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Diversity and distribution of Chironomidae (Insecta: Diptera) of protected areas in North Tunisia

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ABSTRACT

Key-words: *Diptera, Chironomidae, wadi, biodiversity, Tunisia*

In North Africa seasonal streams called wadi are an unique habitats with serve hydrological and thermal regime. Non-biting midges take an important part of freshwater biodiversity in North Africa. We present new data on the distribution and diversity of Chironomidae in North Tunisia. Larvae, pupal exuviae and adult males of chironomids were collected from a various freshwater ecosystems from May 2005 till April 2006. The aim of this study was to recognize the pattern of midge diversity in North Tunisia to estimate ecological value of running waters in the region. In total, 79 taxa were identified. Nearly all of the taxa were typical representatives of the Palaearctic and Mediterranean complexes. The majority of the investigated sites belonged to the protected areas in North Tunisia, such as the Ichkeul National Park, the Kroumerie Mountains and the El Feija National Park, part of the Intercontinental Reserve of the Mediterranean Biosphere. Altitudinal zonation of the communities composition was found in the lowland (<250 m a.s.l.) and upland zone (>250 m a.s.l.) with maximum of 760 m. Whereas among the data 39 species are recorded from Tunisia for the first time, the species richness of Chironomidae is higher than previously estimated.

RÉSUMÉ

Diversité et distribution des Chironomidae (Insecta, Diptera) au Nord de la Tunisie

Mots-clés : *Diptera, Chironomidae, oueds, la biodiversité, la Tunisie*

En Afrique du Nord, les cours d'eau saisonniers (Wadis) sont les habitats uniques qui servent les régimes hydrologiques et thermaux. Les chironomidae prennent une part importante dans la biodiversité des eaux douces d'Afrique du Nord. Nous présentons de nouvelles données sur la répartition et la diversité des Chironomidae au Nord de la Tunisie. Les larves, les pupes, les exuvies et les mâles adultes de chironomes sont recueillies dans divers wadis de mai 2005 à avril 2006. L'objectif de cette étude est de reconnaître le modèle de diversité de cette famille dans le Nord de la Tunisie pour estimer sa valeur écologique dans la région. Au total, 79 taxons ont été identifiés. La plupart des taxons sont des représentants typiquement paléarctique et méditerranéens. Les sites étudiés appartenaient aux réserves dans le Nord de la Tunisie : le parc d'Ichkeul, les montagnes de Kroumirie et le Parc El Feij. La zonation altitudinale de la composition des communautés a été trouvé dans la plaine (<250 m) et dans la zone de montagne (>250 m) avec un maximum de 760 m. Considérant qu'avec 39 espèces de Chironomidae enregistrées pour la première fois en Tunisie, la richesse spécifique est plus élevée que dans les estimations précédentes.

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INTRODUCTION

In the Mediterranean Region, freshwater biological communities and their ecological traits are constrained by great seasonal variability of temperature and rainfall (Bonada *et al.*, 2007; Chaib *et al.*, 2011). The hydrological conditions, strongly variant among seasons with many rivers and streams (known in North Africa as wadi) having intermittent flows, cause a strong shift between lotic and lentic conditions through the year (Pinto, 1994; Morais *et al.*, 2004).
Up till now, North African streams or wadis have been scantily studied; particularly their ecological aspects are scarcely known (*e.g.* Lounaci *et al.*, 2000a; Chaib *et al.*, 2011). Nevertheless, some studies on benthic communities have been carried out recently (*e.g.* Lounaci *et al*., 2000b; Arab *et al*., 2004; Belaidi *et al*., 2004; Zrelli *et al*., 2011; Touaylia *et al*., 2011). New data show certain peculiar features of Northern African lotic ecosystems: wadis are strongly influenced by the unique Mediterranean climate (*i.e.* extreme temperature values during summer and short extreme flooding events in winter, followed by long periods of drought). As a result, fauna of wadi is relatively poor compared to continental European Mediterranean regions (Giudicelli *et al*., 1985; Lounaci *et al*., 2000a).
The chironomid fauna of North-West Africa has been relatively well recognized in Marocco (Kettani *et al*., 2001; Kettani and Langton, 2011), Algeria (Moubayed *et al*., 2007; Chaib *et al*., 2011, 2013) while much less in Tunisia (Boumaiza, 1988). All faunistic data on non-biting midges in Tunisia have been summarized in the checklist of Chironomidae species recorded in this country (Boumaiza and Laville, 1998). Currently, there is available only a faunistic list of taxa from the country. That is why there is a need for a broad investigation which would explain not only the faunistic connection of Tunisian chironomid fauna, but also the ecological relation between larval communities and the environment. As wadis are widely spread, flowing water habitats in North Africa, the midge diversity strongly depends on their existence. North Tunisia is highly diversified due to geographical conditions – from high mountains to lowland deserts, which results in climatic variability in this region. We wish to present new data on Chironomidae of North Tunisia with many new species recorded in the country. The preliminary analysis of non-biting midge biodiversity and the general geographical pattern of its distribution are also presented.

