

Origin and evolution of pathogenicity in Chaetothyriales



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Disease-causing Fungi

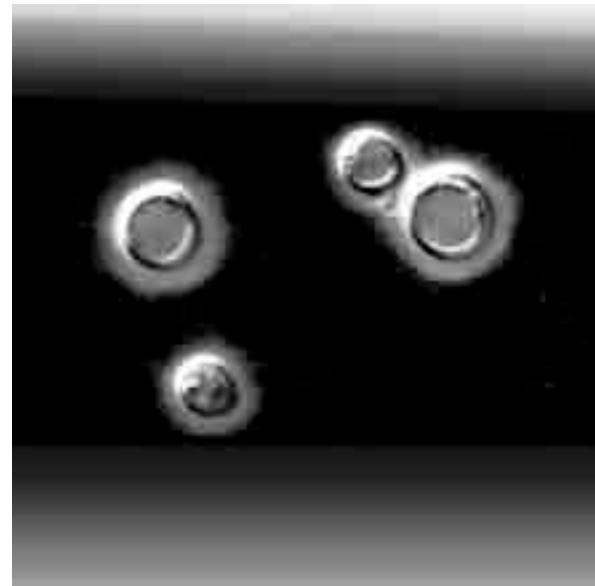
Opportunists or true pathogens?

- Opportunists: cause disease in a host whose resistance is lowered
- True pathogens: can also infect healthy patients

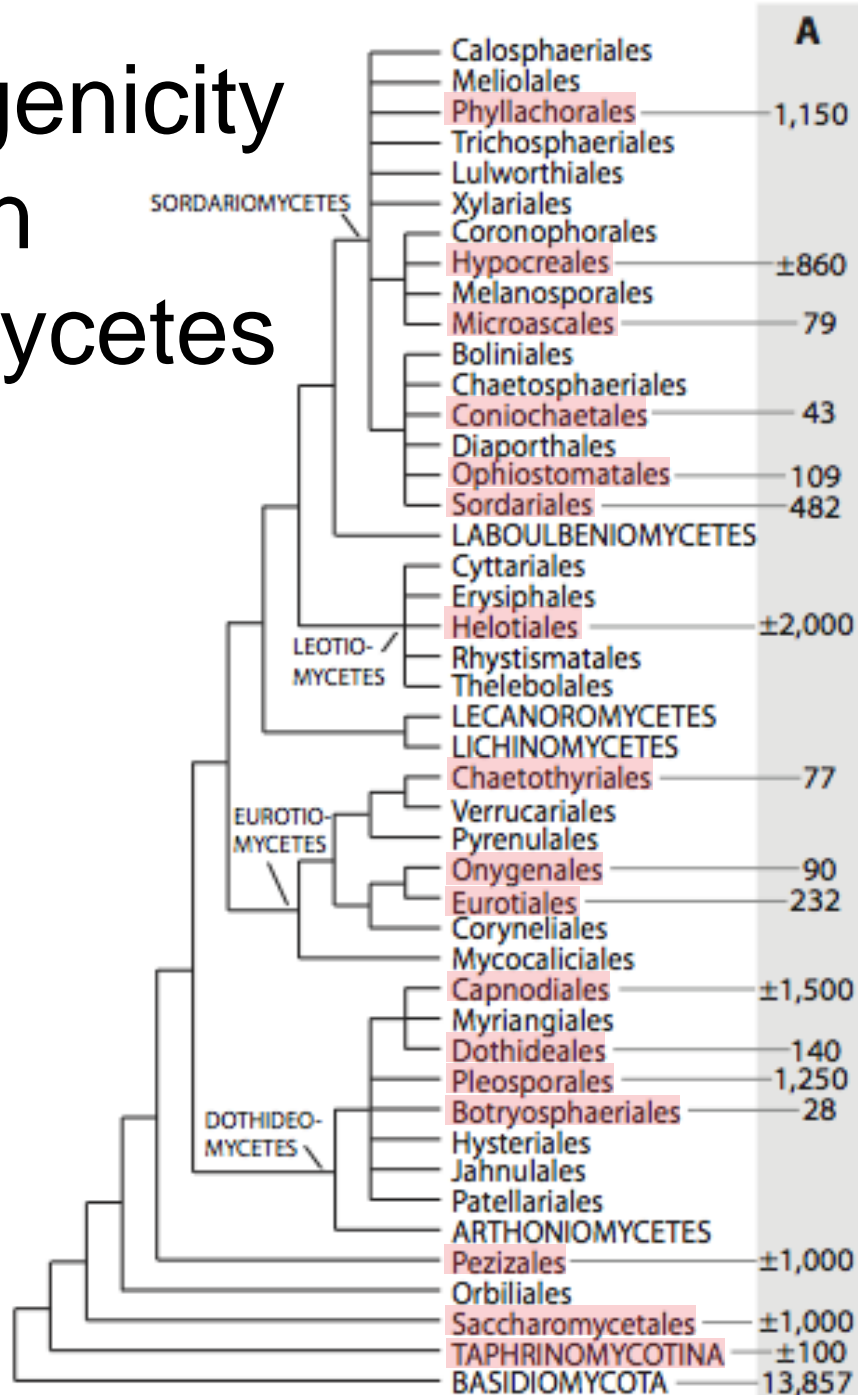
Factors favorizing virulence

Factors favorizing the fungal adhesion, colonization, dissemination, ability to survive in the host and elude its immune response; but also ability to damage the host.

