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REGULAR PAPER

First records of Protosteloid Amoebae (Eumycetozoa) from the Democratic Republic of the Congo

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Background – The first records of Protosteloid Amoebae in the Democratic Republic of the Congo are discussed in the present paper. This survey on Protosteloid Amoebae is the first from Central Africa; the previous records for the African continent were restricted to Egypt, Kenya, Malawi, Tanzania and Uganda.
Methods – Aerial litter samples, collected in 2010 during the "Boyekoli Ebale Congo" expedition in the Congo River basin between the cities of Kisangani and Bumba, were put into culture on wMY medium, a weak malt yeast nutrient agar medium.
Results – The aerial litter cultures revealed 23 species representing 70% of the total number of species described worldwide. Two of these taxa, *Schizoplasmodiopsis reticulata* and *Schizoplasmodium seychellarum*, are new records for the African continent. The isolate LHI05 was observed for the first time on a substrate collected outside Hawai'i. In addition, 5 unknown taxa were observed. A selection of micrographs is presented of the new recorded species, the unknown taxa and all their related species observed in this study.
Conclusion – The high species diversity observed on a limited number of samples suggests that the investigated region is, together with Hawai'i, one of the world's tropical hotspots for Protosteloid Amoebae.

Key words – Aerial litter, Central Africa, D.R. Congo, Eumycetozoa, Protosteloid Amoebae, Species richness.

INTRODUCTION

Protosteloid Amoebae, formerly known as protostelids (Olive 1967), represent a small group of thirty three species described worldwide (Spiegel et al. 2007b). Identification of the taxa is primarily based on morphologic characters of the microscopic fruiting bodies or sporocarps, composed of a translucent stalk supporting one or more spores. Protostelids were traditionally placed together with and considered as progenitors of two other groups of fruiting amoebae, myxomycetes and dictyostelids in the Eumycetozoa (Olive 1975). Recent molecular phylogenetic research by Shadwick et al. (2009) revealed that protostelids are not monophyletic and that only a few of them are related to myxomycetes and dictyostelids. Furthermore the study showed that the protostelids can be divided into at least seven non-related but well supported clades, some showing affiliations with known groups of non-fruiting amoebae, scattered throughout the Amoebozoa. For this reason it was proposed to replace the name protostelids by Protosteloid Amoebae (Shadwick et al. 2009).

Data about the distribution and ecology of these protists is not spread evenly throughout the world. North America and Europe are relatively well studied, but from other temperate regions less data are available (Ndiritu et al. 2009, de Haan 2011). Most parts of the tropics are still unexplored. A few surveys have been published from South and Central America, e.g. Costa Rica (Stephenson & Moore 1998, Moore & Stephenson 2003) and Puerto Rico (Stephenson et al. 1999, Moore & Spiegel 2000). Data have been published from Australia (Powers & Stephenson 2006), and results from a series of surveys from Hawai'i have been presented (Spiegel et al. 2007a). Few records were known from Africa, restricted to Egypt and Uganda (Olive & Stoianovitch 1969, 1972, 1976) until recently Ndiritu et al. (2009) published a large survey carried out in the Aberdare region, Kenya, including some data from Malawi and Tanzania.

The present paper deals with the first records of Protosteloid Amoebae from the Democratic Republic of the Congo (D.R. Congo).

Aerial litter samples were collected during a multidisciplinary expedition in D.R. Congo in May and June 2010. The expedition was an initiative of the Congo 2010 Consortium composed of three Belgian and two Congolese institutes, the

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Royal Museum for Central Africa, the Royal Belgian Institute of Natural Sciences and the National Botanic Garden of Belgium, the University of Kisangani and the National Herbarium of D.R. Congo at Yangambi (www. congobiodiv. org). It was organized in 2010 on the occasion of the international year of biodiversity and of the 50th anniversary of the independence of D.R. Congo. The expedition was given the title "Boyekoli Ebale Congo", Lingala for "the study of the Congo River" (Congo Biodiversity Initiative 2010). The aim of the expedition was to assess the biodiversity in and along the Congo River between the cities of Kisangani and Bumba in locations with almost no to extreme anthropogenic impact. The aim of the present study was to ascertain whether Protosteloid Amoebae were present in the studied locations and if so, to obtain the highest possible number of species from the chosen substrate type – aerial litter. An important goal was to observe rare taxa, because little is known of their morphological variation, distribution and substrate preferences.

