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RESEARCH ARTICLE

Epiphytic diatoms on herbarium material from the Central Forest phytogeographic region of the Democratic Republic of the Congo

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Background and aims – Epiphytic diatoms are excellent bio-indicators of the present and past ecological condition of aquatic ecosystems. In order to reconstruct the diatom history and to evaluate its diversity in the Democratic Republic of the Congo, epiphytic diatoms were sampled from herbarium specimens of aquatic plants deposited at the National Herbarium of the Congo at Yangambi (YBI) and at the herbarium of Meise Botanic Garden (BR).
Material and methods – In YBI, nine specimens belonging to the Nymphaeaceae, three to the Ceratophyllaceae, and 12 to the Lentibulariaceae collected in the Central Forest phytogeographic region were sampled for diatom investigation. In addition, nine *Nymphaea lotus* specimens were sampled in BR. Semi-quantitative analyses were performed by light microscopy on permanent diatom slides.
Key results – Analyses of the epiphytic diatom communities on YBI and BR materials showed a large diversity of 132 species belonging to 44 genera. Taxa belonging to the genus *Eunotia* were relatively the most abundant in all studied samples followed by *Frustulia saxonica* and a *Desmogonium* sp. The diatom communities on *Nymphaea* were as varied as on *Ceratophyllum*, while on *Utricularia*, a significant lower diversity was observed. The Trophic Diatom Index (TDI) and Generic Diatom Index (GDI) showed that the water quality in the Central Forest phytogeographic region was overall good during the 20th century. They point to oligotrophic conditions for the running waters with a slight increase towards more mesotrophic conditions from the 1950s onwards. The only sample in the present study indicating mesotrophic condition was from a swamp.
Conclusions – The results on the epiphytic diatoms present on herbarium material can serve as a basis for sustainable management of aquatic ecosystems in D.R. Congo. In absence of an in-depth knowledge of the species and their ecological preferences, a genus-based TDI and IDG have proven to be valuable tools for water quality monitoring in tropical Africa.

Keywords – Aquatic plants; Bacillariophyta; Congo Basin; diatoms; D.R. Congo; herbarium; water quality; Yangambi.

INTRODUCTION

First reports on diatoms from the Democratic Republic of the Congo (D.R. Congo) date back to the end of the 1930s (Zanon 1938). Since the second half of the 20th century, some diatom investigations have been carried out but these studies remain at the preliminary inventory step (Taylor & Cocquyt 2016; Cocquyt et al. 2019). Moreover, samples from D.R. Congo for diatom investigation were rarely collected making historic materials scarce. However, this can be compensated, at least partly for epiphytic diatoms, thanks to herbarium specimens of aquatic plants. Diatoms growing on the submerged parts of the aquatic plants are dried together

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with the plants. The herbarium specimens, stored in museum and botanic garden collections, may still contain epiphytic diatoms, provided that they were not thoroughly rinsed beforehand. This hidden diversity on herbarium specimens is a real goldmine for diatomists.

Studies on epiphytic diatoms from herbarium material are rather rare (e.g. Denys 2003, 2007; Vogel et al. 2005). For tropical Africa, only one article can be reported (i.e. Cocquyt & De Wever 2002) that discusses diatoms sampled on herbarium material of *Nymphaea caerulea* Savigny, *Potamogeton pectinatus* L., *P. schweinfurthii* A.Benn., *Najas horrida* A.Braun ex Magnus, and *Cyperus laevigatus* L. collected in Lakes Naivasha and Sonachi in Kenya. However, the available number of herbarium specimens was too small to provide additional information on the history of Lake Naivasha during the past century.

Diatoms, characterized by their siliceous cell wall, can provide a lot of information on water quality (Baars 1983; Bellinger & Sigee 2010; Dalu et al. 2016) and are excellent bio-indicators for the health of aquatic ecosystems (Ndiritu et al. 2006; Suroy 2013; Tremblay 2015; Lavoie et al. 2018). Moreover, they can give information on the history

