

# Paleotemperature reconstruction in tropical Africa using fossil Chironomidae (Insecta: Diptera)

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Received: 14 October 2008 / Accepted: 27 April 2009 / Published online: 16 May 2009  
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**Abstract** Fossil assemblages of chironomid larvae (non-biting midges) preserved in lake sediments are well-established paleothermometers in north-temperate and boreal regions, but their potential for temperature reconstruction in tropical regions has never before been assessed. In this study, we surveyed sub-fossil chironomid assemblages in the surface sediments of 65 lakes and permanent pools in southwestern Uganda (including the Rwenzori Mountains) and central and southern Kenya (including Mount Kenya) to document the modern distribution of African

chironomid communities along the regional temperature gradient covered by lakes situated between 489 and 4,575 m above sea level (a.s.l.). We then combined these faunal data with linked Surface-Water Temperature (SWTemp: range 2.1–28.1°C) and Mean Annual Air Temperature (MATemp: range 1.1–24.9°C) data to develop inference models for quantitative paleotemperature reconstruction. Here we compare and discuss the performance of models based on different numerical techniques [weighted-averaging (WA), weighted-averaging partial-least-squares (WAPLS) and a weighted modern analogue technique (WMAT)], and on subsets of lakes with varying gradient lengths of temperature and other environmental variables. All inference models calibrated against MATemp have a high coefficient of determination ( $r_{\text{jack}}^2 = 0.81\text{--}0.97$ ), low maximum bias (0.84–2.59°C), and low root-mean-squared error of prediction (RMSEP = 0.61–1.50°C). The statistical power of SWTemp models is generally weaker ( $r_{\text{jack}}^2 = 0.77\text{--}0.95$ ; maximum bias 1.55–3.73°C; RMSEP = 1.39–1.98°C), likely because the surface-water temperature data are spot measurements failing to catch significant daily and seasonal variation. Models based on calibration over the full temperature gradient suffer slightly from the limited number of study sites at intermediate elevation (2,000–3,000 m), and from the presence of morphologically indistinguishable but ecologically distinct taxa. Calibration confined to high-elevation sites (>3,000 m) has poorer error statistics, but is less susceptible to biogeographical

**Electronic supplementary material** The online version of this article (doi:[10.1007/s10933-009-9339-2](https://doi.org/10.1007/s10933-009-9339-2)) contains supplementary material, which is available to authorized users.

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and taxonomic complexities. Our results compare favourably with chironomid-based temperature inferences in temperate regions, indicating that chironomid-based temperature reconstruction in tropical Africa can be achieved.

**Keywords** Chironomids · East Africa · Midges · Temperature · Transfer function

## Introduction

Paleoclimate research in tropical Africa over the past 40 years has established the broad outlines of glacial/interglacial hydrological change across the continent (Gasse et al. 2008) and the approximate magnitude of temperature change since the Last Glacial Maximum (Hostetler and Clark 2000; Tierney et al. 2008). The thermal history of tropical Africa during the Holocene is still largely unknown, however, and therefore also the mechanisms responsible for temperature variation at sub-orbital time scales (Powers et al. 2005; Tierney et al. 2008). The present scarcity of trustworthy reconstructions of Holocene temperature change in Africa makes it difficult to test and validate the performance of climate models in hind-casting past African climate change, calling into question their performance in predicting future climate change. A new paleothermometer based on the composition of membrane lipids found in crenarchaeotal bacteria (TEX<sub>86</sub>; Schouten et al. 2002) shows promise for application on sedimentary records from very large lakes (Powers et al. 2005; Tierney et al. 2008), but its application in the much more common smaller lakes is complicated (Blaga et al. 2009). Consequently, there is an urgent need for other temperature proxies that, at least in hydrologically open systems, are unaffected by simultaneous changes in lake moisture balance.

In this study, we aim to develop fossil assemblages of African chironomid larvae (Insecta: Diptera: Chironomidae, or non-biting midges) preserved in lake sediments as a moisture-independent paleothermometer. The influence of temperature on the geographical and altitudinal distribution of chironomid species has been recognised since the early work of Brundin (1949). Since the early 1990s, numerous chironomid inference models, based on calibration in lowland as

well as mountain lakes, have been developed to quantitatively reconstruct summer air and/or water temperature (Olander et al. 1997; Heiri and Lotter 2005; Porinchu et al. 2007). Their subsequent use in down-core studies shows that, at least in north-temperate regions, midge assemblages have responded sensitively to most known climatic fluctuations of the last 15,000 years, including the Gerzensee Oscillation and Younger Dryas cold event in the late Glacial period, the early Holocene Optimum, and the Little Ice Age (Brooks 2006; Walker and Cwynar 2006). Several studies have demonstrated response of midge communities to the subtle temperature changes that occurred since the early twentieth century (Larocque and Hall 2003; Porinchu et al. 2007). Current efforts to improve chironomid-based paleotemperature reconstruction are primarily concerned with the choice between local air and surface-water temperatures as the basis to calibrate chironomid distribution (Livingstone et al. 1999). Current studies also focus on assessment of the relative influence of environmental variables other than temperature (Heiri et al. 2003; Velle et al. 2005), and reduction of model prediction errors, which are usually  $\sim 1.5$ – $2.0^{\circ}\text{C}$  (Brooks 2006). However, these developments have been limited geographically to north-temperate and boreal regions (Brooks 2006; Walker and Cwynar 2006), and to the equivalent climate zones in the southern Hemisphere (Woodward and Shulmeister 2006; Dieffenbacher-Krall et al. 2007; Rees et al. 2008). This study is the first to assess the potential of fossil chironomid assemblages for paleotemperature reconstruction in the tropics.

In this study, we surveyed sub-fossil chironomid assemblages in the surface sediments of 65 lakes and permanent pools in southwestern Uganda (including the Rwenzori Mountains) and central and southern Kenya (including Mount Kenya) to document the modern distribution of African chironomid communities along the regional temperature gradient covered by lakes situated between 489 and 4,575 m above sea level (a.s.l.). We then combined these faunal data with linked Surface-Water Temperature (SWTemp: range  $2.1$ – $28.1^{\circ}\text{C}$ ) and Mean Annual Air Temperature (MATemp: range  $1.1$ – $24.9^{\circ}\text{C}$ ) data to develop inference models for quantitative paleotemperature reconstruction. Here we compare and discuss the performance of models based on different numerical techniques and on subsets of lakes with

varying gradient lengths of temperature and other environmental variables.

## Materials and methods

### Field and laboratory methods

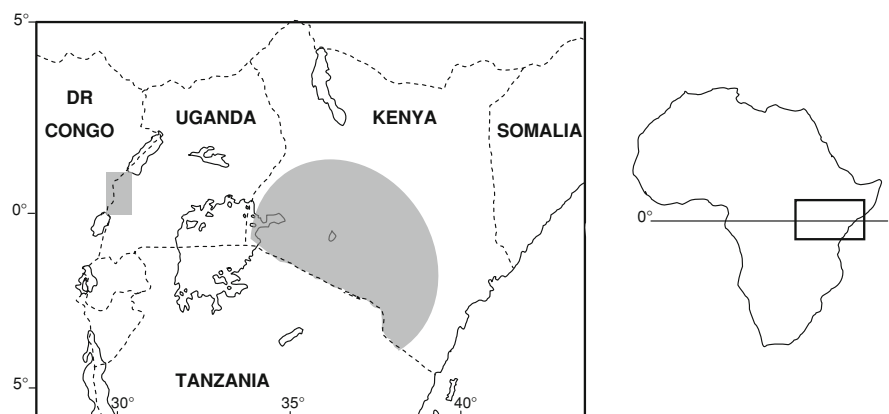
Fieldwork in Uganda and Kenya between 2000 and 2007 yielded surface-sediment samples from 57 lakes and permanent pools in southwestern Uganda, including the eastern slope of the Rwenzori Mountains, and central and southern Kenya. In addition, Alayne Street-Perrott provided eight samples from mid- and high-elevation lakes on Mount Kenya. This modified EL-EM calibration data set (Eggermont et al. 2006), comprises 65 freshwater lakes (conductivity <500  $\mu\text{S}/\text{cm}$ ) ranging in altitude between 489 and 4,575 m a.s.l., with MATemp between 1.1 and 24.9°C, and SWTemp between 2.1 and 28.1°C (Fig. 1; Table 1 in Supplementary material). Low- and mid-elevation sites sampled in western Uganda are mostly small maar crater lakes. In Kenya, we also sampled large tectonic lakes, smaller lakes on the floor and shoulders of the Rift Valley, and shallow lakes and swamps. In the Rwenzori, we sampled 28 high-elevation lakes and permanent pools on the Ugandan side of the range (Eggermont et al. 2007 b). Mount Kenya is represented by six glacier-fed lakes and tarns (Eggermont and Verschuren 2007). Anticipating the use of weighted-averaging regression techniques (Birks 1995), we selected lakes with a fairly even distribution across the regional temperature gradient. Still, the EL-EM data set includes only few lakes between 2,000 and 3,500 m, due to scarcity

of such intermediate-elevation lakes in Kenya and Uganda. Sites were also chosen to be shallow ( $\sim 75\%$  are less than 15 m deep) and fresh to minimize the influence of secondary environmental gradients and to optimize the relationship between annual mean air and surface-water temperature ( $r = 0.99$ ,  $P < 0.001$ ).

Inference models were developed for the full calibration data set of 65 lakes (EL-EM data set) and for the subset of 35 lakes located close to or above 3,000 m a.s.l., and display MATemp and SWTemp values of <10–12°C and <13°C, respectively. This reduced calibration data set is referred to as the East African Mountain lakes (EM) data set. Temperature calibration with high-elevation lakes only is intended to improve model sensitivity in the coldwater range and to obtain more reliable species optima and tolerances for high-elevation taxa that cannot be distinguished reliably from related lowland taxa (Eggermont and Verschuren 2007). We also constructed models based on Rwenzori lakes only (28 of our 35 mountain sites) to eliminate the biogeographical complexity caused by taxa that so far appear to be endemic to Mount Kenya.

