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Taxonomic implications of antheridial variability in forty-five watermold isolates: a statistical analysis

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Abstract – Morphological variability of sexual features used in watermold identification renders species identifications difficult and calls into question the taxonomic utility of these characters. Herein I have employed chi square statistical analysis to quantify antheridial character state distribution differences between replicate pairs of 38 isolates representing the saprolegniaceous genera *Achlya*, *Saprolegnia*, *Thraustotheca,* and 7 non-sporulating watermolds. Thirty-nine of 45 pairs differed at or below the P=.05 significance level, suggesting that current morphological species concepts are inadequate, at least for *Achlya* and *Saprolegnia*.

Key words – *Oomycota*, *Saprolegniaceae*, morphology, systematics

Introduction

Identification of watermolds (*Saprolegniales*, *Oomycota*) belonging to the genera *Achlya* Nees and *Saprolegnia* Nees has long been problematic owing principally to extensive morphological overlap among recognized species (Johnson et al. 2002). Hulvey et al. (2007) studied this problem in 55 isolates of *Saprolegnia* and demonstrated that little correlation exists between species boundaries based on sexual morphology and those based on gene sequence analysis.

More recently, Sheffer & Padgett (2008) demonstrated that variations in oospore diameter, oospore centricity, antheridial origin, and antheridial appression among subcultures of a single *Saprolegnia* isolate were as great as those that have been used to separate different species in other studies. Their study called into question the taxonomic validity of these sexual characters. The principal unanswered question arising from their report was whether or not the isolate in question was aberrant or demonstrated a degree of variability that applied to the genus or family as a whole. The present study was designed to answer this larger question by assessing the extent of antheridial variability between replicate colonies of 45 isolates belonging to *Achlya* (26 isolates),

Saprolegnia (10 isolates), *Thraustotheca* Humphrey (2 isolates), and non-sporulating watermolds (7 isolates).

Materials and methods

I extracted the data for the current investigation from a much broader study (hereafter referred to as the 'master study') aimed at reevaluating the taxonomic foundation of the family *Saprolegniaceae*. In the course of the master study, approximately 490 watermolds either were acquired from culture collections (the Centraalbureau voor Schimmelcultures [CBS] and the American Type Culture Collection [ATCC]) or isolated from soil samples collected in Italy, Australia, Costa Rica, Canada, Hawaii, and the continental United States. All culture numbers cited herein (Table 1) refer to stocks maintained, during the master study, in the watermold culture collection at the University of North Carolina Wilmington.

All isolations from soil were made using standard methods (Johnson 1956, Seymour 1970) as modified below. Soil samples (10 g) were dispensed into disposable 15 × 100 mm Petri dishes, flooded with distilled water (DW), and baited with sterile, shelled hemp seeds (hs). Culture plates then were incubated at room temperature until watermold colonies developed. Axenic cultures subsequently were derived by single spore or hyphal tip isolations from gross cultures and maintained on hs in water and on Difco corn meal agar (CMA) in preparation for microscopic analysis.

Morphological characterization of all axenic isolates required 10 replicate, DW-grown subcultures for each watermold. These were initiated first by infesting 10 sterile hs for 24 h at the edge of CMA-grown colonies then transferring individual, infested hs to separate Petri dishes containing 20 mL of DW. After incubation at room temperature for 24 to 48 h isolates were identified to genus by observing zoosporangial discharge from 10 primary sporangia. Incubation then continued for up to 14 days until mature oogonia and antheridia were visible.

As asexual and sexual features matured through time, individual colonies (of the 10 replicates for each isolate) were harvested for morphological characterization; qualitative data were recorded on separate data sheets (one data sheet per replicate subculture). These observations were made using Olympus phase contrast microscopes with 400× magnification. During data collection we attempted to record 50 observations for all sexual characters presented by a particular colony at the time of harvest, but rarely were unobstructed views of this number available.

Of the 490 watermolds acquired in the master study we identified all that produced zoosporangia to genus. About half of the axenic cultures subsequently produced sexual features, but few were good fits to described species. I limited isolates for the present statistical analysis to those with 50 character state observations of the same sexual character on each of two separate data sheets (i.e. from two separate replicate colonies of the same isolate). Ultimately only 45 isolates met this criterion and of those only two sexual characters (antheridial origin and antheridial appression) consistently presented the required sample sizes. Thirty-five cultures qualified for statistical analysis for both antheridial characters and the remaining 10 qualified for one character. Hereafter the two replicates for each qualifying culture are referred to as a 'replicate pair' (RP).

All RPs in the test pool presented 3 character states for antheridial origin – monoclinous, diclinous, and androgynous – , and 3 for antheridial appression – apical, lateral, and projections (illustrations in Johnson 1956). Members of each RP were compared for uniformity of character state distribution (for both characters) using Chi square statistical analysis. For calculation purposes I used the mean value (per character state) as the 'expected' value for the particular RP. This necessitated doubling each 'calculated' Chi square to derive the value used for comparison to the appropriate tabular Chi square value. I considered P=.05 to be the maximum level for statistical significance.