- Thermotolerance
- Presence of a capsule
- Presence of melanin
- Growth forms
- Proteases
- Binding molecules
- Extracellular enzymes



Pathogenicity in ascomycetes



B

A: number of species
B: number of pathogens

5



37

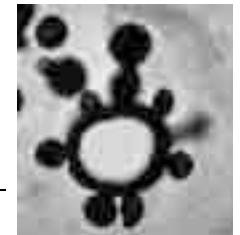
16



9

2

14



7

33

57

60



6

6

51

2



1

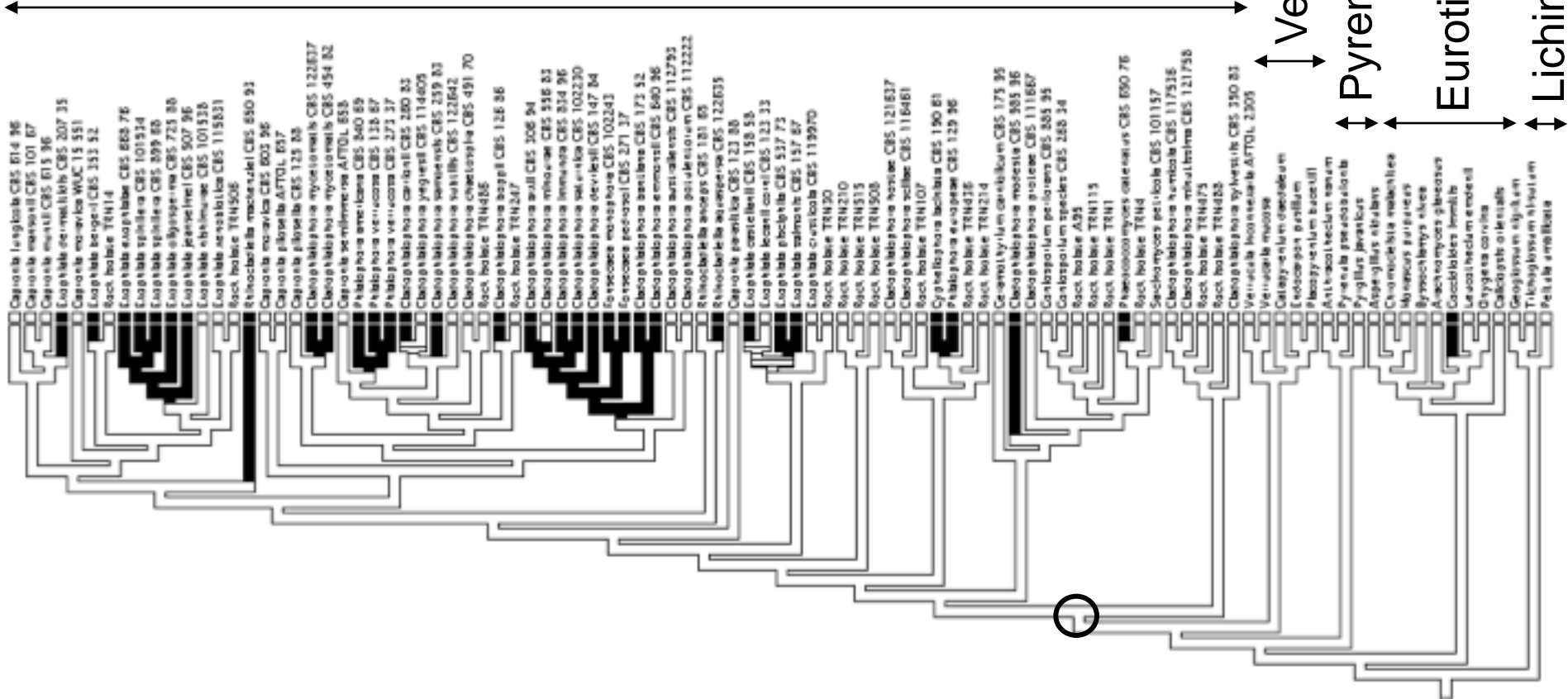
16

1

37

Pathogenicity in Chaetothyriales

Chaetothyriales



- human pathogen
- not pathogen

Verrucariales

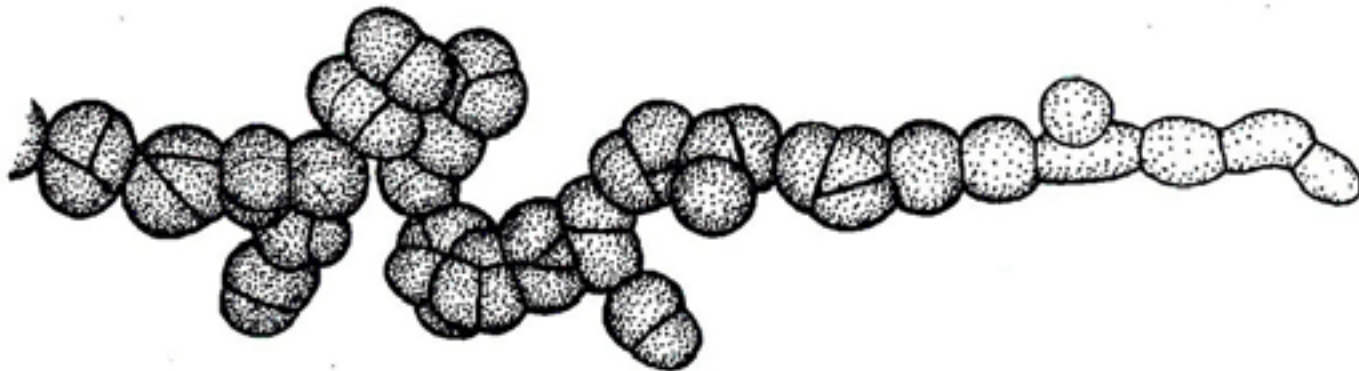
Pyrenulales

Eurotiales

Lichinales

Factors favorizing virulence in Chaetothyriales

