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ABSTRACT

We used the globally widespread genus *Ophiognomonia* as a model system to investigate climatic niche patterns in fungi, characterizing the climatic profiles of 28 species with seven temperature and seven precipitation variables. Using a novel version of Spatial Evolutionary and Ecological Vicariance Analysis (SEEVA), designed to deal with continuous and correlated variables, we examined well-sampled phyletic splits of a multi-gene phylogeny. We evaluated the degree to which phyletic divergence has been associated with climatic niche divergence between sister lineages, permitting elucidation of climatic associations in evolutionary context. From the 14 inter-correlated climatic variables, we extracted four principal axes, accounting for 93.2% of the climatic variation, with axes broadly labeled as: polarity, tropicality, winter mildness, and aridity. We also analyzed the two single variables maximum monthly temperature and precipitation. We detected climatic associations that were compatible with both niche-conservatism and niche-divergence within the phylogeny, and different cladistic bifurcations associated with different climatic splits. As might have been anticipated, geographic separation (or lack thereof) of phylogenetic splits was correlated with climate niche divergence (or conservation). This elaborated SEEVA method provides a visual and statistically solid basis for characterizing climatic niche divergence that should prove useful for elucidation of many other taxonomic groups.

Key Words: adaptation – climate history – evolutionary ecology – niche evolution – phylogeny–precipitation – SEEVA – Sordariomycetes – temperature

INTRODUCTION

Fungi arose in the Proterozoic, and are among the oldest terrestrial eukaryotes (Berbee & Taylor, 1993, 2010; Taylor & Berbee 2006); they are now distributed across much of the planet. They have been subjected to continuous (and planet-wide) climatic change since their origins, and their current distributions align with both historical and current climatic diversification of the planet itself. Notwithstanding that obvious reality, our current understanding of climatic impacts on fungal evolution remains severely limited (Scherm *et al.,* 1994; Sutherland *et al.,* 1997; Harvell *et al.,* 2002; Dukes *et al.,* 2009). Tedersoo *et al.* (2014) was the first study to show that climatic factors can be used to predict fungal richness and community composition at a global scale and that soil fungi follow general biogeographic patterns. To elucidate the evolutionary impact of geographically dispersed, long-term and large-scale climatic changes on fungal phylogeny, we need to begin by investigating how the phyletic patterns among extant taxa align with current climatic patterns.

Both abiotic and biotic context define the ecological niche, within which a species can survive (Hutchinson, 1957). The actual niche is a result of ancestral adaptations and constraints, from the time of separation - onward (Prinzing *et al.*, 2001). A pattern of extant species occupying ecological niches resembling those of their ancestors is referred to as phylogenetic niche conservatism (Harvey & Pagel, 1991; Wiens *et al.*, 2010), and this can be detected through careful analysis of climate variables throughout a reconstructed phylogeny of an organismal clade. Some authors view niche conservatism as important in speciation (Riedl, 1966; Hodgson, 1986; Hua & Wiens 2013), but others view it as a minor issue (Stebbins, 1975; Cronquist, 1988; Walter & Breckle, 1991; Bennett, 1997). Our own work has shown, for example, that broad scale ecological diversification is associated with phyletic radiation in three genera

(*Macrocarpaea, Prepusa, Senaea*) of the angiosperm family Gentianaceae (Struwe *et al*., 2009, 2011).

The family Gnomoniaceae consists of ten genera, and has been subjected to detailed molecular phylogenetic, host association, and morphological analyses (Mejía *et al*., 2008, 2011a,b,c; Sogonov *et al*., 2008; Walker *et al*., 2010, 2012). Fungi in this family are commonly found on deciduous trees in many temperate and high elevation tropical biomes. We report here on species of the fungal genus *Ophiognomonia* (Gnomoniaceae, Diaporthales, Ascomycota), which are generally inconspicuous to the naked human eye, forming dark microscopic spots on hardwood leaves and twigs; these spots contain long-necked perithecia, the sexual life stage (Supplemental Fig. S1). Its asexual life stage is often symptomatic on hardwood deciduous trees as various leaf blotch diseases. The northernmost member of the genus (*O. rosae*) has been found at latitude 66.26 N in Finland and the southernmost (*O. tucumanensis*) at latitude 26.85 S in Argentina (Walker *et al.*, 2012). Species of *Ophiognomonia* have been described as endophytes, pathogens, and saprobes on a diverse range of tree species used commercially for nuts, lumber, and landscaping, but which are also common in natural habitats. Two of the most notable pathogens causing significant economic and ecological damage are *O. leptostyla* (walnut anthracnose) and *O. clavigignenti-juglandacearum* (butternut canker; Neely & Black, 1976; Berry 1981; Juhasova *et al.*, 2006; Rossman *et al.*, 2007; Belisario, 2008; Broders & Boland, 2012; Broders *et al.*, 2014). *Ophiognomonia clavigignenti-juglandacearum* has reached epidemic levels in North America, threatening the existence of butternut (*Juglans cinerea*). Individual *Ophiognomonia* species infect only a narrow range of host species, but while taxonomic host association is strongly correlated with phylogenetic pattern within *Ophiognomonia*, there are also

notable host jumps, presumably involving novel host adaptations, as seen in a previous SEEVA-aided analysis (Walker *et al*., 2014).

