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## A phylogeny for the plant pathogen *Piptoporellus baudonii* using a multigene data set

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### ABSTRACT

*Piptoporellus baudonii* is proposed as a new combination for *Laetiporus baudonii* in the Polyporales (Basidiomycota) based on morphological and molecular features. This parasitic macrofungus attacks cashew trees, *Eucalyptus*, cassava, *Tectona*, and some indigenous trees in southern regions of Tanzania and poses a serious threat to agroforestry and livelihood conditions in the area. Phylogenetic trees were produced from partial sequences of three rDNA gene regions and a portion of translation elongation factor 1-alpha (*TEF1*) gene of *Laetiporus baudonii* for comparisons with samples from the antrodia clade. Our results reveal a strongly supported group of *L. baudonii* with *Piptoporellus* in Fomitopsidaceae. *Piptoporellus baudonii* shares many morphological features with other members of *Piptoporellus* but differs from them in having broadly ellipsoid or rarely ovoid basidiospores. Both morphological and phylogenetic evidence justify the placement of *L. baudonii* in *Piptoporellus* together with the three other known species in the genus.

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## INTRODUCTION

*Laetiporus baudonii* (Pat.) Ryvarden (Ryvarden 1991) is a large parasitic polypore fungus well known in Africa. Reports suggest that the species afflicts a wide range of economically important plants in various parts of Africa (Van der Westhuizen 1973), including Congo (Patouillard 1914), on *Manihot* in Madagascar (Heim 1931), on *Cassia siamea*, *Khaya senegalensis*, and *Citrus* species in Ghana (Ofosu-Asiedu 1975), in tea plantations in Malawi (Rattan and Pawsey 1981), on *Eucalyptus* in South Africa (Van der Westhuizen 1973), on *Acacia tortilis* in Kenya, on *Anacardium occidentale* (Cashew tree) in Tanzania, and on various indigenous plants (Sijaona 2007). Death of the host plants is associated with the appearance of large bright orange-yellow poroid basidiomata at the bases of trunks and on the ground among the infected trees. These fruit bodies develop from a pseudosclerotial tissue composed of a mat of coarse creamy-yellowish mycelium and sand grains that cover the roots and underground parts of the stems of afflicted trees (Van der Westhuizen 1973). Root infection causes early symptoms of loss of the deep green color of leaves and the yellowing of leaves on individual branches, followed by frequent and rapid wilting of the leaves (Rattan and Pawsey 1981).

*Laetiporus baudonii* belongs to the order Polyporales (Basidiomycota), which includes more than 1800 species (Kirk et al. 2008). Most species in this order occur on wood. Seven major clades have been recognized in the Polyporales—the core polyporoid, residual polyporoid, phlebioid, gelatoporia, tyromyces, fragiliporia, and antrodia clades (Binder et al. 2005; Zhao et al. 2015; Justo et al. 2017). Justo et al. (2017) assigned family names to 18 clades in their formal classification. The antrodia clade encompasses many genera, including *Laetiporus* Murrill and *Phaeolus* (Pat.) Pat. in Laetiporaceae Jülich and *Piptoporus* P. Karst. and *Fomitopsis* P. Karst. in Fomitopsidaceae Jülich.

Since it was first described from Congo, *Polyporus baudonii* Pat. (Patouillard 1914) has been less well understood in a systematic framework that has resulted in the naming of several taxonomic synonyms (Westerhuizen 1973). In a study investigating the morphology of fruit bodies and cultures isolated from a parasitic fungus found in South Africa, and comparison with the descriptions of the type specimens of *Polyporus baudonii* and *Phaeolus manihotis* R. Heim, Van der Westhuizen (1973) observed morphological similarities between the studied parasitic fungus and *Phaeolus manihotis*, with the former generally having larger basidiomata than the latter. Van der Westhuizen (1973) confirmed the morphological

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resemblance between *Phaeolus manihotis* and *Polyporus baudonii* as suggested by Browne (1968). The study also referred to the fungus as *Polyporus baudonii* due to the observation of a dimitic hyphal system that differed from that of the type species of *Phaeolus* (Donk 1960), which has a monomitic hyphal system. Furthermore, Van der Westhuizen (1973) observed complex morphological features that did not match those of any known genus at that time. Because of this, Ryvarden described the new genus *Pseudophaeolus* Ryvarden, with *Ps. baudonii* (Pat.) Ryvarden as the type (Ofosu-Asiedu 1975). Later, Ryvarden (1991) accepted *P. baudonii* in *Laetiporus* as *L. baudonii* (Pat.) Ryvarden.

The association of a polyporoid macrofungus with wilting of cashew nut trees, cassava, and *Eucalyptus* trees in the Mtwara and Lindi regions of southern Tanzania has been reported (Sijaona 2003, 2007). Morphologically, this fungus resembled *L. baudonii*. In order to ascertain its identity, and given the inconsistencies in classifications based on morphological features, we incorporated molecular data for an accurate placement. This study aims to ascertain the phylogenetic position of *L. baudonii* using a four-gene region data set based on analyses of partial sequences from three nuc rDNA gene regions and translation elongation factor 1-alpha (*TEF1*).