- Melanin (Dixon et al. 1987, 1991, Schnitzler et al. 1999, Feng et al. 2001)
- Meristematic growth (Mendoza et al. 1993, Szaniszlo et al. 1993, Karuppayil & Szaniszlo 1997)
- Growth at 37 ° C (Liu et al. 2004)

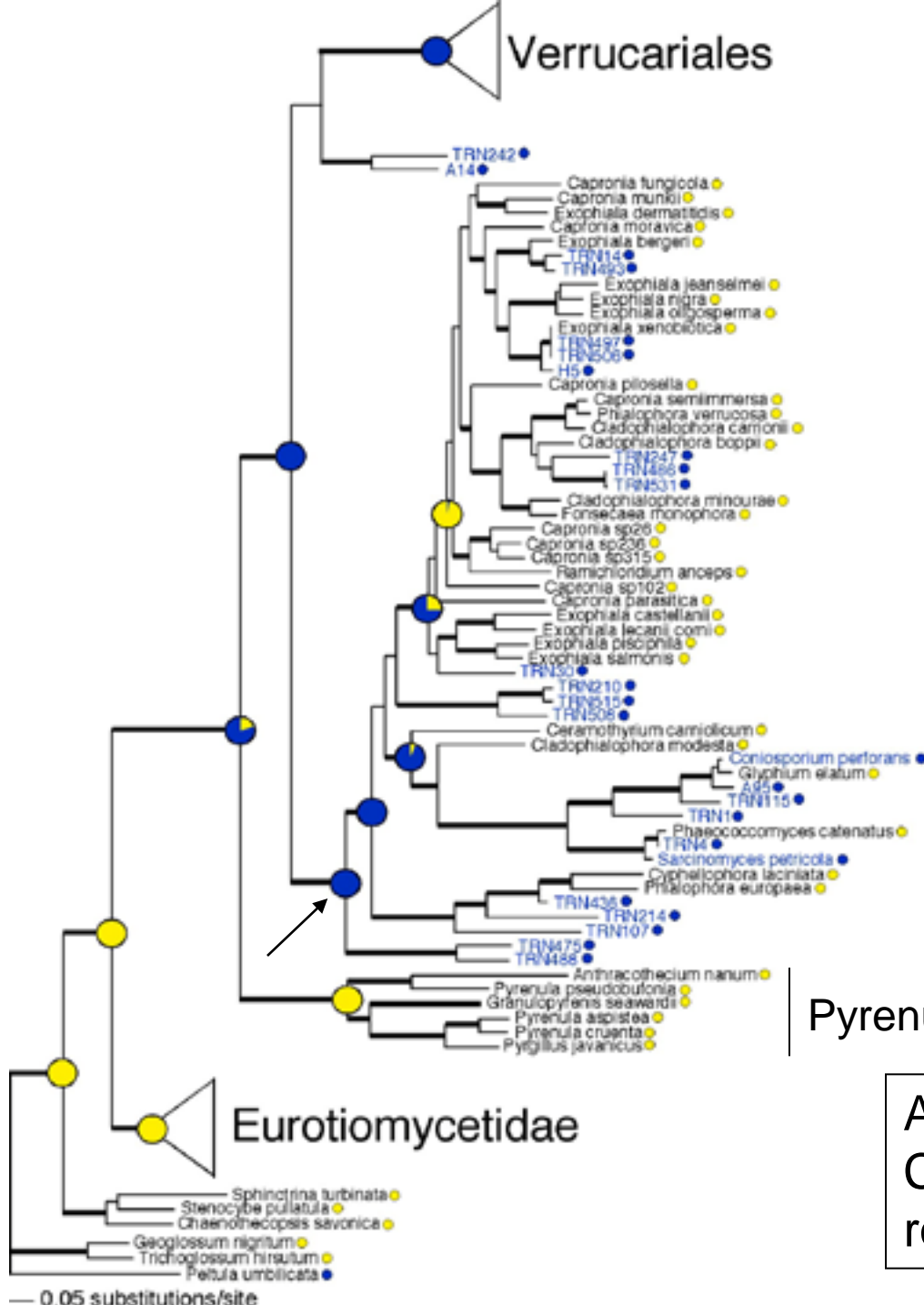


Substrates

- Rock substrate
- Other substrates

Chaetothyriales

Pyrenulales



Ancestor of Verrucariales and Chaetothyriales reconstructed as rock-inhabiting

Research question

Which of the following the potential virulence factors are likely to be involved in the evolution of pathogenicity in Chaetothyriales?