STUDY AREA

North Tunisia includes two mountain chains: the Tell (Kroumir and Mogods Mountains) and the Dorsal (Châambi Mountains) (Ben Ayed, 1993).
The humid area is limited to the Kroumir Mountains, leading to the development of a dense forest (Zielhofer and Faust, 2008). Annual rainfall increases from the north to the south with most rains during winter; some northern regions get an annual rainfall average of more than 1300 mm per year (*e.g.*: the northwestern region of Tabarka). In contrast, the regions in the far south receive less than 100 mm of rain per year (Ben Jemaa *et al*., 1998). Water bodies are unevenly distributed within the country, with the northern part holding 60% of the total water resources (Houcine *et al*., 1999). The precipitation decreases from the north to the south, with most of the rainfall in winter (Ben Jemaa *et al*., 1998). Water resources are unevenly distributed within the country; the northern part, covering an area of only 17% of the territory, has 60% of the total water resources (Houcine *et al.*, 1999).
According to the subdivision of hydrographic nets in the northern region of Tunisia by the Commissariat Régional au Développement Agricole (CRDA), the total wadi system belongs to three different basins (Figure 1): (i) The North-West Basin is primarily agricultural land with an area of 6512 km^2. This basin is also full of large water reservoirs with 366 million m^3 (21 dams and 39 lakes) in the extreme North-West of Tunisia; it is distinguished by the wettest climate in the country with annual precipitation reaching 1000 mm on the coast (near Tabarka 1050 mm) and more than 1500 mm in Ain Draham. (ii) The Basin of Beja-Ichkeul, situated in the extreme north of Tunisia and forming the most advanced point of Africa, with an area of 7490 km^2. It has two types of climate: a humid climate with rainfall of 600 mm to 1200 mm (north);

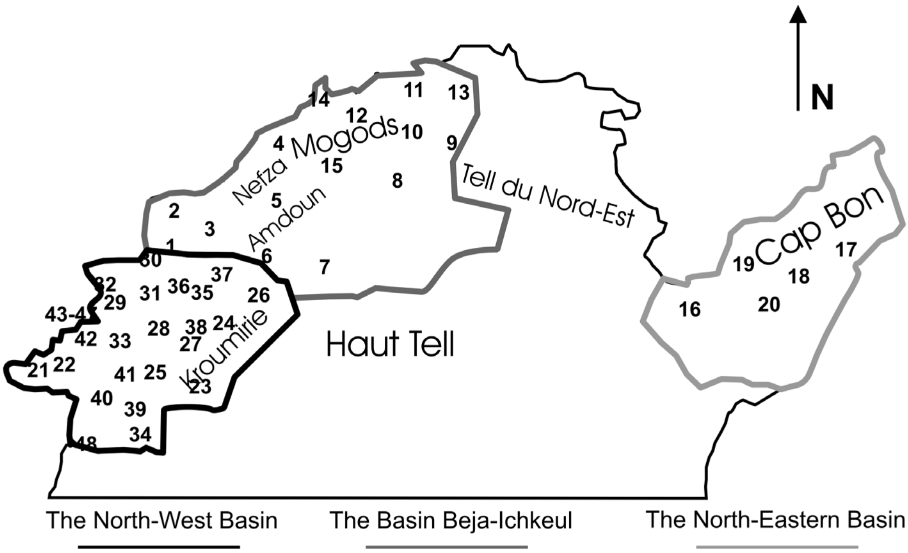

Figure 1
Map of the study area. Numbers of study sites according to Appendix I.

a relatively dry climate with precipitation of 350–450 mm (south); and iii) the North-Eastern Basin in the Cap Bon region, covering an area of 6570 km^2, with an annual precipitation of 750 mm.

MATERIALS AND METHODS

Forty-eight sampling sites were chosen among the three basin area on the basis of water permanence, with samples taken monthly, from May 2005 till April 2006. The study sites were distributed over three major watersheds. The positions, coordinates and altitudes of the sampling sites are indicated in Figure 1 and Appendix I chironomid larvae and pupae exuviae were collected with a Surber net (300 μm mesh size, 50 cm width) (Surber, 1937). Ten samples were collected opposite to the current along the sampling station, in the middle of the current and near the banks, then merged together for the statistical analysis. It was also randomly collected larave from submerged stones with a total surface area of 1 m^2 at each site. Adults were collected with a entomological net near a wadi banks. The material was first preserved in 10% formaldehyde, and later transferred to 70% ethyl alcohol for sorting and final conservation. The species identification was based on imagines, prepupae, where the pupal characters were morphologically visible, and larvae. The keys for larvae determination were used (Brooks *et al*., 2007; Epler, 2001; Ferrarese, 1983; Ferrarese and Rossaro, 1981; Nocentini, 1985; Rossaro, 1982; Wiederholm, 1983), along with the keys for Palaearctic pupal exuviae (Langton, 1984; Langton and Visser, 2003; Wilson and Ruse, 2005) and for adult males (Langton and Pinder, 2007). There are no keys for Chironomidae published from the Tunisian region. Besides Langton and Visser (2003), the above-mentioned keys are from Europe, the Western Holarctic, the North Mediterranean or the whole Holarctic region and allow to identify chironomids only to genera. Thus, most of the material was identified only to the genus level or morphotypes and to the species level when possible. The community analysis was done with PRIMER 5 software. To examine faunistic differences between the studied wadis and basins, the data were transformed to the presence-absence matrix. The within sites Bray-Curtis similarity index 93 was calculated. The SIMPER analysis was performed to reveal characteristic taxa for the two main community types (A and B) separated with Non-Metric Multidimensional Scaling (NMDS). To estimate how the measured diversity reflects the real species richness in the region, the rarefaction curve was counted for all the samples with PAST software.

