

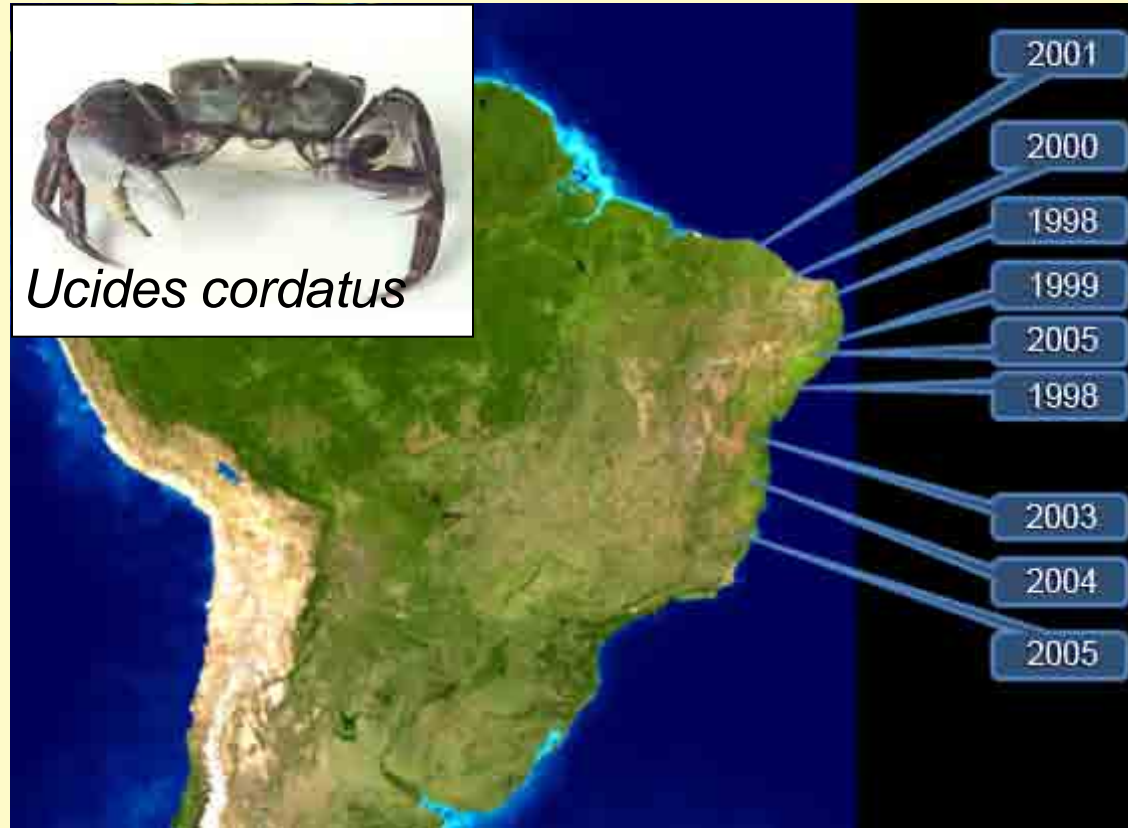
# **Causative agents of Lethargic Crab Disease (LCD) in mangrove land crab *Ucides cordatus* (Ocypodidae) in Brazil**

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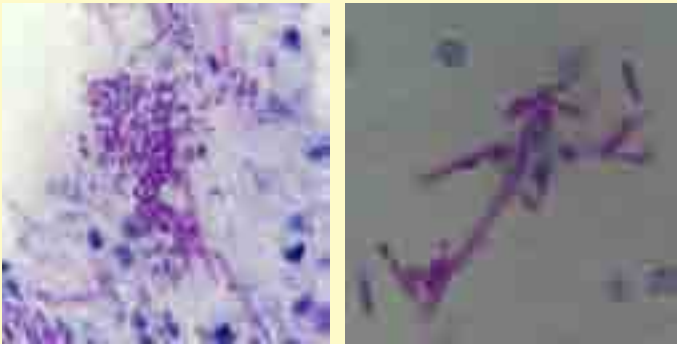
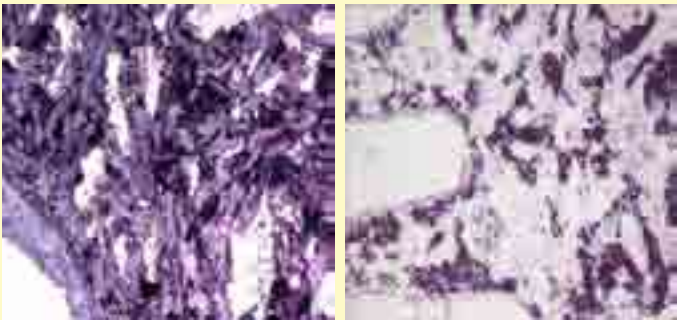


# Lethargic Crab Disease (LCD)

- Systemic infection
- Extensive epizootic mortality in mangrove crab along brazilian coast