- Formation of muriform cells
- Presence of melanin
- Growth at 37 ° C
- Growth at 40 ° C

Methods

1. Multigene phylogeny of the Chaetothyriales using a taxon sampling representative of the ecological diversity of this group.
 - nucLSU, nucSSU, mtSSU, *RPB1*
 - about 80 taxa

Methods

2. Culture experiments to code all taxa included in our study

Presence or absence of melanin:

=> visual examination of the cultures

Ability to form muriform cells:

=> culture experiments: in-vitro induction with calcium and low pH

Ability to grow at 37 ° C:

=> culture experiments

Ability to grow at 40 ° C :

=> culture experiments

Methods

3. Reconstruct evolutionary history and test for correlated evolution

Mesquite (parsimony and likelihood methods)

BayesTraits (likelihood method)

Methods

- Null hypothesis: characters are not correlated (independent or I)
- Alternative hypothesis: characters are correlated (dependent or D)

$$LR = -2 \ln [L(I)/(D)]$$

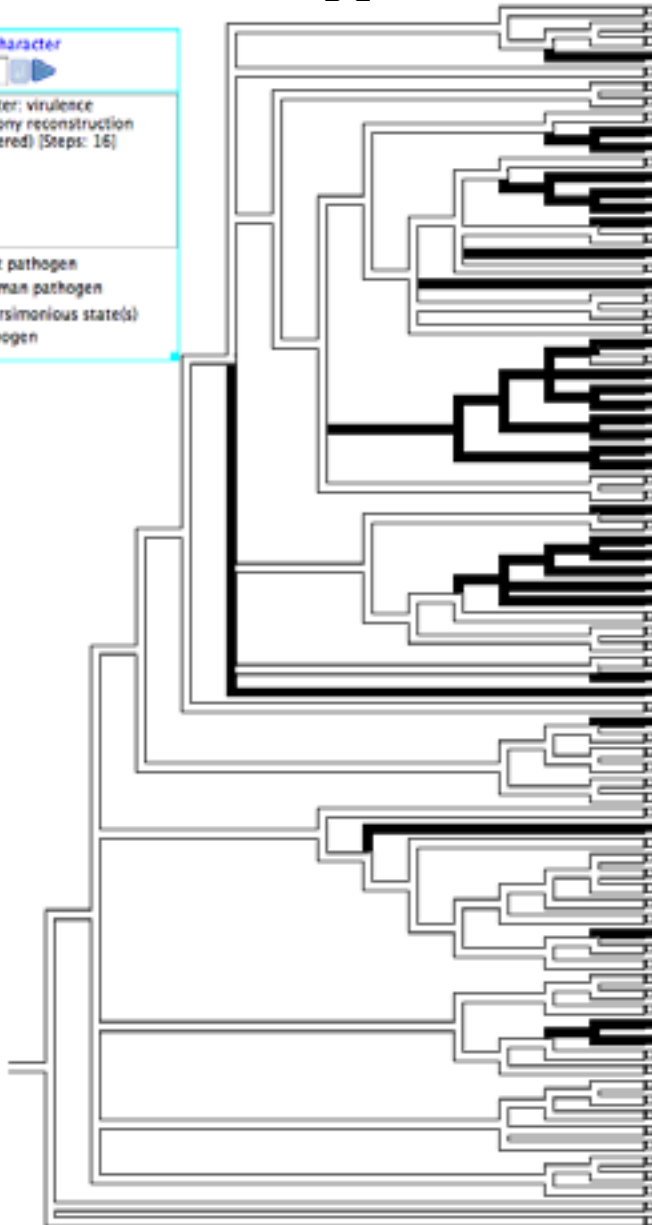
Pathogenicity and melanization

Trace Character
1

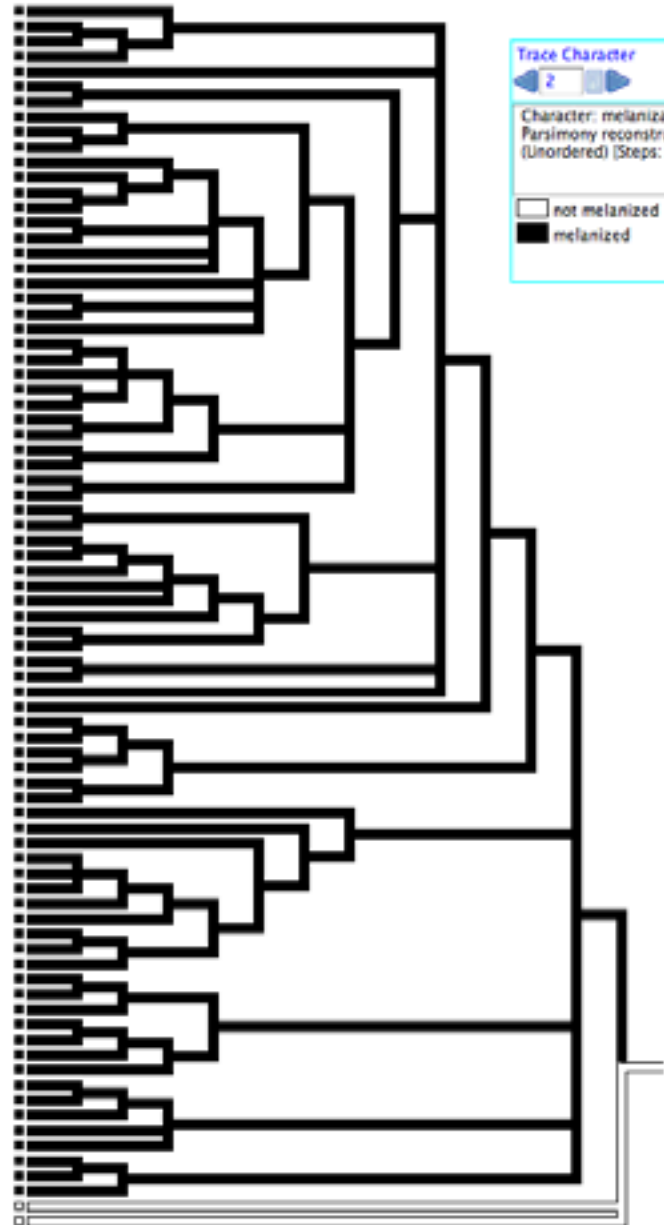
Character: virulence
Parsimony reconstruction
(Unordered) (Steps: 16)

not pathogen
 human pathogen

Most parsimonious state(s)
not pathogen



- Capronia fungicola CBS 614 96
- Capronia mairouxi CBS 101 67
- Capronia munkii CBS 615 96
- Exophiala dermatitidis CBS 207 35
- Capronia moravica WUC 15 551
- Capronia moravica CBS 603 96
- Capronia pilosella AFTOL 657
- Capronia pilosella CBS 125 88
- Cladophialophora mycetomatis CBS 122637
- Cladophialophora mycetomatis CBS 454 82
- Capronia semimmersa AFTOL 658
- Phialophora americana CBS 840 69
- Phialophora verrucosa CBS 138 67
- Phialophora verrucosa CBS 273 47
- Cladophialophora carnioni CBS 260 83
- Cladophialophora yegresii CBS 114405
- Cladophialophora samoensis CBS 259 83
- Cladophialophora subtilis CBS 122642
- Cladophialophora boppii CBS 126 86
- Cladophialophora chaetospira CBS 491 70
- Rock isolate TRN489
- Rock isolate TRN247
- Cladophialophora arxii CBS 306 94
- Cladophialophora minorae CBS 556 83
- Cladophialophora devriesii CBS 147 84