## MATERIALS AND METHODS

**Study site.**—The material studied originated from the Mtwara region in southern Tanzania (ca. 10°10'–11°30'S to 37°58'–40°25'E), where cashew is a major cash crop that is frequently affected by this fungus. The region is mostly characterized by a high relative humidity (87% in Feb–Jun, which is usually the long rainy season), whereas the lowest relative humidity of 63% occurs in Sep–Oct. Temperatures vary with cold months (Jun–Sep) with an average of 19.5 C, whereas hot months reach above 30 C (Sep–Dec). The soils are sandy loam or red clay soil, receiving an annual rainfall of 900–1200 mm (Sijaona 2003; Sijaona and Shija 2005).

**Field sampling.**—An unidentified fungus was first observed by the staff at the Naliendele Agricultural Research Institute (NARI). A field trip was arranged by the first author during the rainy season of 2011. Basidiomata were collected, and each collection locality was recorded using Global Positioning System (GPS) (Tibuhwa et al. 2010). Prior to collecting, mushrooms were photographed in situ (FIG. 1A–D). Field identification features such as sporocarp shape, color, smell, and color changing on bruising were recorded.

Collected samples were brought to the Department of Molecular Biology and Biotechnology Laboratory at the University of Dar es Salaam (UDSM) where parts of the mushrooms were oven dried at 50 C for 8 h. Vouchers were deposited at UDSM and UPS herbaria, the latter registered in Index Herbariorum (Thiers [continuously updated]). Color descriptions were based on Kornerup and Wanscher (1962).

Four collections of *Pseudophaeolus baudonii* (Pat.) Ryvarden (one from Zimbabwe, one from Senegal, one from Ghana, and one from Uganda) were obtained from the herbarium at Oslo University.

**Microscopy.**—Microscopic characters were recorded from specimens preserved by dehydration using silica gel and later observed in a 10% ammonium solution in an aqueous solution of Congo red. Twenty measurements of basidiospores and basidia were analyzed statistically, with the results presented as (min)A–SD–A+SD(max), where min is smallest observed value, A is the arithmetic average, SD is the standard deviation, and max is largest observed value for the measured specimen. Basidiospore characters, which include their size, shape, and reactions to Melzer's reagent, were observed. The hyphal system (monomitic, dimitic, or trimitic) was studied as well as the type of septa (simple septa or clamped septa in generative hyphae).

**Molecular study.**—This part of the study was carried out at the Department of Organismal Biology, Uppsala University. Total DNA was extracted from the inner part of the basidiomata, preferably from the hymenium to avoid contamination, following the protocol of the Plant Genomic DNA Extraction Kit (Qiagen, Hilden, Germany). Diluted ( $10^{-1}$ – $10^{-3}$ ) and undiluted DNA was used for polymerase chain reaction (PCR) amplifications. For herbarium material, nested PCR was employed due to low yields from initial PCR. The 5' end of the nuc 28S rDNA (28S), nuc rDNA internal transcribed spacer region ITS1–5.8S–ITS2 (ITS), a portion of nuc 18S rDNA (18S), and a portion of *TEF1* were amplified. Primers used for amplification and sequencing included LR0R, LR5, and LR7 (Vilgalys and Hester 1990) for 28S; ITS1F (Gardes and Bruns 1993), 5.8S (Vilgalys and Hester 1990), ITS3, ITS4, and ITS5 (White et al. 1990) for ITS; NS1, NS7, and NS4 for 18S (White et al. 1990); and EF1-983F, EF-2218R, and EF1-1567R for *TEF1* (Rehner and Buckley 2005). For PCR amplification, we used the AccuPower PCR PreMix (Bioneer, Daejeon, Korea), adding 3  $\mu$ L diluted or undiluted DNA, 1.5  $\mu$ L of each primer (10  $\mu$ M), and



**Figure 1.** *Piptoporellus baudonii* in situ. A. Basidioma occurring on *Eucalyptus* (Tibuhwa 1098.2014). B. Turtle eating an old fruiting body. C. Basidioma attached to the root of *Eucalyptus* (Tibuhwa 1098.2014). D. Dying *Eucalyptus* with basidioma (Tibuhwa 1098.2014) forming at the base of the tree.

water to a total volume of 20  $\mu$ L. Thermal cycling parameters were as described in Savić and Tibell (2009) and touchdown PCR as in Matheny (2005) for *TEF1*. Amplification products were visualized on 1.5% agarose gels stained with gel red, and PCR products were purified using Illustra ExoStar buffer (GE Healthcare, UK) diluted 10 $\times$ , following the manufacturer's protocol. Sequencing was carried out by Macrogen ([www.macrogen.com](http://www.macrogen.com)).

