Sónia Raquel Quinás Serra

From taxonomy to multiple-trait bioassessment: the role of Chironomidae in separating naturally poor from disturbed communities

Tese de doutoramento em Biociências, ramo de especialização Ecologia de Bacias Hidrográficas,
orientada pela Doutora Maria João Feio, pelo Doutor Manuel Augusto Simões Graça e pelo Doutor Sylvain Dolédec
e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

Agosto, 2016

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Universidade de Coimbra

From taxonomy to multiple-trait bioassessment: the role of Chironomidae in separating naturally poor from disturbed communities

Da taxonomia à abordagem baseada nos multiatributos dos taxa: função dos Chironomidae na separação de comunidades naturalmente pobres das antropogenicamente perturbadas

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LEHNA

Aos meus amados pais,
sempre os melhores e mais dedicados amigos

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Abstract

Chironomidae (Diptera) have a worldwide distribution, being found over a wide range of habitats. Their larvae thrive in almost every possible freshwater habitat, with representatives also in terrestrial and marine environments, representing a major macroinvertebrate component in terms of abundance and richness. However, Chironomidae are generally neglected in community studies mainly because of difficulties in species larval identification. This compromises also the recognition and use of Chironomidae in trait-based approaches, which promote an indirect measurement of functional integrity in fresh waters. The aim of this thesis was to fill this latter gap by: (1) building a trait database for European Chironomidae at the genus level (Chapter I); (2) evaluating if the developed database at the genus level provides additional information to a commonly used European database developed at the subfamily and tribe level for Chironomidae (Chapter II); (3) testing Chironomidae traits relevance in bioassessment through their ability to discriminate naturally different stream types and segregate least disturbed from disturbed sites (Chapters III and IV); (4) comparing trait information gathered in the new European database (Chapter I) with similar information gathered for North America for the same genus and species, and simultaneously testing for trait variability between continents (Chapter V).

In Chapter I the general methodology for the construction of the Chironomidae trait database at the genus level is described. The trait database was developed gathering existing information in literature for 744 species and 178 genera, considering 186 trait categories from 37 traits divided in two major domains: Eltonian - related to organism function and influence over its environments; and Grinnellian - associated to organism requirements and performance in its environments.

In Chapter II traits at the genus level (from Chapter I) and the existing ones at the subfamily level (tribe at most; Tachet et al. 2010) are compared. This comparison showed that there are significant differences in trait information gathered at different taxonomic levels, thus questioning the use of the Chironomidae subfamily level in ecological, functional and biomonitoring studies. Subfamilies are very heterogeneous in terms of traits, exposing the evolutionary divergence in each subfamily. The distances between subfamilies given by their traits are also not in agreement with the most accepted phylogenetic subfamily relatedness, indicating a divergence from the traits of a common ancestry.

In Chapter III Chironomidae taxonomic composition at the genus level, unlike the subfamily level, allowed the segregation of different stream types: permanent medium elevation, permanent lowland, and south temporary. Different Chironomidae assemblages manifest different trait composition for Eltonian traits (emergence season, maximal body size, overwinter diapause stage, substrate relation), but also morphological traits (body setae, mentum, Lauterborn organs, premandible brush, claws of anterior parapods). Morphological traits seem interesting tools that would avoid the laborious identification of genus that rely on multiple minute structures, enabling the trait assessment through the observation of few Chironomidae structures.

In Chapter IV it is tested whether using Chironomidae taxonomic and trait compositions allow the segregation of disturbed sites under multiple anthropogenic stressors (subjected to hydromorphological and physicochemical alterations) from least-disturbed sites in Mediterranean temporary streams. Traits were analysed individually and also grouped into life-history strategies. Both methods enabled a significant segregation between disturbed and least-disturbed sites. Individual traits that were significantly different between sites were also those that are evolutionarily interrelated and used to define Chironomidae life-history trait strategies.

Finally, in Chapter V, North American and European Chironomide traits (Eltonian and Grinnellian) are compared considering some common genus and species. There is some intraspecific trait variability that can result from divergence or trait plasticity. This finding questions the generalized use of large databases irrespective of the region for which they were developed but reveals also the need for a standardized procedure in the collection of relevant traits, able to manifest trait variability.

In conclusion, in this study the first comprehensive European Chironomidae trait database at genus level was developed and tested, which can be hereafter used in ecological studies. Chironomidae are promising bioindicators that should be considered in bioassessment of streams, especially where they are among the dominant taxa.

Keywords: Diptera, bioassessment, fuzzy coding, traits, life-history strategies, river types, disturbance, Holarctic.

Resumo

Os Chironomidae (Diptera) apresentam uma distribuição mundial, sendo encontrados numa enorme diversidade de habitats. Suas larvas prosperam em praticamente todos os habitats de água doce, representando em termos de abundância e riqueza um importante componente dos macroinvertebrados. Porém, na maioria dos ambientes a família está muito pouco estudada sendo, frequentemente, negligenciada em estudos de comunidade, devido às dificuldades inerentes à identificação das suas larvas. Isto compromete também o reconhecimento e uso dos seus atributos (‘traits’) biológicos, fisiológicos e ecológicos, em análises de múltiplos atributos, cada vez mais utilizados como forma de avaliar indirectamente a integridade funcional dos ecossistemas.

Este trabalho teve como objectivo preencher esta lacuna: (1) construindo uma base de dados de atributos para os Chironomidae Europeus ao nível do género (Capítulo I); (2) avaliando se existem diferenças nos atributos quando reunidos a diferentes níveis taxonómicos (Capítulo II); (3) testando a relevância dos atributos dos Chironomidae na avaliação biológica, pela capacidade de discriminar rios naturalmente diferentes e na segregação de locais afetados por diferentes níveis de perturbação (Capítulo III e IV); (4) testando a existência de variabilidade de atributos entre Europa e a América do Norte comparando os atributos de géneros e espécies de distribuição Holártica (Capítulo V).

No Capítulo I, é descrita a metodologia seguida para a construção da base de dados de atributos dos Chironomidae ao nível do género. A base de dados foi desenvolvida reunindo informação de literatura para 178 géneros baseada em 744 espécies, considerando 186 categorias de 37 atributos, distribuídos por dois domínios: o ‘Eltonian’, relacionado com a função dos organismos e sua influência nos seus ambientes; e o ‘Grinnellian’, associado aos requisitos ambientais dos organismos.

No Capítulo II, os atributos dos Chironomídeos Europeus reunidos ao nível do género (Capítulo I) são comparados com os existentes para o nível da subfamília/tribo de uma base de dados de uso comum. Ocorreram diferenças significativas entre os atributos reunidos a diferentes níveis taxonómicos, questionando o uso dos Chionomídeos ao nível da subfamília em estudos ecológicos e na biomonitorização. As subfamílias revelaram heterogeneidade nos atributos dos seus taxa, expondo a divergência evolutiva dentro de cada subfamília. Porém, a distância entre as subfamílias reflectida pelos atributos dos seus taxa não espelhou os padrões filogenéticos mais aceites dentro dos Chironomidae.

No Capítulo III, a composição taxonómica dos Chironomidae ao nível do género, ao contrário do nível da subfamília, permitiu a segregação de rios Portugueses com diferentes tipologias: permanentes de elevação média e de planície do Centro e Norte, e temporários do Sul. A composição em atributos dos Chironomidae característica de cada tipo também se revelou diferente. Os tipos de rio foram separados por atributos ‘Eltonian’ (e.g., estação de emergência, tamanho máximo do corpo, relação para com o substrato); e por atributos morfológicos (e.g., sedas no corpo, mento, órgão de Lauterborn). Os atributos morfológicos prometem ser boas ferramentas para contornar problemas associados à identificação laboriosa dos géneros, que requer a observação de inúmeras estruturas minutas, permitindo a avaliação das condições ambientais pela observação de um número reduzido de estruturas.

No Capítulo IV, tanto a composição taxonómica como a de atributos dos Chironomidae permitiram a segregação de locais perturbados sob a influência de várias pressões (alterações hidromorfológicas e físico-químicas) de locais menos perturbados em rios Mediterrânicos temporários. Os atributos dos Chironomidae foram analisados individualmente como unidades independentes mas também combinados em estratégias de vida. As duas abordagens permitiram a discriminação significativa dos locais, atendendo ao seu nível de perturbação. Os atributos individuais que se revelaram importantes na segregação foram os que se encontram combinados nas estratégias consideradas.

Finalmente, no Capítulo V, compararam-se os atributos, tanto ‘Eltonianos’ como ‘Grinnellianos’ de alguns géneros e espécies comuns aos continentes Norte Americano e Europeu. Foi revelada a existência de variabilidade nos atributos entre os continentes, que pode ser resultados de plasticidade ou divergência dos atributos. Estes resultados colocam em causa a utilização de uma base de dados para regiões diferentes daquela para a qual foi desenvolvida, revelando também a necessidade de estandardizar procedimentos para a elaboração das bases de atributos.

Neste estudo foi desenvolvida e testada a primeira base de dados de atributos dos géneros de Chironomidae Europeus, que poderá ser usada em estudos ecológicos. Os Chironomidae devem ser bioindicadores considerados na avaliação da qualidade dos rios, especialmente onde constituem os insetos dominantes.

Palavras-chave: Diptera, bioavaliação, codificação ‘fuzzy’, atributos, estratégias de vida, tipos de rios, perturbação, Holártico.

Résumé détaillé

La famille des Chironomidae (Dipteres) présente une large distribution mondiale dans une large gamme d'habitats. Ses représentants ont colonisé presque tous les habitats d'eau douce et on trouve également des Chironomidae dans des environnements terrestres et marins. Cette famille représente ainsi une composante majeure des macroinvertébrés benthiques en termes d'abondance et de richesse. De part leur position trophique, les Chironomidae dominent souvent les communautés non perturbés en termes d'abondance et de biomasse, et représentent plus de 50% des espèces de macroinvertébrés benthiques dans les eaux stagnantes et courantes. Dans les zones profondes des lacs eutrophes et les cours d'eau très impactés par les activités humaines, c'est souvent la seule famille d'insectes aquatiques qui perdure. De ce fait, la famille des Chironomidae comprend un grand nombre de genres et d'espèces présentant des préférences écologiques diversifiées. De plus, la prédominance et la richesse spécifique des Chironomidae en font une source d'énergie très importante pour les prédateurs (vertébrés et invertébrés) et leur confère un rôle majeur dans le flux d'énergie dans les écosystèmes aquatiques. De ce fait, les Chironomidae pourraient avoir un rôle non négligeable dans la mise en place des conditions de référence pour la bioévaluation des rivières et dans la détection des cours d'eau plus et moins impactés, et pourraient fournir une information sur le fonctionnement des écosystèmes aquatiques. Cependant, les Chironomidae sont généralement délaissés dans l'étude de la structure de la communauté des cours d'eau, principalement à cause des difficultés d'identification des stades larvaires aquatiques des espèces.

Les approches basées sur les traits fournis par la littérature sont basées sur la théorie de «l'habitat templet» selon laquelle, l'habitat fournit le cadre environnemental sur lequel les espèces forgent des stratégies à travers leur adaptation dans tous les types d'environnements. Conceptuellement, les conditions environnementales représentent des filtres qui, en éliminant les espèces ayant les combinaisons de traits les moins adaptées, participent à la mise en place des communautés. Comme les traits reflètent la performance des organismes dans des conditions environnementales données, l'objectif principal des analyses basées sur les traits est d'acquérir une compréhension plus mécaniste des relations entre les espèces et leur habitat. Dans les deux dernières décennies, la liaison espèces-trait-environnement a été explorée par de nombreux auteurs dans des études où la composition des traits a été analysée individuellement ou combinées en termes de stratégies. Ainsi, l'utilisation des traits dans les approches d'écologie des communautés (écologie fonctionnelle) s'est développée très rapidement, offrant l’avantage d’une relative indépendance des réponses vis-à-vis de la biogéographie des taxons. En d’autres termes, un trait tel que la respiration branchiale se rencontre dans les milieux aquatiques indépendamment de la région biogéographique alors même que l’identité des taxons change de manière importante entre régions.

L'identification taxonomique des Chironomidae au niveau de la famille ou de la sous-famille généralement utilisée dans les programmes de biomonitoring et le manque d'information spécifique sur les traits des taxons de ce groupe ne permet pas une utilisation fiable dans l'évaluation de la structure des communautés. Leur utilisation dans les approches basées sur les traits biologiques qui permettent une mesure indirecte de l'intégrité fonctionnelle des eaux douces est également peu pertinente à ce niveau d’identification taxonomique. Le but de cette thèse est de remédier à ces problème liés à l’identification et de proposer des développements méthodologiques et conceptuels permettant de mieux intégrer les Chironomidae dans les diagnostics écologiques des eaux courantes: (1) en élaborant une base de données des traits pour les genres de Chironomidae européens (chapitre I), (2) en évaluant la pertinence et la précision des informations supplémentaires fournies par cette base de données en comparaison des informations existantes dans la base de données européenne couramment utilisée et en quantifiant les traits des sous-familles et des tribus de Chironomidae (chapitre II) ; (3) en testant la pertinence de l'utilisation des traits des genres de Chironomidae dans le diagnostic écologique des eaux courantes en examinant leur capacité à différencier des types de cours d’eau naturellement différents ou différant suivant leur niveau de perturbation (chapitres III et IV) ; (4) en évaluant la variabilité intercontinentale des traits de Chironomidae par une comparaison des informations sur les traits recueillies dans la base de données des genres de Chironomidae proposée dans cette thèse (chapitre I) avec des informations similaires recueillies en Amérique du Nord, pour les mêmes genre et espèces (chapitre V).

Au chapitre I, on décrit la méthodologie générale utilisée pour la construction de la base de données de traits des genres de Chironomidae. Cette base de données a été développée en collectant les informations existantes en particulier dans la littérature européenne (~150 références couvrant différents types d'habitats d'eau douce et zones biogéographiques du Paléarctique) sur 744 espèces et 178 genres et en considérant 186 catégories de 37 traits divisés en deux grands domaines (Cf. Tachet et al. 2010): les traits biologiques (Eltonian), liés à la fonction et l'influence des organismes sur leur environnement, et les traits écologiques (Grinnellian), associés aux exigences et performances des organismes dans leur environnement. L'information des traits a été compilée en suivant une procédure de codage flou qui quantifie l'affinité d'un taxon à une catégorie de trait, en utilisant le nombre de références citant cette affinité comme score d'affinité du taxon pour la catégorie de trait.

Au chapitre II, on compare les traits au niveau du genre (décrits au chapitre I) avec ceux précédemment décrits au niveau de la sous-famille (selon Tachet et al. 2010) en utilisant une analyse multivariée type de Analyse des Correspondances Floues (FCA; Chevenet et al. 1994). On a montré des différences significatives entre les informations données par les traits suivant le niveau taxonomique considéré (genre ou sous-famille). Ce résultat remet en question l'emploi des sous-familles Chironomidae dans les diagnostics écologiques et fonctionnelles. Les sous-familles sont très hétérogènes en termes de traits révélant une divergence évolutive entre chaque sous-famille ainsi que des distances entre traits qui ne sont pas en accord avec la relation phylogénétique la plus acceptée pour les sous-familles. Les exceptions sons les Orthocladiinae et Chironominae, considérés comme des groupes sœurs du point de vue évolutif, et qui sont positionné proches sur l'arbre construit à partir des traits biologiques (qui devrait être plus conservateur).

Au chapitre III, on compare des sites d'étude établis dans deux zones climatiques contrastées du territoire portugais: les sites de rivières permanentes dans une zone du nord sous l'influence du climat atlantique tempéré et les sites de rivières temporaires dans une zone du sud sous l'influence du climat méditerranéen. Les trois types de rivière considérés (pérenne à élévation moyenne, pérenne en plaine et intermittente) divergent en termes d'élévation, de température annuelle moyenne, de précipitation annuelle moyenne et de lithologie. Une analyse multivariée de type «non-metric multidimensional scaling» (NMDS) a été utilisé pour décrire les différences de composition en Chironomidae entre les types de rivières et, l'importance statistique de ces différences a été déterminée à l'aide d'un test de type PERMANOVA. L'analyse et le test ont été réalisés à deux niveaux différents de résolution taxonomique: sous-famille et genre. Pour identifier les genres Chironomidae dominants dans chaque type de rivière, nous on avons a utilisé l'analyse des pourcentages de similarité entre espèces (SIMPER). Enfin, la matrice traits-par-sites a été analysée en utilisant une FCA. On montre que l'utilisation de la composition taxonomique des Chironomidae au niveau du genre permet de distinguer plus clairement les types de ruisseaux naturels (non impactés) en comparaison d'une utilisation des sous-familles de Chironomidae. De plus les genres de Chironomidae présents dans les assemblages peuvent montrer des traits différents non seulement pour les caractères biologiques (saison d'émergence, taille maximale du corps, stade de diapause hivernale, relation avec le substrat), mais aussi pour les traits morphologiques (concernant les soies corporelles, le mentum, les organes de Lauterborn, la brosse des premandibules, les griffes des parapodes antérieures). Les assemblages de Chironomidae des cours d'eau d' élévation moyenne diffèrent de ceux de plaine et des cours d'eau temporaires par la saison d'émergence et la diapause hivernale, avec la présence de plus larves qui émergent en été, et peu stades larvaires manifestant diapause en hiver dans cours d'eau d' élévation moyenne, ce qui reflète des adaptations des genres de Chironomidae à la température et les régimes de variation de débit. Les assemblages de Chironomidae des cours d'eau temporaires se distinguent de ceux des cours d'eau permanents par la relation au substrat et la taille maximale du corps, avec la diminution de la proportion de larves de taille intermédiaire et l'augmentation de la proportion de fouisseurs dans les cours d'eau temporaire, ce qui peut être attribué à une adaptation genres de Chironomidae aux sédiments instables et au régime d'écoulement irrégulier. De plus, les assemblages de Chironomidae des cours d'eau temporaires et permanents se distinguent également par des différences dans les traits morphologiques, comme les soies du corps (associés à la mobilité et à la relation avec le substrat) et les organes lauterbon (structures sensorielles importantes dans la recherche de refuge et nourriture), ce qui peut être relié aux différences d'hétérogénéité spatiale et temporelle des deux types de cours d'eau. Les traits morphologiques sont apparus comme des outils intéressants pour le diagnostic écologique, car ils se basent uniquement sur l'observation de quelques structures des larves de Chironomidae et permettent d'éviter l'identification laborieuse des genres qui repose sur de multiples structures minuscules.

Au chapitre IV, on compare la capacité de la composition taxonomique et de la composition en traits des assemblages de Chironomidae pour discriminer des sites perturbés par de multiples facteurs de pressions anthropiques (altérations hydromorphologiques et physico-chimiques) et des sites moins perturbés, dans des cours d'eau intermittents méditerranéens. Les traits sont analysés individuellement et en groupes de stratégies d'histoire de vie. On a effectué une analyse en composantes principales (ACP) pour analyser la ségrégation des sites basés sur l'abondance des taxons de Chironomidae. Une analyse de type SIMPER (similitude de Bray-Curtis) a été réalisée pour déterminer quels genres de Chironomidae contribuent le plus à la similitude des sites moins perturbés et perturbés. Les genres de Chironomidae, représentants de chaque groupe (moins perturbés/perturbés), ont été sélectionnés et leurs tableaux trait-par-sites ont été analysés à l'aide d'un PCA. La composition taxonomique et la composition en traits biologiques permettent de discriminer les sites en fonction de leur niveau de perturbation de manière semblable. La perturbation induit une augmentation prévisible des Chironomidae émergeant préférentiellement en hiver et possédant de l'hémoglobine et diminution prévisible des racleurs, ainsi qu'une l'augmentation moins prévisible de la proportion de taxons présentant des cycles de vie longs avec quelques générations par an. Des stratégies d'histoire-de-vie correspondant aux Chironomidae multivoltins de taille moyenne qui n'investissent pas dans l'hémoglobine et ne présente pas de forte synchronisation printanière pour l'émergence des adultes, adaptés aux eaux stagnantes et avec un dynamisme temporelle réduit (pas de changements soudains) sont favorisées sous conditions perturbées. Ces deux méthodes (traits et stratégies) permettent une séparation significative entre les sites perturbés et moins perturbés. Les traits individuels qui différent significativement entre les sites sont aussi ceux qui ont un lien évolutif entre eux et qui sont utilisés pour définir des stratégies de traits d'histoire de vie des Chironomidae.

Finalement, le au chapitre V on compare les traits biologiques (Eltonian) et écologiques (Grinnellian) pour les genres et espèces communs aux zones Nearctique (base de données de l'Amérique du Nord (Vieira et al. 2006)) et Paléarctique (base de données Européenne développée dans cette thèse). Des genres et espèces communes aux deux bases de données ont été sélectionnés et les traits communs ont été regroupés dans les mêmes catégories de traits. Compte-tenu des différences de codage, les affinités ont été transformées en présence/absence et divisés en traits biologique/fonctionnel et écologiques. Les genres et les espèces communes ont été analysés par une FCA. Les différences observées entre les bases de données concernent tous les types de traits, mais le niveau de concordance entre les continents est plus faible pour les traits biologiques/fonctionnels que pour les traits écologiques. La variabilité intra-spécifique des traits observée entre les continents, peut résulter de la divergence ou de la plasticité du trait. Ce résultat questionne l'utilisation généralisée des grandes bases de données indépendamment de la région dans laquelle elles ont été développées, et révèle aussi la nécessité d'une procédure normalisée pour la collection de traits pertinents qui sont en mesure de manifester cette variabilité.

En conclusion, dans cette étude, on a développé et testé la première base de données européenne de traits des genres de Chironomidae au niveau du genre en utilisant les informations biologiques de la littérature plus récente. Les Chironomidae sont des bioindicateurs prometteurs qui devraient être pris en compte dans le diagnostic écologique des cours d'eau, en particulier là où ils représentent l'un des taxons les plus dominants.

Mots-clés: Diptères, bioindication, codage flou, traits, stratégies d'histoire de vie, types de ruisseaux, perturbation, Holarctique.

General Introduction

Habitat Templet theory and trait-based approaches

Human activities, in a rapidly developing world, pressure and deteriorate natural ecosystems leading to loss of their natural diversity. The environmental assessment becomes thus essential in the identification and quantification of the effects of human disturbance over the ecosystems. The growing need to measure the integrity of freshwater systems has determined an extensive collection of biological monitoring approaches measured at different organizational levels, from the sub-organism to the ecosystem level (Norris & Norris 1995). The majority of these approaches addresses structural composition of communities (abundance, richness, species composition) inhabiting the system, considering various elements of biota, including benthic macroinvertebrates. Furthermore the functional assessment of freshwater integrity has been done through the measurement of processes occurring in the freshwater systems, such as: community metabolism, leaf little breakdown and secondary production (see review in Dolédec & Statzner 2010 and references therein). Indirect approaches have also been developed to assess ecosystem processes through, for example, invertebrates multiple traits.

The use of traits is grounded on the Habitat Templet theory (Southwood 1977) according to which the habitat provides the ‘templet’ that forges strategies and attributes of species through evolution in all kinds of environments. Thus environmental conditions have been conceptualized as trait filters, acting in the elimination of the least suited combination of traits; thus, only the species with the set of traits that passes through those environmental filters make part of the community (Scarsbrook & Townsend 1993, Poff 1997). As a consequence, organisms display a set of naturally selected and co-adapted traits that guarantee individual fitness, survival and reproduction in their habitats; governing their ability to deal with the environmental constraints and opportunities, and reflecting their performance and evolutionary adaptation to those habitats (Townsend & Hildrew 1994, Statzner et al. 2010). Following this rationale, there is a link between species traits and the environment, allowing the forecast of presence/absence of traits favoured by the particular environmental conditions; locations with similar environmental conditions are expected to share similar trait composition (Townsend & Hildrew 1994, Townsend et al. 1997, Dolédec et al. 2006).

Because traits reflect organism performance in the system, analysing trait composition holds the promise of a better understanding of the role of biodiversity in the maintenance of ecosystem processes and services and ultimately in the system function.

Thus, changes due to anthropogenic disturbance can also be reflected in trait composition, supposedly mirroring changes in the ecosystem functions (Gayraud et al. 2003, Diaz & Cabido 2001, Diaz et al. 2013).

Multiple trait-based (MTB) approaches were developed as an indirect measure of the functional integrity of the ecosystem, providing additional metrics and promising a better mechanistic perspective on the cause and level of impairment (e.g., Archaimbault et al. 2005, Vieira et al. 2006, Culp et al. 2011). MTB present other advantages, such as facilitating regional comparisons as traits vary less across different biogeographical regions, contrary to taxonomy-based approaches since taxa assemblages vary across those regions (e.g., Statzner et al. 2004, Bonada et al. 2007a). Another potential advantage of MTB approaches is related to the fact that some traits may not require the identification of taxa, such as morphological traits like body size, which is associated to organisms' growth.

One of the biggest challenges of the approach is the need of trait information for taxa, additionally a consistent trait definition and standardized collection (Statzner & Bêche 2010, Baird et al. 2011). The species-trait-environment linkage has been explored by several authors in the past few decades by: establishing methods to express quantitatively the diversity of traits in communities (e.g., Chevenet et al. 1994); creating general databases that quantify the association between taxa and traits.

There are several trait databases with information for several aquatic insect and non-insect taxa (e.g., Poff et al. 2006 and Vieira et al. 2006 for North America; and Tachet et al. 2010 for Europe, completed by Bonada & Dolédec 2011 for Mediterranean area). Tachet et al. (2010) is often used in the identification of freshwater macroinvertebrate taxa, and also offers a trait database for some of those taxa. It classifies traits of freshwater macroinvertebrates into three fundamental categories: biological (e.g., life cycle, dispersion, reproduction, feeding strategies), physiological and ecological (e.g., habitat preferences of temperature, substrate, hardness, alkalinity). Each trait includes several trait categories, and trait information is quantified through the affinity of a taxa to a given trait category using a fuzzy coding approach (Chevenet et al. 1994). Instead, Vieira et al. (2006) used a binary coding for mutually exclusive categories. Fuzzy coding however is considered a more realistic representation for taxa at higher levels of taxonomic resolution, being also able to offer a perspective on trait plasticity inside a given taxon.

Notwithstanding its growing use, the term 'trait' is still context-sensitive (Violle et al. 2007, Dolédec & Statzner 2010, Menezes et al. 2010, Schmera et al. 2015). The consequence is the existence of various definitions and traits grouped in different sets, as for example: biological, ecological, physiological, functional, performance, demographic traits (e.g., Usseglio-Polatera et al. 2000a, Violle et al. 2007, Tachet et al. 2010). A more restrained concept directs the use of the term traits only for physiological, morphological and life-history related attributes of organisms, that can be measured at the individual level without reference to external conditions (Violle et al. 2007, Verberk et al. 2013). According to this concept, preferences of species, referred as ecological traits (see Usseglio-Polaterra et al. 2000a) should not be consider as a trait itself, instead as a result of the interaction of the organism's traits with their environment.

With the growing need of functional approaches and the advantages associated to the use of traits, MTB approaches have become frequent in bioassessment, analysing trait compositional patterns of various components of biota including macroinvertebrates (Dolédec & Statzner 2010, Menezes et al. 2010, Culp et al. 2011, Feio & Dolédec 2012). These approaches have been described as able to significantly discriminate between various causes and different levels of anthropogenic disturbance (Dolédec et al. 1999, Usseglio-Polatera & Beisel 2002, Gayraud et al. 2003, Dolédec & Statzner 2008). The linkage between species-traits and environment was discussed in many studies (Townsend & Hildrew 1994, Poff 1997, Statzner et al. 1997, Townsend et al. 1997) but often this binding is complex, since species display traits combined in different ways, obscuring the casual mechanisms and making predictions difficult.

More recently, Verberk et al. (2013) exposed the main problems associated to trait-based approaches that deal with traits as independent units, which ultimately translates into a low discriminatory power and poor mechanistic understanding (the original promise of the method). According to these perspectives traditional MTB approaches have conceptual problems, as natural selection operates on species (organism carrying multiple traits) and not at the level of each single trait. As a consequence, species performance in its environment is dependent on a suitable combination of traits: out of all theoretically possible traits' combinations, only a few combinations (strategies) will actually favour the survival and thriving of a species in a particular environment. Traits are then phylogenetically linked by evolution and trait-combination is context dependent with the possibility of trade-offs (Resh et al. 1994, Usseglio-Polatera et al. 2000b, Poff et al. 2006). With these conceptual changes, new trait-based approaches have also been developed, using traits combined into a priori strategies or tactics defining groups of taxa with the same set of combined traits, reflecting the complex traits interrelation with the promise of exposing stronger trait (strategy)-environment relationships (Verberk et al. 2008a,b, Verberk et al. 2013).

Bioassessement: why Chironomids are neglected

Among aquatic communities, benthic macroinvertebrates have been widely used as bioindicators of stream health presenting various methodological advantages. These communities are ubiquitous, abundant and gather high taxa richness relatively easy to sample and identify. Being highly diverse, communities respond to a wide range of environmental conditions. Sensitivities of taxa to common pressures (e.g., organic enrichment) are well known. In addition, the sedentary nature of aquatic invertebrates and their life cycles, which can extend to one year or more, make them good indicators of localized conditions, integrating a temporal perspective of the environmental conditions found in their habitats (Bonada et al. 2006a).

Within macroinvertebrates, the high species richness of Chironomidae larvae in freshwater systems, when compared to other benthic macroinvertebrates, allows a wide range of responses to different environmental conditions and stresses, since the family includes taxa that are able to live in different environmental conditions (e.g., Cranston 1995a, Lencioni et al. 2007). Beyond that, Chironomidae larvae are virtually present in all freshwater systems where they may contribute to more than half of macroinvertebrate richness, occurring also in semi-terrestrial/terrestrial, and marine habitats. They have also been found in extreme habitats such as glacial streams, hot springs, sub-desert steppes), or in rather unusual aquatic habitats such as thin layers of water flowing over a rock face, water in rot-hole of trees and even in leaf axes of plants (Armitage et al. 1995, Cobo & Blasco-Zumeta 2001, Vallenduuk & Moller Pillot 2007, Moller Pillot 2009, 2013). In running waters they are sometimes the only insect present (e.g., extremely polluted streams) and the only group common to all streams in a study.

As a result, Chironomidae have already been used as bioindicators. Some species are currently exploited in ecotoxicity tests due to their easy laboratory maintenance and relatively short life cycles (Vermeulen 1995, De Haas et al. 2002, Sánchez & Tarazona 2002, Carew et al. 2007). Morphological abnormalities of Chironomid larvae as a result of ontogenic instability determined by environmental stress have also been used in pollution assessment (Servia et al. 2004, Odume et al. 2012). Finally, in lakes, Chironomidae have been used as paleoindicators of past environmental changes (e.g., temperatures, dissolved oxygen) using the fully sclerotized head capsules that remain intact in the sediments over time (Porinchu & MacDonald 2003, Brodersen et al. 2008).

In spite of their potential to monitor changes in environmental conditions, Chironomidae have been particularly neglected in lotic systems in favour of other insect groups like Ephemeroptera, Plecoptera and Trichoptera (EPT), which are considered more sensitive to pollution (Calle-Martínez & Casas 2006, Kenney 2009). This is mainly related to Chironomidae systematic and taxonomy and the lack of congruence between classifications of their different life history stages (Oliver 1971, Lindeberg 1980, Webb 1980).

Identification of species even genera based on immature forms requires preparation of samples and mounting specimens onto microscopic slides. The time consuming taxonomic identification of larvae has discouraged the inclusion of Chironomidae at higher levels of taxonomic resolution in regular biomonitoring programs. As a result, larvae are usually identified only at the family level, subfamily or tribe, assuming that all taxa included in these higher taxonomic groups are ecologically equivalent. Because of this lack of investment, information on responses of Chironomidae species and genera to anthropogenic disturbance is still lacking or is insufficient (Rosenberg 1992). In other terms, a significant part of macroinvertebrate biodiversity of water bodies is regularly neglected and underestimated.

Given the diversity of Chironomidae species aggregated as a single taxon, the family is seen as highly tolerant, the averaging result of the ubiquity of the family. According to Wymer & Cook (2003), information lost in community studies by low taxonomic resolution is proportional to the diversity of the taxa considered. A higher taxonomic resolution (genus, species) of Chironomidae could thus enhance the sensitivity of ecological studies using benthic communities (Hawkins & Norris 2000, Wymer & Cook 2003). This is particularly relevant when considering systems naturally poor in other macroinvertebrate groups, where Chironomidae dominate in abundance and richness (e.g., lowland streams).

The practical difficulties linked to Chironomidae identification led ecologists to the development of alternative approaches such as the Chironomid Pupal Exuvial Technique, CPET, that make use of the pupal exuviae to deduce information on local larval chironomid assemblages (Ruse 1995, Hughes & Furse 2001, Raunio et al. 2007, Raposeiro et al. 2011). Using pupal exuviae, the genus level is relatively easy to attain without high expertise. However, this method is highly dependent on the emergence period. Moreover, exuviae may drift away from emergence sites (Hardwick et al. 1995, Raunio et al. 2007). Finally, CEPT sampling is not comparable to other macroinvertebrates sampling.

Chironomidae in freshwater ecosystems

A group with diversified biological characteristics

Chironomidae (Insecta: Diptera) are holometabolous insects with a four-stage life cycle: egg, larva, pupae, and imago (adult) (Figure 1). In general, the first three stages are aquatic. Pupae and imago have a relatively short duration; almost solely supported by the energy stored during larval stages. The egg-masses (or very rarely isolated eggs) are generally laid at the surface of freshwater bodies (e.g., stones, plants); however, different egg-laying behaviours are determinant for the larval distribution, which can be very specialized (e.g., cattle dung; phytotelmata, water bodies held by terrestrial plants).