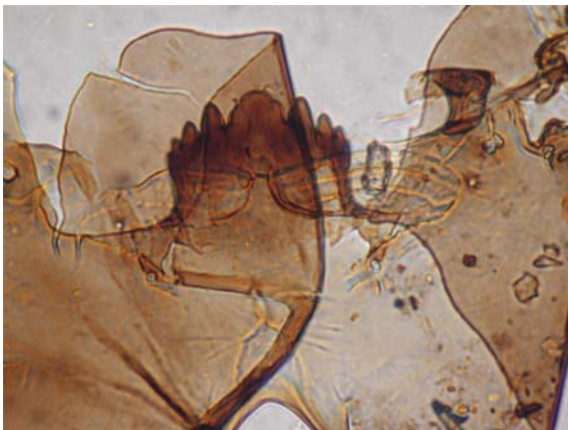
The organic content of the sediment was estimated using the loss-on-ignition technique (Dean 1974), and expressed as the percent weight loss after combustion at 550°C for 4 h. Chironomid preparation followed Walker (2001) with sediments being washed through a 100  $\mu\text{m}$  sieve. Head capsules picked from the residue were mounted in glycerine on microscope slides. Identification was done at 100–400 $\times$ , by reference to Eggermont and Verschuren (2004a, 2004b) and Eggermont and Verschuren (2007). Mount Kenya specimens resembling the *Chironomus* near *imicola* Kieffer presented in Eggermont and Verschuren

**Fig. 1** Map of East Africa with study areas indicated in grey



(2007) are here treated as the distinct morphotype *Chironomus* type Mount Kenya, because of its heavier sclerotization, strongly fused first and second lateral mentum teeth, and more distinct size difference between the 4th and 5th lateral teeth of the mentum than the lowland populations. We here define *Diamesa* type East Africa as a group taxon possibly including the three known African *Diamesa* species (*D. kenyae* Freeman, *D. freemani* Willassen and Cranston, and *D. rwenzoriensis* Freeman: Willassen and Cranston 1986), as early instars and worn specimens cannot be unequivocally identified to species (Eggermont and Verschuren 2007). We further recognize one new morphotype designated Tanytarsini indet. type Kanganyika (Fig. 2), of which only the mentum and ventromental plates have been found so far. The characteristic features of this taxon are a mentum with weakly notched median tooth, probably because of wear, and four pairs of lateral teeth gradually decreasing in size. The median tooth is  $\sim 2.5$  times as wide as the 1st laterals; ventromental plates are compact ( $\sim 3$  times as long as wide) and about as wide as the mentum, with slightly curved anterior margin and a broad band of striae taking up the middle third. Criteria for counting fragmentary fossils followed Walker (1987). The volume of sediment analysed was adjusted to yield the  $\sim 50$ – $80$  head capsules desired for numerical analysis (Quinlan and Smol 2001).

Except for eight Rwenzori lakes, which had an air temperature logger placed directly at the site, MATemp data were estimated using region-specific linear relationships between elevation and temperature



**Fig. 2** Subfossil remains (mentum and ventromental plates) of larval Tanytarsini indet. type Kanganyika

( $P < 0.001$  in all cases). For low- and mid-elevation lakes in Uganda, we derived MATemp data from the Global Historical Climatology Network (GHCN-Monthly) data base (time series from the 1930s to 2006; four stations) and Global Summary of the Day (GSOD) data base (time series from 1957 to 2006; 10 stations). For low- and mid-elevation lakes in Kenya, we used the GHCN data base (time series from the 1930s to 2006; three stations) and GSOD-data base (time series from 1957 to 2006; 24 stations). For Mount Kenya, we used free air temperatures retrieved from the National Center for Environmental Prediction (NCEP)/National Center for Atmospheric Research (NCAR) Re-analysis R1 by extracting temperature and geopotential height data from the grid cell closest to Mount Kenya ( $0^{\circ}\text{N}/37.5^{\circ}\text{E}$ ) on all mandatory pressure levels between 400 hPa ( $\sim 7,500$  m) and 1,000 hPa ( $\sim 50$  m), averaged over the period 1968–1996. Finally, for the Rwenzori we used StowAway<sup>®</sup> Tidbit<sup>™</sup> temperature logger data (2005–2008 at 2-hour resolution) from 16 locations between 1,766 and 4,555 m elevation. NCEP-NCAR Re-analysis data for Mount Kenya were corrected  $0.14^{\circ}\text{C}$  upward, the mean difference between NCEP-NCAR Re-analysis data for Rwenzori and our own Rwenzori temperature logger data. In 38 lakes, SWTemp, pH, surface-water oxygen (SWO<sub>2</sub>) and specific conductivity (K25) are single (mid-lake) measurements taken at 10 cm water depth with a Hydrolab Quanta multi-probe at the time of sediment sampling. In the 28 lakes surveyed twice or more within a sampling period of maximum 2.5 years, we used the average of all measurements made. For the remaining four sites (Salomon's pool, Zaphana's pool, Balengekania's pool and Lake Irene) lacking direct temperature data, SWTemp was estimated using the significant regression between SWTemp and elevation at the other Rwenzori sites. Total nitrogen (TN), phosphorus (TP), dissolved organic and inorganic carbon (DOC, DIC) and Chl*a*-values are only available for the Rwenzori data set, measured following procedures described in Eggermont et al. (2007b).

## Numerical methods

### Data screening

Ordination and inference-model development consider all chironomid taxa with two or more

occurrences in the respective calibration data set (Eggermont et al. 2006), but exclude the four broad categories of unidentifiable fossils (Tanypodinae indet., Orthoclaudiinae indet., Chironomini indet., and Tanytarsini indet.) since these represent only a minor fraction of the recovered fauna (1.2%) and do not contain much ecological information. We did include the juvenile morphotypes ‘Pentaneurini larvula’ and ‘Chironomini larvula types 1 and 2’ (Eggermont and Verschuren 2004a, 2004b) because these are too abundant to ignore (10.8% of all fossils), and excluding them from the modelling might produce too optimistic prediction errors. Samples were declared outliers and deleted from further analyses if their residual distance to the first axis in a Detrended Canonical Correspondence Analysis (DCCA; ter Braak 1986) with MATemp or SWT as sole constraining variable was within the extreme 5% of all residual distances (Birks et al. 1990a, b).

#### *Ordinations and species turnover*

A Detrended Correspondence Analyses (DCA; Hill and Gauch 1980) was used for initial data screening. DCCA with down weighting of rare taxa and MATemp or SWTemp as the sole constraining variable was performed to assess their strength as explanatory variables for chironomid species distributions (Eggermont et al. 2006). Limited availability of uniform environmental data other than MATemp and SWTemp across the entire EL-EM data set precluded comprehensive analysis of the relationship between chironomid distribution and their modern environment. We also assessed the biological importance of water depth, conductivity, and pH, which had variable total numbers of sites in the analyses. Values for water depth and conductivity were log-transformed because of their skewed distribution. To assess whether a particular environmental variable explained a significant ( $P \leq 0.05$ ) portion of the variation in the faunal data, we carried out a series of CCAs with that variable as sole constraining variable (999 unrestricted permutations). Only variables which explained a significant portion of the variation in the faunal data and had a variance inflation factor (VIF)  $< 20$  were retained for subsequent analyses. We then assessed the unique and independent explanatory power of each variable through a series of CCAs with that environmental parameter as sole

constraining variable and the other variables treated as co-variables.

Availability of a fairly uniform set of environmental data (elevation, MATemp, SWTemp, water depth, conductivity, pH, DOC, DIC, TN, TP, %LOI and Chl $a$ ) for all 28 Rwenzori study lakes allowed more in-depth evaluation of the relative importance of these environmental variables in structuring chironomid communities. Values for water depth, conductivity, TP and Chl $a$  were log-transformed. Assessment of the explanatory power of each environmental variable again proceeded through a series of CCAs, as detailed above. All ordinations were done in CANOCO version 4.5 (ter Braak and Smilauer 1998), using square-root-transformed chironomid percentage data.

Chironomid species turnover along the temperature gradient was explored by calculating Cody’s diversity measure  $\beta_c = (G + L)/2$  (Cody 1975), where  $G$  and  $L$  are the number of taxa gained and lost in successive intervals of 5.0°C.

#### *Taxon response models and transfer functions*

MATemp and SWTemp optima and tolerances for all retained taxa were estimated as, respectively, weighted averages and weighted standard deviations (Birks et al. 1990a) using C2 version 1.3.4. (Juggins 2003). The shape of each taxon’s response to MATemp or SWTemp was assessed by hierarchically fitting a series of taxon-response models using the Huisman-Off-Fresco (HOF) programme version 2.3 (Oksanen 1998). Transfer functions were developed in C2 version 1.3.4. (Juggins 2003) using algorithms based on weighted-averaging (WA), weighted-averaging partial-least-squares (WA-PLS) and the weighted modern analogue technique (WMAT) (Birks 1995). Following Eggermont et al. (2006), the statistical performance of inference models produced by applying each of these numerical methods to the various calibration data sets was compared using the jack-knifed coefficient of determination between observed and predicted temperature ( $r_{\text{jack}}^2$ ), the root-mean-squared error of prediction ( $\text{RMSEP}_{\text{jack}}$ ), and mean and maximum biases. To find the simplest adequate WA-PLS model, we required that ‘useful’ additional partial-least-squares components should further reduce RMSEP by at least 5% (Birks 1998). In WMAT, we selected the model with the lowest number of closest analogues that yielded the best



jack-knifed error estimates, using squared chi-square distance as the dissimilarity coefficient. All numerical analyses with the exception of the taxon response modelling were based on squared-root transformed percentage data in an attempt to stabilise variances and to optimise the ‘signal’ to ‘noise’ ratio in the data set.

## Results

### Air-temperature inference models (MATemp models)

#### *EL-EM calibration data set (MATemp range: 1.1–24.9°C)*

The unscreened calibration data set comprises 6,613 chironomid remains recovered from 65 lakes ( $95 \pm 27$  fossils per sample), of which 96.8% were identified to a total of 81 different taxa. None of the lakes were deleted as outliers. Thirteen taxa with fewer than two occurrences were removed, and eight taxa were paired two-by-two as *Corynoneura* cf. *coronata*/near *dewulfi*, *Dicrotendipes septemmaculatus*/near *pilosimanus*, *Harnischia* type *Tanganyika*/near *curtilamellata* and *Polypedilum* type *Bandasa*/type *Narasha*, given limited discriminatory features between them (Eggermont and Verschuren 2004a, b; Eggermont and Verschuren 2007). The screened EL-EM calibration data set thus contains distributional information on 64 taxa, which together account for 99.1% of all identifiable fossils (Table 1).

The correlation between DCA axis 1 sample scores and MATemp is highly significant ( $r = 0.95$ ;  $P < 0.001$ ), suggesting a strong relationship between compositional change in the chironomid assemblages and MATemp. Similarly, the fairly high  $\lambda_1/\lambda_2$  ratio of 2.409 in a DCCA with MATemp as sole constraining variable indicates that MATemp represents a major gradient in the faunal data. Still, the latter analysis also reveals a significant secondary gradient as evidenced by the second axis (4.8 SD) being longer than the first (4.6 SD).

Of the 64 taxa considered, 52 (81.3%) display a symmetrical or skewed unimodal response to MATemp (HOF response types IV and V); 10 (15.6%) display sigmoidal decreasing (IIId) or increasing (IIi)

responses suggesting that their true temperature optima lie below or above the sampled range; and two taxa (3.1%) show no statistically significant relationship with MATemp (Table 1 and 2).