The geographic distribution of *Ophiognomonia* is a complex manifestation of spatial and temporal availability of host plants and suitable ecological context, within which both host species and fungal pathogen can persist and thrive (Peterson *et al.*, 2011; Tedersoo *et al.,* 2014). We investigated the correlation of climatic and taxonomic patterns in this group, by deploying a multi-dimensional Spatial Evolutionary and Ecological Vicariance Analysis (henceforth SEEVA; Struwe *et al.*, 2011), newly modified to allow for treatment of continuous quantitative variables that may (or may not) be independent. We hypothesized that climatic niche-based divergence has contributed to cladogenesis in *Ophiognomonia.* Our goals were to: (1) discover patterns of climatic niche conservation, niche shifts, and niche expansion between sister lineages, within the scope of a geographically widespread evolutionary lineage, and to (2) extend our ability to evaluate climatic associations in evolutionary context.

MATERIALS AND METHODS

Selection of Specimens

We used 178 wild-collected herbarium specimens from the United States National Fungus Collections (BPI), as well as cultures from the Centraalbureau voor Schimmelcultures (CBS) in our dataset, incorporating all species included in the phylogenetic analysis by Walker *et al*. (2012; Supplemental Table S1, Supplemental Fig. 2). Species determinations on herbarium sheets or culture labels were verified using morphology and ITS rDNA sequencing (Walker *et al.,* 2014), and only specimens with locality data fulfilling the quality criteria described in Struwe *et al*. (2011) were included. Specimens were georeferenced to four decimal places

(decimal degrees) on Google Maps (http://maps.google.com), using a custom Java script, as described in St. Clair (2012; javascript:void(prompt(",gApplication.getMap().getCenter())).

Ophiognomonia Phylogeny

The phylogenetic topology utilized in this study is a cladistic tree from biosystematic reconstruction of specimen data, without regard to climate, and includes 43 *Ophiognomonia* species (Walker *et al.* 2012). All nodal designations and contrasts are listed on the phylogenetic tree in Supplemental Figure 2. Three major Superclades were found within this genus, Superclade A_1 (N_{A1} = 75 specimens), Superclade B_1 (N_{B1} = 41 specimens), and Superclade C_1 (N_{C1} = 58 specimens) [Fig. 1; Walker *et al.* 2012]. Node D_2 separates Superclade C (N_C = 58) from Superclades A & B (N_{A+B} = 116). Each Superclade had strong bootstrap and posterior probability branch support (Walker *et al.*, 2012), but there was weaker support for the basal splitting order among Superclades A, B and C themselves, and especially weak internal branch support within Superclade C (Fig. 1). The SEEVA results for all superclades can be found in supplemental tables 2–5. To concentrate attention on the best defined and best replicated portion of the genus, we present the SEEVA results from Superclades A and B here (see rationale below).

Phyletic Nodes Chosen for Statistical Exploration

Small sample sizes are an unavoidable reality in research based on uncommon, relatively obscure, and rarely collected species, and it is important to assess phylogenetic diversity with robust methods. At the same time, scientific research should not be restricted to only well-known, well-collected taxa, especially when less known taxa might have important ecological, evolutionary, or economic effects. Many terminal taxa in our study are represented by just the few specimens that have ever been collected from those taxa. Meaningful statistical evaluation

of most of the terminal splits in Figure 1 is not presently possible, so we have restricted formal SEEVA analysis to six pairwise phylogenetic splits (A_1 - A_4, A_6 and A_9) within Superclade A and four pairwise phylogenetic splits (B_1 - B_3 and B_5) within Superclade B, each of which meets three requirements, ensuring at least a modicum of phylogenetic and statistical credibility: (1) 60% maximum likelihood bootstrap support (MLBS), (2) a minimum of ($N = 3$) specimens per taxon, and (3) each tested node involving at least 11 specimens.

Climatic Variables Selected for SEEVA

We acquired climate variable data for the time period between 1950 and 2000 from the website WorldClim v1.4 (Hijmans *et al.,* 2005), which we downloaded in 2.5 arc-minute format. We deployed a set of 14 (candidate) BIOCLIM variables for SEEVA analyses: (1) annual mean temperature (BIO1), (2) maximum temperature of the hottest month (BIO5), (3) minimum temperature of the coldest month (BIO6), (4) mean temperature of the wettest quarter (BIO8), (5) mean temperature of the driest quarter (BIO9), (6) mean temperature of the hottest quarter (BIO10), (7) mean temperature of the coldest quarter (BIO11), (8) annual precipitation (BIO12), (9) precipitation of the wettest month (BIO13), (10) precipitation of the driest month (BIO14), (11) precipitation of the wettest quarter (BIO16), (12) precipitation of the driest quarter (BIO17), (13) precipitation of the hottest quarter (BIO18), (14) precipitation of the coldest quarter (BIO19). These variables were chosen to sample the climatic extremes. We used the program DIVA-GIS v7.5 (Hijmans *et al*., 2012) to extract data for the selected BIOCLIM variables for each specimen's point location, imported them into Microsoft Excel, for further transfer to the SEEVA-R module in R (Reginato, 2016). The phylogenetic position of each species is represented by one or more records that include both geolocation variables and 14 climatic