Table I
Comparison of the number of Chironomidae species recorded in North Tunisia with the work of Boumaiza and Laville (1988).

Subfamily	Boumaiza and Laville, 1988	This study
Diamesinae	1	3
Prodiamasinae	1	0
Tanypodinae	8	12
Orthocladiinae	27	26
Chironominae	33	38
Total	70	79

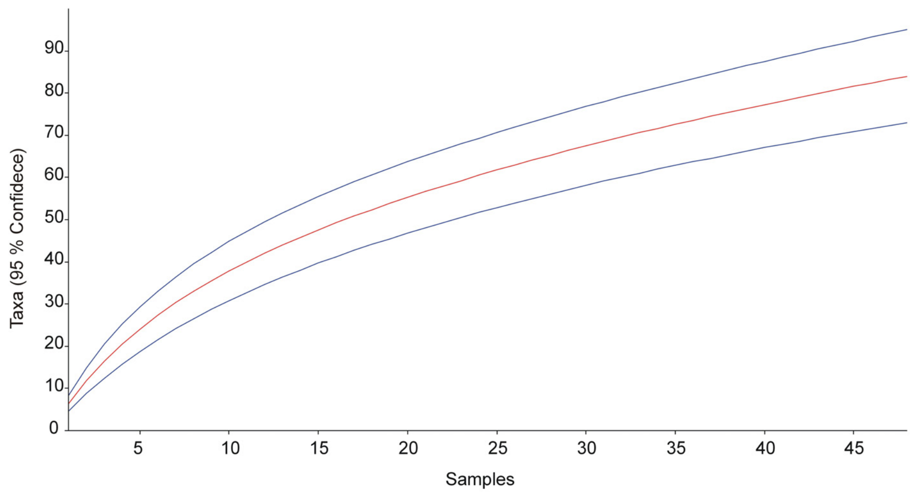

Figure 2
Rarefaction curve of the presented new data on Chironomidae occurrence in North Tunisia.

RESULTS

> *NEW FAUNISTIC DATA*

Appendix II presents a list of the Chironomidae recorded from the 48 sites in the study area. These new data increase the number of species recorded in North Tunisia to 117. Table I presents the comparison of Boumaiza and Laville (1988) and our data. Both studies show similar proportions in the number of species among subfamilies with strong dominance of Chironominae. This research adds 39 new species of Chironomidae from Tunisia not recorded earlier (Boumaiza and Laville, 1988). As more than 49% of all the taxa recorded in this study are new for the study area and the number of recorded species quickly increase with any new data added (Figure 2), the present investigation predicts also the high potential of the Chironomidae diversity in the region.

> *GEOGRAPHICAL PATTERN*

Among the 79 identified taxa, 21 species (27%) are reported from the North-Eastern Basin, including 7 species so far recorded in Tunisia only in this region. In the Basin of Beja Ichkeul, 51 species (64%) were found with 15 species recorded only in this area of Tunisia, 58 species (73%) were identified in the North West Basin with 24 taxa found only in this part of the country.

The number of species varies between the three regions. The highest number of taxa in the North-Eastern Basin was found in the Chiba wadi (st. 18) (9 species). In the Basin of Béja-Ichkeul, the number of recorded taxa exceed 10 only in the Tetria (st. 1), Sejenane (st. 12)