**Alignments and phylogenetic analyses.**— Additional sequences from GenBank (TABLE 1) were chosen to reconstruct a multigene alignment including as much taxonomic coverage of Fomitopsidaceae and Laetiporaceae following Han et al. (2016) and Song et al. (2018). Alignments were performed using MAFFT 7 online (<https://mafft.cbrc.jp/alignment/server/>; Katoh et al. 2019) and viewed and

manually adjusted, where necessary, using AliView 1.26 (Larsson 2014). Ambiguously aligned regions were excluded prior to phylogenetic analyses. We retained only the 5.8S region of ITS for the combined data set, since the neighboring regions (ITS1 and ITS2) were poorly aligned. A conflict among single-locus data sets was considered significant if a well-supported monophyletic group (posterior probability [PP]  $\geq 0.95$ ) was found to be well supported as nonmonophyletic when different loci were used. Further analyses were carried out after concatenation using SequenceMatrix (Vaidya et al. 2011).

The best-fit model of DNA evolution for the analyses, for both individual codon positions and genes, was obtained using Akaike information criterion (AIC) as implemented in MrModeltest 2.3 (Nylander 2004). The GTR+I+G model was employed across sites for 28S, 18S, and the 2nd codon of *TEF1*. For the 1st codon of *TEF1*, the model F81+I+G was applied, whereas HKY+G was

**Table 1.** Species, sample numbers, and GenBank accession numbers of sequences used in this study; new sequences in bold.