# Lethargic Crab Disease (LCD)

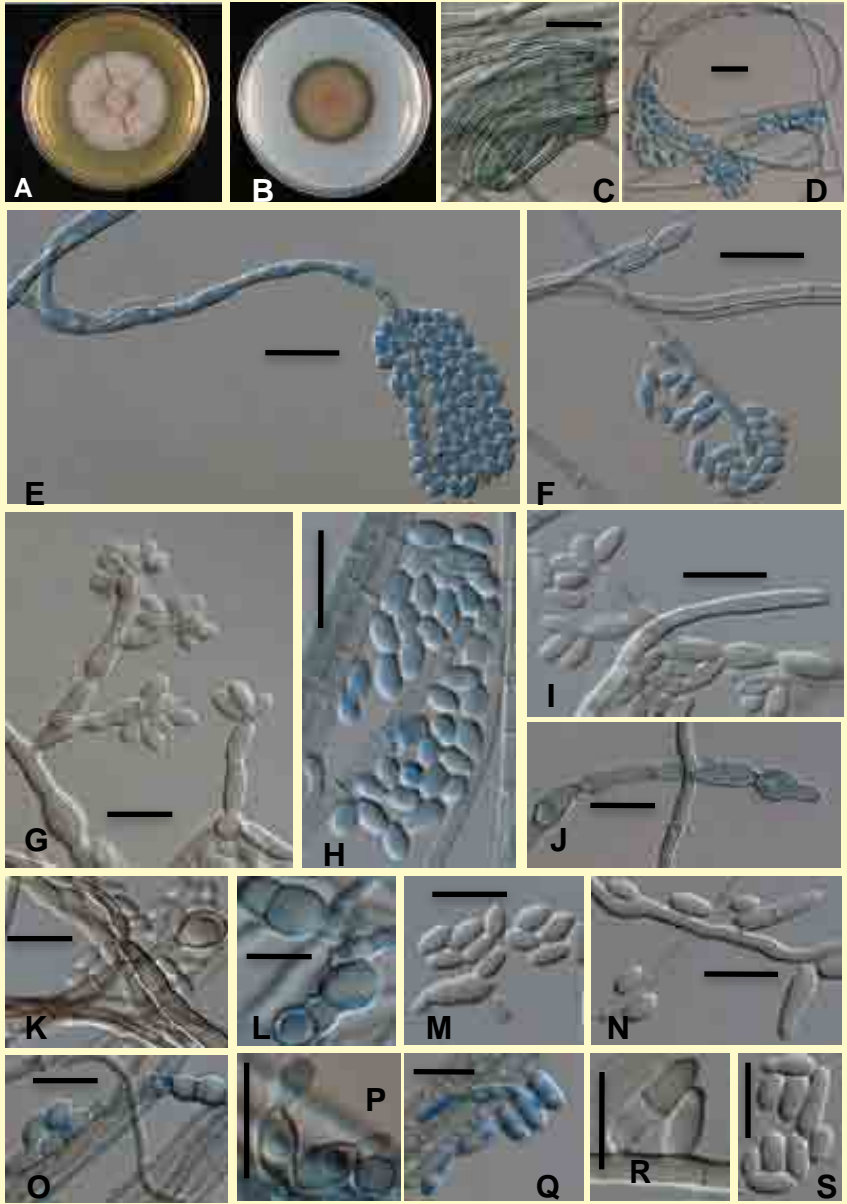


Disseminated infection

The main signs:

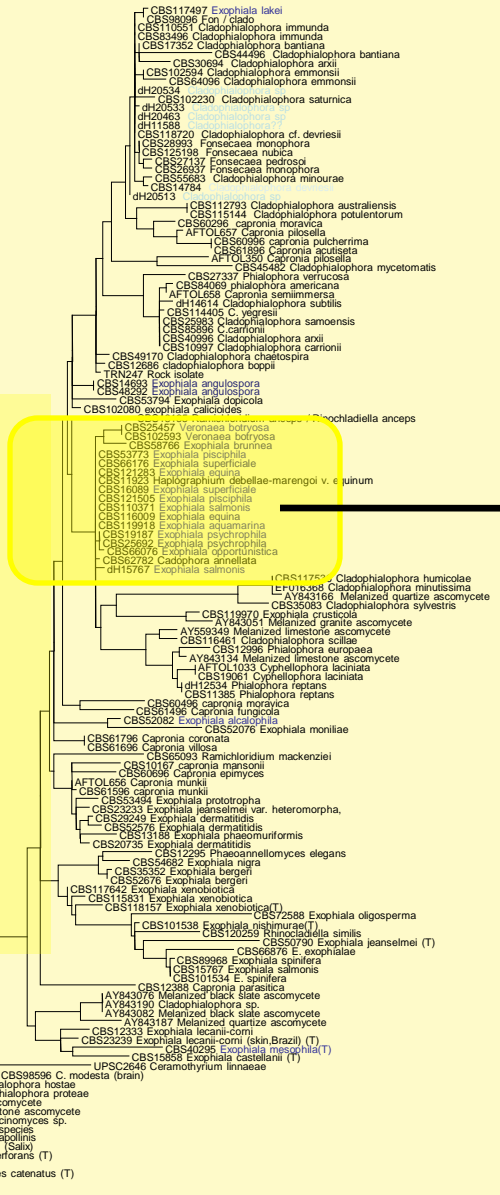
- weak motor control
- causing lethargy and poor balance
- tetany

*Exophiala cancerae* - CBS 120420 type strain, Mangrove crab, Brazil.



*Exophiala* species:

- E. cancerae*
- E. halophila*
- E. alcalophila*
- E. angulospora*
- E. salmonis*
- E. pisciphila*
- E. equina*
- E. psychrophila*
- E. aquamarina*
- E. opportunistica*
- E. superficiale*
- V. botryosa*



0.005

# Strains of *Exophiala cancerae*

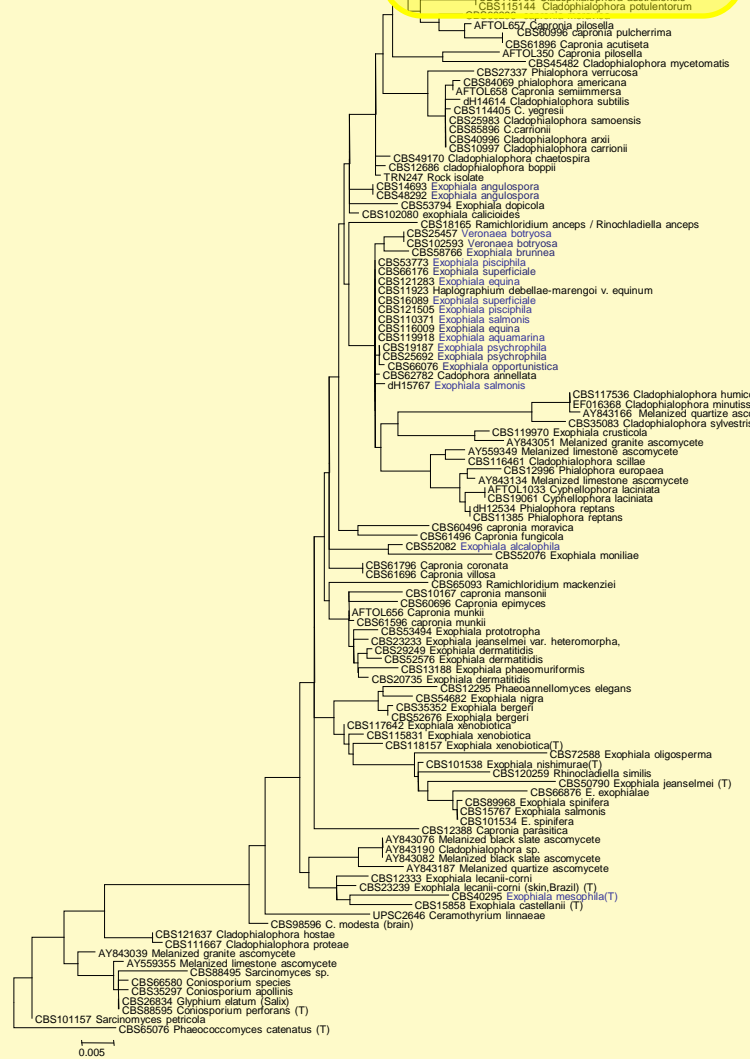
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<b>Strain number</b>	<b>Source</b>	<b>Region</b>
GHPO R40	drinking water tower	Germany
CBS 120532	crab	Brazil
<b>CBS 120420 (T)</b>	<b>crab</b>	<b>Brazil</b>
CBS 119920	toad liver, frog	Israel
Det. M154/ 2007	nail	The Netherlands
UWFP 724	clinical isolate	USA, Univ. Washington
GHP 2419	skin diabetes	Germany
dH 12901	water	Germany
dH 12895	water	Germany
CBS 117491	clean water from CIP tank	Netherlands
CBS 115142	fruit drink	Australia