MATERIALS AND METHODS

The region visited during the expedition in 2010 is located in the Congo River basin between the cities of Kisangani (Tshopo district, Orientale province) and Bumba (Mongala district, Équateur province) (Virgilio et al. 2011). Additional localities along three of the major tributaries of the Congo River, the rivers Itimbiri, Lomami and Aruwimi, were also explored. The sampling locatities were situated at an elevation of 357–382 m above sea level. The climate in this region is tropical, hot and humid, type Af of the Köppen-Geiger climate classification system (Kottek et al. 2006), the average annual maximum temperature is 29°C, the minimum 21°C, and the annual relative humidity is 86%. The average monthly precipitation in Basoko, a town situated in the center of the visited region, is highest in April (200 mm) and in October (216 mm), the driest month is January with an average of 70 mm (data from www.weatherbase.com). Sampling took place in May (avg. ppt. 176 mm), towards the end of the rainy season.

Samples were taken at five localities: Yaekela, Engengele, Kona 1, Kona 2 and Yafira. The habitats ranged from tropical lowland rainforest with no, almost pristine, over moderate to extensive anthropogenic impact (table 1). We considered occasional selective tree cutting as moderate, and forest fallows as extensive anthropogenic impact.

Thirteen samples of aerial litter or dead primary tissue still attached to living plants for the culturing of Protosteloid Amoebae were collected by M. de Haan and C. Cocquyt (table 1). Aerial litter and ground litter are the microhabitats with the highest species abundance and diversity of Protosteloid Amoebae (Moore & Spiegel 2000, Spiegel et al. 2004). As such for this survey aerial litter was given preference to ground litter. Moreover cultures of the latter substrate type are known to be very rapidly overgrown with moulds thus hindering observation. All collected material was air-dried on site in separate paper bags. Each sample was put into culture and examined in the laboratory of the National Botanic Garden of Belgium by M. de Haan. Duplicates of the samples were sent to the Department of Biological Sciences, University of Arkansas (USA) to be cultured and examined by A. Tice, G. Zahn and F.W. Spiegel.

The method of culturing by Spiegel et al. (2007b) after Olive (1975) was followed. Each substrate sample was cut into pieces of about 1 cm wide and 2 cm long and soaked in sterile water for 30 min. About eight pieces or strips per sample were distributed evenly on a 9 cm Petri dish containing a weak malt yeast nutrient agar medium (1 liter of wMY medium: 0.002 g malt extract, 0.002 g yeast extract, 0.75 g K_2HPO_4 and 15 g agar in distilled water) representing one primary isolation plate (PIP). For each sample a minimum of three PIPs were prepared for observation in order to obtain as many taxa as possible.

After an incubation-period of three days at room temperature the PIPs were examined under a compound microscope (x200 and x400 magnification) every three days during the first three weeks, once a week for the next 8 weeks and from then on once a month. Photographs were taken by M. de Haan with a digital single-lens reflex camera (Olympus E-3) mounted on the microscope (Pro.Way, BK 5000) using plan infinity x20 or x40 objectives. A copy of all images taken during this study will be deposited at the National Botanic Garden of Belgium and the "Centre de Surveillance de la Biodiversité" (CSB) in Kisangani (D.R. Congo). The collected samples are deposited in the National Botanic Garden of Belgium and duplicates of these samples in the Department of Biological Sciences, University of Arkansas, USA.

Results of the PIP counts performed at the laboratory of the National Botanic Garden of Belgium and at the department of Biological Sciences, University of Arkansas (USA) were collated. Species abundances were determined and divided in four classes according to Ndiritu et al. (2009). A record of a taxon on one strip corresponds with a positive observation made in one field of view. The relative abundance is the percentage of the number of records made on all strips and is divided by total number of strips made on the PIPs from all the samples. Relative abundance classes are > 10% abundant (A); 10-5% common (C); 4.9–1% occasional (O); < 1% rare (R).

The names of the Protosteloid Amoebae and myxomycetes species follow the nomenclature proposed by Lado (2005–2013). The names of the substrate species are consistent with the nomenclature of *The Plant List* (2010–2013).

RESULTS AND DISCUSSION

The taxa observed during the present study are listed in table 2 with their respective relative species abundance and occurrence on the specific substrate samples. The different substrate species are presented per locality in table 1, with the respective Protosteloid Amoebae taxa observed on the PIPs. A selection of micrographs of the new recorded species, the unknown taxa and all their related species observed during this study, is provided on figure 1.