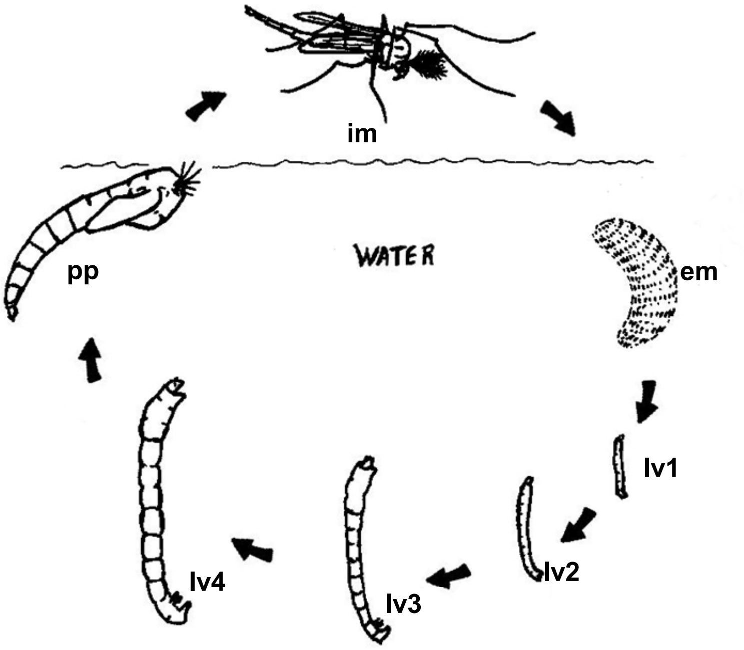

Figure 1 Chironomidae life cycle. **im**, imago (adult); **em**, egg-mass; **lv1**, 1st instar larva; **lv2**, 2nd instar larva; **lv3**, 3rd instar larva; lv4, 4th instar larva; **pp**, pupa.

Fresh water is the primary habitat of larvae, which constitute the longest phase of Chironomidae life cycle (Pinder 1983, Armitage et al. 1995, Coffman & Ferrington 1996). Larvae pass through 4 instars. The first-instar is usually overlooked because of their small size and pelagic habits. In contrast, the last larval instars are the ones collected by traditional benthic sampling devices. The fourth instar larvae have well-developed, sclerotized non-retractile head capsule. Their mandibles operate in an oblique to horizontal plane. The elongated segmented body has paired prolegs (parapods) above the first thoracic and bellow the terminal abdominal segments. A paired procerci is present at the terminal abdominal segment as the anal tubules important in the ionic regulation (Figure 2). *Chironomus* sp. and some relatives often have haemolymph-filled abdominal ventral tubules originally associated with respiration. It should be noted that most morphological and taxonomic observations are confined to the final-instar larvae (fourth-instar), although most structures are already defined in the earlier instars (Armitage et al. 1995).

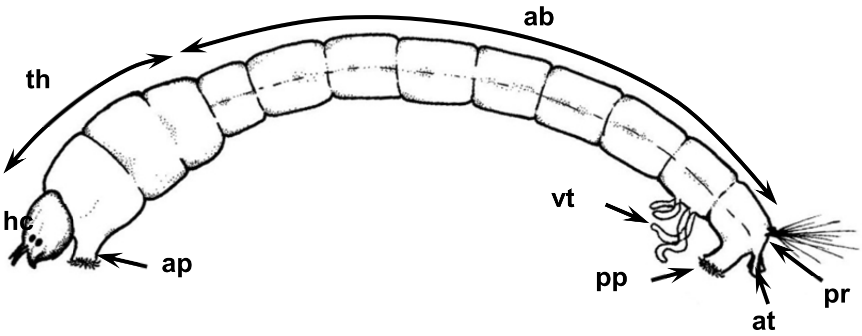

Figure 2 *Chironomus* sp. larva. **ab**, abdomen (9 body segments); **ap**, anterior parapods; **at**, anal tubules; **hc**, head capsule; **th**, thorax (3 body segments); **pp**, posterior parapods; **pr**, procercus (pl. procerci); **vt**, ventral tubules.

Chironomidae gather 15 to 20 thousand species distributed through different habitats over all major biogeographic regions of the world. Nevertheless, this number is probably underestimated since a substantial number of insect species remain undescribed (Armitage et al. 1995, Coffman and Ferrington 1996). Richness estimations rely on faunistic inventories made at the country level, but the investment made within each country is not the same. The underestimation of Chironomidae global richness is substantially aggravated by the taxonomic challenge associated to their identification, the number of taxa with unknown stages, the high evolutionary plasticity, the undisclosed level of endemism and the lack of adequate guidance and specialists in some regions of the world (Armitage et al. 1995).

The family include widespread species (e.g., *Chironomus plumosus*; Gunderina 2012) but also species with a confined distribution (e.g., *Sergentia baicalensis* endemic from Baikal Lake in Russia; Proviz 2008). It gathers a high richness in running waters from headwater to mouth withstanding a broad gradient of environmental variables with a shift in Chironomidae assemblages (Lindegaard & Brodersen 1995). For example, 27 species can be found in cold pristine Alpine and pre-Alpine springs representing 45% of the macroinvertebrate species (Lencioni et al. 2011); and in small Pyrenean streams 60 species were reported (Lavandier 1979). Chironomidae are also important in number and richness in agricultural stream sites (~40% of species) and also in urban lowland streams (~31% of species) together with Oligochaeta (~21% of species) (Lenat & Crawford 1994).

Chironomidae are highly adaptable, resistant and resilient. In temporary Mediterranean rivers their communities change very quickly, after periods of drought or flood, they are often the first colonizers, given their high fecundity and relatively short life cycles (Langton & Casas 1999, Frouz et al. 2003, Calle-Martínez & Casas 2006, Punti et al. 2007, 2009, Marziali et al. 2010). There is even one species (*Polypedilum vanderplanki*) that exhibits anhydrobiosis, a biological state extremely resistant to drought. Individuals of this species are able to withstand inside a tubular nest of mud, gently dropping the water body content to ~3% of the body weight. This allows larvae to survive during long dry periods in semiarid regions of central Africa (Kikawada et al. 2005).

The ubiquity of Chironomidae larvae is due to a high behavioural and physiological diversity, manifested for example in the construction of tubes, the presence of haemoglobin, and many other features that may not be reflected unequivocally in morphology. Chironomidae are also highly diverse in terms of feeding behaviours. The family includes predators (e.g., most Tanypodinae) or consumers of algae, or organic particles, largely contributing to detritus processing when other macro-consumers are absent. They are in turn an important food source for almost all other invertebrate, fish, bird, and even amphibian consumer. This way, Chironomidae constitute an important energy link to upper levels (Berg 1995). Estimates of Berg & Hellenthal (1992) have attributed 80% of the total insect secondary production to Chironomidae, in a third-order woodland stream.

How to quantify chironomidae traits?

Few attempts have been made to compile and quantify Chironomidae traits at higher resolutions levels (but see Franquet 1996 for Europe, and Vieira et al. 2006 for North America) and few studies have used Chironomidae traits at these levels (e.g., Van Kleef et al. 2015). As a result Chironomidae traits in Europe are available only at the subfamily or tribe level; whereas for other groups, such as Trichoptera or Ephemeroptera, this information goes up to genus or species level (Gayraud et al. 2003).

Some Chironomidae taxa are often the first colonizers after extreme events, such as droughts or floods (Marziali et al. 2010), which is generally attributed to a suitable set of Chironomidae traits related with life history, size and dispersal together with reproduction and resistance (Oliver 1971, McLachlan 1985, Wotton et al. 1992, Armitage et al. 1995). However few and contradictory information exists for individual taxa regarding these traits (but see Vallenduuk & Moller Pillot 2007, Moller Pillot 2009, 2013).

Not only there is a lack of knowledge on the traits that distinguish Chironomidae taxa but there is also a general unaware about the degree of plasticity of traits displayed by taxa, namely those that are in populations apart. A Chironomidae imago is able to migrate given its relatively good flight ability, although the distance travelled is conditioned by the short duration of this adult stage, determining a relative geographic isolation. Thus, intraspecific differentiation is expected to occur and the same species, widespread across various biogeographic regions, may present different ecological tolerances (Gunderina et al. 2009). Such plasticity varies greatly among taxa, but also according to the habitat, since various aspects such as emergence and hatching respond to different external cues (Langton 1995, Tokeshi 1995a, Prat & Rieradevall 1995). Life history traits may also rapidly change in few generations under environmental stress (e.g., metal contamination; Postma et al. 1995).

This lack of organized information for Chironomidae traits motivates the non-use of Chironomidae information at higher taxonomic resolution, including MTB approaches, perpetuating the omission of potential valuable information enclosed by the family, and justifying a rigorous scrutiny of the freshwater species of the group.

General aims and thesis outline

The main objectives of this thesis were thus: 1) to improve trait information about European Chironomidae traits; 2) to build a European database based on existing data and bibliography; and 3) to test its relevance for bioassessment.

To accomplish these main aims, the following tasks were undertaken:

- Assemble traits related to biological, physiological and ecological characteristics of European Chironomidae larvae at genus and species level, following mostly European literature for different freshwater systems and construction of a database, creating a European Chironomidae trait database (Chapter I).
- Compare the information provided by the new database, for European Chironomidae data at genus and species level, with the one existent for lower levels of taxonomic resolution (subfamily, tribe at most) in a global macroinvertebrate trait database (Tachet el at. 2010) (Chapter II).
- Identify Chironomidae larvae to finer taxonomic levels (genus and species whenever possible) collected in references sites located in two contrasting climatic areas of Portugal (central-north streams with Atlantic climate and south Mediterranean streams) and in least-disturbed and disturbed sites from southern Mediterranean temporary streams.
- Evaluate Chironomidae taxonomic and trait composition in the discrimination of rivers with different typology: permanent (central-north medium elevation streams and lowland streams) and temporary streams (south Mediterranean temporary streams). Test also the potential use of morphological traits in the distinction between these different river types (Chapter III).
- Evaluate Chironomidae taxonomic and trait composition and life history strategies at higher levels of taxonomic resolution in the discrimination of human disturbance (Chapter IV).
- Compare European and North American Chironomidae trait databases, considering the common taxa and exploring the possibility of divergence and intraspecific variability or plasticity between continents (Chapter V).

The accomplishment of these tasks generated the five chapters (I-V) composing this PhD thesis. In Chapter I describes the general methodology that leaded to the elaboration of the European Chironomidae trait database; the first product of this study. For the other four chapters a scientific paper was produced. All papers are currently published or submitted for publication. The trait database at the genus level was provided in the first scientific paper as supplementary material. General integrative conclusions are presented in the end of the thesis, addressing the most relevant findings of the study. Furthermore some suggestions and relevant new questions that emerged during the work and that can be addressed in future research are presented. This PhD thesis is expected to contribute to the knowledge on Chironomidae, encouraging the future integration of more trait data in the developed database by other authors, and the more regular use of genera or species level information of the family in European biomonitoring programs.

Publications

This thesis is based on the following published or submitted publications:

Chapter I and II

Serra S.R.Q., Cobo F., Graça M.A.S., Dolédec S., Feio M.J., 2016. Synthesising the trait information of European Chironomidae (Insecta: Diptera): Toward a new database. Ecological indicators. 61: 282-292. (Providing the developed trait database as supplementary material available online)

Chapter III

Serra S.R.Q., Graça M.A.S., Dolédec S., Feio M.J.. Chironomidae genera and respective traits are relevant to discriminate permanent and temporary rivers. (Submitted to Annales de Limnologie - International Journal of Limnology)

Chapter IV

Serra S.R.Q., Graça M.A.S., Dolédec S., Feio M.J.. Chironomidae traits and life history strategies as indicators of anthropogenic disturbance. (Submitted to Environmental Monitoring and Assessment)

Chapter V

Serra S.R.Q., Graça M.A.S., Dolédec S., Feio M.J.. Chironomidae of Holarctic region: comparison of traits between North America and Europe. (Submitted to Hydrobiologia)

Chapter I:

Building a new European Chironomidae database: general methodology

Chapter I: Building a new European Chironomidae database: general methodology

Chironomidae diversity

The family Chironomidae includes a high diversity of species and genus. Worldwide estimates point to more than 15-20 thousand species of Chironomidae, in Europe the estimates point to more than 1.2 probably around 1.4 thousand species (Armitage et al. 1995, Merrit & Cummins 1996, Carles-Tolrá Hjorth-Andersen 2002, Saether & Spies 2013). A high diversity within this group may be found in various areas: there are for example 202 species known in southern Alpine running waters (Rossaro et al. 2006), 81 species in springs of the Italian Prealps and Alps (Lencioni et al. 2012), and 58 species in the Rhine river (Klink 1989).

There is no European taxonomic database specific for Chironomidae family and it is difficult to know the exact number of existing species. For example, considering Spanish and Portuguese Mainland and Balearic Islands, there are several inventories for Chironomidae: Carles-Tolrá Hjorth-Andersen (2002) mentions a total of 481 species; Iberfauna (2005) mentions just 448 species; whereas Saether & Spies (2013) mentions 498 species. As can be seen these inventories do not necessarily mean more species recorded over time instead reflect some disagreement.

Country-by-country inventories are fundamental for the knowledge of diversity and distribution at wider geographical scales. However the artificial political units defined by countries are in the base of the differentiated investment in ecological biodiversity studies being also dependent on the existence of experts and consistent bibliographic guidance. Chironomidae biodiversity in Great Britain is very well known given the early efforts and accumulated expertise (e.g., Webb 1980, Armitage et al. 1995). While knowledge of Chironomidae Mediterranean fauna, for example, is still very recent, in the 90’s new species first captured and recorded in Spanish mainland were still presented to the scientific community (*Hydrobaenus cranstoni*, Langton & Cobo 1992; *Brillia pudorosa*, Cobo et al. 1995). Additionally Mediterranean regions are characterized by patterns of high richness and endemism for insect taxa given the glacial-interglacial cycles from Pleistocene that guaranteed more stable conditions in southern Europe devastating populations in the north (Laville & Reiss 1992, Blondel & Aronson 1999).

Regarding trait information, European Chironomidae traits are in general available only for some subfamilies and tribes (Tachet et al. 2010), whereas for other groups, such as Trichoptera or Ephemeroptera, this information goes up to genus or species level (Gayraud et al. 2003). The use of family level however, does not allow for a realist analysis of Chironomidae as it disregards the diversity of functions they assume in their habitats and their influence in ecosystem processes.

Few works have attempted to code information on Chironomidae traits at finer taxonomic levels (but see Franquet 1996). Thus the aim of this work was to build a trait database (biological, physiological and ecological aspects) at finer levels of taxonomic resolution for the Chironomidae family of Europe. The development of such trait database for Chironomidae required first gathering a comprehensive list of species and genera present in Europe, and then the definition of the list of relevant traits and categories to be described taking into account the information available in the literature.

Trait compilation

The trait information was compiled following Franquet (1996). The affinity of a taxon to a specific trait category was quantified using the number of references citing the link: taxon-category. The higher the number of references expressing this link the greater the affinity of that taxon to that particular trait category. A taxon may also present affinities to different categories of a trait. If a species does not have affinity to a certain trait category, according to a given reference, the reference is maintained with a zero associated; if another work states the affinity towards that category, an additional work should be found to support one or both; in the final database both citations are maintained allowing the update and a more reasoned choice.

European Chironomidae list

The European Chironomidae list was initially based on various European references (e.g., Illies 1978, Wiederholm 1983, Andersen et al. 2013). Yet, because of the lack of consensus, ultimately the Chironomidae faunistic list was defined following Fauna Europaea database (Saether & Spies 2013), which was developed and is maintained by experts in the area.

The starting point to the construction of the database was then a list containing a total of 194 Chironomidae genus (126 of which can occur in Iberian Peninsula, therefore Spanish and Portugal mainland) and 1262 species (from which 497 recorded in Iberian Peninsula) distributed by 8 different Subfamilies: Buchonomyiinae, Chironominae, Diamesinae, Orthocladiinae, Podonominae, Prodiamesinae, Tanypodinae, and Telmatogetoninae (Saether & Spies 2013).

Literature consulted

The Chironomidae species information found covered a wide geographic area, different categories of water bodies (freshwater lentic and lotic, brackish waters), and different elevations and latitudes. Many Chironomidae taxa have a Holarctic distribution (Cranston & Oliver 1987) however only studies from the European continent and regions around (Palaearctic region) were used in the database to diminish intraspecific variation generated by geographical barriers responsible for isolation and populations divergence. Despite the good flight ability of Chironomidae adults the distance travelled is conditioned by the short duration of the adult stage. This results in a certain degree of geographical isolation which in turn leads to an intraspecific population differentiation that may be reflected in different ecological tolerances of taxa widespread across various biogeographic regions (Gunderina et al. 2009).

Some studies consulted refers to other regions of the world, such as Nearctic or Neotropical areas (e.g., Oliver & Roussel 1983a, Cranston 2000), because they provide reviews for relevant Chironomidae groups from Palearctic. Whenever possible, studies that described first a species (as n .sp.) for Europe were exploited (e.g., Langton & Moubayed 2001). In addition, books such as the Armitage et al. (1995) and Merrit & Cummins (1996) containing general information about the family or from different geographic areas were used only to support and justify the selection of trait variables and categories. In general, the species and genus with a wider distribution in Europe are also the most frequently mentioned in the literature, which means that the species more widely distributed in Europe assumed a higher importance in the characterization of European genus traits, against rare or confined species.

List of traits in the database

The initial trait list was composed by the 21 biological, physiological and ecological traits (119 categories) following the compilation of Tachet et al. (2010) for European freshwater macroinvertebrates. According to Tachet et al. (2010) biological traits include: maximum body size, life cycle duration, number of generation per year, aquatic stage, reproduction type, dispersal, resistance form, feeding habits, food preferences. The physiological traits comprehend: respiration type, temperature and pH preferences, trophic degree, saprobic values, and salinity. And the ecological traits include: elevation, longitudinal and transversal distribution, microhabitat and flow preferences, and mode of locomotion/position relative to substrate. As Chironomidae larvae are usually collected in their last stages of development, traits were gathered for the fourth instar.

In a final stage, various traits from Tachet et al. (2010) were redefined and/or refined by maintaining more or less the same categories or changing categories completely considering the information available for Chironomidae in the literature (e.g., food type, respiration). In addition, 16 new traits, specific for Chironomidae, were added: emergence season; flight period; emergence duration; number of eggs per egg-mass; length of larval development; hibernation phase/instar; distance travelled in aquatic and/or aerial habitat; tube construction; presence/absence of haemoglobin; chlorinity; oxygen saturation preferences; depth preferences; general/gross habitat; optimal temperature of emergence; and type of migration (Table I. 1). Some of these were previously described for Chironomidae by Franquet (1996).

Table I. 1 Traits gathered for the Chironomidae family only, based on available information.

Trait	Number of categories	Categories
Emergence season	4	Winter; spring; summer; autumn
Flight period	4	Winter; spring; summer; autumn
Emergence duration	2	Short period (some hours to few days; <15 days); long period (several days; >15 days)
Number of eggs per egg-mass	4	<100; 100-500; 500-1000; >1000
Length of larval development (months)	9	≤1; 2; 3; 4; 5; 6; 7; 8; ≥9
Hibernation phase/instar (overwinter diapause)	5	Egg; 1st instar; 2nd instar; 3rd instar; 4th instar
Distance travelled in aquatic habitat (m)	4	<10; 10-100; 100-1000; >1000
Distance travelled in aerial habitat (m)	4	<10; 10-100; 100-1000; >1000
Tube construction	3	Tube absent; tube without shape (unorganized, gelatinous); rigid tube (or case)
Haemoglobin	2	Presence; absence
Chlorinity (g Cl $^{-1}$)		<0.3; 0.3-1; 1-3; 3-10; >10
Dissolved oxygen preferences (%)	4	Stable always > 50%; unstable 10-50%; <5% for few hours; rotting (in summer almost daily) <5%
Depth preferences (m)	3	Profundal habitat; Indifferent and/or medium depth; shallower habitats (litoral and sublitoral lakes or rivers)
General/gross habitat	7	Lotic; lentic; creeks and brooks; small streams; large rivers; semi-terrestrial; terrestrial
Optimal temperature of emergence (ºC)	5	≤6; 7-9; 10-12; 13-15; ≥16
Type of migration	2	Horizontal; vertical

Since there are many different types of traits and distinction is not always straightforward (see Violle et al. 2007) the final traits selected were divided in two domains: Eltonian and Grinnellian traits following the terminology of Soberón (2007), Devictor et al. (2010) and Mondy & Usseglio-Polatera (2014) (Table I. 2). Eltonian traits are a proxy to biological traits and are related to the functional role of the taxon in the ecosystem, focusing on its influence in the environment (e.g., emergence season, body size, feeding habits). The Grinnellian traits are related fundamentally with non-interactive (scenopoetic) variables, therefore traits associated to taxon responses to particular resources or environmental conditions, requirements and performance in habitats.

The final list of all traits and respective categories (20 Eltonian traits with 86 categories and 17 Grinnellian traits with 98 categories) can be seen in Appendix Table A1.

Table I. 2 Eltonian and Grinnellian list of traits in the European database.

Eltonian traits	Grinnellian traits
Dispersal	Elevational preferences
Distance travelled in aerial habitat	Chlorinity
Distance travelled in aquatic habitat	Current velocity preferences
Emergence duration	Depth preferences
Emergence season	Food type
Feeding habits	General/gross habitat
Flight period	Longitudinal distribution along stream channel
Haemoglobin	Optimal temperature of emergence
Hibernation phase/instar	Oxygen saturation preferences
Length of larval development	pH preferences
Life cycle duration	Salinity preferences
Maximal body size of the 4th instar larva	Saprobity
Number of eggs per egg-mass	Substrate preferences
Potential number of generation per year	Temperature preferences
Reproduction type	Transversal distribution along stream channel
Resistance forms/habits	Trophic status preferences
Respiration (number of tracheas)	Type of migration
Substrate relation/Locomotion	
Tube construction	
Type of aquatic stages	

Content of the database

Approximately 150 references were considered to describe Chironomidae traits of European genus. The list is presented in Appendix Table A2, and includes publications between 1931 and 2013 and includes mainly scientific papers and books, but also some few PhD theses.

The database contains entries for 178 Chironomidae genera and 744 species distributed among eight subfamilies: Buchonomyiinae, Chironominae, Diamesinae, Orthocladiinae, Podonominae, Prodiamesinae, Tanypodinae, and Telmatogetoninae (listed in Appendix Table A3). This corresponds to ~92% of the total European genera considered, including information for ~59% of species, distributed proportionally to their diversity in each subfamily (Figure I. 1) with just few subfamilies with less information (Podonominae and Telmatogetoninae).

Many European species do not have any trait information in the database. Yet genera with a large number of species generally have entries for more than one species, usually for those species with a wider distribution. Therefore, genera are relatively well characterized attending to the species that represent them in the database, allowing the use of genus level in further studies. The genera (and respective species) without any information, and therefore not included in the database, are listed in Table I. 3.

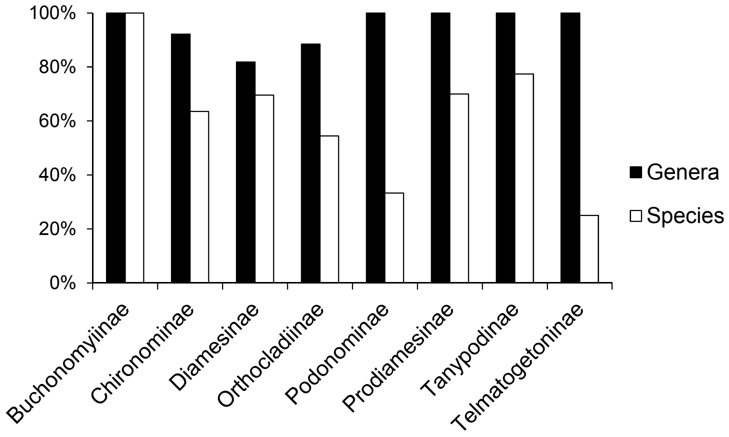

Figure I. 1 Percentage of genera and species from each subfamily incorporated in the database.

Table I. 3 List of genus and respective species not covered by the database.

Family	Genus	Species
Chironominae	*Baeotendipes* *Carbochironomus* *Nilomyia* *Olecryptotendipes* *Synendotendipes*	*Baeotendipes noctivagus* (Kieffer 1911) *Carbochironomus improvisus* Reiss & Kirschbaum 1990 *Nilomyia aculeata* Kieffer 1921 *Olecryptotendipes macropodus* (Lyakhov, 1941) *Synendotendipes abranchius* (Lenz 1955) *Synendotendipes dispar* (Meigen 1830) *Synendotendipes impar* (Walker 1856) *Synendotendipes kaluginae* Durnova 2010 *Synendotendipes lepidus* (Meigen 1830)
Diamesinae	*Arctodiamesa* *Pagastia*	*Arctodiamesa appendiculata* (Lundstroem 1915) *Pagastia orientalis* (Chernovskii 1949)
Orthocladiinae	*Arctosmittia* *Bavarismittia* *Boreosmittia* *Corynoneurella* *Lappokiefferiella* *Molleriella* *Neobrillia* *Prosmittia* *Tavastia*	*Arctosmittia biserovi* Zelentsov 2006 *Bavarismittia reissi* Saether 1995 *Boreosmittia inariensis* Tuiskunen 1986 *Boreosmittia karelioborealis* Tuiskunen 1986 *Corynoneurella paludosa* Brundin 1949 *Lappokiefferiella platytarsus* Tuiskunen 1986 *Molleriella calcarella* Saether & Ekrem 1999 *Neobrillia longistyla* Kawai 1991 *Prosmittia jemtlandica* (Brundin, 1947) *Prosmittia rectangularis* Tuiskunen, 1985 *Tavastia alticrista* Stur & Wiedenbrug 2005 *Tavastia australis* Tuiskunen 1985 *Tavastia yggdrasilia* Bodin, Lundstroem & Paasivirta 2008

The missing genera are represented in Europe by very few species (in many cases only one) and some are confined to European islands or small regions: *Carbochironomus* is represented by only one species from Germany; *Nilomyia* and *Neobrillia*, both with one species, were found in Romania; *Molleriella* appeared in Netherland (Saether & Spies 2013); *Boreosmittia* and *Lappokiefferiella* are both restricted to the North Europe (Finland and Norway); *Bavarismittia* is not clearly defined; and *Arctodiamesa* and *Arctosmittia* are only mentioned in Novaya Zemlya (Russia) by Saether & Spies (2013).

Eltonian traits that have more information for genera include: aquatic stages with information for 97% of the genera and maximal body size of the 4th instar larvae for 94% of the genera (Figure I. 2).

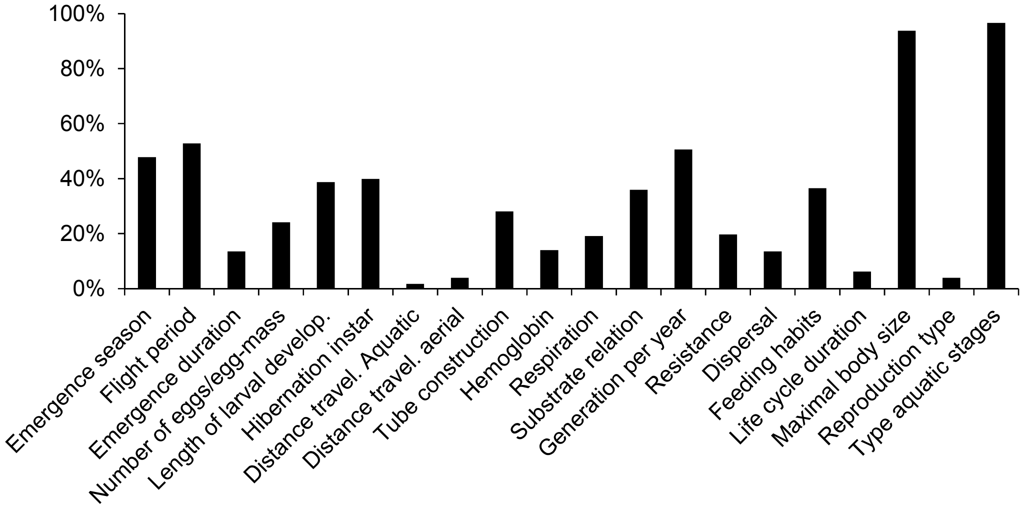

Figure I. 2 Percentage of genera included in the database with entries for each Eltonian trait.

The Grinnellian traits with more information are: general habitat with information for 90% of the genera; and transversal distribution along the stream channel with information for 93% (Figure I. 3). In general, Grinnellian traits have information for more genera.

Traits with information for 50% of the genera or above include 4 Eltonian traits and 11 Grinnellian traits. The 4 Eltonian traits are: flight period, potential number of generation per year (or voltinism), maximal body size and aquatic stages. Grinnellian traits that include more information for the genera include: oxygen saturation, depth, pH, substrate, salinity, elevational and current preferences, general habitat, food type and transversal and longitudinal distribution along stream. Grinnellian traits are in fact those with more information available in literature. This means that less is known on biological and physiological features of Chironomidae taxa. Thus, in the future, these are the characteristics that require more attention, since they are the most commonly used in functional characterization of the systems.

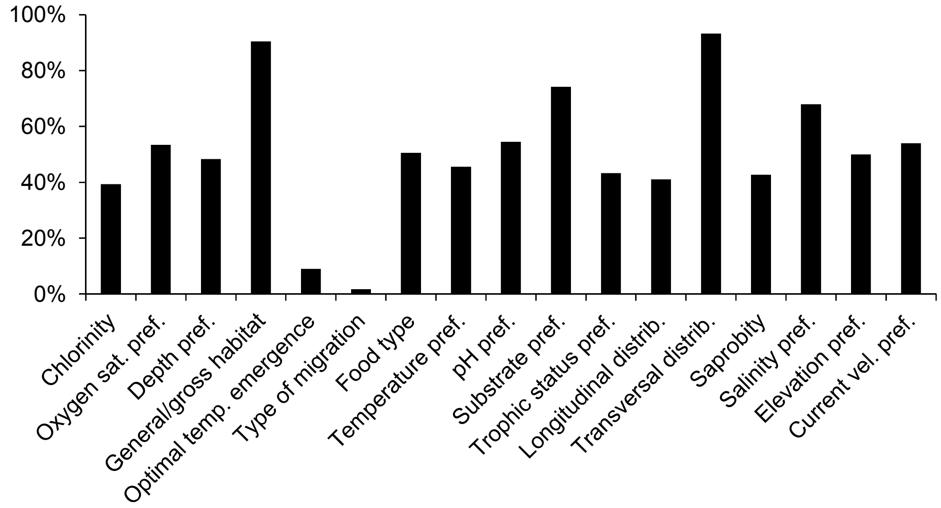

Figure I. 3 Percentage of genera included in the database with entries for each Grinnellian trait.

Finally, the way the database is constructed, with the citations in for each taxon-category link, presents long-term advantages allowing the correction of possible entries or the addition of new information (including taxa) by Chironomidae experts. Therefore its information can easily evolve and be update.

Chapter II:

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

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

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.

Keywords: Chironomidae, fuzzy coding, bioassessment, Eltonian traits, Grinnellian traits.

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 & Blasco-Zumeta 2001, Vallenduuk & Moller Pillot 2007, Moller Pillot 2009, 2013).

Chironomidae richness worldwide is estimated at 20000 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 & Ferrington 1996).

In fresh waters, the Chironomidae family can account for ~50% of the macroinvertebrate community (Armitage et al. 1995, Coffman & 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 & 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 & González 1990, 1991, Lindegaard & Brodersen 1995, Puntí et al. 2009) and according the lake typology (Saether 1979, Brodersen & 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 & 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*, *Orthocladius thienemanni* and *Zavrelimyia melanura*; Vallenduuk & Moller Pillot 2007, Marziali et al. 2010, Moller 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 & Quinlan 2006, Marziali et al. 2010, Prat et al. 2013) or low levels of dissolved oxygen (e.g., *Procladius* sp. and *Eukiefferiella claripennis*; Bazzanti & 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 & 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 (Dolédec & Statzner 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 (Archaimbault et al. 2005, Culp et al. 2011, Feio & Dolédec 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).

Methods

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 & Moller Pillot 2007, Moller Pillot 2009, 2013) we followed the Fauna Europaea database (http://www.faunaeur.org/ see Saether & Spies 2013) with 194 and 1261 genera and species entries distributed among eight subfamilies: Buchonomyiinae, Chironominae, Diamesinae, Orthocladiinae, Podonominae, 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 elevations 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 (Appendix Table A1). A set of 16 additional traits specific to Chironomidae larvae included emergence season, flight period, emergence duration, number of eggs per egg mass, length of larval development, hibernation phase/instar, distance travelled in aquatic and/or aerial habitat, tube construction, presence/absence of haemoglobin, oxygen saturation preferences, chlorinity, depth preferences, optimal temperature of emergence, general/gross habitat, and type of migration (see Appendix Table A1 and

Table I. 1 for the 16 additional set of Chironomidae traits). 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 & 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.

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 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 & Chessel 1987, Ter Braak 1988) and tested its significance against simulated values obtained after 999 permutations of the rows of the trait-composition arrays.

Chironomidae subfamily trait relatedness

FCA 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 & Nei 1987, Studier & 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. 2007a,b) and 'ape' libraries (Paradis et al. 2004, Paradis 2012) implemented in R freeware (R Core Team 2015).

Results

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 II. 1 and see in the information supplied as supplementary data in

Serra et al. 2016). 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 Appendix Table A1). 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, elevational, 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.

Table II. 1 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	

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*, *Lappokiefferiella*, *Molleriella*, *Neobrillia*, *Prosmittia*, *Tavastia*, *Baeotendipes*, *Carbochironomus*, *Nilomyia*, *Olecryptotendipes*, *Synendotendipes* (see Table I. 3).

The distribution of trait categories varied greatly across subfamilies for some traits (food types, salinity preferences, longitudinal distribution; Figure II. 1a-c), whereas for other traits, all Chironomidae behaved in a very similar way (e.g., elevational preferences, pH tolerance, number of generation per year; Figure II. 1d-f).