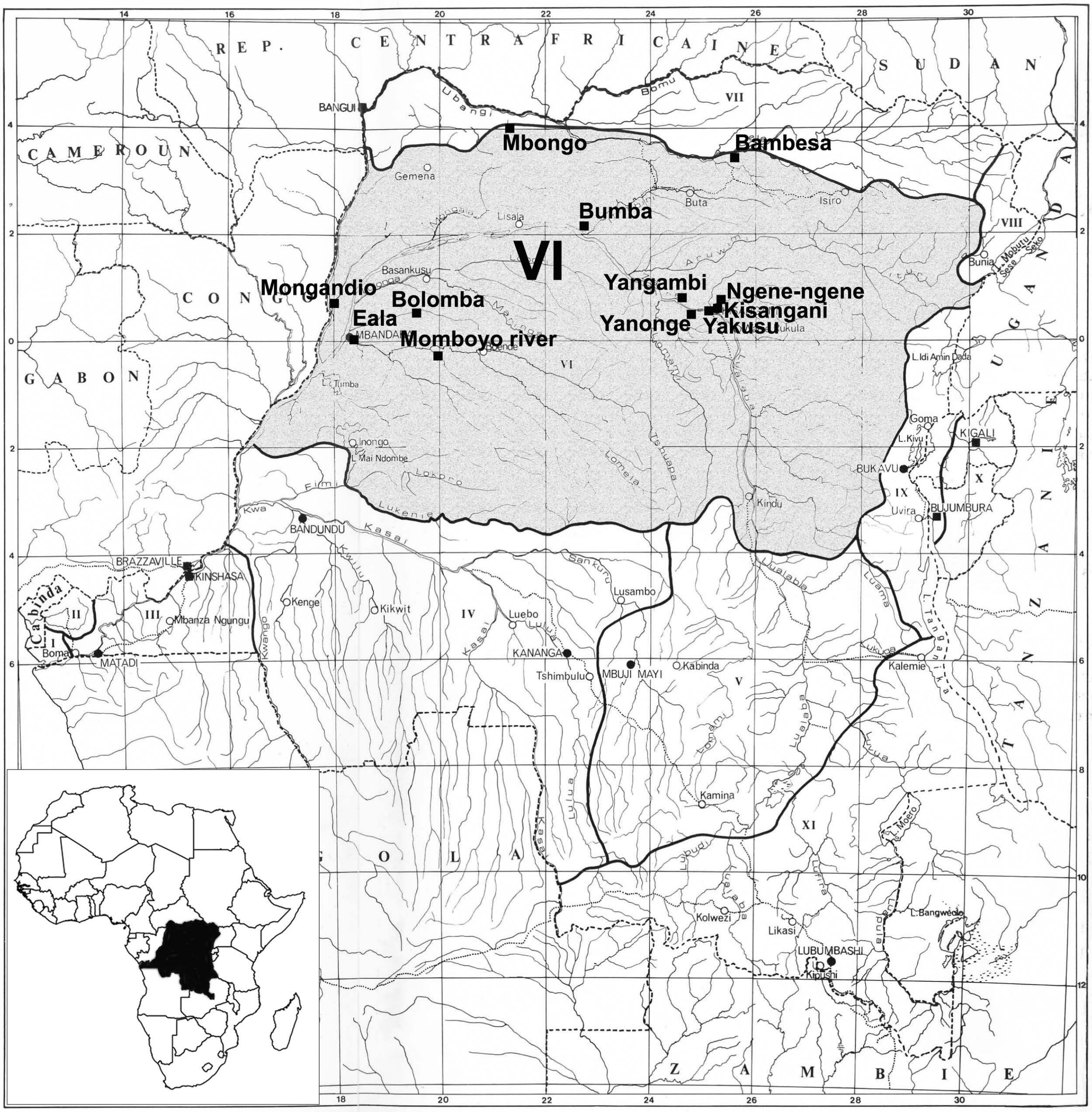

Figure 1 – Map of D.R. Congo with indication of the Central Forest phytogeographic region (VI) (after Bamps 1982), coloured light grey, and the collecting localities of the macrophytes present in the herbaria of YBI and BR. Inset: map of Africa with of D.R. Congo in black.

of an aquatic ecosystem (Round et al. 1990; Kucki 2009), including changes in physical and chemical composition of the water (Golama 1996; Sawaiker & Rodrigues 2017) through which climate changes can be studied.

In D.R. Congo, and particularly in the Kisangani and Yangambi area, diatoms and water quality assessment studies are fragmentary. In the 1990s, a small number of studies on algae from streams, rivers, and ponds was conducted in the Kisangani (Symoens & van der Werff 1993; Golama 1996; Compère 1998) and Yangambi area (Symoens & van der Werff 1996). However, research on diatoms did not get much attention in D.R. Congo despite the urgent need for sustainable management of biodiversity and aquatic ecosystem health. Fortunately, a change has been observed in recent years, to which the Boyekoli Ebale Congo 2010 expedition has undoubtedly played an important role (Cocquyt et al. 2019).

To demonstrate the importance of diatom research in the Congo Basin, this study was conducted, covering the Central Forest phytosociological region (number VI, Forestier central, fig. 1) according to Bamps (1982) and in particular the Yangambi region. Bamps (1982) defined the Central Forest region based on an earlier work by Robyns (1948), which was the first attempt to classify the Congo based on plant communities.

In this paper, the epiphytic diatom communities of the Central Forest region during the 20th century, since the first collection in 1907, are investigated. Species richness and diversity through time as well as water quality are discussed. It is investigated whether a relationship can be demonstrated between the observed changes in the diatom communities and changes in ecological conditions of the sampling sites.

MATERIAL AND METHODS

Diatoms were sampled from herbarium material of aquatic plants present in the National Herbarium of the Congo at Yangambi (YBI), housed at the "Institut national pour l'Étude et la Recherche Agronomique (INERA), Centre de Recherche de Yangambi", and in the herbarium of Meise Botanic Garden (BR) in Belgium. Initially, the aim was to study only epiphytic diatoms on aquatic plants from the Yangambi Biosphere Reserve, but as the number of available specimens was restricted, our study area was extended to the Central Forest phytogeographic region (Bamps 1982). The area covers a large part of the Congo Basin, roughly between 16°35′E in the most western part and 30°27′E in the most eastern part, and 4°N in the north and 4°S in the south (fig. 1). The Yangambi Biosphere Reserve is located between 25°07′E and 30°27′E, and 0°45′N and 1°08′N, at about 100 km northwest of Kisangani, in the Tshopo province. In YBI, 24 herbarium specimens from plants of the family Nymphaeaceae, Ceratophyllaceae, and Lentibulariaceae were sampled (table 1). However, diatoms were almost absent in two samples YBI_ALG00126 (*Ceratophyllum*) and YBI_ALG00131 (*Utricularia*), with less than five valves observed, and were not taken into account in the rest of this paper. In BR, the study was restricted to the Nymphaeaceae and 12 herbarium specimens were selected (table 1) out of 19 in order to have samples from the region of Yangambi (6), Eala (4), and Kisangani (2). Because of the absence of geographic coordinates, the collecting locations are given approximatively on the map represented in fig. 1. Many of the samples were collected from streams or rivers in the Central Forest phytogeographic region. Two samples came from a pond (YBI_ALG00120 at Bambesa, CCA 4495 at Ngene-Ngene), three from a swamp (YBI_ALG00112 at Yakusu, YBI_ALG00135 at Yangambi, CCA 4488 at Yambao), and one from a marshy meadow (YBI_ALG00136 at Mongandio). Because the information available on the sample localities and habitats is limited, we cannot say with certainty if they are pristine or not. However, given the intensive agricultural activities in the region during the colonial period (Drachoussoff et al. 1991), we are convinced that most collecting was done in accessible areas already disturbed by human activities including deforestations. Samples for diatom analysis from YBI materials were given a unique number (starting with YBI_ALG) and deposed in the newly established algae herbarium at INERA Yangambi and as part of YBI. Diatoms samples from BR material were given a CCA (Christine Cocquyt) collection number until a BR barcode number can be assigned.

