

DNA-Based Characterization Of Wood-, Butt- And Root-Rot Fungi From The Western Pacific Islands

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INTRODUCTION AND METHODS

Although the islands of the western Pacific comprise a hotspot of species, including fungi, a large number of these species have not been catalogued or documented in the scientific literature on an island to island basis. Butt- and root-rot fungi were collected from infected wood and fruiting bodies of diverse tropical trees from forest, agricultural, and urban sites during an outreach/survey trip to USA-affiliated islands in the western Pacific including Palau, Yap, Guam, Saipan, Pohnpei, and Kosrae (Figure 1). Collections focused on the aggressive brown root-rot pathogen, *Phellinus noxius* (Figure 2), although other collections were made when possible. These fungi were subsequently cultured from fruiting bodies (e.g. Figure 3) or infected wood. DNA of each cultured isolate was extracted and partial sequences of the internal transcribed spacer (ITS1-5.8S-ITS2) region (hereafter ITS) were amplified with PCR at the Moscow Forestry Sciences Laboratory (USDA Forest Service-Rocky Mountain Research Station). PCR products were sequenced, and edited sequences were compared to the GenBank® database using the nucleotide BLAST®. All

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living collections made on these islands are currently archived at the Moscow Forestry Sciences Laboratory Fungal Culture Collection for ongoing genetic analyses.

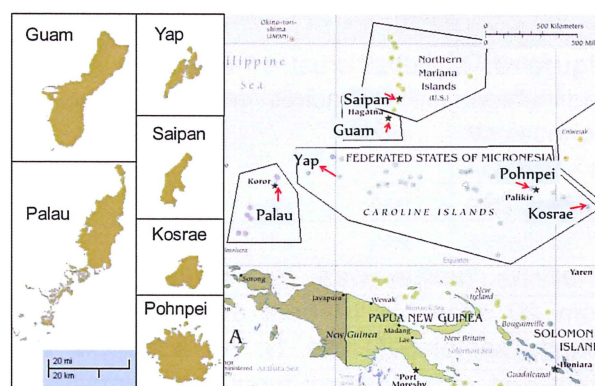


Figure 1. Fungi were collected from the U.S.A.-affiliated islands of Palau, Yap, Guam, Saipan, Pohnpei and Kosrae.



Figure 2. Basidiocarps of *Ganoderma australe* complex on *Casuarina equisetifolia* from Saipan (top left) and Guam (bottom left and right).

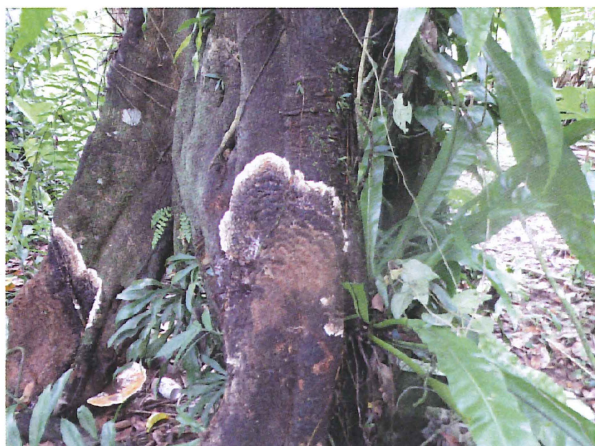


Figure 3. Mycelial crust of *Phellinus noxius* and unidentified bracket fruiting body in Pohnpei.

RESULTS

Phellinus noxius was successfully isolated from 20 sites on 10 host species on all 6 islands sampled (Table 1). An additional 24 butt-, root- and wood-rot fungi were tentatively identified to species, genus or family from 31 sites across the 6 islands using the ITS region of rDNA (Table 2). In the process, non-targeted, tree/fungus-associated fungi from 28 sites across all islands were also tentatively identified to genus: *Lasiodiplodia*, *Rhytidhysterium*, *Hypocrea* / *Trichoderma*, *Fusarium*, *Valsa*, *Chaunopycnis*, *Pestalotiopsis*, *Conidiocarpus*, and *Pseudoallescheria*.

DISCUSSION & FUTURE WORK

DNA sequences of the ITS region of the rDNA proved useful to identify *P. noxius* isolated from infected wood and fruit bodies. These sequences also narrowed the identification of other fungi to the species, genus, or family level. Depending on how a species is defined, often entries with more than 2% difference in base pair identity are considered to be different species (e.g., see Lindner et al. 2011). We specifically targeted fungi with obvious fruit bodies or other morphological indicators, and

still nine isolations obtained in this project did not meet the 2% criteria (i.e., maximum identity score was less than 98%). Many of the 'closest match' entries in GenBank® had no species-level or even genus-level names attached to them. While the ITS region cannot always differentiate species or even genera, it is useful in preliminary identifications because GenBank® has more fungal sequences of the ITS region than any other gene region. Using this tool, taxa were defined as well as possible into a table that could assist in prioritizing additional research needs.