158 measures,. The data for all specimens and their locational climatic profiles are provided in
159 Supplementary Table 1 (SOAR Accession http://dx.doi.org/doi:10.7282/T3ZS2ZZM).

160 *SEEVA for Continuous Variables*

161 SEEVA combines data from all specimen records and uses non-parametric
162 (permutational) statistical tests for comparison of daughter subclades, tests that are (by
163 construction) statistically independent for the different nodes (Struwe *et al*., 2011). The null
164 hypothesis for each test is no divergence of the ecological feature under consideration between
165 sister clades emerging from a particular nodal split, whereas significant divergence indicates a
166 convincing pattern of ecological divergence between the modern members of the two clades
167 ascending from that particular split (Struwe *et al*., 2011). SEEVA has been deployed in earlier
168 studies to analyze both qualitative and quantitative variables, but until now, we have defined
169 quartile classes for the quantitative variables, treated as qualitative, unranked categories (Struwe
170 *et al.,* 2011). To improve that treatment for the continuous climatic predictor variables deployed
171 here, accounting for both direction and magnitude of differences, we have transformed each of
172 the 14 BIOCLIM variables into a scaled version of the non-parametric Mann-Whitney-Wilcoxon
173 rank test (Thompson *et al*., 2014).

174 Briefly, consider a particular node in Superclade A, defining a pair of derivative
175 subclades (A_1 & A_2) with sample sizes (N_1 & N_2). Rank the $\underline{N} = (N_1 + N_2)$ values for the
176 continuous variable in question from smallest to largest (1, ⋯ , N), and then sum the ranks
177 within each of the subclades separately (denoted R_1 & R_2), and convert those rank sums to
178 median rank values within each of the subclades, denoted as U_1 & U_2, and computed as:

179 $$U_1 = R_1 - N_1 \cdot (N_1 - 1) / 2 \qquad \text{and} \qquad U_2 = R_2 - N_2 \cdot (N_2 - 1) / 2 \ . \qquad [1]$$

At issue is the extent to which those median ranks (U_1 & U_2) are different, as well as the direction of that difference. We scale the test so that if all Subclade-A_1 members have higher ranks than Subclade-A_2 members, the criterion ($Z_{1\text{-}2} = +1$), but if all Subclade-A_2 members have higher ranks than Subclade-A_1 members, the criterion ($Z_{1\text{-}2} = -1$), accomplished by defining:

$$Z_{1\text{-}2} = (U_1 - U_2) / (N_1 \cdot N_2) \qquad \text{and} \qquad Z_{2\text{-}1} = (U_2 - U_1) / (N_1 \cdot N_2) \quad . \qquad [2]$$

These simple transforms scale the MWW-test [–1, +1]; we measure how consistently the rank-order measure for subclade A_1 is greater (or less) than that for subclade A_2, with the null hypothesis expectation of 'no difference' coded as ($Z_{1\text{-}2} = 0 = Z_{2\text{-}1}$). We permute specimens between subclades (holding N_1 and N_2 constant) to test that null hypothesis. A function to calculate the Z-test for quantitative variables in SEEVA was written for the R language (Paradis *et al.*, 2004; Schliep, 2011; R Core Team 2016). The R package for SEEVA, SEEVA-R, including wrapper functions for data handling and plotting, is now available (see Reginato, 2016). SEEVA-R uses functions of the R packages ape and phangorn (Paradis *et al.,* 2004; Schliep, 2011).

Principle Components Rotation

The difficulty with using large numbers of different climatic predictors, of course, is that they are typically correlated, and cannot realistically be viewed as independent, even sampled across the planet. That is certainly the case here, and we present the correlation matrix for the 14 ranked features (Supplemental Table S2). To convert 14 correlated climate features into a smaller number of independent but coherent climatic signals across the broad geographic expanse occupied by the genus *Ophiognomonia*, we converted the rank orders of the 178 specimens for each of the 14 climatic features, via a Principle Components Analysis (PCA),

implemented in R. We extracted 14 PCA-axes, their loadings, and their relative fractions of the total climatic variation (Supplemental Table S3). Our climatic panoply is essentially four-dimensional for the locations of the (N = 178 specimens, S = 43 species) examined here. Using the loadings for these four principle axes, we computed the PCA-scores for each specimen, and then rank-scaled each of the resulting PCA-axes from minimum (1) to maximum (178). The final set of four PCA variables provides tests that are independent of each other, both for any given node and across nodes. To allow for multiple independent tests at different phylogenetic nodes, we used Bonferroni correction (Rice, 1989) to provide a conservative 'experiment-wise' error rate (α). We deployed SEEVA for six nodes in Superclade A (resulting in critical value, $\alpha_A = 0.0085$) and four nodes in Superclade B ($\alpha_B = 0.0127$).