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*Cladophialophora*  
*Fonsecaea*

CBS117497 *Exophiala lakei*  
 CBS98096 *Fon / clado*  
 CBS110551 *Cladophialophora immunda*  
 CBS33496 *Cladophialophora immunda*  
 CBS17352 *Cladophialophora bantiana*  
 CBS44496 *Cladophialophora bantiana*  
 CBS30694 *Cladophialophora arxii*  
 CBS102594 *Cladophialophora emmonsii*  
 CBS44096 *Cladophialophora emmonsii*  
 dh20534  
 CBS102230 *Cladophialophora saturnica*  
 dh20533  
 dh20483  
 dh11568  
 CBS118720 *Cladophialophora cf. devriesii*  
 CBS28993 *Fonsecaea monophora*  
 CBS125198 *Fonsecaea nubica*  
 CBS27137 *Fonsecaea pedrosoi*  
 CBS29537 *Fonsecaea monophora*  
 CBS55583 *Cladophialophora minourae*  
 CBS14784  
 dh20513  
 CBS112793 *Cladophialophora australiensis*  
 CBS115144 *Cladophialophora poluicorum*



0.005

*Cladophialophora* sp.



*Cladophialophora devriesii*

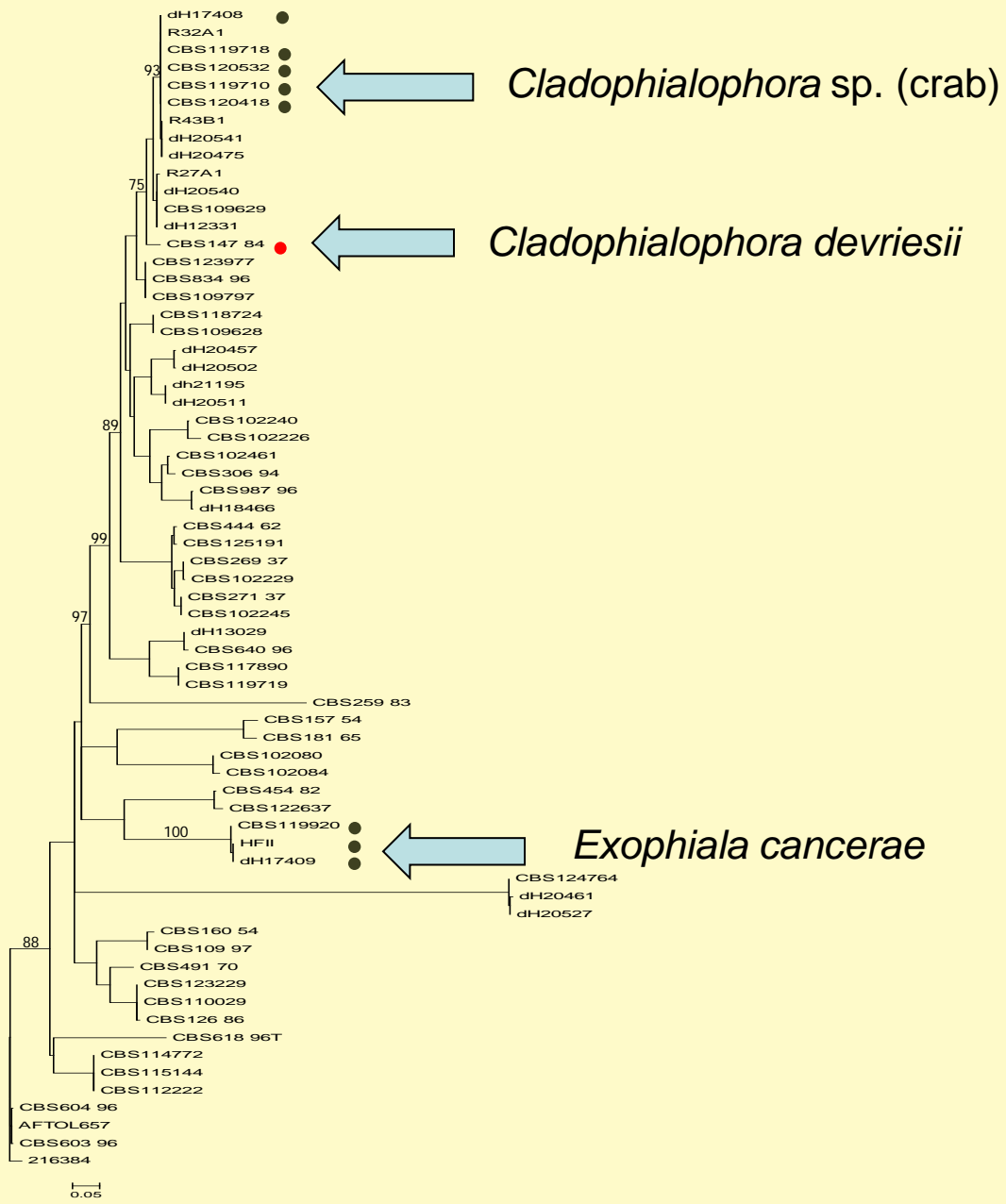


co-infection by *Cladophialophora* sp.

the first case was described from human  
- Cayman Island (Gonzalez *et al.* 1984,  
Mitchell *et al.* 1990).

