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First Survey Of Aquatic Microbial Fungi-Like *Pythiaceae* Predominantly Colonizing The South-Mediterranean Freshwater Wetlands.

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ABSTRACT

In order to contribute identifying the aquatic bioactive microbial resources occurring in North-eastern Algeria wetlands, we established this first survey of saprotrophic *Oomycetes* colonizing the most important freshwater inland ecosystem in the National Park of El Kala, named Oubeira Lake. The latter is a South-Mediterranean subtropical water-plane extended on 2200 ha, representing the largest Ramsar site of the region. In the total absence of authorities control policy, the lake is anarchically exploited in diverse agricultural and fishing activities. The biodiversity and occurrence of *Oomycetes* dominated by the *Pythiaceae* family taken from water, sediments and associate lake materials in decomposition, play a vital role in the aquatic habitat auto-purification. Molecular phylogeny based on rDNA ITS sequences have been highly significant to distinguish 66 aquatic indigenous *Pythiaceae* taxa, with a large predominance of the genus *Pythium*, almost isolated from sediments and plant materials in decomposition. *Pythium* and its related genera *Phytophthora* and *phytopythium* herein identified are reputed to efficiently contribute in the quick recovery of the ecosystem against repeated Eutrophication threats. Typical Mediterranean elements, adapted to the warm subtropical climate have been identified and some morphological and behavioural overlap between neighbours seems to be stimulated by temperate conditions.

Keywords: Oubeira Lake, freshwater, Molecular diversity, Mediterranean autochtonous, *Pythium*, *Phytophthora*, *Phytopyhthium*.

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INTRODUCTION

Oubeira Lake has been listed since 1982 as a Ramsar Site. The endoreic freshwater lake is one the most important stopover of migrant birds in the NorthAfrican few wetlands. It is classified as a world patrimony for hosting rare and endemic biota, particularly a belt of rare and very rare helophytes essential for water-birds nesting; we mention: *Trapa natans* and *Nuphar lutea*, unique presence in Algeria (Boumezbeur, 2003). Through time, the lake could oppose many Eutrophication menaces, and found back its stability by simple auto-purification mechanisms (Meddour *et al.*, 2001, 2008, 2009). The microbial activity in such aquatic ecosystems is efficiently turning the cycle of organic material. Concerning our chosen site of study and similar ecosystems role within the National Park of El Kala, no available data are yet registered about these bioactive microbiota. In the current work, we take the initiative to identify the indigenous saprophytic *Pythiaceae*, to open the way towards understanding their occurrence and behaviour.

These fungi-like protists represent the main decomposing machine of dead organic materials in freshwater ecosystems, regarding their distinct enzymatic profile. They are systematically known as *Oomycota*, typified by the *Pythiaceae,* which are worldwidespread active biodegradation agents; they count more than 140 indexed species (Kageyama *et al*., 2014). Almost saprophytic, occupying a considered nutritional position in aquatic and wet terrestrial habitats; their chitinolytic, keratinolytic or cellulolytic properties characterize of obligate biotrophes and/or occasional plant and animal parasites; furthermore, they can parasite algae and true fungi; many are essentially utilized in fungal diseases bio-control (Vallance et al., 2009).

Their taxonomy based on morphological descriptions of sporangia and zoospores, *inter alia*, with the aid of Middleton 1943, Waterhouse 1968, Van der Plaats-Niterink 1981 and Dick 1990 dichotomy keys, lets a lack of consistency on the most important morphological characteristics leading to identification errors (Levèsque and de cock, 2004). The development of molecular biology by the beginning of the 2000s permitted the study of evolutionary relationships and history between living lineages. Among the most useful molecular fingerprints, the internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of fungi and fungilike phyla, with the most clearly defined barcode gap between inter- and intraspecific variation. Comparing to other markers such as LSU and SSU, ITS is formally proposed for adoption as the primary fungal barcode marker to the Consortium for the Barcode of Life (Schoch et *al*., 2012). Primarily, the PCR primers developed by White et al. 1990 universally amplified a highly variable region through all *taxa*, including Oomycetes. The advantages and limitations of the ITS region for phylogeny were reviewed since then (Bruns, 2001).

Since 2000 Paul Bernard and his researchers group have described up to 45 new species from different ecosystems in France, Tunisia, Morocco, Turkey, Spain; he was the first to identify oomycetes in Algeria, where he could describe 15 species of *Pythium*, in drier western and Saharian areas.

The current work permitted to describe non biotrophic Oomycetes ranged in *Pythium* and its closer relatives *Phytophthora* and *Phytopythiyum* genera, occurring in both water and sediments of the Oubeira lake. The investigation is mainly based on the phylogenic analysis of rDNA ITS *locci*, extracted from 66 selected isolates mycelia.

