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Species of Saprolegniales in water and soil samples of moist areas declared as laminar natural monuments by the administrative district of Kassel, Germany with special consideration of species occurring on amphibians

Abstract

We examined the diversity of species of the Saprolegniales in water and soil samples of 12 different moist areas, officially declared as laminar natural monuments by the administrative district of Kassel. Water samples of 4 different running water bodies, 6 stagnant water bodies and 2 additional areas, in which both water body types prevail, were examined in spring 2004. In order to isolate aquatic Oomycetes, the baiting method was used and additionally, some isolates could also be cultivated. The hereby presented studies of simultaneously recorded measurement readings from the aforementioned moist areas, also enabled us to conduct a limnological-chemical characterisation of the examined water bodies.

A total of 11 species of aquatic Oomycetes belonging to the order of Saprolegniales could be isolated from the water and/or soil samples. *Aphanomyces laevis* DE BARY, *Saprolegnia ferax* (GRUITH.) THURET, *Dictyuchus* LEITGEB spp. and *Saprolegnia* NEES spp. were, according to references, already been detected on species of Amphibia occurring in the examined water bodies. *Brevilegnia bispora* COUCH and *Geolegnia inflata* COKER and J. V. HARV. were obtained only from the soil samples. All species were isolated with respect to their seasonal occurrence in spring. A conspecifity of *Calyptralegnia achlyoides* (COKER and COUCH) COKER and *C. riparіensis* HÖHNK can be assumed on morphological criteria.

Zusammenfassung

Wir untersuchten die Diversität von Saprolegniales-Arten in Wasser- und Bodenproben aus 12 verschiedenen, als flächenhafte Naturdenkmale ausgewiesenen Feuchtgebieten des Landkreises Kassel. Im Frühjahr 2004 wurden Wasserproben aus 4 verschiedenen Fließgewässern, 6 Stillgewässern und von 2 zusätzlichen Flächen, in denen Still- und Fließgewässer vorkommen, untersucht. Um aquatische Oomyceten zu isolieren, wurde die Ködermethode verwendet; außerdem konnten einige Arten in Kultur genommen werden. Die dargestellten Studien von zeitgleich in diesen Feuchtgebieten erhobenen limnologischen Messwerten, ermöglichten uns außerdem eine limnologisch-chemische Charakterisierung der untersuchten Gewässer.