Arranged according to their temperature optima, East African Chironomidae can be sorted into five ecological groups (Fig. 3a; Table 2). Group 1 taxa not recorded above  $\sim 1,500$ – $1,800$  m (MATemp  $\geq 17.0^\circ\text{C}$ ). This group represents one-third of the fauna, with an average MATemp optimum of  $22.6 \pm 0.9^\circ\text{C}$ . Group 2 taxa show a broader distribution with respect to temperature but still are restricted to low and middle elevations up to  $\sim 2,500$ – $3,000$  m (MATemp  $\geq 10.0^\circ\text{C}$ ). This group constitutes about one-third of the fauna, with an average MATemp optimum of  $19.8 \pm 2.7^\circ\text{C}$ . Group 3 taxa are restricted to elevations above approximately  $3,000$ – $3,500$  m (MATemp  $\leq 10.0^\circ\text{C}$ ). This group contains 15% of the taxa, all with a temperature optimum below  $6.5^\circ\text{C}$  (on average  $4.4 \pm 1.7^\circ\text{C}$ ). Group 4 taxa occur both in lowland sites ( $<1,500$ – $1,800$  m) and in the Alpine zone ( $>3,800$ – $4,000$  m), but not at intermediate altitudes. This group comprises two taxa only (*Paramerina* cf. *ababae* and *Pentaneurini* larvula), both having an optimum of  $\sim 21.0^\circ\text{C}$ . Group 5 taxa are present at basically all elevations up to  $\sim 4,000$ – $4,500$  m. The approximately 15% of taxa making up this group have their optimum within the broad range of  $4.0$ – $21.0^\circ\text{C}$  (on average  $11.6 \pm 5.6^\circ\text{C}$ ). Overall, the major faunal transition occurs at  $\sim 1,500$ – $1,800$  m, (i.e. the lower limit of the montane forest zone; MATemp  $\sim 17.0$ – $20.0^\circ\text{C}$ ), and approximately  $3,000$  m, (i.e. the lower limit of the Ericaceous zone; MATemp  $\sim 10.0^\circ\text{C}$ ). This is also reflected in high local species turnover values ( $\beta_c$ ) of 12 and 16, respectively, compared to lower numbers along other parts of the gradient (Table 3 in Supplementary material).

Distribution of subfamilies along the altitudinal temperature gradient also reveals distinct trends (Table 2). Chironominae and Tanypodinae are most abundant in number of taxa, and individuals at sites below  $3,400$ – $3,800$  m, (i.e. MAT above  $6^\circ\text{C}$ ), whereas Orthoclaadiinae and Diamesinae are more abundant in the lower temperature range.

Summary statistics of inference models based on different numerical methods are listed in Table 3. Figure 4a shows chironomid-inferred versus observed MATemp for these models, and the distribution of

**Table 1** Descriptive statistics for the various calibration data sets

	EL-EM dataset	EM dataset	Rwenzori dataset
Number of samples	68	35	28
N2 for samples			
Minimum	1.04	1.40	1.40
Median	3.35	2.94	2.82
Maximum	12.51	7.65	6.41
Number of taxa	64	20	11
N2 for taxa			
Minimum	1.05	1.29	1.99
Median	4.77	4.73	7.32
Maximum	23.06	27.25	25.58
DCA			
$\lambda_1$	0.872	0.720	0.400
Gradient length axis 1 (SD)	5,926	3,984	2,065
%Variance explained by axis 1	19.5	30.4	36.7
$\lambda_2$	0.268	0.254	0.141
Gradient length axis 2 (SD)	3,275	2,764	1,601
%Variance explained by axis 2	6.0	10.7	12.9
DCCA			
$\lambda_1$	0.812 (0.825)	0.379 (0.449)	0.280 (0.262)
Gradient length axis 1 (SD)	4.595 (5.239)	2.397 (2.891)	1.605 (1.609)
%Variance explained by axis 1	18.2 (18.5)	16.0 (19.0)	21.8 (20.1)
$\lambda_2$	0.337 (0.318)	0.330 (0.310)	0.204 (0.198)
Gradient length axis 2 (SD)	4.775 (4.004)	3.745 (3.094)	1.629 (1.712)
%Variance explained by axis 2	7.5 (7.1)	13.9 (13.0)	18.8 (18.1)
$\lambda_1/\lambda_2$	2.409 (2.594)	1.148 (1.448)	1.373 (1.323)
Taxon responses	Out of 64	Out of 20	Out of 11
Skewed unimodal (V)	20 (21)	3 (5)	1 (5)
Symmetric unimodal (IV)	32 (32)	8 (5)	5 (2)
Sigmoidal increasing (III)	6 (10)	3 (5)	0 (0)
Sigmoidal decreasing (IID)	4 (1)	3 (1)	3 (2)
Null model (I)	2 (0)	3 (4)	2 (2)
Ecological category	Out of 64	Out of 20	Out of 11
Category 1	20	0	0
Category 2	25	3	0
Category 3	11	11	8
Category 4	2	0	0
Category 5	6	6	3

prediction residuals. WMAT(4) and simple WA generally yielded the poorest results ( $r_{jack}^2 = 0.96-0.97$ ; RMSEP = 1.6–1.8°C; maximum bias = 2.7–3.0°C). The most obvious feature in their performance plots is the fairly large data gap between ~6.0 and 18.0°C. Samples within this gap feature fairly high prediction residuals, reflecting the model’s modest ability to track

temperature changes in that range. WA-PLS(2) only slightly improves upon WA and WA-PLS(1), with a higher  $r_{jack}^2$  (0.97) and lower RMSEP (1.5°C, a 7.4% reduction). The distribution of residuals resembles that of WMAT(4) and WA but the maximum bias is slightly smaller (2.6°C). This improvement is at least partly due to the better spread of cold-water lakes

**Table 2** Weighted-averaging MATemp optima and tolerances for the chironomid taxa present in the EL-EM, EM, and Rwenzori-only calibration data set

Name	Cat.	N0		N2		Opt		Tol		HOF				
		EL-EM	EM	RWE	EM	RWE	EL-EM	EM	RWE	EL-EM	EM	RWE		
<b>Diaesinae</b>														
<i>Diaesa</i> type East Africa group	3	14	14	14	10.8	12.9	4.1	4.1	1.3	1.3	1.3	IV	IV	
<b>Tanypodinae</b>														
<i>Ablabesmyia</i> type Murusi	1	17			8.8		22.0		1.4			IV		
<i>Ablabesmyia</i> type Baringo	1	3			2.7		23.8		1.1			V		
<i>Clinotanypus</i> near <i>claripennis</i>	1	4			2.9		22.8		1.1			IV		
<i>Larsia</i> type Kitandara	3	11	11	11	5.6	9.3	5.2	4.9	0.9	0.9	0.9	IV	IV	
<i>Paramerina</i> cf. <i>ababae</i>	4	14			8.3		21.1		1.7			III		
<i>Procladius</i> <i>brevipetiolatus</i>	2	30	3	3	9.7	2.5	17.2	8.4	6.1	1.7		IV	IV	
<i>Tanypus</i> near <i>guttatipennis</i>	1	15			6.9		23.5		1.9			III		
Pentaneurini indet. type Naivasha	2	5			4.8		17.5		3.1			IV		
Pentaneurini larvula	4	15			10.1		21.8		1.9			III		
Tanypodinae indet. type Naivasha	2	4	1	1	1.3	1.0	11.6	10.0	8.6	2.1		IV	V	
<b>Orthocladinae</b>														
<i>Chaetocladus</i> <i>melaleucus</i>	3	28	28	26	12.9	19.8	18.1	2.2	2.6	2.7	1.3	1.5	1.5	IIId
<i>Corynoneura</i> cf. <i>coronata</i> /near <i>dewulfi</i>	1	11			5.7		22.7		1.9			III		
<i>Cricotopus</i> type East Africa group	1	9			3.1		23.3		1.4			V		
<i>Linnophyesminimus</i> (Epler) group	5	44	33	28	23.1	27.3	25.6	4.2	3.8	3.7	2.9	1.7	1.5	I
<i>Nanocladus</i> near <i>saetheri</i>	1	10			5.6		23.2		0.7			IV		
<i>Parametrioctenus</i> type Tanganyika	2	6			4.4		20.8		2.4			IV		
near <i>Paraphaenocladus</i> type OI bolossat	5	12	7	6	6.8	6.3	5.5	10.4	5.3	4.9	6.2	1.8	0.9	IV
<i>Paratrichocladus</i> type Hausburg Tarn	3	7	7	5	3.4	4.9	3.0	3.7	3.8	5.7	2.5	2.2	0.7	IIId
<i>Paratrichocladus</i> type Simba Tarn	3	8	8	6	2.2	4.7	4.8	1.5	2.4	3.9	1.8	1.7	1.4	IIId
<i>Psectrocladius</i> <i>viridescens</i>	2	3			1.6		20.8		0.7			V		
<i>Pseudosmittia</i> type Tanganyika	5	16	11	10	10.9	10.0	9.0	8.0	4.4	4.8	6.9	1.2	0.7	I
<i>Thienemanniella</i> cf. sp.D of Epler	1	6			3.6		23.8		0.9			V		
Orthocladinae indet. type Amboseli NP Pool 3	2	4			1.4		20.8		2.7			V		