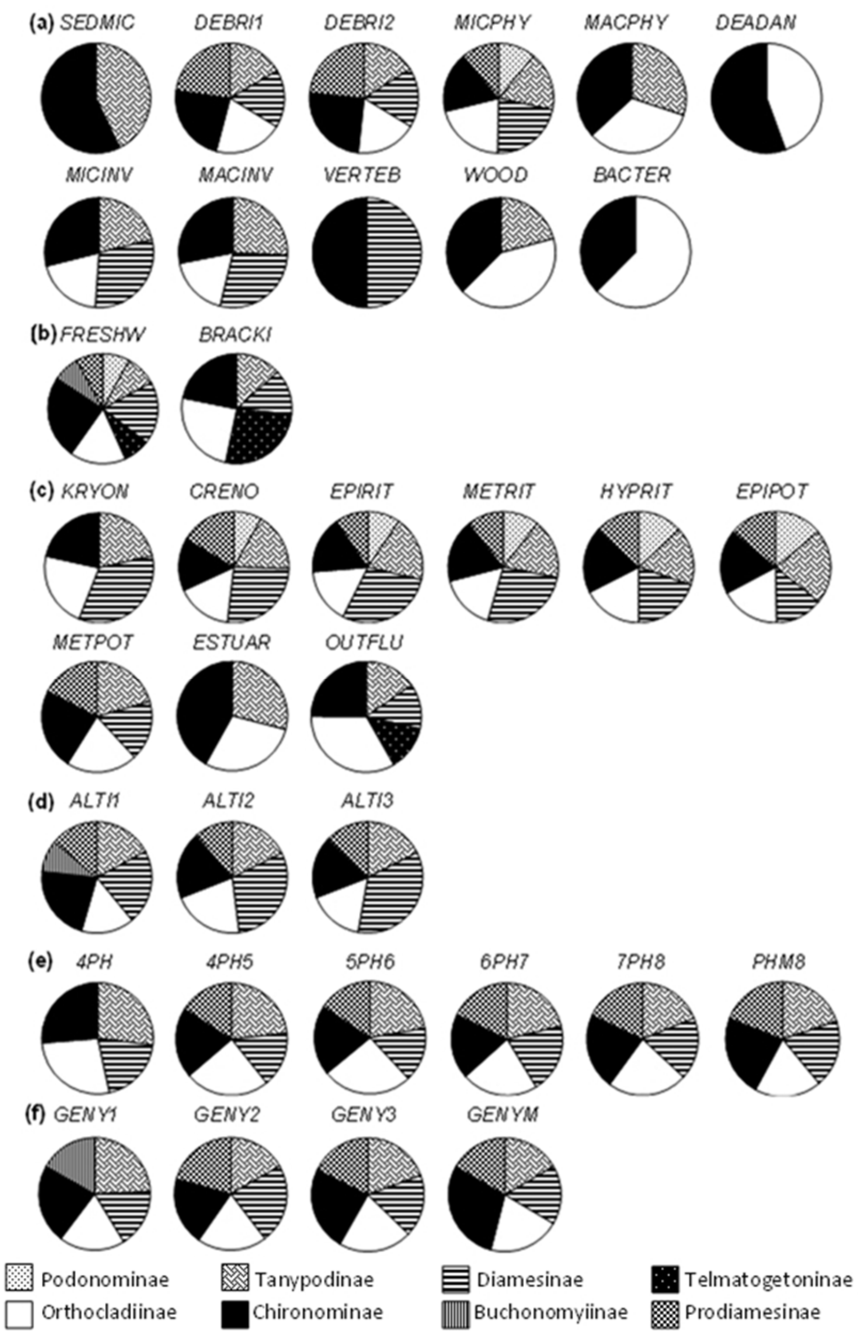

Figure II. 1 Proportions of subfamilies for selected traits and respective categories (see Appendix Table A1 for acronyms) with: (a) food type; (b) salinity preferences; (c) longitudinal distribution; (d) elevational preferences; (e) pH tolerance; and (f) number of generations per year.

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 elevations 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 <15ºC.

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 one generation per year, whereas Prodiamesinae are characterised by at least two generations per year, and Chironominae have higher affinities for more than three generations per year (Figure II. 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 Figure II. 2. For example, Chironominae (Chrn; Figure II. 2) and Diamesinae (Dmsn; Figure II. 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; Figure II. 2) whereas *Kloosia* sp. and *Lauterborniella* sp. had high affinities to small size categories (SIZE 2; Figure II. 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 (DEBRI1 and 2; Figure II. 2) and live microphytes (MICPHY; Figure II. 2). Within the Diamesinae subfamily, despite its lower 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; Figure II. 2), whereas *Protanypus* sp. showed larger sizes (SIZE 4; Figure II. 2). Considering food types, *Diamesa* sp. generally consume living microphytes such as diatoms (MICPHY; Figure II. 2) whereas *Potthastia* sp. feed on detrital particles (DEBRI1 and 2; Figure II. 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).

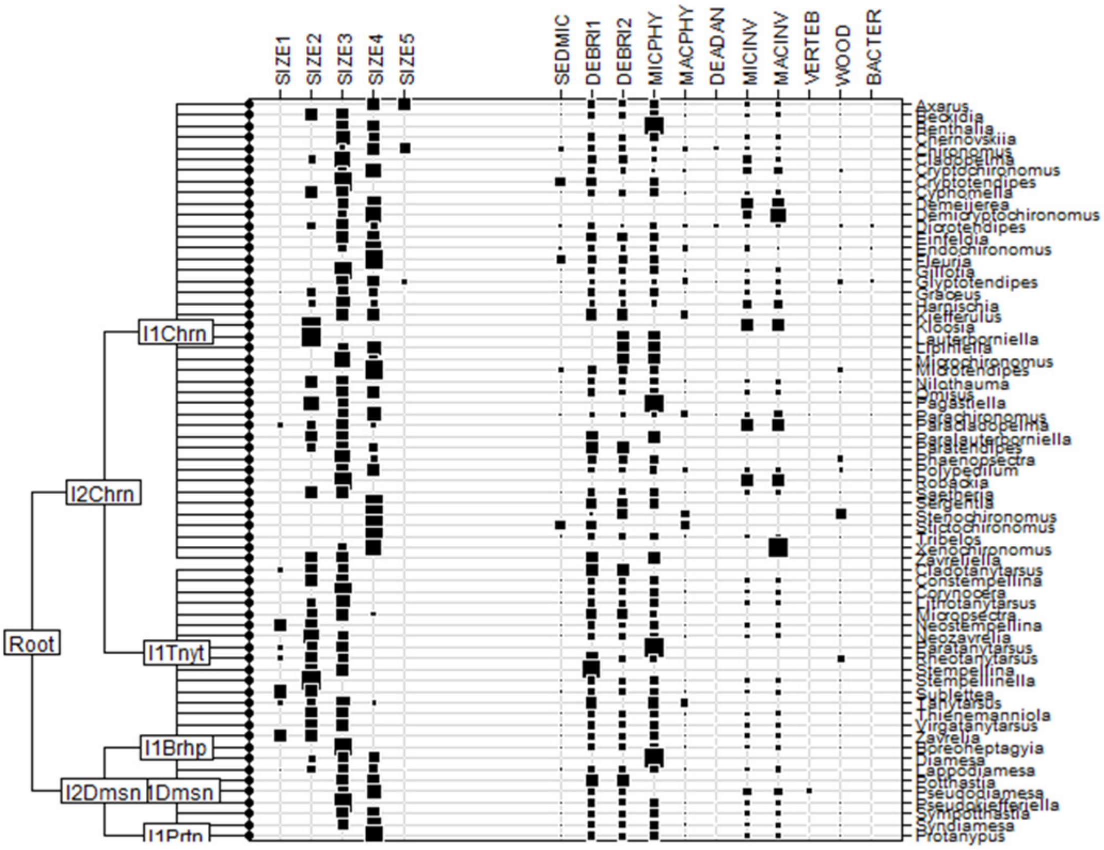

Figure II. 2 Representation of two traits (maximal body size of the 4th larval instar and food type) and their categories (see Appendix Table A1 for acronyms) for two subfamilies (Chironominae and Diamesinae). The information is presented against a taxonomic tree with two taxonomic levels: l1 (tribe) and l2 (subfamily). Chironominae (l2 Chrn) are represented in the top leaf of the cluster by two tribes (Chironominii, l1 Chrn; and Tanytarsinii, l1Tnyt). Diamesinae are represented in the below leaf by three tribes (Boreoheptagyiini, l1 Brhp; Diamesini l1 Dmsn; and Protanypodini, l1 Prtn). The size of each square is proportional to the frequency of the corresponding trait category (on top) for a given genus (at right).

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* = 0.001; Figure II. 3a, Table II. 2). 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), whereas elevational 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).

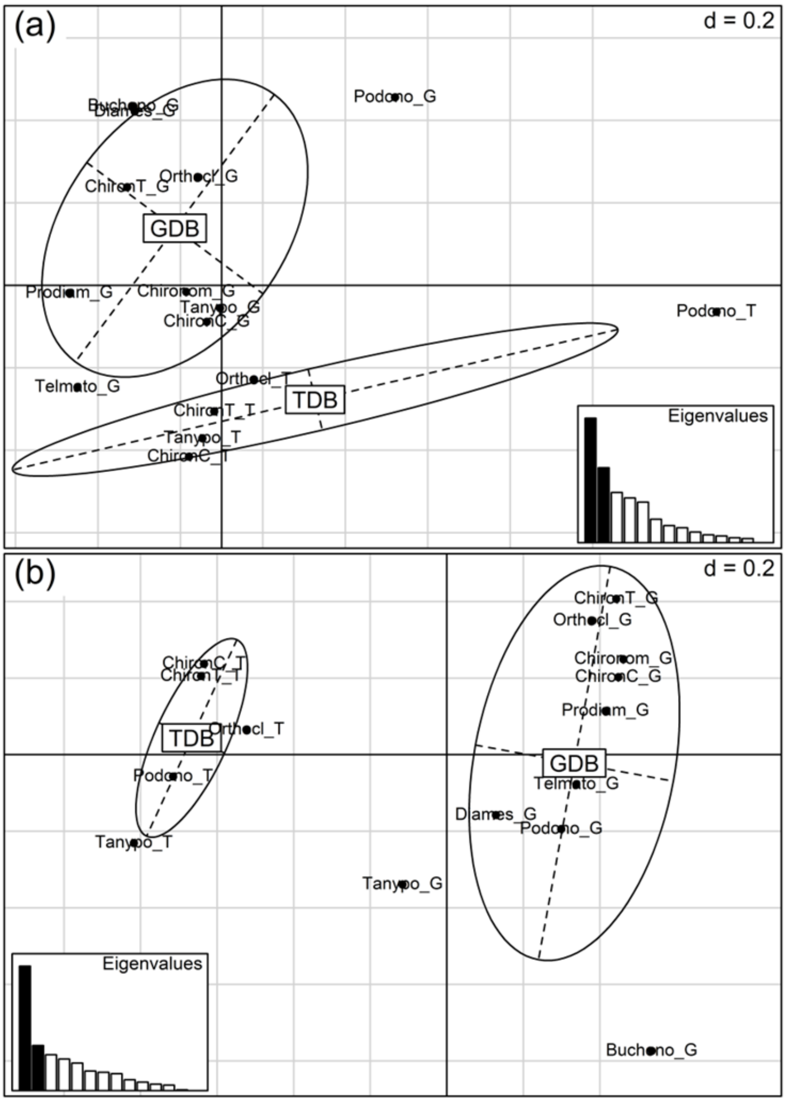

Figure II. 3 Fuzzy correspondence analysis (FCA) plot performed on: (a) Grinnellian traits; and (b) Eltonian traits of the two databases; considering the information existing at the subfamily level and few tribe levels for Chironominae. The information is grouped by database with GDB, the European database developed using the genus information averaged at the subfamilies and tribe levels; and TDB, the database from Tachet et al. (2010) at the subfamily and tribe level. Subfamilies are identified by their first five letters; tribe levels are identified by first four letters corresponding to subfamily plus one letter for the corresponding tribe (Bucho– Buchonomyiinae; Chiro– Chironominae; ChirC– Chironomini; Diame– Diamesinae; Ortho– Orthocladiinae; Podon– Podonominae; Prodi– Prodiamesinae; Tanyp– Tanypodinae; ChirT– Tanytarsini; Telma– Telmatogetoninae); followed by _G for GDB plot, or _T for the TDB plot. Ellipses include 80% of the points for readability.

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

Table II. 2 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 preferences	0.079	0.131
pH preferences	0.050	0.112
Trophic status preferences	0.009	0.068
Saprobity	0.091	0.014
Salinity preferences	0.043	0.030
Elevational preferences	0.134	0.007
Longitudinal distribution	0.183	0.052
Transversal distribution	0.398	0.271
Substrate preferences	0.319	0.081
Current velocity preferences	0.059	0.050
Food type	0.135	0.086
Eigenvalues	0.137	0.082

Table II. 3 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**
Maximal body size	0.043	0.066
Life cycle duration	0.334	0.035
Voltinism	0.164	0.428
Type of aquatic stages	0.019	0.043
Reproduction type	0.756	0.084
Dispersal	0.066	0.024
Resistance forms/habits	0.792	0.017
Feeding habits	0.139	0.099
Respiration	0.004	0.000
Substrate relation/Locomotion	0.158	0.102
Eigenvalues	0.248	0.090

Chironomidae subfamily trait relatedness

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

The accuracy of the tree assessed through the bootstrap analysis give confidence to the group formed by Orthocladiinae 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.

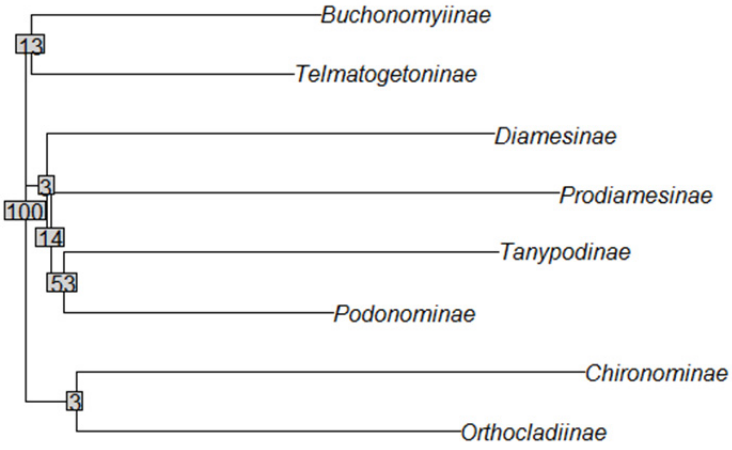

Figure II. 4 Trait relatedness tree estimated among Chironomidae subfamilies using neighbour-joining, given the Euclidean distances of their FCA coordinates. Values associated to each node represent the percentage of partitions present in bootstrap trees.

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 & Norris 2000, Rabeni & Wang 2001). Some authors fully agreed with the family-level and its ability to detect impairment (Móra et al. 2008), whereas 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 & 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* spp.) and sensitive (e.g., *Diamesa* spp.) taxa to human impacts (Armitage et al. 1995, King & Richardson 2002, Lencioni et al. 2012). One main problem for bioassessment purposes arises from the difficulties 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 & Holopainen 1995).

A total of 16 European genera belonging to Diamesinae, Orthocladiinae and Chironominae subfamilies lacked information (Serra et al. 2016 supplementary material). The inability to characterise these genera may be due to their limited distribution so far (e.g., *Molleriella*, *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 gathered.

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 & Norris 2000, King & Richardson 2002).

It is common to find autoecological information at the subfamily level mentioning faunistic patterns along environmental gradients (e.g., longitudinal, elevational) such as the greater abundance of Diamesinae and Orthocladiinae upstream, giving place to Tanypodinae and Chironominae downstream (Prat et al. 1983, Bitušík 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 & 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 & Moller 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. (2000a) 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; Figure II. 5a) and molecular phylogeny studies (Cranston et al. 2010, 2012; Figure II. 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.

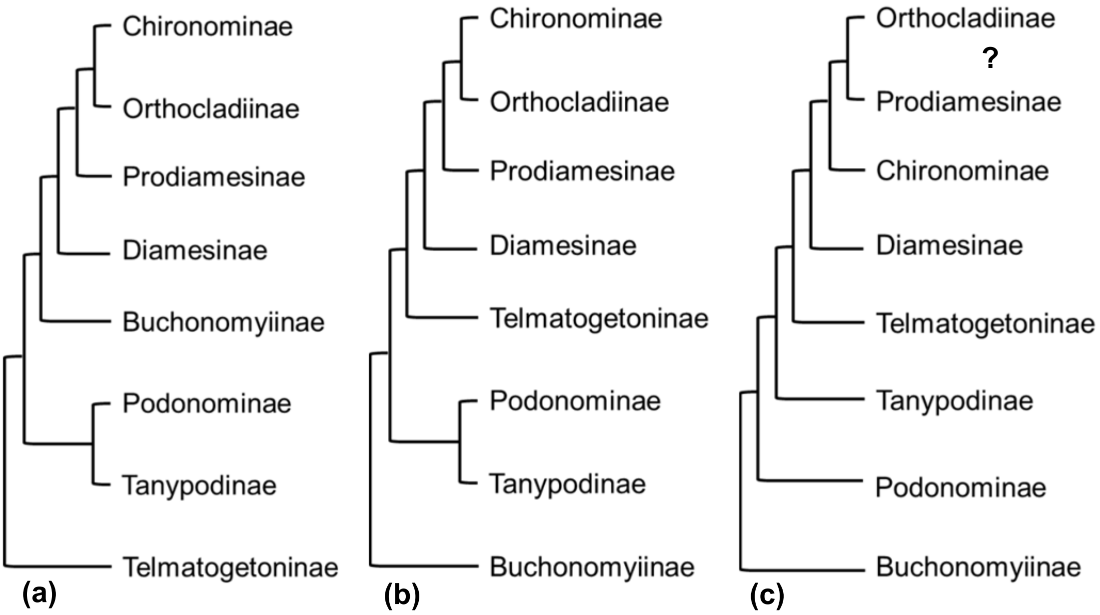

Figure II. 5 Subfamily relationships among Chironomidae subfamilies given by: (a) cladistic analyses using parsimony of morphological characters of adults, pupae and larvae(Saether 2000); and (b) and (c) molecular phylogenies by Cranston et al. (2010, 2012), respectively.

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.

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 subfamily 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.

Supplementary material: We provided data in Serra et al. (2016) that correspond to the Chironomidae trait database at the genus level. The database gathers information for a total of 178 Chironomidae genera from 744 species information distributed among eight subfamilies: Buchonomyiinae, Chironominae, Diamesinae, Orthocladiinae, Podonominae, Prodiamesinae, Tanypodinae, and Telmatogetoninae. Information was gathered from ca. 150 references that are listed in the database file. The affinities in the trait database are indicated by the bibliographic references that mention the attribute of the taxa.

Chapter III:

Chironomidae genera and respective traits are relevant to discriminate permanent and temporary rivers

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Abstract

Chironomidae taxa are diverse and present a wide variety of ecological preferences. Thus, they have a high potential in the establishment of reference conditions for rivers bioassessment and in providing functional information, especially when other macroinvertebrate are poorly represented. Yet, prompted by a taxonomic difficulties and poor knowledge of traits, they are neglected in bioassessment programs and kept at coarser taxonomic levels, reducing discrimination power. Here, we compared the efficiency of Chironomidae at subfamily and genus levels and their biological traits (Eltonian and morphological) in the distinction between permanent (medium elevation and lowland streams) and Mediterranean temporary streams. We established a priori predictions on the expected Chironomidae trait categories in each stream type, conferring the best adaptations to their particular environmental constraints. Genus composition (not subfamily) and respective trait categories differed among the 3 stream types. Both Eltonian and morphological traits identified differences between stream types. Among Eltonian traits, emergence season and overwinter diapause segregated permanent medium elevation from lowland and temporary streams, reflecting adaptations to temperature and flow regime variations. Substrate relation and size distinguished temporary from the permanent streams, interpreted as an adaptation to unstable sediments and irregular flow regime. Morphological traits associated to mobility and foraging (e.g., body setae, Lauterborn sensory organs) discriminated temporary from permanent streams, reflecting differences in temporal and spatial heterogeneity. These morphological characteristics offer alternatives to the use of Chironomidae traits in bioassessment and ecological studies, relying on the observation of few characteristics and dismissing high taxonomic expertise.

Keywords: Diptera, traits, flow regime, bioassessment, reference conditions.

Introduction

Climate, geomorphology and hydrological regime influence water quality, quantity of food resources, dominant substrate, temperature, and dissolved oxygen available in space and time. As a result, stream and river habitats will naturally support a particular diversity of benthic macroinvertebrate communities, with specific structure and functions (Poff 1996, Heino et al. 2007). Evaluating their biological and ecological integrity requires accounting for this natural variance, according to the Reference Condition Approach

(Reynoldson et al. 1997). For this reason, grouping rivers and streams with similar environmental and biological characteristics into river types, is the basis of ecological monitoring programs in Europe (Water Framework Directive; European Commission 2000).

Headwater temperate streams, with well oxygenated waters and diverse habitats usually support high macroinvertebrate diversity, including Ephemeroptera, Plecoptera and Trichoptera (EPT), which are widely used bioindicators (Karr 1991, Rosenberg & Resh 1993). In contrast, rhithral habitats are usually restricted in temperate lowland streams, resulting in communities that are naturally deprived in EPT (Allan 1995, Hawkins & Norris 2000, Harrison et al. 2004), which does not necessarily mean poor water quality. Mediterranean rivers are highly seasonal in terms of discharge, resulting frequently in flow intermittence. Macroinvertebrates must have adaptive mechanisms and behaviours to survive drought and floods (desiccation-resistance and dispersal). Here EPT taxa are uncommon while Odonata, Coleoptera, and Heteroptera are more frequent (Bonada et al. 2007b).

Independently of the river type, Chironomidae (Diptera) are always present as one of the most conspicuous and ecological diverse invertebrates. However, the ubiquity of this family is normally confounded with a high tolerance to pollution and other pressures (e.g., *Chironomus* spp.; Armitage et al. 1995), while in fact this family includes very sensitive taxa, as the *Diamesa* spp., *Zavrelimyia* spp. and *Stilocladius montanus* (Brown et al. 2007, Lencioni et al. 2012, 2013). Nevertheless, they have been neglected as environmental indicators in running waters because of their difficult identification, not being considered at higher levels of taxonomic resolution in regular biomonitoring programs (Wymer & Cook 2003, Calle-Martínez & Casas 2006, Móra et al. 2008). For that reason they are often identified to family or subfamily levels in community ecology or bioassessment studies. Such practice may result in the failure to detect ecological differences especially in systems naturally low in EPT taxa where Chironomidae can be diverse (Wymer & Cook 2003, Greffard et al. 2011). For example in Mediterranean streams, Chironomidae at genera or species level distinguished siliceous headwaters, middle elevation streams, calcareous and temporary streams (Puntí et al. 2007, 2009).

As an alternative and complement to taxonomy-based approaches, multiple-trait based (MTB) approaches have been increasingly used in the assessment of river and stream integrity (Statzner et al. 2008, Dolédec & Statzner 2010, Feio & Dolédec 2012). MTB approaches cannot only reveal the cause of impairment but also potentially offer a functional perspective of this impact, unravelling mechanisms beneath the structuring of stream communities (Usseglio-Polatera et al. 2000b, Bady et al. 2005, Devin et al. 2005, Statzner et al. 2005).

Eltonian traits are related to organisms’ functional role and their impact in the ecosystems including life cycle aspects, physiological and behavioural characteristics (Serra et al. 2016). In addition to biological traits common to all benthic macroinvertebrates, Chironomidae have also morphological - specific traits, such as: body setae, type of antennae and Lauterborn organs related to feeding, defence and locomotion. Previous studies with terrestrial organisms have shown relation between morphological traits and species in the environment (Makkonen et al. 2011, Astor et al. 2014).

Here we evaluate the relevance of Chironomidae genera, Eltonian (Table III. 1) and morphological traits (Table III. 2, Figure III. 1) in the segregation of stream types (permanent, medium elevation and lowland; and temporary). We hypothesize that Chironomidae taxonomic and trait information at genus level, without considering another macroinvertebrate component, will show a clear segregation between different stream types, unlike level subfamily, due to the large number of genera with different ecological preferences included in the family. In addition, for each stream type, we predicted the trait categories that are expected to be favoured attending to the dominant habitat characteristic and explain the respective the rationale in Table III. 3.

Table III. 1 List of Eltonian traits, their categories and codes used the analyses.

Traits	Trait categories	Code
Emergence season	Winter	EMWINT
	Spring	EMSPRI
	Summer	EMSUMM
	Autumn	EMAUTU
Emergence period [a]	Short period (hours to few days; <15 d.)	EDSHORT
	Long period (several days; >15 d.)	EDWIDE
Life cycle duration[a]	≤1 year	LCEQ1
	>1 year	LCMO1
Flight period[a]	Winter	FLYWINT
	Spring	FLYSPRI
	Summer	FLYSUMM
	Autumn	FLYAUTU
Reproduction type[a]	Eggs in clutches	CLUTCH
	Asexual reproduction	ASEXU
Number of eggs per egg-mass[a]	≤500	EGGMAS1
	>500	EGGMAS2
Length of larval development	Always short (≤ 3 months)	DEVLARVS
	Longer (>3 months)	DEVLARVI
Overwinter diapause/ Hibernation stages	Egg	DIAEGG
	≤ 2 larval stages	DIA2IN
	≥ 2 larval stages	DIAMIN
Tube construction	Tube absent	TUBNON
	Tube without shape, unorganized	TUBUNO
	Tube rigid	TUBRIG
Respiration type (tracheas)	12 tracheas	TRACH1
	6 tracheas	TRACH2
	3 tracheas	TRACH3
Haemoglobin[a]	Present	HBPRES
	Absent	HBNONE
Substrate relation	Free living	FREELV
	Burrower	BURROW
	Miner	MINER
	Fixed (substrate or plants)	FIXED
Number of generations per year/ Voltinism	1	GENY1
	2	GENY2
	3	GENY3
	>3	GENYM
Resistance forms and habits to avoid desiccation[a]	Cocoons	RFCOC
	Resistant instars	RFINST
	Diapause or quiescence	RFDIAP
	Deeper penetration in substrate during dryness	RFSUB
Dispersal[a]	Passive	PASSIV
	Active	ACTIV
Feeding habits	Fine sediment eater	DEFEE
	Shredder	SHR
	Scraper, grazer	SCR
	Filter	FFEEDT
	Predator	PRED
	Symbiotic life	SIMB
Maximal body size of the 4th larval stage (mm)	<2.5	SIZE1
	>2.5-5	SIZE2
	>5-10	SIZE3
	>10-20	SIZE4
	>20-40	SIZE5

[a] Traits not used in further analysis.

Table III. 2 List of morphological traits, their categories and codes used in the analyses (see details on morphological traits in Figure III. 1).

Traits	Categories	Code
Body setae	Setal tufts in abdomen or lateral fringe of swim-setae	TUFFR
	Body setae pale and indistinct	SIND
	Simple long body setae	SETP
	Seta absent at least on abdomen	SETA
Mentum	Simple or absent	SABS
	Double-walled plate without teeth	DNOT
	D-w plate with ≤13 teeth	D13T
	D-w plate with >13 teeth	DTM
Type of Antenna/length	Retractable into head capsule	ARETR
	Less than ½ head capsule	AHALF
	At least ½ head capsule	ALONG
Lauterborn organs	Indistinct	LOI
	Small, much shorter than flagellum	LOS
	Moderate to large, if small in pedicel longer than flagellum	LOL
Premandible brush	Present	PMBP
	Absent	PMBA
Anal Tubules	Present	ATUP
	Absent	ATUA
Claws of posterior parapods	All Simple	CPSIM
	Some larger with fine spinules/spines	CPSPI
	At least some serrated	CPSER
Claws of anterior parapods	All Simple	CASIM
	Some larger with fine spinules/spines	CASPI
	At least some serrated	CASER
Procercus	Shorter than wide	PROCS
	Longer than wide	PROCL

Table III. 3 Trait categories predicted to be favored in each stream type and the rationale associated (N1, permanent medium elevation; L, permanent lowland; S1, temporary Mediterranean).

	Stream Types			
	Permanent		Temporary	
Traits	**N1**	**L1**	**S1**	**Rationale**
Emergence season	Summer	All year	Spring and summer	When temperatures are sufficiently high (Armitage et al. 1995).
Emergence period	Short period	Long period	Short period	In short periods when temperatures are not so cold at higher elevations. In the short periods between disturbances.
Life cycle duration	Long	Short and long	Short	Shorter cycles after disturbances (Bonada et al. 2007a).
Reproduction type			Asexual	Better resilience after disturbances through asexual reproduction (unnecessary presence of a sexual partner) (Bonada et al. 2007a).
Length of larval development	Long	Short	Short	Temperature is a major controlling factor in larval growth together with food availability (Armitage et al. 1995).
Overwinter diapause/ Hibernation stage	Higher number of stages with diapause	Lower number of stages with diapause	Higher number of stages with diapause	Photoperiod and temperature control larval diapause influencing timing of emergence which in colder streams is much more limited (Tokeshi 1995a). A diapause can also benefit Chironomidae in unstable environments.
Tube construction	Tube absence	Tube (without shape and rigid)	Tube (without shape)	Chironomidae can more easily obtain oxygen from water by undulating their bodies within their silken tubes or substrate burrows (Armitage et al. 1995). Rigid tubes can prevent predation where substrate offers few refuges.
Respiration type (tracheas)	Lower number of tracheas	Higher number of tracheas	Higher number of tracheas	Lowers levels of dissolved oxygen will require higher efficiency in obtaining but also distribution of oxygen in the body by the abdominal tubules; tracheal gills (Cranston 1995b).
Haemoglobin	Absence	Presence	Presence	Levels of dissolved oxygen are expected to be higher in N1 streams, where riffles are more common. Haemoglobin in Chironomidae have ‘higher affinity’ to oxygen allowing some Chironomidae to live in low oxygen environments (Armitage et al. 1995).
Substrate relation	Free living and fixed	Burrowers	Burrowers, free living	See tube construction. Smaller substrate favours burrowers. Pools favour free living swimmers (Bonada et al. 2007a). Permanent action of flow favours attachment.
Voltinism	Lower number of generations y^{-1}	Higher number of generations y^{-1}	Higher number of generations y^{-1}	The number of generation is known to vary, less than 1 generation per year was mainly reported for species inhabiting cold Holarctic regions (Tokeshi 1995a).
Resistance forms/habits			Cocoons, deeper penetration in substrate	Resistant forms and behaviours against dryness should allow Chironomidae to face severe droughts.
Dispersal	Aquatic passive	Aquatic passive and active	Aerial active	Flow cessation favour flying (Bonada et al. 2007a, Feio & Dolédec 2012); whereas continuous flow favours aquatic dispersion which in low current zones may even be active.
Feeding habits	Shredder	Fine-sediment eater	Scraper and fine-sediment eater	Higher input of large litter in higher elevation streams. Abundant periphyton algae accessible by scraping if substrate is available and small organic particulate matter collected from sediment in unstable substrate (Bonada et al. 2007a, Feio & Dolédec 2012).
Maximal body size of the 4th larval stage	Medium	Medium to large	Small and large	Permanent action of flow forces excludes very large sizes; whereas slow flow and pools allow large sizes. Higher resilience of smaller sizes after disturbances in temporary streams (with floods or droughts) (Bonada et al. 2007a, Feio & Dolédec 2012).

Methods

Study area, selection of stream types and sites

The study sites were located in two contrasting climatic areas of the Portuguese territory: a northern area under the influence of Atlantic-temperate climate and southern area under the influence of Mediterranean climate. In the northern Atlantic-temperate climate region two types of permanent streams were studied: N1, medium elevation streams, located in the Mondego and Vouga catchments; and L, lowland streams, located in the Lis and Tagus catchments. N1 streams were characterized by elevations lower than 600 m a.s.l., (Mean± SD: 343± 223 m a.s.l.), low mean annual temperatures (12-13 ºC), relatively high mean annual precipitation (1193± 352 mm) and siliceous lithology (acid rocks, igneous nature). The permanent L type included low elevations streams (44± 44 m a.s.l.) characterized by relatively high mean annual temperatures (15 °C), low annual rainfall (941±118 mm) and mixed geology (limestone and siliceous nature). Under the Mediterranean climate, samples were collected in south temporary streams (S1) in the Guadiana River catchment. These streams were located at low elevations (183± 75 m a.s.l.), in an area with high annual temperatures (16ºC), dry summers and with low and irregular winter precipitation (628± 86 mm), and mixed geology (limestone and siliceous nature). A total of 25 benthic macroinvertebrate samples were collected from the selected stream types during springs of 2011 and 2012: 10 in N1, 8 in L and 7 in S1; sites were characterized according to abiotic variables presented in Table III. 4. All sites were considered Least Disturbed (Stoddard et al. 2006, Feio et al. 2014) considering the water quality, hydromorphological conditions and land use.

Table III. 4 Abiotic variables recorded in each site and transformation applied in the Principal Components Analysis.

Variables	Units	Transformation
Latitude	WGS-84 *decimal* coordinates	ln(x+1)
Elevation	m	ln(x+1)
Conductivity	uS cm^{-1}	ln(x+1)
mean annual Precipitation [a]	mm	ln(x+1)
mean annual Temperature [a]	ºC	ln(x+1)
Drainage area	km^2	ln(x+1)
Lithology (categorical)[a]	Sedimentary (1) Sedimentary+Metamorphic (2) Plutonic (3)	square root

[a] Data from Portuguese Environmental Agency (2007) available at http://sniamb.apambiente.pt/Home/Default.htm

Sampling collection, processing and mounting

Benthic macroinvertebrate samples were collected in the study sites with a kick net (500 µm mesh size; 0.25 m × 0.25 m opening) following a multi-habitat protocol (INAG 2008). Each sample was fixed with formalin (4%). Chironomidae larvae were separated and preserved in ethanol (70%). Chironomidae individuals were then divided into morphotypes and counted under a stereomicroscope. Then, larvae were digested in caustic potash (KOH, 10%) at 85 ºC for about 15-20 minutes and then washed for 5 minutes in distilled water removing the muscle and soft tissue. Afterwards they were dehydrated for 3 minutes in 70% ethanol, followed by 3 minutes in 96% ethanol. Finally, heads and bodies were mounted separately in slides with Euparal medium (according to Andersen et al. 2013) and identified to the lowest possible taxonomic level under the microscope (400-1000× magnification), according to the guides for Palearctic/Holarctic Chironomidae taxa (e.g., Cranston 1982, Lencioni et al. 2007, Andersen et al. 2013). The collection of Chironomidae from Mediterranean rivers and an existing preliminary key from Prat and Rieradevall supported the identification (Prat & Rieradevall 2014).