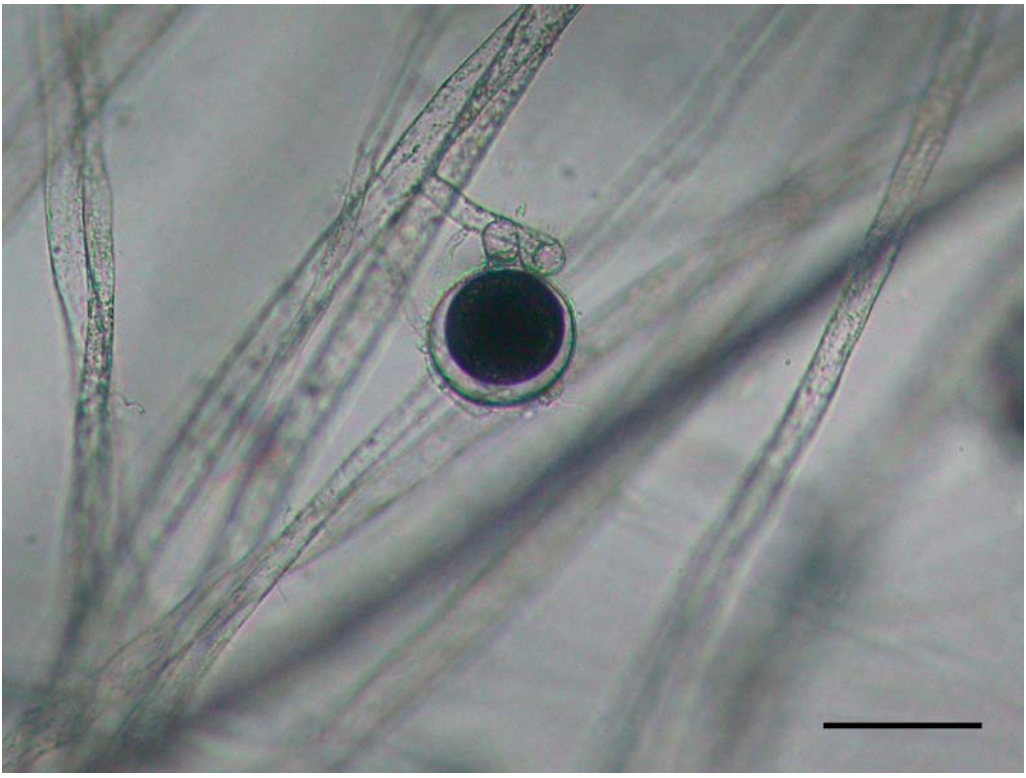

Fig. 1: *Calyptralegnia* sp. of sample 184 with androgynous antheridia, scale 70 µm.

Insgesamt konnten 11 Arten aquatischer Oomycetes der Ordnung Saprolegniales aus den Wasser- und/oder Bodenproben isoliert werden. Nach Literaturangaben sind *Aphanomyces laevis* DE BARY, *Saprolegnia ferax* (GRUITH.) THURET, *Dictyuchus* LEITGEB spp. und *Saprolegnia* NEES spp. bereits von Amphibienarten, welche in den untersuchten Gewässern vorkommen, isoliert worden. *Brevilegnia bispora* COUCH und *Geolegnia inflata* COKER und J. V. HARV. konnten lediglich in Bodenproben nachgewiesen werden. Alle Arten wurden unter Berücksichtigung ihres saisonalen Vorkommens im Frühjahr isoliert. Eine Konspezifität von *Calyptralegnia achlyoides* (COKER und COUCH) COKER und *C. riparіensis* HÖHNK kann auf Grund von morphologischen Kriterien angenommen werden.

Introduction

An occurrence of saprolegniaceous species on diseased or moribund fish as well as on amphibians has been reported since the Middle Ages (SPARROW in CLAUSZ 1974). An early finding of *Achlya* NEES sp. upon a dead frog was documented e. g. by HINE (1878) and recently in 1998 by RIETHMÜLLER (2000). RIETHMÜLLER & LANGER (2005b) examined the seasonal occurrence of Saprolegniales and Leptomitales in Lake Auesee and in the River Fulda in Kassel, under consideration of fishpathogenic species. Only few authors have studied the occurrence of Oomycetes on the different life stages of amphibians e. g. FORD et al. (2004), BLAUSTEIN et al. (2003), ROBINSON et al. (2003), KIESECKER et al. (2001a & 2001b), CZECZUGA et al. (1998) and LEFCORT et al. (1997). In Germany, no overall research has been carried out on the potential of isolated Saprolegniales parasitis-

ing species of Amphibia. Hence, a discussion of the species of Oomycetes under consideration of a possible infection in different life stages of amphibians is still underrepresented and therefore studied here.

Some publications on the occurrence of aquatic Oomycetes in water samples in the state of Hesse are already available (RIETHMÜLLER et al., submitted & RIETHMÜLLER et al. 2006, RIETHMÜLLER & LANGER 2005a), and KELLNER (2004, unpublished) examined the occurrence of amphibians in 12 different moist areas, declared as laminar natural monuments by the administrative district of Kassel in spring 2004. Finally, considering the sole biodiversity of aquatic Oomycetes in water and soil samples and putting them in correlation with water parameters and their seasonal occurrence in spring, in the examined moist areas, will further the knowledge obtained so far from fundamental research on fugal diversity in important regional freshwater ecosystems.

According to DICK (2001), the genus *Calyptralegnia* COKER contains two species, *C. achlyoides* and *C. riparіensis*. Based on our obtained isolates, the author's opinion is, that these two species are conspecific.