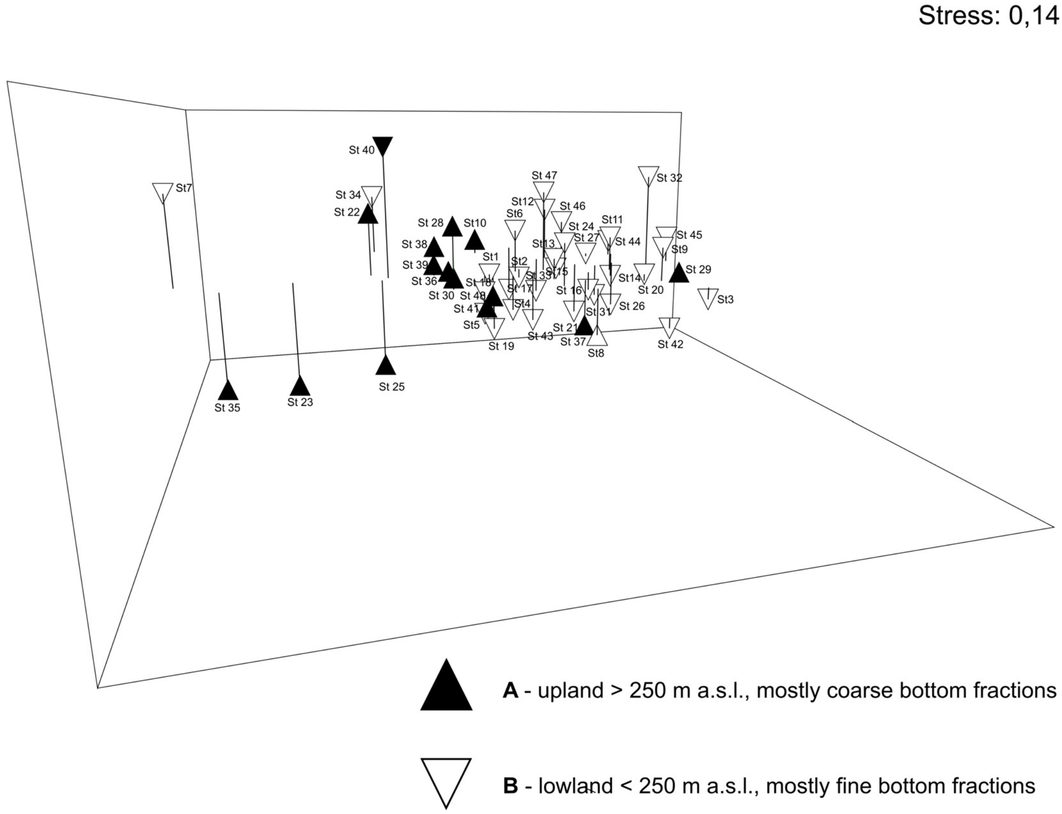

Figure 3
Results of NMDS analysis.

and Kloufi (st. 13) (15, 13 and 17 species respectively). The highest number of taxa was found in 117 the North-West Basin in the Ennour wadi (st. 39) (26) and the wadis of Sallen (st. 48) and Mrig (st. 41) (10 and 9 respectively). The NMDS analysis (Figure 3) has indicated that there are two main types of communities in North Tunisia. Community type A dominates in upland areas from *ca.* 250 m a.s.l. to more than 750 m a.s.l. on coarse sediments (frequently rock blocks and pebbles). This type is more diverse, as the sites differ in the fauna of chironomids more strongly than in type B. *Rheocricotopus effusus*, *Larsia atrocincta*, *Zavrelimyia nubila* and *Procladius* spp. are more frequent in mountain and foothill streams (Table II). According to Moller Pillot (2009) and Moller Pillot and Klink (2003), *R. effusus* inhabits small, fast flowing streams of relatively high trophy, with good oxygen conditions, and often occurs on the hard bottom. Community type B (Figure 3) dominates in lowland areas up to 250 m a.s.l., frequently on the soft bottom with fine mineral fractions. Fauna of the North Tunisia lowlands is less diverse. This group includes taxa more tolerant to oxygen deficiency. They are found mainly in small streams with a strong current (*Orthocladius frigidus*, *Macropelopia nebulosa*), but present also in larger, slow-flowing and stagnant waters (*Polypedilum nubeculosum*-type, *Chironomus plumosus*) (Table II) (Vallenduuk and Moller Pillot, 2007; Moller Pillot, 2009; the authors' personal observations).

DISCUSSION

The midge communities in North Algeria also differ according to the sediment structure. Chaib *et al.* (2013) have pointed out that in the North African water systems, hard substrate (*e.g.* cobbles) offers suitable conditions (interstitial space to survive a low water level and accumulate organic matter, a substructure for peryphiton) for midge larvae. In the Algerian river systems, chironomid fauna is more diverse in hard bottomed streams. In wadis, where fine

Table II
Results of SIMPER analysis.

Group A
Average similarity: 14.84

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Rheocricotopus effusus **(Walker, 1856)**	1.93	2.73	0.43	18.41	18.41
Larsia atrocincta **(Goetghebuer, 1942)**	1.47	2.68	0.30	18.06	36.47
Procladius **sp**	4.33	2.32	0.45	15.64	52.11
Zavrelimyia nubila **(Meigen, 1830)**	2.0	1.58	0.52	10.69	62.80
Orthocladius frigidus **(Zetterstedt, 1838)**	0.53	0.79	0.21	5.32	68.12
Phaenopsectra flavipes **(Meigen, 1818)**	1.53	0.74	0.24	4.99	73.11
Ablabesmyia longistyla **(Fittkau, 1962)**	0.53	0.74	0.21	4.98	78.08
Chironomus plumosus **(Linné, 1758)**	0.40	0.56	0.22	3.79	81.87
Rheopelopia ornata **(Meigen, 1838)**	0.33	0.43	0.17	2.87	84.74
Stictochironomus maculipennis **(Meigen, 1818)**	0.60	0.40	0.14	2.70	87.44
Micropsectra atrofasciata **(Kieffer, 1911)**	0.67	0.35	0.17	2.38	89.82
Cryptochironomus **sp.**	0.40	0.32	0.10	2.14	91.96