A total of twenty two species of Protosteloid Amoebae and one minute myxomycete, *Echinostelium bisporum*, which is usually treated as a Protosteloid Amoeba (Ndiritu et al. 2009), were observed on the PIPs. This represents 70% of the thirty three species known worldwide, which is a high

Table 1 – Sample localities with the collection date, coordinates, vegetation type, sample number and its corresponding substrate and the Protosteloid Amoebae recorded (abbreviations of the taxa are given in table 2), number of species (Spp), taxa of the *Protostelium mycophaga* complex (Pm s.l.) and unknown taxa (N.N.) per sample.
Level of anthropogenic disturbance indicated as follows in the column with information on the vegetation: 1 = none, 2 = moderate, 3 = extreme.

Locality	Collection date	Coordinates	Vegetation	Sample number	Substrate	Taxa	Spp	Pm s.l.	N.N.
Yaekela	4 May 2010	0.81183° N 24.28276° E	clearing in tropical lowland rain forest that is regularly flooded – 3	MdH-DRC-Proto001	*Placodiscus* sp. (Sapindaceae)	Ca, Eb, Eo, Pm, Pn, Sps, Sr, Si	8	1	0
Yaekela	4 May 2010	0.81183° N 24.28276° E	clearing in tropical lowland rain forest that is regularly flooded – 3	MdH-DRC-Proto002	*Musanga cecropioides* R.Br. (Utricaceae)	Ca, Eb, Eo, Ez, Mp, Pa, Pm, Pn, Sa, Se, Sps, Sv, Si, Sp2	13	1	1
Yaekela	4 May 2010	0.81199° N 24.28249° E	small banana plantation in tropical lowland rain forest – 3	MdH-DRC-Proto003	*Musa* sp. (Musaceae)	Ca, Cr, Eb, Eo, Ez, Ng/Ct, Pa, Partic, Pm, Pmvc, Pml, Pmr, Pn, Ppyr, Sa, Sps, Sc, Si, Sv, Ta, Sp1, Sp5	17	4	2
Engengele	10 May 2010	2.05898° N 22.70203° E	flooded river bank with reed (*Phragmites* sp.) – 3	MdH-DRC-Proto004	*Irvingia smithii* Hook.f. (Irvingiaceae)	non observed	0	0	0
Kona 1	11 May 2010	2.04096° N 22.78805° E	tropical lowland rain forest – 2	MdH-DRC-Proto005	*Microsorum punctatum* (L.) Copel. (Polypodiaceae)	Ca, Cr, Eb, Eo, Ng/Ct, Pa, Partic, Pm, Pmvc, Pmr, Pn, Sa, Sps, Sr, Sv, Sc, Ss, Se, Si, Ta, Sp2, Sp3, Sp6	18	3	3
Kona 1	11 May 2010	2.04096° N 22.78805° E	tropical lowland rain forest – 2	MdH-DRC-Proto006	*Chromolaena odorata* (L.) R.M.King & H.Rob. (Compositae)	Ca, Eb, Pa, Pm, Sa, Sps, Se, Si	8	1	0
Kona 1	11 May 2010	2.04096° N 22.78805° E	tropical lowland rain forest – 2	MdH-DRC-Proto007	*Rhabdophyllum* sp. (Ochnaceae)	Ca, Eo, Mp, No, Pa, Pm, Sa, Sps, Sr, Sv, Sc, Se, Si, Ta, Sp4, Sp5	14	1	2
Kona 1	11 May 2010	2.04046° N 22.78787° E	tropical lowland rain forest – 2	MdH-DRC-Proto008	*Elaeis guineensis* Jacq. (Arecaceae)	Ca, Eo, Pm, Pn, Sps, Si	6	1	0
Kona 1	12 May 2010	2.04096° N 22.78805° E	tropical lowland rain forest – 2	MdH-DRC-Proto011	*Musa* sp. (Musaceae)	Ca, Eb, No, Pa, Pm, Pml, Pn, Sps, Sc, Si	9	2	0
Kona 2	12 May 2010	2.05188° N 22.79409° E	tropical lowland rain forest – 1	MdH-DRC-Proto009	*Cola* sp. (Malvaceae)	Ca, No, Pm, Sa, Sps, Sv, Sc, Si, Ta	9	1	0
Kona 2	12 May 2010	2.05188° N 22.79409° E	tropical lowland rain forest – 1	MdH-DRC-Proto010	Maranthaceae	Ca, Ez, Pa, Pf, Pm, Pml, Sa, Sps, Sv, Sc, Si, Ta	11	2	0
Kona 2	12 May 2010	2.05604° N 22.79514° E	tropical lowland rain forest – 1	MdH-DRC-Proto012	*Millettia* sp. (Leguminosae)	Ca, Eb, Mp, Ng/Ct, No, Pm, Ss, Ta	8	1	0
Yafira	31 May 2010	0.70273° N 24.20061° E	tropical lowland rain forest, dominated by *Gilbertiodendron* sp. – 1	MdH-DRC-Proto013	unknown tree	Ca, Sa, Sps, Sr	4	0	0

Table 2 – List of the taxa observed in the present study, conventional abbreviations of each of the recorded taxa, relative abundances divided into four classes: > 10% abundant (A); 10–5% common (C); 4.9–1% occasional (O); < 1% rare (R) (abundance classes according to Ndiritu et al. 2009) and occurrence on the specific substrate samples.
The numbers of the substrates correspond with the substrate number as given in table 1.