MATERIALS AND METHODS

Sampling and isolates processing

Around 36°.81842N, 8°.44179E coordinates related to Oubeira Lake, sampling units were taken from superficial water, sediments and organic dead materials rotten by aquatic microflora biodegradation.

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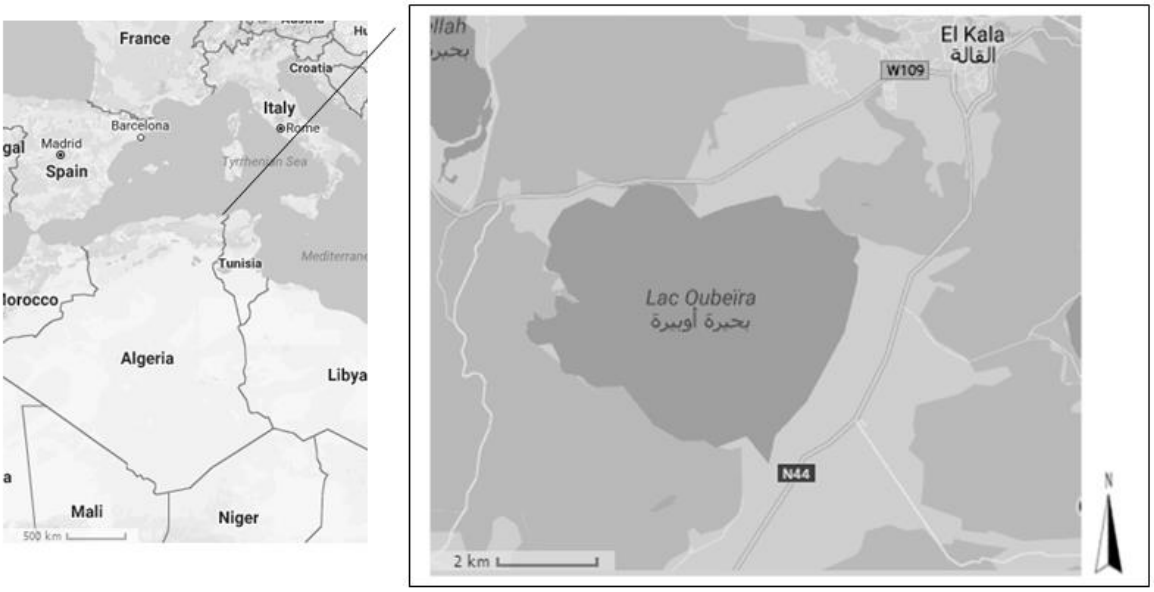

Figure 1: Satellite localisation of Oubeira Lake (extreme Northeastern Algeria). ©2016Google, Map Data ©2016 AND Image Landsat ©SPOT IMAGE, date: 10.04.2013)

They were picked during the period 2011-2013, at low depth from five different water input points, up to 500 meters distant from each other. Microbiologic labour consisted in crumbling solid samples into about 25 mm^2 pieces of dead organic materials as leaves and insects in decomposition, and sediments, then plating them on modified water agar medium (Samson *et al*., 2004), to which we added Ampicillin, Nystatin, Pimaricyn, Rifampicyn and Pentachloronitrobenzene (Eckert and Tsao, 1962).

Water samples were processed by the baiting method (Ali, 2007), using sterilized wheat seeds, to attract swimming zoospores in the water samples, using 18 cm diameter glass Petri dishes; then transferred after 5 days on water agar. Colony selection were based on culture aspect showing non sporulate whitish mycelium with soft texture and typical oomycetal growth (Erwin and Ribeiro, 1996). Mycelial specimens were collected in sterile 1.5 ml microtubes, then placed at -20° C for at least 12 hours, up to complete freezing for further analyses. We selected 66 isolates whose whitish mycelia adequate to the genus *Pythium* and similar Oomycetes.