Small samples of the herbarium specimens (about 1 cm^2) were taken from the leaf blade and petiole with little damage to the herbarium specimens. Permanent slides for diatom investigation were made after oxidation of the material with hydrogen peroxide (30%) on a hot plate for at least 2 hours. Prior to embedding in Naphrax (refraction index 1.71), the cleaned material was rinsed three to five times with distilled water and centrifuged at 3000 rpm during 10 minutes at the University of Kisangani (UNIKIS) (D.R. Congo) or decanted after settling during 24 hours at Meise Botanic Garden. Diatom investigation was done using a Zeiss Axio plan microscope equipped with a Toupcam digital camera at UNIKIS and an Olympus BX51 microscope equipped with differential interference contrast (DIC) and an Olympus UC30 digital camera at Meise Botanic Garden.

For the identification of diatom genera and species, literature on African diatoms was consulted (e.g. Zanon 1938; Gasse 1986; Golama 1996; Cocquyt & Taylor 2015; Taylor & Cocquyt 2016; Taylor et al. 2016).

Water quality is discussed on the basis of two indices: the Trophic Diatom Index (TDI) (Kelly et al. 2001) and the Generic Diatom Index (GDI) (Rumeau & Coste 1988). Both indices were calculated based on genus level identifications using OMNIDIA software v.5.0 (Lecointe et al. 1993). The same software was used to calculate the Shannon diversity index (H) and evenness (J) (Hill 1973). Principal component analysis (PCA) was performed in R v.3.3.2 (R Development Core Team 2016) after square root transformation of the percentual data.

RESULTS

Diatom species richness in the Central Forest phytogeographic region, D.R. Congo, based on the studied herbarium materials, showed 132 species distributed among 44 genera (table 2).

Table 1 – Overview of the diatom samples (unique collection number) collected from aquatic macrophytes present in the herbarium of Yangambi (YBI) and the herbarium of Meise Botanic Garden (CCA number in BR). The information given on the collector, collector number, collecting date, and locality of the macrophytes is according to the herbarium label. The plant organ sampled is added.

Collection number	Species	Collector	Collector number	Collecting date	Locality	Plant organ
YBI_ALG00112	*Nymphaea lotus* L.	J. Louis	8524	21 Mar. 1938	swamp (Yakusu)	leaf and petiole
YBI_ALG00113	*Nymphaea lotus* L.	J. Louis	7941	18 Mar. 1938	Tutuku island (Yangambi)	leaf and petiole
YBI_ALG00116	*Nymphaea lotus* L.	J. Louis	100	11 Sep. 1935	Momboyo River	leaf and petiole
YBI_ALG00117	*Nymphaea lotus* L.	J. Léonard	435	27 Aug. 1946	Bamanya (Eala)	leaf and petiole
YBI_ALG00118	*Nymphaea lotus* L.	K. Kalanda	31	25 Oct. 1973	Isalowe river (Yangambi)	leaf and petiole
YBI_ALG00119	*Nymphaea lotus* L.	Ph. Gerard	4502	2 May 1960	Mbangana river (Bambesa)	leaf and petiole
YBI_ALG00120	*Nymphaea lotus* L.	Ph. Gerard	2916	8 May 1957	pond (Bambesa)	leaf and petiole
YBI_ALG00121	*Nymphaea lotus* L.	P. Bamps	326	4 Feb. 1959	mouth Lubilu river (Yangambi)	leaf
YBI_ALG00122	*Nymphaea lotus* L.	P. Bamps	641	24 Jun. 1959	mouth Lubilu river (Yangambi)	leaf and petiole
YBI_ALG00123	*Ceratophyllum demersum* L	R. Gutzwiller	491	21 Jan. 1955	Lokoma island (Yangambi)	leaf
YBI_ALG00125	*Ceratophyllum demersum* L.	J. Léonard	1622	24 Jan. 1948	Yangambi	leaf
YBI_ALG00126	*Ceratophyllum demersum* L.	A. Léonard	1070	11 Aug. 1958	Yangambi	leaf
YBI_ALG00127	*Utricularia* sp.	J. Louis	16259	29 Oct. 1939	Botukula swamp (Yanonge)	leaf and petiole
YBI_ALG00128	*Utricularia benjaminiana* Oliv.	C. Evrard	5072	17 Oct. 1958	Botukula swamp (Yanonge)	leaf and petiole
YBI_ALG00129	*Utricularia inflexa* Forssk.	J. Léonard	970	6 Nov. 1946	Banalia and Ilelonge (Eala)	leaf and petiole
YBI_ALG00130	*Utricularia inflexa* Forssk.	J. Léonard	434	27 Aug. 1946	Bamanya (Eala)	leaf and petiole
YBI_ALG00131	*Utricularia inflexa* Forssk.	C. Evrard	5966	16 Mar. 1959	Ikelemba (Bolomba)	leaf and petiole
YBI_ALG00132	*Utricularia inflexa* Forssk.	C. Evrard	5071	17 Oct. 1958	Ikelemba (Bolomba)	leaf and petiole
YBI_ALG00133	*Utricularia inflexa* Forssk.	C. Evrard	3402	3 Feb. 1958	Loeke river, Aketi road (Bumba)	leaf and petiole
YBI_ALG00134	*Utricularia inflexa* Forssk.	C. Evrard	1741	4 Sep. 1955	Mbangana river (Mbongo)	leaf and petiole
YBI_ALG00135	*Utricularia inflexa* Forssk.	D. Bolema	515	21 Mar. 1961	swamp (Yangambi)	leaf and petiole
YBI_ALG00136	*Utricularia mannii* Oliv.	R. Germain	4738	1 Feb. 1949	marshy meadow (Mongandjo)	leaf and petiole
YBI_ALG00137	*Utricularia foliosa* L.	R. Germain	1605	3 Oct. 1943	surroundings of Eala	leaf and petiole
YBI_ALG00138	*Utricularia foliosa* L.	C. Evrard	3405	3 Feb. 1958	Loeke river, Aketi road (Bumba)	leaf and petiole
CCA 4488	*Nymphaea lotus* L.	J. Louis	3635	19 Mar. 1937	Yambao, swamp	leaf and petiole
CCA 4495	*Nymphaea lotus* L.	A.K. Apema	45	29 Jul. 1987	Ngene-Ngene, fish pond	leaf and petiole
CCA 4496	*Nymphaea lotus* L.	P. Bamps	326	4 Feb. 1959	mouth Lubilu river (Yangambi)	leaf and petiole
CCA 4497	*Nymphaea lotus* L.	P. Bamps	641	24 Jun. 1959	mouth Lubilu river (Yangambi)	leaf and petiole
CCA 4510	*Nymphaea lotus* L.	J. Léonard	435	27 Aug. 1946	Bamanya, near Eala	leaf and petiole
CCA 4511	*Nymphaea lotus* L.	J. Louis	2107	31 May 1936	Lolifa, Ruki river	leaf and petiole
CCA 4512	*Nymphaea lotus* L.	J. Louis	7941	18 Mar. 1938	Tutuku island (Yangambi)	leaf and petiole
CCA 4514	*Nymphaea lotus* L.	J. Louis	9474	21 May 1938	mouth Bohonde (Yangambi)	leaf and petiole
CCA 4515	*Nymphaea lotus* L.	L. Louis	16174	9 Oct. 1939	Yangambi, Lubilu river	leaf and petiole
CCA 4517	*Nymphaea lotus* L.	F. Szafranski	1013	2 Jan. 1982	Batshamaleko, Kisangani	leaf and petiole
CCA 4518	*Nymphaea lotus* L.	L. Pynaert	884	19 Jan. 1907	Eala	leaf and petiole
CCA 4520	*Nymphaea lotus* L.	F. Vermoessen	2351	26 May 1919	Eala	leaf and petiole