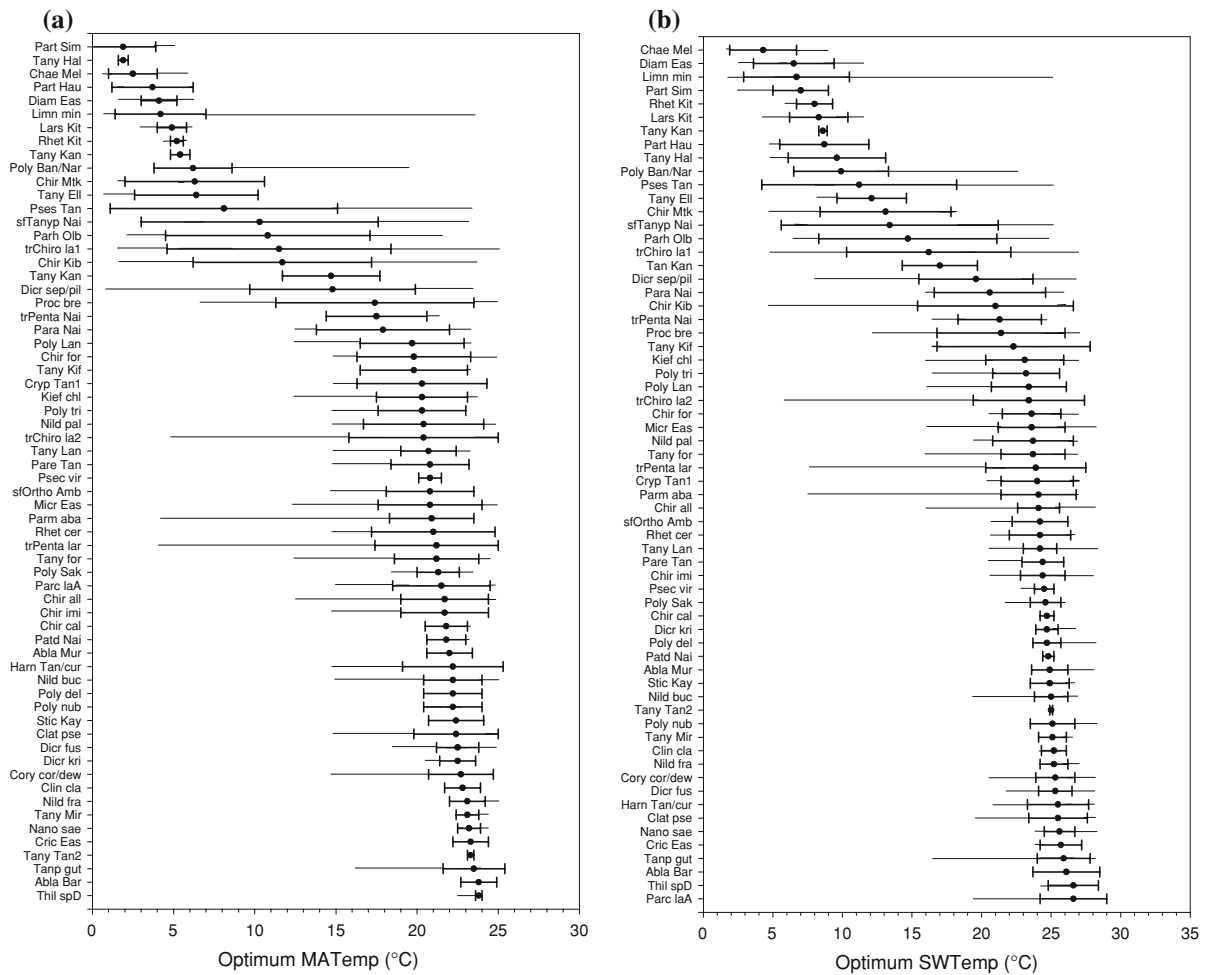


Table 2 continued

Name	Cat.	N0			N2			Opt			Tol			HOF		
		EL-EM	EM	RWE	EL-EM	EM	RWE	EL-EM	EM	RWE	EL-EM	EM	RWE	EL-EM	EM	RWE
<b>Chironomini</b>																
<i>Chironomus</i> near <i>alluaudi</i>	2	23			8.2			21.7			2.7			IV		
<i>Chironomus</i> type Kibos	5	21	4		13.0	3.7		17.6	6.8		5.6	4.5		V	I	
<i>Chironomus</i> near <i>calipterus</i>	1	4			3.2			21.8			1.3			V		
<i>Chironomus</i> near <i>imicola</i>	2	12			3.9			21.7			2.7			IV		
<i>Chironomus</i> type Mt. Kenya	3	5	5		3.8	4.5		6.3	6.1		4.3	4.2		V	V	
<i>Chironomus formosipennis</i>	2	10			4.9			19.8			3.5			IV		
<i>Cryptochironomus</i> type Tanganyika group 1	2	5			4.7			20.3			4.0			IV		
<i>Dicrotendipes fusconotatus</i>	1	21			9.7			22.5			1.3			IV		
<i>Dicrotendipes septemmaculatus</i> /near <i>pilosimanus</i>	5	28	6		10.8	4.4		14.9	8.2		5.1	3.2		V	III	
<i>Dicrotendipes kribiicola</i>	1	7			3.5			22.5			1.1			IV		
<i>Hamishia</i> type Tanganyika/near <i>curtilamellata</i>	2	5			4.0			22.2			3.1			III		
<i>Kiefferulus chloronotus</i>	2	19			13.3			20.3			2.8			IV		
<i>Microchironomus</i> type East Africa group	2	18			5.0			20.8			3.2			IV		
<i>Microchironomus deribae</i>	2	2			2.0			14.8			2.6			V		
<i>Nilodorum brevivibacca</i>	2	20			11.6			22.2			1.8			III		
<i>Nilodorum brevipalpis</i>	2	17			8.1			20.4			3.7			V		
<i>Nilodorum fractilobus</i>	1	5			1.7			23.1			1.1			V		
<i>Parachironomus</i> cf. Larva A of Harrison	2	12			6.9			21.5			3.0			V		
<i>Parachironomus</i> type Naivasha	2	9			6.9			17.9			4.1			IV		
<i>Paratendipes</i> type Naivasha	1	2			1.6			21.8			1.2			V		
<i>Polypedium</i> near <i>deletum</i>	1	12			3.7			22.2			1.8			IV		
<i>Polypedium</i> cf. <i>nubifer</i>	1	10			6.7			22.2			1.8			IV		
<i>Polypedium</i> cf. <i>trigonus</i>	2	7			4.2			20.3			2.7			V		
<i>Polypedium</i> near <i>wittei</i> type Langanjo	2	12			6.3			19.7			3.2			IV		
<i>Polypedium</i> near <i>wittei</i> type Saka	1	7			2.9			21.3			1.3			IV		

Table 2 continued

Name	Cat.	N0		N2		Opt		Tol		HOF									
		EL-EM	EM	RWE	9	EL-EM	EM	RWE	9.6	7.3	EL-EM	EM	RWE	6.0	5.0	3.4	2.2	0.5	EL-EM
<i>Polypedilum</i> type Bandasa/Narasha	5	17	12	9	9.2	9.6	7.3	6.7	6.0	5.0	3.4	2.2	0.5	V	V	V	V		
Near <i>Stictochironomus</i> type Kayihara	1	5			4.6			22.4			1.7			V					
Chironomini larvula type 1	5	28	7		9.5	6.0		11.3	6.6		6.6	3.8		IV			III		
Chironomini larvula type 2	5	27	2		13.5	1.8		20.6	8.9		4.2	2.5		V			III		
Tanytarsini																			
<i>Cladotanytarsus pseudomancus</i>	2	27			13.3			22.4			2.6			V					
<i>Rheotanytarsus</i> near <i>ceratophylli</i>	2	5			4.0			21.0			3.8			IV					
Near <i>Rheotanytarsus</i> type Kitandara	3	5	2	5	4.0	4.7	4.7	5.1	5.1	5.1	0.5	0.6	0.6	IV	IV	IV	IV		
<i>Tanytarsus formosanus</i>	2	23			10.6			21.2			2.6			IV					
<i>Tanytarsus</i> type Langano	2	11			4.8			20.7			1.7			III					
Tanytarsini indet. type Kanganyika	3	2	2	2	2.0	2.0	2.0	5.4	5.4	5.4	0.6	0.6	0.5	I	I	I	I		
Tanytarsini indet. type 2 of Tanganyika	1	2			1.8			23.3			0.2			IV					
Tanytarsini indet. type Ellis	3	3	3		1.1	1.6		6.4	5.4		3.8	3.5		IV			IV		
Tanytarsini indet. type Hall Tam	3	2	2		1.0	1.3		1.9	1.9		0.3	0.3		IV			IV		
Tanytarsini indet. type Kifuruka	1	3			1.9			19.8			3.3			V					
Tanytarsini indet. type Mirambi	1	8			3.9			23.1			0.7			IV					



**Fig. 3** Range (*thin lines*), weighted-average MATemp (a) and SWTTemp (b) optima (*solid circles*) and tolerances (*thick lines with ticks*) of the 64 EL-EM chironomid taxa. Taxon codes as in Eggermont et al. (2006)

(MATemp < 7.5°C) and warm-water lakes (MATemp > 15.0°C) along the 1:1 regression line.

*EM calibration data set (MATemp range: 1.1–10.2°C)*

This unscreened calibration data set comprises 3,013 chironomid fossils recovered from 35 lakes (91 ± 31 fossils per sample), of which 96.6% were identified to 23 taxa. None of the lakes were deleted as outliers, but three taxa with fewer than two occurrences were removed. The screened EM calibration data set thus contains 20 taxa together accounting for 99.8% of all identifiable fossils (Table 1). The correlation between DCA axis 1 sample scores and MATemp is highly

significant ( $r = 0.82$ ;  $P < 0.001$ ), but DCCA with MATemp as sole constraining variable does reveal a significant secondary gradient, (i.e. gradient length of axis 1 (2.4 SD) is smaller that of axis 2 (3.8 SD), and the  $\lambda_1/\lambda_2$  ratio is fairly low (1.148; Table 1).

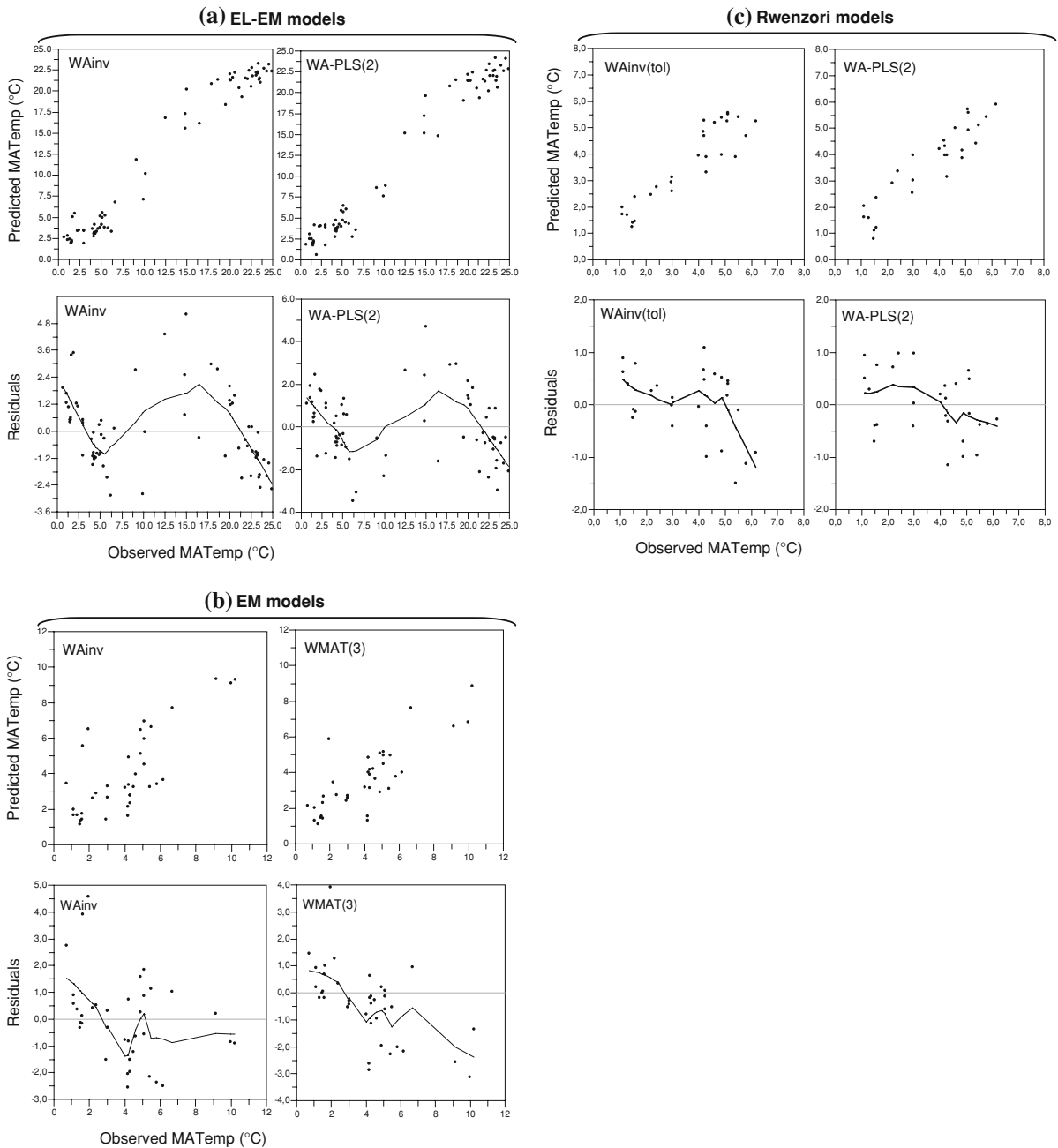
Of the 20 taxa included here, 11 (55.5%) display a HOF response type IV or V; six (30.0%) display a HOF response type IId or Ili, and three taxa (15%) display no statistically significant relationship (Table 1). The largest change in optima in this smaller dataset occurs for previously defined group 3 and 5 taxa such as *Procladius brevipetiolatus* (8.4 vs. 17.2°C), *Dicrotendipes septemmaculatus/near pilosimanus* (8.2 vs. 14.9°C) and Chironomini larvula type 2 (8.9 vs. 20.6°C; Table 1 and 2).