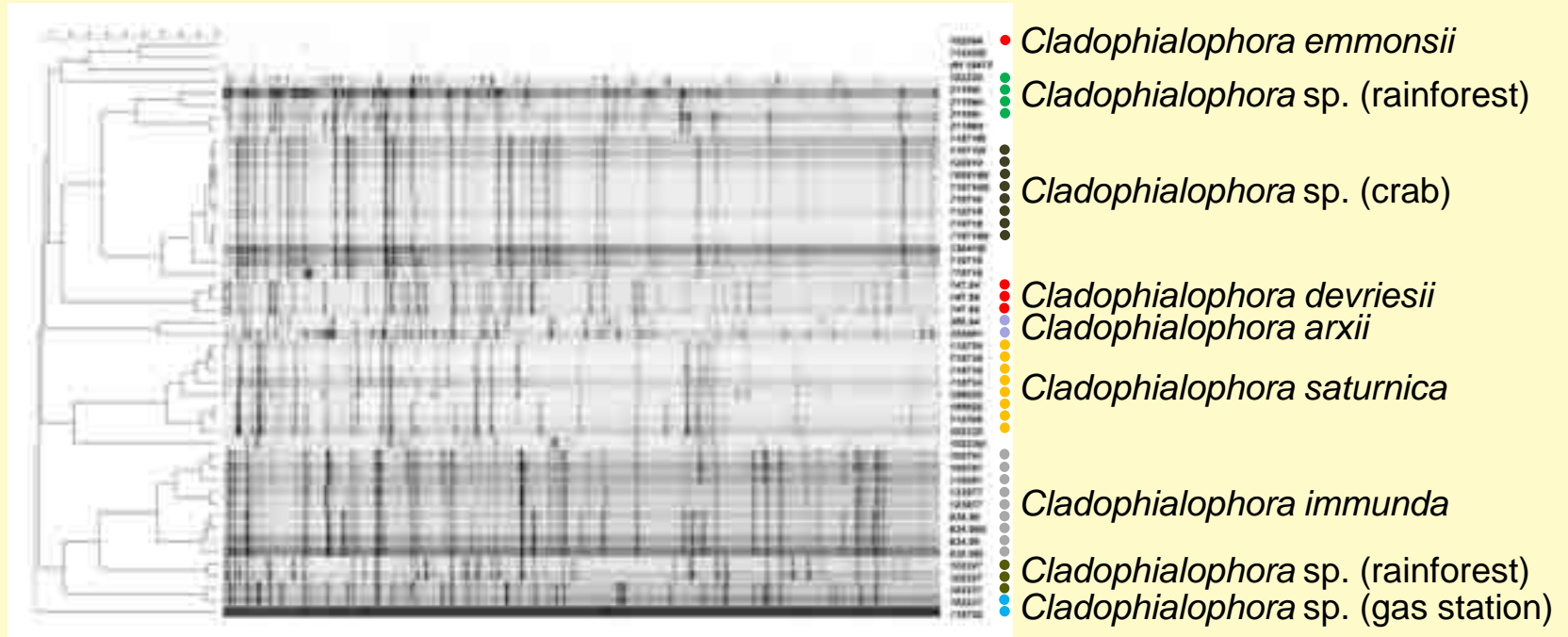
ITS – Tree: RaxML version 7.0.4 (model GTR)

- crab
- human



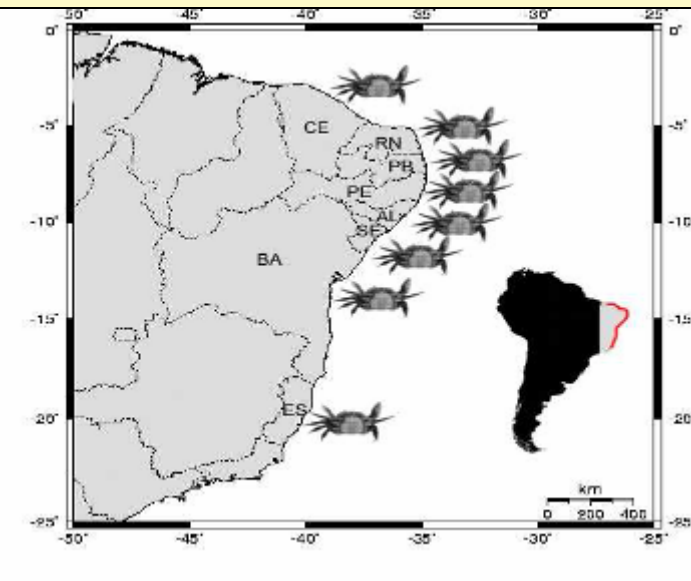


# AFLP profile of sibling species



# Isolation from the mangrove

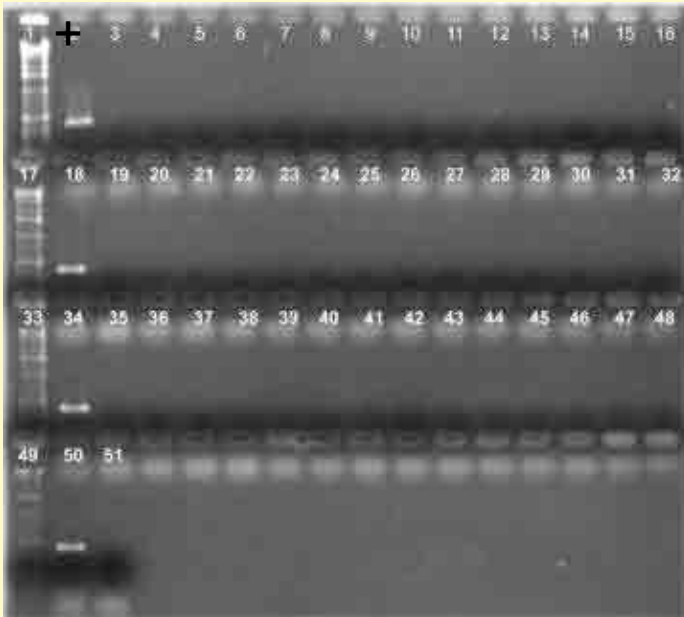
(Flotation method – Vicente *et al.* 2008, *Studies in Mycology* 61: 137–144)



# Isolation from mangrove - endemic area

- 141 environmental isolates, morphologically:
  - 51 *Exophiala*-like
  - 90 *Cladophialophora*-like
- species-specific primer

