

Exophiala Infection in Pacific Bonito (*Sarda chiliensis lineolata*) in a public aquarium

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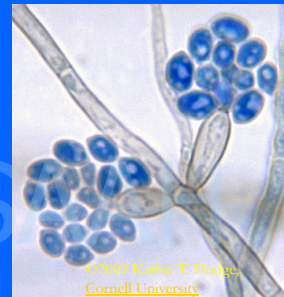
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⁴ Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio



Exophiala species

- Septate, dark-walled fungi (dematiaceous, phaeoid, melanized, “black yeast”)
- Multiple species, high molecular diversity
- Saprophyte; but cutaneous, subcutaneous, systemic mycosis (phaeohyphomycosis, chromoblastomycosis)
- Affects humans and animals (fish, invertebrates, cats, dogs, reptiles, amphibians)
- ID by culture & PCR
- Ubiquitous in environment
- Worldwide distribution
- Opportunistic pathogen
- Emerging pathogen

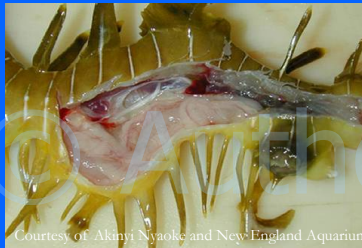


Exophiala Infection in Fish

- FW and MW fish
- Cultured and captive fish
- Anorexia, weakness, lethargy, Skin ulcers, muscle necrosis, exophthalmia, piping, abnormal buoyancy/swimming, kidney & coelom distension, nodules on internal organs, prolapsed hemorrhagic vent, mycetoma,



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Courtesy of Akinyi Nyaok and New England Aquarium

- Most isolated cases; mass mortalities also
- Stress, trauma, immunosuppression
- Contamination (e.g., water, food, soil, rock salt)
- *E. salmonis*, *E. pisciphila*, *E. psychophila*, *E. jeanselmei*-like, *phialophora*-like, *E. angulospora*, *E. xenobiotica*

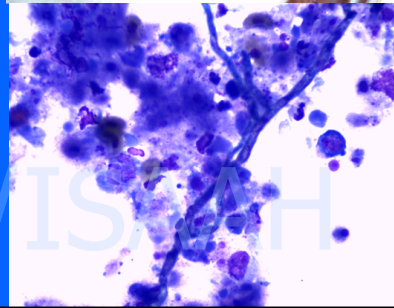
Bonito Case History

- Wild-caught off S. California
- For the Outer Bay Exhibit
- Quarantine started 5/28/10
- Fed sardine, squid, gel diet
- 48 pacific bonitos, 21 pacific barracuda in the tank
- Unremarkable entrance exam
- 2 healthy survey animals necropsied 5/28/10
 - Coccidia in liver
 - *Photobacterium damsela* in kidney (1)
- No prophylactic medical treatment
- Two sudden deaths 6/30/10