**Table 3** Summary statistics for chironomid-based MATemp-models and SWTemp models (in brackets) using the various data sets

Model type	$r^2_{\text{jack}}$		RMSEP			Mean bias <sub>jack</sub>			Max bias <sub>jack</sub>			RMSEP as % of gradient		
	EL-EM	EM	RWEN	EM	RWEN	EL-EM	EM	RWEN	EL-EM	EM	RWEN	EL-EM	EM	RWEN
Inverse														
WA	<b>0.97</b> ( <b>0.95</b> )	<b>0.63</b> (0.66)	0.76 (0.68)	<b>1.46</b> (2.32)	0.78 (1.63)	<b>-0.01</b> ( <b>-0.01</b> )	<b>0.07</b> (0.10)	-0.01 (-0.02)	<b>2.69</b> ( <b>3.21</b> )	<b>2.32</b> (5.02)	0.86 (3.89)	<b>6.8</b> ( <b>7.5</b> )	<b>16.0</b> (14.3)	15.3 (17.0)
WA <sub>tol</sub>	0.95 (0.92)	0.61 ( <b>0.69</b> )	<b>0.84</b> ( <b>0.76</b> )	<b>2.06</b> ( <b>2.59</b> )	<b>0.64</b> ( <b>1.40</b> )	-0.16 (-0.17)	0.01 ( <b>0.17</b> )	-0.03 ( <b>-0.08</b> )	4.31 (8.84)	3.34 ( <b>5.56</b> )	<b>1.02</b> ( <b>3.09</b> )	8.7 (9.8)	16.9 ( <b>13.8</b> )	<b>12.5</b> ( <b>14.6</b> )
Classic														
WA	0.97 (0.95)	0.65 (0.68)	0.77 (0.69)	1.63 (2.00)	0.84 (1.83)	-0.01 (-0.01)	0.09 (0.12)	-0.01 (-0.02)	2.82 (3.36)	2.42 (5.81)	0.69 (3.89)	6.8 (7.6)	16.3 (15.4)	16.5 (19.1)
WA <sub>tol</sub>	0.95 (0.92)	0.63 (0.70)	0.84 (0.77)	2.08 (2.67)	0.66 (1.49)	-0.16 (-0.18)	0.01 (0.21)	-0.03 (-0.08)	4.45 (9.12)	2.69 (5.07)	0.85 (2.97)	8.7 (10.2)	16.0 (14.2)	12.9 (15.5)
WA-PLS(1)	0.97 ( <b>0.95</b> )	0.61 ( <b>0.66</b> )	0.76 (0.68)	1.62 ( <b>1.97</b> )	0.78 (1.64)	-0.03 ( <b>-0.06</b> )	0.07 ( <b>0.03</b> )	-0.04 (-0.10)	2.70 ( <b>3.26</b> )	2.72 ( <b>5.25</b> )	0.86 (3.79)	6.8 ( <b>7.5</b> )	16.6 ( <b>14.6</b> )	15.3 (17.1)
WA-PLS(2)	<b>0.97</b> (0.95)	<b>0.68</b> (0.60)	<b>0.85</b> ( <b>0.77</b> )	<b>1.50</b> (2.12)	<b>0.61</b> ( <b>1.39</b> )	<b>-0.02</b> (-0.01)	<b>0.12</b> (0.22)	<b>0.01</b> ( <b>-0.05</b> )	<b>2.59</b> (2.81)	<b>2.92</b> (5.97)	<b>0.84</b> ( <b>2.37</b> )	<b>6.3</b> (8.1)	<b>15.2</b> (15.7)	<b>12.0</b> ( <b>8.0</b> )
MAT*	0.96 (0.95)	0.81 (0.80)	0.81 (0.75)	1.86 (2.02)	0.80 (1.52)	0.02 (0.35)	0.28 (0.54)	0.41 (0.51)	3.73 (2.18)	1.76 (3.87)	1.40 (4.10)	7.8 (7.7)	11.8 (11.4)	15.7 (15.8)
WMAT*	<b>0.96</b> ( <b>0.95</b> )	<b>0.81</b> ( <b>0.81</b> )	<b>0.82</b> ( <b>0.77</b> )	<b>1.76</b> ( <b>1.98</b> )	<b>0.78</b> ( <b>1.47</b> )	<b>0.08</b> ( <b>0.33</b> )	<b>0.23</b> ( <b>0.45</b> )	<b>0.39</b> ( <b>0.44</b> )	<b>3.02</b> ( <b>1.55</b> )	<b>1.53</b> ( <b>3.73</b> )	<b>1.39</b> ( <b>3.90</b> )	<b>7.4</b> ( <b>7.5</b> )	<b>11.6</b> ( <b>11.1</b> )	<b>15.3</b> ( <b>15.3</b> )

Numerical methods include weighted-averaging (WA) and tolerance-downweighted WA (WA<sub>tol</sub>) using inverse and classic deshrinking, partial least squares regression (PLS), weighted-averaging partial least squares regression (WA-PLS), and the Modern Analogue Technique (MAT). Listed are the coefficient of determination ( $r^2$ ) between predicted and observed values, the root-mean-squared error of prediction (RMSEP), mean and maximum bias, the percent RMSEP change after adding an additional partial-least-squares component, and the RMSEP as % of the gradient. All statistics are based on leave-one-out (jack-knifing) cross-validation. Model outputs in bold type indicate the best model for each numerical method

\* (W)MAT4 for EL-EM models; (W)MAT3 for EM- and Rwenzori models



**Fig. 4** Chironomid-based MATemp-inference models using the EL-EM data set (a), the EM data set (b), and the Rwenzori data set (c); all models use square-root transformed species percentage data. Illustrated are models based on WA, WA-PLS and/or WMAT, with scatter plots of predicted versus observed

MATemp (*top row*) and of the residuals (predicted minus observed value) versus observed MATemp (*bottom row*). Trends in the residuals are highlighted with a LOESS smoother (span = 0.45)

Compared to the EL-EM-based models, the  $r_{jack}^2$  of the EM models is lower (0.63–0.81 vs. 0.96–0.97), but the RMSEP and maximum bias are slightly reduced

(1.1–1.5°C and 1.5–2.9, respectively); yet RMSEP expressed as a percentage of the total gradient is higher (11.6–16.0% vs. 6.3–7.4%; Fig. 4b, Table 3). All

models have largely the same residual trends, with values below  $\sim 3.0^{\circ}\text{C}$  slightly overestimated and values above  $\sim 3.0^{\circ}\text{C}$  somewhat underestimated. WMAT(3) with  $r_{\text{jack}}^2 = 0.81$ , RMSEP =  $1.1^{\circ}\text{C}$ , and maximum bias =  $1.5^{\circ}\text{C}$  appears to be the best model.

*Rwenzori-only calibration (MATemp range: 1.1–6.2°C)*

This unscreened calibration data set comprises 2,113 chironomid fossils recovered from 29 lakes ( $77 \pm 13$  fossils per sample), of which 95.2% were identified to 23 taxa. Mahoma, the only Rwenzori lake located below 3,400 m a.s.l., was deleted as an outlier in the DCCA. This lake holds five taxa (making up 35.3% of total fossil abundance) not present in any of the other Rwenzori lakes. Seven taxa with fewer than two occurrences were removed, thus the screened Rwenzori-only calibration data set contains 11 taxa together accounting for 99.0% of all identifiable fossils (Table 1). The correlation between DCA axis 1 sample scores and MATemp is significant ( $r = 0.68$ ;  $P < 0.001$ ). Yet, DCCA with MATemp as sole constraining variable does point to an important secondary gradient, as evidenced by axis 2 being as long as axis 1 (both 1.6 SD), and a fairly small  $\lambda_1/\lambda_2$  ratio (1.373; Table 1).

Of the 11 taxa included here, seven (63.6%) display a HOF response type IV or V, three (27.3%) display a HOF response type II<sub>d</sub> or II<sub>i</sub>, and two taxa (18.1%) display no statistically significant relationship (Table 1, 2). Compared to the EM estimates, the largest shifts in optima seem to occur for group 3 taxa occurring both on Mount Kenya and in the Rwenzori but in different relative abundances, such as *Paratrichocladius* type Hausburg Tarn (5.7 vs.  $3.8^{\circ}\text{C}$ ) and *Paratrichocladius* type Simba Tarn (3.9 vs.  $2.4^{\circ}\text{C}$ ).

The  $r_{\text{jack}}^2$  of models based on Rwenzori-only calibration (0.82–0.85) is lower than that of EL-EM-based models, but higher than that of the EM-based models. The RMSEP and maximum bias are reduced to  $0.6\text{--}0.8^{\circ}\text{C}$  and  $0.8\text{--}1.4^{\circ}\text{C}$ , respectively, but are still higher than in EL-EM-based models when expressed as a percentage of the total gradient (Table 3). The trend in the residuals differs slightly among models: in WA and WA-PLS(2), values are generally overestimated at the lower end of the gradient ( $<5^{\circ}\text{C}$ ) and overestimated at the high end; in WMAT(3), all values are underestimated (Fig. 4c).