DNA extraction, Amplification and purification

DNA was extracted using GenEluteTM plant Genomic DNA Miniprep Kit (Sigma-Aldrich) and finally stored at -20 °C according to Manufacturer's specifications (Ginetti *et al*., 2014). ITS region (Internal Transcribed Spacer) of the ribosomal DNA was amplified using universal primers ITS-6 (5' GAA GGT GAA GTC GTA ACA AGG 3 ') (Cooke et al. 2000) and ITS-4 (5 'TCC TCC GCT TAT TGA TAT GC 3') (White et al. 1990). Subsequent PCR was accomplished by using the following program: step (1) initial denaturing for 3 min at 95 °C; step (2) denaturing for 30 sec at 95 °C; step (3) annealing for 30 sec at 55 °C; step (4) extension for 1 min at 72 °C; step (5) final extension for 5 min at 72 °C. The steps 2-4 were repeated 35 times (White et al. 1990). The PCR products were quantified by Electrophoresis on Agarose gel, then finally cleaned-up using Thermo-Fisher-Scientific kit, by adding to 5µl of it, a mix of 0.5µl Exonuclease 1 and 1µl Fast-up alkaline phosphate according to manufaturer's specifications (Ginetti *et al*., 2014).

Sequence analyses

Amplicons were sent for sequencing, then sequences were checked in Chromas Lite v. 211, then assembled, analyzed and edited in Geneious v.8.1. Multiple alignment was performed using Mafft 7 online aligner, by choosing Q-INS-i strategy setting and 1PAM=2k for scoring matrix. Then, we chose Maximum Parsimony and Minimum Evolution methods with default parameters, to obtain the best representation from the produced trees (Baldwin, 1992). Bootstrap statistics and branch lengths were computed with Mega 6

(Tamura *et al*., 2013). Maximum Likelihood and posterior probability based with Mr. Bayes strategy were computed on TOPAli program.

RESULTS

De Novo Assembly permitted to select 111 rDNA ITS data of around 700 bp length, including 44 reference sequences and 66 subjects from the current study, which we deposited on Genebank under the accession numbers labeled from KU588196 to KU588267 (table 1) and 44 reference sequences resulting from Blast operation (table 2).

Table 1: Selected isolates from different substrates sampled in the Oubeira Lake. Accession numbers were accorded to rDNA ITS1 and 2 amplified regions of the extracted genomic DNA

Isolate code	*Genus*	*GB Accession number*	*Substrate*
LK-1	*Phytophthora*	*KU588202*	*Sediments*
LK-2	*Phytophthora*	*KU588200*	*Dead plant materials*
LK-3	*Phytophthora*	*KU588201*	*Dead plant materials*
LK-4	*Phytophthora*	*KU588199*	*Dead plant materials*
LK-5	*Phytophthora*	*KU588198*	*Dead plant materials*
LK-6	*Phytophthora*	*KU588197*	*Dead plant materials*
LK-7	*Phytophthora*	*KU588196*	*Dead plant materials*
LK-8	*Phytopythium*	*KU588203*	*Dead plant materials*
LK-9	*Phytopythium*	*KU588206*	*Dead plant materials*
LK-10	*Pythium*	*KU588253*	*Sediments*
LK-11	*Phytopythium*	*KU588204*	*Dead plant materials*
LK-12	*Pythium*	*KU588251*	*Sediments*
LK-13	*Phytopythium*	*KU588205*	*Sediments*
LK-14	*Phytopythium*	*KU588209*	*Sediments*
LK-15	*Phytopythium*	*KU588208*	*Sediments*
LK-16	*Phytopythium*	*KU588207*	*Sediments*
LK-17	*Pythium*	*KU588249*	*Sediments*
LK-18	*Pythium*	*KU588231*	*Birds feather*
LK-19	*Pythium*	*KU588261*	*Sediments*
LK-20	*Pythium*	*KU588257*	*Sediments*
LK-21	*Pythium*	*KU588220*	*Dead plant materials*
LK-22	*Pythium*	*KU588265*	*Sediments*
LK-23	*Pythium*	*KU588247*	*Sediments*
LK-24	*Pythium*	*KU588216*	*Dead plant materials*
LK-25	*Pythium*	*KU588217*	*Dead plant materials*
LK-26	*Pythium*	*KU588219*	*Dead plant materials*
LK-27	*Pythium*	*KU588215*	*Dead plant materials*
LK-28	*Pythium*	*KU588252*	*Sediments*
LK-29	*Pythium*	*KU588212*	*Dead insects*
LK-30	*Pythium*	*KU588566*	*Sediments*
LK-31	*Pythium*	*KU588228*	*Dead plant materials*
LK-32	*Pythium*	*KU588238*	*Birds feather*
LK-33	*Pythium*	*KU588218*	*Dead plant materials*
LK-34	*Pythium*	*KU588250*	*Sediments*
LK-35	*Pythium*	*KU588260*	*Sediments*
LK-36	*Pythium*	*KU588234*	*Birds feather*
LK-37	*Pythium*	*KU588224*	*Dead plant materials*
LK-38	*Pythium*	*KU588223*	*Dead plant materials*
LK-39	*Pythium*	*KU588258*	*Sediments*
LK-40	*Pythium*	*KU588255*	*Sediments*
LK-41	*Pythium*	*KU588241*	*Birds feather*
LK-42	*Pythium*	*KU588248*	*Sediments*