***E. cancerae*: 0/51**

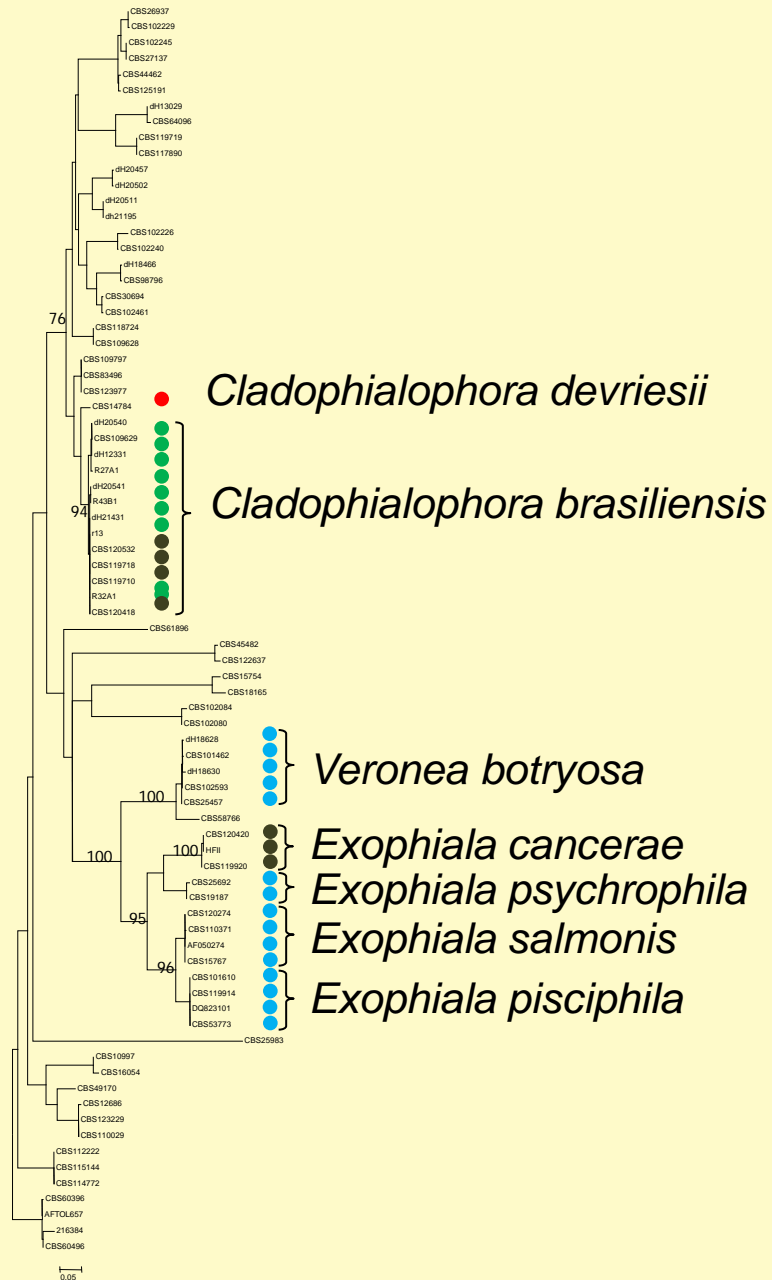


***C. brasiliensis*: 10/90**

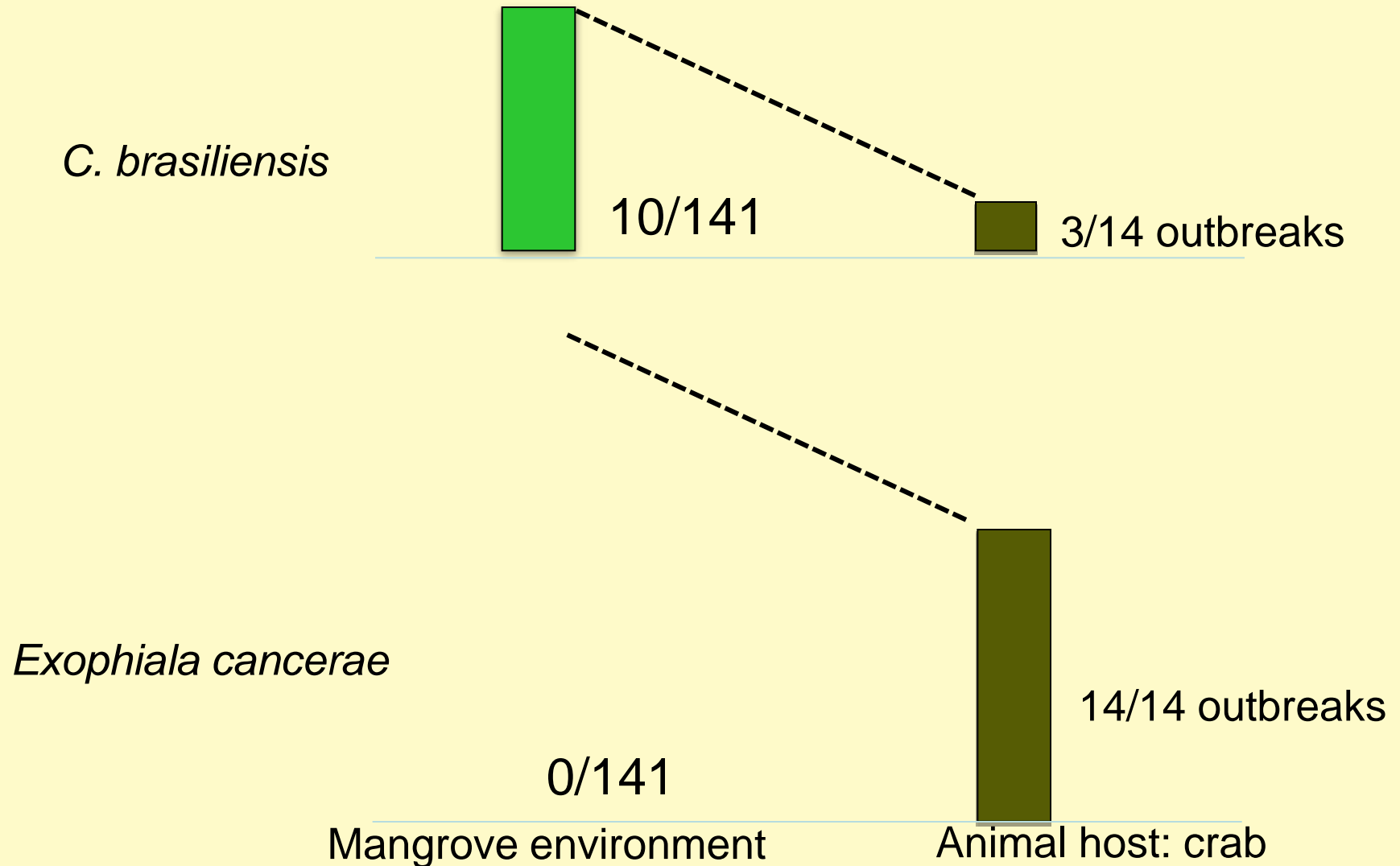


# ITS – Tree: RaxML version 7.0.4 (model GTR)

- crab
- environment
- human
- waterborn



# Frequencies mangrove environment / crab of sibling species



# Pathogenicity tests

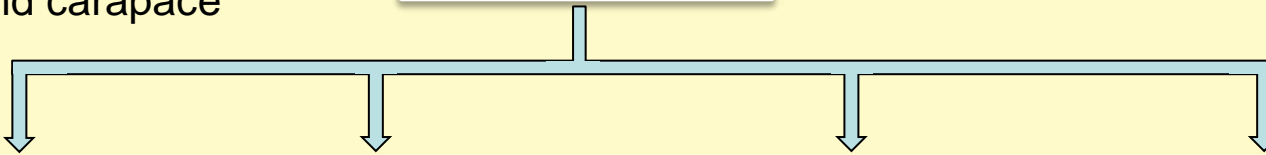
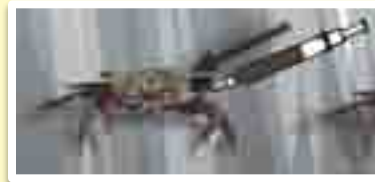
Healthy specimens  
from outside LCD-stricken area



Animals were housed for  
one week acclimation and  
absence of overt disease



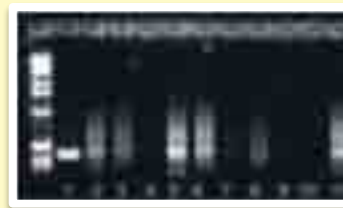
Injections were given in the  
arthrodial membrane at the  
juncture of the pereopod basis  
and carapace



Mortality rate



Histopathology



Specific marker



Re-isolation

# Pathogenicity tests

The crabs were divided in 3 groups per strain evaluated (1 treatment and 2 controls):