- Cladophialophora immunda CBS 834 96
- Cladophialophora saturnia CBS 102230
- Fonsecaea monophora CBS 102243
- Fonsecaea pedrosii CBS 271 37
- Cladophialophora bartiana CBS 173 52
- Cladophialophora emmonsii CBS 640 96
- Cladophialophora australiensis CBS 112793
- Cladophialophora potulientorum CBS 112222
- Exophiala bergii CBS 353 52
- Rock isolate TRN14
- Exophiala exophialae CBS 668 76
- Exophiala spinifera CBS 101534
- Exophiala spinifera CBS 899 68
- Exophiala jansselmei CBS 107 96
- Exophiala oligosperma CBS 725 88
- Exophiala nishimurae CBS 101538
- Exophiala xenobiotica CBS 115831
- Rock isolate TRN506
- Rhinocladiella anceps CBS 181 65
- Rhinocladiella aquaspersa CBS 122615
- Rhinocladiella mackenziei CBS 650 93
- Capronia parasitica CBS 123 88
- Exophiala castellanii CBS 158 58
- Exophiala lecanii CBS 123 33
- Exophiala piiciphila CBS 537 73
- Exophiala salmonis CBS 157 67
- Exophiala crusticola CBS 119970
- Rock isolate TRN30
- Ceramothyrium carniolicum CBS 125 95
- Cladophialophora modesta CBS 985 96
- Cladophialophora proteae CBS 111657
- Coniosporium perforans CBS 885 55
- Coniosporium species CBS 268 34
- Rock isolate A95
- Rock isolate TRN115
- Rock isolate TRN1
- Phaeoocomyces catenatus CBS 650 76
- Rock isolate TRN4
- Sarcinomyces petricola CBS 101157
- Cladophialophora hostae CBS 121637
- Cladophialophora scillae CBS 116461
- Rock isolate TRN107
- Cyphellophora lacinata CBS 190 61
- Phialophora europaeae CBS 129 96
- Rock isolate TRN416
- Rock isolate TRN214
- Cladophialophora humicola CBS 117536
- Cladophialophora minutissima CBS 121758
- Rock isolate TRN475
- Cladophialophora tylerstris CBS 350 83
- Rock isolate TRN489
- Rock isolate TRN210
- Rock isolate TRN515
- Rock isolate TRN508
- Placocarpus schaeferi AFTOL 2289
- Verrucula inconexaria AFTOL 2305



Trace Character
2

Character: melanization
Parsimony reconstruction
(Unordered) (Steps: 1)

not melanized
 melanized

Correlation test

Character pairs	Mesquite		BayesTraits		Null hypothesis of independence between characters
	LR	P-value	LR	P-value	
Pathogenicity and melanization	2.27	0.03	2.12	>0.1	Not rejected