RESULTS

Climatic Features are Generally Correlated

Analysis of the 14 correlated BIOCLIM variables, each converted to rank-ordered form, demonstrated that the useful dimensionality of the set was closer to four uncorrelated variables than the fourteen BIOCLIM features recorded. We constructed a principal components analysis, and extracted the variances, proportions of variation accounted for, and feature loadings associated with each of those first four PC-axes, collectively accounting for 93.2% of the climatic variation, sampled across the vast geographic expanse occupied by the genus *Ophiognomonia* (Supplementary Table S3). The major axis, PC-1 (labeled *polarity*), accounted for 44.5% of total climatic variation, loaded negatively on all features, and captured the overall climatic trends associated with increasing polarity. The second axis, PC-2 (labeled *winter mildness*), accounted for 23.6% of the variation, and loaded positively on temperature and negatively on precipitation. The third axis, PC-3 (labeled *tropicality*) accounted for 14.0% of the

variation, loaded positively and strongly on summer temperatures and precipitation, negatively but modestly on winter temperatures and precipitation. Finally, PC-4 (labeled *aridity*) accounted for 11.1% of the climatic variation, loaded positively on temperature, and negatively on precipitation. The other ten PC-axes accounted for 6.8% of total climatic variation, were poorly established, and of questionable utility. Complete SEEVA results for all 14 single Z-variables, as well as those for the first four PC axes, are presented for all (10) tested nodes within SubClades A and B in Supplementary Table S4. We report our tests of the first four PC-axes in what follows, but will also present (T_{max}) Hottest Month and (P_{max}) Wettest Month. These two variables were minimally correlated with each other, over the full dataset, but the both indicate the considerable range of climatic conditions spanned by the genus *Ophiognomonia.* The full set of *Z*-values (14 BIOCLIM variables, plus 4 PCA axes) is presented in (Supplementary Table S5).

Superclade A

Within Superclade A, we present the SEEVA results (as barcharts) for the selected six Superclade A nodes (Fig. 2). Since there are six independent nodal bifurcations being tested here, our Bonferroni test is significant only if $P < 0.0085 = \alpha_A$, and we have flagged significant climatic features (or axes) as (∗). Each node-specific barchart shows the results for all four PCA-axes, as well as those for *temperature* (T_{max}) and *precipitation* (P_{max}) extremes. The deepest (and well sampled) nodal split A_1 (clade A_2, occupying warmer and wetter climates than clade A_6) yields significant values for all six climatic features, from *polarity* (PC-1: $Z = -0.55$) to *precipitation* (P_{max}: $Z = +0.37$), to *winter mildness* (PC-2: $Z = +0.47$), to *temperature* (T_{max}: $Z = +0.63$), (all significant =∗), but shows no compelling pattern for either *tropicality* (PC-3: $Z = +0.14$) or *aridity* (PC-4 : $Z = -0.01$). This deep bifurcation involves moderate to strong climatic

niche divergence among these 15 species, all of which occur primarily on Fagales host tree species of the Northern Hemisphere, indicating compelling climatic separation, in spite of geographic clustering and relatively tight host fidelity.

Node A_2, separating *Ophiognomonia otanii* from Clade A_3, contains five species primarily in East Asia, occurring mostly on *Castanea* and *Quercus* in the Fagaceae, aside from *O. setacea,* which has an expanded host range on the Proteales and Sapindales (Walker *et al.,* 2014). *Ophiognomonia otanii* occurs in more temperate, cooler, and wetter environments than the other four species in Clade A_3. Our test criteria ($Z_{otani\text{-}A3}$) show strongly suggestive (though non-significant) patterns of climatic-based niche divergence for *precipitation* (P_{max}: $Z = +0.60$), *winter mildness* (PC-2: $Z = +0.41$), *aridity* (PC-4: $Z = -0.66$), and *tropicality* (PC-3: $Z = -0.68$), but showed no credible separation for either *temperature* (T_{max}: $Z = +0.07$) or *polarity* (PC-1: $Z = -0.06$). Despite unbalanced sampling, SEEVA yields results that collectively suggest a degree of climatic niche separation, in spite of a relatively narrow host and geographic ranges.