Table 2 – Number of species and infraspecific taxa and the relative importance (%) of each genus in proportion to the number of genera (gen.) and to the number of valves counted (counts) observed in the material sampled from YBI and BR, covering the time period from 1907 to 1987. The total number for each genus is the most probable number assuming that most of the taxa observed in the YBI and BR materials were identical.

Genus	Material from YBI			Material from BR			Total		
	Number of species and infraspecific taxa	Relative abundance of the genus		Number of species and infraspecific taxa	Relative abundance of the genus		Number of species and infraspecific taxa	Relative abundance of the genus	
		gen.	counts		gen.	counts		gen.	counts
Achnanthes Bory	1	1.1	0.06	–	–	–	1	0.8	0.04
Achnanthidium Kütz.	2	2.2	0.05	3	2.6	0.36	3	2.3	0.17
Actinella F.W.Lewis	1	1.1	0.04	–	–	–	1	0.8	0.02
Adlafia Gerd Moser, Lange-Bert. & Metzeltin	–	–	–	2	1.7	1.65	2	1.5	0.62
Aulacoseira Thwaites	2	2.2	0.47	2	1.7	0.97	2	1.5	0.65
Bacillaria J.F.Gmel.	1	1.1	0.01	–	–	–	1	0.8	0.01
Brachysira Kütz.	1	1.1	0.01	1	0.9	0.04	2	1.5	0.02
Caloneis Cleve	1	1.1	0.28	3	2.6	0.20	1	0.8	0.25
Cavinula D.G.Mann & Stickle	–	–	–	1	0.9	0.01	1	0.8	0.02
Cocconeis Ehrenb.	3	3.4	0.40	1	0.9	0.04	3	2.3	0.27
Craticula Grunow	–	–	–	2	1.7	0.08	2	1.5	0.03
Cyclotella Kütz.	1	1.1	0.01	1	0.9	0.02	1	0.8	0.01
Cymbopleura (Krammer) Krammer	1	1.1	0.11	–	–	–	1	0.8	0.07
Desmogonium Ehrenb.	2	2.2	8.04	1	0.9	0.54	2	1.5	5.27
Diadesmis Kütz.	–	–	–	2	1.7	0.12	2	1.5	0.05
Diploneis (Ehrenb.) Cleve	–	–	–	1	0.9	0.04	1	0.8	0.02
Encyonema Kütz.	2	2.2	0.54	4	3.4	1.05	4	3.0	0.73
Eolimna Lange-Bert. & W.Schiller	–	–	–	1	0.9	0.12	1	0.8	0.08
Eunotia Ehrenb.	22	24.7	59.5	26	22.2	65.16	26	19.7	61.47
Fallacia Stickle	–	–	–	1	0.9	0.02	1	0.8	0.01
Fistulifera Lange-Bert.	–	–	–	1	0.9	0.16	1	0.8	0.01
Fragilaria Lyngb.	1	1.1	3.73	1	0.9	0.26	1	0.8	2.45
Fragilariforma D.M.Williams & Round	2	2.2	0.07	2	1.7	0.08	2	1.5	0.08
Frustulia Rabenh.	1	1.1	0.06	2	1.7	5.72	2	1.5	8.43
Geissleria Lange-Bert. & Metzeltin	–	–	–	1	0.9	0.04	1	0.8	0.02
Gomphonema Ehrenb.	8	9.0	6.99	6	5.1	0.36	8	6.1	4.57
Halamphora (Cleve) Levkov	–	–	–	1	0.9	0.04	1	0.8	0.02
Humidophila R.L.Lowe et al.	–	–	–	2	1.7	2.34	2	1.5	0.87
Iconella Jurilij	3	3.3	0.34	1	0.6	0.04	1	0.8	0.30
Luticola D.G.Mann	2	2.2	0.20	3	2.6	0.40	3	2.3	0.28
Mayamaea Lange-Bert.	–	–	–	1	0.9	0.16	1	0.8	0.06
Navicula Bory	2	2.2	1.87	5	4.3	1.51	5	3.8	1.75
Neidium Pfitzer	2	2.2	0.33	5	4.3	0.64	5	3.8	0.45
Nitzschia Hassall	3	3.4	1.65	4	3.4	2.96	7	5.3	2.13
Nupela Vyverman & Compère	–	–	–	3	2.6	0.60	3	2.3	0.38
Orthoseira Thwaites	1	1.1	0.04	1	0.9	0.01	1	0.7	0.02

Table 2 (continued) – Number of species and infraspecific taxa and the relative importance (%) of each genus in proportion to the number of genera (gen.) and to the number of valves counted (counts) observed in the material sampled from YBI and BR, covering the time period from 1907 to 1987. The total number for each genus is the most probable number assuming that most of the taxa observed in the YBI and BR materials were identical.