40 ° C					Not rejected
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Ⓟ Pathogenicity and melanization independent or not

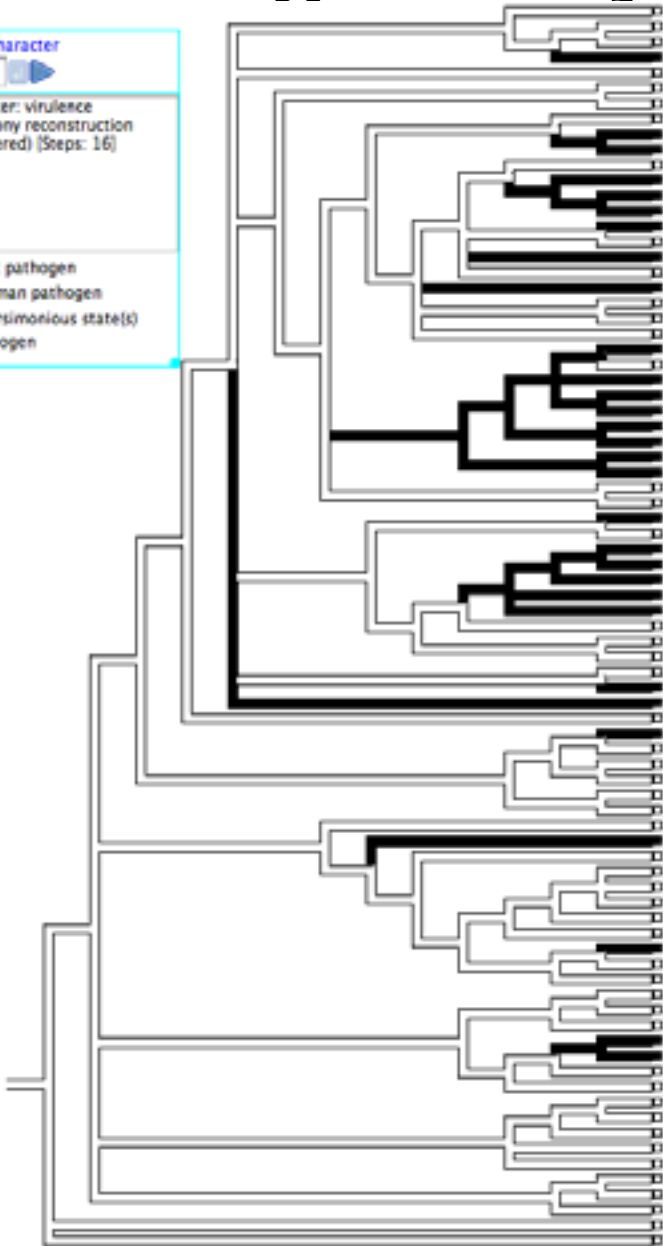
Pathogenicity and muriform cells

Trace Character

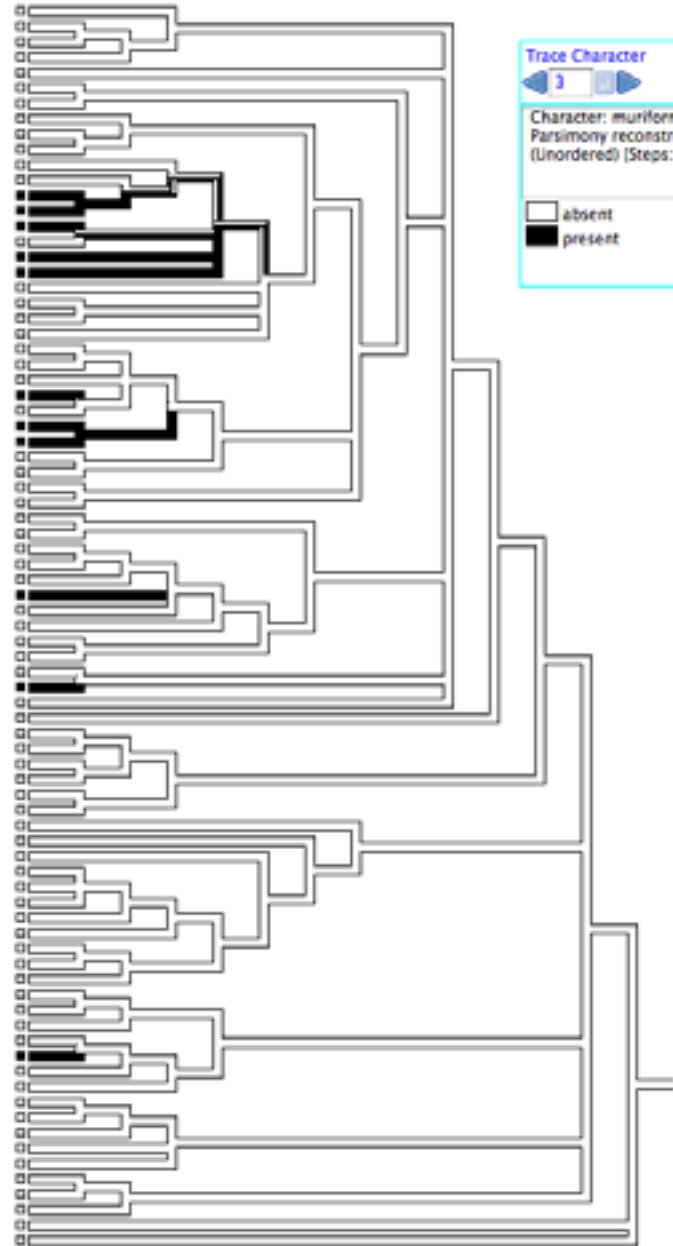
1

Character: virulence
 Parsimony reconstruction
 (Unordered) (Steps: 16)