Node N_{A3} has a similar host set (*Castanea* and *Quercus* in Fagales, Proteales, Sapindales) and geographic range (all found in Japan) as Node N_{A2}. Node N_{A3} ($Z_{kobayashii\text{-}A4}$) shows suggestive patterns of climate-based (though non-significant): specialization for *precipitation* (P_{max}: $Z = +0.70$), *winter mildness* (PC-2: $Z = +0.37$), *aridity* (PC-4: $Z = -0.31$), *polarity* (PC-1: $Z = -0.62$), and *tropicality* (PC-3: $Z = -0.74$), but no supported separation for *temperature* (T_{max}: $Z = +0.17$). In general, *Ophiognomonia kobayashii* occupies warmer (less polar, PC-1), more temperate (PC-3) and wetter (P_{max}) locations than do the other three species within Clade A_4. Again, the sampling is quite unbalanced, and the results are more suggestive than statistically compelling.

Node N_{A4} ($Z_{setacea\text{-}A5}$) shows compelling patterns of strong niche divergence for *precipitation* (P_{max}: $Z = -0.83*$), *winter mildness* (PC-2: $Z = -0.39$), *polarity* (PC-1: $Z = -0.47$), *tropicality* (PC-3: $Z = -0.90*$), *aridity* (PC-4: $Z = -0.90$), but no separation for *temperature* (T_{max}: $Z = -0.03$). Relative to the two *Ophiognomonia* species in Clade A_5, *O. setacea* occupies an expanded host and geographic range in non-tropical (PC-3) and arid (PC-4, P_{max}) climates.

Node N_{A6} separates *Ophiognomonia* species in Clades A_7 and A_8, collected from a worldwide distribution on Fagales host plants, except for two species in Clade A8 that infect *Prunus* (cherries and relatives, Rosales). Species in Clade A_7 are found within temperate climates (PC-3) with heavy precipitation (P_{max}), relative to Clade A_8. Sampling is quite unbalanced, so statistical power is limited, but formal analysis shows non-trivial ($Z_{A7\text{-}A8}$) patterns of climatic niche divergence for *precipitation* (P_{max}: $Z = +0.74$), *polarity* (PC-1: $Z = -0.50$), *temperature* (T_{max}: $Z = +0.23$), *winter mildness* (PC-2: $Z = -0.20$), *aridity* (PC-4: $Z = -0.38$), and *tropicality* (PC-3: $Z = -0.98*$).

Node N_{A9} ($Z_{A10\text{-}A12}$) shows patterns of niche-conservatism and a general absence of separation for *aridity* (PC-4: $Z = +0.11$), *precipitation* (P_{max}: $Z = +0.02$), *polarity* (PC-1: $Z = -0.04$), *winter mildness* (PC-2: $Z = -0.05$), though suggestive separation for *tropicality* (PC-3: $Z = -0.31$) and *temperature* (T_{max}: $Z = -0.45$). Species delimited by Node N_{A9} occur mostly on the Fagales, except for *Ophiognomonia japonica* and *O. michiganensis* (both in Clade A_{10}), which occur on *Prunus* (Rosales). Species in Clade A_{12} were not documented in North America, whereas those in Clade A_{10} are found worldwide. No compelling pattern of niche-based specialization was observed, in spite of strong allopatry of these species.

293 *Superclade B*

294 Node N_{B1} shows ($Z_{B2\text{-}B5}$) statistically compelling patterns of niche divergence for
295 *temperature* (T_{max}: Z = +0.91∗), *tropicality* (PC-3: Z = +0.78∗), *winter mildness* (PC-2: Z =
296 +0.68∗), and *polarity* (PC-1: Z = –0.85∗), but no supported separation for either *aridity* (PC-4: Z
297 = +0.14) or *precipitation* (P_{max}: Z = –0.14; Fig. 3). This pattern indicates strong divergence at
298 this deep nodal split for species that are both physically and climatically separated into warmer
299 and wetter climates near the equator (for Clade B_2) or colder and drier (for Clade B_5) locations
300 nearer the poles. The four *Ophiognomonia* species in Clade B_2 occur on *Carya* and *Juglans*
301 (hickories and walnuts, Juglandaceae), except for *O. lenticulispora* (on *Prunus*) in North
302 America, whereas the nine species in Clade B_5 occupy a worldwide distribution and a diverse
303 taxonomic range of hosts (Walker *et al*., 2012, 2014).

304 Patterns suggestive of strong niche conservatism are evident at Node N_{B2} ($Z_{vasiljevae\text{-}B3}$) for
305 *precipitation* (P_{max}: Z = + 0.12), *tropicality* (PC-3: Z ± 0.00), *winter mildness* (PC-2: Z = –0.12),
306 *aridity* (PC-4: = –0.12), and *polarity* (PC-1: Z = –0.18), though there was minor separation for
307 *temperature* (T_{max}: Z = –0.27). The four species in Clade B_2 are distributed across eastern North
308 America and occur on diverse plant lineages of the Fagales and Rosales. These species (Clade
309 B_2) appear to exhibit patterns of strong temperature or precipitation limitations, limiting their
310 dispersal abilities and entraining narrow habitat ranges.