Trait composition

Morphological traits were analysed in an exploratory way, since functional morphology of Chironomidae is still mostly unfamiliar. Each identified genera was characterized according to morphological traits. General larval morphology important in the distinction of each morphological trait is shown in Figure III. 1. The morphological traits selected were those related to potential functional role of Chironomidae in the ecosystem: structures such as the mentum, premandibles, antennae, Lauterborn organs that articulate with the head capsule (Figure III. 1a,b,e-g), involved in feeding and stimuli perception (Cranston 1995b); body setae, claws of parapods, and procercus (Figure III. 1b,d), related with locomotion, substrate relation and feeding behaviour of individuals (Coffman & Ferrington 1996); and anal tubules were related with active salt absorption (Cranston 1995b).

Eltonian trait information was based on Serra et al. (2016) trait database. For both types of traits, the affinity of a taxon to a given trait category was quantified following Franquet (1996): the higher the number of references in European literature associating a taxon to a trait category, the greater is its affinity. Affinity scores given trait categories were converted into: '0 no affinity, '1' low, '2' medium, '3' high affinity. Trait affinity scores were standardized to sum 1 for each taxon-trait combination, following the fuzzy coding procedure (Chevenet et al. 1994), ensuring that all taxa have the same weight in further analyses. Traits Eltonian with missing values for more than 50% of

Chironomidae taxa present in samples were not used in further analyses (marked in Table III. 1) whereas all morphological traits had information for at least 50% of the Chironomidae taxa. The trait profiles of sites were obtained by computing the cross-product between Chironomidae taxa abundance (transformed in ln[x+1]) and their traits (Eltonian and morphological traits separately). This cross product was then rescaled by trait to get the proportion of individuals that have a given trait category in a given site. This resulted in a trait abundance matrix that allowed comparing the trait composition of sites across stream types.

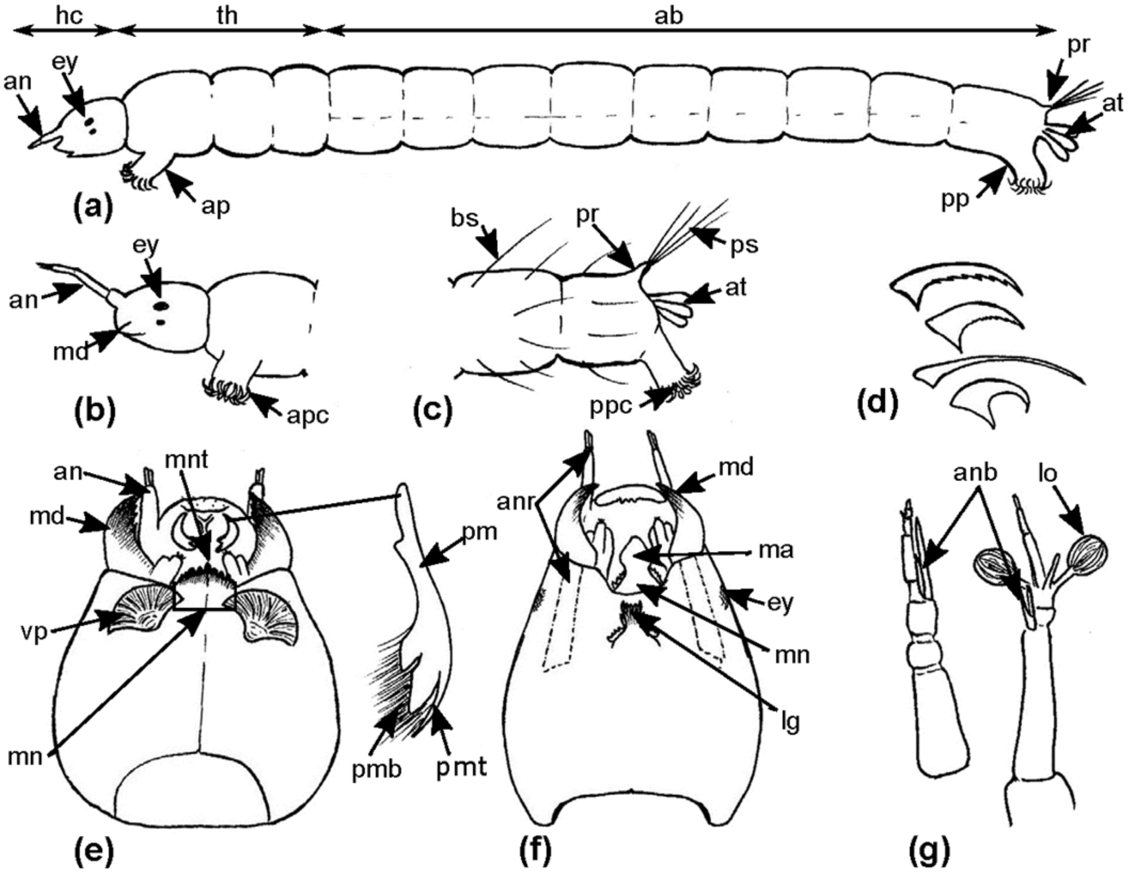


Figure III. 1 General larval morphology of Chironomidae: (a) Body structure, lateral view; (b) Anterior part of the body, lateral view; (c) Posterior part of the body, lateral view; (d) Different types of claws of parapods; (e) Head capsule of Chironominae, ventral view and Premandibule; (f) Head capsule of Tanypodinae, ventral view; (g) Antenna types with and without Lauterborn organs. **ab**, abdomen (9 body segments); **an**, antenna; **anb**, antennal blade; **anr**, antenna retractable; **ap**, anterior parapods; **apc**, anterior parapod claws; **at**, anal tubules; **bs**, body seta; **ey**, eyespot; **hc**, head capsule; **lg**, ligula of the prementum; **lo**, Lauterborn organ; **ma**, M-appendage; **md**, Mandible; **mn**, mentum; **mnt**, mentum median tooth; **pm**, premandible; **pmb**, premandible brush; **pmt**, premandible apical tooth; **pp**, posterior parapods; **ppc**, porterior parapod claws; **pr**, procercus; **ps**, procercal seta; **th**, thorax (3 body segments); **vp**, ventromental plate.

Data analyses

A Principal Component Analysis (PCA) was performed on environmental variables after their transformation for normality (Table III. 4) to confirm the differences between stream types. In addition, Permutational Univariate Analysis of Variance (PERMANOVA using 999 permutations) was used to test for the statistical significance of the differences (permanent N1, permanent L and temporary S1) (PRIMER 6 + PERMANOVA package).

With Chironomidae composition, the non-metric multidimensional scaling analysis (NMDS) was used (ln[x+1] transformation; Bray-Curtis similarity coefficient) to depict differences among stream types (permanent N1, permanent L and temporary S1). Similarly to above, the statistical significance of differences was determined with a PERMANOVA (999 permutations). NMDS and PERMANOVA were executed considering 2 different levels of taxonomic resolution: subfamilies and genera. To assess the dominating Chironomidae genera in each stream type, we used Similarity Percentages of species analysis (SIMPER; cut-off cumulative percentage of 90%; PRIMER 6 + PERMANOVA package).

To investigate taxa redundancy among Eltonian or morphological traits and within traits (between trait-categories), we performed a centred PCA on each trait separately (FPCA). We measured the correlation between 2 traits using the Rv-coefficient, which is a multidimensional equivalent of the ordinary correlation coefficient between 2 variables (Robert & Escoufier 1976). Correlation ≥ 0.85 between traits were removed from further analysis.

The trait-by-site matrix was analysed using a Fuzzy Correspondence Analysis (FCA) enabling the joint ordination of sites and trait categories (Chevenet et al. 1994). The variance explained by a trait for separating sites is known as correlation ratio (i.e., the highest the correlation ratio, the highest the separation of sites across trait categories). The trait separation across stream types was assessed through a between-class analysis (class: stream type; Dolédec & Chessel 1987, Ter Braak 1988). We tested the significance of the trait composition variance across stream types against simulated values obtained after 999 permutations of the rows of the trait-composition array. Individual differences between stream types in each trait category were assessed; a Kruskal-Wallis was performed. Trait categories showing significant differences between types were subsequently tested using Dunn's test of multiple comparisons to ascertain between which streams types significant differences occur.

Trait composition was analysed using ‘ade4’ library (Thioulouse et al. 1997, Chessel et al. 2004, Dray et al. 2007a,b) in R freeware (R Core Team 2015).

Results

Environmental conditions

Permanent N1, permanent L and temporary S1 streams were segregated by the PCA (Figure III. 2) based on their environmental characteristics. The first-two axes explained 74% of the total variance (PC1= 48% and PC2= 26%). Differences between stream types groups were statistically significant (PERMANOVA: Pseudo-F = 18.558, *P*<0.001).

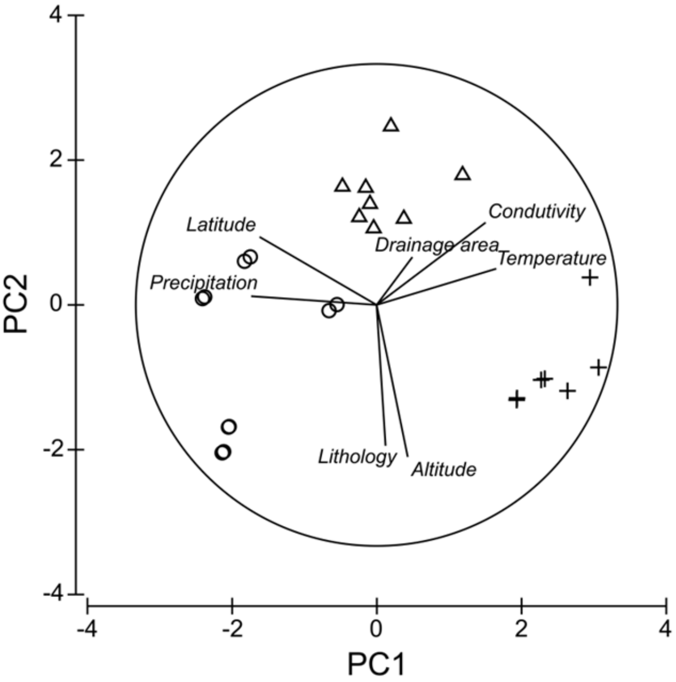


Figure III. 2 Principal component analysis of the study sites attending to their abiotic characterization. The stream types are identified as: circles, permanent medium elevation streams, N1; triangles, permanent lowland streams, L; plus, south temporary streams, S1 (transformations in Table III. 4).

Taxonomic composition

From the samples collected for this study ~ 8.5 thousand Chironomidae individuals were studied. The three stream types differed according to Chironomidae composition (pairwise tests in Table III. 5). Considering the abundance of Chironomidae taxa identified at the subfamily level, significant differences occurred between stream types (PERMANOVA: Pseudo-F global test = 3.466, *P*= 0.004; Figure III. 3a). Pairwise tests showed significant differences between temporary (S1) and permanent streams, (N1 and L) but not within permanent streams (Table III. 5). Yet, at the genus level the segregation between all types was statistically significant (PERMANOVA: Pseudo-F global test = 2.962, *P*= 0.001; Figure III. 3b, Table III. 5).

Table III. 5 PERMANOVA pairwise test results on differences between stream types (N1, permanent medium elevation; L, permanent lowland; S1, temporary) at subfamily and genus levels.

	Subfamily			Genus		
Groups	***t***	**Simul-*P***	**perms**	***t***	**Simul-*P***	**perms**
S1≠N1	1.932	0.023	966	2.097	0.001	970
S1≠L	2.025	0.017	933	1.629	0.001	935
N1≠L	1.604	0.085	992	1.352	0.028	985

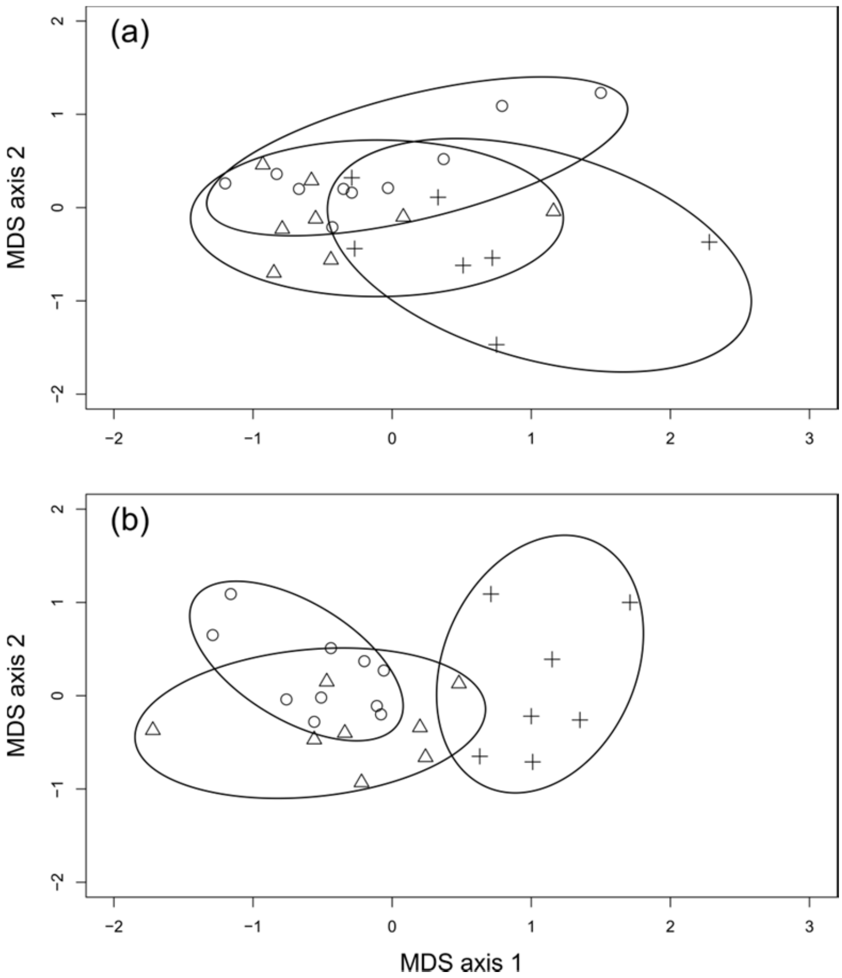


Figure III. 3 Non-metric multidimensional scaling analyses based on Chironomidae composition (Bray Curtis similarity; log-abundance) at: (a) subfamily level; (b) genus level. The stream types are identified as: circles - permanent medium elevation streams, N1; triangles - permanent lowland streams, L; plus - south temporary streams, S1.

Taking into account the most contributive taxa of each stream type (cumulative contribution up to 90% to within stream type similarity), six taxa were common to permanent streams N1 and L, while only three were common to N1 and temporary streams S1 or L and temporary streams S1 (Table III. 6). The genus *Tvetenia* (Orthocladiinae was the only taxa common to the 3 lists (Table III. 6). In permanent N1 streams, *Conchapelopia* sp. (Tanypodinae) had the highest contribution to similarity (38%) followed by *Rheotanytarsus* sp. (Tanytarsini; 13%), *Parametriocnemus* sp. (Orthocladiinae; 10%) and *Polypedilum* sp. (Chironomini; 8%). Therefore, Tanypodiinae was the representative subfamily of this stream type (38%), followed by both Chironominae and Orthocladiinae (with 27% and 26% respectively). In L streams, the most representative genera were *Parametriocnemus* sp. (Orthocladiinae; 23%) and *Polypedilum* sp. (Chironomini; 15%), followed by *Tvetenia* sp. (Orthocladiinae, 9%). *Prodiamesa olivacea* was the only Prodiamesinae selected as an important contributor and was exclusive of lowland streams; however Orthocladiinae was the most contributive subfamily of this stream type (46%), followed by Chironominae (35%), Tanypodinae (8%) and Prodiamesinae (2%). In temporary streams (S1) the most contributive taxa were *Potthastia* gr. *gaedii* (Diamesinae; 16%) and Orthocladiinae specimens from the undistinguished group of *Cricotopus-Orthocladius-Paratrichocladius* (*CriOrtPar*; 15%), followed by *Cladotanytarsus* sp. (Tanytarsini) and *Ablabesmyia longistyla* (Tanypodinae). Overall, Orthocladiinae was the most contributive subfamily for within S1 temporary streams similarity (37%; including *Corynoneura* sp. which was only found in this type of streams), followed by the Chironominae (28%), Diamesinae (16%) and Tanypodinae (11%).

In our samples, we collected six genera not mentioned in Fauna Europaea (Saether & Spies 2013) for Portugal mainland: three Orthocladiinae, *Paracladius* sp., *Epoicocladius* sp. and *Hydrobaenus* sp. found in L, N1 and S1 streams, respectively; two Chironomini, *Paratendipes* sp. found in L and S1 streams and *Tribelos* sp. found in S1 streams; and the Prodiamesinae *Odontomesa* sp. uniquely collected in some L streams.

Trait-by-taxa arrays

From an initial list of 17 Eltonian traits, 12 remained after removing the correlated traits and those with a low amount of information (Table III. 1). Traits such as emergence period, life cycle duration and reproduction type were available for very few taxa present in the study sites (less than 15%) reducing the initial list. Six traits cumulate 65% of the total variability of the FPCA performed on all traits: length of larval development, tube construction, presence of haemoglobin, respiration type (tracheas). Haemoglobin and respiration type were correlated (0.86), so only the respiration type was used in further analysis as it explains better the data variability and shows higher segregation between trait categories when compared with haemoglobin. Flight period and emergence season were only slightly correlated (about 0.38), although a correlation is expected as a product of a causal relationship, as flight period follows the emergence. Therefore, only emergence season was maintained; as it had information for more taxa and explained better the data variability.

Among the 12 morphological traits no relevant redundancy occurred between traits and therefore they were all kept for further analyses (Table III. 2).

Table III. 6 Percentage of the most contributive taxa, generated by a SIMPER analysis for each group of stream types N1, permanent medium elevation; L, permanent lowland; S1, temporary) considering Chironomidae abundance (ln[x+1] transformation; cut-off level for cumulative contributions up to 90%).

Taxa	**Subfamily**	**N1 (%)**	**L (%)**	**S1 (%)**
Tvetenia sp.	Orthocladiinae	5.6	10.5	8.8
Conchapelopia sp.	Tanypodinae	37.6	5.3	
Parametriocnemus sp.	Orthocladiinae	10.3	22.5	
Rheotanytarsus sp.	Chironominae	13.5	5.5	
Polypedilum sp.	Chironominae	8.3	15.0	
Brillia bifida	Orthocladiinae	4.0	4.5	
CriOrtPar Orthocladiinae [a]	Orthocladiinae	3.2		15.0
Tanytarsus sp.	Chironominae	2.5		3.9
Rheocricotopus chalybeatus	Orthocladiinae		2.6	4.7
Cladotanytarsus sp.	Chironominae		2.5	14.6
Potthastia gr. *gaedii*	Diamesinae			15.9
Ablabesmyia longistyla	Tanypodinae			11.1
Corynoneura sp.	Orthocladiinae			8.5
Stictochironomus sp.	Chironominae			6.1
Cryptochironomus sp.	Chironominae			3.3
Eukieferiella spp.	Orthocladiinae	2.9		
Virgatanytarsus sp.	Chironominae	2.9		
Micropsectra sp.	Chironominae		6.2	
Cricotopus gr. *bicinctus*	Orthocladiinae		3.3	
Macropelopia	Tanypodinae		3.0	
Phaenopsectra sp.	Chironominae		3.0	
Brillia longifurca	Orthocladiinae		2.7	
Paratanytarsus sp.	Chironominae		2.3	
Prodiamesa	Prodiamesinae		2.0	

Trait-by-sites arrays

Eltonian traits

The first-two axes of the FCA extracted 47.9% of the total variability (64% with FCA axis 3). The Eltonian trait that best explained the between-type FCA variance along the first axis was tube construction (17%), followed by overwinter diapause stage and substrate relation (10% and 7% respectively); along the second axis, overwinter diapause stage and respiration type (12% and 7% respectively) explained more variance than the remaining. In the third axis, substrate relation, winter diapause and maximal body size are the traits that explained more of the between- type FCA variance (between 8 and 5%). The FCA performed on the Eltonian trait-by-site array showed low but significant differences among stream types (17% of variance explained by stream type, simulated-*P*= 0.008; Figure III. 4a, Table III. 7). Traits that differed the most among stream types were overwinter diapause and to a lesser extent substrate relation. The Eltonian traits that differentiated stream types were emergence season, overwinter diapause stage, substrate relation and maximal body size of the 4th larval stage (Kruskal-Wallis test; Table III. 8a).

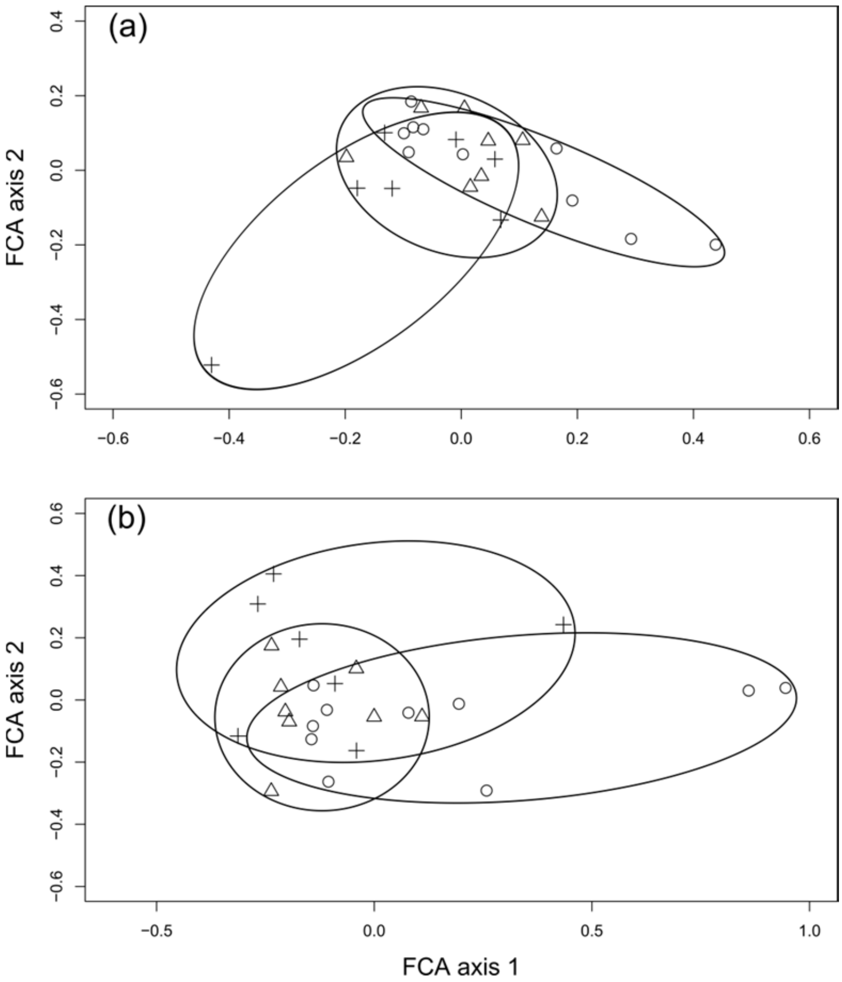


Figure III. 4 First-two axes of the Fuzzy Correspondence Analyses performed on the traits-by-sites array using: (a) Eltonian traits; (b) morphological traits. Each point represents a study site. Sites are grouped by stream type identified as: circles - permanent medium elevation streams, N1; triangles - permanent lowland streams, L; plus- south temporary streams, S1.

The proportion of individuals for several Eltonian trait categories differed significantly across stream types (Dunn's test of multiple comparisons; Table III. 8a); a higher proportion of individuals emerge during summer in permanent N1 streams (EMSUMM; Figure III. 5a); a larger proportion of individuals with ≤ 2 larval stages with overwinter diapause rather occurred in N1 streams (DIA2IN; Figure III. 5b) whereas in L1 and S1 have a significantly larger proportion of individuals with ≥ 2 larval stages and overwinter diapause (DIAMIN; Figure III. 5c); the proportion of burrowers (BURROW; Figure III. 5d) was higher in temporary streams (S1); and the proportion of animals with an intermediate size (5-10mm SIZE3; Figure III. 5e) was significantly lower in temporary rivers S1.

Table III. 7 Correlation ratios (multiplied by 1000) obtained for the first-3 axes of a FCA performed on the Eltonian trait-by-sites array and respective eigenvalues (multiplied by 1000) and variance extracted by each axis (%).

	Axes		
Traits	**F1**	**F2**	**F3**
Emergence season	5	3	3
Length of larval development	8	36	24
Winter diapause	97	121	65
Tube construction	172	15	3
Respiration type (tracheas)	50	67	39
Substrate relation	67	22	75
Generations per year	38	7	5
Feeding habits	45	11	3
Maximal body size	26	9	53
Eigenvalues	57	32	30
Variance extracted (%)	30.4	17.5	16.1

Table III. 8 Kruskal-Wallis and Dunn's tests used for analyzing differences among permanent and temporary stream types for Eltonian traits and their categories (N1, medium elevation north streams; L, permanent lowland north streams; S1, south temporary streams).

Trait category	**Code**	**Chi-squared**	***p***	**Dunn's test significance (*p*<0.05)**
(a) Eltonian traits:				
Emergence season	EMSUMM	6.32	0.042	N1≠L; N1≠S1
Overwinter diapause stage	DIA2IN	13.403	0.001	N1≠L; N1≠S1
	DIAMIN	9.086	0.011	N1≠L; N1≠S1
Substrate relation	BURROW	11.143	0.004	S1≠L; S1≠N1
Maximal body size	SIZE3	13.920	0.001	S1≠L; S1≠N1
(b) Morphological traits:				
Body setae	SIND	12.045	0.002	S1≠L; S1≠N1
	SETP	7.279	0.026	N1≠L
Mentum	DTM	12.123	0.002	N1≠L; N1≠S1
Lauterborn organs	LOI	7.493	0.024	S1≠L; S1≠N1
Premandible brush	PMBP	8.054	0.018	N1≠L; N1≠S1
	PMBA	8.054	0.018	N1≠L; N1≠S1
Claws of anterior parapods	CASIM	11.468	0.003	N1≠L; N1≠S1
	CASER	7.495	0.024	N1≠L; N1≠S1

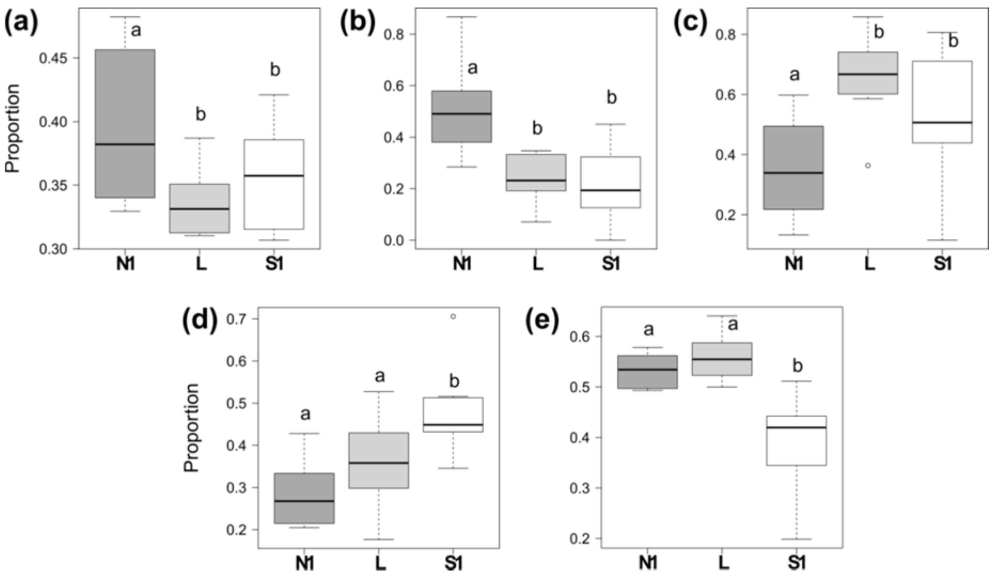


Figure III. 5 Eltonian trait categories that contribute to differences between stream types (N1, permanent north medium elevation streams; L, permanent lowland streams; S1, south temporary streams): (a) summer as emergence season, EMSUMM; (b) ≥ 2 larval stages with overwinter diapause, DIA2IN; (c) ≤ 2 larval stages with overwinter diapause, DIAMIN; (d) burrowers, BURROW; (e) intermediate size 5-10mm, SIZE3. Dunn's test results are shown as 'a' and 'b' associated to each box. Stream types not sharing the same letter are significantly different.

Morphological traits

The first-two axes of the FCA were able to extract 60% of total variability of data. The morphological traits that explained most of the between-type FCA variance along the first axis were mentum and type of antenna (each 25%) followed by claws of the anterior parapod and Lauterborn organs (15 and 11% respectively); while along the second axis, mentum explained 10% of the variance, followed by the 9% explained by the anal tubules. In the third axis, procercus is the trait that explained more of the between- type FCA variance (5%). FCA performed on the morphological trait-by-site array showed significant differences between stream types (20% of variance explained, simulated *P*= 0.008; Figure III. 4b, Table III. 9). Morphological traits that most differed among stream types included mentum (considering type and total number of teeth) and claws of the anterior parapod.

Considering morphological trait categories, proportions of some trait categories varied significantly between stream types (Kruskal-Wallis and Dunn's tests; Table III. 8b): the proportion of individuals with pale/indistinct setae (SIND; Figure III. 6a) was higher in temporary streams (S1); proportion of individuals with long body setae (SETP; Figure III. 6b) was higher in N1 streams than in L streams; the proportions of mentum holding a higher number of teeth (DTM; Figure III. 6c) and having a premandible brush (PMBP; Figure III. 6e) were lower in N1 streams; indistinct Lauterborn organs (LOI; Figure III. 6d) was more frequent in S1 temporary streams; finally N1 streams were characterized by a significantly lower proportion of taxa with simple claws (CASIM; Figure III. 6f) and a significantly higher proportion of taxa with serrated claws (CASER; Figure III. 6g).

Table III. 9 Correlation ratios (multiplied by 1000) obtained for the first-2 axes of the Fuzzy Correspondance Analysis performed on the morphological trait-by-sites array and respective eigenvalues (multiplied by 1000) and variance extracted by each axis (%).

	Axes		
Traits	**F1**	**F2**	**F3**
Body setae	41	51	28
Mentum	246	101	8
Antenna type	246	53	18
Lauterborn organs	113	16	28
Premandible brush	58	0	1
Anal tubules	19	89	0
Claws of posterior parapods	49	7	38
Claws of anterior parapods	150	29	26
Procercus	67	5	50
Eigenvalues	110	39	22
Variance extracted (%)	44.1	15.7	8.7

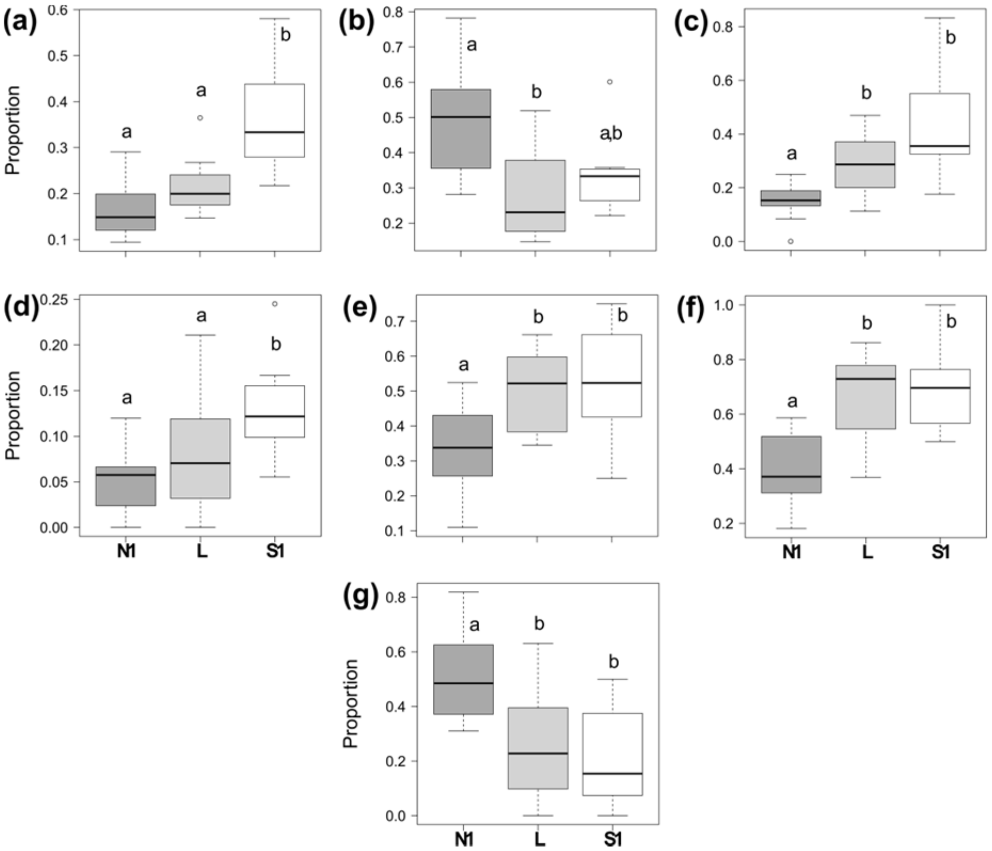


Figure III. 6 Morphological trait categories that contribute to differences among stream types (N1, permanent north medium elevation streams; L, permanent lowland streams; S1, south temporary streams): (a) body setae pale/indistinct, SIND; (b) long body setae, SETP; (c) mentum with more than 13 teeth, DTM; (d) Lauterborn organs indistinct, LOI; (e) presence of premandible brush, PMBP; (f) simple claws of anterior parapod, CASIM; (g) claws of anterior parapod serrated, CASER. Dunn's test results are shown as letters 'a' and 'b' associated to each box. Stream types not sharing the same letter are significantly different.

