a measure of long-term environmental stress in a polluted lake. *Water Air Soil Pollut.* 33, 435–442.
- Bitušik, P., Svitok, M., Dragúňová, M., 2006. The actual longitudinal zonation of the River Hron (Slovakia) based on chironomid assemblages (Diptera, Chironomidae). *Acta Univ. Carol. Biol.* 50, 5–17.
- Brodersen, K.P., Lindegaard, C., 1999. Classification, assessment and trophic reconstruction of Danish lakes using chironomids. *Freshw. Biol.* 42, 143–157.
- Brodersen, K.P., Quinlan, R., 2006. Midges as palaeoindicators of lake productivity, eutrophication and hypolimnetic oxygen. *Quat. Sci. Rev.* 25, 1995–2000.
- Brooks, S.J., 2006. Fossil midges (Diptera: Chironomidae) as palaeoclimatic indicators for the Eurasian region. *Quat. Sci. Rev.* 25, 1894–1910.
- Cavender-Bares, J., Kozak, K.H., Fine, P.V.A., Kembel, S.W., 2009. The merging of community ecology and phylogenetic biology. *Ecol. Lett.* 12, 693–715. <http://dx.doi.org/10.1111/j.1461-0248.2009.01314.x>.
- Chessel, D., Dufour, A.B., Thioulouse, J., 2004. The ade4 Package—I: One-table Methods. *R News* 4, 5–10.
- Chevenet, F., Dolédec, S., Chessel, D., 1994. A fuzzy coding approach for the analysis of long-term ecological data. *Freshw. Biol.* 31, 295–309. <http://dx.doi.org/10.1111/j.1365-2427.1994.tb01742.x>.
- Cobo, F., Blasco-Zumeta, J., 2001. Quironómidos (Diptera: Chironomidae) de la estepa subdesértica de los Monegros (Zaragoza, España). *Zapateri* 9, 43–47.
- Cobo, F., González, M.A., 1990. Las comunidades de Quironómidos (Diptera: Chironomidae) del río Ulla (NW de España). *Limnetica* 6, 109–118.
- Cobo, F., González, M.A., 1991. Étude de la dérive des exuvies nymphales de Chironomidés dans la rivière Sar (NO. Espagne). *Spixiana. J. Zool.* 14, 193–203.
- Cobo, F., Soriano, O., González, M.A., 2001. Inventario de los Quironómidos (Diptera: Chironomidae) de Portugal. *Nova Acta Scientifica Compostelana (NACC. Biologia)* 11, 225–248.
- Coffman, W.P., 1995. Conclusions. In: Armitage, P.D., Cranston, P.S., L.C.V., Pinder, L.C.V. (Eds.), *The Chironomidae: the Biology and Ecology of Non-biting Midges*. Chapman & Hall, London, UK, pp. 436–447.
- Coffman, W.P., Ferrington Jr., L.C., 1996. Chironomidae. In: Merritt, R.W., Cummins, K.W. (Eds.), *An Introduction to the Aquatic Insects of North America*, third edition. Kendall/Hunt Publishing Company, Dubuque, pp. 635–643.
- Cranston, P.S., Hardy, N.B., Morse, G.E., 2012. A dated molecular phylogeny for the Chironomidae (Diptera). *Syst. Entomol.* 37, 172–188. <http://dx.doi.org/10.1111/j.1365-3113.2011.00603.x>.
- Cranston, P.S., Hardy, N.B., Morse, G.E., Puslednik, L., McCluen, S.R., 2010. When molecules and morphology concur: the 'Gondwanan' midges (Diptera: Chironomidae). *Syst. Entomol.* 35, 636–648.
- Culp, J.M., Armanini, D.G., Dunbar, M.J., Orlofske, J.M., Poff, N.L., Pollard, A.I., Yates, A.G., Hose, G.C., 2011. Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. *Integr. Environ. Assess. Manag.* 7, 187–197. <http://dx.doi.org/10.1002/ieam.128>.