Group B
Average similarity: 16.65

Species	Av. Abund	Av. Sim	Sim/SD	Contrib%	Cum.%
Procladius **sp.**	2.39	4.36	0.50	26.19	26.19
Chironomus plumosus **(Linné, 1758)**	1.18	3.29	0.51	19.76	45.96
Orthocladius frigidus **(Zetterstedt, 1838)**	0.61	1.54	0.32	9.23	55.19
Polypedilum nubeculosum **-type**	3.70	1.33	0.29	7.99	63.18
Macropelopia **sp. (Meigen, 1818)**	0.97	1.18	0.28	7.09	70.28
Chironomus salinarius **(Kieffer, 1915)**	0.58	0.62	0.22	3.75	74.03
Cryptochironomus **sp.**	1.52	0.46	0.17	2.76	76.79
Natarsia **sp. (Meigen, 1804)**	0.58	0.41	0.16	2.46	79.25
Dicrotendipes nervosus **(Staeger, 1839)**	0.39	0.40	0.16	2.40	81.65
Rheocricotopus effusus **(Walker, 1856)**	1.15	0.39	0.16	2.33	83.98
Tvetenia calvescens **(Edwards 1929)**	0.58	0.35	0.16	2.12	86.10
Procladius choerus **(Meigen, 1804)**	0.36	0.31	0.14	1.86	87.96
Einfeldia pagana **(Meigen, 1838)**	0.24	0.29	0.14	1.75	89.71
Phaenopsectra flavipes **(Meigen, 1818)**	0.67	0.23	0.14	1.38	91.09

Groups B & A
Average dissimilarity = 87.43
SIMPER
Similarity percentages - species contributions
Parameters
Transform: presence/absence
Cut off for low contributions: 90.00%
Factor groups
A - upland >250 m a.s.l., mostly coarse bottom fractions
B - lowland ⩽ 250 m a.s.l., mostly fine bottom fractions

and ultrafine mineral fractions dominate, the current is usually strong while a compacted bottom fraction does not provide larvae with shelter when discharge is high or during habitat desiccation. Lower transparency and low concentration of dissolved oxygen favor more resistant species. Chaib *et al.* (2011) have proved that seasonality plays a fundamental role in the composition of Chironomidae communities in the southern Mediterranean region. They have concluded that harsh climatic conditions in North-African wadis require resistance to disappearance of suitable habitats, high temperature and lentic conditions. Our study in

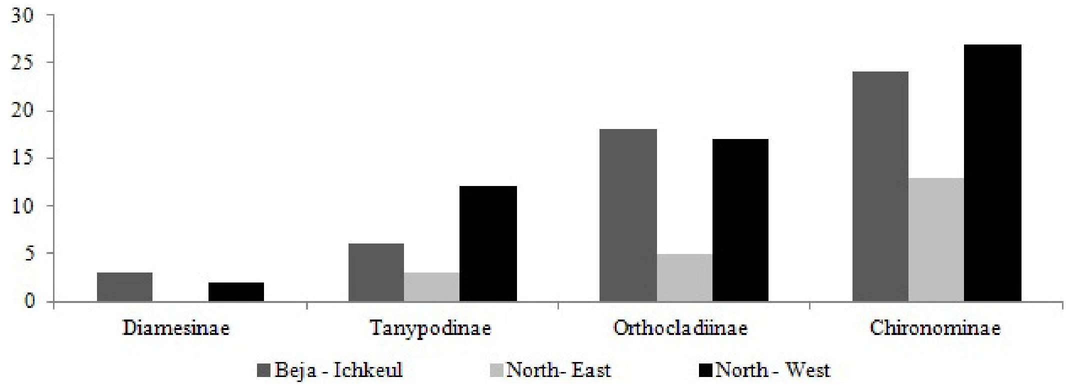

Figure 4
Total number of species within major subfamilies identified in three geographic areas of North Tunisia.

North Tunisia indicates that, in fact, lowland flowing waters are inhabited mainly by eurytopic taxa (*Procladius*, *C. plumosus*, *P. nubeculosum*-type) while in highland wadis many rheophilous, stenotopic species, requiring good water quality, such as *Larsia*, *Rheopelopia*, *Phaenopsectra*, *Stictochironomus,* occur.

Among the three study areas, only 8 taxa are relatively common and widespread (*M. nebulosa*, *Procladius* sp., *O. frigidus*, *R. effusus*, *C. plumosus*, *Eukiefferiella calvescens*, *Dicrotendipes nervosus*, *Polypedilum laetum*). As the North Tunisia water system is characterized by the diversity in hydrological, morphometric, geological and geographical conditions, the fauna of each region is specific and the localities with the same species recorded were situated at a distance of no more than 380 km from each other. In contrast to the Algerian river system (Chaib *et al.*, 2013), for instance in the Moroccan Rif region (Kettani and Langton, 2011), Chironominae is the subfamily richest in the number of recorded species. The *Cricotopus* species are widespread in Algeria and Morocco (Kettani *et al.*, 2001; Chaib *et al.*, 2013), *C. bicinctus, C. sylvestris, C. vierriensis* and *C. trifascia*, as well as *Polypedilum cultellatum*, have not been, or are only rarely noted, in North Tunisia. The North-West Basin fauna stands out with the highest number of species of Orthocladiinae, Tanypodinae and Chironominae (Figure 4). Similarly to the localities in the high Atlas Mountains (Kettani *et al.*, 2001), in the North-Eastern Basin Diamasinae are relatively well represented, while in the Ichkeul National Park Orthocladiinae are represented by a higher number of species than in the other areas. The Orthocladiinae (*Eukiefferiella coerulescens*, *Tvetenia discoloripes*, *Orthocladius frigidus*, *Orthocladius rivulorum*, *Rheocricotopus effusus*, *Rheocricotopus fuscipes*) and Tanytarsini (mainly *Tanytarsus* spp. and *Micropsectra atrofasciata*) included mostly rheophilic species (Moller Pillot, 2009; Moller Pillot and Klink, 2003). This is correlated with high frequency of orthoclads in the North-West Basin, where there are many upland and foothill streams with high slopes and a strong current (Laville, 1981). Other Chironominae (tribe Chironomini with *Chironomus*) are common in lentic and polluted waters such as the Béja (st. 6) and Kloufi wadis (st. 13) in the Beja Ichkeul Basin, Kasseb (st. 24), Ellil aval (st. 31), Sallen (st. 48) in the North-Western Basin and Chiba (st. 18), Méliane (st. 19) in the North-Eastern Basin site. Diamesinae occur only in the North-West Basin and the North-Eastern Basin water systems. The Diamesinae is a rather small subfamily, found mainly in high mountain areas, and cold, ultraoligotrophic, *e.g.* glacier fed, streams (Rossaro and Lencioni, 1999). Habitats typical for this subfamily are very scarce in Tunisia.