311 When evaluating Node N_{B3} ($Z_{micromegala\text{-}B4}$), the two species in Clade B_4 and
312 *Ophiognomonia micromegala* show climate-based niche conservatism for *winter mildness* (PC-
313 2: Z = +0.18), *aridity* (PC-4: Z = +0.11), *temperature* (T_{max}: Z = –0.25), *precipitation* (P_{max}: Z =
314 – 0.25), and *tropicality* (PC-3: Z = –0.39), but a suggestive pattern for *polarity* (PC-1: Z =

315 +0.46). These three species occur on two host genera (*Carya* and *Prunus*) from a narrow
316 geographic range on the eastern coast of the United States.

317 Node N_{B5} ($Z_{B6\text{-}B9}$) shows niche divergence for *temperature* (T_{max}: Z = +0.68∗), *winter*
318 *mildness* (PC-2: Z = +0.51), *tropicality* (PC-3 Z = +0.36), and *polarity* (PC-1: Z = –0.31), but no
319 strong separation for *aridity* (PC-4: Z = –0.02) or *precipitation* (P_{max}: Z = –0.05). Species in
320 Clades B_6 and B_9 have truly global distributions (North America, Europe, Asia), occurring on a
321 broad range of host species of the angiosperm orders of Rosales, Fagales, Malvales, and
322 Laurales. Species in Clade B_6 show patterns of temperature based specialization in niches with
323 warm temperatures during the hottest month and increased winter mildness in warmer climates,
324 whereas those species in Clade B_9 show the reverse pattern.

325 **DISCUSSION**

326 Understanding how evolutionary patterns are associated with climatic patterns and how
327 this may impact both current and future distributions of different species will impact our
328 understanding of long-term specialization, adaptation, and phylogenetic separation into diverging
329 climate niches, thus informing biodiversity conservation management, and potentially impacting
330 agricultural trade-based decisions, especially in light of ongoing climate change. Our objective
331 here was to understand the patterns of climatic niche divergence that now characterize sister
332 lineages within an economically important group of pathogenic fungi. Climatic variables are
333 certainly not the only factors influencing the current distribution of fungal species and their plant
334 hosts, but as one of the most important sets of variables that influence survival of organisms their
335 evolutionary importance cannot be denied.

Here we illustrate the extent to which broad geo-climatic patterns are associated with nodal bifurcations within the phylogenetic tree itself, on the premise that a speciation event may well have been associated with adaptive responses to climate (climatic niche vicariance). While we cannot implicate climatically vicariant speciation *per se*, currently observable patterns of climatic association almost surely reflect climatically-associated evolutionary divergence. For example, species delimited by node N_{A1} (separating subclades A_2 and A_6, Fig. 2) represent a worldwide distribution of the genus *Ophiognomonia* that colonize hosts in the angiosperm families of Betulaceae, Fagaceae, Platanaceae, Rosaceae, and Sapindaceae (Walker *et al.,* 2014). Upon close examination, species in subclade A_6 tend to occur closer to the poles in colder and drier environments, when compared with those in subclade A_2, which occur in warmer and wetter conditions, closer to the equator (PCA-1, PCA-2; Fig. 2). The fungal patterns would appear to be a consequence of the more northerly distribution for some host plant families, suggesting climate-based niche specialization in distinct environments for the two fungal Superclades. Whether genetic divergence accompanied or followed speciation (reproductive isolation), the current climatic distinction is almost surely not coincidental.

The evolution of a biological trait shared by closely related species is typically a complex process, however, and detailed understanding requires a comprehensive analysis of phylogenetic patterns, population-level processes, evolutionary biology, and ecological data (Wiens *et al.*, 2010). The niches of closely related species have been hypothesized to evolve (differentiate increasingly) during the speciation process (aka niche evolution; Warren *et al.,* 2008). To understand the evolutionary biology of an organism in any depth, it is useful to understand whether the environment influenced the speciation event itself or whether the derivative species adapted to different habitats, subsequent to the development of reproductive isolation itself.