Material and methods

We examined water and soil samples of 12 different moist areas declared as laminar natural monuments by the administrative district of Kassel for the occurrence of aquatic Oomycetes. In April and March 2004, samples of 4 running water bodies ("Quellmulde des oberen Rinnbachtals", "Zwischen Hollenberg und Rinderplatz", "Feuchtgebiet hinter'm Eichbeutel" and "Quellarm der Lohbeeke") 6 stagnant water bodies ("Auf der Holunder", "Feuchtgebiet Klein Calden", "Der Kampteich", "Der nasse Wolkenbruch", "Am Rinderplatz" and "Das Deesenfeld") and additionally of 2 areas, in which both water body types are present ("Die Bruchwiesen" and "Der weiße Born") were examined on the occurrence of species of Amphibia and aquatic Oomycetes. A limnological-chemical characterisation of the examined water bodies, which was carried out simultaneously, was obtained by measuring water parameters directly at the water bodies and by carrying out further analyses in the lab.

All water samples were collected from surface water into polyethylene bottles (0.5 L content) for the isolation of aquatic Oomycetes; 1 L of water was taken additionally for chemical

Table 1: Chemical-physical methods.

Parameter	References
Content of chlorophyll-a	DEV L16 DIN 38412 L 16
BSB5	EN 25814
0_2 content and saturation	Oxi 320/SET, WTW according to EN 25814 G 22
Water temperature	Oxi 320/SET, WTW
Conductivity	LF 320/SET, WTW
PH value	Digital pH-meter 646, Knick, pH-electrode, Schott
Total hardness in Ca^{2+}	DIN 38406-3
Total hardness in Mg^{2+}	DIN 38406-3
Carbonate hardness	SCHWOERBEL (1994)
NO_3-N	DIN 38405 D29
NO_2-N	EN 26777 D10
NH_4^+-N	DIN 38406 E5-1
O-$P0_4^{3-}$-P	EN 1189 D11
Total PO_4-P	EN 1189 D11
Use of $KMn0_4$	EN ISO 8467 H5
Chloride	DIN 38405 D1-1
Legend: BSB5: Biochemical oxygen content after 5 days	

analyses. The soil samples were collected in clean plastic bags. For the detection of aquatic Oomycetes from the collected water samples, the baiting technique was used. Aliquots of 50 mL of the collected water samples were portioned into each of 10 petri dishes containing some seeds of *Sesamum indicum* L. which had previously been boiled for 8 minutes. For the recovery of aquatic fungi from the soil, 5 g of each sample was portioned into each of 10 petri dishes containing boiled sesame seeds and were then covered with 50 mL of autoclaved

Name of moist area	Type of water body	Sampling date	Nr. of sample AR	Nr. of sample KK	T_w	Ca^{2+}	Mg^{2+}	Carbonate hardness
					° C	mg L^{-1}	mg L^{-1}	°d KH
Quellmulde des oberen Rinnbachtals	RW	16.03.2004	183	1	8,2	123	15	19,6
Die Bruchwiesen	RW/SW	16.03.2004	184	241	10,9	123	16	18,2
Auf der Holunder	SW	16.03.2004	185	803	11,5	59	9	4,8
Feuchtgebiet Klein Calden	SW	23.03.2004	188	125	8,2	380	159	12,9
Zwischen Hollenberg und Rinderplatz	RW	23.03.2004	189	128	7,0	145	138	15,7
Der weiße Born	RW/SW	23.03.2004	190	132	8,6	101	137	16,8
Der Kampteich	SW	23.03.2004	191	406	9,7	25	4	2,8
Der nasse Wolkenbruch	SW	30.03.2004	192	778	5,8	8	10	2,5
Feuchtgebiet hinter'm Eichbeutel	RW	30.03.2004	193	94	9,2	113	31	13,2
Am Rinderplatz	SW	20.04.2004	246	214	9,8	52	9	5,0
Quellarm der Lohbeeke	RW	27.04.2004	247	129	11,7	n.m.	n.m.	20,2
Das Deesenfeld	SW	27.04.2004	248	779	13,5	n.m.	n.m.	20,7