Results

Table 1 presents results of Chi square comparisons of all 45 RPs. In all cases I made the conservative assumption that any RP for which one character had insufficient data for comparison (less than 50 observations) did not differ for that character. This being the case, when results for all genera were combined I found that only 6 of 45 RPs had no significant differences for either character. Of the remaining 39 all exhibited differences in at least one character. Furthermore 15 of 45 exhibited significant differences for both characters.

Separating results by genus revealed that all 10 RPs of *Saprolegnia* differed with respect to one or both characters, both RPs of *Thraustotheca* differed for one character, and all seven non-sporulating RPs differed for one or both characters. *Achlya* RPs were the least variable, yet 12 of 26 pairs differed with respect to one character while 8 differed for both.

Discussion

In light of the present data (Table 1), it is apparent that the statistically significant variability reported by Sheffer & Padgett (2008) was not aberrant but may be typical not only for *Saprolegnia* but also for *Achlya*. I am keenly aware that drawing sweeping conclusions based on data for only two sexual characters is risky. Consequently, I visually inspected raw data (from the master study described above) for other watermolds that did not qualify for the present analysis and found comparable variability in oogonial and oospore characters.

I carefully reviewed historical monographs of saprolegniaceous genera (Coker 1923, Coker & Matthews 1937, Johnson 1956, Scott 1961, Seymour 1970) and found no mention of statistical tests ever having been applied to assess variability of taxonomic characters. Clearly results reported herein demonstrate that this omission represents a serious taxonomic problem that introduces an unacceptable level of subjectivity into identifications of *Achlya* and *Saprolegnia* isolates.

New watermold species currently are being erected at an alarmingly rapid pace (e.g. Steciow 2001a,b, 2002, 2003a,b, Steciow & Elides 2002a,b,c, Steciow

Table 1. Chi square significance levels per replicate pair for antheridial characters

Genus	Stock culture number^	Antheridial origin	Antheridial appression
Achlya	223	**	**
Achlya	234	ID[b]	**
Achlya	243	ID	***
Achlya	246	P>.25	***
Achlya	247	P>.1	***
Achlya	267	*	***
Achlya	276	**	***
Achlya	281	***	P>.25
Achlya	287	P>.25	***
Achlya	313	ID	***
Achlya	326	*	***
Achlya	342	P>.25	***
Achlya	347	*	***
Achlya	362	***	***
Achlya	367	*	ID
Achlya	418	**	***
Achlya	451	**	P>.1
Achlya	455	**	P>.1
Achlya	456	P>.1	P>.25
Achlya	460	P>.1	P>.1
Achlya	462	P>.1	P>.25
Achlya	463	P>.05	P>.25
Achlya	465	P>.25	***
Achlya	469	P>.25	ID
Achlya	485	P>.25	P>.1
Achlya	487	**	*
Saprolegnia	105	ID	***
Saprolegnia	217	***	ID
Saprolegnia	254	***	P>.25
Saprolegnia	257	P>.25	*
Saprolegnia	262	**	***
Saprolegnia	280	**	P>.25
Saprolegnia	284	***	P>.25
Saprolegnia	361	***	***
Saprolegnia	383	*	P>.1
Saprolegnia	472	**	***
Thraustotheca	60	ID	***
Thraustotheca	325	ID	***
Unknown[a]	277	P>.25	*
Unknown	291	***	ID
Unknown	292	**	***
Unknown	299	***	**
Unknown	380	*	*
Unknown	382	P>.1	***
Unknown	424	***	*

^ UNC-W watermold culture collection. [a] Unknowns did not produce zoosporangia. [b] ID = insufficient data for statistical comparison, * indicates P≤.05, ** indicates P≤.01, *** indicates P≤.001.

et al. 2007, Steciow & Marano, 2008, Paul & Steciow 2004, 2008, Johnson et al. 2005, Amal et al. 2006, Sati & Paliwal 2006), yet no descriptions have been accompanied by morphological variability assessments. Continuing this practice inevitably will render watermold taxonomy progressively more problematic.

Recent literature (e.g. Leclerc et al. 2000, Bouzenzana et al. 2006, Hulvey et al. 2007, Dieguez-Uribeondo et al. 2007, Fregeneda-Grandes et al. 2007) reflects a gratifying expansion both of biochemical and gene sequence information that no doubt will be of great value in comprehensive revision of *Oomycete* taxonomy. Such studies, however, represent only the start of a necessary baseline that must develop more fully before meaningful revision can emerge. Few scientists would argue with the paradigm that genes determine biochemistry, which determines morphology. I must infer, therefore, that the variability reported herein reflects some currently unknown disconnect between genes and morphology that renders present concepts of *Achlya* and *Saprolegnia* species inadequate.

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