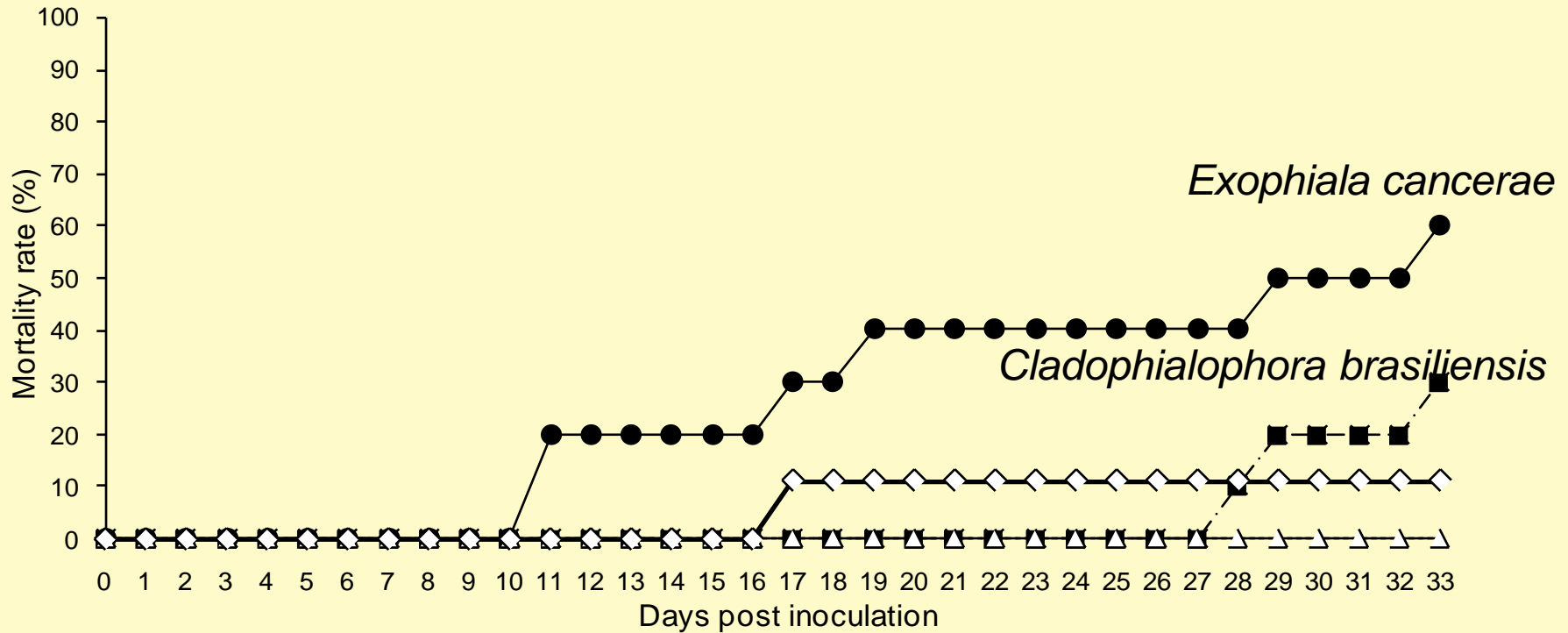


(1) specimens were injected with 1 mL inoculum solution;

(2) specimens were submitted to injection of 1 mL of saline solution (2.5%);

(3) crabs were held in the experimental aquaria without manipulations, after having suffered the needle inoculation stress (without any solution).

# Pathogenicity tests



Symbols:

●, Infected group (*Exophiala cancerae*); ■, Infected group (*Cladophialophora* sp.)  
◇, Control group (saline solution); △, Control group



# Conclusions

- The *E. cancerae* is considered as the main agent since it was present in all events of Lethargic Crab Disease
- The *C. brasiliensis* takes an advantage of weak crabs affected by *E. cancerae*, suggesting an existence of an opportunistic infection
- Relative frequency host / environment is a useful parameter to predict virulence of the fungus