CONCLUSIONS

In conclusion, our studies in three different areas of North Tunisia show a high regional diversity of Chironomidae with 127 taxa recorded. Similarly to other regions of the southern

Mediterranean Coast (Chaib *et al.*, 2011, 2013; Kettani *et al.*, 2001; Kettani and Langton, 2011), mainly typical Paelearctic taxa, common also in Europe, occur in North Tunisia. Its geological structure and relief provide a place for diverse habitats of temporary streams (wadi), stagnant waters and wetlands. The outspread water systems maintain rich chironomid fauna. It should be noted that the present study, if continued and intensified, should uncover even a much higher Chironomidae diversity in the Tunisian river systems. This is indicated by the rarefaction analysis (Figure 2), which shows a steep increase of taxa when any new data are added and shows no saturation at present.

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Appendix I*. Sampling sites within the investigated area with their coordinates, altitude (Alt) and type of substrate: Bl - Blocks, Gl - Pebbles, Gr - gravel, Sg - sand, Li - silt, Ar - clay, V - vase).*

Sites	Name of Station	Latitude N	Longitude E	Alt. (m a.s.l.)	Substrate	Stream Permanace
The basin of Beja = Ichkeul						
St 1	Wadi Titria	36°57′793″	08°57′894″	78	Gr. + Sg.	permanent
St 2	Wadi el Maaden	36°58′188″	09°05′100″	32	Gl. +Gr. + Sg. + Li. + Ar.	permanent
St 3	Wadi Bouzenna	37°00′077″	09°05′338″	33	V. + Gr. + Ar + Li	permanent
St 4	Wadi Tamra	37°23′642″	09°06′245″	54	Bl. + Sg. + Gl.	permanent
St 5	Wadi Chatt Zwaraa	37°25′842″	09°06′263″	49	V. + Gr. + Ar. + Li.	permanent
St 6	Wadi Béja	36°45′380″	06°51′390″	190	V. + Gr. + Ar. + Li.	permanent
St 7	Wadi Ksar Mezouar	36°46′692″	09°20′344″	232	Sg. + Li. + V. + Gr.	permanent
St 8	Wadi Joumine amont	36°57′762″	09°31′478″	99	Bl. + Sg. + Gl.	permanent
St 9	Wadi Joumine aval	36°52′450″	07°20′090″	30	V. + Ar. + Li.	permanent
St 10	Wadi El Melah	37°06′250″	07°12′100″	15	Bl. + Sg. + Gl. + Ar.	permanent
St 11	Wadi Douimis	37°12′051″	09°37′465″	9	Gl. + Sg. + Ar.	permanent
St 12	Wadi Sejenane	37°07′219″	09°15′801″	108	Sg. + Li. + V. + Gr. + Bl.	permanent
St 13	Wadi El Kloufi	37°11′985″	09°34′237″	13	V. + Ar. + Li.	seasonal
St 14	Wadi Ziatine	37°12′459″	09°13′976″	5	Sg. + Gr. + V.	permanent
St 15	Wadi Magsbaya	37°03′421″	09°13′820″	138	Bl. + Gl. +Sg. + Li.	permanent
The North-eastern basin						
St 16	Wadi Abid	36°52′500″	08°19′380″	10	Sg. + V. + Li. + Gr.	permanent
St 17	Wadi Lebna	36°41′623″	08°24′856″	42	Sg. +V. + Gr.	permanent
St 18	Wadi Chiba	36°41′510″	08°26′150″	70	Sg. + V. + Gl.	permanent
St 19	Wadi Méliane	36°43′590″	10°15′202″	8	Li.+ Ar.	permanent
St 20	Wadi Zaouit el Megaiez	36°56′397″	08°44′259″	16	Bl.+Gl.+ Gr.	permanent
The Northwest basin						
St 21	Wadi Medjerda amont	36°27′100″	06°05′059″	192	Bl.+ Sg. + V.	permanent
St 22	Wadi El Mouadjen	36°55′676″	06°01′100″	760	Bl.+ Sg. + V.	permanent
St 23	Wadi Mellegue	36°23′480″	08°45′980″	200	Bl.+ Sg. + Ar.	permanent
St 24	Wadi Kasseb	36°66′105″	06°67′575″	135	Sg. + V.	permanent
St 25	Wadi Mliz	36°27′584″	08°33′586″	181	Gl.+Gr.+Sg.	permanent
St 26	Wadi Hammam Sayala	36°40′423″	09°93′581″	232	Gr.+ Li.	permanent
St 27	Wadi Ghrib	36°36′964″	08°41′139″	260	Sg. + Li + V.+ Gr.	permanent
St 28	Wadi Ghézala	36°38′525″	06°21′590″	236	Gl. +Gr. + Sg. + Li. + Ar.	permanent
St 29	Wadi Labgaa	36°44′812″	08°44′350″	446	Sg. + Gr. + Ar.	permanent
St 30	Wadi Ellil amont	36°41′411″	08°44′363″	246	Bl. + Gl. + Sg. + V.	permanent
St 31	Wadi Ellil aval	36°41′244″	08°44′219″	237	Bl.+ Gl.+ Gr.	permanent
St 32	Wadi Chaabet el Magroun	36°37′820″	06°21′890″	252	Bl.+ Gl. + Sg.	permanent
St 33	Wadi Saboun	36°39′501″	08°41′337″	261	Gl.+ Gr.	permanent
St 34	Wadi Barbar	36°40′351″	08°32′451″	187	Gl.+ Gr.+ Sg.	permanent
St 35	Wadi EdDmène	36°43′416″	08°41′474″	630	Bl. + Gl. + Sg.	permanent
St 36	Wadi Berbeg	36°44′588″	08°41′489″	558	Bl.+ Gl.+Gr.+Sg.	permanent
St 37	Wadi Lasfar	36°46′383″	08°46′348″	482	Gl.+ Gr.+ Sg.	permanent
St 38	Wadi Bransia	36°45′871″	08°45′091″	584	Gl.+ Gr.+ Sg.	permanent
St 39	Wadi Ennour	26°48′004″	08°39′539″	394	Gl.+ Gr.+ Sg.	permanent
St 40	Wadi Ain Gnaa	36°33′533″	08°47′252″	137	Li.	seasonal
St 41	Wadi Mrig	36°45′605″	08°41′270″	577	Gr.+ Li.	seasonal
St 42	Wadi El Kébir	36°55′252″	08°45′252″	10	V. + Li. + Gl. + Ar.	permanent
St 43	Wadi El Amor amont	36°54′255″	08°44′512″	30	Bl. + Gl. + Sg.	permanent
St 44	Wadi El Amor aval	36°55′303″	08°44′425″	14	Sg. + Li.	permanent
St 45	Wadi Rennagha	36°51′620″	08°43′245″	62	Bl. + Gl. + Sg. + Li.	permanent
St 46	Wadi Bouterfes	36°55′251″	08°50′579″	24	Bl. + Gl. + Sg. + Li.	permanent
St 47	Wadi Mlila	36°46′172″	08°34′459″	150	Bl.+ Gl.+ Gr.+ Li.	permanent
St 48	Wadi Sallen	36°46′123″	08°34′491″	410	Bl.+ Gl.+ Gr.+ Li.	permanent

