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Five thousand years of tropical lake sediment DNA records from Benin

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ABSTRACT

Until now, sedimentary DNA (*sed*DNA) studies have only focused on cold and temperate regions were DNA is relatively well preserved. Consequently, the tropics, where vegetation is hyperdiverse and natural archives are rare, have been neglected and deserve attention. In this study, we used next-generation sequencing to barcode *sed*DNA from Lake Sele, localized in the tropical lowlands of Benin (Africa), and compared the taxonomic diversity detected by DNA analyses with pollen assemblages. Plant *sed*DNA was successfully amplified from 33 of the 34 successfully extracted samples. In total, 43 taxa were identified along the 5000 years spanned by the sediment: 22 taxa were identified at the family level and 21 at the genus level. The plant diversity recovered through *sed*DNA from Lake Sele showed a specific local signal and limited overlapping with pollen. Introduced plants, grown and cultivated close to the water, such as sweet potato, were also well recorded by *sed*DNA. It appears, therefore, to be a promising approach to studying past diversity in tropical regions, and could help in tracking the introduction and history of agriculture. This is the first time this method has been used in the field of domestication and dissemination of several specific crops, and the results are very encouraging.

1. Introduction

Plant sedimentary DNA (*sed*DNA) has recently emerged as a promising proxy for paleoecological reconstructions over thousands of years, complementary to pollen and macrofossils analysis (Thomsen and Willerslev, 2015; Parducci et al., 2017). However, until now, paleoenvironmental plant *sed*DNA analyses have essentially been conducted in temperate and boreal zones (Willerslev et al., 2003; Haile et al., 2009; Jørgensen et al., 2012; Parducci et al., 2013, 2017; Giguet-Covex et al., 2014). The oldest *sed*DNA records were recovered from the permafrost of Siberia (Willerslev et al., 2003) and Northern Scandinavia (Parducci et al., 2012). In more temperate zones, the published results generally span over the last 10,000 years (Anderson-Carpenter et al., 2011; Giguet-Covex et al., 2014; Pansu et al., 2015; Alsos et al., 2016), as the studied lakes have accumulated sediments since the last deglaciation. While the taxonomic resolution of *sed*DNA is not necessarily better than pollen or macroremain counts (Matisoo-Smith et al., 2008; Parducci et al., 2013; Pedersen et al., 2013), they nevertheless allow the detection of specific taxa either poorly or not recorded by classical paleoecological methods. The analysis of plant *sed*DNA has enabled multiple improvements to our paleoecological knowledge, such as the discovery that coniferous trees survived the last glaciation in Northern Scandinavia despite this not being detected by pollen analysis (Parducci et al., 2012), and the description of long-term vegetation changes determined by human activities and pasture (Giguet-Covex et al., 2014; Pansu

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et al., 2015).

Few studies have explored *sed*DNA in tropical regions (Epp et al., 2010; Stoof-Leichsenring et al., 2012), and only one, from a high-altitude lake, analyzed long-term variations in terrestrial plant DNA (Boessenkool et al., 2014). The initial focus on high-latitude or high-altitude areas is related to the fact that DNA degradation increases at high temperatures (Allentoft et al., 2012), so the absence of publications on plant *sed*DNA analyses in low-altitude and latitude sites might merely be due to the absence of trials or a failure of these trials (Parducci et al., 2017). Despite the relatively short time period examined, the recovery of diatom *sed*DNA dating back 200 years by Stoof-Leichsenring et al. (2011) in Lake Naivasha, Kenya, is particularly interesting as water temperatures rarely fall below 20 °C (in Britton et al., 2007). In the two high-altitude lakes studied by Boessenkool et al. (2014) *sed*DNA sequences were obtained from 4500-year-old samples. With the help of a reference library covering the majority of the afro-alpine flora, the authors were able to identify several taxa at a better/more precise taxonomic level than pollen analyses, but with fewer species detected. If DNA can be preserved in sediments from warm climates, the method could be an important proxy to identify early agriculture and farming in the tropics. In Africa, for example, the onset of the cultivation of pearl millet (*Pennisetum glaucum*) or sorghum (*Sorghum bicolor*) is poorly understood (Fuller et al., 2014) because traditional bioproxies such as pollen, and phytoliths are inadequate (phytolith and pollen of African grasses cannot be differentiated into cereals and wild grasses), and charred grains are rare. *Sed*DNA may also be a valuable tool to complement and alleviate the time constraints of phylogeography studies, the study of the geographic distribution of genetic lineages within species (e.g. for *Aucoumea klaineana* in Born et al., 2008), or to track the history of specific vegetation types badly recovered by more conventional methods, for instance the Marantaceae forests (Cuni-Sanchez et al., 2016).