# Acknowledgments

- CAPES: financial support

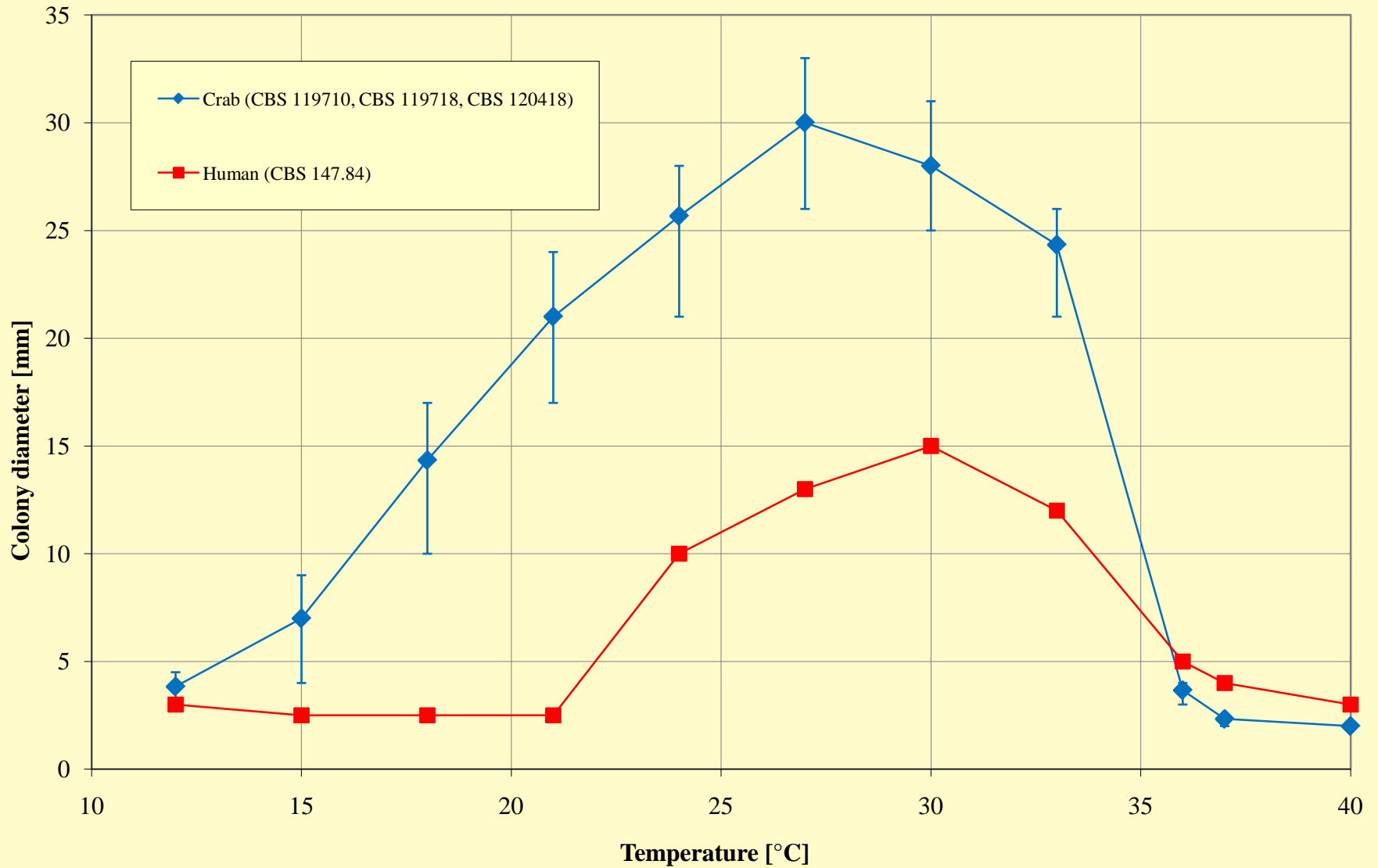


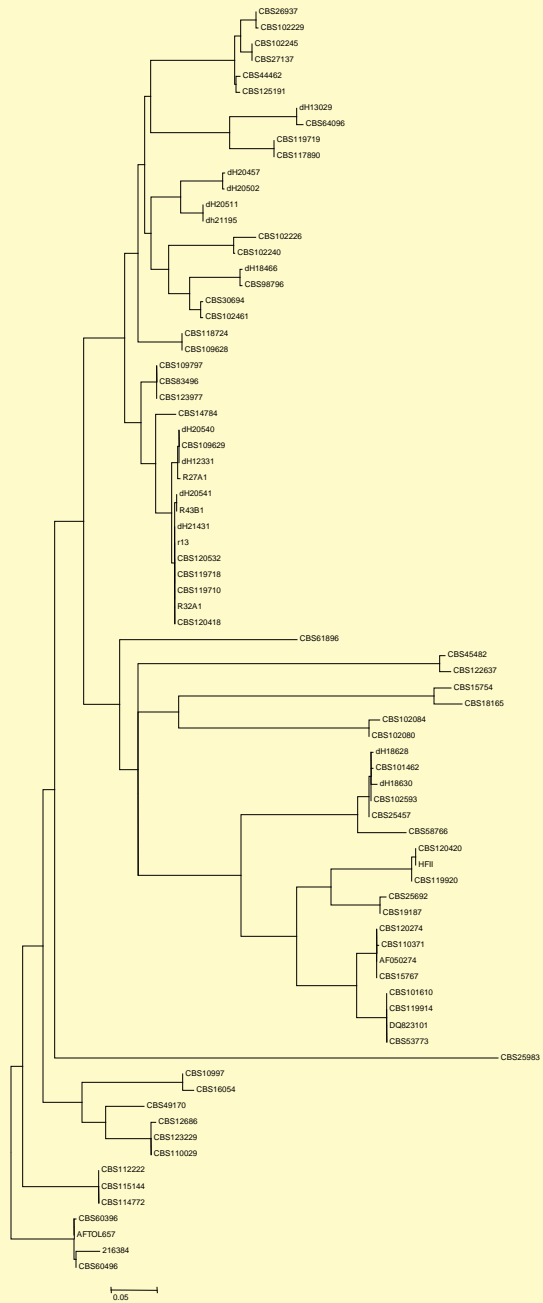
- GIA group (Grupo Integrado de Aqüicultura e Estudos Ambientais) - Brazil
- BYP group/CBS