The best model seems to be the one obtained with WA-PLS(2):  $r_{\text{jack}}^2 = 0.85$ , RMSEP =  $0.6^{\circ}\text{C}$ , maximum bias =  $0.8^{\circ}\text{C}$ .

*Water-temperature inference models (SWTemp models)*

For these models we only discuss model performance itself, since the pattern of species distribution along the SWTemp gradient is broadly similar to that along the MATemp gradient (Table 2 in Supplementary material). The EL-EM calibration data set spans a SWTemp range between 1.8 and  $28.1^{\circ}\text{C}$ . EL-EM-based models using simple WA with inverse deshrinking, WA-PLS(1) or WMAT(4) yield comparable results with regard to  $r_{\text{jack}}^2$  and RMSEP (0.95 and  $2.0^{\circ}\text{C}$ , respectively), but maximum bias is smallest in the latter ( $1.6^{\circ}\text{C}$  vs.  $3.3^{\circ}\text{C}$ ; Table 3). The trend in the residuals is also fairly similar among all models, with values below  $5.0\text{--}7.0^{\circ}\text{C}$  being overestimated, and values of ca.  $7.0\text{--}13.0^{\circ}\text{C}$  generally being underestimated (Fig. 1a in Supplementary material). Overall, the statistical performance of the SWTemp models is poorer than in the MATemp models, (i.e. RMSEP and maximum bias are on average  $0.4^{\circ}\text{C}$  and  $\sim 0.6^{\circ}\text{C}$  higher, respectively).

The EM calibration data set spans a SWTemp range between 1.8 and  $18.0^{\circ}\text{C}$ . Here, WMAT(3) produces the best model ( $r_{\text{jack}}^2 = 0.81$ , RMSEP =  $1.8^{\circ}\text{C}$ , maximum bias =  $3.7^{\circ}\text{C}$ ). The EM-based SWTemp models generally produce poorer error statistics compared to those based on the EL-EM data set, and they are also less powerful compared to the EM-based MATemp models (Table 2; Fig. 1b in Supplementary material). The overall trend in the residuals is fairly similar to that of the EM-based MATemp models.

The Rwenzori-only calibration data set spans a SWTemp range between 1.8 and  $11.4^{\circ}\text{C}$ . The best model is the one obtained with WA-PLS(2) ( $r_{\text{jack}}^2 = 0.77$ , RMSEP =  $1.4^{\circ}\text{C}$ , maximum bias =  $2.4^{\circ}\text{C}$ ). The Rwenzori-only SWTemp models generally produce poorer error statistics compared to those based on the EL-EM data set ( $r_{\text{jack}}^2 = 0.95$ , RMSEP =  $2.0^{\circ}\text{C}$ , maximum bias =  $1.6\text{--}3.3^{\circ}\text{C}$ ), and they are also less powerful than Rwenzori-only MATemp models ( $r_{\text{jack}}^2 = 0.82\text{--}0.85$ , RMSEP =  $0.6\text{--}0.8^{\circ}\text{C}$ , maximum bias =  $0.8\text{--}1.4^{\circ}\text{C}$ ; Table 3 and Fig. 1c in Supplementary material).



Secondary environmental gradients

CCAs of the EL-EM data set constrained to one variable at a time showed that besides MATemp and SWTemp, which explained 18.2 and 18.5% of the variance, respectively, all three other variables tested [log(water depth), pH, and log(conductivity)] contributed a significant part of the faunal variation (5.8%,  $P < 0.01$ ; 12.4%,  $P < 0.01$ ; 13.6%,  $P < 0.01$ , respectively; Table 4 in Supplementary material). When the effect of the other variables is partialled out, MATemp, SWTemp, log(depth) and pH seem to make an unique, independent contribution to the variance (5.0, 4.5, 3.9 and 3.5%, respectively) that is also significant ( $P < 0.05$ ).

CCAs of the Rwenzori dataset showed that seven variables (altitude, MATemp, SWTemp, DOC, TN, log TP, and log Chl $a$ ) each contributed a significant part of the faunal variation in the data set (Table 5 in Supplementary material), but altitude was removed from further analyses due to a VIF >20. Given missing dissolved oxygen data for Rwenzori pools, SWO $_2$  and BWO $_2$  could only be tested for the Rwenzori lakes; CCAs with these environmental variables and the Rwenzori lake data set did not reveal any significant relationship. When the effects of DOC, TN, log TP and LOI were partialled out, MATemp and SWTemp still explained a significant ( $P < 0.05$ ) amount of the total variance in the data set (10.9 and 10.7%, respectively). This was not the case for the other variables, (i.e. unique independent contributions were not significant; Table 4).

The eigenvalues of the first two CCA axes restricted to the six significant variables were 0.272 and 0.138, respectively; and they captured 22.9 and 11.5% of the variance in the data set (Table 6 in Supplementary material). Correlation coefficients indicate that CCA axis 1 is most strongly correlated with MATemp, SWTemp, %LOI and DOC, and that CCA axis 2 is mainly correlated with logTP. The CCA bi-plots (Fig. 5a–b) show that *Diamesa* type East Africa, *Paratrichocladius* type Simba Tarn, *Limnophyes minimus* group and *Chaetocladus melaleucus* typify the relatively colder clear-water lakes and high-elevation pools (plotted in or close to the right quadrants), whereas the other taxa prevail in relatively warmer waters, stained by DOC (plotted in

**Table 4** Summary of the partial CCAs based on the Rwenzori data set

Variable	Covariable(s)	%variance	
MATemp	None	21.8***	
	SWTemp	6.1	
	DOC	10.6*	
	TN	14.9***	
	log(TP)	20.7***	
	LOI	19.6***	
	DOC,TN, log(TP), %LOI	10.9*	
	SWTemp, DOC, log(TP), TN, LOI	3.7	
	SWTemp	None	20.1***
		MATemp	4.3
DOC		10.2*	
TN		12.4*	
log(TP)		18.1***	
LOI		9.7*	
DOC,TN, log(TP), %LOI		10.7*	
MATemp, DOC, log(TP), TN, LOI		3.4	
DOC	None	16.1***	
	MATemp	5.2	
	SWTemp	5.6	
	TN	10.7***	
	TP	10.1***	
	LOI	4.0	
	All	7.1	
	TP	None	12.7*
		MATemp	11.4*
SWTemp		10.3*	
TN		7.4	
DOC		12.9*	
LOI		10.2*	
TN	All	6.5	
	None	12.3***	
	MATemp	4.6	
	SWTemp	3.7	
	TP	7.0	
	DOC	6.7	
	LOI	5.3	
LOI	All	0.9	
	None	17.6***	
	MATemp	6.0	
	SWTemp	6.7	
	TN	11.0*	
	TP	15.3***	

**Table 4** continued

Variable	Covariate(s)	%variance
	DOC	6.9
	All	7.1

The significance is based on 999 unrestricted Monte Carlo permutations ( $P < 0.05^*$ ,  $P < 0.01^{**}$ ,  $P < 0.001^{***}$ )

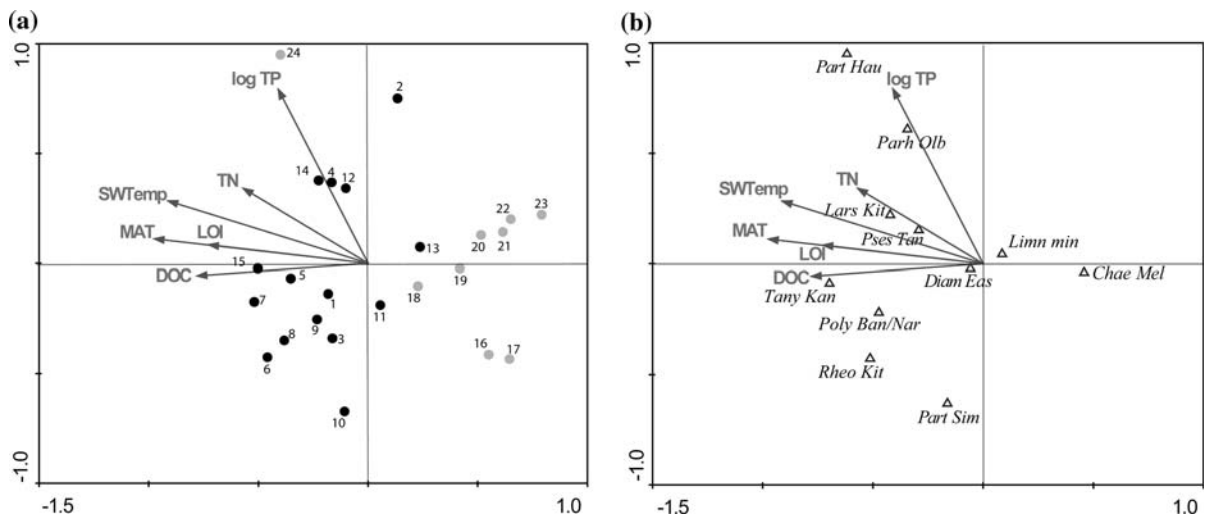
the left quadrants). Moreover, taxa such as near *Paraphaenocladus* type Ol Bolossat and *Pseudosmittia* type Tanganyika (plotted in the left upper quadrant) characterise waters relatively rich in nutrients, whereas taxa such as *Polypedilum* type Banda/Narasha and *Rheotanytarsus* type Kitandara are more typical for nutrient-poor waters.