Species name	Sample no.	GenBank accession numbers				Reference
		ITS	28S	18S	TEF1	
<i>Amyloporia carbonica</i>	Cui 12212	KR605816	KR605755	KR605917	KR610745	Han et al. 2016
<i>A. xantha</i>	Cui 9901	KC951168	KT968827	KR605918	KR610746	Cui and Dai 2013
<i>Antrodia heteromorpha</i>	Dai 12755	KP715306	KP715322	KR605908	KP715336	Chen and Cui 2015
<i>A. macra</i>	Eriksson 1967	KR605810	KR605749	KR605909	KR610739	Han et al. 2016
<i>A. serialis</i>	Cui 10519	KP715307	KP715323	KR605911	KP715337	Han et al. 2016
<i>A. serpens</i>	Dai 7465	KR605813	KR605752	KR605913	KR610742	Han et al. 2016
<i>A. tanakae</i>	Cui 9743	KR605814	KR605753	KR605914	KR610743	Han et al. 2016
<i>A. variiformis</i>	CBS 309.82	DQ491418	AY515344			Kim et al. 2007
<i>Buglossoporus eucalypticola</i>	Dai 13660	KR605808	KR605747	KR605906	KR610736	Han et al. 2016
<i>B. quercinus</i>	JV 0906/15-J	KR605800	KR605739	KR605898	KR610729	Han et al. 2016
<i>Coriolopsis polyzona</i>	Cui 11040	KR605824	KR605767	KR605932	KR610760	Han et al. 2016
<i>Daedalea africana</i>	O 15372	KP171196	KP171216	KR605871	KR610704	Han et al. 2015
<i>D. allantoidea</i>	Dai 13612A	KR605795	KR605734	KR605892	KR610723	Han et al. 2016
<i>D. circularis</i>	Cui 10134	JQ314352	KP171221	KR605876	KR610709	Li and Cui 2013
<i>D. dickinsii</i>	Yuan 1090	KR605790	KR605729	KR605878	KR610711	Han et al. 2016
<i>D. hydroides</i>	Lindblad 3679	KP171203	KP171225	KR605881		Han et al. 2015
<i>D. quercina</i>	Dai 2260	KR605792	KR605731	KR605885	KR610718	Han et al. 2016
<i>D. radiata</i>	Cui 8624		KR605732	KR605889	KR610721	Han et al. 2016
<i>D. stevensonii</i>	O 10543	KP171212	KP171235	KR605891		Han et al. 2015
<i>Fibroporia albicans</i>	Cui 9464	KC456250	KR605758	KR605920	KR610748	Chen et al. 2015
<i>F. radiculosa</i>	Cui 2790	KC456248	KR605761	KR605923	KR610751	Chen et al. 2015
<i>F. radiculosa</i>	Cui 11404		KR605760	KR605922	KR610750	Chen et al. 2015
<i>F. citrina</i>	Cui 10497	KT895886	KT988993			Han et al. 2016
<i>Fomitopsis iberica</i>	O 10810	KR605771	KR605710	KR605842	KR610676	Han et al. 2016
<i>F. duresscens</i>	O 10796	KF937292	KF937294	KR605834	KR610669	Han et al. 2014
<i>F. meliae</i>	Dai 10035	KR605774	KR605713	KR605847	KR610683	Han et al. 2016
<i>F. nivosa</i>	JV 0509/52-X	KR605779	KR605718	KR605853	KR610686	Han et al. 2016
<i>F. palustris</i>	Cui 7597	KP171213	KP171236	KR605854	KR610687	Han et al. 2015
<i>F. pinicola</i>	Cui 10312	KR605781	KR605720	KR605856	KR610689	Han et al. 2016
<i>Kusaghioporia usambarensis</i>	J. Hussein 01/16		MH010044	MH010046	MH048871	Hussein et al. 2018
<i>K. usambarensis</i>	J. Hussein 01/17		MH010045		MH048869	Hussein et al. 2018
<i>Laetiporus ailaoshanensis</i>	Dai 13567	KX354470	KX354498	KX354535	KX354623	Song et al. 2018
<i>L. ailaoshanensis</i>	Dai 13256	KF931289	KF931317	KX354537	KX354625	Song et al. 2018
<i>L. cincinnatus</i>	Dai 12811	KF951291	KF951304	KX354516	KX354516	Song et al. 2018
<i>L. cincinnatus</i>	JV 0709/168-J		KF951305	KX354517	KX354606	Song et al. 2018
<i>L. conifericola</i>	JV 0709/81J	KF951292	KF951327	KX354531		Song et al. 2018
<i>L. cremeiporus</i>	Dai 10107	KF951281	KF951301	KX354515	KX354604	Song et al. 2018
<i>L. cremeiporus</i>	Cui 10586	KF951277	KF951297	KX354513	KX354602	Song et al. 2018
<i>L. gilbertsonii</i>	TJV2000/101	EU402553	EU402528	KX354542	AB472668	Song et al. 2018
<i>L. huroniensis</i>	MI-14	EU402573	EU402539		AB472672	Song et al. 2018
<i>L. medogensis</i>	Cui 12219	KX354472	KX354500	KX354538	KX354626	Song et al. 2018
<i>L. montanus</i>	Dai 15888	KX354466	KX354494	KX354530	KX354619	Song et al. 2018
<i>L. sulphureus</i>	Cui 12389	KR187106	KX354487	KX354519	KX354608	Song et al. 2018
<i>L. sulphureus</i>	Z.R.L. CA08		KX354507	KX354546	KX354634	Song et al. 2018
<i>L. versisporus</i>	Yuan 6319	KX354475	KX354503	KX354541	KX354629	Song et al. 2018
<i>L. xinjiangensis</i>	Dai 15825	KX354465	KX354493	KX354527	KX354616	Song et al. 2018
<i>L. zonatus</i>	Cui 10404	KF951283	KF951308	KX354551	KX354639	Song et al. 2018
<i>Phaeolus schweinitzii</i>	AFTOL-ID 702		AY629319	AY705961	DQ028602	Matheny et al. 2007
<i>P. schweinitzii</i>	FP-102447-Sp	KC585368	KC585197	AF026598		Ortiz-Santana et al. 2013
<i>P. schweinitzii</i>	FP-133218-Sp	KC585369	KC585198	U59087		Ortiz-Santana et al. 2013
<i>P. schweinitzii</i>	OKM-4435-T	KC585370	KC585199	KX354553		Ortiz-Santana et al. 2013
<i>Piptoporellus baudonii</i>	<b>Tibuhwa 09.2018/JMH 01/19</b>	<b>MT447066</b>	<b>MT447069</b>	<b>MT447063</b>	<b>MT452549</b>	This study
<i>P. baudonii</i>	<b>Tibuhwa 09.2018/JMH 02/19</b>	<b>MT447067</b>	<b>MT447070</b>	<b>MT447064</b>	<b>MT452550</b>	This study
<i>P. baudonii</i>	<b>L. Ryvarden 25098/JMH 03/19</b>	<b>MT447068</b>	<b>MT447071</b>	<b>MT447065</b>	<b>MT452551</b>	This study
<i>P. hainanensis</i>	Dai 13714	KR605806	KR605745	KR605904	KR610735	Han et al. 2016
<i>P. soloniensis</i>	Dai 11872	KR605804	KR605743	KR605902	KR610731	Han et al. 2016
<i>P. soloniensis</i>	Cui 11390	KR605803	KR605742	KR605901	KR610733	Han et al. 2016
<i>P. triqueter</i>	Dai 13121	KR605807	KR605746	KR605905	KR610738	Han et al. 2016
<i>Pycnoporellus fulgens</i>	CA-20	KC585385	KC585218	KX354554		Ortiz-Santana et al. 2013
<i>Rhodofomitopsis lilacinogilva</i>	Schigel 5193	KR605773	KR605712	KR605846	KR610680	Han et al. 2016
<i>R. feei</i>	Ryvarden 37603	KC844850	KC844855	KR605838	KR610670	Han and Cui 2015
<i>Rubellofomes cystidiatus</i>	Cui 5481	KF937288	KF937291	KR605832	KR610667	Han et al. 2014
<i>R. minutisporus</i>	Rajchenberg 10661	KR605777	KR605716	KR605850		Han et al. 2016
<i>Trametes suaveolens</i>	Cui 11586	KR605823	KR605766	KR605931	KR610759	Han et al. 2016
<i>Ungulidaedalea fragilis</i>	Cui 10919	KF937286	KF937290	KR605840	KR610674	Han et al. 2014
<i>Wolfiporia cocos</i>	CBK-1		KX354689	KX354690	KX354688	Song and Cui 2017

implemented for both the 3rd codon of *TEF1* and 5.8S. Bayesian inference (BI) was conducted using MrBayes 3.2.6, and branch support was estimated by the posterior probability (PP) (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). Two independent runs were executed, each with four Markov chains for 10 million generations, sampling trees every 100 generations. A 25% threshold was used as the burn-in. The Markov chain Monte Carlo (MCMC) analysis converged well in advance of the burn-in threshold, and chain mixing was found to be satisfactory as assessed by using Tracer 1.5 (Drummond et al. 2012).