LK-43	*Pythium*	*KU588211*	*Dead insects*
LK-44	*Pythium*	*KU588262*	*Sediments*
LK-45	*Pythium*	*KU588229*	*Dead plant materials*
LK-46	*Pythium*	*KU588230*	*Dead plant materials*
LK-47	*Pythium*	*KU588263*	*Sediments*
LK-48	*Pythium*	*KU588214*	*Water*
LK-49	*Pythium*	*KU588213*	*Dead insects*
LK-50	*Pythium*	*KU588256*	*Sediments*
LK-51	*Pythium*	*KU588243*	*Birds feather*
LK-52	*Pythium*	*KU588233*	*Birds feather*
LK-53	*Pythium*	*KU588232*	*Birds feather*
LK-54	*Pythium*	*KU588237*	*Birds feather*
LK-55	*Pythium*	*KU588222*	*Dead plant materials*
LK-56	*Pythium*	*KU588236*	*Birds feather*
LK-57	*Pythium*	*KU588227*	*Dead plant materials*
LK-58	*Pythium*	*KU588246*	*Birds feather*
LK-59	*Pythium*	*KU588235*	*Birds feather*
LK-60	*Pythium*	*KU588221*	*Dead plant materials*
LK-61	*Pythium*	*KU588225*	*Dead plant materials*
LK-62	*Pythium*	*KU588264*	*Sediments*
LK-63	*Pythium*	*KU588267*	*Sediments*
LK-64	*Pythium*	*KU588266*	*Sediments*
LK-65	*Pythium*	*KU588239*	*Birds feather*
LK-66	*Pythium*	*KU588259*	*Sediments*

Table 2: Closest indexed Pythiaceae references from NCBI database; references are all recently published

Genbank indexed species	Phylogeny	ITS Accession numbers	Ressources	Location
Phytophthora inundata	*Phytophthora Clade 6*	*KC201295*	*Ginetti,B. et al. 2012*	*Italy*
Phytophthora humicola	*Phytophthora Clade 6*	*JQ757060*	*Ginetti,B. et al. 2012*	*Italy*
Phytophthora rosacearum	*Phytophthora Clade 6*	*HQ261664*	*Robideau,G.P. et al. 2011*	-
Phytophthora drechsleri	*Phytophthora Clade 6*	*KF444068*	*Ford,B. and Balci,Y.2013*	*Italy*
Phytophthora Pgchlamydo	*Phytophthora Clade 6*	*KJ755194*	*Hansen,E.M. et al. 2014*	-
Phytophthora gonaappodyides	*Phytophthora Clade 6*	*KF444065*	*Ford,B. and Balci,Y.2013*	*Italy*
Phytophthora CAL2011b	-	HQ643355	*Robideau,G.P. et al. 2011*	-
Phytopythium chamaehyphon	*ex Pythium Clade K*	HQ643374	*Robideau,G.P. et al. 2011*	-
Phytopythium helicoides	*ex Pythium Clade K*	HQ643382	*Robideau,G.P. et al. 2011*	-
Pythium amazonianum	*Pythium Clade K*	HQ261728	*Robideau,G.P. et al. 2011*	-
Pythium vexans	*Pythium Clade K*	HQ643954	*Robideau,G.P. et al. 2011*	-
Pythium sylvaticum	*Pythium Clade F*	HQ643845	*Robideau,G.P. et al. 2011*	-
Pythium parocaendrum	*Pythium Clade F*	HQ643734	*Robideau,G.P. et al. 2011*	-
Pythium kandovanese	*Pythium Clade E*	KP723168	*Chenari Bouket,A. et al.*	*Iran*