## Discussion

### Distribution of East African chironomids in relation to temperature

Temperature is known to exert strong control on many aspects of chironomid biology and ecology, including rate and success of egg hatching, larval growth and feeding rates, pupation and emergence, and flight and swarming behaviour of the adults

(Brooks 2006). Evidently, chironomid species turnover along latitudinal and altitudinal gradients is a worldwide phenomenon, but it has never before received proper attention in tropical regions. The five ecological categories distinguished in the present East African data set largely coincide with those described by Eggermont and Verschuren (2007), but the greater number of lakes and pools above 2,900 m a.s.l. in the present study (35 vs. 11) allows delineation of warm stenothermal taxa (Group 1; 20 taxa) and warm eurythermal taxa (Group 2; 24 taxa). Group 3 taxa, on the other hand, must be cold stenothermal species adapted to the harsh climatic conditions prevailing above 3,000 m elevation: substantial daily temperature variation, night-time freezing and mean annual temperatures below 10°C. With the recovery of one new taxon (*Tanytarsini* indet. type Kanganyika) and separation of *Chironomus* near *imicola* from *Chironomus* type Mount Kenya (see earlier), group 3 now includes ten taxa, or 50% of the total species richness in East African high-mountain waters (>2,900 m a.s.l.). The disjunct distribution of the two morphotaxa belonging to group 4 (*Pentaneurini* larvula and *Paramerina* cf. *ababae*) suggests that its lowland and high-mountain populations actually represent different species, which at present cannot be reliably separated. Similarly, all group 5



**Fig. 5** Sample biplot (a) and species biplot (b) of the CCA based on the Rwenzori data set and the six significant variables. *Black circles*: Lake samples (1 = Batoda, 2 = Kopello, 3 = Bigata, 4 = Africa, 5 = Kanganyika, 6 = Katunda, 7 = Lower Kachope, 8 = Middle Kachope, 9 = Upper Kachope, 10 = Upper Kitandara, 11 = Lower Kitandara, 12 = Bujuku,

13 = Lac du Speke, 14 = East Bukurungu, 15 = Nsuranja); *grey circles*: Pool samples (16 = Irene, 17 = Balengekania, 18 = Zaphania, 19 = Ruhandika, 20 = Tuna Noodle, 21 = Mbahimba, 22 = Kamsongi, 23 = Muhesi, 24 = Mutinda). Abbreviations for species follow Table 3

taxa (*Limnophyes minimus* group, *Pseudosmittia* type Tanganyika, near *Paraphaenocladus* type Ol Bolossat, *Polypedilum* type Bandasa/Narasha, *Chironomus* type Kibos, *Dicrotendipes septemmaculatus/pilosimanus*, Chironomini larvula type 1 and 2) likely include more than one biological species (Eggermont and Verschuren 2007). Overall, the major faunal transition occurs at approximately 1,500–1,800 m, the lower limit of the montane forest zone, and most importantly, at approximately 3,000 m, the lower limit of the Ericaceous zone. These patterns are consistent with other studies identifying the tree-line as an important ecological boundary controlling chironomid distribution (United States: Porinchi and Cwynar 2000; New Zealand: Woodward and Shulmeister 2006; Swiss Alps: Lotter et al. 1997; Heegaard et al. 2006).

Afro-tropical warm stenothermal and warm eurythermal taxa (groups 1 and 2) are dominated by representatives of the Chironomini (25 out of 45 taxa), whereas cold stenothermal taxa are dominated by highly sclerotized forms of Diamesinae, Orthocladiinae and Tanytarsini (9 out of 10 taxa). This chironomid-temperature relationship at subfamily and tribe level largely parallels that in the Holarctic region, visible along both elevational (Walker and Mathewes 1989; Lotter et al. 1997; Bigler et al. 2006) and latitudinal gradients (Walker et al. 2003; Larocque et al. 2006), strongly supporting the notion that this relationship may be apparent worldwide (Lindegaard 1995). However, whereas cold-water chironomid communities of boreal and alpine lakes in Nearctic and Palaearctic regions also show striking similarities at the genus level (Thienemann 1954), the genus composition in Afro-alpine lakes is distinctly different. For example, genera such as *Pseudodiamesa*, *Heterotrissocladius*, *Microspectra*, and *Corynocera* appear to be absent in tropical Africa. The Afro-alpine fauna is also very different from its Tasmanian (with high numbers of *Telmatopelopia*, *Parakiefferella*, *Paralimnophyes* and *Cladotanytarsus*; Rees et al. 2008) and New Zealand counterparts (typified by *Chironomus*, *Tanytarsus* and *Macropelopiini*; Woodward and Shulmeister 2006; Dieffenbacher-Krall et al. 2007).

Discussing the uniqueness of the Afro-alpine chironomid fauna in relation to lowland African lakes and alpine lakes in temperate regions, Eggermont and Verschuren (2007) concluded that

chironomid communities in glacier-fed lakes on Africa's highest mountains are potentially unique on a continental scale. That study also revealed that only a small fraction of the cold-stenothermous taxa was shared between Mount Kenya and Rwenzori, but given that only about 15% of potential habitat on the two mountains was sampled at that time, they could not conclusively address the degree of local endemism. Having sampled virtually all lakes and permanent pools on the Ugandan side of Rwenzori for this study, our results now point to a substantial degree of local endemism. For example, Tanytarsini indet. type Hall Tarn, Tanytarsini indet. type Ellis and *Chironomus* type Mount Kenya appear to be restricted to Mount Kenya. Conversely, *Larsia* type Kitandara, Tanytarsini indet. type Kanganyika and *Rheotanytarsus* type Kitandara have so far only been recorded on Rwenzori. Afro-tropical *Diamesa* is also known to show species differentiation between mountains (*D. kenyae* and *D. freemani* on Mount Kenya, *D. rwenzorensis* in the Rwenzori; Willassen and Cranston 1986). Indeed, unambiguous fossil *D. rwenzorensis* have so far been recorded only in Rwenzori (Eggermont and Verschuren 2007), whereas instantly recognizable fossil *D. kenyae* have so far been found only on Mount Kenya (Hallaert 2005). Moreover, the distribution of near *Paraphaenocladus* type Ol Bolossat is atypical in that it occurs in both the Rwenzori and the lowlands of Kenya and Uganda, while being absent from high-elevation sites on Mount Kenya (Hallaert 2005). Other aspects of differentiation between the two mountains include *Paratrachocladus* type Simba Tarn, *P.* type Hausburg Tarn, Tanytarsini indet. type Hall Tarn and *Chironomus* type Mount Kenya prevailing in high-elevation sites on Mount Kenya, whereas sites at similar elevation in the Rwenzori are dominated by *Chaetocladus melaleucus*, *Limnophyes minimus* group and/or *Polypedilum* type Bandasa/Narasha.

Performance and applicability of African chironomid-based temperature inference models

#### *General performance and model selection*

Over the past 10 years, a substantial number of regional and trans-continental chironomid-based transfer functions have been created for reconstruction of air and surface water temperatures. Judged by statistical performance parameters, the WA, WA-PLS

and WMAT models produced in this study compare favourably with these earlier efforts. If models are compared based on the RMSEP relative to the total sampled temperature range, our EL-EM data set has even the best error statistics produced so far, and models using the Rwenzori-only calibration data set also rank among the best.

Still, some features in the models deserve special attention. First, EL-EM residual plots (Fig. 4a; and Fig. 1a in Supplementary material) show four ‘edge effects’ instead of the usual two, namely both at the gradient ends and at the edges of the gap in the middle of the temperature range (between  $\sim 6.0$  and  $18.0^{\circ}\text{C}$  in the MATemp models, and between  $10.0$ – $20.0^{\circ}\text{C}$  in the SWTemp models). Edge effects are inherent to WA-based methods because there is always a tendency to pull predicted values towards the mean of the training set, inevitably leading to overestimation of low and underestimation of high observed temperatures (ter Braak and Juggins 1993). The uncommon pattern in the residuals here is likely due to scarcity of lakes at intermediate elevation, between 2,000 and 3,900 m a.s.l. WA-PLS uses the residual structure in the biological data not only to improve species estimates, but also to reduce as far as possible any bias in the results. In our case, this technique may simply correct for the numerical artifact, and thus not really produce a better model in terms of paleoenvironmental inferences (Eggermont et al. 2006).

Other factors may also be responsible for under- and over- estimation in various parts of the EL-EM temperature gradient. First, presence of apparently eurythermic taxa, such as the group 4 and 5 taxa defined earlier, may contribute to this effect. For example, the temperature of Hausburg Tarn (at the low end of the gradient) is substantially overestimated likely because *Chironomus* type Kibos and Chironomini larvula type 1 make up a considerable part ( $\sim 17.0\%$ ) of its chironomid fauna. Similarly, Ol Bolossat (at the right-side edge of the gap) is considerably overestimated possibly because *Limnophyes minimus* group and near *Paraphaenocladius* type Ol Bolossat constitute  $\sim 18.0\%$  of the fauna. Essentially, EL-EM estimates will be less reliable in lowland situations where the proportion of the latter is high because their optima are biased towards lower values due to higher occurrence in mountain lakes). In mountain sites with substantial numbers of

*Chironomus* type Kibos and Chironomini larvula type 1, their optimum is biased towards higher values due to higher occurrence in lowland lakes. A similar problem has been noted in the New Zealand calibration dataset of Woodward and Shulmeister (2006). They found *Chironomus* to be well-represented both in high-altitude, cool oligotrophic lakes and low-altitude, warm, eutrophic lakes. As such, this taxon was found to have an intermediate optimum, overestimating cool temperatures in the high-elevation sites and underestimating warm temperatures of lowland lakes. Secondly, one could expect that inferences for lakes directly receiving glacial melt-water will be consistently lower than observed air temperature (Brooks 2006). The effect of this is not readily visible in our data set, at least not for the glacial lakes on Mount Kenya whose values are always overestimated, not underestimated. Finally, several early authors (Hann et al. 1992; Warner and Hann 1987) attributed poor estimation of temperature to the confounding influence of other environmental variables (water depth, trophic conditions, substrate composition and organic content), discussed further below.

Only a few studies have evaluated the consequences on model performance when gradients are shortened or extended (Lotter et al. 1997; Walker et al. 1997; Porinchu et al. 2007). In principle, larger training sets may allow better estimates of species’ optima and tolerances in relation to important environmental variables and may also increase the chance of finding good modern analogues. Yet, extending a gradient also increases geographical and environmental heterogeneity so secondary gradients may become more important, and it also introduces the problem of multiple analogue scenarios (Lotter et al. 1997). It thus appears that there may be a critical size for a calibration data set after which model improvement due to better estimates of taxon optima is outweighed by the inaccuracies introduced by heterogeneity in the data. We previously noted biogeographical problems associated with group 4 and 5 taxa, and differences between Mount Kenya and Rwenzori, which in this study we attempted to correct by limiting the calibration data set to a smaller region. Walker et al. (1997) noted that by reducing the gradient length in this way, apparent error statistics are improved but the real errors remain. Indeed, although RMSEP of all models is smaller

when confining the dataset to mountain lakes, RMSEP expressed as a percentage of the gradient is larger than in EL-EM models. The question therefore arises which models are most appropriate for reconstruction of past temperature in East Africa. In our opinion, model selection should depend on the particular lake site, the time window of interest, and the expected range and amplitude of temperature change. For example, EL-EM models may prove most valuable for reconstructions in lakes located near the 3,000 m ecotone, both on short (recent warming) and long time scales (Holocene and earlier). In such lakes, one could expect distinct shifts between typical highland and lowland faunas (Eggermont and Verschuren 2007). In high-elevation Rwenzori sites (>3,800 m) then again, Rwenzori-only models may produce the most reliable results, especially when focusing on small-amplitude temperature changes. The applicability of EM-based models is more questionable, as long as the issue of endemicity has not been satisfactorily resolved. Still, in the absence of a temperature model based on an extensive set of Mount Kenya lakes, the EM models may provide a reasonable alternative for reconstructions in high-elevation sites on Mount Kenya.