Taxa observed in the present study	Abbreviation	Relative abundance class	Occurrence on substrate (MdH-DRC-Proto)
Cavostelium apophysatum L.S.Olive	Ca	A	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
Clastostelium recurvatum L.S.Olive	Cr	O	3, 5
Echinosteliopsis oligospora Reinhardt & L.S.Olive	Eo	C	1, 2, 3, 5, 7, 8
Echinostelium bisporum (L.S.Olive & Stoian.) K.D.Whitney & L.S.Olive	Eb	C	1, 2, 3, 5, 6, 11, 12
Endostelium zonatum (L.S.Olive & Stoian.) W.E.Benn. & L.S.Olive	Ez	O	2, 3, 10
Microglomus paxillus L.S.Olive & Stoian.	Mp	O	2, 7, 12
Nematostelium gracile (L.S.Olive & Stoian.) L.S.Olive or *Ceratiomyxella tahitiensis* L.S.Olive & Stoian.	Ng/Ct	O	3, 5, 12
Nematostelium ovatum (L.S.Olive & Stoian.) L.S.Olive & Stoian.	No	O	7, 9, 11, 12
Protosporangium articulatum L.S.Olive & Stoian.	Partic	R	3, 5
Protosteliopsis fimicola (L.S.Olive) L.S.Olive & Stoian.	Pf	R	10
Protostelium arachisporum L.S.Olive	Pa	A	2, 3, 5, 6, 7, 10, 11
Protostelium mycophagum var. *mycophagum* L.S.Olive & Stoian.	Pm	A	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12
Protostelium mycophagum var. *crassipes* L.S.Olive & Stoian.	Pmvc	R	3, 5
Protostelium mycophagum type little	Pml	O	3, 10, 11
Protostelium mycophagum type repeater	Pmr	O	3, 5
Protostelium nocturnum Spiegel	Pn	O	1, 2, 3, 5, 8, 11
Protostelium pyriformis L.S.Olive & Stoian.	Ppyr	R	3
Schizoplasmodiopsis amoeboidea L.S.Olive & K.D.Whitney	Sa	C	2, 3, 5, 6, 7, 9, 10, 13
Schizoplasmodiopsis pseudoendospora L.S.Olive, M.Martin. & Stoian.	Sps	A	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 13
Schizoplasmodiopsis reticulata L.S.Olive & Stoian.	Sr	O	1, 5, 7, 13
Schizoplasmodiopsis vulgaris L.S.Olive & Stoian.	Sv	O	2, 3, 5, 7, 9, 10
Schizoplasmodium cavostelioides L.S.Olive & Stoian.	Sc	O	3, 5, 7, 9, 10, 11
Schizoplasmodium seychellarum L.S.Olive & Stoian.	Ss	O	5, 12
Soliformovum expulsum (L.S.Olive & Stoian.) Spiegel	Se	O	2, 5, 6, 7
Soliformovum irregularis (L.S.Olive & Stoian.) Spiegel	Si	A	1, 2, 3, 5, 6, 7, 8, 9, 10, 11
Tychosporium acutostipes Spiegel, D.L.Moore & J.Feldman	Ta	C	3, 5, 7, 9, 10, 12
Proto-DRC-Sp1	Sp 1	R	3
Proto-DRC-Sp2 = LHI05	Sp 2	O	2, 5
Proto-DRC-Sp3	Sp 3	R	5
Proto-DRC-Sp4	Sp 4	R	7
Proto-DRC-Sp5	Sp 5	O	3, 7
Proto-DRC-Sp6	Sp 6	R	5

yield considering that only thirteen samples were collected. A few isolated records are known from Egypt and Uganda (Olive & Stoianovitch 1969, 1972, 1976). The only other published study on Protosteloid Amoebae from Africa (Ndiritu et al. 2009) is a large survey in East Africa, where eighteen species (including *E. bisporum*) were observed on forty six aerial litter samples, and a total of twenty one when including the results from forty nine ground litter and forty nine bark samples. Other surveys in tropical forests of Costa Rica (Stephenson & Moore 1998, Moore & Stephenson 2003), Puerto Rico (Stephenson et al. 1999, Moore & Spiegel 2000) and Australia (Powers & Stephenson 2006) have produced sixteen, thirteen and eight species respectively. Presently Hawai'i holds the highest species diversity; all thirty three described Protosteloid Amoebae have been recorded from this tropical island (Spiegel et al. 2007a).