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Pythium rostratum	*Pythium Clade E*	HQ643767	*Robideau,G.P. et al. 2011*	-
Pythium rostratifringens	*Pythium Clade E*	KF806440	*Moschard,M. et al. 2013*	*France*
Pythium aphanidermatum	*Pythium Clade A*	HQ643442	*Robideau,G.P. et al. 2011*	-
Pythium deliense	*Pythium Clade A*	KF836356	*Paul,B et al. 2013*	*Thailand*
Pythium monospermum	*Pythium Clade A*	HQ643442	*Robideau,G.P. et al. 2011*	-
Pythium adhaerens	*Pythium Clade A*	HQ643415	*Robideau,G.P. et al. 2011*	-
Pythium porphyrae	*Pythium Clade A*	HQ643753	*Robideau,G.P. et al. 2011*	-
Pythium chondricola	*Pythium Clade A*	HQ643499	*Robideau,G.P. et al. 2011*	-
Pythium arrhenomanes	*Pythium Clade B*	HQ643472	*Robideau,G.P. et al. 2011*	-
Pythium vanterpolii	*Pythium Clade B*	HQ643950	*Robideau,G.P. et al. 2011*	-
Pythium inflatum	*Pythium Clade B*	HQ643566	*Robideau,G.P. et al. 2011*	-
Pythium valencianum	*Pythium Clade B*	EU003443	*Coffey,M.D. et al. 2007*	*Spain*
Pytium plurosporum	*Pythium Clade B*	HQ643749	*Robideau,G.P. et al. 2011*	-
Pythium angustatum	*Pythium Clade B*	HQ643437	*Robideau,G.P. et al. 2011*	-
Pythium catenulatum	*Pythium Clade B*	KM061655	*Sarowar,M.N. et al 2014*	*UK*
Pythium BP2013k	*Pythium Clade B*	KF836354	*Robideau,G.P. et al. 2011*	-
Pythium CAL2011f	*Pythium Clade B*	HQ643814	*Robideau,G.P. et al. 2011*	-
Pythium AL2010	*Pythium Clade B*	HQ261734	*Robideau,G.P. et al. 2011*	-
Pythium caudatum	*Pythium Clade B*	HQ643136	*Robideau,G.P. et al. 2011*	-
Pythium capillosum	*Pythium Clade B*	HQ643483	*Robideau,G.P. et al. 2011*	-
Pythium flevoense	*Pythium Clade B*	HQ643539	*Robideau,G.P. et al. 2011*	-
Pythium sukuiense	*Pythium Clade B*	HQ643836	*Robideau,G.P. et al. 2011*	-
Pythium aquatile	*Pythium Clade B*	HQ643446	*Robideau,G.P. et al. 2011*	-
Pythium oopapillum	*Pythium Clade B*	HQ643717	*Robideau,G.P. et al. 2011*	-
Pythium pachycaule	*Pythium Clade B*	HQ643727	*Robideau,G.P. et al. 2011*	-
Pythium cf dictyosporum	*Pythium Clade B*	HQ643495	*Robideau,G.P. et al. 2011*	-
Pythium aff diclinum	*Pythium Clade B*	HQ643417	*Robideau,G.P. et al. 2011*	-
Pythium groupF	*Pythium Clade B*	HQ643789	*Robideau,G.P. et al. 2011*	-
Pythium coloratum	*Pythium Clade B*	HQ643504	*Robideau,G.P. et al. 2011*	-

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Pythium lutarium	*Pythium Clade B*	HQ643682	*Robideau,G.P. et al. - 2011*
Pythium disstocum	*Pythium Clade B*	KM061701	*Sarowar,M.N. et al UK 2014*

Qualitative Diversity and substrate distribution

Qualitative molecular identification highlighted up to 49 *Pythium*, 10 *Phytopythium* and 07 *Phytophthora* species; few repeated sequences were eliminated and a total number of 66 data was retained to make the object of the current study.

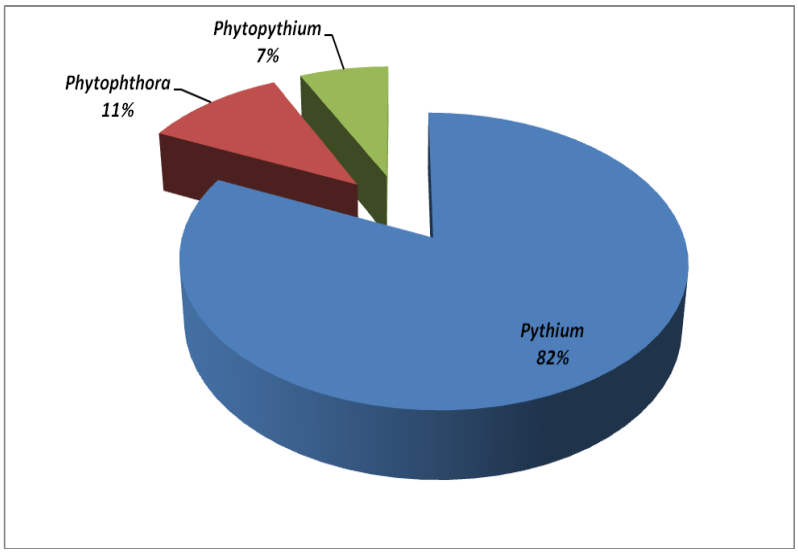

Figure 2: Graphical distribution of highlighted *Pythiaceae*, with a clear predominance of the genus *Pythium*

The qualitative distribution represented in the figure 2 show more diversity in *Phytophthora* compared to *Phytopythium* identification, and a large invasion of the ecosystem by *Pythium* population.