#### *MATemp vs. SWTemp models*

All studies where both surface-water and air-temperature inference models have been developed show that the statistical relationship between the composition of modern chironomid assemblages and air temperature is as strong as or stronger than for surface-water temperatures. Various papers have discussed both ecological and statistical reasons for the generally poorer performance of surface-water temperature models (Brooks 2006; Walker and Cwynar 2006), and they are consistent with findings in this study. Whereas water temperature only influences the immature stages, air temperature impinges directly on both the adult and larval stage because of the relationship which generally holds between air and water temperature (Livingstone et al. 1999). Furthermore, air temperature data are often available at much higher temporal resolution and quality and can be interpolated both horizontally and vertically. In contrast, single surface-water measurements are prone to large and unpredictable random variation within and between days/months. Although repeat measurements

in a subset of our lakes indicate muted seasonality (Table 1 in Supplementary material), incorporating more accurate estimates of surface water temperatures, possibly based on annual time series, could possibly improve model performance.

Warner and Hann (1987) and Hann et al. (1992) suggested that the littoral, sublittoral and profundal components of a fossil chironomid fauna must be evaluated independently when making inferences about past temperatures, as they are differently buffered from temperature change. More specifically, they argued that midge larvae are benthic organisms and therefore only those living in very shallow water will be exposed to surface-water temperatures, whereas deepwater chironomids in stratified lakes are isolated from the direct effects of changing air temperature. Evaluation of this concern in our data set requires analysis of thermal profiles. Temperature differences between surface and bottom waters are on average 2.0°C for lowland lakes and 1.3°C for mountain lakes (Table 1 in Supplementary material). Most of the latter are continuously or discontinuously polymictic (*sensu* Eggermont et al. 2007b). Deep (>15 m) lowland lakes in the EL-EM calibration data set that are permanently stratified (Bugwagi, Challa, Ekikoto, Kamweru, Kyaninga, Mursi, Nkugute and Wankenzi; D. Verschuren et al. unpublished data) have a fully anoxic hypolimnion below ~10 m water depth, and a temperature gradient within the epilimnion of no more than ~2.0°C. Hence, the profundal zone in these lakes is likely devoid of chironomids, even of anoxia-tolerant taxa such as *Chironomus* (Eggermont and Verschuren 2004b). In summary, most taxa encountered in our data set are truly exposed to ambient temperatures close to those at the water surface, and this accounts at least partially for the fairly good performance of the SWTemp models.

#### Environmental control on African chironomid distribution

##### *Chironomid distributions in the EL-EM data set*

Ordination of the EL-EM data set revealed that temperature (MATemp, SWTemp), water depth, conductivity (salinity) and pH each explained a significant part of the variation in chironomid assemblages; we suspect DOC and nutrients to also play a role but, as said, limited availability of such



data did not allow us to evaluate this. In other studies, water depth has often been found to have a statistically significant influence on chironomid faunal composition (Bigler et al. 2006; Eggermont et al. 2007a). Walker et al. (1991) argued that in boreal and temperate lakes much of this relationship could be due to the influence of lake volume on surface-water temperature with the deepest lakes usually being the coldest. Also, in these regions, deep lakes will generally be stratified in summer and cool temperatures will be maintained in the hypolimnion during the entire annual cycle. This provides a habitat for cold-stenothermous chironomids that is not available in shallower lakes. The coupling between water temperature and lake depth is obviously complex and different between high- and low-latitude lakes. In our tropical data set, the deepest lakes are the warmest ( $r = -0.42$ ;  $P < 0.001$ ), but this likely has a purely geological explanation. The cold mountain lakes formed by glacier activity are generally shallower than the warm lowland lakes which are maar craters or of tectonic origin. Yet, since the effect is still significant when partialling out the effect of temperature, we suggest that basin-morphometric influence on oxygen and habitat availability is also an important mechanism underlying the chironomid-depth relationship in our dataset.

The significant effect of conductivity in our dataset is at least partially due to the fact that coldwater lakes at high elevation are generally more dilute than the warm lowland lakes (elevation-conductivity:  $r = -0.46$ ,  $P < 0.001$ ; temperature-conductivity:  $r = 0.49$ ,  $P < 0.001$ ). This is confirmed by the observation that, when partialling out the effect of temperature, conductivity explains a considerably lower proportion of the faunal variance. Indeed, influence of salinity on chironomid distribution in the freshwater range ( $<500 \mu\text{m}$ ) is known to be fairly modest (Eggermont et al. 2006).

Various surveys have shown that pH is a significant variable in explaining chironomid distribution. The pH directly affects the physiology of aquatic organisms by influencing ionic balance and enzyme function (Jernelöv et al. 1981), and comes into effect mostly when it is near or below 5 (Olander et al. 1997). In many datasets (Lotter et al. 1997; Porinchu et al. 2007), pH does not seem to have a significant effect on chironomid distribution, likely because the sampled pH gradient was too small. In our data set, pH varied

between 4.30 (Lake Nsuranja) and 9.75 (Lake Kibengo), with the most acidic lakes confined to Rwenzori. Here, acidity is primarily due to the presence of humic substances leached from organic top soil and bogs (Eggermont et al. 2007b). Indeed, pH appears to be strongly correlated with altitude ( $r = -0.76$ ,  $P < 0.001$ ) and thus temperature ( $r = 0.77$ ,  $P < 0.001$ ). Although pH explains a considerably lower portion of the variance in the data set when temperature is partialled out, its unique independent contribution is still significant (Table 4 in Supplementary material).

#### *Chironomid distribution in the Rwenzori data set*

Rwenzori lakes can largely be separated in two categories (Eggermont et al. 2007b): (1) lakes located near or above 4,000 m, with at least some direct input of glacial melt-water and surrounded by rocky catchments or alpine vegetation; and (2) lakes located mostly below 4,000 m, remote from glaciers and surrounded by Ericaceous vegetation and/or bogs. The former group are mildly acidic to neutral clear-water lakes with often above-average dissolved-ion concentrations. They are (ultra-) oligotrophic to mesotrophic and phosphorus-limited. The latter group are mildly to strongly acidic waters stained by dissolved organic carbon, and thus show more modest transparency. Given this fairly distinct division, the question arises whether these differences in physical and chemical parameters among Rwenzori lakes are also reflected in the chironomid species composition. Unfortunately, the Rwenzori dataset contains significant correlations between elevation (temperature) and catchment-related physico-chemical parameters (Eggermont et al. 2007b) making it difficult to disentangle their individual effects.

Ordination results show that chironomid distribution in Rwenzori lakes is mainly structured by temperature (MATemp, SWTemp), DOC, LOI of the surface sediments, and nutrients (defined as TN, TP). In several other studies, in the arctic zone (Gajewski et al. 2005; Larocque et al. 2006) as well as mountain areas (Lotter et al. 1997; Bigler et al. 2006), DOC has been reported as a major environmental variable influencing chironomid assemblage's composition. In the Rwenzori study sites, DOC derives mainly from allochthonous sources (Eggermont et al. 2007b). Allochthonous organic matter constitutes a vital food source for littoral food webs, especially in oligotrophic



lakes (Vinebrook and Leavitt 1998). Bacterial production in lakes, and hence the quality of detritus as food source for insects, also depends on the inputs of allochthonous humic material (Bergström and Jansson 2000). Additionally, DOC influences a number of other key parameters for lake ecosystems such as light penetration (Vincent and Roy 1993), thermal stratification (Scully 1998) and lake pH (Wetzel 2001). The control of DOC on Rwenzori chironomid distribution is thus understandable. However, in the ordination plot (Fig. 5), DOC and temperature were represented by vectors that pointed in the same direction, indicating that separation of DOC and temperature effects on chironomid distribution might be difficult.

Similar to other surveys (Bigler et al. 2006; Porinchu et al. 2007), sedimentary organic matter content (LOI) was also found to be an important structuring variable. Ecologically, this is due to species-specific feeding habits and variable abilities of different chironomid species to burrow into soft-organic or stiff-minerogenic sediments (Danks 1971), among other factors. In the current Rwenzori dataset, LOI is positively correlated with temperature ( $r = 0.76$ ;  $P < 0.001$ ) in a way that the lakes with most minerogenic sediments are also the coldest. Close coupling of these variables is obviously related to the decrease in productivity of colder lakes and the low input of organic matter from their catchments.

Although the nutrient gradient in our Rwenzori dataset was fairly small, both TN and TP explained a significant portion of the variance in the chironomid assemblages, similar to other training sets (Heiri and Lotter 2005). Again, as revealed by variance partitioning (Table 4), there seems to be close coupling with other variables such as temperature, DOC and LOI.

### Future developments

Notwithstanding promising error statistics, our current temperature inference models based on East African Chironomidae suffer from (1) underrepresentation in the calibration data set of sites at elevations between 2,000 and 2,900 m a.s.l., (2) presence of morphologically similar but ecologically distinct taxa in different parts of the temperature gradient, and (3) the confounding influence of variables other than MATemp (or SWTemp) on the relationship between chironomid distribution and

temperature. Sites within this elevation range in East Africa that could be sampled to resolve the first problem are located in the Mount Elgon and Virunga region (Uganda-Rwanda). The second problem is more difficult to tackle because many morphological features possibly allowing separation between closely related species, such as antenna, labrum, etc., are often poorly preserved on fossil specimens. Integration of morphological, ecological and karyotypic studies on the various life stages would allow discriminating individual species within higher taxonomic units such as *Limnophyes minimus* (Epler) group and *Dicrotendipes septemmaculatus/pilosimanus*. Work should then focus on exploring previously unstudied head capsule features that are preserved in fossil material, to identify different species in the fossil record. The third problem is inherent to the use of biological proxies, but collection of a more extensive suite of uniform environmental data would certainly be a major step toward better understanding secondary gradients affecting chironomids in tropical Africa. Other possible improvements to our dataset include more accurate measurements of surface-water temperatures. Finally, inclusion of more Mount Kenya sites may elucidate some biogeographical issues, and lead to more accurate paleotemperature reconstructions in this mountain region.

**Acknowledgments** This research was sponsored by the Fund for Scientific Research (Flanders—Belgium; project G0528.07), the Institute for the Advancement of Scientific-Technological Research in Industry (Flanders-Belgium), the US National Science Foundation (grant 7999-06), the Salomon fund (Brown University), the Leopold-III Fund for Nature Exploration and Conservation (Belgium) and the Stichting ter Bevordering van het Wetenschappelijk Onderzoek in Afrika. Fieldwork was conducted under Uganda NCST research clearances EC540 and NS21, Uganda Wildlife Authority permits UWA/TBDP/RES/50, and the Kenya government research permit MOES/001/11C. We thank all people involved in the fieldwork, and Uganda Wildlife Authority and Kenya Wildlife Service for permission to sample lakes in national parks. A. Street-Perrott is thanked for providing additional surface-sediment samples. H.E. is postdoctoral fellow with the Fund of Scientific Research (Flanders-Belgium).

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