Discussion

Here we showed that within macroinvertebrates, the structure and abundance of Chironomidae alone reflects the natural differences of physical and chemical conditions between permanent and temporary streams and also between different types of permanent streams (lowland vs medium elevation streams). Differences in Chironomidae composition between stream types were also found by Puntí et al. (2007) in the Mediterranean streams from the NE of Spain. Yet, identifying Chironomidae at subfamily level did not allow for a full segregation of stream types (no differences between different types of permanent streams), showing the importance of a lower taxonomic resolution in ecological studies (Lenat & Resh 2001) and questioning previous recommendations for the elimination of Chironomidae from bioassessment protocols or their use at family or subfamily level (e.g., Rabeni & Wang 2001, Móra et al. 2008).

Water temperature, current velocity, substrate type and food availability are critical factors in the distribution of macroinvertebrates in general and Chironomidae in particular (Prat et al. 1983, Lindegaard & Brodersen 1995, Rossaro et al. 2006, Lencioni et al. 2007). Here, we also detected changes in Chironomidae composition between the 2 permanent stream types, which are probably related to differences in the distance to the sea, granulometry, water temperature and elevational gradient. Yet, the major differences in Chironomidae communities reflected the latitudinal and climatic gradient: south temporary Mediterranean streams (S1) from Northern permanent streams with Atlantic temperate climate (L and N1). These differences challenge the notion of Chironomidae as environmental generalist taxa, the practical result of keeping their use at coarser taxonomic levels, as it has been often argued (King & Richardson 200) but still unable to counter the constant neglect of the family.

Regarding, the most representative taxa of each stream type, our study confirmed some information from previous studies in the Iberian Peninsula but brought also additional knowledge on genera distribution and tolerances. In the permanent medium elevation streams, *Conchapelopia* sp. (Tanypodinae) had a high contribution to the similarity between streams. This is consistent with their association with faster-flowing waters or well-oxygenated lake habitats (Vallenduuk & Moller Pillot 2007). *Virgatanytarsus* sp. (Chironominae, Tanytarsini) was also representative of these streams, which is in accordance with studies by Puntí et al. (2009) that refer an elevation optimum below 500 m. In both permanent stream types (N1 and L) the genera *Parametriocnemus* sp. (Orthocladiinae) and *Polypedilum* sp. (Chironomini) were relatively well represented. This is consistent with other studies that showed that these genera are present at higher but also lower elevations (Moller Pillot 2013, Rossaro et al.

2006). Prodiamesinae subfamily was found only in permanent lowland rivers (L), represented by 2 genera: *Prodiamesa* sp. and *Odontomesa* sp. In both cases, previous references (Moller Pillot 2013) justify this distribution: *Prodiamesa* are well adapted to rivers with deposition of organic matter; while *Odontomesa* are free-living passive filter-feeders that drive water in and out by peristaltic movements, and feed mainly on unicellular algae retained by specialized mouthparts, requiring low current velocities and sandy bottoms.

Mediterranean temporary rivers are characterized by a marked seasonality in the flow regime, alternating large floods and severe droughts (Gasith & Resh 1999, Puntí et al. 2007). In our study, the Diamesinae (*Potthastia* gr. *gaedii*) dominated temporary streams assemblages. This family is commonly associated to cold fast-flowing waters, however the Dimesinae common in studied samples, *Potthastia gaedii,* has also been reported in mid and lowland river sections with stony bottoms (Puntí et al. 2007, Moller Pillot 2013). Accordingly, this genus occurs in our permanent rivers (both in medium and low elevation) but surprisingly it was only considered a representative species in temporary streams. The high number of individuals found in our temporary streams must be related with the availability of microhabitats required by *Potthastia gaedii* bottom dwellers, as our temporary streams are characterized by great sediment heterogeneity.

Corynoneura sp. (Orthocladiinae) was considered a representative species of our Mediterranean temporary rivers but not of the remaining types. However, within this genus, the congeneric species have different ecological preferences: various *Corynoneura* species (*C. lobata* and *C. scutellata*) are specifically associated to headwaters, mid-high elevations, siliceous substrates and low temperature; while *C. coronata* for example occurred at carbonate waters with higher temperatures (Puntí et al. 2009). This is an example where a lower level of taxonomic resolution (species) would contribute to the discrimination of types.

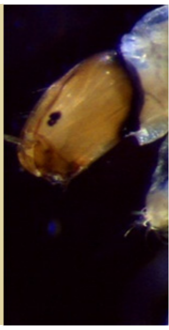
Predicted Eltonian and morphological Chironomidae traits disclosed differences among stream types. That was expectable, as abiotic characteristics (e.g., photoperiod and temperature) that differ among our stream types, are known to influence the selected traits, such as larval growth, diapause, emergence of adults, also duration of life cycles and emergence period. Yet, regarding the direction of change, some of our hypotheses were confirmed while others were contradicted (see Table III. 3). Burrowing should increase the resistance against droughts and flood events (Bonada et al. 2007a). Small body size is generally associated to a fast reproduction and development, conferring resilience to disturbance (e.g., *Corynoneura* sp.). Simultaneously large body sizes are advantageous in stagnant pools during the low flow period (Bonada et al. 2007a). And effectively, our results show that temporary streams were segregated by their significantly higher proportion of burrowers and lower proportion of intermediate sizes. The proportion of Chironomidae emerging in summer was significantly higher in rivers with high elevations under the influence of the Atlantic climate, where temperatures are lower throughout the year. However patterns of diapause do not meet the predictions: we expected higher proportions of taxa with a longer larval overwinter diapause (DIAMIN) in colder streams to face temperature challenges, but this trait category was much higher in L and S1, characterized by higher temperatures through the year. The condition of dormancy and torpor in Chironomidae is however controversial: Armitage et al. (1995), Vallenduuk & Moller Pillot (2007), Moller Pillot (2009, 2013) mention its occurrence whilst Andersen et al. (2013) do not considered it. Our results suggest that diapause may be used to face colder temperatures but maybe also high temperatures (aestivation). In fact, more autoecological studies in Chironomidae are needed to clarify diapause and emergence patterns relating these with temperature (Goddeeris 1990); information about traits related with resistance but also resilience by dispersal and colonization is still scarce for many diptera including Chironomidae (Delettre 1988, Delettre & Morvan 2000). Although, it is recognize that many times Chironomidae become the first colonizers after natural disturbance determined by floods and droughts (Marziali et al. 2010).

In this study, we investigated the ecological relevance of Chironomidae morphological traits, which are absent in general macroinvertebrate trait databases. Studies on other groups of organisms have been relating the occurrence of taxa with specific morphological traits to their habitats. For example, Makkonen et al. (2011) found that larger-sized Collembola with a higher number of oecelli and body pigmentation were favoured by dryness, showing their resistance to desiccation. The great advantage of such morphological traits is that they do not require the identification of individuals and the existence of previous taxonomic studies and taxonomic keys on local fauna. These traits could thus allow ecological interpretations free of taxonomic errors. On the other hand, their use may have a limited application as they are not shared with other groups of macroinvertebrate groups. To enlarge their use to more families would require the identification of analogous structures that would play the same key role in the environment. In fact, some Chironomidae morphological traits reflected ecological differences in the 3 stream types. Chironomidae with long body setae were more frequent in northern permanent streams distinguishing them from the lowland streams, whereas short setae were more frequent in temporary streams. In northern streams, setae allow organisms to anchor to coarse substrate, whereas in temporary streams small setae allow burrowing. Chironomidae without teeth on the mentum were also more frequent in northern permanent streams. The mentum architecture together with other structures of the head capsule reflect evolutionary adjustments of feeding habits but also to other activities such as silk production and construction of tubes or cases (Armitage et al. 1995). Here, the lower sclerotization of mentum (fewer teeth) may facilitate swallowing all preys whereas numerous teeth may facilitate particles entrapment and substrate surface scraping, and thus opportunistic omnivores. Taxa with inconspicuous Lauterborn organs were more frequent in temporary streams, distinguishing them from the two other types. Lauterborn organs are sensory structures located in the second antennal segment or at its apex. These organs allow Chironomidae to actively explore their habitat, especially in foraging activities. However, at the same time, these thin-walled sensors are particularly vulnerable to the surroundings, and thus the reduction of these organs is known to occur in terrestrial Chironomidae (Cranston 1995b). So, the possible vulnerability of Lauterborn organs may be a reason for their reduction in highly variable temporary streams under Mediterranean climate, but little information is available on this subject. Claws and length of the Chironomidae posterior parapods have an important function on fast current and unstable substrate for stabilizing Chironomidae bodies (Lencioni et al. 2007). In fact, Chironomidae with serrated claws of the anterior parapod were more frequent in permanent northern streams where current velocities are higher.

This study highlights the importance of the use of at least genus level in ecological studies and to define robust reference conditions for bioassessment. In addition, we found that Chironomidae morphological traits could be an interesting tool for ecological studies, as they also highlighted differences between stream types. The use of traits as morphological characteristics related to functions that Chironomidae perform in their habitat, can avoid their laborious identification which depends on the observation of numerous minute structures, relying instead on the observation of fewer morphological characteristics that in some cases don’t even need the use of microscope, opening alternatives to the need of strong taxonomic expertise. Yet, the poor knowledge on the relation between morphological characteristics (e.g., antennal blade, procercus setae) and their ecological functions highlights the need for further research. Finally, future tests should test the relevance of Eltonian and morphological traits in distinguishing different impairment levels.

Chapter IV:

Chironomidae traits and life history strategies as indicators of anthropogenic disturbance



Chapter IV: Chironomidae traits and life history strategies as indicators of anthropogenic disturbance

Abstract

In freshwater ecosystems Chironomidae are currently considered indicators of poor water quality because the family is often abundant in degraded sites. However, it incorporates taxa with a large ecological and physiological diversity and different sensitivity to impairment. Yet, the usual identification of Chironomidae at coarse taxonomic levels (family or subfamily) masks genus and species sensitivities. In this study, we investigate the potential of taxonomic and functional (traits) composition of Chironomidae to detect anthropogenic disturbance. In this context, we tested some a priori hypotheses regarding the ability of Chironomidae taxonomic and trait compositions to discriminate Mediterranean streams affected by multiple stressors from least-disturbed streams. Both taxonomic and Eltonian trait composition discriminated sites according to their disturbance level. Disturbance resulted in the predicted decrease of scrappers and increase of Chironomidae emerging in winter and with haemoglobin, and unpredicted increase of the proportion of taxa with longer life cycles and few generations per year. LHS corresponding to medium-sized multivoltine Chironomidae that do not greatly invest in haemoglobin and lack strong spring synchronisation, adapted to water bodies with reduced temporal dynamism (no sudden changes) were favoured under disturbed conditions. Results indicate that Chironomidae genus and respective traits could be a useful tool in the structural and functional assessment of Mediterranean streams. The ubiquitous nature of Chironomidae should be also especially relevant in the assessment of water bodies naturally poor in other groups such as the Ephemeroptera, Plecoptera and Trichoptera, such as the lowland rivers with sandy substrates, lakes or reservoirs.

Keywords: Diptera, bioassessment, biological traits, life-history strategies.

Introduction

The community structure (taxa distribution and abundance) of benthic invertebrates is commonly used in the ecological assessment of rivers (Oliveira & Cortes 2006, Dolédec & Statzner 2010, Hawkins et al. 2010, Chang et al. 2014). Within benthic macroinvertebrates, the importance of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa is often emphasized on the premise that high-quality streams generally host high EPT richness. However, in some rivers, EPT richness can be naturally reduced due to specific hydrological conditions such as those prevailing in Mediterranean temporary rivers (Bonada et al. 2006b, 2007a). In contrast, Chironomidae, which encompass an important fraction of the macrozoobenthos in most of freshwater systems and can be the only insects present in naturally poorly diverse streams, are usually disregarded mainly due to taxonomic challenges (Hawkins & Norris 2000). In Mediterranean streams, Chironomidae assemblages are known to shift quickly, due to their high resistance and resilience, high fecundity and relatively short life cycles, and dispersion capabilities; as a result, Chironomidae are often the first colonizers after periods of drought or flood (Langton & Casas 1999, Calle-Martínez & Casas 2006, Puntí et al. 2007, 2009, Marziali et al. 2010). Because of the presence of haemoglobin in several species (e.g., *Chironomus plumosus, C. riparius*), which confer them high tolerance to low oxygen concentrations, the family is often used as an indicator of bad water quality (Moller Pillot 2009). Consequently, sensitive Chironomidae taxa, indicating clean water, have not been incorporated in Saprobic systems or received low scores (poor sensitivity) in biological indices such as the BMWP (Hawkes 1998) despite their known sensitivity to anthropogenic disturbance (Saether 1979, Wiederholm 1981, Seire & Pall 2000, Lencioni et al. 2012) and attempts to develop a Chironomidae based index (Lindegaard 1995).

The growing need for tools that provide, not only a structural assessment of aquatic communities but that also link patterns to processes, gave rise to multiple-trait based approaches (Statzner et al. 2001, Statzner & Bêche 2010, Menezes et al. 2010). Consequently, the ability of macroinvertebrate communities' traits to detect of various kinds of impairment has been evaluated in aquatic ecosystems including multiple interacting stressors (e.g., Charvet et al. 1998, Vieira et al. 2004, Feio & Dolédec 2012, Lange et al. 2014, Mondy & Usseglio-Polatera 2014, Greenwood & Booker 2016). In parallel, studies defining states or strategies as a combined product of evolution emphasized that traits were not evolutionary independent (Poff et al. 2006, Verberk et al. 2008a,b, 2013). To date, none of these studies has paid attention to the Chironomidae traits at a fine identification level in the context of stream environmental monitoring (but see Franquet 1996, Van Kleef et al. 2015, Serra et al. 2016).

In the present study, we tested the efficiency of Chironomidae taxonomic and trait compositions as indicators of anthropogenic disturbance in Mediterranean streams. We first established and tested a priori hypotheses on the direction of changes in individual Eltonian traits in the presence of multiple stressors (Table IV. 1). Eltonian traits include biological traits related to organisms' functional role and their impact in the ecosystems including life cycle aspects, physiological and behavioral characteristics (Devictor et al. 2010, Mondy & Usseglio-Polatera 2014). This first approach should offer a complete set of traits responding to the various stressors. Secondly, we established and tested a priori hypotheses on the distribution of Life History Strategies (LHS as proposed by Van Kleef et al. 2015; see Table IV. 2) in disturbed and least-disturbed sites. LHS are groups of taxa that possess similar trait combinations and that were first tested as indicators of acidification in lakes. Here we tested this approach for Chironomidae and their response to multiple-stressors. This approach is expected to reflect how the individual traits are linked and which combinations favor Chironomidae in the presence of disturbance.

Table IV. 1 Predictions of changes in Chironomidae trait proportions in disturbed sites affected by multiple stressors (nutrient increase, oxygen depletion, sedimentation and hydromorphologic changes, habitat homogenization). Categories favored by disturbance ***bold italicized***.

Trait	Categories	Rationale
Maximal body size of the 4th instar (mm)	***<2.5***, ***<2.5-5***, <5-10, <10-20, >20-40	Smaller organisms are favoured - reduced availability of stony and plant habitats diminish the occurrence of refuges; large-sized organisms are more exposed and susceptible to flow (Reice 1991, Statzner 2008).
Life cycle duration	***≤1 year***, >1 year	Increase in short-lived chironomid species due to nutrients enrichment (Cross et al. 2005)
Voltinism	Semivoltine, univoltine, ***plurivoltine***	High number of generations per year - nutrient enrichment and higher biomass turnover rates (Cross et al. 2005).
Resistance forms/habits	***Cocoons***, ***diapause or quiescence***, none	Response against droughts (Bonada et al. 2007a,b) and depletion of oxygen favoured.
Locomotion/ substrate relation	Swimmer, ***burrower, interstitial, temporary attachment***	Animals able to escape flow are favoured - absence of stable substrates diminishes availability of refuge (Statzner 2008).
Feeding habits	***Deposit-feeder***, shredder, scraper, ***active filter-feeder***, ***predator***	Organisms living in impaired sites should feed on small particles (seston) brought from upstream sites - high amounts of nutrients may increase the productivity of planktonic algae that can be used as food supply (Statzner 2008); absence of periphytic growth and low coarse detrital food supply disfavour scrappers and shredders. Predation increases with the absence of refuges and high abundances of invertebrate preys (Reice 1991)
Emergence season	Winter, ***spring***, summer, autumn	Spring synchronization is one way to avoid periods of low oxygen in summer (Van Kleef et al. 2015) which assumes importance in natural temporary Mediterranean rivers, in disturbed sites emergence should be altered.
Length of larval development (months)	***<4***, >4	Together with spring synchronization may avoid periods of stress (depletion of oxygen) not investing time and energy in other strategies, having a high intrinsic rate of development (Van Kleef et al. 2015)
Tube construction	Tube absent, ***tube without shape (unorganized)***, ***tube rigid***	Tube construction in soft sediments avoid the impact of sedimentation, allowing supply of oxygen and food, flushing out metabolites and carbon dioxide not needed by the organism (Armitage et al. 1995), also make Chironomidae less conspicuous for predators (Van Kleef et al. 2015)
Haemoglobin	***Present***, absent	Types of respiration that require more dissolved oxygen are expected to decrease - higher concentrations of nutrients lead to the increase in primary productivity and reduced oxygen content during night (Cross et al. 2005, 2006, Yuan 2010). Haemoglobin may confer tolerance to low oxygen availability (Van Kleef et al. 2015)

Methods

Study area, sites and environmental characterization

The study sites are located in the south of Portugal, Alentejo, a Mediterranean-climate area characterized by dry summers, irregular but intense rainfall in winter and total annual precipitation below 600 mm yr^{-1}. Stream sites (three least-disturbed and three disturbed) were selected from two similar temporary streams at elevations between 200 and 250 m a.s.l.. Disturbed sites, which are located downstream of water treatment plant that processes industrial wastewater, have high conductivity, nitrate and sulphate concentrations, and low dissolved oxygen concentrations (Feio et al. 2010). In addition, disturbed sites are subjected to hydromorphological alterations including fine sediment deposition whereas least-disturbed sites are characterized by a high diversity of habitats (sand, cobles and macrophytes; Feio et al. 2010). Water physical and chemical parameters (pH, dissolved oxygen, conductivity, temperature, nitrates, and sulphates) were recorded in early spring of 2013 and 2014 at the six stream sites.

Chironomidae sampling and identification

Benthic macroinvertebrates were collected in early spring of 2013 and 2014 using a kick-net (500 µm mesh size; 0.25 m × 0.25 m opening). Sampling followed a multi-habitat approach protocol, which covers all habitats (riffles, macrophytes beds, pools) proportionally to their abundance, and comprised a 6 × 1m kicking (INAG 2008). Samples were fixed with formalin (4%) and Chironomidae were sorted in the laboratory and preserved in ethanol (70%). Chironomidae identification was performed sequentially. In a first step, individuals were grouped into morphological types and counted under a stereomicroscope. Morphotypes are smaller groups of taxa (usually genera), within Chironomidae, that share similar morphological features related for example to body setae, head capsule colour, antenna length, position and number of eye spots (Prat & Rieradevall 2014). In a second step, Chironomidae were mounted on microscopic slides under the stereomicroscope. To do this, larval specimens were digested in caustic potash (KOH, 10%) at 85ºC in a water bath for 15-20 minutes; digested tissues were then washed in distilled water for about five minutes. Specimens were then dehydrated: three minutes in 70% ethanol, followed by three minutes in 96% ethanol. Heads and bodies were separated and mounted using Euparal medium (Andersen et al. 2013). When a morphotype gathered more than 200 individuals, only a representative portion was mounted (≥50%). In a last step, Chironomidae larvae were identified under the microscope (magnification: 400-1000×) to the highest possible taxonomic resolution, frequently to genus but also species and species-groups level, following Palearctic/Holarctic guides for Chironomidae taxa (e.g., Cranston 1982, Lencioni et al. 2007, Andersen et al. 2013, Prat & Rieradevall 2014).

We used genus level identification in data analyses because the available information on traits was predominantly available at that level. Exceptions included the genera of *Cricotopus* and *Orthocladius,* which represent particular morphological groups (groups of species) inside each genus rather than the genus itself. In this study *Cricotopus* included taxa belonging only to the *sylvestris, trifascia* and *bicinctus* groups; whereas *Orthocladius* included *Orthocladius* (*Euorthocladius) rivulorum* and *Orthocladius (Symposiocladius) lignicola.* All the other *Cricotopus* and *Orthocladius* were gathered in a wider morphological undistinguished group of three genera *Cricotopus-Orthocladius-Paratrichocladius* (*CriOrtPar*).

Trait composition

Chironomidae Eltonian traits were taken from Serra et al. (2016) genus level database (Table IV. 1, from Appendix Table A1). Trait-by-sites arrays were further computed as the cross-product between the relative abundance (ln[x+1]) of Chironomidae taxa and each trait profile (see e.g., Gayraud et al. 2003). As a result, trait-by-sites arrays contained the relative abundance of each trait category in each least-disturbed and disturbed sites.

Life History Strategies (LHS)

Six Chironomidae life history strategies (LHS A-F, see Table IV. 2) have been proposed by Van Kleef et al. (2015) to evaluate natural recovery of acidified lakes based on Eltonian traits expected to be relevant under environmental changes: haemoglobin, tube building and larval development. These traits in turn interact with other traits such as feeding habits, voltinism, spring emergence (spring synchronization) and maximal body size. In addition, we made a priori predictions on the expected changes in proportions of each LHS in the disturbed sites, compared to the least-disturbed sites (Table IV. 2). Chironomidae taxa were attributed to each of the six LHS, which were reinterpreted and adjusted according to our own database (Serra et al. 2016). We expected that Chironomidae having haemoglobin, constructing tubes and with higher number of generations: (1) the presence of haemoglobin allows Chironomidae to live in water with poor oxygen content, (2) the oxygen concentration inside the tube may increase significantly compared with surroundings due to the larval body movements and their opening above the level of sediments (Armitage et al. 1995). In some cases, the number of generations may compensate the absence of other adaptations to stress (e.g., haemoglobin) by the high number of individuals present in a site, resulting in a better chance of species survival and allowing organisms to attain adult size and dispersing to more favourable environments earlier avoiding adverse conditions. According to this rationale, we expected that, in disturbed sites, the proportions of individuals with: (1) LHS-E should increase; (2) the proportions of individuals with LHS-B and C should decrease; and (3) the proportions of LHS-A, D and F should remain similar. Genera that could not be assigned to a LHS category were included in an unknown category (LHS-U) due to the absence of data about their biological traits, the existence of various affinities to different categories (high variability to given trait), or because the combination of traits in the genus did not fit in any defined strategy.

Table IV. 2 Chironomidae life-history strategies (LHSs) based on different combination of biological traits and predictions made for disturbed sites in comparison to least-disturbed sites. **Size**, maximum length of 4th instar larvae; **Volt**, number of generations per year/ voltinism; **Spr**, Spring synchronization; **Hb**, Haemoglobin; **Tub**, tube construction; **Feed**, primary feeding habit; **Taxa**, total number of taxa attributed to each LHS.

LHS	Size[a]	Volt	Spr[b]	Hb[b]	Tub[b]	Feed[c]	Taxa	Predictions	Example
A	S	>2	a	p/a	a	A	1	=	*Corynoneura* sp.
B	M	>2	a	p/a	p/a	A, D, C	11	Decrease	*Ablabesmyia* sp.
C	M	1-3	p	a	p/a	A, C	2	Decrease	*Psectrocladius* sp.
D	M	2-3	a	p	p/a	A	3	=	*Cladotanytarsus* sp.
E	L	1-3	p	p	p	A, D	8	Increase	*Chironomus* sp.
F	L	1-2	p	p	a	C	4	=	*Cryptochironomus* sp.
U[d]	-	-	-	-	-	-	3		-

[a] S, small <5mm; M, medium 5-12mm; L, large >8 mm

[b] a, absent; p, present

[c] A, algivorous; D, detritivorous; C, carnivorous

[d] LHS- U, unknown life history strategy category

Data analysis

Environmental characterization

We performed a Principal Component Analysis (PCA) on the abiotic data to confirm the discrimination of sites regarding their levels of anthropogenic disturbance (i.e., disturbed vs. least-disturbed sites).

Trait redundancy

We computed Rv-coefficient (the multivariate equivalent of a R^2 for tables; Robert & Escoufier 1976) to measure the correlation between each pair of trait-by-taxa array. This allowed eliminating possible redundancy among traits and testing for potential correlations between biological and morphological traits. Biological traits with correlation ≥ 0.85 with morphological traits were removed from further analysis.

Taxonomic and trait composition

Patterns of richness and abundance in least-disturbed and disturbed sites were explored and significant differences were accessed by a Kruskal-Wallis rank sum test. We also performed a Principal Component Analysis (PCA) on the abundance (ln[x+1]) of Chironomidae taxa to analyse the segregation of sites based on species abundance and we used it to derive multivariate scores of least-impaired and impaired sites based on taxa composition. In a following step, we used a SIMPER analysis (Bray-Curtis similarity) to determine which Chironomidae genera were representative of least-disturbed and disturbed sites, i.e., contributed the most to the within-group similarity (up to 90% cumulative contribution), assuming that taxa should be best adapted to one or the other level of disturbance due to their traits. The taxa selected based on untransformed data did not add more information so finally we selected those taxa contributing the most to groups similarity based on presence/absence data.

Based on those selected taxa, we performed a PCA on trait-by-sites arrays (ln[x+1]) to yield multivariate scores of least-disturbed and disturbed sites (Cundari et al. 2002, Sârbu & Pop 2005). To test for the degree of discrimination between least-disturbed and disturbed sites considering Chironomidae taxonomic and trait composition, we used between-class analysis (see e.g., Dolédec & Chessel 1987, Lebreton et al. 1991). The significance of the explained variance was tested against simulated values obtained after 999 permutations of the rows of the taxa- or trait-composition arrays (Monte-Carlo test; see e.g., Romesburg & Marshall 1985). Finally, considering trait categories individually, differences between least-disturbed and disturbed sites were assessed for each trait category using a Kruskal-Wallis rank sum test.

Life History Strategies

We tested our predictions on the LHS with a Permutational Multivariate Analysis of Variance (Bray-Curtis distances) on Chironomidae LHSs abundances (ln[x+1]) present in least-disturbed and disturbed sites. A SIMPER analysis was used to assess which LHSs were important in the discrimination between least-disturbed and disturbed sites (Bray-Curtis dissimilarities).

Statistics and graphical outputs were computed using R freeware (R Core Team 2015). Taxa and trait compositions were specifically analysed with ‘vegan’ (Oksanen et al. 2015) and ‘ade4’ libraries (Thioulouse et al. 1997, Chessel et al. 2004, Dray et al. 2007a,b) and statistical package PRIMER+PERMANOVA v6 (PRIMER-E Ltd.; Clarke & Gorley 2006).

Results

Environmental characterization

The first-two axes of a PCA performed on environmental data explained 99.7% of the total variance (93.6% and 3.1% of the total variance, fort the first and second axis, respectively). PCA axis 1 segregated disturbed from least-disturbed sites. Disturbed sites had higher SO_4^{2-} concentrations (Mean± SD: 526.8± 151.1 vs. 28.0± 0.5 mg l^{-1},), higher conductivity (1307± 369 vs. 262± 67 µS cm^{-1}), and higher NO^{3+} concentrations (6.7± 2.0 vs. < 0.02 mg $^{l-1}$) (Figure IV. 1) whereas dissolved oxygen was higher in the least-disturbed sites. A Monte-Carlo test revealed a highly significant variance of environmental data explained by the disturbance (variance explained= 89.7%, simulated-*P*= 0.002).

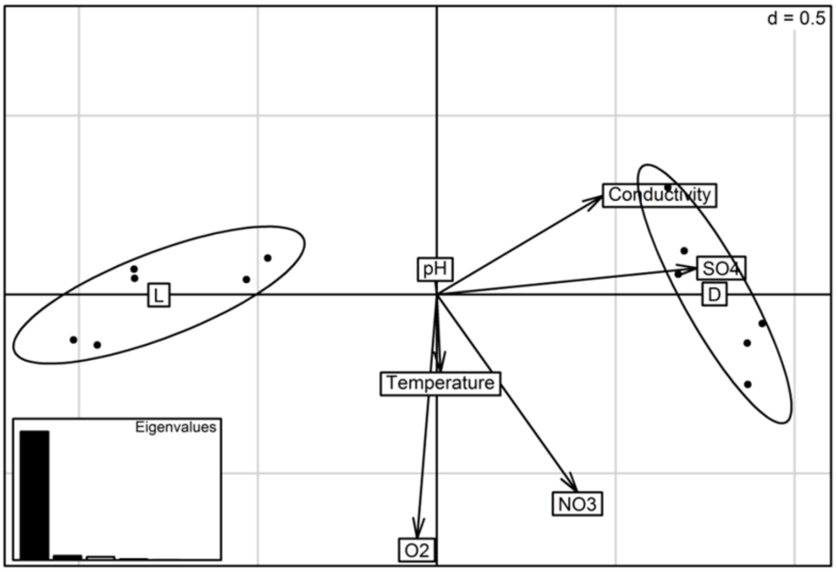


Figure IV. 1 First-two axes PCA biplot according to abiotic conditions in least-disturbed (L), and disturbed (D) sites (dots). The histogram of eigenvalues is inserted.

Taxonomic composition

Approximately 2300 individuals were identified. Chironomidae were more abundant in disturbed sites (*P*= 0.016; Figure IV. 2) but richness was not significantly different from least-disturbed sites (*P*= 0.292; Figure IV. 2). The first-two axes of a PCA performed on Chironomidae composition explained 59.9% of the total variance (PC1 41.7% and PC2 18.2%; Figure IV. 3) A Monte-Carlo test showed that 32.8 % of the variance of the Chironomidae composition was significantly explained by the degree of disturbance (simulated-*P*= 0.001).

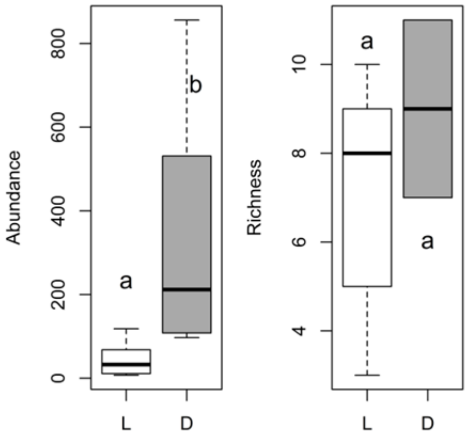


Figure IV. 2 Chironomidae abundance and richness in least-disturbed (L) and disturbed (D) sites. Kruskal-Wallis rank sum test results are shown as letters ‘a’ and ’b’ associated to each box (different letters indicate significant differences).

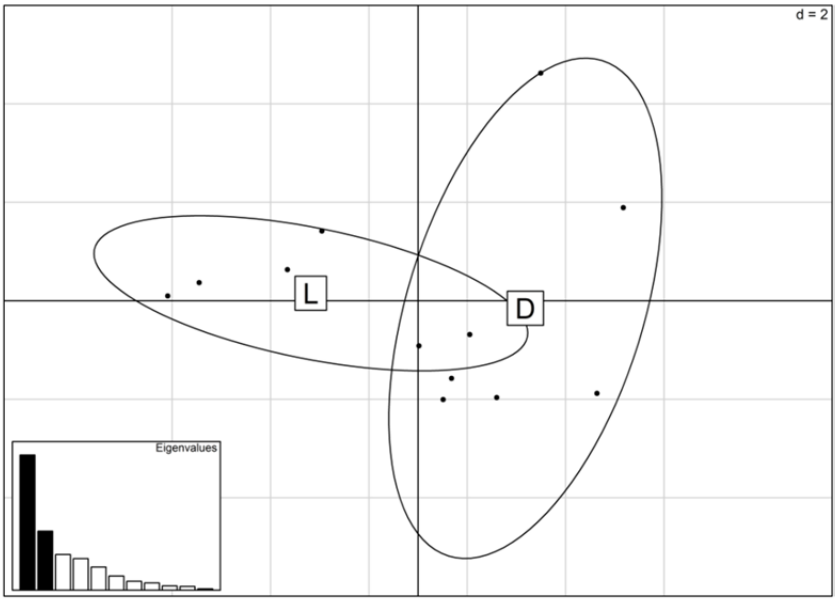


Figure IV. 3 First-two axes of a PCA performed on the Chironomidae taxonomic composition of least-disturbed (L) and disturbed (D).

Taxa that contributed most to the similarity within least-disturbed and within disturbed sites and comprising up to 90% of cumulative contribution included five taxa (*Cricotopus*, CriOrtPar, *Othocladius*, *Potthastia*, *Sympotthastia*; Table IV. 3). *Corynoneura* and *Paratanytarsus* contributed exclusively in least-disturbed sites, whereas *Tvetenia*, *Chironomus*, *Eukiefferiella*, and *Rheocricotopus* were relevant only in disturbed sites. *Chironomus* was the only genera that did not occur in least-disturbed sites.

Table IV. 3 Average frequency and percentage of taxa that contribute most to the similarity within least-disturbed (L; with average similarity = 29.7%) and disturbed (D; average similarity = 66.2%) sites, considering Chironomidae presence/absence generated by a SIMPER analysis (cut-off level for cumulative contributions up to 90%).