The objective of this study is to explore the preservation of plant DNA in the sediments of a low-elevation tropical lake over the last 5000 years, and to test the pertinence of *sed*DNA analysis to record past changes in plant diversity and agriculture practices on the shoreline of the lake. The DNA of wild and cultivated plants is generally incorporated into the soils (Yoccoz et al., 2012), and may be transported to the lake by erosion during rainfall. We chose Lake Sele (south of Benin) because the shorelines are flat, with large parts being used for seasonal agriculture during the dry season when the lake level is low. Furthermore, its sediments have already been studied for fossil pollen content (Salzmann and Hoelzmann, 2005), allowing comparison between these records and the *sed*DNA.

2. Material and method

2.1. Study site

Lake Sele in Benin is located about 1 km east of the Ouémé River (7° 9′19.29″N, 2°26′25.57″E; Fig. 1) at an altitude of less than 10 m above sea level, with a mean annual temperature of 28 °C (Hijmans et al., 2004); and though it is not directly fed by the river it is within the same riverbed. The lake is elongated and shallow at around. 2.5 km in length, 1 km wide (at its maximum point), and with a maximum water depth of <1.5 m during the dry season (Salzmann and Hoelzmann, 2005). Nowadays, during the dry season, the shorelines are partially covered with Cyperaceae and the water hyacinth *Eichhornia crassipes* (Pontederiaceae; introduced from South America) (Salzmann and Hoelzmann, 2005). The shorelines are generally cultivated when the lake level is low. Agricultural parcels are present all around the lake forming a belt ~200–300 m wide, which are partially or totally submerged during the wet season. The main cultures are sweet potatoes (*Ipomoea batatas*, Convolvulaceae), the red variety planted close to the water and the white variety further away; also cultivated away from the shoreline are cassava (*Manihot esculenta*, Euphorbiaceae), maize (*Zea mays*, Poaceae), and peanuts (*Arachis hypogaea*, Fabaceae). Pigeon pea (*Cajanus cajan*, Fabaceae), bananas (*Musa sp.*, Musaceae), tomatoes (*Solanum lycopersicum*, Solanaceae), and other legumes are cultivated in the upper part of the shoreline, along with coconut (*Cocos nucifera*, Arecaceae) and oil palms (*Elaeis guinensis*, Arecaceae). The natural vegetation belongs to the Guinean transition zone with its mosaic of forests and savannas (White, 1983). The main trees of the residual forest stands, include *Triplochiton scleroxylon* (abachi, Sterculiaceae), *Celtis* spp. (Cannabaceae), and Ulmaceae, *Milicia excelsa* (iroko, Moraceae).

2.2. Sampling

We collected five meters of sediment cores from the deepest part of the lake (N7.15,537° E2.44,106°). The two upper meters of the sediment were collected with an Uwitec Gravity corer (63 mm inner diameter), in order to get the longest possible section in one tube to minimize contamination; the three lowers meters were taken with a modified Livingston piston corer (47 mm inner diameter), which permitted us to reach the deepest sediments in one meter sections. The whole sediment core was kept closed in either plastic (Uwitec) or aluminum (Livingston) tubes and stored for several days in an air-conditioned room in Benin, before being moved to a cold chamber (4 °C) in Montpellier, France. Sampling for DNA extraction was performed one month later after arrival in France. Seven AMS ^{14}C analyses were carried out on terrestrial macroremains (seeds and piece of plant leafs) by the Poznan Radiocarbon Laboratory. The ^{14}C ages were converted to calendar years using CLAM software (Blaauw, 2010) and the age-depth model was generated using BACON software (Blaauw and Christen, 2011). A hiatus of sedimentation was detected between 4400 years cal. BP and 3100 years cal. BP, as previously indicated by Salzmann and Hoelzmann (2005) (AMS ^{14}C dates and age-depth model in supplementary data: Appendix S3).

2.3. Samples for DNA analysis

From the sediment core, we sampled 50 slices each 2 cm thick. Sampling of core slices was carried out at the University of Savoie. To avoid DNA contamination, particularly with regards to water circulation along the coring tube, 10 mm was removed from the edge of each sediment slice. The tools (knives, spoons, pliers) were cleaned after each sampling with distilled water, then alcohol and inflamed. DNA extraction, PCR set-up and PCR amplification steps were later performed in three separate rooms at the University Grenoble Alpes, which is specifically dedicated to ancient DNA studies. *Sed*DNA extraction targeted extracellular DNA (Pansu et al., 2015). For each sediment slice, we mixed approximately 15 g of sediment with 15 ml of saturated phosphate buffer (Na2HPO4; 0.12 M; pH ≈ 8) for 15 min. The mixture was then centrifuged (10 min at 10,000 g). The resulting supernatant (12 ml) was transferred to Amicon® Ultra-15 10 K Centrifugal Filter Devices (Millipore) and centrifuged (20 min at 4000 g) to concentrate *sed*DNA. Of the resulting concentrate, 400 µl were kept as a starting material for the following extraction steps, using the NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany), skipping the cell lysis step and following the manufacturer's instructions (Taberlet et al., 2012b). Three extraction controls were performed.

The *sed*DNA extractions and amplifications were performed in laboratories specifically dedicated to ancient DNA studies in the University Grenoble-Alpes, France.