Maximum likelihood (ML) estimates were carried out by RAxML 8.2.10 using the GTR+G+I model of site substitution (Stamatakis 2014). Branch support was obtained by bootstrapping 1000 replicate data sets (Hillis and Bull 1993). Sequence alignments and phylogenetic trees were deposited in TreeBASE (submission ID 26318).

## RESULTS

**Phylogenetic analyses.**—A total of 12 new sequences from three specimen vouchers were generated (TABLE 1). BLAST results from GenBank (accessed 23 Apr 2019), using BLASTn with “discontiguous megablast” (for cross-species comparison, searching with coding sequences) with sequences in this study, showed the highest sequence similarity to *Piptoporus soloniensis* (ITS query cover 97% and identity 76%).

The analyses contained a total of 249 sequences representing 64 species of the “antrodia clade,” including the additional sequences downloaded from GeneBank and two species from the “core polyporoid” clade as outgroups. No significant incongruence among single gene trees was detected; hence, the four matrices were concatenated. The concatenated data matrix contained 2859 unambiguously aligned sites. The phylogeny of the “antrodia clade” and the position of *Piptoporellus baudonii* were inferred from four data sets: 58 sequences of 5.8S, 66 of 28S sequences, 63 of 18S, and 62 sequences of *TEF1*. The combined analysis recovered a phylogeny with two distinct clades representing Fomitopsidaceae and Laetiporaceae (FIG. 2). Clade annotations follow Han et al. (2016). Three samples of *Laetiporus baudonii* clustered (shown as *Piptoporellus baudonii*, comb. nov., see below) with several samples of *Piptoporellus*, including the type of the genus *P. soloniensis*, in the Fomitopsidaceae. This arrangement was strongly supported and necessitated the new taxonomic placement of *Laetiporus baudonii* in *Piptoporellus*.

## TAXONOMY

*Piptoporellus baudonii* (Pat.) Tibuhwa, Ryvarden & S. Tibell, comb. nov.

Mycobank MB835378

≡ *Polyporus baudonii* Pat., Bull Soc Mycol Fr 30:337. 1914 (basionym).

≡ *Pseudophaeolus baudonii* (Pat.) Ryvarden, Trans Br Mycol Soc 65:285. 1975.

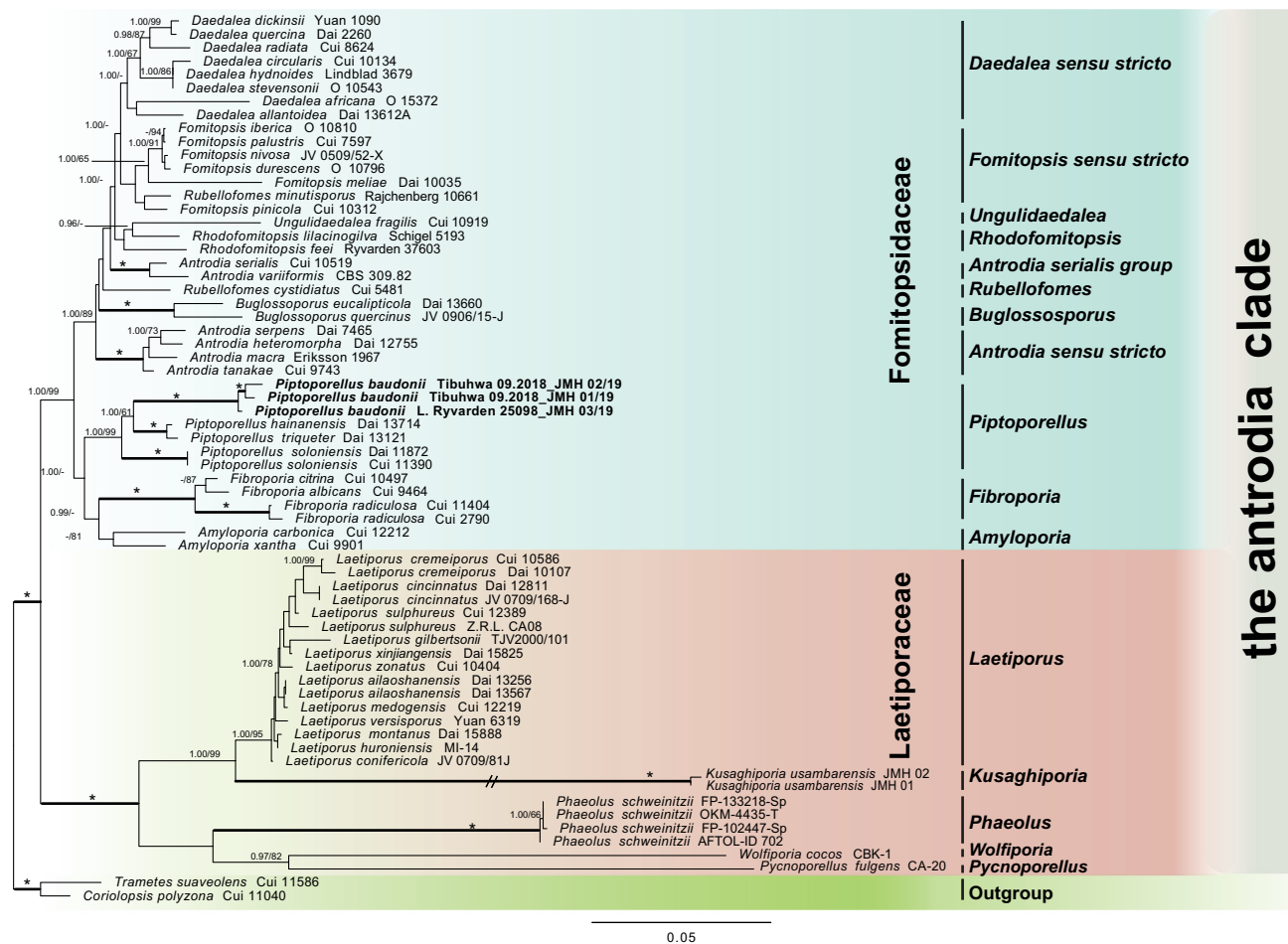
≡ *Laetiporus baudonii* (Pat.) Ryvarden, Syn Fung (Oslo) 5:215. 1991.