Nutritional preferences toward different substrates capturing Oomycetes in the lake are shown in figure 3.

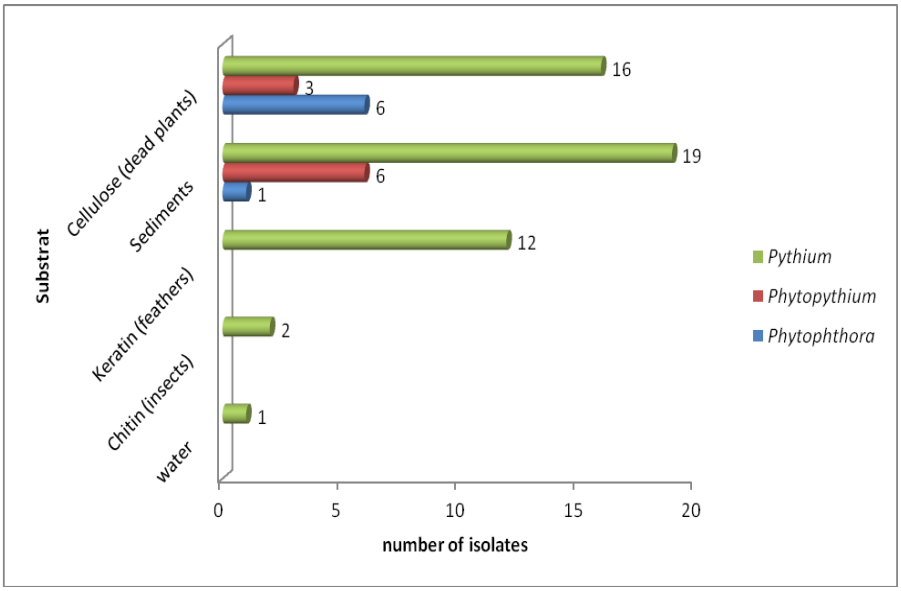

Figure 3: Graphical distribution of highlited *Pythiaceae*, isolated from five different substrates

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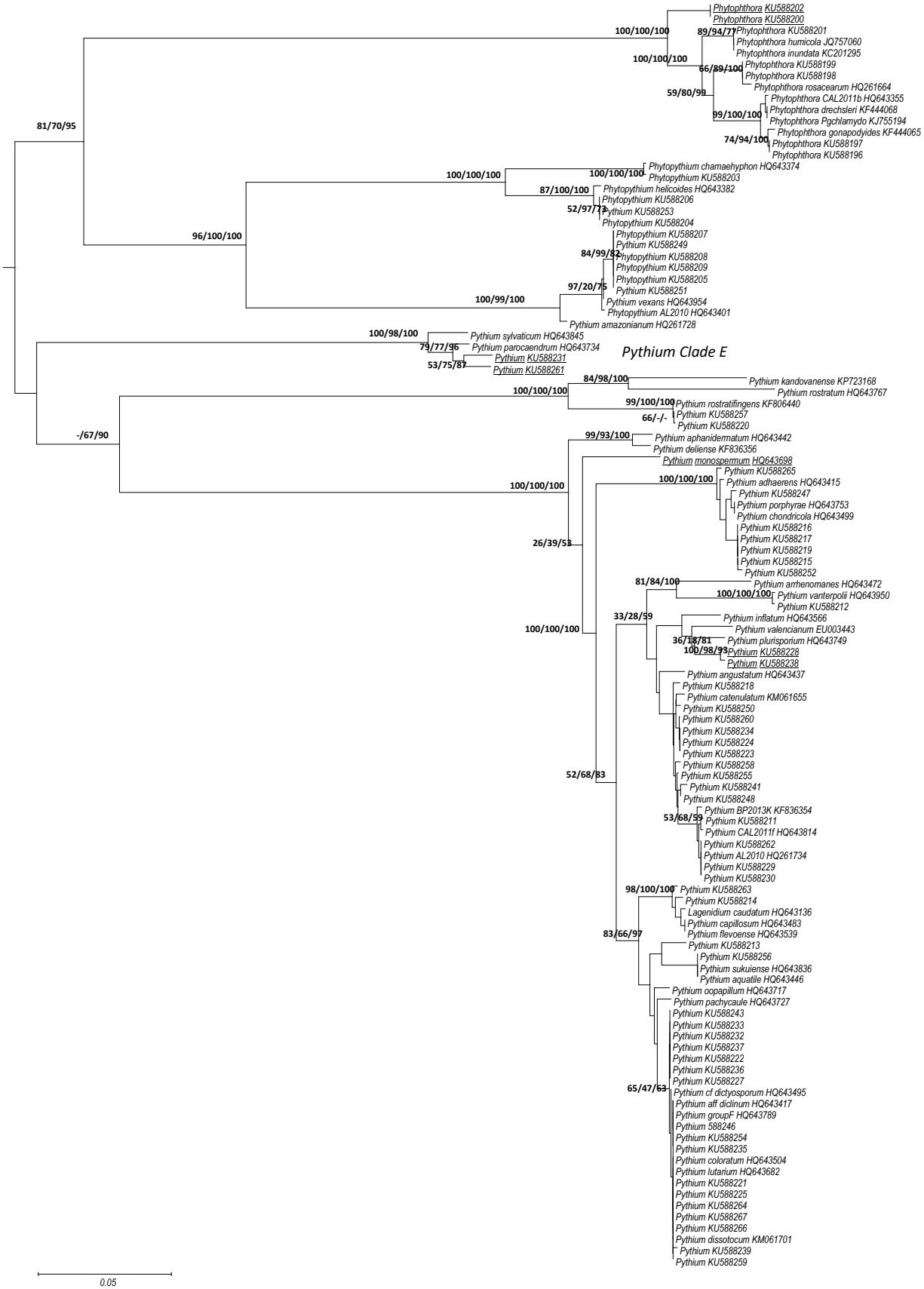