Genus	Material from YBI			Material from BR			Total		
	Number of species and infraspecific taxa	Relative abundance of the genus		Number of species and infraspecific taxa	Relative abundance of the genus		Number of species and infraspecific taxa	Relative abundance of the genus	
		gen.	counts		gen.	counts		gen.	counts
Pinnularia Ehrenb.	10	11.2	4.36	12	10.3	3.50	12	9.1	4.06
Placoneis Mereschk.	1	1.1	0.01	1	0.9	0.08	1	0.8	0.04
Planothidium Round & Bukhtiyarova	1	1.1	0.10	2	1.7	0.16	2	1.5	0.12
Sellaphora Mereschk.	3	3.4	0.35	3	2.6	9.08	3	2.3	3.58
Stauroneis Ehrenb.	4	4.5	0.16	5	4.3	0.40	5	3.8	0.25
Staurosira Ehrenb.	2	2.2	0.02	2	1.7	0.14	3	3.8	0.07
Staurosirella D.M.Williams & Round	–	–	–	1	0.9	0.50	1	2.3	0.19
Ulnaria Compère	3	3.4	0.03	1	0.9	0.30	3	2.3	0.03
Number of genera	30			40			44		
Number of species	89			118			132		

Eighty-nine species and infraspecific taxa, belonging to 30 genera, were observed in the material sampled from YBI and covering the time period from 1935 to 1973 (table 2).

For the BR materials, a longer time period was studied. However, a selected number of *Nymphaea* specimens were sampled for diatom study, the oldest dating back to 1907, the most recent sampled in 1987. In these materials from BR, 118 species and infraspecific taxa were observed, distributed over 40 genera. *Eunotia* was the most diverse genus with 26 species, followed by *Pinnularia* with 12 species, and by *Gomphonema* with 6 species, accounting for 22.2, 10.3, and 5.1% respectively of the diatom species diversity (tables 2, 3). The *Nymphaea* material from YBI showed a diversity of 58 species and infraspecific taxa: *Eunotia* was the most diverse genus with 15 species (25.9%) followed by *Pinnularia* with 8 species (13.8%), and *Gomphonema* with 5 species (8.6%) (table 3).

From the *Nymphaea* collection in BR, 12 herbarium specimens were analysed. Of these, four were also present in YBI, namely *Louis 7941* from 1938, *Léonard 435* from 1946, and *Bamps 326* and *Bamps 641* from 1959 (see table 1).

The analyses of the YBI and the BR materials were done using different microscopes, the Olympus BX51 in Meise Botanic Garden equipped with DIC, and by two different persons (first and third author respectively). Of the 58 species, belonging to 22 genera, observed in these nine materials (supplementary file 1), some smaller species (*Adlafia*, *Fistulifera*, and *Mayamaea*) were not detected in the YBI analyses, but these were always present in low quantities (less than 1.5%) in the BR analyses. On the other hand, other species were observed during the valve enumerations in YBI and not in BR materials, e.g. *Actinella*, *Eunotia pierrefuseyi*, and *Stauroneis*. However, this does not affect the quality of the YBI analyses, which is also confirmed by the positive correlations of the relative abundances between the two studies: 0.553, 0.997, 0.995, and 0.953 on genus level and 0.562, 0.813, 0.762, and 0.899 ($p = 0.01$) on species level respectively.

Bamps collected *Nymphaea* plants at the same locality (mouth of the Lubilu River, Yangambi) during two different months of the same year, on 2 Feb. and 24 Jun. 1959. The correlation between both collections is significant (between 0.709 and 0.950, $p = 0.01$). On the other hand, comparison of the Lubilu River collections with the collection of Léonard at Bamanya near Eala in 1946 was also significant but with lower values (comprised between 0.458 and 0.643, $p = 0.01$).

Within the genus *Eunotia*, one species was dominant in most of the samples, reaching relative abundances up to 74% (YBI_ALG00122, *Bamps 641* collected in 1959) of the total diatom community. The identity of this species is currently under investigation. In a number of samples, *Frustulia saxonica* Rabenh. peaked with relative abundances of 26.7 and 27.8% in material from YBI and BR respectively (YBI_ALG00117 and CCA 4510, *Léonard 435* collected in August 1946). *Sellaphora* cf. *pupula* (Kütz.) Mereschk. reached a relative abundance of 17.6% in a *Nymphaea* sample collected by Bamps (*641*) in June 1959, but only in the BR material (CCA 4497), not in the YBI material (YBI_ALG00122) where it was not observed during the counts.

Species richness in the *Nymphaea* samples varied between 9 and 54 (supplementary file l). Highest taxonomic diversity was observed in samples from Yangambi, taken near the mouth of the Bohonde river (CCA 4514, 54 species) and on Tutuku island (CCA 4512, 52 species).

For the other Yangambi samples, species and infraspecific diversity ranged between 9 and 30 for the YBI materials (9,

Table 3 – Number of species and infraspecific taxa for each genus observed in the *Nymphaea* material sampled from YBI and BR and the relative importance (%) of each genus in proportion to the number of genera (gen.) and to the number of valves counted (counts), covering the time period from 1935 to 1973.