Basidiomata 20–35 cm wide, pileate, stipitate, and seldom effused-reflexed forming shelves clustered in rosettes up to 85(–110) cm wide, total weight of up to 15 kg; upper surface of pileus bright orange-yellow (5A5–6) when young and fresh, rusty brown (7DE7) upon aging, surface soft without crust, with a few faint concentric brown zones. When aging the basidiomata undergo autolysis. Hymenophore poroid, pore surface concolorous with the upper surface of the pileus or slightly paler. Context fleshy, light ochraceous (2A4–5).

Hyphal system dimitic. Generative hyphae thin-walled, 2–3.5  $\mu\text{m}$  wide, hyaline, septate with clamp connections. Binding hyphae relatively thick-walled, 2–3  $\mu\text{m}$  wide, hyaline, nonseptate with arbori-shaped branching. Basidiospores broadly ellipsoid, or rarely ovoid, 6–7(–7.5)  $\times$  (3–)3.5–4(–4.5)  $\mu\text{m}$ , ave. = 6.5  $\pm$  0.34  $\times$  3.75  $\pm$  0.25  $\mu\text{m}$ , smooth, thin-walled, hyaline, inamyloid. Basidia clavate, (7–)12.5–17.5(–23)  $\times$  6.5–7  $\mu\text{m}$ , hyaline, thin-walled, with 2–4 sterigmata. Cystidia not found.

*Ecology and distribution:* On a wide range of hosts (TABLE 2); for additional host trees, see Van der Westhuizen (1973) and Rattan and Pawsey (1981). Basidiomata occurring during the rainy season (Nov–May). Widely distributed in Africa and Madagascar (Kile 2000); also recorded from Yemen (Al-Fatimi et al. 2005).

*Specimens examined:* TANZANIA. MTWARA PROVINCE: Naliendele cashew farm, on wilting cashew tree in the vicinity of other wilting eucalyptus trees in cleared farm, 17 Apr 2011, *Tibuhwa 1067.2011*. (UPS, UDSM); Naliendele cashew farm, 25 Apr 2014, *Tibuhwa 1096.2014* (UDSM); Naliendele, in *Eucalyptus* artificial plantations, 30 Apr 2014, *Tibuhwa 1098.2014* (UDSM); Kitangari, 10°37'18.6"S, 39°20'19.5"E, 360 m, growing on the roots of wilting cashew tree, 9 Jan 2018, *S. Amandus, Tibuhwa 09.2018* (UDSM; DNA isolations: JMH 01/19, JMH 02/19). GenBank: ITS = MT447066, MT447067; 28S = MT447069, MT447070; 18S = MT447063, MT447064; *TEF1* = MT452549, MT452550. ZIMBABWE. CENTRAL PROVINCE: Mazowe Botanic Reserve, ca. 20 km north of



**Figure 2.** ML tree of *Piptoporellus* and related genera in the “Antrodia clade” inferred from a combined data set of 5.8S+28S+18S+TEF1. The tree was rooted with two species from the Polyporaceae. Branches are labeled with ML bootstrap values >50% and BPP values >0.95. Branches in bold indicate a support of PP  $\geq$ 0.95 and bootstrap  $\geq$ 70%. An asterisk above a bold branch indicates 100% bootstrap and 1.0 PP. The branch with a double-slash is shortened. Clade names follow Han et al. (2016).