Figure 4: molecular Phylogram of 66 isolates from Oubeira Lake and 44 references, constructed with the combined Minimum Evolution, RAxML and Mr. Bayes methods (the bootstrap values are respectively indicated at the branch points), analyzing ITS1 5.8S subunit and ITS2 locci of the rDNA (Cook et al. 2000)

It demonstrates that sediments, and less abundantly plant and animal substrates are overcharged of *Pythiums* species, whereas *Phytophthora* are phytopathogens, more involved in either decomposing dead plant materials or causing the death by themselves. *Phytophthora* and *Phytopythium* were not found in animal materials neither swimming in free water samples. *Phytophthora* includes the largest number of soilborne species (Ahumada *et al*., 2013); accordingly, pathogens against vegetable cultures surrounding the ecosystem could occur during rives movements in wet seasons rather than direct irrigation from the lake.

Sediments are a favourable reservoir of the largest diversity of *Pythium* species showing the result of a very high biodegradation activity shifting into what we call the vase. Dead plant submerged materials host the highest amount of Oomycetes, whence all the identified *Phytophthora* isolates and the majority *Phytopythium* species.

Phylogeny

Pythiales are Oomycetes known for occupying a middle evolutionary position, between the most primitives saprophytic aquatic *Saprolegniales*, and advanced animal/plant pathogen *Peronosporales* (Gaüman, 1918). Phylogenic analyses reconstructed the phylogram represented in figure 3, with 06 distinct clusters; ITS data were highly significant to show a large diversity among the studied lineages. 49 isolates are grouped within 36 known species of the genus *Pythium*, arranged in clades A, B, E, F, and further clade K as described in Levesque and de Cock 2004; the evolution history narrates an earlier separation of the genus *Pythium* from both *Phytophthora* and *Phytopythium* sharing more recent common ancestor, and which respectively including 08 and 09 isolates from the Oubeira Lake.

DISCUSSION

Pythium diversity

Based on molecular systematic analyses, *Pythium* taxonomy was designated by Lévesque & de Cock (2004), to be arranged into 11 clades labelled from A to K; the latter has been recently renamed as *Phytopythium* . These clades are basically supported by the morphological taxonomic key of Van Der Plaats-Niterink (1981). This classification is strongly supported by morphological characteristics showing an evolution history of the most primitive species with globose internally proliferating sporangia of the subclades E and G, to perfect globose sporangia within subclades J, I and F, then contiguous grouped in subclades D and C to filamentous inflated and perfect filamentous sporangia forming subclades A and B; the latter represent the most evolved lineages known as *Vanterpolii* subclade B1 (Lévesque and De Cock, 2004).

The limits between this dominant genus and its relatives in the current study, are drawn by the clade F lineage including P. *Sylvaticum* and P. *Parocaendrum*; two isolates from the lake, namely KU588261 and KU588231 are bunched in the same clade, but highly diverge from each other and from the most similar references. The antagonistic action against pathogen Fungi of animals and plants characterizing clade F members (El Yassimi *et al*., 2004), makes the subject of a new study of the possibility to utilise them as biocontrol agents against agro-forestal Mycoses.