Nr. of sample AR	Conductivity	Cl^-	pH value	O_2 content	O_2 saturation	BSB5	Use of $KMn0_4$	Chlorophyll-a content
	µS cm^{-1}	mg L^{-1}		mg L^{-1}	%	mg L^{-1}	mg L^{-1}	µg L^{-1}
183	887	19,9	7,9	10,9	93	2,2	2,3	n.m.
184	699	36,9	6,8	7,0	63	1,3	1,7	n.m.
185	383	31,9	6,5	7,7	70	0,0	14,0	7,6
188	1665	17,0	7,6	11,4	97	0,0	2,4	18,6
189	835	24,8	8,1	11,7	97	2,7	2,2	n.m.
190	638	17,0	7,8	11,3	97	2,7	1,1	n.m.
191	1654	14,9	7,1	12,1	107	0,0	4,0	13,4
192	n.m.	5,7	5,7	1,9	15	0,0	9,7	10,8
193	700	44,7	8,2	15,5	135	4,2	1,8	n.m.
246	436	28,4	7,4	13,1	115	4,3	6,2	2,0
247	660	10,6	7,8	9,3	85	9,1	1,2	n.m.
248	363	19,9	7,1	9,4	91	0,0	12,2	4,7

Nr. of sample AR	Nr. of sample KK	NH_4^+-N	NO_3^--N	NO_2^--N	o-$P0_4^{3-}$-P	$P0_4$-P		
		mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}		
183	1	0,00	2,31	0,00	0,011	0,036		
184	241	0,00	0,83	0,00	0,004	0,027		
185	803	0,00	0,00	0,00	0,040	0,112		
188	125	0,00	0,50	0,00	0,010	0,032		
189	128	0,00	7,29	0,00	0,021	0,028		
190	132	0,00	9,27	0,00	0,017	0,024		
191	406	0,00	1,44	0,00	0,007	0,037		
192	778	1,55	0,00	0,00	0,013	0,191		
193	94	0,20	7,37	0,00	0,109	0,131		
246	214	0,03	0,00	0,00	0,010	0,045		
247	129	0,03	2,62	0,00	0,000	0,010		
248	779	0,56	0,00	0,00	0,323	0,383		

Legend:
RW: running water body, SW: stagnant water body, RW/SW: running water with stagnant water, n.m.: not measured, BSB5: biochemical oxygen content after 5 days

Table 2: Limnological-chemical water parameters of the investigated moist areas.

Fig. 2: *Calyptralegnia* sp. of sample 184 with diclinous antheridia, scale 70 µm.

distilled water. The petri dishes were incubated in natural light and at room temperature for 4 weeks. During this time, the zoospores colonised the sesame seeds and grow out with hyphae. The seeds were then examined every day under a light microscope thus identifying the species. The recovered isolates were cultivated on a glucose-peptone agar medium, according to WILLOUGHBY (1997) and modified by adding 0,5 g L^{-1} Sodium Benzylpenicillin or 0,5 g L^{-1} Streptomycin Sulfate at approx. 50 ° C. The strains were examined by means of light microscopy and identified with JOHNSON et al. (2002), SEYMOUR (1970), JOHNSON (1956), SCOTT (1961); the nomenclature followed DICK (2001). For the discussion of the conspecifity of *Calyptralegnia achlyoides* and *C. ripariensis*, based on morphological aspects, JOHNSON et al. (2002), HÖHNK (1953), COKER & COUCH (1923) and COKER (1927) were used.

Results

Together with 11 isolated species in the spring samples, some isolates attributable to the genera *Achlya*, *Aphanomyces* DE BARY, *Brevilegnia* COKER & COUCH (Saprolegniales), *Saprolegnia* NEES, and *Pythium* PRINGSHEIM (Pythiales), could be isolated as well. They could only be identified on a generic level, do not count as species and thus are treated as isolates. According to table 3, *Aphanomyces laevis*, *Saprolegnia ferax*, *Dictyuchus* spp. and *Saprolegnia* spp. could already be detected on amphibians. Interestingly, *Brevilegnia bispora* and *Geolegnia inflata* could only be found in soil samples. A conspecifity of *Calyptralegnia achlyoides* and *C. ripariensis* based on morphological criteria of the obtained isolates “184, Die Bruchwiesen” (figures. 1 to 3) and “247 Quellarm der Lohbeeke“ can be assumed.