An average of 9.5 species (range 0–18) was observed on the thirteen substrates from D.R. Congo which is very high. When the unknown taxa are included the average increases to 10.1 (range 0–21). Three plates were prepared per sample.

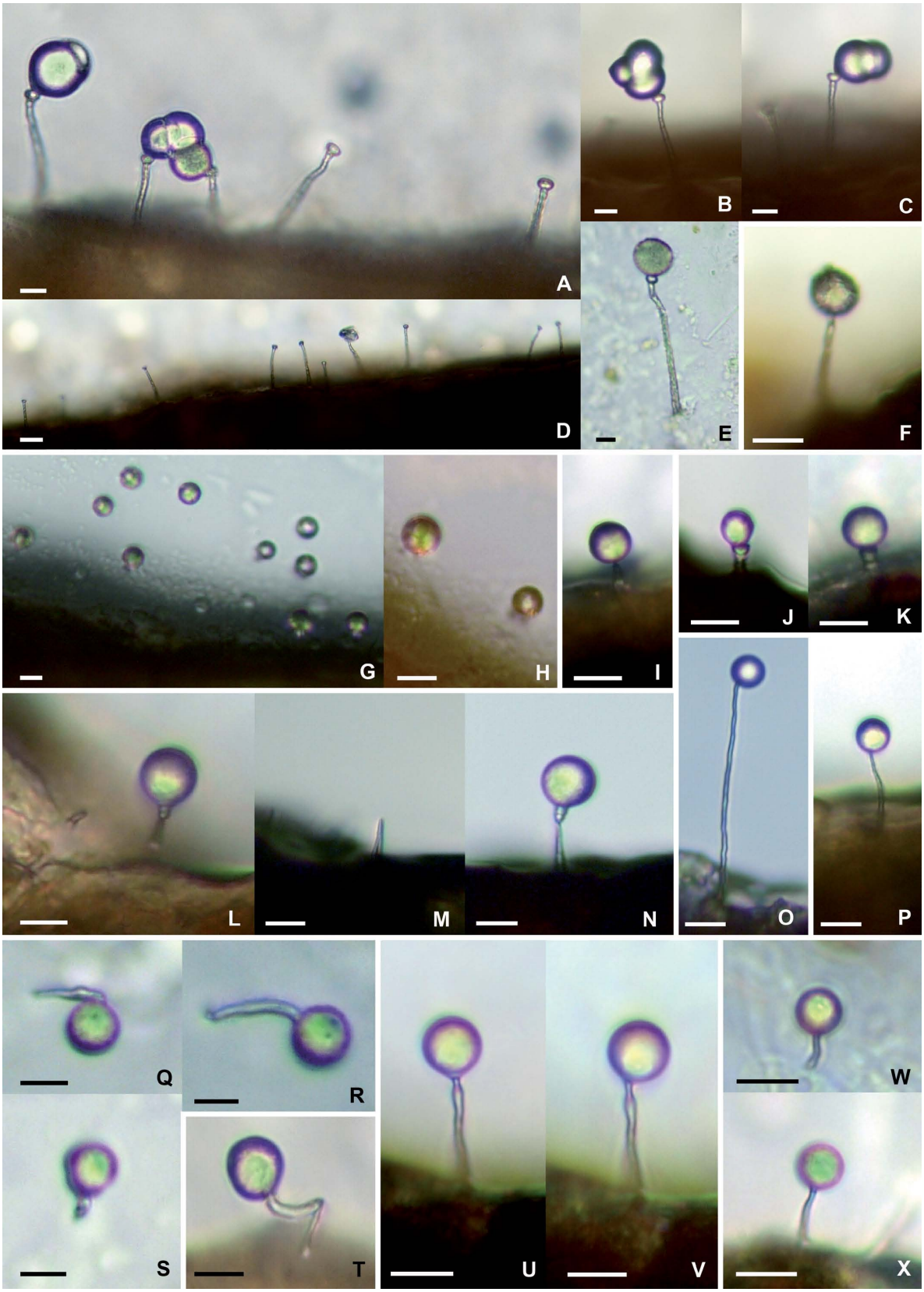

Figure 1 – Images taken from the PIPs of the new records for Africa, of the unknown taxa and their related species observed in this study: A–E, *Schizoplasmodium seychellarum*; F, *Schizoplasmodiopsis reticulata*; G & H, Proto-DRC-sp1; I, *Microglomus paxillus*; J & K, *Cavostelium apophysatum*; L–N, Proto-DRC-sp2; O, Proto-DRC-sp6; P, *Protosteliopsis fimicola*; Q–S, *Clastostelium recurvatum*; T, Proto-DRC-sp3; U & V, Proto-DRC-sp4; W & X, Proto-DRC-sp5. Scale bar = 10 µm, except D = 50 µm.

When the substrates yielded an initial high species number and/or interesting taxa, additional plates were set up to confirm observations. In a few cases more taxa were added to the species list of a particular substrate. A reason for the high overall species yield could be the ideal weather conditions at the time of sampling, with heavy rainfall every 3 to 5 days and dry weather in between. Most samples were collected 2 to 3 days after rainfall. The only sample from which no observations of Protosteloid Amoebae or myxomycetes were made originated from a tree, *Irvingia smithii*, growing on the flooded bank of the river Itimbiri. Apparently the collected leaves were still too fresh, there was still a green hue from chlorophyll that was not entirely degraded. All cultures made from this sample were overgrown with moulds within a few days, probably due to the constant high humidity of the substrate.

Substrates from different plant species supported varying number of Protosteloid Amoebae. Highest species numbers were obtained from *Microsorium punctatum*, *Musa* sp. (banana tree), and *Rhabdophyllum* sp. respectively yielding eighteen, seventeen and fourteen species, and in addition three, two and two of the unknown taxa respectively (table 1). *Musa* sp. was collected in two different locations: one from a small field in the forest near the village of Yaekela with extensive anthropogenic influence and another one from Kona 1 which is situated about 215 km north-west from Yaekela, at the entrance of the lowland tropical forest with moderate human interference, i.e. occasional selective tree cutting. This sample from Kona 1 yielded nine species. At the moment it is not possible to attribute the role of human interference in these locations when it comes to determining species diversity of Protosteloid Amoebae (see Ndiritu et al. 2009).