Genus	Material from YBI			Material from BR		
	Number of species and infraspecific taxa	Relative abundance of the genus		Number of species and infraspecific taxa	Relative abundance of the genus	
		gen.	counts		gen.	counts
Achnanthidium	2	3.4	0.12	3	2.6	0.55
Actinella	1	1.7	0.01	–	–	–
Adlafia	–	–	–	2	1.7	2.47
Aulacoseira	1	1.7	0.56	2	1.7	1.46
Brachysira	–	–	–	1	0.9	0.07
Caloneis	1	1.7	0.17	3	2.6	0.24
Cavinula	–	–	–	1	0.9	0.07
Cocconeis	–	–	–	1	0.9	0.07
Craticula	–	–	–	2	1.7	0.13
Cyclotella	–	–	–	1	0.9	0.07
Cymbopleura	1	1.7	0.03	–	–	–
Desmogonium	2	3.4	8.50	1	0.9	0.45
Diadesmis	–	–	–	2	1.7	0.13
Diploneis	–	–	–	1	0.9	0.07
Encyonema	2	3.4	0.86	4	3.4	1.55
Eolimna	1	1.7	0.01	1	0.9	0.07
Eunotia	15	25.9	60.25	26	22.2	54.31
Fallacia	–	–	–	1	0.9	0.07
Fistulifera	–	–	–	1	0.9	0.07
Fragilaria	–	–	–	1	0.9	0.07
Fragilariforma	1	1.7	0.06	2	1.7	0.132
Frustulia	1	1.7	8.64	2	1.7	6.82
Geissleria	–	–	–	1	0.9	0.07
Gomphonema	5	8.6	6.33	6	5.1	0.24
Halamphora	–	–	–	1	0.9	0.07
Humidophila	–	–	–	2	1.7	3.00
Iconella	–	–	–	1	0.6	0.45
Luticola	2	3.4	0.31	3	2.6	0.48
Mayamaea	–	–	–	1	0.9	0.07
Navicula	2	3.4	3.61	5	4.3	2.26
Neidium	2	3.4	0.53	5	4.3	0.60
Nitzschia	2	3.4	1.94	4	3.4	4.39
Nupela	–	–	–	3	2.6	0.85
Orthoseira	–	–	–	1	0.9	0.07
Pinnularia	8	13.8	6.39	12	10.3	4.08
Placoneis	1	1.7	0.01	1	0.9	0.07
Planothidium	–	–	–	2	1.7	0.13
Sellaphora	3	5.2	0.56	2	1.7	12.52
Stauroneis	3	5.2	0.19	5	4.3	0.42
Staurosira	1	1.7	0.03	2	1.7	0.21
Staurosirella	–	–	–	1	0.9	0.76
Ulnaria	1	1.7	0.01	1	0.9	0.07
Number of genera	22			40		
Number of species	58			118		

18, 21, and 30) and between 11 and 33 for the BR materials (11, 17, 22, 26, and 33) (table 3).

Compared to other substrata, *Nymphaea* has an epiphytic diatom community as varied as on *Ceratophyllum* (YBI materials, mean of 20.3 (n = 9) and 21.3 (n = 2) respectively). The diatom community on *Utricularia*, on the other hand, was less diverse (mean 11.3, n = 11). No trend in increasing or decreasing species and infraspecific diversity could be observed for the period 1935–1973. As already mentioned, species and infraspecific richness was higher on the BR material due to different equipment, and a mean of 28.1 taxa (n = 12) was calculated. (supplementary file 1).

Through time, the highest species and infraspecific richness was be observed between 1935 and 1960, while similar richness could be noticed before and after this period, from 1907 to 1935 and from 1960 to 1987 respectively (fig. 2).

The Shannon diversity index (H) varied between 0.33 and 4.03 and the evenness (J) between 0.13 and 0.87 (fig. 3, supplementary files l, 2). Lowest H and J values were observed in 1935, highest in 1938, both diatom communities on *Nymphaea lotus* from Momboyo River and Tutuku Island at Yangambi respectively. Through time, we can observe the same trend for the diversity (fig. 2) as for the species richness (fig. 3A), with highest values for the period between 1935 and 1960. During this period the diatom communities showed also their highest evenness (fig. 3B, supplementary files 1, 2).

Results of a PCA performed on the relative abundances of species and infraspecific taxa, after square root transformation, could not indicate significant differences between the sample localities nor any changes over time (supplementary file 3). The first two axes are explaining 30.41 and 10.48 percent of the variance respectively.

The TDI, calculated at the genus level, ranged between 0.1 and 29.2 for the YBI materials (fig. 4, supplementary file 4) and between 0.1 and 39.6 for the BR materials. Based on a scale of 0 to 100, lower values represent better water quality: TDI < 20: free of organic pollution, > 21 TDI < 40: some evidence of organic pollution. For GDI, the score for water quality is on a scale of one to five, where smaller values indicate lower water quality. The GDI values showed the same results as for the TDI (fig. 4, supplementary file 4), i.e. the samples with the highest TDI also had the lowest GDI scores.

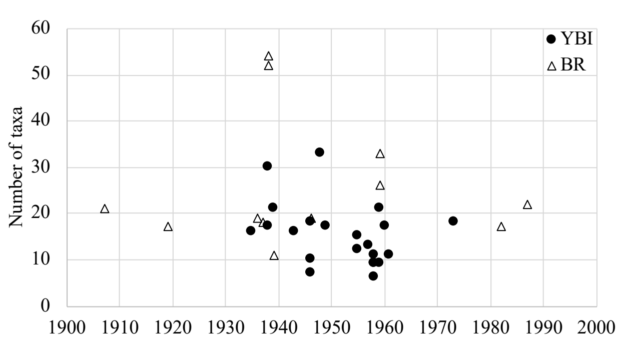

Figure 2 – Overview of the number of specific and infraspecific diatom taxa observed on the macrophytes present in the herbaria of YBI (black circle) and BR (triangle) between 1907 and 1987.

DISCUSSION

Overall, this study shows that a high diatom species richness was present on the aquatic macrophytes from the Central Forest phytogeographic region present in YBI and BR, both on the genus and the species level. The highest species and infraspecific numbers have been observed between 1935 and 1960. However, similar richness could be noticed before and afterwards (fig. 2). This period coincides to the expansion of agricultural projects in the Belgian Congo, which declined after the independence in 1960.

The higher number of observed genera and specific and infraspecific taxa in the BR materials (40 and 118 respectively) relative to the YBI materials (30 and 89 respectively) may be due to the resolution of the microscopes used, the first being equipped with differential interference contrast optics. The observation of *Adlafia* spp., *Craticula submolesta* (Hust.) Lange-Bert., *Mayamaea* sp., and *Nupela* spp. points in this direction. On the other hand, species observed in the YBI materials have not been seen during the diatom investigations of the BR material, e.g. *Achnanthes inflata* (Kütz.) Grunow, *Actinella* sp., *Amphora copulata* (Kütz.) Schoeman & R.E.M.Archibald, *Bacillaria paxillifera* (O.F.Müll.) Hendey, *Cymbopleura amphicephala* (Nägeli) Krammer, *Orthoseira* sp., and *Ulnaria* sp. Notwithstanding