- Devictor, V., Clavel, J., Julliard, R., Lavergne, S., Mouillot, D., Thuiller, W., Venail, P., Villéger, S., Mouquet, N., 2010. Defining and measuring ecological specialization. *J. Appl. Ecol.* 47, 15–25. <http://dx.doi.org/10.1111/j.1365-2664.2009.01744.x>.
- Dolédec, S., Chessel, D., 1987. Rythmes saisonniers et composantes stationnelles en milieu aquatique I. – Description d'un plan d'observation complet par projection de variables. *Acta Oecol. Oecol. Generalis* 8, 403–426.
- Dolédec, S., Stutzner, B., 2010. Responses of freshwater biota to human disturbances: contribution of J-NABS to development in ecological integrity assessment. *J. N. Am. Benthol. Soc.* 29, 286–311. <http://dx.doi.org/10.1899/08-090.1>.
- Dray, S., Dufour, A.B., Chessel, D., 2007. The ade4 Package—II: Two-Table and KTable Methods. *R News* 7, 47–52.
- Efron, B., Halloran, E., Holmes, S., 1996. Bootstrap confidence levels for phylogenetic trees. *Proc. Natl. Acad. Sci. U. S. A.* 93, 13429–13434.
- Feio, M.J., Dolédec, S., 2012. Integration of invertebrate traits into predictive models for indirect assessment of stream functional integrity: a case study in Portugal. *Ecol. Indic.* 15, 236–247. <http://dx.doi.org/10.1016/j.ecolind.2011.09.039>.

- Franquet, E., 1996. Occupation d'un espace fluvial par les Diptères Chironomidés à l'aval du Rhône: répartition des espèces selon la nature du substrat et les conditions de débit. Université Lyon, Villeurbanne, France, pp. 1.
- Hawkins, C.P., Norris, R.H., 2000. Chapter 15 – Effects of taxonomic resolution and use of subsets of fauna on the performance of RIVPACS-type models. In: Wright, J.F., Sutcliffe, D.W., Furse, M.T. (Eds.), *Assessing the Biological Quality of Freshwaters RIVPACS and Other Techniques*. Freshwater Biological Association, Ambleside, Cumbria, UK, pp. 217–228.
- Illies, J., 1978. *Limnofauna Europaea. A Checklist of the Animals Inhabiting European Inland Waters with Accounts of their Distribution and Ecologu (except Protozoa)*, 2nd ed. Gustav Fisher Verlag, Stuttgart.
- King, R.S., Richardson, C.J., 2002. Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. *J. N. Am. Benthol. Soc.* 21, 150–171, <http://dx.doi.org/10.2307/1468306>.
- Kraft, N.J.B., Cornwell, W.K., Webb, C.O., Ackerly, D.D., 2007. Trait evolution, community assembly, and phylogenetic structure of ecological communities. *Am. Nat.* 170, 271–283.
- Laville, H., Vinçon, G., 1991. A typological study of Pyrenean streams: comparative analysis of the Chironomidae (Diptera) communities in the Ossau and Aure Valleys. *Verh. Intern. Ver. Limnol.* 24, 1775–1784.
- Lenat, D.R., Resh, V.H., 2001. Taxonomy and stream ecology – the benefits of genus- and species-level identifications. *J. N. Am. Benthol. Soc.* 20, 287–298.
- Lencioni, V., Marziali, L., Rossaro, B., 2007. I Ditteri Chironomidi. *Morfologia, tassonomia, ecologia, fisiologia e zoogeografia*. Museo Tridentino di Scienze Naturali. Trento. (Quaderni del Museo Tridentino di Scienze Naturali, 1).
- Lencioni, V., Marziali, L., Rossaro, B., 2012. Chironomids as bioindicators of environmental quality in mountain springs. *Freshw. Sci.* 31, 525–541, <http://dx.doi.org/10.1899/11-038.1>.