		Type of water body	RW	RW /SW	SW	SW	RW	RW /SW	SW	SW	RW	SW	RW	SW
		Nr. of sample AR	183	184	185	188	189	190	191	192	193	246	247	248
Species/isolates of samples:		Nr. of sample KK	1	241	803	125	128	132	406	778	94	214	129	779
Achlya americana HUMPHREY														w
A. racemosa HILDEBRAND			s	ws	w				w	w		ws		w
Achlya NEES spp.									w			w		ws
***Aphanomyces laevis* DE BARY**						**ws**	**w**							
Aphanomyces DE BARY sp.					w									
Brevilegnia bispora COUCH												s		
Brevilegnia COKER and COCH sp.				w										
Calyptralegnia COKER sp.				w									w	
***Dictyuchus* LEITGEB spp.**				**w**						**w**		**w**		
Geolegnia inflata COKER and J. V. HARV.										s				
Saprolegnia diclina HUMPHREY									w					
***S. ferax* (GRUITH.) THURET**				**ws**		**w**		**w**		**w**	**ws**	**w**		**w**
S. glomerata (THIESENHAUSEN) LUND					w									
S. megasperma COKER					w									
***Saprolegnia* NEES spp.**				**w**	w	**w**					**ws**		**ws**	**w**
Pythium PRINGSHEIM spp.			w	w	w		w	w						w
Species of amphibia:	**Isolates of Oomycetes:**	**References:**												
Bufo bufo (L.)				x		x	x	x	x	x		x		x
	Dictyuchus spp.	*												
	S. ferax	*												
	eggs with *S.* spp.	**												
Rana temporaria L.				x		x	x				x	x	x	x
	Dictyuchus spp.	*												
	S. ferax	*												
	eggs with *S.* spp.	**												
Green frog species complex						x	x					x		x
acc. to (BLAB & VOGEL 1996):														
1. *Rana esculenta* L.														
2. *Rana lesonae* CAMERANO														
3. *Rana ridibunda* PALLAS														
	Aphan. laevis	*												
	Cal. achlyoides	*												
	Dictyuchus spp.	*												
	S. ferax	*												
Salamandra salamandra (L.)							x							x
Triturus alpestris (LAURENTI)			w	x			x				x	x	x	
Triturus cristatus (LAURENTI)										x				
Triturus helveticus (RAZOUMOWSKY)				x			x	?				x		
Triturus vulgaris (L.)				x			x	?		x		x		
	Aphan. laevis	*												
	Caly. achlyoides	*												
	Dictyuchus spp.	*												
	S. ferax	*												

Legend: s: soil, w: water, RW: runnning water body, SW: stagnant water body, RW/SW: running water with stagnant water
bold: infection possible acc. to literature
References: *: CZECZUGA (1998), **: ROBINSON et al. (2003)

Table 3: Species/isolates of aquatic Oomycetes and amphibians of the examined moist areas under consideration of these isolates of Saprolegniales, which infect amphibians, according to references.