Below an annotated account is given of each species encountered during the present study, with special attention to their taxonomy, distribution and ecology.

Cavostelium apophysatum is the most common species recorded in the study, present on twelve of the thirteen substrates, closely followed by *Protostelium mycophagum sensu lato* and *Schizoplasmodiopsis pseudoendospora* each present on eleven samples. *Soliformovum irregularis* was observed on ten substrates (table 1). *C. apophysatum* is one of the most common species in tropical areas (Ndiritu et al. 2009), which is confirmed in the present study. *Schizoplasmodiopsis pseudoendospora* and *Soliformovum irregularis* are both very common in surveys from all parts of the world. The former species tends to prefer higher temperatures and the latter shows a preference for a cooler and wetter climate (Ndiritu et al. 2009, Aguilar et al. 2011).

Protostelium mycophagum is common in all climate zones especially on aerial litter, but most likely it represents a complex comprising subspecific taxa and/or cryptic species (Spiegel et al. 2007b, Ndiritu et al. 2009). Further in-depth molecular phylogenetic study will reveal more on the presence or absence of possible cryptic species. One variety, *P. mycophagum* var. *crassipes* and at least two distinct forms, conventionally designated as Pm "little" and Pm "repeater" (Spiegel et al. 2007b) were observed respectively three and two times on different substrates (table 2). *P. mycophagum* var. *mycophagum* is usually observed forming large colonies, although in this study only small colonies of a few dozen and even solitary fruiting bodies were seen.

Protostelium arachisporum is more common in tropical areas (Ndiritu et al. 2009), and was observed on seven substrates. Different forms of *P. arachisporum* with differences in spore shape and stalk length were observed in isolated colonies on four of the substrates. Here too, phylogenetic research is needed to demonstrate whether it concerns a very variable species or a species complex.

Protostelium pyriformis is not uncommon in tropical regions. However, it was found in our study on only one sample, a banana tree from Yaekela.

Another species that is expected to be more common in samples from the tropics is *Nematostelium gracile*. Aguilar et al. (2011) noted that this species shows a preference for high annual but seasonal rainfall. In the present study it was observed in three samples, *Musa* sp. from Yaekela, *Microsorium punctatum* and *Millettia* sp. from Kona 1 and 2 respectively. These results are far from conclusive because we were not able to differentiate *Nematostelium gracile* from *Ceratiomyxella tahitiensis* without the trophic stage in culture.