Appendix II. *List of Chironomidae recorded in North Tunisia. * New taxa for North Tunisia. Stage determination: L - larvae, PrP - prepupae, P - pupae, A - adult males.*

Species	Basin Beja-Ichkeul	North-Eastern basin	North-West basin	Stage determination	Boumaiza and Laville, 1988
DIAMESINAE					
Diamesa starmachi (Kownacki and Kownacka, 1970)*	16, 19		28	L	
Potthastia gaedii (Meigen, 1838)	1, 5		48, 44, 46	L	E
Pseudodiamesa branickii (Nowicki, 1837)*	1			P	
TANYPODINAE					
Ablabesmyia longistyla (Fittkau, 1962)	2, 6, 10, 12, 13		28, 30, 34, 39	PrP	p
Clinotanypus nervosus (Meigen, 1818)*			39	P	
Larsia atrocincta (Goetghebuer, 1942)*			23, 25, 35, 39, 41, 48	P	
Macropelopia sp.	2, 9, 12, 13, 14	16, 20	21, 26, 29, 31	L	E
Natarsia sp.	1, 9	16	32, 39, 45, 46	L	
Procladius choreus (Meigen, 1804)*	6		27, 29, 39, 41, 44, 46, 47	A	
Procladius sp.	2, 4, 5, 10, 13, 14,15	16, 17	21, 26, 27, 30, 31, 33, 36, 38, 39, 41, 43, 44, 46, 48	L	E
Rheopelopia ornata (Meigen, 1838)			28, 38, 40	P	
Tanypus punctipennis (Meigen, 1818)			41, 42	A	
Thienemannimyia northumbrica (Edwards, 1929)			40	A	E
Thienemannimyia pseudocarnea (Murray, 1976)*			39	P	
Zavrelimyia nubila (Meigen, 1830)*	1, 6		22, 28, 36, 38, 39	P	
ORTHOCLADINAE					
Bryophaenocladius sp.			39	L	
Chaetocladius sp.	11			L	
Cricotopus fuscus (Kieffer, 1909)*			39	L	
Cricotopus sp.	15			L	
Cricotopus sylvestris (Fabricius, 1794)	9, 13		42	P	E
Eukiefferiella brevicalcar (Kieffer, 1911)*			24	P	E
Eukiefferiella claripennis (Lundbeck, 1898)*	13			L	
Eukiefferiella coerulescens (Kieffer, 1926)			36, 43	P	E
Eukiefferiella minor (Edwards, 1929)*	12			PrP	
Gymnometriocnemus sp.			39	L	
Orthocladius frigidus (Zetterstedt, 1838)	1, 3, 6, 8, 11, 12, 15	16	27, 29, 31, 36, 37, 39, 44, 45	A	E P
Orthocladius oblidens (Walker, 1856)*	8		47	P	
Orthocladius rivicola (Kieffer, 1911)*	5, 12, 13		33, 41	PrP	