not pathogen
 human pathogen
 Most parsimonious state(s)
 not pathogen



- Capronia fungicola CBS 614 96
- Capronia maresouii CBS 101 67
- Capronia murkii CBS 615 96
- Exophiala dermatitidis CBS 207 35
- Capronia moravica WUC 15 551
- Capronia moravica CBS 603 96
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- Cladophialophora yegresii CBS 114405
- Cladophialophora samoensis CBS 250 83
- Cladophialophora subellii CBS 122642
- Cladophialophora boppii CBS 126 86
- Cladophialophora chaetoptera CBS 491 70
- Rock isolate TRN486
- Rock isolate TRN247
- Cladophialophora anxii CBS 306 94
- Cladophialophora minourae CBS 358 83
- Cladophialophora devriesii CBS 347 84
- Cladophialophora immunda CBS 834 96
- Cladophialophora saturnica CBS 102230
- Fonsecaea monophora CBS 102245
- Fonsecaea pedrosi CBS 271 57
- Cladophialophora bantiana CBS 173 52
- Cladophialophora emmonsii CBS 640 96
- Cladophialophora australiensis CBS 112793
- Cladophialophora potulenterum CBS 112222
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- Exophiala spinifera CBS 101534
- Exophiala spinifera CBS 899 68
- Exophiala jeanselmei CBS 507 96
- Exophiala oligosperma CBS 725 88
- Exophiala nishimurae CBS 101518
- Exophiala xenobiotica CBS 115831
- Rock isolate TRN506
- Rhinocladiella anceps CBS 181 65
- Rhinocladiella aquaspersa CBS 122635
- Rhinocladiella mackenziei CBS 650 95
- Capronia parasitica CBS 123 88
- Exophiala castellani CBS 158 58
- Exophiala lecanii corni CBS 123 33
- Exophiala pycnophila CBS 337 71
- Exophiala salmonis CBS 157 67
- Exophiala crusticola CBS 119970
- Rock isolate TRN30
- Ceramothyrium carmolicum CBS 175 95
- Cladophialophora modesta CBS 985 96
- Cladophialophora proteae CBS 111667
- Coniosporium perforans CBS 885 95
- Coniosporium species CBS 268 34
- Rock isolate A95
- Rock isolate TRN115
- Rock isolate TRN1
- Phaeoocomyces catenatus CBS 650 76
- Rock isolate TRN4
- Sarcinomyces petricola CBS 101157
- Cladophialophora hostae CBS 121637
- Cladophialophora soliae CBS 116461
- Rock isolate TRN107
- Cyphellophora laciniata CBS 190 61
- Phialophora europaea CBS 129 96
- Rock isolate TRN436
- Rock isolate TRN214
- Cladophialophora humicola CBS 117536
- Cladophialophora minutissima CBS 121758
- Rock isolate TRN475
- Cladophialophora sylvestrus CBS 350 83
- Rock isolate TRN488
- Rock isolate TRN210
- Rock isolate TRN515
- Rock isolate TRN508
- Placocarpus schaeferi AFTOL 2289
- Verrucula inconexaria AFTOL 2305



Trace Character

3

Character: muriform cells
 Parsimony reconstruction
 (Unordered) (Steps: 9)

absent
 present

Correlation test

Character pairs	Mesquite		BayesTraits		Null hypothesis of independence between characters
	LR	P-value	LR	P-value	
Pathogenicity and melanization	2.27	0.03	2.12	>0.1	Not rejected
Pathogenicity and muriform cells	7.98	<0.001	16.00	<0.005	Rejected at the 1% level
40 ° C					Not rejected

↳ Pathogenicity and muriform cells correlated

Pathogenicity and growth at 37 °C

Trace Character

1

Character: virulence
 Parsimony reconstruction
 (Unordered) [Steps: 16]

not pathogen
 human pathogen

Trace Character

4

Character: growth at 37 °C
 Parsimony reconstruction
 (Unordered) [Steps: 14]

absent
 present



Correlation test

Character pairs	Mesquite		BayesTraits		Null hypothesis of independence between characters
	LR	P-value	LR	P-value	
Pathogenicity and melanization	2.27	0.03	2.12	>0.1	Not rejected
Pathogenicity and muriform cells	7.98	<0.001	16.00	<0.005	Rejected at the 1% level
Pathogenicity and growth at 37 ° C	10.99	<0.001	20.00	<0.001	Rejected at the 1% level
40 ° C

↳ Pathogenicity and growth at 37 ° C correlated

Pathogenicity and growth at 40 °C

Trace Character

1

Character: virulence
Parsimony reconstruction
(Unordered) [Steps: 16]