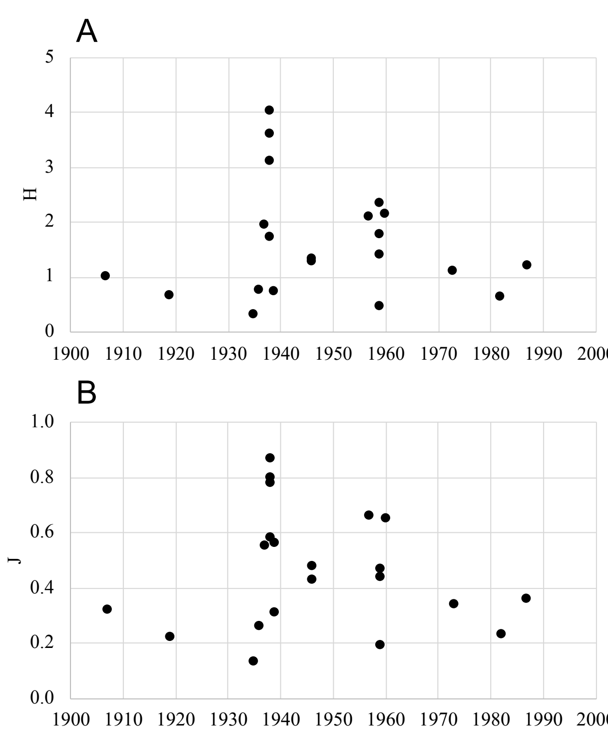

Figure 3 – Overview of the Shannon diversity index and the Evenness values as derived from the diatom analyses (genus level) of macrophytes, present in the herbaria of YBI and BR between 1907 and 1987. **A**. Shannon diversity index (H). **B**. Evenness (J).

these differences, there was a high positive correlation of the relative abundances between three of the four duplicate herbarium specimens in YBI and BR (0.997, 0.995, and 0.953) on genus level and 0.813, 0.762, and 0.899 ($p = 0.01$) on species level.

The observation that *Eunotia* is the dominant and by far the most diverse genus in both YBI and BR materials is not surprising (table 2). All the waterbodies in the Central Forest phytogeographic region are characterised by acidic waters (Golama 1996), which is an ideal habitat for *Eunotia*.

The diatom assemblages in the Central Forest phytogeographic region differ from those reported on herbarium materials from Lake Naivasha (Cocquyt & De Wever 2002). In the Congo materials, the genus *Eunotia* was the most important genus, while in the Lake Naivasha materials, *Gomphonema gracile* Ehrenb. and *Epithemia gibberula* (Ehrenb.) Kütz. (as *Rhopalodia gibberula* (Ehrenb.) O.Müll.) were the most important species, and *Eunotia pectinalis* (Kütz.) Rabenh. was only relatively important in material collected in 1909 (Cocquyt & De Wever 2002). In D.R. Congo, the materials were collected in acidic rivers with very low conductivity (Golama 1996 and several other unpubl. res.). In Lake Naivasha, on the other hand, pH is neutral to slightly alkaline ranging between 6.71 and 8.97 (Keyombe & Waithaka 2017). With regard to the aquatic plants collected in Lake Naivasha, the conductivity varied between 366 and 899 $\mu S.cm^{-1}$ (Verschuren 1996 in Cocquyt & De Wever 2002). The higher pH and conductivity in Lake Naivasha explain the importance of *Epithemia gibberula*, a species that has a pH optimum of 8.2 and a fairly high salt tolerance (Cholnoky 1968). On the other hand, some tropical African diatoms such as *Gomphonema zairense* and *Iconella ebalensis* (synonym: *Surirella ebalensis* Cocquyt & J.C.Taylor), both described from D.R. Congo and observed in this study, were not observed in the Lake Naivasha materials. Although the ecology of these species is not well known, up to now, they are only reported from acidic rivers.

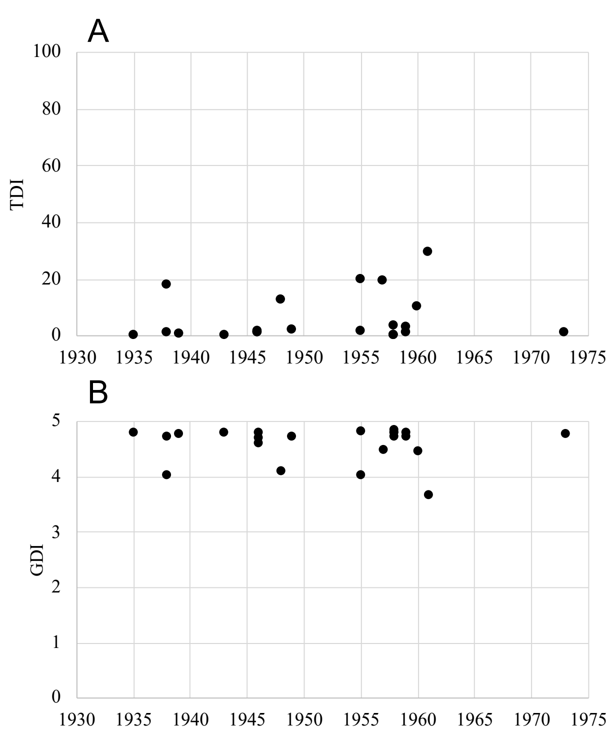

Figure 4 – Overview of the Trophic Diatom Index (TDI) and Generic Diatom Index (GDI) values as derived from the diatom analyses (genus level) of macrophytes present in the herbarium of YBI between 1935 and 1973. **A**. TDI. **B**. GDI.

Most of the diatoms observed during this study were also reported by Symoens & van der Werff (1993) from water samples taken in the Tshopo River. This river is also located in the Central Forest phytogeographic region and flows through Kisangani, located about 100 km from Yangambi and upstream the Congo River. Notwithstanding the larger size of the Tshopo River compared to the small streams and rivers in the Yangambi region, their pH and conductivity are rather similar. Goloma (1996) mentioned a pH of 6.6–6.8 and a conductivity of 38.7–48.5 $\mu S.cm^{-1}$ for the Tshopo River at the end of the 1980s. Recently, a pH of 5.6–6.6 (mean 6.3) and a conductivity of 33.2–46.1 $\mu S.cm^{-1}$ (mean 39.3) was measured upwards the cataracts on this river near the electricity plant at Kisangani (Kisuba 2019). A recent study on epiphytic diatoms of the Tshopo River (Kisuba 2019), showed that 61.3% of the observed diatom valves belong to *Eunotia*, followed by *Gomphonema* with 18.2%. Other genera never attained relative abundances higher than 6%.