	L sites		**D sites**	
	Average frequency	**Contribution (%)**	**Average frequency**	**Contribution (%)**
Potthastia	0.67	21.48	1.00	15.29
Corynoneura	0.67	18.78	0.17	*
Cricotopus	0.67	16.89	1.00	15.29
CriOrtPar	0.67	16.89	1.00	15.29
Paratanytarsus	0.50	8.44	0.33	*
Orthocladius	0.50	7.50	0.67	6.27
Sympotthastia	0.33	2.63	0.67	6.09
Chironomus	0.00	*	1.00	15.29
Tvetenia	0.17	*	0.83	9.99
Eukiefferiella	0.33	*	0.67	6.12
Rheocricotopus	0.33	*	0.50	2.94

Trait composition

For the 11 selected taxa (Table IV. 3), none of the traits were correlated ≥ 0.70, so all traits were kept in further analyses. The first-two axes of a PCA performed on the trait composition of sites explained 92.0% of the total variability. The difference between disturbed and least-disturbed sites was significant (Monte-Carlo test: explained variance= 0.53, simulated-*P*= 0.008). The trait categories with the highest correlations with the axes and segregating disturbed from least-disturbed sites (Figure IV. 4) belonged to: life cycle duration, voltinism, feeding habits, emergence season, tube construction and haemoglobin (Table IV. 4). The Kruskal-Wallis tests confirmed that categories within all these traits but tube construction differed significantly between the disturbed and least-disturbed sites (Figure IV. 5, Table IV. 4). Least-disturbed sites had more taxa that live less than one year (Figure IV. 5a); and more taxa that had more than one generation per year (plurivoltine) (Figure IV. 5c); contrarily, in disturbed sites there were more semivoltine taxa living more than one year (Figure IV. 5a-b), a pattern that went against the one initially predicted (Table IV. 1). Furthermore, disturbed sites had fewer scrapers, which confirm our predictions (Figure IV. 5d) but also fewer active filter feeders (Figure IV. 5e). Chironomidae taxa emerging in winter were more frequent in disturbed sites (Figure IV. 5f) whereas taxa emerging in autumn were more frequent in least-disturbed sites (Figure IV. 5g). Taxa with haemoglobin were more frequent in disturbed sites (Figure IV. 5h), thus following the predictions.

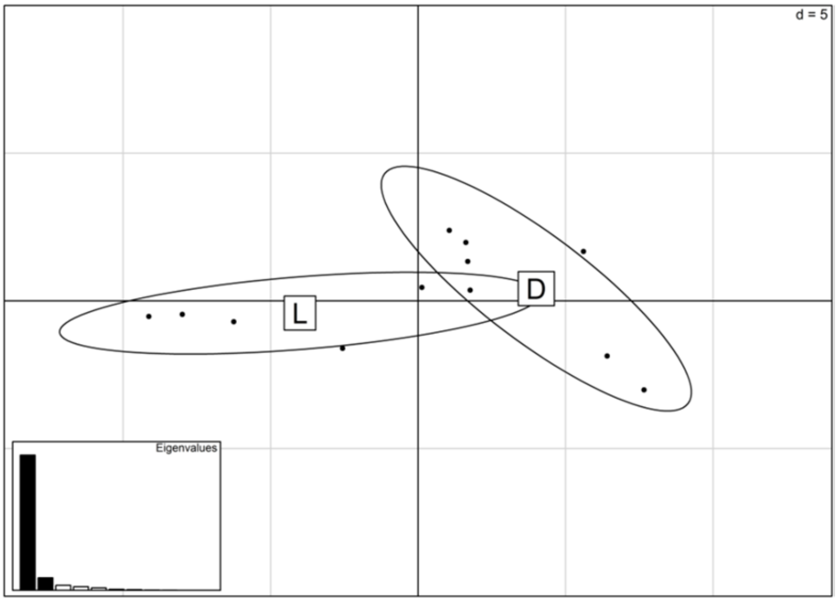


Figure IV. 4 First-two axes of a PCA performed on Eltonian Chironomidae trait composition in least-disturbed (L) and disturbed (D) sites.

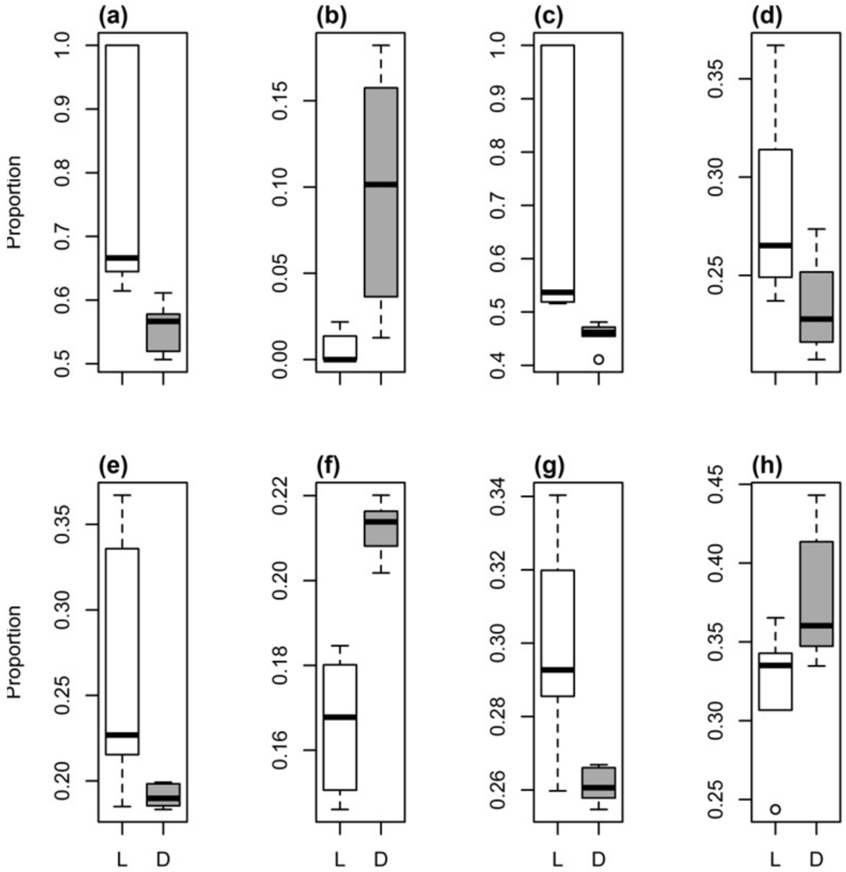


Figure IV. 5 Chironomidae trait categories of selected taxa showing significant differences between least-disturbed (L) and disturbed sites (D). (a) Life cycle duration ≤ 1 year. (b) Semivoltine. (c) Plurivoltine. (d) Scraper. € Active filter-feeder. (f) Winter emergence. (g) Autumn emergence. (h) Haemoglobin present.

Table IV. 4 Correlation of each trait category with the first-two axes of a PCA performed on Chironomidae traits. Results of Kruskall-Wallis rank sum test used to test differences between least-disturbed and disturbed sites in Chironomidae trait category relative abundance.

Trait	Categories	PC1	PC2	Kruskal-Wallis chi-squared	*P*
Maximal body size of the 4th instar	<2.5 mm	0.261	-0.863	2.657	0.103
	<2.5-5 mm	0.913	0.002	0.923	0.337
	<5-10 mm	0.985	-0.066	0.926	0.336
	<10-20 mm	0.987	0.045	1.641	0.200
	>20-40 mm	0.708	0.564	0.643	0.423
Life cycle duration	≤1 year	0.996	-0.005	8.337	0.004
	>1 year	0.978	-0.139	8.337	0.004
Voltinism	Semivoltine	0.429	0.760	6.802	0.009
	Univoltine	0.986	-0.083	0.412	0.521
	Plurivoltine	0.997	-0.022	8.337	0.004
Resistance forms	Cocoons	0.917	-0.257	0.231	0.631
	Diapause/quiescence	0.987	-0.010	1.641	0.200
	None	0.762	-0.020	0.923	0.337
Locomotion/ substrate relation	Swimmer	0.701	0.547	2.573	0.109
	Burrower	0.936	0.292	2.077	0.150
	Interstitial	0.987	-0.112	1.256	0.262
	Temporary attachment	0.964	-0.118	2.084	0.149
Feeding habits	Deposit-feeder	0.984	-0.142	3.102	0.078
	Shredder	0.995	-0.066	0.026	0.873
	Scraper	0.994	0.005	4.333	0.037
	Active filter-feeder	0.992	-0.076	4.333	0.037
	Predator	0.945	-0.251	0.231	0.631
Emergence season	Winter	0.986	-0.068	8.308	0.004
	Spring	0.996	-0.075	0.923	0.337
	Summer	0.993	-0.091	3.103	0.078
	Autumn	0.998	-0.047	5.769	0.016
Length of larval development	≤4 months	0.993	-0.073	0.641	0.423
	>4 months	0.893	0.291	0.641	0.423
Tube construction	Tube absent	0.580	0.416	0.410	0.522
	Tube without shape	0.991	-0.031	0.410	0.522
	Tube rigid	0.977	-0.079	0.103	0.748
Haemoglobin	Present	0.936	0.138	4.333	0.037
	Absent	0.995	-0.067	4.333	0.037

Life History Strategies

The LHS (ln[x+1] transformed abundance) were significantly different between least-disturbed and disturbed sites (explained variance= 0.23, *P*= 0.029; Figure IV. 6). The LHS-B was the life history strategy that most contributed (25.7%) to the differences between least-disturbed and disturbed sites (*P*= 0.004) followed by the unknown (22.5%), LHS-E (17.1%), LHS-C (12.5%), LHS-F (9.2%), LHS-A (8.9%), and finally the LHS-D (4.2%).

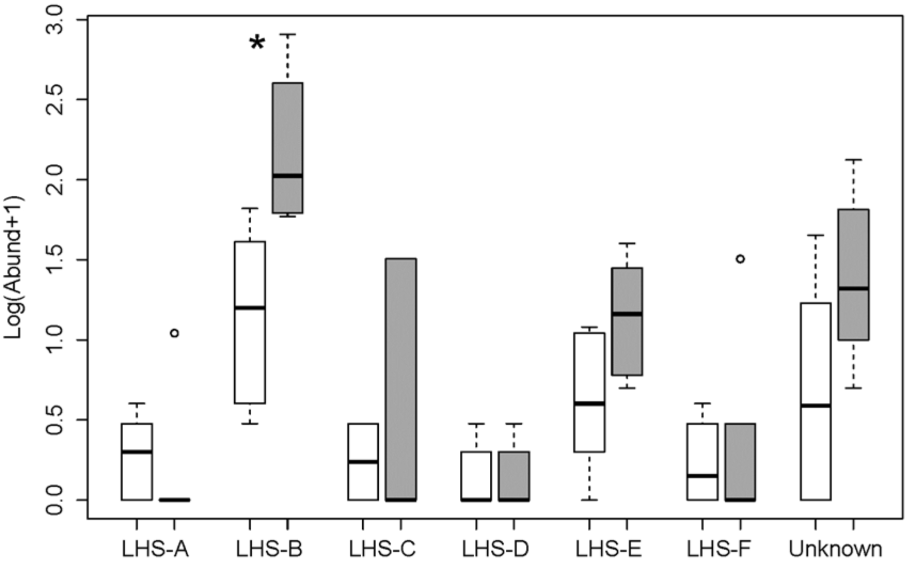


Figure IV. 6 Abundance (ln[x+1]) of taxa from each LHS considered in least disturbed (white) and disturbed (dark grey) sites. * Marks the LHS-B as significantly different between least-disturbed and disturbed sites.

Discussion

Our study aimed at evaluating the relevance of Chironomidae as functional and structural indicators of rivers to assess anthropogenic disturbance. Indeed, chironomids were more abundant in disturbed sites whereas taxa richness was equally well represented in disturbed and least-disturbed sites. Despite a similar number of taxa, compositional differences in Chironomidae assemblages occurred: *Chironomus* sp. (Chironominae) was exclusive of disturbed sites and differences in abundance of Diamesinae genera (e.g., *Potthastia* and *Sympotthastia*), Orthocladiinae (e.g., *Corynoneura* and *Cricotopus*), and Chironominae (Tanytarsini, *Paratanytarsus*) attested the difference between disturbed and least-disturbed sites. This result agrees with previous studies that showed the sensitivity of Chironomidae taxonomic composition to anthropogenic disturbance resulting from nutrients enrichment (Camargo et al. 2004, Maasri et al. 2008, Stewart et al. 2014). *Chironomus* (*C. riparius*) and *Cricotopus* (e.g., *C. bicinctus*) are both known tolerant and dominant in Mediterranean systems (Calle-Martínez & Casas 2006, Chaib et al. 2011). *Chironomus riparius* species is well known by its tolerance to low pH and oxygen and by its occurrence in organically polluted streams and they are inclusively used as bioindicators of freshwater contamination through the evaluation of anatomical deformities (Servia et al. 2000, Stuijfzand et al. 2000, De Haas et al. 2005, De Haas & Kraak 2008, Moller Pillot 2009). Also life-history traits of *Chironomus riparius* (e.g., larval development time, hatchability of the eggs) have been shown to change in the presence of metal contamination, rapidly evolving in response to external conditions being very adaptable to changes in the environment (Postma et al. 1995).

The trait composition of Chironomidae communities alone was able to separate disturbed from least-disturbed sites. However, the direction of change was sometimes contrary to our predictions. For example, those Chironomidae with more than one generation per year and with shorter life cycles were counter-intuitively favoured in the least-disturbed sites whereas those with longer life span were more frequent in disturbed sites. Yet, this might be a confounding effect caused precisely by the high adaptability of *Chironomus* (9% of Chironomidae total abundance but exclusive from disturbed sites), and *Cricotopus* (55% of the total abundance of disturbed sites) to changing environmental conditions, namely in the life cycle duration (Bazzanti et al. 1997, Casas & Langton 2008), as these taxa may have few or many, but also one or less generations per year (Tokeshi 1995a). Emergence season is also a trait that can change to confer a better adaptability to the external conditions for genus such as *Chironomus* and *Cricotopus* which colonize temporary habitats (Bazzanti et al. 1997, Tokeshi 1995a). Other studies showed also that nutrient enrichment resulted in an increase of invertebrate abundance, biomass and secondary production with life spans less than one year (Cross et al. 2006). Nitrogen and phosphorus affect algal and microbial assemblages, and consequently primary productivity and decomposition rates of aquatic ecosystems (Hillebrand 2002, Ferreira et al. 2015). Therefore, a change in nutrient concentrations may have immediate effect on individual consumption, growth and fecundity of macroinvertebrates (Borer et al. 2006, Yuan 2010, Snell-Rood et al. 2015). This individual performance in turn will likely affect population and community structure and functions. However, these changes are difficult to predict given the complexity of food-web interactions and the multiple stressors interactions as those existing in our study sites (Statzner & Bêche 2010, Wagenhoff et al. 2012).

As we expected, Chironomidae requiring more oxygen were less abundant in disturbed sites due to the lower oxygen levels and sedimentation. On the opposite side, the presence of haemoglobin and ‘waving’ behaviour of bodies manifested by many Chironomidae larvae, allow a better supply of oxygen even in environments with reduced levels of dissolved oxygen (Armitage et al. 1995).

Regarding feeding habits, in disturbed sites the reduced amount of hard substrates or the deposition of fine sediments over them, could prevent epiphytic growth and therefore the proportions of scrapers and grazers were expected to be lower when compared to least-disturbed sites. In fact, scrapers were among the most representative taxa in least-disturbed sites but not in the disturbed ones. In opposition, in disturbed sites, organisms that fed on small organic particles were expected to thrive in disturbed stream, which was not confirmed. However, Chironomidae active filter feeders could also be negatively affected by inorganic fine in disturbed sites, as already demonstrated with filter-feeding macroinvertebrates such as Hydropsychidae (Wood et al. 2005). Feeding traits proved to be very sensitive to the microhabitats (e.g., substratum availability) in a Mediterranean river reflecting the primary food sources present (Manfrin et al. 2016).

Regarding emergence patterns it seems that there was no strong spring synchronization in our Mediterranean sites, independently of the disturbance level. Emergence of Chironomidae may occur virtually all through the year it varies from species to species according to environmental conditions. Generally univoltine species tend to emerge during spring (sometimes summer) whereas species with two or more generations may emerge from spring to autumn, spring emergence however tend to be stronger in response to the rising in temperature and to the increasing photoperiod of spring (Tokeshi 1995a). Spring synchronization allows the schedule of mating, guarantee egg viability and hatching, development and growth. Organisms in least-disturbed and disturbed sites seem to emerge all over the year with some reduced numbers in autumn for disturbed sites and winter for least-disturbed sites. In our least-disturbed Mediterranean sites Chironomidae emergence increases from winter to summer, being almost equally high in summer and autumn decreasing slightly during this last season. In disturbed sites there is also a slight increase towards the summer but not so pronounced as in least-disturbed sites because the emergence is significantly higher in winter and significantly lower in autumn when compared with least-disturbed sites. Despite of an increase in emergence beginning in winter towards the summer in both sites, patterns of emergence were much less different over the seasons in disturbed sites. This indicates that in disturbed sites environmental conditions are more homogeneous over the year. In fact, the industrial effluent results in a more continuous flow over the year, altering the natural pattern of summer droughts.

When analysing the traits combinations by way of life history strategies (as proposed by Verberk et al. 2008 a,b, 2013, Van Kleef et al. 2015) medium-sized multivoltine taxa (LHS-B) were generally prominent in our sites and more abundant in disturbed sites. These taxa do not greatly invest in haemoglobin and have no strong spring synchronization but their development is still fairly rapid, including tube building algivorous and detritivorous taxa, and also free-living carnivorous. Because they are relatively vulnerable to oxygen depletion this may indicate that this is not the most disruptive factor distinguishing disturbed sites from least-disturbed but others such as nutrient enrichment as well as hydromorphological alterations may better explain these Chironomidae trait assemblage patterns. Ultimately, disturbance leads to functional homogenization as seen by the increase of Chironomidae taxa having the same strategies, which reflects the impoverishment of the habitats and food resources available. The percentage of taxa with unknown LHS (LHS-U) is relatively high (22.5%) because of the abundance of the group *CriOrtPar* (which account for taxa inside the genera *Cricotopus*, *Orthocladius*, or *Paratrichocladius*). Given the diversity inside each genus we decided not to attribute them to any LHS. However, most of these taxa probably belong to the LHS-B, which would increase even more differences between least-disturbed and disturbed sites.

In our study we showed that Chironomidae taxonomic and trait assemblages at genus level were able to distinguish disturbance levels in Mediterranean streams. The most representative taxa in least-disturbed sites and in disturbed sites offered a set of traits that supports the defined life-history strategies. Therefore, independent analyses of traits individually and the use of combined-trait methods may complement each other: the first that may be important in the identification of a set of traits (regardless the direction of their individual response) that respond to a multiple stressor scenario whereas the second reflects the evolutionary linkage between the traits, offering an integrated perspective of functional changes occurring in the system. Finally, due to the high abundances of Chironomidae, the changes in trait proportions as a result of anthropogenic disturbance may mirror changes in ecosystem functions.

Chapter V:

Chironomidae of Holarctic region: comparison of traits between North America and Europe

Chapter V: Chironomidae of Holarctic region: comparison of traits between North America and Europe

Abstract

Chironomidae (Diptera) are widespread, abundant, diverse and ubiquitous, and include genus and species that are distributed across the Holarctic region. However, the geographical barriers between continents should have resulted in intraspecific population differentiation with reflection on individual biological and ecological traits. Our aim was to test for potential differences in Chironomidae species/genus and traits between the Nearctic and Palearctic regions. We compared the Chironomidae trait information gathered in two databases; one database was developed in Europe and the other in North America. Common genus and species of both databases were selected and the common traits were adjusted into the same trait categories. Data were transformed into presence/absence and divided into Eltonian (biological/functional) and Grinnellian traits (ecological). Common genera and common species were analysed using Fuzzy correspondence analysis (FCA). Differences between databases occur for all trait domains. Yet, Eltonian traits showed lower level of concordance than Grinnellian traits at the species level. Different biological characteristics in the Nearctic and Palearctic regions may indicate that Chironomidae have different adaptions to similar ecological environments due to intraspecific variability or even trait plasticity.

Keywords: Diptera; ecological traits; Palearctic; functional traits; Nearctic; regional traits.

Introduction

Chironomidae (Diptera) are widely distributed over the world exploiting a wide range of environmental conditions (e.g., temperature, pH, oxygen concentration, salinity, current velocity; Cranston 1995a). Chironomidae larvae are the most abundant aquatic insect family in freshwaters, representing frequently more than half of the macroinvertebrates species (Oliver & Roussel 1983b, Marziali et al. 2010). Their ubiquity is due to a variety of behavioural and physiological adaptations (e.g., tube construction, presence of haemoglobin) despite the apparently modest morphological differences (Armitage et al. 1995). These characteristics may confer them an important role in freshwater ecosystem functioning.

Many Chironomidae taxa have a Holarctic distribution. The Holarctic region is composed by the Nearctic and Palaearctic regions, which were originated during the Laurasia and Gondwana separation, in the Jurassic. Some studies support the unified view of this region, indicating a high number of shared genus and species, and a relatively low level of endemism identified in each area (Cranston & Oliver 1987). Ferrington (2008) reviewed the global diversity of Chironomidae in freshwater systems and 181 genera and 1321 species were associated to the Palaearctic region, whereas 211 genus and 1092 species were associated to the Nearctic regions. About 71-74% of the genera may occur in both regions but the number of species shared is not accurately mentioned in literature (Ashe et al. 1987, Cranston & Oliver 1987).

Currently it is recognized that biodiversity should not just reflect the number of species, but also their functional attributes. Macroinvertebrate traits are increasingly used in the functional assessment of streams, providing a mechanistic perspective on the effect of different impacts (e.g., Statzner et al. 2001, Dolédec & Statzner 2008, Feio et al. 2015). Notably, few trait-based studies have used Chironomidae information at finer taxonomic levels (but see Van Kleef et al. 2015) to which the absence of adequate databases greatly contributes. One of the main reasons limiting knowledge on Chironomidae ecology and species distribution relies on their difficult taxonomy (e.g., Lindeberg 1980, Oliver & Roussel 1983b), particularly for immature stages (structurally very similar), which is the longest phase of Chironomidae life cycle (Pinder 1983, Armitage et al. 1995).

Given its high representation and high richness in freshwater systems, recently, we attempted to fill this gap by promoting a European Chironomidae trait database developed at the genus level (Serra et al. 2016). Comparable trait information was collected by the United States Environmental Protection Agency (USEPA 2012) for freshwater macroinvertebrates. The database comprises entries for Chironomidae genus and species using data from for example Beck (1977), Vieira et al. (2006) and Yuan (2006). In spite of the existing information, few studies have addressed intercontinental variability (Lindeberg 1980). Yet, Chironomidae taxonomic and ecological uncertainties are stronger for distant allopatric populations, such as those from Europe and North America. Such variability has been tackled from the molecular point of view by several authors (e.g., Kiknadze et al. 1996, Guryev & Blinov 2002, Martin et al. 2002, Gunderina et al. 2009). However, despite the link between genetic variability and the phenotypic expression, it is unknown how this variability is reflected in the traits of species or in the plasticity of these attributes. In addition, it is important to understand the validity of large trait databases for different regions, and to establish data collection methods that reflect trait plasticity. Thus, in this paper we compare for the first time the European and North American Chironomidae traits aiming at: 1) understanding whether common species and genus share the same traits and functions in the Palaearctic and Nearctic regions; 2) identifying which traits contribute more to the difference between Chironomidae of the two regions, exposing the possible trait plasticity of taxa; 3) identifying potential relevant traits lacking in databases.

Methods

Trait data from European (EU) and North American (N.A) Chironomidae were gathered from two databases: Serra et al. (2016) and USEPA (2012) respectively. Common genera and species and common traits were first identified. The traits included biological and physiological characteristics, and ecological requirements. These traits were grouped into Eltonian and Grinnellian traits following Serra et al. (2016). Eltonian traits (proxy to biological traits) are related with the functional role of taxa in the ecosystem (e.g., body size, voltinism, feeding habits). Grinnellian traits are related with the taxa response to particular resources and therefore with their requirements and performance over various environmental conditions (e.g., pH, temperature, and food preferences). The N.A database gathered trait information using mainly a binary coding (yes/no) identifying trait existence/nonexistence of an affinity to a trait category (non-mutually exclusive), or by directly attributing nominal trait categories to taxa given a specific trait variable. The EU database expressed affinities of a genus to a specific trait category, quantifying the number of references liking a genus to a given trait category. The information used to characterize a genus was based on the respective species information whenever possible. Since the affinity of taxa to each trait was presented in different ways in the two databases, all affinities were harmonized by conversion to “0”/”1” data, being “1” the affinity of a given taxa to a trait category and “0” no affinity (or missing values if “0” for all categories of a trait).

Initial comparisons were implemented at the genus level enclosing the information of all species available in each database, to allow for a potentially higher number of traits and taxa to be used in data analyses. Trait patterns with information for more than 50% of common genera of the databases were analysed using Fuzzy Correspondence Analysis (FCA; Chevenet et al. 1994) for each group of traits separately (Eltonian and Grinnellian). A between-class analysis (Dolédec & Chessel 1987, Ter Braak 1988) was used to assess the degree of discrimination between trait databases, defining N.A and EU databases as factor partitioning the rows (Chironomidae genera). The significance of the separation was obtained by generating simulated values after permutation of the rows of the genus trait-composition arrays (999 permutations).

In a second phase, databases were compared at species level to allow for finer resolution comparisons. Within the total of common species, the best-described species (with information for at least 50% of trait information available) were selected to compare their trait-category affinities using a FCA to assess the significance of discrimination between databases. In addition, percentages of discordance were estimated, considering the total number of species-trait combination that revealed the affinity towards the same trait categories in both databases, and the number of divergent combination in comparison with all combinations of species traits selected. This approach allowed clarifying whether differences between databases reflected some trait divergence or the absence of information.

Statistics and graphical outputs required R freeware (R Core Team 2015), using ‘ade4’ library (Thioulouse et al. 1997, Chessel et al. 2004, Dray et al. 2007a,b).

Results

Database description

The North American database included 168 Chironomidae genera and 275 species (80% of total genera and 25% of total species listed for Nearctic region; Ferrington 2008) whereas the European database includes 178 Chironomidae genera based on the information for 650 species (92% of the genera and 52% of the species listed for Europe; Saether & Spies 2013). A total of 136 Chironomidae genera were common to the two databases (76.4% and 81.0% of genera present in the EU and the N.A databases, respectively) whereas they had only 64 species in common (8.4% and 23.3% of species presented in the EU and the N.A databases, respectively). The list of common genera and species to both databases can be seen in Table V. 1.

The N.A database included information for a higher number of Eltonian than Grinnellian traits (n= 31 and n= 21 respectively) whereas the opposite happens in the EU database (n= 20 against n= 36 respectively). Traits in both databases included mainly information at the genus level due to the limited information on species, especially in the N.A database (see Table V. 2). In average, Eltonian traits are described for 47 genus and 9 species in the EU database (corresponding to 34.3 and 14.5% of common genus and species, respectively) and for 20 genus and 4 species in the N.A database (14.9 and 5.9% of common genus and species, respectively). Grinnellian traits are described for 87 genus and 28 species in the EU database (63.6 and 43.7% of common genus and species, respectively) and 45 genus and 19 species in the N.A database (31.6 and 26.6% of common genus and species respectively).

Several traits lacked information for at least 50% of the 136 genera common to both databases. A total of 4 Eltonian traits (development time, type of dispersal, aerial distance travelled and diapause) gathered information for less than 50% of genera common to both databases, whereas 3 Eltonian (emergence season, voltinism, maximal body size) and 2 Grinnellian traits (salinity and elevational preferences) had no information available in the N.A database for at least 50% of genera common to both databases.

Table V. 1 Chironomidae genera and species common to the European and North American databases. The total number of taxa in each subfamily is presented at the end of each list in parentheses.

Subfamily	List of genera (*n*)	List of Species(*n*)
Chironominae	*Axarus*, *Chernovskiia*, *Chironomus*, *Cladopelma*, *Cladotanytarsus*, *Constempellina*, *Cryptochironomus*, *Cryptotendipes*, *Cyphomella*, *Demeijerea*, *Demicryptochironomus*, *Dicrotendipes*, *Einfeldia*, *Endochironomus*, *Glyptotendipes*, *Harnischia*, *Kiefferulus*, *Kloosia*, *Lauterborniella*, *Lipiniella*, *Microchironomus*, *Micropsectra*, *Microtendipes*, *Neozavrelia*, *Nilothauma*, *Omisus*, *Pagastiella*, *Parachironomus*, *Paracladopelma*, *Paralauterborniella*, *Paratanytarsus*, *Paratendipes*, *Phaenopsectra*, *Polypedilum*, *Pseudochironomus*, *Rheotanytarsus*, *Robackia*, *Saetheria*, *Sergentia*, *Stempellina*, Stempellinella, *Stenochironomus*, *Stictochironomus*, *Sublettea*, *Tanytarsus*, *Tribelos*, *Xenochironomus*, *Zavrelia*, *Zavreliella* (49)	*Chironomus plumosus*, *Chironomus riparius*, *Chironomus tentans*, *Cryptotendipes darbyi*, *Demicryptochironomus vulneratus*, *Dicrotendipes nervosus*, *Glyptotendipes barbipes*, *Glyptotendipes paripes*, *Harnischia curtilamellata*, *Lauterborniella agrayloides*, *Microtendipes pedellus*, *Microtendipes rydalensis*, *Parachironomus frequens*, *Parachironomus monochromus*, *Parachironomus tenuicaudatus*, *Paracladopelma undine*, *Paralauterborniella nigrohalteralis*, *Paratendipes albimanus*, *Polypedilum apicatum*, *Polypedilum convictum*, *Polypedilum fallax*, *Polypedilum laetum*, *Polypedilum scalaenum*, *Polypedilum tritum*, *Phaenopsectra flavipes*, *Phaenopsectra punctipes*, *Robackia demeijerei*, *Saetheria tylus*, *Sergentia coracina*, *Xenochironomus xenolabis*, *Cladotanytarsus mancus*, *Cladotanytarsus vanderwulpi*, *Tanytarsus curticornis* (33)
Diamesinae	*Boreoheptagyia*, *Diamesa*, *Potthastia*, *Pseudodiamesa*, *Sympotthastia*, *Syndiamesa* (6)	*Potthastia gaedii*, *Potthastia longimanus* (2)
Orthocladiinae	*Acricotopus*, *Brillia*, *Bryophaenocladius*, *Cardiocladius*, *Chaetocladius*, *Clunio*, *Corynoneura*, *Cricotopus*, *Diplocladius*, *Doncricotopus*, *Epoicocladius*, *Eukiefferiella*, *Euryhapsis*, *Georthocladius*, *Gymnometriocnemus*, *Halocladius*, *Heleniella*, *Heterotanytarsus*, *Heterotrissocladius*, *Hydrobaenus*, *Krenosmittia*, *Limnophyes*, *Mesosmittia*, *Metriocnemus*, *Nanocladius*, *Oliveridia*, *Orthocladius*, *Parachaetocladius*, *Paracladius*, *Paracricotopus*, *Parakiefferiella*, *Paralimnophyes*, *Parametriocnemus*, *Paraphaenocladius*, *Paratrichocladius*, *Parorthocladius*, *Psectrocladius*, *Pseudorthocladius*, *Pseudosmittia*, *Psilometriocnemus*, *Rheocricotopus*, *Rheosmittia*, *Smittia*, *Stilocladius*, *Symbiocladius*, *Synorthocladius*, *Thienemanniella*, *Tokunagaia*, *Trissocladius*, *Tvetenia*, *Zalutschia* (51)	*Brillia flavifrons*, *Corynoneura celeripes*, *Corynoneura lobata*, *Cricotopus bicinctus*, *Cricotopus sylvestris*, *Cricotopus tremulus*, *Cricotopus trifascia*, *Cricotopus vierriensis*, *Cricotopus intersectus*, *Diplocladius cultriger*, *Epoicocladius flavens/ephemerae*, *Eukiefferiella brehmi*, *Eukiefferiella claripennis*, *Eukiefferiella coerulescens*, *Eukiefferiella devonica*, *Eukiefferiella gracei*, *Heterotrissocladius marcidus*, *Nanocladius rectinervis*, *Orthocladius lignicola*, *Synorthocladius semivirens*, *Tvetenia bavarica*, *Tvetenia calvescens*, *Tvetenia discoloripes* (23)
Podonominae	*Boreochlus*, *Parochlus* (2)	(0)
Prodiamesinae	*Monodiamesa*, *Odontomesa*, *Prodiamesa* (3)	*Odontomesa fulva*, *Prodiamesa olivacea* (2)
Tanypodinae	*Ablabesmyia*, *Apsectrotanypus*, *Arctopelopia*, *Clinotanypus*, *Conchapelopia*, *Derotanypus*, *Guttipelopia*, *Hayesomyia*, *Krenopelopia*, *Labrundinia*, *Larsia*, *Macropelopia*, *Meropelopia*, *Monopelopia*, *Natarsia*, *Nilotanypus*, *Paramerina*, *Procladius*, *Psectrotanypus*, *Rheopelopia*, *Tanypus*, *Telopelopia*, *Thienemannimyia*, *Trissopelopia*, *Zavrelimyia* (25)	*Ablabesmyia monilis*, *Hayesomyia senata*, *Procladius culiciformis*, Tanypus punctipennis (4)
Total taxa	136	64

Table V. 2 Number of genera (gn) and species (sp) having available trait information (136 genera; 64 species) in European (EU) and North American (N.A) databases.