- Lindgaard, C.B., Brodersen, K.P., 1995. Distribution of Chironomidae (Diptera) in the River Continuum. In: Cranston, P. (Ed.), *Chironomids: From Genes to Ecosystems*. CSIRO Publications, Melbourne, pp. 257–271.
- Marziali, L., Armanini, D.G., Cazzola, M., Erba, S., Toppi, E., Buffagni, A., Rossaro, B., 2010. Responses of chironomid larvae (Insecta, Diptera) to ecological quality in mediterranean river mesohabitats (South Italy). *River Res. Appl.* 26, 1036–1051, <http://dx.doi.org/10.1002/rra.1303>.
- Mondy, C.P., Usseglio-Polatera, P., 2014. Using fuzzy-coded traits to elucidate the non-random role of anthropogenic stress in the functional homogenization of invertebrate assemblages. *Freshw. Biol.* 59, 584–600, <http://dx.doi.org/10.1111/fwb.12289>.
- Móra, A., Csépes, E., Tóth, M., Dévai, G., 2008. Spatio-temporal variations of macroinvertebrate community in the Tisza river (NE Hungary). *Acta Zool. Acad. Sci. Hung.* 54, 181–190.
- Mousavi, S.K., 2002. Boreal chironomid communities and their relations to environmental factors – the impact of lake depth, size and acidity. *Boreal Environ. Res.* 7, 63–75.
- Paradis, E., 2012. *Analysis of Phylogenetics and Evolution with R*, 2nd ed. Springer, New York.
- Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290.
- Penttinen, O.-P., Holopainen, I.J., 1995. Physiological energetics of a midge, *Chironomus riparius* Meigen (Insecta, Diptera): normoxic heat output over the whole life cycle and response of larva to hypoxia and anoxia. *Oecologia* 103, 419–424, <http://dx.doi.org/10.1007/BF00328679>.
- Pillot, H.K.M.M., 2009. *Chironomidae Larvae – Biology and Ecology of the Chironomini*, vol. II. KNNV Publishing, The Netherlands.
- Pillot, H.K.M.M., 2013. *Chironomidae Larvae – Biology and Ecology of the Aquatic Orthocladinae*, vol. III. KNNV Publishing, The Netherlands.
- Poff, N.L., Olden, J.D., Vieira, N.K.M., Simmons, M.P., Kondratieff, B.C., 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. *J. N. Am. Benthol. Soc.* 25, 730–755, [http://dx.doi.org/10.1899/0887-3593\(2006\)025\[0730:FTNONA\]2.0.CO;2](http://dx.doi.org/10.1899/0887-3593(2006)025[0730:FTNONA]2.0.CO;2).
- Prat, N., Puig, M.A., González, G., Millet, X., 1983. Chironomid longitudinal distribution and macroinvertebrate diversity along the Llobregat River (NE Spain). *Mem. Am. Entomol. Soc.* 34, 267–278.
- Prat, N., Rieradevall, M., Barata, C., Munné, A., 2013. The combined use of metrics of biological quality and biomarkers to detect the effects of reclaimed water on macroinvertebrate assemblages in the lower part of a polluted Mediterranean river (Llobregat River, NE Spain). *Ecol. Indic.* 24, 167–176.
- Puntí, T., Rieradevall, M., Prat, N., 2009. Environmental factors, spatial variation, and specific requirements of Chironomidae in Mediterranean reference streams. *J. N. Am. Benthol. Soc.* 28, 247–265, <http://dx.doi.org/10.1899/07-172.1>.
- Rabeni, C.F., Wang, N., 2001. Bioassessment of streams using macroinvertebrates: are the Chironomidae necessary? *Environ. Monit. Assess.* 71, 177–185.
- Raunio, J., Heino, J., Paasivirta, L., 2011. Non-biting midges in biodiversity conservation and environmental assessment: findings from boreal freshwater ecosystems. *Ecol. Indic.* 11, 1057–1064.
- Rosenberg, D.M., 1992. *Freshwater biomonitoring and Chironomidae*. *Neth. J. Aquat. Ecol.* 26, 101–122.