**Table 2.** Host tree range for *Piptoporellus baudonii*.

Family	Species	English vernacular name	Swahili (Bantu) vernacular name	Makonde <sup>a</sup> (Bantu) vernacular name	Growth form
Myrtaceae	<i>Corymbia citriodora</i> Hook		Makaratusi	—	Tree
Myrtaceae	<i>Eucalyptus tereticornis</i> Sm.	Forest Red Gum	Makaritusi	—	Tree
Annonaceae	<i>Annona senegalensis</i> Pers		Mchekwa	Mtopetope	Shrub/tree
Annonaceae	<i>Uvaria gracilipes</i> N. Robson		Msofu	Msofu	Shrub
Bignoniaceae	<i>Markhamia zanzibarica</i> (Bojer ex DC.) K. Schum.	Bell bean tree	Mtandavala	Mtandavala	Small tree
Anacardiaceae	<i>Anacardium occidentale</i> L.	Cashew tree	Mkorosho	Mkorosho	Tree crop
Euphorbiaceae	<i>Manihot esculenta</i> Crantz	Cassava (yuca, mogo, manioc, tapioca etc.)	Muhogo	Mmogo	Shrub
Fabaceae	<i>Cassia</i> sp. 1		Mjohoro	Mnyadi	Shrub
Fabaceae	<i>Cassia</i> sp. 2		—	Mnemblembe	Shrub
Fabaceae	<i>Julbernardia globiflora</i> (Benth.) Troupin		Mtongo	Mchenga	Tree
Discoraceae	<i>Discores sansibarensis</i> Pax.		Mn'goko	Mng'oko	Woody vine
Euphobiaceae	<i>Croton</i> sp.		—	Mnyao	Tree
Fabaceae	<i>Albizia adianthifolia</i> (Schumach.) W. Wight.		Mgelenge	Mtanga	Tree
Poaceae	<i>Panicum maximum</i> Jacq.		Nyasi	Nambole	Grass

<sup>a</sup>The Makonde are an ethnic group in southeast Tanzania and northern Mozambique.

Harare, on the ground, 16 Jan 1988, *L. Ryvardeen* 25098 (O; DNA isolation JMH 03/19). GenBank: ITS = MT447068; 28S = MT447071; 18S = MT447065; *TEF1* = MT452551. SENEGAL. CAP-VERD: 13 Oct 1984, *J. Moen* (O). UGANDA: Rwenzori Mountains, 2600 m, on liana, 10 Nov 2007, *C. Decock* (O). GHANA. GREATER ACCRA: locality: Cassava Farm, on tuber of Cassava, Jan 2011, *Mary M. Apetergbor* (O).

*Remarks:* The edibility is unknown, although it was once found eaten by a wild tortoise (FIG. 1B).

## DISCUSSION

*Laetiporus baudonii* has had a controversial taxonomic history regarding its generic placement and species recognition as reported previously by Van der Westhuizen (1973). Here, we infer the phylogenetic position of the species using molecular data from four gene regions. In our analyses, *L. baudonii* from Zimbabwe clustered with two isolates from Tanzania to form a highly supported and distinct species-level lineage within *Piptoporellus* of the antrodia clade (FIG. 2). Phylogenetic evidence thus justifies the incorporation of *Laetiporus baudonii* in *Piptoporellus* along with other sampled members of the genus—*P. soloniensis*, *P. hainanensis*, and *P. triqueter*. Morphologically, *P. baudonii* is similar to other *Piptoporellus* in having annual, pileate, or stipitate basidiomata, similar pileus coloration, a dimitic hyphal system, thin-walled basidiospores, and presence of clamp connections. However, *P. baudonii* differs from the other *Piptoporellus* species by spore shape (Han et al. 2016).

For more than 10 years, farmers in Mtwara, Tanzania, have noticed fungal infections in plantations of cashew. Farmers also observed that when the fungus was found beneath a tree, the leaves of the tree were chlorotic and completely wilted. Defoliation and wilting of the attacked trees continued until the whole tree succumbed, with the wilt spreading to adjacent trees. However, *P. baudonii* has low host specificity. During our study, we observed its occurrence on a wide range of trees, including *Eucalyptus*, cashew tree, cassava trees, *Tectona* trees, and indigenous trees, in southern Tanzania (see TABLE 2). Researchers at the Naliendele Agricultural Research Institute, Mtwara, have confirmed these observations. A clear indication of local fungal infection was seen in plantations of cultivated *Tectona grandis* and *Eucalyptus* trees, where distinct patches of dry and dying trees were noted. Pathogenicity studies of *P. baudonii* and possible mitigation measures are ongoing.

*Piptoporellus baudonii* has a deep impact on agroforestry and ecology of the affected areas. Cashew is the main cash crop for more than 380 000 households in southeastern Tanzania, whereas cassava is their major

staple food. Infection by *P. baudonii*, which attacks both their main cash crop and main staple food apart from other cultivated and wild forest trees, is of great concern in this region and a threat to agroforestry. There is concern that a collapse in this system would entail complex and multifarious socioeconomic problems. Mitigation procedures are essential to inhibit spread of the disease caused by *P. baudonii*.