**Thank you for your  
attention !!!**



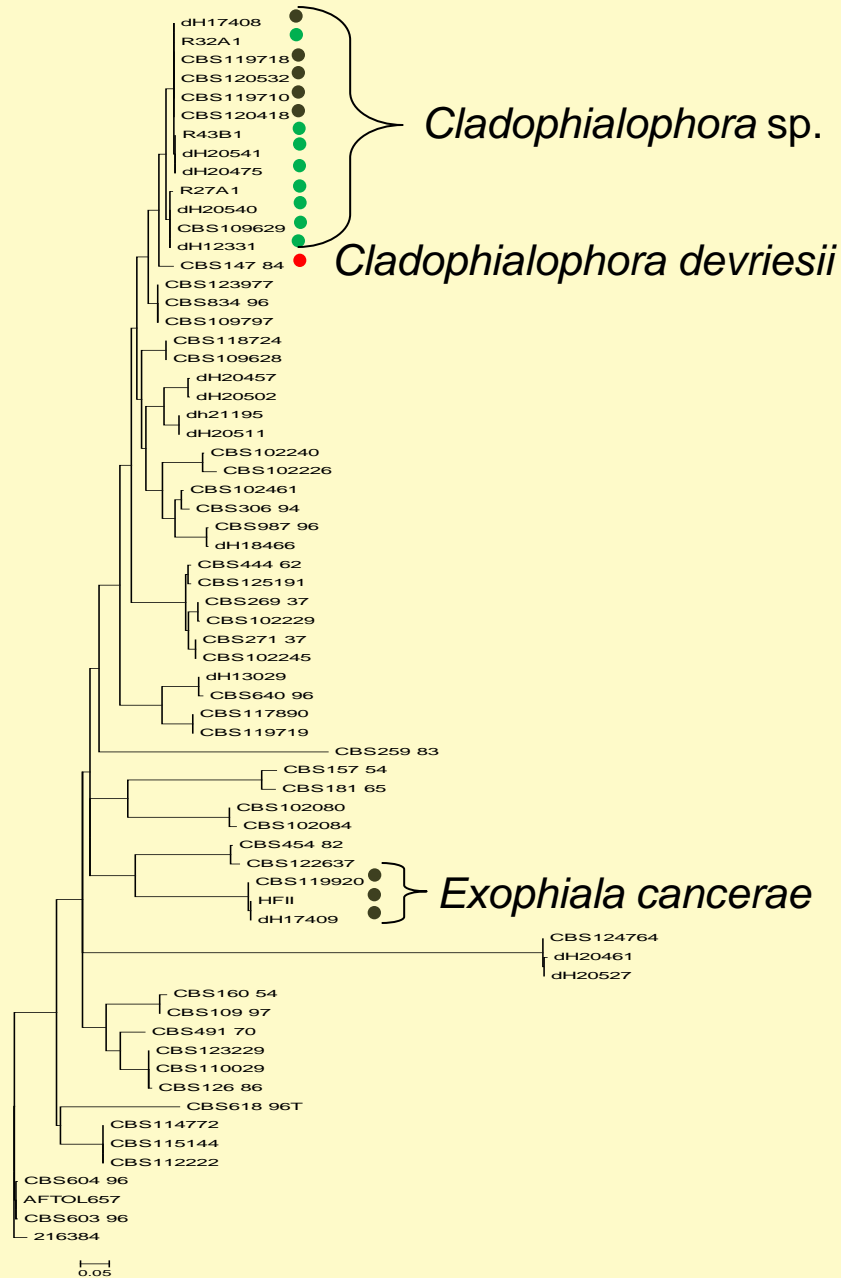
# Temperature test





# ITS – Tree: RaxML version 7.0.4

- crab
- environment
- human



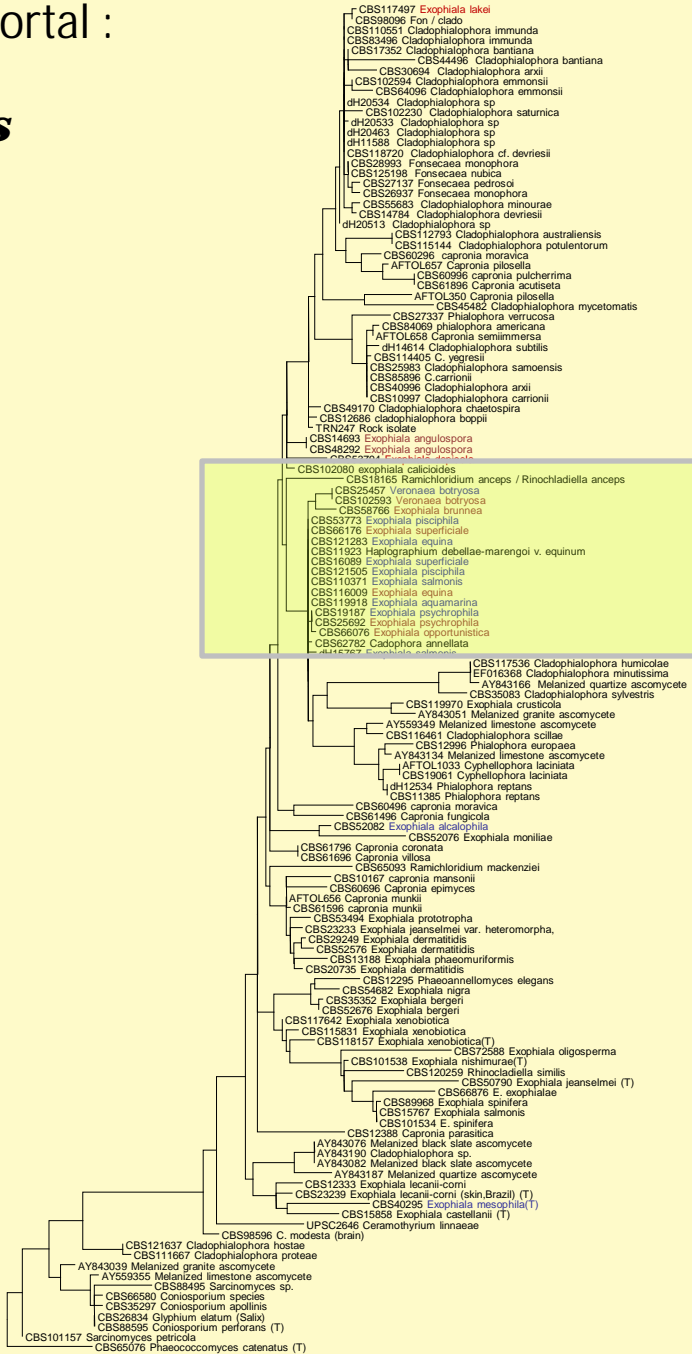
SSU RaxML ver.7.0.4 in cipres portal :

# order *Chaetothyriales*

Waterborne clade



salmonis clade:  
*E. angulospora*  
*E. halophila*  
*E. alcalophila*  
*E. pisciphila*  
*E. aquamarina*  
*E. equina*  
*E. superficiale*  
*E. salmonis*  
*E. opportunistica*  
*E. psychrophila*  
*E. cancerae*  
*V. botryosa*



Temperature