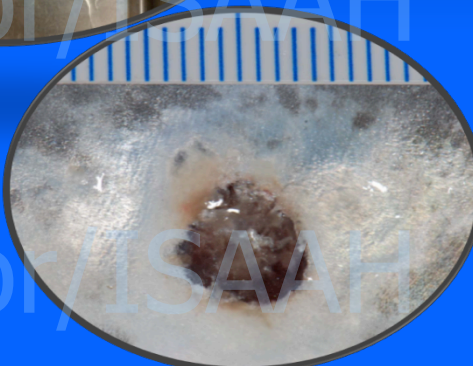


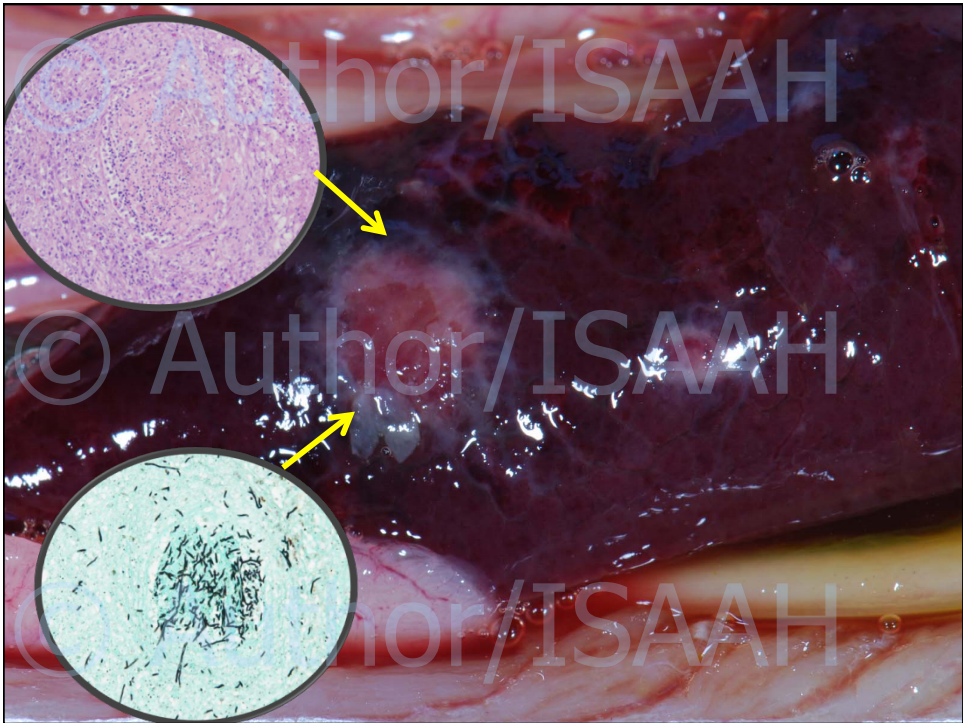
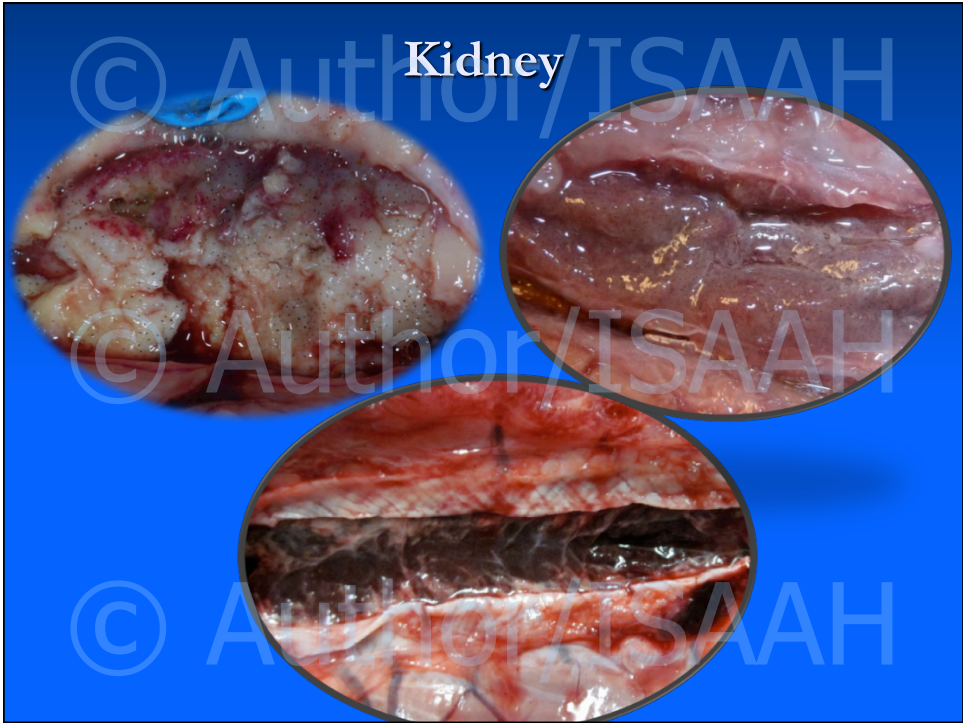
Diagnostic tests

- Gross necropsy
- Cytology, skin scraping, gill biopsy
- Histopathology
- Skin and kidney cultures
 - Bacteriology
 - Mycology:
- Fungus identification
 - Culture
 - PCR
- Viral culture



Skin Ulcer



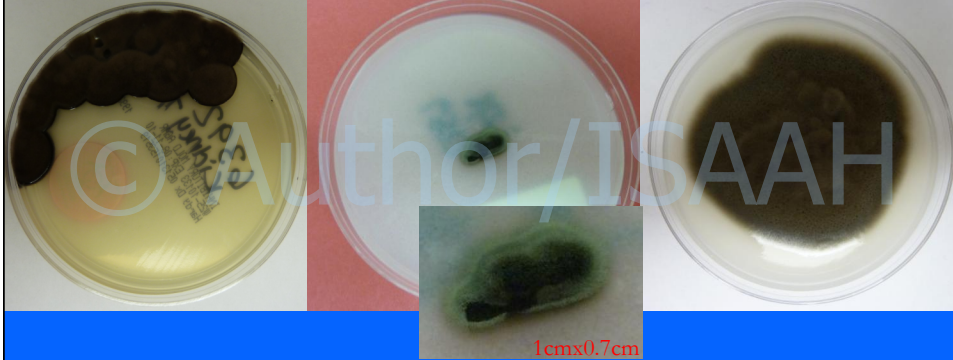


Initial Fungal Culture (UC Davis)

Inhibitory mold agar @ 30 C
(5wks)

Potato flake agar @ 30C
(1wk)