Table 1. Collection and identification of *Phellinus noxius* on different hosts and islands.

Island	Host	Sites sampled	Highest Maximum ID Score	Closest GenBank Match Accession #
Guam	<i>Guamia mariannae</i>	3	98%	KF233592
Guam	<i>Macaranga thompsonii</i>	2	98%	KF233592
Guam	<i>Ochrosia mariannensis</i>	1	98%	KF233592
Kosrae	<i>Artocarpus altilis</i>	1	99%	JQ003227
Kosrae	<i>Sonneratia</i> sp.	1	98%	JQ717347
Palau	unknown stump	1	97%	HQ400705
Pohnpei	<i>Artocarpus altilis</i>	2	98%	JQ717347
Pohnpei	<i>Ficus tinctoria</i>	1	98%	KF233592
Saipan	<i>Delonix regia</i>	5	98%	KF233592
Saipan	<i>Mangifera</i> sp.	1	99%	JQ003227
Yap	<i>Mangifera</i> sp.	1	99%	KF233592
Yap	<i>Inocarpus</i> sp.	1	95%	KF233592

As time permits, the fruiting bodies collected in this project will be identified morphologically. Morphological identifications will be then connected to the ITS sequences from the fruit bodies or cultures and entered into GenBank®. Additional molecular work investigating the population genetics of *P. noxius* is also underway.

On islands dependent on global commerce, it is vitally important to have species lists for each island in order to avoid unwanted introductions of invasive species. Destructive

species such as *P. noxius* should be tracked and monitored to prevent further spread within an island's shores as well as reduce island-to-island spread. Results from this project emphasize the need for further collection and identification efforts in the western Pacific to provide baseline information on current species distribution within and among islands. These efforts must combine morphological and molecular identification with subsequent entry

into public databases such as GenBank®. Reliable long-term fungal databases for these biologically diverse regions are critical to monitor and manage species movements across the islands. Finally, if possible, maintaining living cultures of these diverse fungi should be maintained to expand options for future analyses as DNA-based and other technologies continue to improve and become more accessible.

Table 2. Collection and identification of wood-associated fungi on different hosts and islands.

Island	Tentative ID	Host	Sites sampled	Highest Max ID Score	Closest GenBank Match Accession #
Guam	Ganoderma austral complex	Casuarina equisetifolia	3	99%	GU213473
Guam	Ganoderma sp.	Casuarina equisetifolia	1	98%	AY569452
Guam	Phillimus sp.	Thespesia populnea	1	94%	EU035311
Kosrae	Ganoderma sp.	Terminalia carolinensis	1	*	
Kosrae	Rigidoporus vinctus	Artocarpus altilis	1	99%	HQ400710
Palau	Coriolopsis sanguinaria	Rhizophora spp.	1	98%	FJ627251
Palau	Fomitiporia sp.	Casuarina spp.	1	95%	GU461943
Palau	Fulvifomes sp.	Rhizophora spp. & unknown mangrove	2	99%	JX104683
Palau	Ganoderma austral complex	Unknown stump	1	99%	FJ582638
Palau	Ganoderma sp.	Rhizophora spp.	1	97%	JN234428
Palau	Psilocybe/ Deconica sp.	Unknown stump	1	91%	HM036648
Pohnpei	Ceriporia lacerata	Artocarpus altilis	1	99%	KF850375
Pohnpei	Earliella scabrosa	Unknown downed log	1	99%	FJ11056
Pohnpei	Phlebia sp.	Unknown stump	1	99%	KC492493
Pohnpei	Rigidoporus microporus	Ficus tinctoria	1	199%	HQ400708
Pohnpei	Rigidoporus vinctus	Artocarpus altilis	1	99%	HQ4000710
Pohnpei	Tinctoporellus epimiltinus	Artocarpus altilis	1	99%	FJ711050
Pohnpei	Wrightoporia sp.	Artocarpus altilis & unknown stump	3	95%	FJ904857
Saipan	Fulvifomes sp.	Casuarina equisetifolia	2	98%	JX104711
Saipan	Ganoderma austral complex	Casuarina equisetifolia	1	99%	FJ392286
Saipan	Phanerochaetaceae	Delonix regia	1	96%	HQ248216
Yap	Cerrena sp.	Areca catechu	1	99%	FJ010208
Yap	Lenzites/ Trametes sp.	Piper betle	2	100%	HQ248217
Yap	Psilocybe/ Deconica sp.	Cocos nucifera	1	92%	HM036648

*Imperfect sequence .Closest matches to Ganoderma spp.; also identified by collector as Ganoderma sp.

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