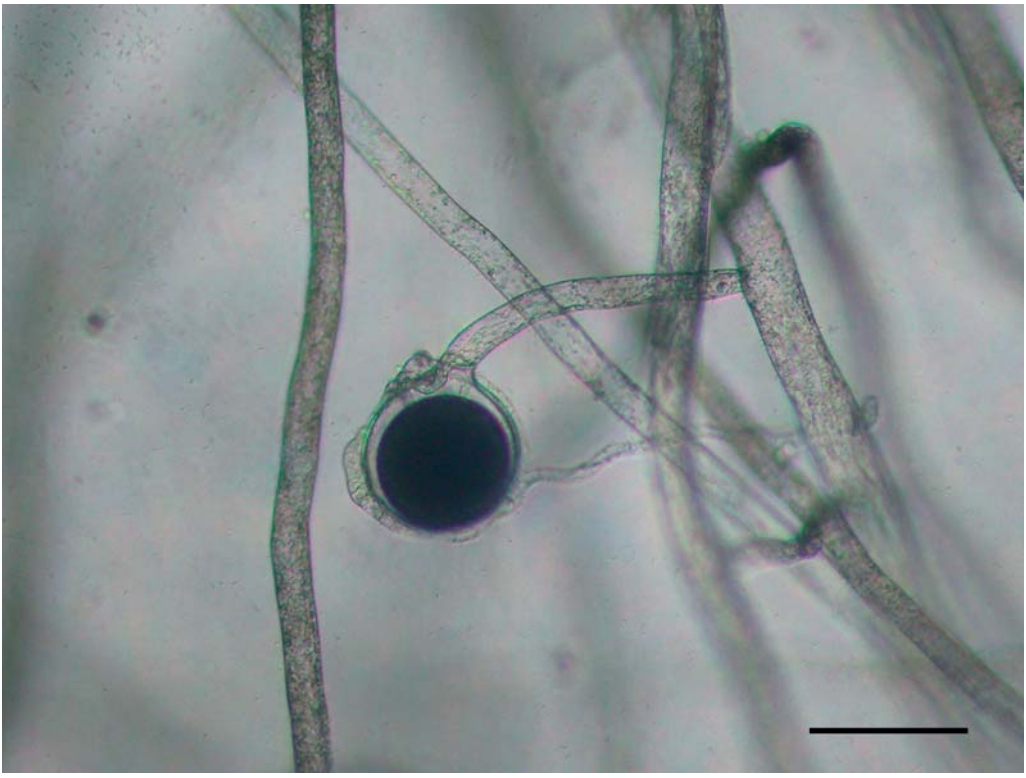

Fig. 3: *Calyptralegnia* sp. of sample 184 with monoclinous antheridia and centric oospores, scale 70 µm.

Discussion

We intend to focus on a new aspect of aquatic limnology, by studying aquatic fungi in correlation to their limnochemical environment. Considering the limnochemical parameters of the examined moist areas, an actual view of the tolerance of the species in water bodies in spring is hereby given. These results are very useful for a characterisation of fungal biodiversity in freshwater ecosystems.

According to table 3, four isolates were already detected on species of amphibians, which occur, according to KELLNER (2004, unpublished) in the examined water bodies. All four mentioned isolates of aquatic Oomycetes normally occur as saprophytes in freshwater and infect amphibians under certain circumstances. We obtained these isolates by the baiting technique with sesame seeds in spring water samples. Hence, an infection of species of Amphibia with these species of Oomycetes is theoretically possible. It can furthermore be assumed that an infection of amphibians with aquatic Oomycetes is dependent on the susceptibility of the amphibians and the simultaneous occurrence of a specific infecting isolate of the parasite. Our studies were restricted to a unique sampling in spring, in which we only collected amphibians with no visible fungal infections. Further studies during other periods of the year will be necessary in order to obtain further results in the question of facultative infection of vertebrate hosts by fungal parasites.

We only examined water samples of moist areas in March and April 2004, merely postulating the occurrence of the obtained species (table 3) in spring. For a comparison of our isolates from this examined region with studies of seasonal occurrences as in RIETHMÜLLER (2000), RIETHMÜLLER et al. (2006), RIETHMÜLLER &

Fig. 4: *Calyptralegnia* sp. of sample 337 with monoclinous antheridia and centric oospores.

LANGER (2005b), further samples in the administrative district of Kassel should be examined.

Including soil samples into studies is always a possibility for obtaining interesting species of Oomycetes. In our soil samples, we found *Brevilegnia bispora* and *Geolegnia inflata*. The corresponding author could never obtain these species during her time of and experience in studying aquatic Oomycetes. In most of the available references, they also occured in soil samples. For *B. bispora* and for *G. inflata* we would like to cite COUCH (1927), DICK & NEWBY (1960 & 1961) and DICK (1963). *B. bispora* could be isolated from soil in Germany by HÖHNK (1952) and *G. inflata* was already isolated from soil taken in Hesse by RICHTER (1936-1937). It seems that these species prefer soil as a habitat.