	EU			**N.A**		
Eltonian	**Trait**	**gn**	**sp**	**Trait**	**gn**	**sp**
Emergence	Season	78	23	Season	17	29
	Duration	23	3	Synchronization	1	0
	Flight period	88	28	Behaviour	3	0
				Primary season	16	2
				Secondary season	16	1
Reproduction/ Oviposition	Eggs per egg-mass	40	11	Fecundity (total number of eggs)	1	1
	Reproduction type	6	1	Egg type	1	1
				Primary oviposition behaviour	1	1
				Secondary oviposition behaviour	1	1
				Hatch time	1	1
Life cycle/ Development	Duration	9	4	Development pattern	8	6
	Length of larval development	64	13	Adult lifespan	1	1
	Voltinism	84	24	Voltinism	22	9
Body size/ Morphology	Maximal body size (4th instar)	133	15	Max body size	16	0
				Measured length	13	0
				Body shape with case	13	0
				Adaptation silk production	15	0
				Adaptation other	2	0
				Attachment	16	29
Mobility/ Dispersal	Aquatic distance travelled	3	0	Drift propensity (early instars)	1	0
	Aerial distance travelled	7	0	Drift propensity (late instars)	5	0
	Type of dispersal	23	4	Adult dispersal distance	0	1
	Migration patterns	3	1	Female dispersal ability	1	0
				Ability to temporarily exit water	2	0
Feeding	Feeding habits	73	23	Primary functional feeding group	113	32
				Primary feeding	89	1
				Secondary feeding	31	1
Locomotion mode/ Substrate relation	Substrate relation	62	2	Primary habit category	105	0
	Tube construction	63	11	Primary locomotion habit	95	0
				Secondary locomotion habit	20	0
Resistance	Hibernation phase/instar	67	14	Diapause	3	0
	Resistance form	31	5			
Respiration	Haemoglobin	41	4			
	Respiration (#tracheas)	34	0			
Grinnellian	**Trait**	**gn**	**sp**	**Trait**	**gn**	**sp**
Elevation	Preferences	88	23	Minimum	40	15
				Maximum	24	3
Food	Food type	92	18			
Oxygen	Oxygen preferences	87	28	Oxygen tolerance	20	32
pH	pH preferences	88	36	pH tolerance	18	30
Current	Current velocity preferences (cm.s-1)	89	33	Current preference categories	23	32
				Current Optima	42	31
				Rheophily	68	32
Salinity	Chlorinity (g.Cl^{-1})	63	16	Salinity tolerance	4	0
	Salinity preferences	104	32			
Temperature	Optimal temperature of emergence (ºC)	16	3	Thermal pref	17	31
	Temperature preferences	73	20	Minimum temperature	16	9
				Maximum temperature	10	7
				Thermal interval	16	31
				Thermal Optima	70	32
				Thermal Tolerance	45	2
				Thermal Indicator	25	1
Habitat	General/gross Habitat	127	52	Primary water body type	136	30
	Substrate preferences	110	45	Microhabitat preferences	79	16
	Longitudinal distribution	92	26	Lateral habitat position	100	8
	Transversal distribution	129	60	Vertical habitat position	61	9
	Saprobity	69	16	Enrichment (organic) tolerance	106	31
	Trophic status preferences	67	24	General turbidity tolerance	24	26
	Depth preferences	91	16			

From the 19 traits with information in both databases a total of 10 traits (Table V. 3 and Table V. 4), gathered information for more than 50% of the genera common to N.A and EU databases. These included 3 Eltonian (flight period, feeding habits and locomotion; Table V. 3), and 7 Grinnellian (preferences for dissolved oxygen, pH, current, temperature, microhabitat, water body type and saprobity; Table V. 4).

Table V. 3 Eltonian traits and respective categories with information for EU and N.A databases. The number of total entries for genera is shown for each database (with respective % facing the number of shared genera).

	Genera (%)			
Eltonian trait	**EU**	**N.A**	**Trait category**	**Code**
Emergence season	78	18	Winter	EMWIN
	(57%)	(13%)	Spring	EMSPR
			Summer	EMSUM
			Autumn	EMAUT
Flight period[a]	88	70	Winter	FLYWIN
	(65%)	(51%)	Spring	FLYSPR
			Summer	FLYSUM
			Autumn	FLYAUT
Voltinism	84	22	Semivoltine (<1gen y^{-1})	SEMI
	(62%)	(16%)	Univoltine (1gen y^{-1})	UNIV
			Bivoltine/ Multivoltine (>1gen y^{-1})	BIMU
Development time	67	11	<3 months	L3M
	(49%)	(8%)	3-6months (inclusive)	B36M
			>6 months	M6M
Maximal body size (4th instar)	133	16	Small (length < 9 mm)	SMALL
	(98%)	(12%)	Medium (length [9-16] mm)	MEDIUM
			Large (length > 16 mm)	LARGE
Type of dispersal	22	5	Passive aquatic	AQUPAS
	(16%)	(4%)	Active aquatic	AQUACT
			Passive aerial	AERPAS
			Active aerial	AERACT
Aerial distance travelled	7	1	<10	DAER1
	(5%)	(<1%)	10 – 100	DAER2
			100 – 1000	DAER3
			>1000	DAER4
Feeding habits[a]	73	118	Fine sediment/ deposit eater, collector-gatherer	CG
	(52%)	(87%)	Shredder	SH
			Scraper/grazer, herbivore	HB
			Filter, Collector-filterer	CF
			Predator	PR
			Parasite	PA
Locomotion[a]	62	116	Free living	FRE
	(46%)	(85%)	Burrower	BUR
			Miner	MIN
			Fixed (substrate or plants)	FIX
Diapause	67	3	Yes	DIAP
	(49%)	(2%)	No	DIAA

[a] Traits with information for more than 50% of genera in each database.

Table V. 4 Grinnellian traits and respective categories with information for EU and N.A databases. The number of total entries for genera is shown for each database (with respective % facing the number of shared genera).

	Genera (%)			
Grinnellian trait	**EU**	**N.A**	**Trait category**	**Code**
Dissolved oxygen preferences[a]	89	76	High (always around 50%)	O2RICH
	(65%)	(56%)	Stable-Intermediate (>50%)	O2STAB
			Low near anoxia	O2LOW
pH preferences[a]	88	75	Acidic	PHACI
	(65%)	(55%)	Intermediate/neutral	PHNEU
			Alkaline	PHALK
Current velocity preferences[a]	89	93	Null	VELO1
	(65%)	(68%)	<25 cm.s^{-1}, slow	VELO2
			> 25 – 50 cm.s^{-1}, moderate	VELO3
			>50 cm.s^{-1}, fast to turbulent	VELO4
Salinity preferences	104	4	Fresh water	FRESH
	(76%)	(3%)	Brackish water	BRACK
Elevational preferences	88	46	<1000m (lowlands)	ALTI1
	(65%)	(34%)	>1000 – 2000m (piedmont)	ALTI2
			>2000m (Alpine)	ALTI3
Temperature preferences[a]	73	80	Psychrophilic <15ºC	TPSYC
	(54%)	(59%)	Thermophilic >15ºC	TTHER
			Eurythermic	TEURY
Microhabitat preferences[a]	110	87	Sand	SAND
	(81%)	(64%)	Silt	SILT
			Gravel	GRAVEL
			Stone, boulder, cobble, pebble	STONE
			Large woody debris, Twigs, roots	WOOD
			Small detritus and organic mud	DETRIT
			Macrophytes, bryophytes, algae, Microphytes	PHYTO
			Invertebrates	MINVER
Saprobity[a]	69	109	Xenosaprobic; intolerant	XENOSAP
	(51%)	(80%)	Oligosaprobic; moderately intolerant	OLIGSAP
			β-mesosaprobic; moderately tolerant	BMESSAP
			α-mesosaprobic; tolerant	AMESSAP
			Polysaprobic ; very tolerant	POLYSAP
Water body type[a]	133	130	River channel	RIVER
	(98%)	(96%)	Lakes	LAKES
			Ponds, pools, disconnected side-arms	POND
			Wetlands, marshes, peat-bog	MARSH
			Crenon, cold and thermal springs	CRENO
			Headwaters streams/ Epirithron	EPIRIT
			Kryon (glacial fead habitats)	KRYON
			Outside fluvial system	OUTFLU
			Temporary waters	TEMPOR

[a] Traits with information for more than 50% of genera in each database.

Database comparison

At the genus level, the first-three axes of a FCA performed on Eltonian traits (with information for more than 50% of genera in both databases; Figure V. 1a) explained 53.4% of the total variance. Correlation ratios (i.e., taxa variance explained by trait categories) revealed that locomotion and feeding habits were the best correlated with the first FCA axis whereas feeding habits were best correlated with the second FCA axis (Table V. 5). A between-FCA analysis of Eltonian traits assessed a low but significant difference between the databases (7.3% variance explained; simulated-*P*= 0.001; Figure V. 1a). Feeding habits and locomotion were the Eltonian traits that contributed the most to this difference.

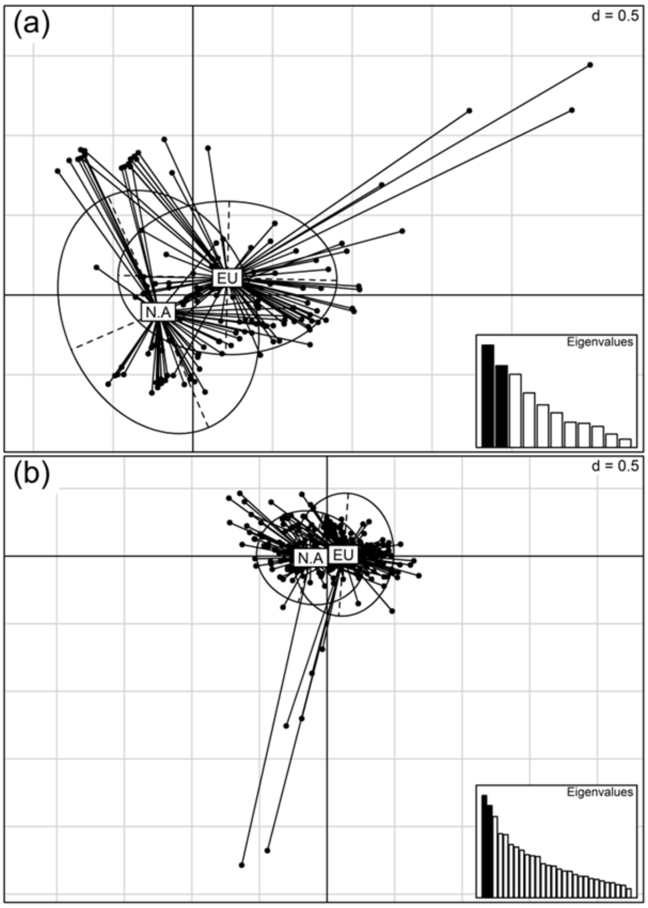


Figure V. 1 Fuzzy Correspondence Analysis (FCA) based on traits of 136 genera common to Europe (EU) and North American (N.A) databases. (a) FCA performed on Eltonian traits. (b) FCA performed on Grinnellian traits. FCA scores for individual genera (small dots) are connected to database centroids (identified by two letters) showing the mean score for each database. Ellipses encompass 67% of the genera along each axis for each database, correlation ratios presented in Table V. 5 and Table V. 6 (Eltonian and Grinnellian respectively).

Table V. 5 Correlation ratios (multiplied by 1000) along the first-three axes of a FCA performed on the Eltonian trait-by-genera and trait-by-species array. Eigenvalue (multiplied by 1000) and variance extracted (%) also are indicated for each axis.

	Trait-by-genera			Trait-by-species		
	Axes			Axes		
Traits	**F1**	**F2**	**F3**	**F1**	**F2**	**F3**
Flight period	9	5	6	198	85	81
Locomotion	339	12	116	91	175	203
Feeding habits	372	559	394	395	385	252
Eigenvalues	240	192	172	228	215	145
Variance extracted (%)	21.2	17.0	15.2	21.4	20.2	16.8

The first-three axes of a FCA of performed on Grinnellian (with information for more than 50% of genera in both databases; Figure V. 1b) explained 26.4% of the total variance. Water body type, current velocity and microhabitat preferences were best correlated with the first FCA axis (Table V. 6). A between-FCA analysis yielded a small but significant difference between the databases (4.4% variance explained; simulated-*P*= 0.001; Figure V. 1b). Traits that contributed the most to the difference were microhabitat preferences and, to a lesser extent, water body type.

Table V. 6 Correlation ratios (multiplied by 1000) along the first-three axes of a FCA performed on the Grinnellian trait-by-genera array and trait-by-species. Eigenvalue (multiplied by 1000) and variance extracted (%) also are indicated for each axis.

	trait-by-genera			**trait-by-species**		
	Axes			**Axes**		
Traits	**F1**	**F2**	**F3**	**F1**	**F2**	**F3**
pH preferences	3	4	2	2	1	6
Temperature preferences	13	14	83	33	298	12
Dissolved oxygen preferences	49	9	16	357	154	7
Saprobity	99	1	5	256	90	70
Microhabitat preferences	107	397	153	82	80	275
Current velocity preferences	122	23	12	149	1	52
Water body type	172	63	180	155	86	171
Eigenvalues	81	73	64	148	101	85
Variance extracted (%)	9.8	8.8	7.8	16.8	11.5	9.6

At the species level, the absence of Eltonian traits information was more evident in EU than in N.A (Figure V. 2). However there was only one species of Tanypodinae (*Hayesomyia senata*) with no information for both Eltonian and Grinnellian traits in the EU database, whereas in the N.A database there was a total absence of information in both trait domains for 9 species, mostly Orthocladiinae (*Diplocladius cultriger*, *Eukiefferiella brehmi*, *E. claripennis*, *E. gracei*, *Heterotrissocladius marcidus, Tvetenia discoloripes*) but also Chironominae (*Polypedilum tritum*) and Diamesinae (*Potthastia gaedii*, *P. longimanus*). Considering the 64 species common to both databases, only in EU database a species, *Chironomus plumosus*, presents information for 100% for both Eltonian and Grinnellian traits database.

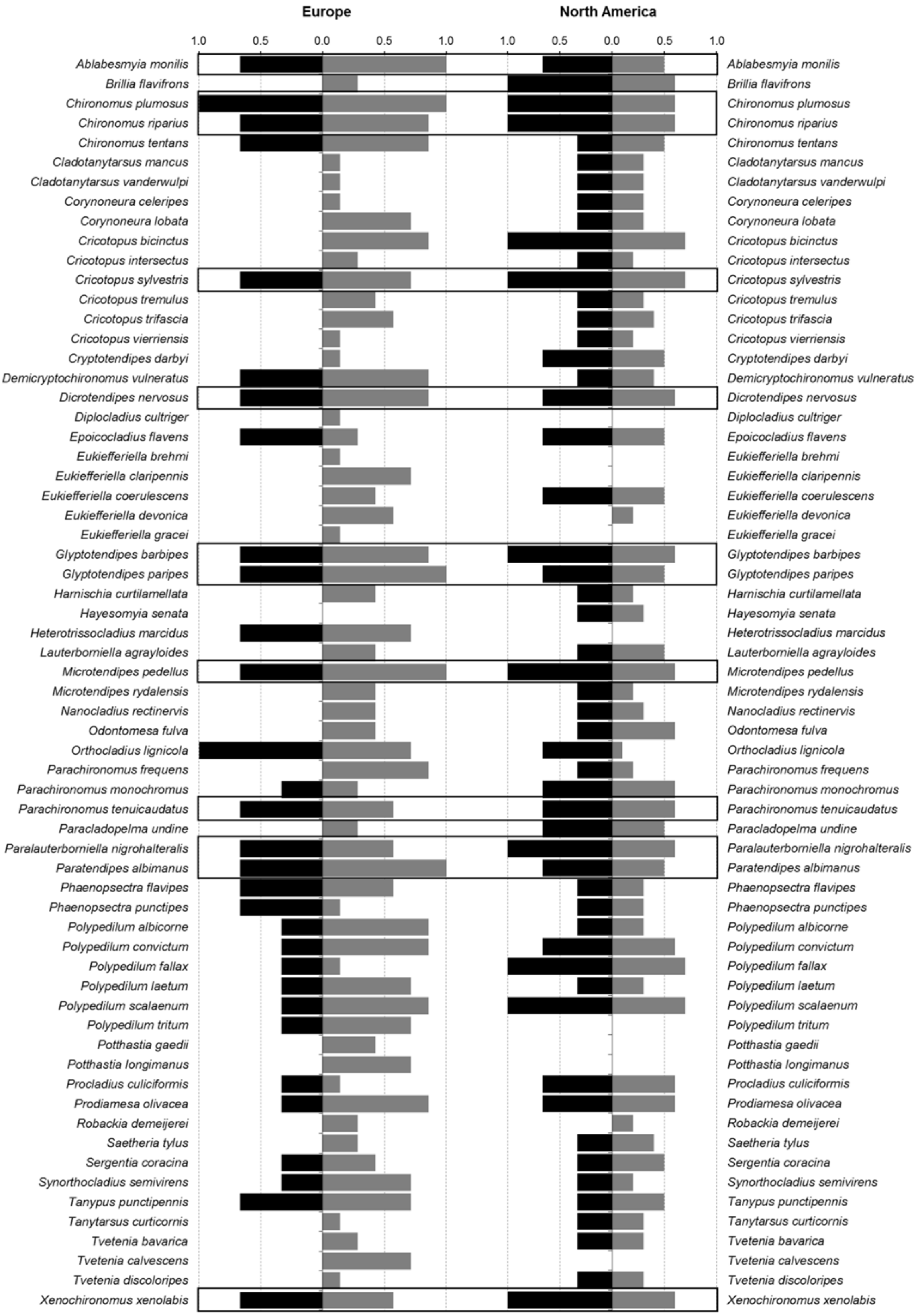


Figure V. 2 Relative proportion of trait information gathered (0 no traits described, 1 all traits described) for the 64 species common to the databases considering the best described Eltonian (3 traits; black) and Grinnellian (7 traits; grey) marked in the Table V. 3 and Table V. 4. Black boxes enclose the species with more than half Eltonian and Grinnellian traits characterized in both databases.

From the 64 species common in both databases, only 12 species had at least 50% information for the selected Eltonian (3) and Grinnellian (7) traits in the two databases, including one Tanypodinae (*Ablabesmyia monilis*), 10 Chironominae (*Chironomus plumosus*, *C. riparius*, *Dicrotendipes nervosus*, *Glyptotendipes barbipes*, *G. paripes*, *Microtendipes pedellus*, *Parachironomus tenuicaudatus*, *Paralauterborniella nigrohalteralis*, *Paratendipes albimanus*, *Xenochironomus xenolabis*) and one Orthocladiinae (*Cricotopus sylvestris*) (Figure V. 2).

The first-three axes of FCA performed on species Eltonian traits (Figure V. 3a) explained 58.5% of the total variance, a between-FCA analysis showed a small but significant difference between the databases (9.9% variance explained; simulated-*P*= 0.001). The traits feeding habits, followed by the flight period and locomotion were those contributing the most to explain data variability (Table V. 5).

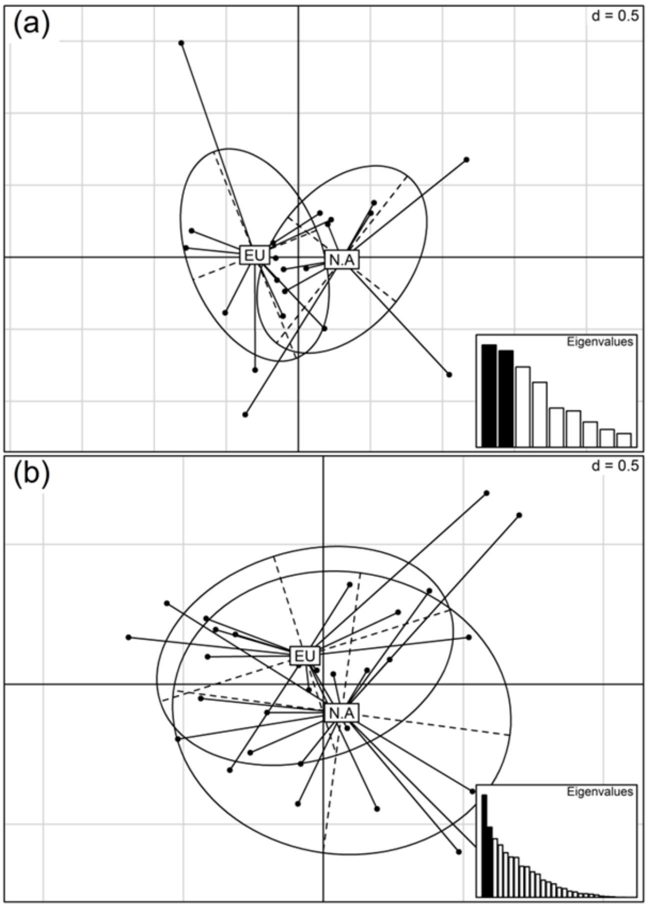


Figure V. 3 Fuzzy Correspondence Analysis (FCA) based on trait information of 12 species common to Europe (EU) and North American (N.A) databases, with information for at least 50% of the ten best described traits of the. (a) FCA performed on Eltonian traits. (b) FCA performed on Grinnellian traits. FCA scores for individual species (small dots) are connected to database centroids (identified by two letters) showing the mean score for each database. Ellipses encompass 67% of the genera along each axis for each database, correlation ratios presented in Table V. 5 and Table V. 6 (Eltonian and Grinnellian respectively).

The first-three axes of a FCA performed on species Grinnellian traits (Figure V. 3b) explained 37.9% of the total variance. Again a between-FCA analysis exposed a small but significant difference between databases (6.5% variance explained; simulated-*P*= 0.005), to which the water body type and microhabitat preferences were the highest contributors (Table V. 6).

When comparing species traits across databases we found: (1) coincident affinities (e.g., Figure V. 4a); (2) totally divergent affinities (e.g., Figure V. 4b,c); (3) partial divergence (e.g., Figure V. 4d); (4) trait information from a database contained into the other (e.g., Figure V. 4e,f); and (5) trait information absent in one of the databases. The first situation occurred in 11.7% of the species traits, for species like *Chironomus plumosus* (with the flight period occurring in all seasons of the year and no specific pH preference), *C. riparius* (collector-gatherer with no specific pH preference), *Glyptotendipes barbipes* (no specific pH preferences and tolerance to organic enrichment), *G. paripes* (no pH preference and slow flow velocity preference), and *Paratendipes albimanus* (no oxygen and temperature preferences). The databases agreed in pH preferences for 5 of the 12 species. A total discordance occurred for 4.2% of the species traits analysed and more often in Eltonian (4 species) than in Grinnellian traits (one species). For example, *Xenochironomus xenolabis* (e.g., Figure V. 4b) is a parasite and collector-filterer in the EU database whereas it is a predator in the N.A database. Similarly, *Microtendipes pedellu*s (e.g., Figure V. 4c), has a winter flight period, and is thermophilic or eurythermic in the N.A database whereas in the EU database it is considered psychrophilic, i.e., the period of flight in all other seasons but winter. The species in the two databases diverge in 11.7% of trait categories' affinities (e.g., Figure V. 4d). The fourth situation happened in: 23.3% of the 120 species-trait combinations studied, for the N.A trait information contained in the EU information (e.g., Figure V. 4e); and 13.3% of the combinations, for the EU contained in the N.A information (e.g., Figure V. 4f). Finally, the absence of information occurred in 19.2% and 15.8% of the species traits in the EU or in the N.A database, respectively. Lack of information on saprobity occurred in both databases for *Parachironomus tenuicaudatus* (0.8% of the species-trait combinations). Only the remaining 11.7% reflect the level of concordance between databases considering the 12 species and 10 traits (and respective 49 categories).

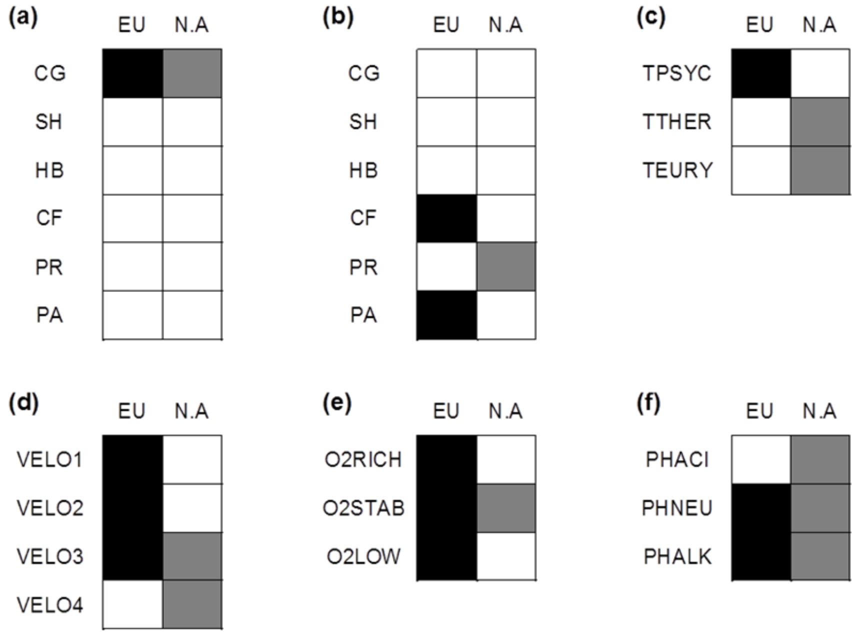


Figure V. 4 Examples of species-trait affinities (EU database in black boxes, N.A database in grey boxes, zero affinity in white) for 6 species. (a) *Chironomus riparius* and feeding habits. (b) *Xenochironomus xenolabis* and feeding habits. (c) *Microtendipes pedellus* and temperature preferences. (d) *Paralauterborniella nigrohalteralis* and current preferences. (e) *Ablabesmyia monilis* and oxygen preferences. (f) *Glyptotendipes barbipes* and pH preferences. See Table V. 3 and Table V. 4 for trait category acronyms.

Discussion

Our study indicates that a same species may have different traits and thus different functional roles and environmental requirements within the Holarctic region, depending on whether they are in the Nearctic or Palaearctic sub-regions. Common species in both sub-regions seem to have also different levels of plasticity, as the variety of categories for which the species has affinity within a trait also vary. This points out to trait filters operating at different spatial scales, from the larger to the local spatial scales (Statzner et al. 2004). Species showed a higher diversity of trait categories in one of the regions studied; Chironomidae species-trait categories from N.A were more frequently contained in the EU information that on its turn gathered a wider possibility of trait categories for the same species.

This is the first study indicating this trait divergence and plasticity for aquatic Chironomidae. Yet, previous studies exposed the trait divergence and plasticity, for various taxonomic groups such as plants (Luo et al. 2015), birds (Bertrand et al. 2016), mammals (e.g., Zhou et al. 2011) and terrestrial invertebrates (e.g., Hochkirch et al. 2008, Lecocq et al. 2013), reflecting local adaptations and patterns of divergence along different geographical scales. There is a link between the environment, the traits of the organisms that inhabit it and their own evolution. Such environment-trait link is disclosed by a parallel evolution reflected in the convergence of the reproductive traits of genetically distinct species of *Enallagma* (Odonata), inhabiting the Nearctic and Palaearctic continents (Stoks et al. 2005).

Trait variability and phenotypic plasticity have been under intense debate (e.g., Hochkirch et al. 2008, Snell-Rood et al. 2015). Organisms' traits are ultimately a product of genetic expression. Differences in traits reflect this genetically accumulated divergence and genetic drift. And even the phenotypic plasticity may itself be genetically determined (Nijhout 2003, Hochkirch et al. 2008, Moczek 2010). Genetic distances between geographically separated populations of species with a widespread distribution like *Chironomus plumosus* or *Chironomus tentans* were shown by many studies (Butler et al. 1999, Kiknadze et al. 1996, Gunderina et al. 2009). Populations from other insect species also reflect the divergence occurring in their populations' genetic structure across different regions, revealing macrogeographic patterns of evolution (e.g., Hemiptera, *Nezara viridula*; Kavar et al. 2006). In some specific cases the divergence is such that populations could be considered as belonging to different species (e.g., Coleoptera, *Colymbetes paykulli*; Drotz et al. 2015).

Usually traits are defined at the individual level and then applied on a wide geographical scale by the development of trait databases. Traits are considered consistent descriptors across large spatial scales (Bêche & Statzner 2009, Statzner & Bêche 2010) the intraspecific variability of traits in quite unknown (e.g., Petchey & Gaston 2006, Griffiths et al. 2016). Individuals within a species may indeed vary considerably attending to the affinity of the traits they exhibit, as result of local adaptation but also of phenotypic plasticity (Bolnick et al. 2011, Albert et al. 2012, Violle et al. 2012, Carmona et al. 2016).

Our results may have also been influenced by two additional factors: (1) differences in the structure of trait databases built in Europe and North America and (2) insufficient information on Chironomidae traits in one or either regions. In fact, for macroinvertebrates, excluding Chironomidae, Statzner & Bêche (2010) showed that the biological traits' knowledge available was quite different in Europe and North America.

There are still a number of challenges associated to the use of trait approaches; one is the standardization of trait definition and collection (Statzner & Bêche 2010, Baird et al. 2011). In fact, there are obvious differences in the Chironomidae databases structure between continents which reflect the absence of standardization in collecting trait information (Culp et al. 2010, Baird et al. 2011, Schmera et al. 2015): (1) there is an unequal list of trait categories included (36 and 52 in EU and N.A databases, respectively); (2) the EU database quantified affinities of a genus to a specific trait category using the number of references as a score liking genus-trait category, similar to the fuzzy coding approach (Chevenet et al. 1994); whereas the N.A database code trait affinities using presence/absence; and (3) the EU database was designed for Chironomidae only and considered traits specific to this family (e.g., presence of haemoglobin) whereas the N.A database was part of a wider trait compilation on N.A freshwater macroinvertebrates. This database includes relevant characteristics that should also be considered for the Holarctic region, related to morphological adaptations and to functions such as drag and silk production, body shape and presence of structures like hooks, hairs, which are partially available for other macroinvertebrates (body size and body flexibility; see Resh et al. 1994, Richoux 1994, Statzner et al. 1994, Tachet et al. 1994, Usseglio-Polatera 1994, Usseglio-Polatera & Tachet 1994) but not for Chironomidae. Another difference (4) is related to the trait categories, as similar traits have different categories in the EU and N.A databases. This is the case of fecundity (type and number of eggs), emergence synchronization and developmental speed from the N.A database; and the traits number of eggs per egg-mass, reproduction type, emergence duration, and time/length of larval development in the EU database. Including them in a common database would require further discussion to identify the most relevant trait categories. For example, the number of eggs may be expressed in eggs per mass or per female. However, further information would be needed to convert one into the other, as the number of eggs per egg-masses vary from few to several thousand and each female may produce one egg-mass (more common) but also more (Vallenduuk & Moller Pillot 2007). Finally (5), discordances in coding affinities for specific trait categories may be related to the interpretation of the available trait and potential biological uncertainties. For example, in the N.A database, *Xenochironomus xenolabis* is considered a predator that engulfs sponges whereas in the EU database it lives in sponges. However, whether in this case it is a parasite or a filter feeder, which benefits from the water movement created by the host, is a matter of debate (Tokeshi 1995c, Moller Pillot 2009).

There are more entries for Grinnellian than for Eltonian traits in the databases. This reflects the wider knowledge we have about the ecological requirements of taxa given their distribution patterns than that on traits related to their ecological functions in the ecosystems (e.g., Statzner et al. 2001, Gayraud et al. 2003). In fact, a higher effort is needed in describing some Eltonian traits to enable ecological large-geographical scale studies. Traits such as voltinism and maximal body size that are not well described for Chironomidae, have been proved to be important for other macroinvertebrates in the discrimination of various types of human impact (Dolédec & Statzner 2008). Also, traits related to Chironomidae resistance are scarce. Yet, for example morphological characteristics like the wings length (McLachlan 1985) are known to reflect dispersion ability and were related to the type of habitat colonized (permanent or temporary) (Delettre 1988).

The information on species is in fact quite uneven, as on the other hand, there are well-described species in both databases, such as the *Chironomus plumosus*. This is the case of a species with a wide distribution and abundance throughout the Holarctic region (Moller Pillot 2009). This species is of easy laboratory maintenance being commonly used in toxicological tests, resulting in a good knowledge on their traits and responses to environmental factors and stressors (McLachlan & Cantrell 1976, Gunderina et al. 2009, Roskosch et al. 2012).

In conclusion, our study indicates differences in the traits associated to a given genus or species within the Holarctic region, which may have resulted from different adaptations to the environment following the historical separation of the Palaearctic and Nearctic sub-regions. However, at least partially, conclusions may be influenced by the reduced information on some common species and traits of Chironomidae. The potential lack of information associated to Chironomidae can only be dismissed by further investigation focused especially on Eltonian traits. Performance traits (production, biomass) besides some other Chironomidae-specific traits should also be part of the investigation. Furthermore, only the standardization of trait information will allow the construction of a common database for Chironomidae for the Holarctic region. This would enable large-scale geographical comparisons and a more realistic contribution of this family to the functional assessment of freshwater systems.

General Conclusions

Trait databases are a fundamental tool for trait-based studies that have been used in the indirect functional assessment of ecosystems. A trait database expressing species trait affinities as the number of references citing specific trait categories to specific taxa ensure future improvement and continued updating of the database. Simultaneously, such a database enables capturing taxa variability, exploring information from different habitats and environmental conditions, and allows the easy identification of taxa with reduced or no information, to which attention should be given in the future.

The trait database produced in this thesis (Chapter I) was the first comprehensive trait database developed for Chironomidae at the genus level, with information gathered at the species level using European references (~150). It covers 92% of European genera based on 59% of European species for a total of 37 traits (with 184 trait categories), with traits related to taxa roles in their habitats (20 Eltonian traits and 86 trait categories) and ecological requirements (17 Grinnellian traits and 98 trait categories). The Grinnellian traits group had generally more information for genera, meaning that it is easier to find information related with Chironomidae environmental requirements and performance along different environmental gradients; whereas information that reflect functional roles of Chironomidae taxa in their habitats is less readily available. The European Chironomidae database can be relevant for ecological studies and has a potential use in biomonitoring. It is provided as supplementary information available online in an academic peer-reviewed journal (Serra et al. 2016).

The trait information obtained in the Chironomidae trait database assembled at the genus level diverges from the information contained in the European database (Tachet et al. 2010) gathered at the subfamily and tribe level (Chapter II). Traits responsible for these differences include life history traits (e.g., life cycle duration, reproduction type) and environmental preferences (e.g., pH, food types). These results indicate that trait information gathered at a higher taxonomic level (family, subfamily, even tribe) disregards the heterogeneity of Chironomidae, assuming a false trait redundancy among taxa inside each coarser taxonomic level. Being widely represented in freshwaters, various Chironomidae subfamilies gather a high species richness and diversity, a result of the extensive adaptive diversification of their traits by the colonization of various habitats. The Eltonian traits, expected to be genetically more conservative (with higher heritability), defined average distances between subfamilies that were not consistent with the most accepted phylogenetic relationships among these subfamilies. Exceptions occurred only for the Orthocladiinae and Chironominae, which are considered sister groups by the most accepted phylogenetic studies and appear together in the traits-based analyses. This exposes the labile nature of the set of traits studied that decrease the phylogenetic signal between related taxa; and also the reduced set of traits defined by categories.