Two rare species, *Schizoplasmodiopsis reticulata* and *Schizoplasmodium seychellarum*, were observed for the first time on substrates from Africa. *Schizoplasmodiopsis reticulata* (fig. 1F), 20–35 µm tall, was recorded on dead leaves from three substrates in three locations. The first substrate, a tree belonging to the genus *Placodiscus*, was collected in the woodland near the village of Yaekela in a clearing with extensive anthropogenic impact, e.g. deforestation through tree cutting and shrub burning. The area is regularly flooded by the Congo River during the rainy seasons. At the time of sampling the ground was still drying. The other two substrates, the epiphytic fern *Microsorium punctatum* and a tree species, *Rhabdophyllum* sp., were collected in the location Kona 1. The third substrate is an unknown tree from a forest dominated by *Gilbertiodendron* sp. near the village Yafira on the opposite bank of the Congo River from Yaekela. *Schizoplasmodiopsis reticulata* is close to *Schizoplasmodiopsis vulgaris*, a much more common species (Spiegel & Feldman 1993). Both species are found on the same substrates. The spore of *S. reticulata* (fig. 1F), 9–12 µm in diameter, has a clearly visible ornamentation of raised ridges. *S. vulgaris* has a reticulum with low ridges, which can be seen as angular irregularities on the spore outline. In some of the observations it was difficult to distinguish these two related species.

Schizoplasmodium seychellarum (fig. 1A–E) was observed on two substrates, the epiphytic fern *M. punctatum* and a tree species belonging to the genus *Millettia*, from Kona 1 and 2. The latter location is a lowland tropical forest with almost no human impact except for hunting purposes. The morphology of the fruiting bodies, 50–85 µm tall, was consistent with the original description (Olive & Stoianovitch 1976). The stalk is robust and almost cylindrical to very slightly tapering towards the distinct ring shaped apex or apophysis. The single spore, 20–25 µm in diameter, is loosely connected to the apophysis with a low ring shaped hylum. When drying the spore wall becomes dented and wrinkled. The spores are discharged by means of lateral droplets that

swell up and burst. Olive & Stoianovitch (1976) remarked that the spore discharge is not always successful; this was indeed observed in our cultures. If there is more than one droplet or if the single droplet is not placed ideally, the spore either stays in position or gets stuck somewhere along the stalk. In that case the spores simply dry up without germinating. Sometimes the droplets did not burst and due to their extra weight the spore simply fell from the apophysis. In most surveys this rare species is found not only in low quantities of colonies but also in less than 1% of the samples, and prefers aerial litter to other substrates (Spiegel et al. 2007b). In both of our observations the large fruitings occurred in a group of about twenty fruiting bodies for a period of no more than two days on one strip of the substrate. It was only observed in PIPs prepared in the laboratory of the National Botanic Garden of Belgium.

The opposite was the case with *Endostelium zonatum* and *Protosporangium articulatum* both observed exclusively in the laboratory of the University of Arkansas. *E. zonatum* was found on three substrates, *Musanga cecropioides*, *Musa* sp. both from Yaekela and an unidentified species of the Maranthaceae family collected in Kona 2. Here also the time of observation was crucial, as *E. zonatum* develops in a limited period of time and is very deciduous. *P. articulatum* is the only species in this genus that is occasionally found on aerial litter; the other four species are known to develop only on bark and wood.

One of the unknown taxa, Proto-DRC-sp1 (fig. 1G & H), 9–11 µm tall, observed on leaves from a banana tree collected near the village of Yaekela, is morphologically similar to *Cavostelium apophysatum* (fig. 1J & K) but its sporangium, 8–9 µm in diameter, appears to have multiple spores. Proto-DRC-sp1 could also be related to small fruitings of the multiple spored *Microglomus paxillus* (fig. 1I) but the stalks are narrow cylindrical and well developed whereas the sporangia of Proto-DRC-sp1 seem to be sessile and resting on a broad base. Until it is observed again in more detail and, preferably, isolated into culture, it is difficult to determine whether its similarity to *C. apophysatum* or *M. paxillus* is due to close relationship or just superficial.