The genus *Calyptralegnia* contains two species, *C. achlyoides* and *C. ripariensis* (DICK 2001). According to JOHNSON et al. (2002), *C. achlyoides* is very near *C. ripariensis* in most of its characteristics, and in oogonium shape and size, oogonial stalk configuration, and oospore measurements hardly could be said to differ from the latter species. The oospores of *C. achlyoides* are centric or subcentric (COKER 1927). They are illustrated as subeccentric for *C. ripariensis* in HÖHNK (1953) although they are identified as eccentric in the text. DICK

Morphology Oospores	***C. achlyoides***	***C. ripariensis***	**Isolate 184 and Isolate 247**	**Isolate 337**
	centric		centric	centric
	subcentric			
		subeccentric		subeccentric
		eccentric		
Antheridia				
	diclinous	diclinous	diclinous	diclinous
	androgynous		androgynous	
		monoclinous	monoclinous	monoclinous

Table 4: A morphological comparison of *Calyptralegnia achlyoides*, *C. ripariensis* and the isolates of *Calyptralegnia* of the samples 184, 247 and 337.

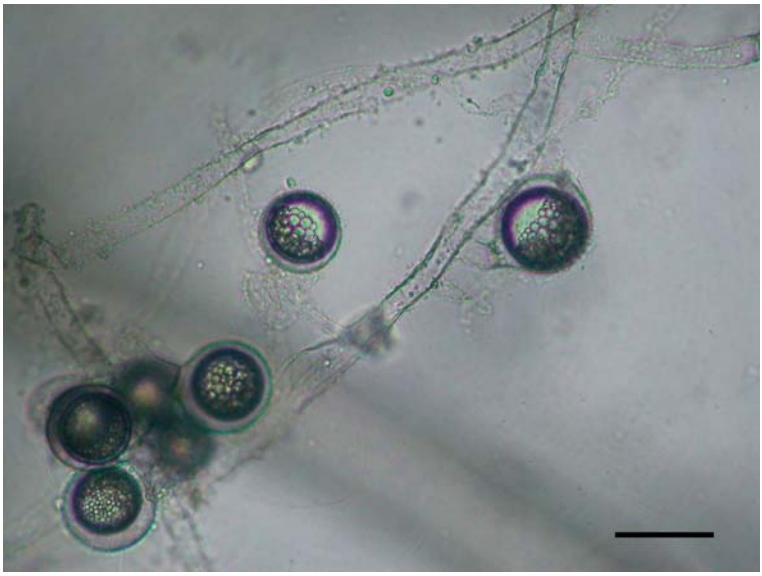

Fig. 5: *Calyptralegnia* sp. of sample 337 with monoclinous and diclinous antheridia and subeccentric oospores, scale 60 µm.

(1969) remarked that within the Saprolegniaceae all gradations exist between centric and eccentric types of oospore structure. Both *C. achlyoides* and *C. ripariensis* have diclinous antheridial branches, androgynous ones are additionally produced by the former (COKER & COUCH 1923), monoclinous ones by the latter (HÖHNK 1953). We do not intend a taxonomic revision of the two species of *Calyptralegnia*, but simply give a postulation for a conspecifity of *C. achlyoides* and *C. ripariensis*, based on morphological criteria of our isolates 184, "Die Bruchwiesen" and 247, "Quellarm der Lohbeeke" of *Calyptralegnia*. Isolate 184 exhibits monoclinous, additionally diclinous and androgynous antheridia and its oospores are centric (figures 1 to 3). Our postulation is additionally supported by an isolate 337 obtained in June 2004 from the River Bauna in Baunatal-Guntershausen, Hesse, which exhibits centric and subeccentric oospores and monoclinous and diclinous antheridia (figures 4 and 5).

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