Potato flake agar @ 30C
(5wk)



- Kidney and skin cultures
- Velvety, olivaceous to gray-black colonies; black reverse
- Microscopic exam
Dark septate, branching fungus; single-borne conidia; anneloconidia

Fungus ID: Culture and molecular ID

- Fungus Testing Laboratory, The University of Texas Health Science Center at San Antonio

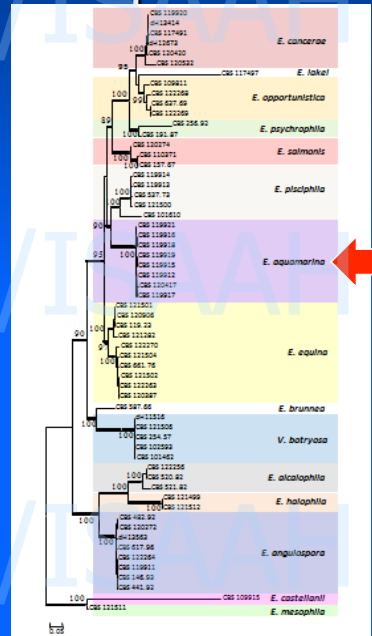


- No match in PCR rDNA sequence → New spp of *Exophiala*

Fungus ID: CBS PCR sequence

- Dr. de Hoog, Centraalbureau voor Schimmelcultures, the Netherlands
- The isolate PCR sequence
 - *E. aquamarina*
 - Salmonis-clade
 - Closely related to *E. pisciphila*
 - Multiple fish isolates
- Comprehensive characterization of waterborne *Exophiala* spp to come (de Hoog upcoming)

The phylogenetic tree adapted from Figure from de Hoog, et al. (upcoming)



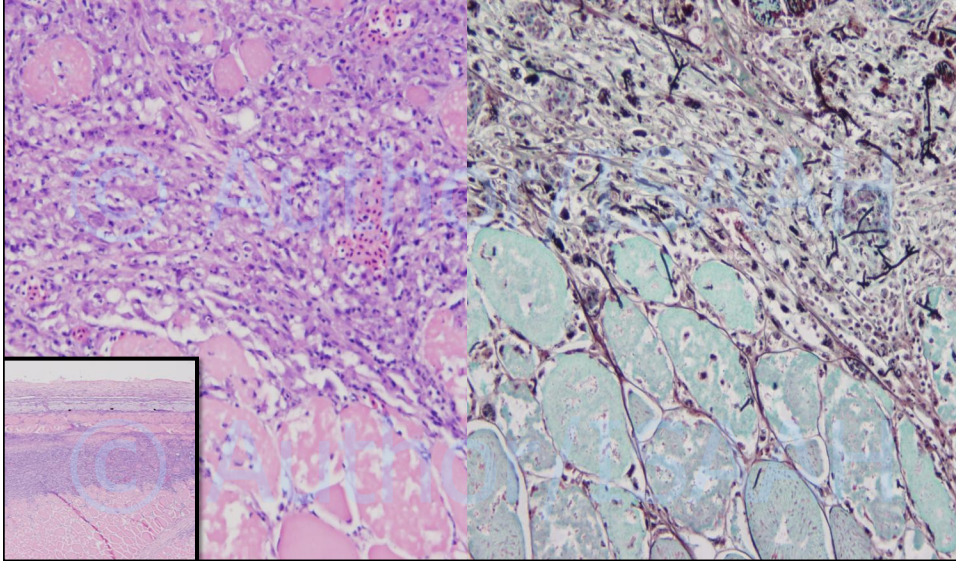
Histopathology

- Disseminated phaeohyphomycosis
- Fungal hyphae: skin, kidney, liver, spleen, stomach
- Granulomatous inflammation
- Angiogenic
- Most severe lesion in kidneys
- The portal of entry: Skin → hematogenous spread



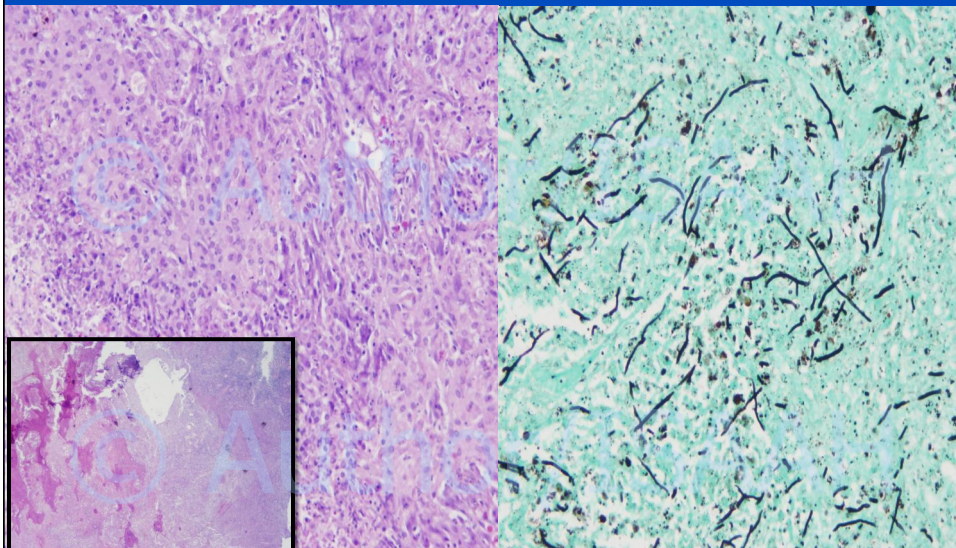
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Skin & Muscle