***Appendix II**. Continued.*

Species	Basin Beja-Ichkeul	North-Eastern basin	North-West basin	Stage determination	Boumaiza and Laville, 1988
Orthocladius rivulorum (Kieffer, 1909)*	14		44	A	
Orthocladius robacki (Soponis, 1977)*	12		33	P	
Orthocladius saxicola (Kieffer, 1911)	15			P	E
Orthocladius saxosus (Tokunaga, 1939)*	12			L	
Orthocladius vaillanti (Langton and Cranston, 1991)*	12			P	
Parametriocnemus stylatus (Sparck, 1923)*		18, 20	28, 32	P	
Paratrichocladius rufiventris (Meigen, 1830)		18		L	E P
Rheocricotopus effusus (Walker, 1856)	1, 2, 4, 5, 8,	16	25, 30, 36, 37, 38, 39, 48	L	P
Rheocricotopus fuscipes (Kieffer, 1909)	1		39	L	P
Stilocladius sp.	11			L	
Thienemanniella sp.			39	L	
Tvetenia calvescens (Edwards, 1929)*	1, 8, 13, 14	16	26, 28, 41, 42	P	
Tvetenia discoloripes (Goetghebuer and Thienemann, 1936)*	1		36	L	
CHIRONOMINAE					
Chironomus calipterus (Kieffer, 1908)		19		A	E P
Chironomus longipes (Staeger, 1839)*		18		A	
Chironomus plumosus (Linné,ă1758)*	1, 2, 4, 5, 6, 11, 13	16, 18, 19, 20	21, 24, 31, 33, 37, 39, 41, 43, 48	A	
Chironomus riparius (Meigen, 1804)*	1, 6, 13	19	24, 30	A	
Chironomus salinarius (Kieffer, 1915)*	1, 2, 9, 13	18, 19	43, 47	A	
Cladotanytarsus sp. E (Epler, 2001)		17		L	
Cryptochironomus albofasiatus (Staeger, 1839)*			25	A	
Cryptochironomus rostratus (Kieffer, 1921)	5		23, 25	A	E P
Cryptochironomus sp.	11	16	32, 44, 46, 47	L	
Dicrotendipes nervosus (Staeger, 1839)*	3, 9, 13	20	24, 28, 41, 42, 48	A	
Dicrotendipes tritomus (Kieffer, 1916) *			21	A	
Einfeldia pagana (Meigen, 1838)*	2, 8	18, 19	39, 43, 48	P	
Endochironomus tendens (Fabricius, 1775)*			39	P	
Glyptotendipes pallens (Meigen, 1804)*	3			P	
Harnischia curtilamellata (Malloch, 1915)*	14		44, 45	PrP	
Micropsectra atrofasciata (Kieffer, 1911)*			29, 38, 48	A	
Microspectra sp.	1		36	L	
Microtendipes chloris (Meigen, 1818)*			39	A	
Microtendipes confinis (Meigen, 1830)		18		P	E P
Parachironomus tenuicaudatus (Malloch, 1915)*	12			A	
Paracladopelma nigritula (Goetghebuer, 1942)*			28	A	
Paratanytarsus dissimilis (Johannsen, 1905)*	12			P	

***Appendix II**. Continued.*

Species	Basin Beja-Ichkeul	North-Eastern basin	North-West basin	Stage determination	Boumaiza and Laville, 1988
Paratendipes sp.	13		39	L	E
Phaenopsectra flavipes (Meigen, 1818)*	2, 6, 12		22, 24, 36, 41, 47, 48	A	E
Polypedilum laetum (Meigen, 1818)	1	18	39	L	E
Polypedilum nubeculosum-type	2, 9, 11, 12, 13, 14, 15	20	27, 33, 36, 38, 39, 42, 45, 48	L	
Polypedilum nubifer-type	6, 12			L	
Polypedilum convictum-type	11, 13		22, 24, 38, 39, 46	L	P
Sergentia sp.	13			L	
Stictochironomus maculipennis (Meigen, 1818)	1, 10	18	39, 40	P	E P
Stictochironomus sp.	2, 9			L	E
Synendotendipes dispar (Meigen, 1830)*	9, 10, 11, 13		24, 27, 48	P	
Tanytarsus sp. form I			34	L	
Tanytarsus sp. form II			34	L	
Tanytarsus sp. form III	9			L	
Tanytarsus sp. form IV			42	L	
Tanytarsus sp. L (Epler, 2001)	13		39	L	
Zavreliella marmorata (van der Wulp, 1859)*			31, 39	A	