359 Patterns suggesting climate-based niche divergence were observed for Node N_{B1} (the split
360 between subclades B_2 and B_5, Fig. 3). Species delimited by node N_{B1} occur on hosts in the
361 Juglandaceae, Lauraceae, Malvaceae, and Rosaceae from a worldwide temperate distribution and
362 show patterns of host conservatism (subclade B_2) and host range expansion (subclade B_5; Walker
363 *et al.,* 2014). Similar host plant ecology may play an important role in facilitating host shifts in
364 similar or overlapping ecological host habitats (Refregier *et al.,* 2008). When comparing
365 subclades B_2 and B_5, PC 1-3 indicate that species in subclade B_2 are located in warmer and
366 wetter habitats, relative to members of subclade B_5, which occur in less tropical areas with more
367 severe winters (Fig. 3). Species in subclade B_2 associate with hosts primarily in the
368 Juglandaceae, except for *O. lenticulispora* (*Prunus* sp., Rosaceae), and occur within warmer and
369 wetter environments, when compared to species in subclade B_5 which associate with hosts in the
370 Rosaceae, Lauraceae, Juglandaceae, and Malvaceae. Perhaps, the expanded host range for
371 species in subclade B_5 (compared with subclade B_2) has allowed these fungi to specialize in
372 unique microhabitats over broad geographic ranges, where plants in the widespread families
373 Lauraceae and Malvaceae are present. A possible explanation may be that by switching hosts the
374 fungus has been provided with a whole new set of climatic options, within which to adapt.
375 The tendency for a species to occupy a niche closely resembling that of its most recent
376 ancestor (and, to a lesser extent, its more distant ancestors) is termed niche conservatism (Wiens
377 & Graham, 2005; Wiens *et al.,* 2010). Stephens and Wiens (2003) predicted that niche
378 conservatism can explain patterns of species richness and distributions along latitudinal and
379 elevational gradients, as well as at the community level. Given the cladistically associated
380 climatic patterns we have observed here, we suggest that climatic niche conservatism has
381 contributed to the patterns we observed at Nodes N_{A9} (Fig. 2) and N_{B2} (Fig. 3). Interestingly,

species in Clade A_9 occur on a broad host range in the Fagales and Rosales with a worldwide distribution, but are found in similar climatic regimes, perhaps attributable to climate-based niche conservatism. Similarly, species in Clade B_2 have a small geographic distribution (eastern US), also compatible with climate-based conservatism.

Patterns of both niche-based conservatism and divergence, in the absence of either spatial or host changes, are present within *Ophiognomonia*. We might hypothesize that host specificity and climatic niche-based divergence have contributed to cladogenesis in *Ophiognomonia*, as well as in other organismal groups (Wooten *et al.,* 2013; Rato *et al.,* 2015). Alternatively, we might take the view that climate has influenced the geographic distribution of several host genera, and that fungal divergence has followed the geographic radiation of their hosts, without any direct effects of climate on fungal evolution. In either case, the use of SEEVA for analysis of climatic data provides a visual and quantitative characterization of niche divergence in the genus *Ophiognomonia*. Further analyses are needed that include a niche analysis of the host species themselves, aimed at elucidating whether the patterns are restricted to the fungi, to their hosts, or to particular fungal-host pairs.

Poleward expansions of plant pests have been demonstrated in the Northern Hemisphere since 1960 (Bebber *et al*., 2013; Boddy *et al*., 2014). Such shifts will also be important for practical agroecosystem management applications, relative to emerging or expanding infectious fungal diseases (Chakraborty & Newton, 2011; Pautasso *et al*., 2012). Similar analyses could be useful in predicting climatic constraints on important host/pathogen relationships, allowing us to predict (and possibly manage) situations reminiscent of the severe declines in American chestnut (*Castanea dentata* (Marsh.) Borkh.) and American elm (*Ulmus americana* L.), due to fungal diseases. More broadly, examining climatic influences on fungal speciation should contribute to

405 our broader understanding of the likely evolutionary trajectories of parasitic organisms in a
406 world of changing climates, growing food insecurities, and increased (and frequently human-
407 assisted) migration of species and pathogens between continents.

408 The use of the new version of SEEVA has helped us analyze and evaluate patterns for
409 complex, multivariate climate data in a biodiversity and phylogenetic context in a way that
410 previously has not been possible in this field. Major climate divergences between clades can be
411 visualized and tracked both on a comparative base between different (not directly related) parts
412 of phylogenies as well as in a temporal scale throughout a phylogeny. For example, three
413 separate clades (A_4, A_6, and B_1) show strong divergence in tropicality (Figs. 2–3), and could be
414 interpreted as possibly convergent climate evolution patterns. Cladistic climate trends can also
415 be visualized within a phylogeny, for example in the nodal comparisons of B_1 to B_5 that show
416 increased severity of winters (winter mildness) and colder temperatures (T_{max}) as a trend for a
417 relatively basal clade B_1 and subclade B_5 (Fig 3). SEEVA provides a novel way of visualizing
418 and thinking about these patterns and underlying events of organismic evolution. With the new
419 digitization efforts of scientific specimens worldwide (e.g. iDigBio, Atlas of Australia, etc.)
420 resulting in millions of specimen-based geolocated data records, new methods in reconstructing
421 large and complex phylogenies, and the combination of the two with climate data, we predict
422 that using SEEVA or similar methods will enhance our knowledge and understanding of the
423 intersection between cladistic patterns, climate changes, and biogeography.

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AUTHOR CONTRIBUTIONS

DMW and LS contributed to the conception and design of the experiment; DMW and LC collected specimens; MR wrote the new package for R; DMW, PES, LS, and MR analyzed data and interpreted results; and DMW, PES, and LS wrote the manuscript.

DATA ACCESSIBILITY

All raw data and result tables for this study have been deposited in the Rutgers University open-access SOAR database (http://soar.libraries.rutgers.edu/) for permanent, free access http://dx.doi.org/doi:10.7282/T3ZS2ZZM. The supplementary tables and figures contain all raw data necessary to repeat the analyses. DNA sequence data were deposited in GenBank, see Walker et al. (2012).