- Rossaro, B., Lencioni, V., Boggero, A., Marziali, L., 2006. Chironomids from Southern Alpine running waters: ecology, biogeography. *Hydrobiologia* 562, 231–246, <http://dx.doi.org/10.1007/s10750-005-1813-x>.
- Saether, O.A., 1979. Chironomid communities as water quality indicators. *Holarct. Ecol.* 2, 65–74.
- Saether, O.A., 2000. Phylogeny of the subfamilies of Chironomidae (Diptera). *Syst. Entomol.* 25, 393–403, <http://dx.doi.org/10.1046/j.1365-3113.2000.00111.x>.
- Saether, O.A., Spies, M., 2013. Fauna Europaea: Chironomidae. In: Beuk, P., Pape, T. (Eds.), *Fauna Europaea: Diptera*. Fauna Europaea version 2.6.2. <http://www.faunaeur.org/> (accessed 29.08.13).
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425.
- Schmidt, S., Wagner, B., Heiri, O., Klug, M., Bennike, O., Melles, M., 2010. Chironomids as indicators of the Holocene climatic and environmental history of two lakes in Northeast Greenland. *Boreas* 40, 116–130, <http://dx.doi.org/10.1111/j.1502-3885.2010.00173.x>.
- Servia, M.J., Cobo, F., González, M.A., 2004. Multiple-traits analysis of fluctuating asymmetry levels in anthropogenically and naturally stressed sites: a case study using *Chironomus riparius* Meigen, 1804 larvae. *Environ. Monit. Assess.* 90, 101–112, <http://dx.doi.org/10.1023/B:EMAS.0000003569.22040.ac>.
- Soriano, O., Cobo, F., Rieradevall, M., Prat, N., 1997. Lista faunística y bibliográfica de los quironómidos (diptera, chironomidae) de la península ibérica e islas Baleares. *Asociación Española de Limnología*, Madrid.
- Studier, J.A., Keppler, K.J., 1988. A note on the neighbor-joining algorithm of Saitou and Nei. *Mol. Biol. Evol.* 5, 729–731.
- Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P., 2010. *Invertébrés d'eau douce*, Nouvelle édition. Centre National de la Recherche Scientifique Press, Paris, France.
- ter Braak, C.F.J., 1988. Partial canonical correspondence analysis. In: Bock, H.H. (Ed.), *Classification and Related Methods of Data Analysis*. North-Holland, Amsterdam, pp. 551–558.
- Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE-4: a multivariate analysis and graphical display software. *Stat. Comput.* 7, 75–83, <http://dx.doi.org/10.1023/A:1018513530268>.
- Usseglio-Polatera, P., Bournaud, M., Richoux, P., Tachet, H., 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. *Freshw. Biol.* 43, 175–205, <http://dx.doi.org/10.1046/j.1365-2427.2000.00535.x>.
- Vallenduuk, H.J., Pillot, H.K.M.M., 2007. *Chironomidae Larvae – General Ecology and Tanipodinae*, vol. I. KNNV Publishing, The Netherlands.
- Vieira, N.K.M., Poff, N.L., Carlisle II, D.M., Moulton, S.R., Koski, M.L., Kondratieff, B.C., 2006. A database of lotic invertebrates traits for North America. U. S. Geological Survey Data Series 187, 1–15 <http://pubs.water.usgs.gov/ds187>.
- Walker, I.R., 2001. Midges: chironomidae and related diptera. In: Smol, J.P., Birks, H.J.B., Last, W.M. (Eds.), *Tracking Environmental Change using Lake Sediments, Volume 4, Zoological Indicators*. Kluwer Academic Publishers, Dordrecht, pp. 43–66.
- Webb, C.O., Ackerly, D.D., McPeck, M.A., Donoghue, M.J., 2002. Phylogenies and community ecology. *Annu. Rev. Ecol. Evol. Syst.* 33, 475–505, <http://dx.doi.org/10.1146/annurev.ecolsys.33.010802.150448>.