As for many other parts of the world, taxonomic keys for Portugal are incomplete and the true Chironomidae richness is still unknown. This study revealed the occurrence of Chironomidae genera that were not previously reported for Portugal mainland in Fauna Europaea (Saether & Spies 2013) including: one Prodiamesinae (*Odontomesa* sp.); three Orthocladiinae (*Paracladius*, *Epoicocladius* and *Hydrobaenus*); and two Chironominae (*Paratendipes* and *Tribelos*). *Paratendipes* sp. and *Hydrobaenus* sp. also stated as present in Spanish mainland have been previously reported in Portugal (montane streams of Serra-da-Estrela; Rieradevall et al. 2007) but are not considered by Saether & Spies (2013). *Odontomesa*, *Paracladius*, *Epoicocladius* and *Tribelos* are reported in Spain (Saether & Spies 2013) but have never been mentioned for Portugal. *Epoicocladius* is a European widespread parasite of nymphs of Ephemera (Moller Pillot 2013), which is a common Ephemeroptera in Portuguese northern rivers. *Paratendipes* sp. was previously found in montane streams of Serra-da-Estrela, Portugal (Rieradevall et al. 2007) and in in southern Mediterranean streams in Spain (Baranov 2014). Yet, here it was also collected in permanent lowland rivers suggesting that they might have wider ecological ranges. The *Hydrobaenus* sp. has been considered a typical member of temporary stream communities, building cocoons to face desiccation (Moller Pillot 2013) and we found it only in temporary streams, however there are also records of its presence in Portuguese montane rivers (Rieradevall et al. 2007). *Tribelos* sp. and *Paratendipes* sp. were exclusively found in temporary rivers and previous studies indicate their preference for stagnant waters (Moller Pillot 2009, 2013), which may occur in these rivers during summers. As far as we know, the present study is the first that reveals the presence of *Odontomesa*, *Paracladius*, *Epoicocladius* and *Tribelos* in Portugal (but were recorded in Spain by Saether & Spies 2013) showing the lack of knowledge on Portuguese Chironomids.

Chironomidae taxonomic composition and Eltonian traits of genera allowed the separation of different types of streams (permanent vs. temporary; and also different types of permanent streams; Chapter III) and disturbed from least-disturbed sites (under multiple stressors; Chapter IV). This is indicative that, contrary to what is commonly recognized among other macroinvertebrate groups, Chironomidae is a highly heterogeneous family. Chironomidae genera composition responded to the natural variability of environmental conditions and anthropogenic stress while taxonomic composition at the subfamily level was not enough to separate stream types. This highlights the need to include Chironomidae at higher levels of resolution in ecological and bioassessment studies.

Among all traits considered, Chironomidae traits related to morphology (maximal body size of the 4th larval instar), life history (voltinism, life cycle duration, emergence season and overwinter diapause), feeding behaviour and substrate relation (feeding habits, locomotion/ substrate relation), and physiology (haemoglobin), reflected natural and anthropogenic environmental differences in taxa distribution (Chapter III and IV). Life cycle duration that generated differences between Serra et al. (2016) (Chironomidae trait database at genus level) and Tachet et al. (2010) (Chironomidae trait database at subfamily/tribe level), have now emerged as important in distinguishing stream types and disturbance levels.

Permanent medium elevation streams differed from permanent lowland and temporary streams by, for example, larger proportions of larvae emerging in summer and with few larvae instars with winter diapause, which reflect adaptations of Chironomidae to low temperatures and strong flow variations. Temporary streams are distinguished from permanent streams by the smaller proportion of larvae with intermediate sizes and burrowing behaviour, reflecting the adaptation to unstable sediment and irregular flow regime. Lowland streams presented an intermediate Eltonian trait composition: intermediate sized larvae are common in these streams comparable to medium elevation permanent, whereas a smaller proportion of taxa with summer emergence make these streams comparable to temporary streams. Temporary disturbed sites compared with temporary least-disturbed sites showed, as predicted, larger proportions of Chironomidae taxa with haemoglobin and a smaller proportion of scrappers. However, disturbed sites also presented unpredictable larger proportions of taxa with longer life cycles and few generations per year, and smaller proportions of taxa with active filter feeding behaviour.

In this study it was shown that Chironomidae morphological traits, which are not frequently used in ecological studies, might provide an alternative to the slow and challenging identification of larvae at the species and genera levels. These traits rely on the observation of only a few small structures and on the existence of trait databases, contrasting with the laborious identification of larvae. It was shown that features related to locomotion and substrate relation (length of body setae, type of claws simple/serrated), foraging and feeding behaviour (mentum architecture, degree of Lauterborn development, type of claws simple/serrated) can be significantly different between specific stream types (Chapter III). For example, Chironomidae from permanent medium elevation streams differed from permanent lowland streams by larger proportions of long body setae; whereas temporary streams showed larger proportions of larvae with indistinct Lauterborn organs. These differences reflect mostly different current and flow regimes, and possibly temperature.

The use of trait life-history strategies (trait combinations that evolved together), instead of analysing traits individually to detect temporary Mediterranean sites affected by multiple-stressors (nutrient enrichment, low dissolved oxygen and deep hydromorphological alterations), was also tested, following an approach proposed by Verberk et al. (2013) and considering Chironomidae strategies identified by Van Kleef et al. (2015). The Chironomidae life-history strategy favoured in disturbed sites corresponded to medium size, multivoltine organisms that do not invest much in haemoglobin but rather in a relatively rapid development and without strong spring synchronized emergence. This strategy includes Chironomidae taxa that are relatively sensitive to hypoxia, tolerating only short periods of oxygen stress, and for which the optimal habitats are water bodies with low dynamics (Chapter IV). The use of traits combined as strategies seems a particularly useful approach in a multiple-stressor scenario, while analysing traits individually may lead to results that contradict a priori predictions by the lack of knowledge of the combined effect of stressors, considering the complex interactions between them and the main functions affected in the system.

Most Chironomidae genera and species are found in both Europe and North America with a recognized Holarctic distribution. The continental divergence of Eltonian and Grinnellian traits of taxa present in both continents was thus analysed (Chapter V). Genera and species occurring in both continents showed intrageneric and intraspecific trait variability, reflecting divergence or plasticity, which should be due to trait filters operating at different scales together with the relative isolation of populations by the large geographical distance. However, the different trait knowledge available in Europe and North America, the absence of a standardized trait definition and collection may have contributed to the divergent information. Eltonian traits related to Chironomidae morphology, dispersion and resistance were found to be scarce in both databases. These traits are particularly relevant for Chironomidae, which are often the first colonizers after periods of natural disturbance (e.g., flood, drought), and the only insects present in highly impacted streams.

This study showed that the use of Chironomidae genus level in ecological studies and bioassessment is advisable, particularly in the case of systems poor in other groups (e.g., lowland rivers, reservoirs) and where it may be the only group capable of distinguishing disturbed from least-disturbed conditions.

Further Perspectives

Chironomidae are very well represented in freshwater systems and are expected to increase in abundance under anthropogenic perturbations. However taxonomic and functional diversity of Chironomidae in fresh waters is often unnoticed. This represents a significant lack of information as the diversity of this family may support crucial functions, for example, in the energy flow supplying upper levels of the trophic network. It is thus important that future studies focus on understanding which Chironomidae genera and species are increasing, being lost and what does this truly means in terms of ecosystems functions and services.

Quicker methods for Chironomidae identification are needed. That could be accomplished by alternative approaches like the Chironomid Pupal Exuvial Technique (CEPT; e.g., Raunio et al. 2007, Raposeiro et al. 2011). This technique presents however some limitations especially in lotic systems. More research is needed to compare collected pupae with the actual community and its true representativeness in different types of streams and rivers. In addition, molecular markers may allow genera or species identification with a relatively accuracy, regardless the life stage, resolving taxonomic ambiguities and improving data quality (Carew et al. 2003, 2007, 2013). Molecular techniques are becoming faster and cheaper with the Next Generation Sequencing (NGS), offering a new perspective for biomonitoring programs and could be especially valuable for taxonomic groups requiring great taxonomic expertise such as the Chironomidae (Pfrender et al. 2010, Hajibabaei et al. 2011, Carew et al. 2013).

The direct observation of morphological traits could allow the use of Chironomidae in bioassessment saving time in identifications. Yet, the link between morphological structures and their functions in the system is poorly understood. Establishing these functional relations is thus important to identify the relevant morphological traits and systematically test their value in the bioassessment.

Chironomidae are frequently mentioned as the first colonizers after extreme events, such as droughts or floods in temporary Mediterranean streams (Marziali et al. 2010). Some Chironomidae taxa are able to use temporary habitats due to behavioural and physiological adaptations that include vertical migration into the substrate, opportunistic behaviour and migration, aestivation or dormancy of eggs and larvae, formation of cocoons or tubes (Armitage et al. 1995). The extreme example is the occurrence of a form of cryptobiosis, the anhydrobiosis of *Polypedilum vanderplanki* (Kikawada et al. 2005). The Chironomidae quick response to change is attributed to a suitable set of traits related with life history but also reproduction and resistance associated in general to the family. However, information is still insufficient regarding many of these traits for most genus and species inside the family; turning also unclear the life history strategies and evolutionary linkage between Chironomidae traits. More research is needed on Chironomidae survival strategies not just for drought by also to cold winter temperatures, and dispersion abilities, which are still poorly described in literature, resulting in the lack of information trait databases (European or North American).

It is also important to explore the traits related to the performance of Chironomidae in the environment, such as biomass and fecundity that do not just reflect fitness of the organism but that should directly or indirectly be a net result of other morpho-physiological traits (Violle et al. 2007). Chironomidae numeric dominance and species richness make them a very important source of energy for predators (vertebrate and invertebrate) and therefore an important component in the energy flow in the lotic ecosystems. In addition very little is known for example about the impact of Chironomidae predators in the invertebrate communities given their small size notwithstanding their frequently high abundance (Tokeshi 1995c). Many production studies also grouped Chironomidae at the family level, neglecting once again their diverse life-history patterns that can go from taxa with one generation per year with synchronous emergence, toward taxa with numerous generations (more than four) with asynchronous emergence and development (Tokeshi 1995a,b). As a consequence Chironomidae production might be much higher than actual estimations (Berg & Hellenthal 1991, 1992, Prat & Rieradevall 1995). It would be important to study the contribution of Chironomidae in the total secondary production in different streams and compare to rivers under different degrees of disturbance. Shifts in Chironomidae assemblages and abundance may have a determinant cascade effect to other trophic levels and strong effect on functions executed by communities.

Since Chironomidae larvae constitute an important food resources for higher trophic levels, it is essential to understand their functional role(s) in the food web to have a realistic perspective of how mass flows through ecological communities. It is known that the family plays a diversity of functional roles in food chains and closely related taxa may exhibit different feeding modes. Chironomidae larvae can be included in various categories considering the feeding habits partially due to differences in mouthparts and tube morphology: collector-gatherers, collector-filterers, scrapers, shredders, engulfers and piercers (Berg 1995). The main food sources are also diverse: algae, detritus (with associated microorganisms), woody debris, macrophytes and invertebrates (including other Chironomidae). There is a considerable plasticity in the feeding behaviour of Chironomidae that contributes to reduce competition between their larvae, and which may be one of the reasons for their success in many different environments (Berg 1995). The plasticity inherent to many Chironomidae taxa enhances the difficulty in assigning them to a feeding category, generating conflicting discrepancies in literature. The use of feeding related traits depends on the definition of primary and secondary feeding habits, but also on the understanding of opportunistic versus selective behaviour of larvae in different environments. In the future, the degree of this plastic behaviour could be assessed by using stable isotopes to signatures for different scenarios.

Another important topic to be developed is related with the intraspecific trait variability in Chironomidae that may come directly from intraspecific diversity or from the phenotypic plasticity of their traits. Trait plasticity may be adaptive as: a) it increases fitness; and b) allows only smaller declines of fitness under adverse conditions. This plasticity could be measured by comparing the actual traits of Chironomidae taxa in various environments with the mean trait value in the trait databases or mean trait across those environments.

In addition, it would be challenging to identify the link between genetic differentiation and phenotypic expression, which subsequently determines organism traits. Genetic differences between distant populations have been disclosed in Chironomidae (Kiknadze et al. 1996, Guryev & Blinov 2002, Martin et al. 2002, Gunderina et al. 2009, Schmidt et al. 2013), but few have been done considering how this genetic differentiation translates into different phenotypic expression of traits. Traits can ultimately manifest these divergence, a result of environmental selective forces together with the reproductive isolation, and help in the distinction of cryptic and sibling species.

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"*It almost seems impossible until it's done*" Nelson Mandela

Appendix

Appendix

Table A1 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		
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
Emergence duration	Short period (some hours to few days; <15 d.)	EDSHORT
	Long period (several days; >15 d.)	EDWIDE
Emergence season	Winter	EMWINT
	Spring	EMSPRI
	Summer	EMSUMM
	Autumn	EMAUTU
Flight period[a]	Winter	FLYWINT
	Spring	FLYSPRI
	Summer	FLYSUMM
	Autumn	FLYAUTU
Haemoglobin	Present	HBPRES
	Absent	HBNONE
Hibernation phase/instar (overwinter diapause)	Egg	HIBEGG
	1st instar	HIBINST1
	2nd instar	HIBINST2
	3rd instar	HIBINST3
	4th instar	HIBINST4
Length of larval development (months)	≤1	DEVLARV1
	2	DEVLARV2
	3	DEVLARV3
	4	DEVLARV4
	5	DEVLARV5
	6	DEVLARV6
	7	DEVLARV7
	8	DEVLARV8
	≥9	DEVLARV9
Number of eggs per egg mass	< 100	EGGMAS1
	100 – 500	EGGMAS2
	500 – 1000	EGGMAS3
	> 1000	EGGMAS4
Tube construction	Tube absent	TUBNON
	Tube without shape, unorganized	TUBUNO
	Tube rigid	TUBRIG
Eltonian adapted from Tachet et al. (2010)		
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	RFCOC
	Resistant stages to desiccation	RFSTA

	Diapause or quiescence	RFDIAP
	None	RFNON
	Deeper penetration in substrate during dryness	RFSUB
Respiration (#tracheas)	12 tracheas	TRACH1
	6 tracheas	TRACH2
	3 tracheas	TRACH3
Substrate relation/ Locomotion	Free living	FREELV
	Burrower	BURROW
	Miner	MINER
	Fixed (substrate or plants)	FIXED
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
	Asexual reproduction	ASEXU
Type of aquatic stages[a]	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
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 (ºC)	≤ 6	6OPTEM
	>7 – 9	7OPTEM9
	>10 – 12	10OPTEM12
	>13 – 15	13OPTEM15
	≥16	OPTEM16
Oxygen saturation preferences[a]	Stable always > 50%	OXSTAB
	Unstable 10-50%	OXUNST
	< 5% for few hours	OXLOW

	Rotting summer daily <5%	OXROTT
Type of migration	Horizontal	MIGHOR
	Vertical	MIGVER
Grinnellian adapted from Tachet et al. (2010)		
Food type[a]	Fine sediment + microorganisms	SEDMIC
	Debris < 1mm	DEBRI1
	Plant debris > 1mm	DEBRI2
	Living microphytes	MICPHY
	Living macrophytes	MACPHY
	Dead animals	DEADAN
	Living microinvertebrates	MICINV
	Living macroinvertebrates	MACINV
	Living vertebrates	VERTEB
	Wood	WOOD
	Bacteria	BACTER
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 fead habitats)	KRYON
pH preferences[a]	<4	4PH
	>4 – 5	4PH5
	>5 – 6	5PH6
	>6 – 7	6PH7
	>7 – 8	7PH8
	≥8	PHM8
Substrate preferences[a]	Stone, boulder, cobble, pebble	STONES
	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
Temperature preferences	Psychrophilic <15ºC	TPSYCH
	Thermophilic >15ºC	TTHERM
	Eurythermic	TEURYT
	Hemistenothermic	THEMIS
Transversal distribution along stream channel[a]	River channel	CHANNEL
	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
Trophic status preferences	Oligotrophic	OLIGTR
	Mesotrophic	MESOTR
	Eutrophic	EUTR
	Hypertrophic (Mesohumic)	HYPTR1
	Hypertrophic (Polyhumic)	HYPTR2
Grinnellian taken from Tachet et al. (2010)		
Elevational preferences[a] (m)	<1000 (lowlands)	ALTI1
	>1000 – 2000 (piedmont)	ALTI2
	>2000 (Alpine)	ALTI3

Current velocity preferences[a] (cm.s-1)	None	VELO1
	<25	VELO2
	> 25 – 50	VELO3
	>50	VELO4
Salinity preferences[a]	Fresh water	FRESHW
	Brackish water	BRACKI
Saprobity	Xenosaprobic	XENOSAP
	Oligosaprobic	OLIGSAP
	β-mesosaprobic	BMESSAP
	α-mesosaprobic	AMESSAP
	Polysaprobic	POLYSAP

[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.

Table A2 List of literature used in the development of the European Chironomidae database.

Citation	Complete reference
Aagaard 1978	Aagaard K., 1978. The chironomids of lake Målsjøen. A phenological, diversity, and production study. Norwegian Journal of entomology. 25:21–37.
Armitage 1986	Armitage P.D., 1986. A redescription of Male *Eukiefferiella* (=*Thalassosmittia*) *atlantica* Stora (Chironomidae, Diptera) Based on type Materials ans recently collected specimens from Tenerife, Canary Islands. Aquatic Insects. 8: 105–109.
Armitage & Tuiskunen 1988	Armitage P.D., Tuiskunen J., 1988. *Thalassosmittia atlantica* (Stora) comb.nov. Description of adult female and immature stages from Tenerife, Canary Islands. (Diptera, Chironomidae). Spixiana. 14: 25–28.
Arslan et al. 2010	Arslan N., Ayık Ö., Şahin Y., 2010. Diversity and Structure of Chironomidae (Diptera) Limnofauna of Lake Uluabat, a Ramsar Site of Turkey, and their Relation to Environmental Variables. Turkish Journal of Fisheries and Aquatic Sciences. 10: 315–322.
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Table A3 List of European genera and species within each subfamily with trait information included in the trait European database.

European Genus (Total=178)	European Species (Total= 744)
Podonominae	
Boreochlus	*thienemanni*
Lasiodiamesa	*bipectinata*
Paraboreochlus	*minutissimus*
Parochlus	Information for genus only
Trichotanypus	Information for genus only
Tanypodinae	
Ablabesmyia	*longistyla; monilis; phatta; dusoleili* (#= 4)
Anatopynia	*plumipes*
Apsectrotanypus	*trifascipennis*
Arctopelopia	*barbitarsis; griseipennis* (#= 2)
Clinotanypus	*nervosus*
Conchapelopia	*intermedia; melanops; pallidula; viator; hittmairorum; triannulata* (#= 6)
Derotanypus	Information for genus only
Guttipelopia	*guttipennis*
Hayesomyia	*senata*
Krenopelopia	*binotata; nigropunctata* (#= 2)
Labrundinia	*longipalpis*
Larsia	*atrocincta; curticalcar* (#= 2)
Macropelopia	*adaucta; fehlmanni; nebulosa; notata* (#= 4)
Meropelopia	Information for genus only
Monopelopia	*tenuicalcar*
Natarsia	*nugax; punctata* (#= 2)
Nilotanypus	*dubius*
Paramerina	*cingulata; divisa* (#= 2)
Pentaneurella	*katterjokki*
Procladius (Holotanypus) or (Psilotanypus)	*choreus; culiciformis; ferrugineus; fimbriatus; islandicus; nudipennis; parvulus; pectinatus; rivulorum; sagittalis; signatus; simplicistilus; suecicus; tatrensis; flavifrons; imicola; lugens; serratus; rufovittatus* (#= 19)
Psectrotanypus	*varius*
Rheopelopia	*eximia; maculipennis; ornata* (#= 3)
Schineriella	*schineri*
Tanypus (Tanypus)	*kraatzi; punctipennis; vilipennis* (#= 3)
Telmatopelopia	*nemorum*
Telopelopia	*fascigera*
Thienemannimyia	*carnea; festiva; fusciceps; geijskesi; laeta; lentiginosa; northumbrica; pseudocarnea; vitellina* (#= 9)
Trissopelopia	*flavida.; longimana* (#= 2)
Xenopelopia	*falcigera; nigricans* (#= 2)
Zavrelimyia	*barbatipes; hirtimanus; melanura; nubila; signatipennis; berberi* (#= 6)
Diamesinae	
Boreoheptagyia	*cinctipes; legeri; monticola; rugosa* (#= 4)
Diamesa (Diamesa)	*aberrata; arctica; bertrami;cinerella; dampfi; goetghebueri; gregsoni; incallida; insignipes; kasymovi; laticauda; latitarsis; lindrothi; longipes; modesta; nowickiana; permacra; starmachi; steinboecki; tenuipes; thomasi; tonsa; vaillanti; valkanovi; veletensis; wuelkeri; zernyi; serratosioi; saetheri; martae* (#= 30)
Lappodiamesa	Information for genus only
Potthastia	*gaedii; longimanus; montium* (#= 3)
Protanypus	*caudatus; forcipatus; morio* (#= 3)
Pseudodiamesa	*branickii; nivosa* (#= 2)
Pseudokiefferiella	*parva*
Sympotthastia	*spinifera; zavreli* (#= 2)
Syndiamesa	*edwardsi; nigra* (#= 2)
Telmatogetoninae	
Telmatogeton	*japonicus*
Thalassomya	Information for genus only
Orthocladiinae	
Aagaardia	*sivertseni*
Abiskomyia	*paravirgo; virgo* (#= 2)
Acamptocladius	*submontanus; reissi* (#= 2)
Acricotopus	*lucens*
Allocladius	*arenarius*
Brillia	*longifurca; bifida; flavifrons* (#= 3)
Bryophaenocladius	*furcatus; muscicola; nidorum; nitidicollis; scanicus; subvernalis; vernalis; faegrii* (#= 8)
Camptocladius	*stercorarius*
Cardiocladius	*capucinus; fuscus; leoni* (#= 3)
Chaetocladius (Chaetocladius)	*acuminatus; acuticornis; dentiforceps; gelidus; gracilis; melaleucus; perennis; piger;*

	tenuistylus; vitellinus; algericus (#= 11)
Clunio	*marinus*
Corynoneura	*arctica; celeripes; celtica; coronata; edwardsi; fittkaui; gratias; lacustris; lobata; magna; scutellata* (#= 11)
Cricotopus (Isocladius) or (Cricotopus) or (Nostococladius)	*albiforceps; algarum; annulator; arcuatus; beckeri; bicinctus; brevipalpis; caducus; coronatus; cumulatus; curtus; ephippium; flavocinctus; fuscus; intersectus; laetus; laricomalis; lestralis; lygropis; magus; ornatus; patens; perniger; pilidorsum; pilosellus; pirifer; polaris; pulchripes; reversus; septentrionalis; similis; suspiciosus; sylvestris; tibialis; tremulus; triannulatus; tricinctus; trifascia; trifasciatus; tristis; vierriensis; levantinus* (#= 42)
Diplocladius	*cultriger*
Doncricotopus	*dentatus*
Dratnalia	*potamophylaxi*
Epoicocladius	*ephemerae*
Eukiefferiella	*boevrensis; brehmi; brevicalcar; claripennis; clypeata; coerulescens; cyanea; devonica; dittmari; fittkaui; fuldensis; gracei; ilkleyensis; lobifera; minor; pseudomontana; similis; tirolensis* (#= 18)
Eurycnemus	*crassipes*
Euryhapsis	Information for genus only
Georthocladius	*luteicornis*
Gymnometriocnemus (Gymnometriocnemus)	*terrestris*
Halocladius (Halocladius) or (Psammocladius)	*braunsi; fucicola; mediterraneus; variabilis; varians* (#= 5)
Heleniella	*dorieri; extrema; ornaticollis; serratosioi* (#= 4)
Heterotanytarsus	*apicalis; brundini* (#= 2)
Heterotrissocladius	*grimshawi; marcidus; scutellatus; subpilosus; brundini; changi; maeaeri* (#= 7)
Hydrobaenus	*lugubris; rufus; conformis; distylus; martini* (#= 5)
Hydrosmittia	*ruttneri*
Krenosmittia	*boreoalpina; camptophleps; hispanica; halvorseni* (#= 4)
Lapposmittia	Information for genus only
Limnophyes	*difficilis; eltoni; gurgicola; habilis; minimus; pumilio; punctipennis; asquamatus; brachytomus; edwardsi; inanispatina; natalensis; ninae; pentaplastus; roquehautensis; schnelli; spinigus* (#= 17)
Mesocricotopus	*thienemanni*
Mesosmittia	Information for genus only
Metriocnemus (Metriocnemus)	*albolineatus; cavicola; eurynotus; fuscipes; hirticollis; inopinatus; picipes; terrester; ursinus; brusti* (#= 10)
Nanocladius	*balticus; parvulus; rectinervis; dichromus* (#= 4)
Oliveridia	*tricornis*
Orthocladius and/or (Eudactylocladius) or (Euorthocladius) or (Mesorthocladius) or (Orthocladius) or (Pogonocladius) or (Symposiocladius)	*fuscimanus; gelidus; olivaceus; almskari; gelidorum; musester; priomixtus; subletteorum; rivicola; rivulorum; saxosus; thienemanni; ashei; calvus; luteipes; telochaetus; abiskoensis; decoratus, dentifer; frigidus; lignicola; oblidens; rhyacobius; rubicundus; ruffoi; smolandicus; wetterensis; consobrinus; halvorseni; holsatus; lunzensis; schnelli; glabripennis; rivinus; stagnicola; maius; nitidoscutellatus; pedestris* (#= 38)
Parachaetocladius	*abnobaeus*
Paracladius	*alpicola; conversus; quadrinodosus* (#= 3)
Paracricotopus	*niger*
Parakiefferiella	*bathophila; coronata; dentifera; fennica; gracillima; nigra; scandica; wuelkeri; bilobata; gynocera; pyrenaica; smolandica; triquetra* (#= 13)
Paralimnophyes	Information for genus only
Parametriocnemus	*boreoalpinus; stylatus; valescurensis* (#= 3)
Paraphaenocladius	*impensus; pseudirritus; exagitans* (#= 3)
Parasmittia	*carinata*
Paratrichocladius	*rufiventris; skirwithensis; micans; gayi; guidalii; nivalis; osellai; pierfrancescoi* (#= 8)
Paratrissocladius	*excerptus*
Parorthocladius	*nudipennis*
Propsilocerus	*lacustris; paradoxus; jacuticus* (#= 3)
Psectrocladius (Allopsectrocladius) or (Mesopsectrocladius) or (Monopsectrocladius) or (Psectrocladius)	*obvius; barbatipes; calcaratus; barbimanus; fennicus; limbatellus; octomaculatus; oligosetus; psilopterus; schlienzi; sordidellus; ventricosus; zetterstedti* (#= 13)
Pseudorthocladius	*berthelemyi; curtistylus; filiformis* (#= 3)
Pseudosmittia	*danconai; gracilis; holsata; obtusa* (#= 4)
Psilometriocnemus	*europaeus*
Rheocricotopus (Psilocricotopus) or (Rheocricotopus)	*chalybeatus; effusus; fuscipes; glabricollis; tirolus; atripes* (#= 6)
Rheosmittia	*languida; spinicornis* (#= 2)
Smittia	*contingens; insignis; rupicola; thalassicola; brevipennis* (#= 5)
Stackelbergina	Information for genus only
Stilocladius	*montanus*

Stygocladius	*multisetosus*
Symbiocladius	Information for genus only
Synorthocladius	*semivirens*
Thalassosmittia	*thalassophila; atlantica* (#= 2)
Thienemannia	*gracilis; fulvofasciata* (#= 2)
Thienemanniella	*clavicornis; majuscula; vittata; acuticornis* (#= 4)
Tokunagaia	*rectangularis*
Trissocladius	*brevipalpis*
Tvetenia	*bavarica; calvescens; discoloripes; verralli* (#= 4)
Vivacricotopus	*ablusus*
Zalutschia	*zalutschicola; humphriesiae; tornetraeskensis* (#= 3)
Chironominae	
Axarus	Information for genus only
Beckidia	*zabolotzkyi*
Benthalia	Information for genus only
Chernovskiia	*macrocera; orbicus* (#= 2)
Chironomus (Chaetolabis) or (Chironomus) or (Lobochironomus)	*aberratus; acidophilus; acerbus; anchialicus; annularius; anthracinus; aprilinus; bernensis; cingulatus; clarus; commutatus; heterodentatus; lacunarius; longistylus; lugubris; luridus; macani; melanescens; melanotus; nuditarsis; obtusidens; parathummi; piger; pilicornis; plumosus; pseudothummi; riihimakiensis; riparius; salinarius; sollicitus; sororius; striatus; tenuistylus; uliginosus; valkanovi; acutiventris; agilis; balatonicus; entis; montuosus; muratensis; nudiventris; pallidivittatus; tentans* (#= 44)
Cladopelma	*bicarinatum; goetghebueri; subnigrum; virescens; viridulum* (#= 5)
Cladotanytarsus (Cladotanytarsus) or (Lenziella)	*amandus; atridorsum; difficilis; dispersopilosus; iucundus; mancus; nigrovittatus; pallidus; teres; vanderwulpi; conversus* (#= 11)
Constempellina	Information for genus only
Corynocera	*ambigua; oliveri* (#= 2)
Cryptochironomus (Cryptochironomus)	*albofasciatus; defectus; denticulatus; obreptans; psittacinus; redekei; rostratus; supplicans; ussouriensis* (#= 9)
Cryptotendipes	*holsatus; nigronitens; pflugfelderi; pseudotener; usmaensis; darbyi* (#= 6)
Cyphomella	*cornea*
Demeijerea	*rufipes*
Demicryptochironomus (Demicryptochironomus); or (Irmakia).	*vulneratus; neglectus* (#= 2)
Dicrotendipes	*fusconotatus; lobiger; nervosus; notatus; pallidicornis; pulsus; tritomus* (#= 7)
Einfeldia	*pagana*
Endochironomus	*albipennis; tendens* (#= 2)
Fleuria	*lacustres*
Gillotia	Information for genus only
Glyptotendipes (Caulochironomus) or (Glyptotendipes) or (Heynotendipes)	*aequalis; anomalus; barbipes; caulicola; foliicola; glaucus; imbecilis; pallens; paripes; signatus; viridis; cauliginellus; ospeli; scirpi* (#= 14)
Graceus	*ambiguus*
Harnischia	*angularis; curtilamellata; fuscimanus* (#= 3)
Kiefferulus (Kiefferulus)	*tendipediformis*
Kloosia	*pusilla*
Lauterborniella	*agrayloides*
Lipiniella	*araenicola; moderata* (#= 2)
Lithotanytarsus	*dadesi; emarginatus* (#= 2)
Microchironomus	*deribae; tener* (#= 2)
Micropsectra	*apposita; aristata; atrofasciata; attenuata; auvergnensis; bodanica; insignilobus; junci; lacustris; lindebergi; lindrothi; notescens; radialis; recurvata; seguyi; styriaca; acuta; borealis; chionophila; fallax; logani; nana; nohedensis; pallidula; uliginosa* (#= 25)
Microtendipes	*brevitarsis; britteni; chloris; confinis; diffinis; nigellus; pedellus; rydalensis; tarsalis* (#= 9)
Neostempellina	*thienemanni*
Neozavrelia	*fuldensis; luteola.* (#= 2)
Nilothauma	*brayi*
Omisus	*caledonicus*
Pagastiella	*orofila*
Parachironomus	*biannulatus; danicus; digitalis; frequens; kuzini; mauricii; monochromus; paradigitalis; parilis; siljanensis; subalpinus; tenuicaudatus; varus; vitiosus; gracilior* (#= 15)
Paracladopelma	*camptolabis; laminatum; mikianum; nigritulum; galaptera; nais; undine; nereis* (#= 8)
Paralauterborniella	*nigrohalteralis*
Paratanytarsus	*austriacus; bituberculatus; brevicalcar; dimorphis; dissimilis; hyperboreus; inopertus; laccophilus; laetipes; lauterborni; natvigi; paralaccophilus; setosimanus; tenellulus; tenuis; abiskoensis; grimmii; penicillatus* (#= 18)
Paratendipes	*albimanus; nubilus; nudisquama* (#= 3)
Phaenopsectra	*flavipes; punctipes* (#= 2)

Polypedilum (Pentapedilum) or (Polypedilum) or (Tripodura) or (Uresipedilum)	*nubens; sordens; tritum; uncinatum; acifer; acutum; albicorne; amoenum; apfelbecki; arundineti; bicrenatum; convictum; cultellatum; laetum; nubeculosum; nubifer; pedestre; pullum; quadriguttatum; scalaenum; tetracrenatum; aegyptium; fallax* (#= 23)
Pseudochironomus	*prasinatus*
Rheotanytarsus	*curtistylus; pentapoda; photophilus; reissi; rhenanus; rioensis*. (#= 6)
Robackia	*demeijerei*
Saetheria	*reissi; tylus* (#= 2)
Sergentia	*coracina; baueri; prima* (#= 3)
Stempellina	*bausei; subglabripennis* (#= 2)
Stempellinella	*brevis; reissi; edwardsi* (#= 3)
Stenochironomus	*fascipennis; gibbus; hibernicus* (#= 3)
Stictochironomus	*crassiforceps; maculipennis; pictulus; rosenschoeldi; sticticus* (#= 5)
Sublettea	Information for genus only
Tanytarsus	*aberrans; aculeatus; anderseni; bathophilus; brundini; buchonius; chinyensis; curticornis; debilis; dispar; ejuncidus; eminulus; fennicus; gregarius; heusdensis; inaequalis; lactescens; latiforceps; lestagei; longitarsis; lugens; medius; mendax; miriforceps; multipunctatus; nemorosus; niger; palettaris; quadridentatus; recurvatus; signatus; sinuatus; telmaticus; usmaensis; volgensis; cretensis; formosanus* (#= 37)
Thienemanniola	Information for genus only
Tribelos	*intextum*
Virgatanytarsus	*arduennensis; triangularis* (#= 2)
Xenochironomus	*xenolabis*
Zavrelia	Information for genus only
Zavreliella	*marmorata*
Buchonomyiinae	
Buchonomyia	thienemanni
Prodiamesinae	
Monodiamesa	*alpicola; bathyphila; ekmani; nitida* (#= 4)
Odontomesa	*fulva*
Prodiamesa	*olivacea; rufovittata* (#= 2)