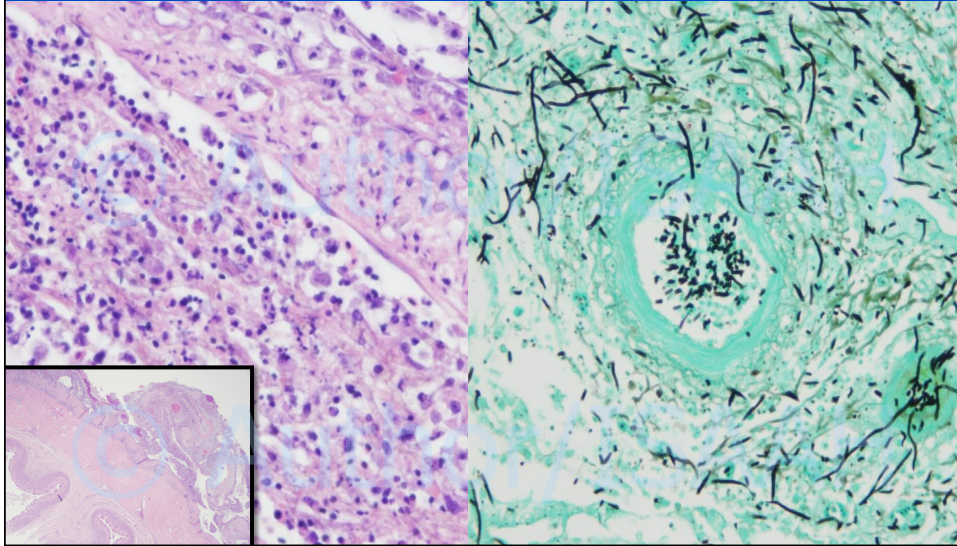


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Kidney



© Author/ISAAH Stomach



© Author/ISAAH Clinical Diagnoses

- Disseminated phaeohyphomycosis
- Sepsis from mixed bacterial infection
 - *Photobacterium damsela* (healthy-kidney, sick – kidney, skin)
 - *Vibrio alginolyticus* (sick kidney, skin)
- Neobenedinia infection
- Coccidiosis (Incidental)



Courtesy of Akinyi Nyaoke and Roger Williams Park

Treatment in Fish

- No approved drug in fish
- Effective drug? Dosage? Duration?
- No effective treatment for fish
 - NEAQ (and Shedd ?)
- Human cases more successful
- Exophiala species-specific tx?
- Antifungal *in-vitro* sensitivity not reliable *in-vivo*
- Long-term Tx required
- Systemic dz more difficult to treat
- Triazole drugs (Itra, posaconazole, voriconazole) more success; some resistance
- Other animal cases:
 - Aldabra tortoise carapace tx (*E. oligosperma*)
 - Cat tail/foot lesion (*Scolecobastidium humicola*)
 - Most euthanised or died



Follow-up and recommendations

Follow-up

- No Rx at this time
- Animals closely monitored
- No additional mortality, abnormal behavior or clinical signs
- Good appetite; active



Recommend:

- Exit PE with random sampling full necropsy
- Disinfect tanks, filter, etc
- Environmental sampling



Prevention and Control

- Preventative medicine; herd health
- Quarantine protocol with strict hygiene, biosecurity; vigilant monitoring
- Isolation of sick & control the spread of dz
- Full necropsy of all dead
- Effective disinfection and sanitation



Conclusion

- Bonito's *Exophiala* similar pathogenesis as in sea dragon
- Low morbidity & mortality in Bonitos (sample size!)
 - Fungal load too low?
 - More time required for clinical signs?
 - Bonito more resistant?
 - Commensal fungus?
 - Strain not as virulent?
- Bonito as a model to study pathogenesis of *exophiala* and immunology
- More information needed:
 - *Exophiala* spp (e.g., pathogenesis, virulence, transmission, prevalence)
 - Tx options?
 - Fish species difference in infectability & survivability
 - Zoonotic potential



Acknowledgement

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