The second unknown taxon observed during this study and annotated as Proto-DRC-sp2 (fig. 1L–N), 25–30 µm tall, was reported for the first time by Shadwick et al. (2009) as isolate LHI05, from ground litter of mixed vegetation collected in native dry/mesic forest in Hawai'i. LHI05 is most likely a new species. Phylogenetic study (Shadwick et al. 2009) revealed a strong affiliation to the *Acanthamoebidae*, more specific to the non-fruiting *Protacanthamoeba bohemica* Dyková, Veverková-Fialová, Fiala & Dvořáková. Consistent characteristics of this deciduous taxon are the pyriform spore, 11.5–12.5 × 13–14 µm, with a distinct, narrow, cylindrical hylum and a short and tapering stalk, and the apex seems to inflate before spore discharge. After the spore is released thorn-like, empty stalks remain on the substrate.

Proto-DRC-sp3 (fig. 1T), 35–40 µm tall, isolated from *Microsorium punctatum* seems to be a form of the uncommon *Clastostelium recurvatum* with an elongated upper stalk segment (fig. 1Q–S). This taxon was observed on the same substrate with its typically short, curved, stalked form, 15–20 µm tall. The spore of *C. recurvatum* is discharged through inflation and rupture of the upper stalk segment, but this could not be observed in either form during the present study. In both cases the sporangium, 9–12 µm in diameter, showed the distinct droplet or pear shaped deformation due to the presence of usually two, exceptionally three to four spores. *C. recurvatum* tends to be more common in the tropics than in temperate regions; it was found on only two of the substrates from D.R. Congo and was only observed in the laboratory of the National Botanic Garden of Belgium.

A single sporangium of isolate Proto-DRC-sp4 (fig. 1U, V), 27 µm tall, found on *Rhabdophyllum* sp., can be related to *C. recurvatum* and Proto-DRC-sp3. The long stalk shows almost no curvature, possibly because the upper section is inflated and preparing to discharge the slightly turbinate spore, 9 × 9.5 µm. In this state the isolate strikingly resembled the equally erect fruiting body as shown in figure 9 accompanying the original description of *C. recurvatum* (Olive & Stoianovitch 1977).

Proto-DRC-sp5 (fig. 1W, X), 10–22 µm tall, from the *Rhabdophyllum* sp. cultures could possibly be an undescribed species of *Protosteliopsis*. Similar sporangia were also observed in cultures from Hawai'i (unpublished data). Aerial litter samples from Belgium (de Haan 2011) produced fruiting bodies with similar features and the stalk in particular has the same gelatinous appearance as that seen in *Protosteliopsis* species. The broadly turbinate spores, 5–8 µm in diameter, seem to be ornamented with a broad-meshed low reticulum. The sometimes faintly curved but always rigid stalk is almost isodiametric from base to apex.

Proto-DRC-sp6, (fig. 1O), 40–60 µm tall, was observed in the cultures from *Microsorium punctatum*. The apex of the long stalk does not narrow abruptly to a fine line, as in *Schizoplasmodiopsis micropunctata* L.S.Olive & Stoian. or *Tychosporium acutostipes*. Proto-DRC-sp6 resembles closely *Protosteliopsis fimicola* (fig. 1P), 15–25 µm tall, which was observed once on a *Maranthaceae* liana in Kona 2. The stalk of Proto-DRC-sp6 and *P. fimicola* is refractile, having a gelatinous aspect except for a cylindrical broadening in the lower part of the stalk. In both taxa the single spore, 8–12 µm in diameter, is spherical and does not appear to have an ornamentations. Normally *P. fimicola* is found on dung, but there are records from aerial litter (Ndiritu et al. 2009). The fruiting bodies observed in the survey from Kenya (Ndiritu et al. 2009) and identified as *P. fimicola*, had long stalks, similar to those of Proto-DRC-sp6.

CONCLUSIONS

All Protosteloid Amoebae records made in this study are new for the D.R. Congo and for Central Africa. Moreover two rare species, *Schizoplasmodiopsis reticulata* and *Schizoplasmodium seychellarum*, are new records for the African continent. The twenty three species observed on the PIPs represent 70% of the number of species described worldwide. Although the thirteen samples collected are too few for a statistical analysis, some conclusions can be drawn from the observations of the D.R. Congo data. Our results demonstrate that the number of plates per sample is crucial to obtain the highest species diversity and number of colonies.

Also important is the time spent on the observation of the plates, including the length of time per observation session, the number of sessions and most of all the timing of the observation. Some species develop and stay visible for only a short period.

No obvious difference in species composition could be observed between substrates collected in habitats with high anthropogenic impact and those with almost no human interference.

More morphological, ecological and phylogenetic data are needed to conclude whether the five unknown taxa can be described as new or assigned to known species.

The visited region in the D.R. Congo merits further study and represents, together with Hawai'i, one of the world's tropical hotspots for Protosteloid Amoebae.

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