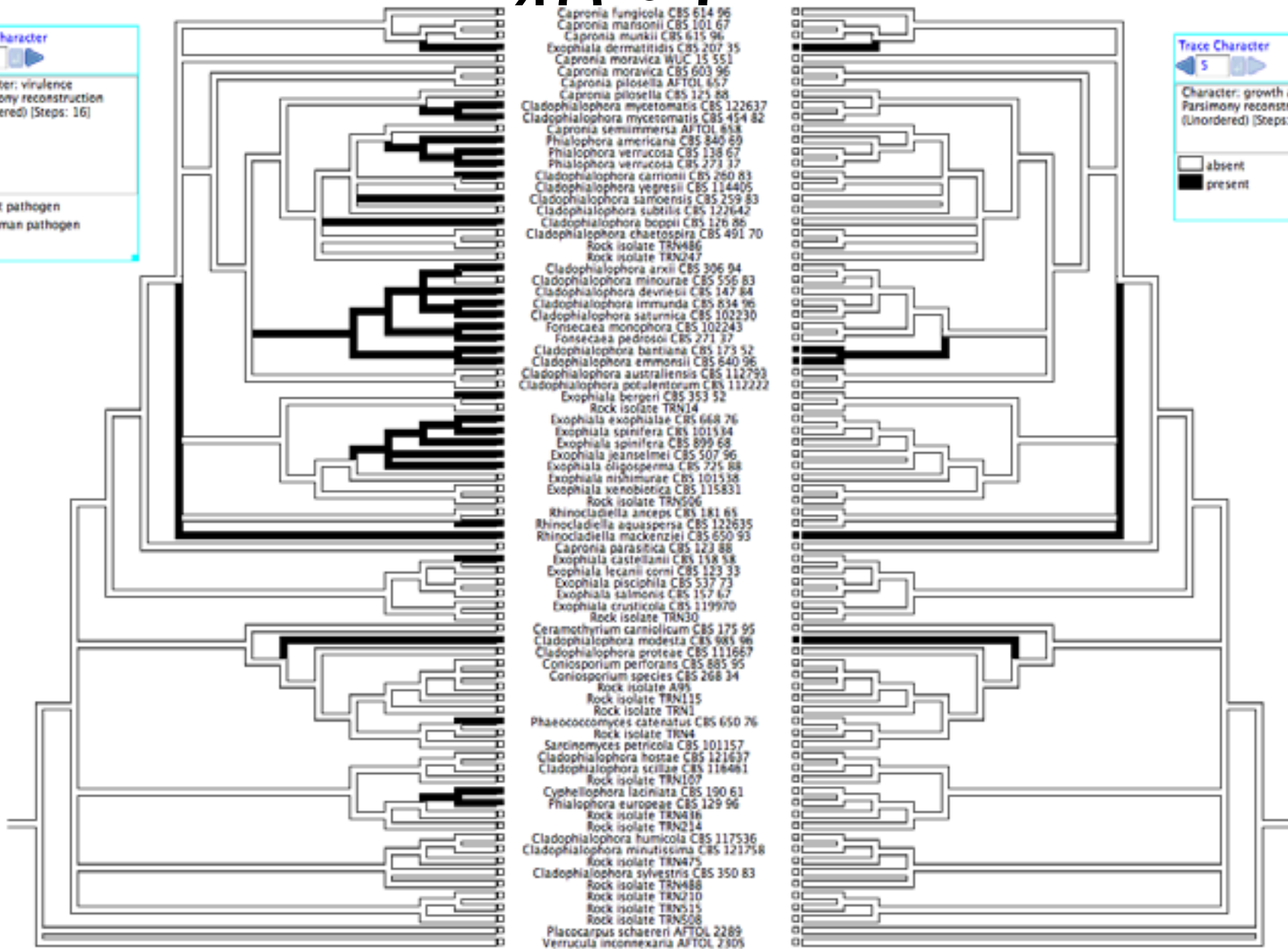
not pathogen
 human pathogen

Trace Character

5

Character: growth at 40 °C
Parsimony reconstruction
(Unordered) [Steps: 4]

absent
 present



Correlation test

Character pairs	Mesquite		BayesTraits		Null hypothesis of independence between characters
	LR	P-value	LR	P-value	
Pathogenicity and melanization	2.27	0.03	2.12	>0.1	Not rejected
Pathogenicity and muriform cells	7.98	<0.001	16.00	<0.005	Rejected at the 1% level
Pathogenicity and growth at 37 ° C	10.99	<0.001	20.00	<0.001	Rejected at the 1% level
Pathogenicity and growth at 40 ° C	5.44	0.01	8.00	>0.01	Not rejected

↳ Pathogenicity and growth at 40 ° C independent or no

Conclusion

- According to our results, the ability to form muriform cells and more importantly, the ability to grow at 37°C are the two main adaptations that drove the evolution of pathogenicity in Chaetothyriales
- The presence of melanin is also most likely involved in pathogenicity, but not sufficient to confer virulence
- The ability to grow at 40°C is probably involved in the pathogenicity of certain strains

Acknowledgments

- Tino Ruibal, Anna Gorbushina for sharing their isolates of rock-inhabiting fungi,
- Samantha Kant for her help with culture experiments,
- CBS collection team.