The highest value for the Shannon diversity index and associated evenness, 4.03 and 0.87 respectively, was found in a sample from 1939 from Tutuku island, followed by a sample from the same year from the mouth of the Bohonde river ($H = 3.60$, $J = 0.78$), both located in the Yangambi region. The Shannon diversity index and associated evenness attained their highest values between 1935 and 1960, the period that coincides to the expansion of agricultural projects in the Belgian Congo, as mentioned before. These very high values are not in line with what we would expect in the studied epiphytic diatom communities with high species richness. We must remark that H and J were calculated in this particular case on genus level data. The obtained results on the diversity index and evenness are not reliable. The diversity index and evenness must be derived at species level, in contrast to TDI and GDI where this can be done at genus level.

TDI and GDI were both developed to evaluate water quality in Europe. The advantage of these two indices is that they can be calculated on species as well as on genus level data. Being able to estimate water quality at the genus level is an important aspect, given that diatoms of the Congo Basin are not well known and identification down to the species level is currently impossible for most taxa. On the other hand, recent publications (e.g. Taylor & Cocquyt 2016) make it possible to recognize diatoms at the genus level making it possible to use the TDI and GDI for water quality monitoring in D.R. Congo and by extension the entire Congo Basin and tropical Africa.

The Trophic Diatom Index, calculated on genus level identifications, showed for the YBI materials (fig. 4, supplementary file 4) that there is almost no eutrophication in most of the waterbodies at the time the aquatic plants were collected. The TDI value of 29.2 obtained for a *Utricularia* sample taken in 1961 can be explained by the sample YBI_ ALG00135 being collected in a swamp where nutrients are retained compared to running waters. A trend of more variability in the index values can be seen from the 1950s onwards. For the Yangambi area, this could likely be due to increased human impact, including deforestation, through the expansion of the research centre of INÉAC (Institut national pour l'étude agronomique du Congo belge), which reached its peak in the 1950s (Drachoussoff et al. 1991; Kambale 2007). This agronomic centre was established in Yangambi in 1933 and became the most important agronomic research centre in the Congo Basin. It changed its name into INERA (Institut national pour l'Étude et la Recherche Agronomique) two years after the independence of D.R. Congo.

In tropical Central Africa, the water quality of small rivers and streams for the Gombe Stream National Park in Tanzania was studied using diatoms and the TDI (Bellinger et al. 2006). Most of these rivers have a neutral to slightly alkaline pH (maximum up to 8.1); only two rivers, the Mtanga and Mkenke, have a pH lower than seven (6.7 and 6.9 respectively) and a low conductivity, 26.3 and 13.8 respectively. Species and genus richness for the Gombe Stream National Park rivers ranged between 10 and 20 and between 6 and 10 respectively. However, these figures cannot be compared with the diversity observed in the Central Forest phytogeographic region as the former concerns epilithic and the latter epiphytic diatom communities.

The TDI values for the watersheds in the Gombe Stream National Park varied between 59 and 90, with no significant differences between forested and deforested watersheds where they enter Lake Tanganyika, and significantly lower values 25 m upstream in the forested watershed (Bellinger et al. 2006). However, the TDI points to eutrophication in all the watersheds. In our study of epiphytic diatoms of the Central Forest phytogeographic region, the highest TDI value calculated was 39.5, which is much lower than the best value observed in one of the watersheds of the Gombe Stream National Park. Even when the TDI values were greater than the theoretical TDI values in deforested watersheds based on the measured phosphorus concentrations as reported by Bellinger et al. (2006), the Gombe Stream National Park waters are still more nutrient-rich than those of the Central Forest phytogeographic region. We must remark, however, that the TDI was calculated at the species level for the Gombe Stream National Park waters. But diatom taxa tolerant to nutrient enrichment were not observed in the Central Forest phytogeographic region, such as *Amphora copulata* (Kütz.) Schoeman & R.E.M.Archibald, or were present only in relatively unimportant numbers, such as *Cocconeis placentula* var. *euglypta*, *Encyonema minutum*, and *Nitzschia* spp. This finding is confirmed by ongoing research on diatoms from rivers and streams in Yangambi and surrounding area.

CONCLUSION

This study provides interesting information on the past diatom communities and diversity of aquatic ecosystems in the Central Forest phytogeographic region, D.R. Congo. Based on two water quality indices developed for European waters, TDI and GDI, information was obtained on the past ecological conditions and the stability of the water quality during the 20th century in this part of the Congo Basin. Overall, the results showed that waterbodies in the Central Forest phytogeographic region in which the aquatic macrophytes were collected did not experience nutrient enrichment during the studied period from 1907 to 1987. Moreover, even at the genus level, TDI and GDI prove to be valuable tools for water quality monitoring in D.R. Congo and by extension the Congo Basin and tropical Africa. However, the genus level is a first approach in anticipation of a species/environmental calibration set for the region to be developed in the future. On species level, the study of herbarium material will play an important role, especially when historical water quality is considered.

SUPPLEMENTARY FILES

Supplementary file 1 – Percentage of valves of each genus observed in the *Nymphaea* materials sampled from YBI and BR and covering the time period from 1907 to 1987. Samples are ordered according to their locality in the Central Forest phytogeographic region from West to East. The sample numbers given are with omission of the prefix YBI_ALG00 for YBI, and of CCA for BR.

https://doi.org/10.5091/plecevo.2021.1763.2429

Supplementary file 2 – Percentage of valves of each genus observed in the material other than *Nymphaea* sampled from YBI and covering the time period from 1939 to 1961. Samples are ordered according to their locality in the Central Forest phytogeographic region from West to East. The sample numbers given are with omission of the prefix YBI_ ALG00.

https://doi.org/10.5091/plecevo.2021.1763.2431

Supplementary file 3 – Ordination (relative genera abundances after square root transformation) showing the first two PCA axes. **A**. Distribution of the genera, abbreviated with the first four letters of the genera names. **B**. Distribution of the samples. The sample numbers given are with omission of the prefix YBI_ALG00 for YBI, and of CCA for BR.

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Supplementary file 4 – Overview of the diatom samples collected from aquatic macrophytes present in the herbarium of Yangambi (YBI) with their respective TDI and GDI values.

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