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580

FIGURE LEGENDS

Figure 1. Phylogenetic topology of *Ophiognomonia* based on maximum likelihood analysis by Walker et al. (2012), using GARLI. Clades A-D are labeled in bold text. Node names are provided under each branch, branch support based on bootstrap support above each branch. Clades A-C were formally analyzed, but only the results from Clades A and B are presented here, see rationale in methods section.

Figure 2. Phylogenetic tree of SuperClade A analyzed in SEEVA for four PCA and two climatic variables. Directionality and climatic interpretation of Z-value scores, ranging from [–1,+1] are listed in bar-chart form. Results from eight nodal bifurcations are not listed on this tree since they did not pass statistical criteria of 60% MLBS and a minimum of ($N = 3$) specimens per taxon.

Figure 3. Phylogenetic tree of SuperClade B analyzed in SEEVA for four PCA and two climatic variables. Directionality and climatic interpretation of Z-value scores, ranging from [–1,+1] are listed in bar-chart form. Results from eight nodal bifurcations are not listed on this tree since they did not pass statistical criteria of 60% MLBS and a minimum of ($N = 3$) specimens per taxon.

SUPPLEMENTARY INFORMATION (ELECTRONIC APPENDICES)

****SUPPLEMENTAL TABLES WILL BE ARCHIVED AS EXCEL FILES SINCE THEY HAVE MANY COLUMNS AND WILL NOT FIT ON A SINGLE PDF PAGE. WE REQUEST THAT THE EXCEL FILES BE EVALUATED DURING THE REVIEW PROCESS.**

Supplemental Table S1. Data for all specimens and their locational climatic profiles.

Supplemental Table S2. Correlation matrix for seven temperature and seven precipitation variables, scaled in rank order form. (1) annual mean temperature (AnnMT), (2) maximum temperature of the hottest month (MxTHM), (3) minimum temperature of the coldest month (MnTCM), (4) mean temperature of the wettest quarter (AvTWQ), (5) mean temperature of the driest quarter (AvTDQ), (6) mean temperature of the hottest quarter (AvTHQ), (7) mean temperature of the coldest quarter (AvTCQ), (8) annual precipitation (AnnPre), (9) precipitation of the wettest month (PreWM), (10) precipitation of the driest month (PreDM), (11) precipitation of the wettest quarter (PreWQ), (12) precipitation of the driest quarter (PreDQ), (13) precipitation of the hottest quarter (PreHQ), (14) precipitation of the coldest quarter (PreCQ).

Supplemental Table S3. 14 PCA-axes (WorldClim Feature Codes), their loadings, and their relative fractions of the total climatic variation. Bold-face text indicates any weight beyond ±0.27. The table is color coded with orange for negative and blue for positive values.

Supplemental Table S4. Results from SEEVA and PCA analyses, node for node for seven temperature measures, seven precipitation measures, and four climatic principal component axes. (1) annual mean temperature (AnnMT), (2) maximum temperature of the hottest month

(MxTHM), (3) minimum temperature of the coldest month (MnTCM), (4) mean temperature of the wettest quarter (AvTWQ), (5) mean temperature of the driest quarter (AvTDQ), (6) mean temperature of the hottest quarter (AvTHQ), (7) mean temperature of the coldest quarter (AvTCQ), (8) annual precipitation (AnnPre), (9) precipitation of the wettest month (PreWM), (10) precipitation of the driest month (PreDM), (11) precipitation of the wettest quarter (PreWQ), (12) precipitation of the driest quarter (PreDQ), (13) precipitation of the hottest quarter (PreHQ), (14) precipitation of the coldest quarter (PreCQ).

Supplementary Table S5. Summarized results of four PCA-axes and two uncorrelated climatic variables for all major *Ophiognomonia* subclade comparisons. Species acronyms are as follows: *O. kobayashii (kob), O micromegala (mi), O. otanii (otan), O. setacea (set), O. vasiljevae (vas).*

Figure S1. Morphology of the genus *Ophiognomonia.* A–B. BPI 882231. Perithecia of *O. asiatica* on leaf petiole and vein of *Quercus serrata*. C. BPI 879565. Perithecium of *O. tucumanensis* extracted from leaf tissue. D. BPI 882213. Asci and ascospores (sexual spores) of *O. sogonovii.* E. BPI 870007. Conidia (asexual spores) of *O. leptostyla.* Scale bars of A–C. = 100 µm, D–E. = 10µm.

Figure S2. Phylogenetic topology of *Ophiognomonia* based on maximum likelihood analysis of the ITS, MS204, and *tef-1α* markers by Walker et al. (2012), using GARLI. Clades A-D are labeled in bold text. Node names are provided under each branch, branch support based on bootstrap support above each branch. GenBank PopSets: 410004380, 410004212, 410004044.