Phylogenic analysis mainly places 38 of our isolates inside Clade B., with 17 isolates similar to *Pythium Vanterpolii, P. arrhenomonas*, *P. Plurisporium*, *P. inflatum*, *P. aungustatum*, *P. catenulatum*, and immediate neighbours *P. Valencianum*, *P. BP2013k*, *P. CAL2011f* and *P. AL2010*. however, the most variated cluster remains *apleroticum* subclade B2 which gathers 21 isolates, related to *P. diclinum*, *P. lutarium*, *P. Group F*, *P. coloratum*, *P. dissotocum*, *P. cf. dictyosporum*, *P. pachycaule*, P. *oopapillum*, *P. sukuiense*, *P. aquatile*, *P. caudatum*, *P. capillosum*, *P. flevoenses*.

The strains KU588238 and KU588228 are 98% identical but present a weak similarity to *P. Valencianum*, a divergent mediterranean species isolated in the Spanish coastal area of Valencia, which never been completely identified by the authors. Afterwards, Paul B. *et al.* 2008, could identify *P. kashmirense*, a homonym of *P. valencianum*, sampled in India during the temperate season. The fact could strongly argue a kind of adaptation to warmer environment, among the species found in the Oubeira Lake.

Pythium chondricola, *porphyrae* and *adhaerens* are representing *Pythium* species from clade A, *P. chondricola*, *P. porphyrae*, and *P. adhaerens* show different host/substrate-specific relationships (Matsumoto *et al*., 1999, Levesque and De Cock, 2004). *Pythium porphyrae* and *Chondricola* are recognized as the only pathogen of red rot algae disease (Kajeiama, 2014), while *P. adhaerens* has been isolated from soil. It's important to precise that clade A includes also *P. aphanidermatum* which morphologically behaves similarly to clade B species in warmer regions by producing filamentous inflated sporangia (Levesque and de cock 2004). *P. aphanidermatum* is harmful for vegetable cultures (Alsheikh *et al*., 2012) and can be causal of tomato diseases cultivated in the watershed. *Pythium monospermum* utilised in this study, shifts in an intermediate branching from clade B towards clade A, and high temperature could be the main mutational stimulator.

Clade E is represented by the isolates KU588220 and KU588257, gathered to *P. rostratifrengens* rather than *P. rostratum* and *P. kandovanense*. Although known as grass pathogens, they behave as non obligate biotrophes. They usually attack cultivated crops at the foliar and root levels, in association with fungal communities (Nzungize *et al.,* 2012).

Phytophthora diversity

While the isolate KU588198 is at 100% identical to *Phytophthora* rosacearum, the taxon KU588202 even contiguous to *Phytophthora* taxa from cade 6, both isolates KU588200 and KU588202 are identical to each other (100% identity supported by RAxML and Mr. Bayes posterior probability methods), and clearly separated from *rosacearum*, *inundata* and *gonapodyides* subclades.

On the basis of its morphology and habitat it was tentatively assigned to Phytophthora species are commonly saprotrophic on plant detritus in ponds and rivers (Jung *et al*., 2015).

Phytopythium diversity

Phytopythium bifurcates into both *chamaehyphon* more related to *Phytophthora*, and *vexans* that still keeps *Pythium amazonianum* and closest taxa from clade K Levesque and de Cock 2004 classifiction; the latter includes more *Phytopythium* isolates found in the Oubeira lake. Besides sharing evolutionary phylogeny, *Phytopythium* behaves in the same way as *Phytophthora* and is associated to plant material biodegradation.
Globally, the identified isolates are classified among the most propagate aquatic microbes all over the world, specifically targeting reticulate structures inside natural niches.

CONCLUSION

The Oubeira Lake hosts widespread oomycetes which mainly occur as non biotrophic or facultative biotrophic microorganisms. They predominantly belong to the genus Pythium, with a high phylogenic diversity, including adapted species to temperate climates. Parasiting true fungi or algae, they can play a crucial role in preventing ecological disturbance, diseases and Eutrophication.

Likely new species are phylogenically separated from their closest indexed relatives, mainly identified in the Mediterranean periphery or comparable areas.

The inventory of native Pythiaceae microbiota predominantly occurring in Oubeira inland freshwaters, has revealed a large distribution of the genus Pythium.

Few Phytophthora and Phytopythium species were identified, with an exclusive preference for decomposing plants and also occurring in sediments, due to their phytopathogenic behaviour.

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