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## LITERATURE CITED

- Al-Fatimi M, Wurster M, Kreisel H, Lindequist U. 2005. Antimicrobial, cytotoxic and antioxidant activity of selected basidiomycetes from Yemen. *Pharmazie* 60:776–780.
- Binder M, Hibbett DS, Larsson KH, Larsson E, Langer E, Langer G. 2005. The phylogenetic distribution of resupinate forms across the major clades of mushroom-forming fungi (Homobasidiomycetes). *Systematics and Biodiversity* 3:113–157.
- Browne FG. 1968. Pests and diseases of forest plantation trees. An annotated list of the principal species occur ring in the British Commonwealth. Oxford, UK: Clarendon Press. 923 p.
- Chen Y-Y, Cui B-K. 2016. Phylogenetic analysis and taxonomy of the *Antrodia heteromorpha* complex in China. *Mycoscience* 57:1–10.
- Chen Y-Y, Li H-J, Cui B-K. 2015. Molecular phylogeny and taxonomy of *Fibroporia* (Basidiomycota) in China. *Phytotaxa* 203:47–54.
- Cui B, Dai Y. 2013. Molecular phylogeny and morphology reveal a new species of *Amyloporia* (Basidiomycota) from China. *Antonie van Leeuwenhoek* 104:817–827.
- Donk MA. 1960. The generic names proposed for Polyporaceae. *Persoonia* 1:173–302.
- Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29:1969–1973.
- Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2:113–118.



- Han ML, Chen YY, Shen LL, Song J, Vlasák J, Dai YC, Cui BK. 2016. Taxonomy and phylogeny of the brown-rot fungi: *Fomitopsis* and its related genera. *Fungal Diversity* 80:343–373.
- Han ML, Cui BK. 2015. Morphological characters and molecular data reveal a new species of *Fomitopsis* (Polyporales) from southern China. *Mycoscience* 56:168–176.
- Han M, Song J, Cui B. 2014. Morphology and molecular phylogeny for two new species of *Fomitopsis* (Basidiomycota) from South China. *Mycological Progress* 13:905–914.
- Han ML, Vlasák J, Cui BK. 2015. *Daedalea americana* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. *Phytotaxa* 204:277–286.
- Heim R. 1931. Le *Phaeolus manihotis* sp. nov., parasite du manioc a Madagascar, et considérations sur le genre *Phaeolus* Pat. *Annales de Cryptogamie Exotique* 4:175–189.
- Hillis DM, Bull JJ. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42:182–192, <http://mafft.cbrc.jp/alignment/server/>
- Hussein JM, Tibuhwa DD, Tibell S. 2018. Phylogenetic position and taxonomy of *Kusaghiporia usambarensis* gen. et sp. nov. (Polyporales). *Mycology* 9:136–144.
- Justo A, Miettinen O, Floudas D, Ortiz-Santana B, Sjökvist E, Lindner D, Nakasone K, Niemelä T, Larsson KH, Ryvarden L, Hibbett DS. 2017. A revised family-level classification of the Polyporales (Basidiomycota). *Fungal biology* 121:798–824.
- Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20:1160–1166.
- Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. *Dictionary of the Fungi*. 10th ed. Wallingford, UK: CABI. 784 p.
- Kile GA. 2000. Woody Root Rots of Eucalypts. In: Keane PJ, Kile GA, Podger FD, Brown BN, eds. *Diseases and pathogens of eucalypts*. Melbourne, Australia: CSIRO Publishing. p. 293–303.
- Kim KM, Lee JS, Jung HS. 2007. *Fomitopsis incarnatus* sp. nov. based on generic evaluation of *Fomitopsis* and *Rhodofomes*. *Mycologia* 99:833–841.
- Kornerup A, Wanscher JH. 1962. *Farver i Farver*. Copenhagen, Denmark: Politikens Forlag.
- Larsson A. 2014. AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30:3276–3278.
- Li H-J, Cui B-K. 2013. Two new *Daedalea* species (Polyporales, Basidiomycota) from South China. *Mycoscience* 54:62–68.
- Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). *Molecular Phylogenetics and Evolution* 35:1–20.
- Matheny PB, Wang Z, Binder M, Curtis JM, Lim YW, Nilsson RH, Hughes KW, Hofstetter V, Ammirati JF, Schoch CL, Langer E, Langer G, McLaughlin DJ, Wilson AW, Frøslev T, Ge Z-W, Kerrigan RW, Slot JC, Yang Z-L, Baroni TJ, Fischer M, Hosaka K, Matsuura K, Seidl MT, Vauras J, Hibbett DS. 2007. Contributions of rpb2 and tef1 to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Molecular Phylogenetics and Evolution* 43:430–451.
- Nylander JAA. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- Oforu-Asiedu A. 1975. A new disease of eucalypts in Ghana. *Transactions of the British Mycological Society* 65:285–289.
- Ortiz-Santana B, Lindner DL, Miettinen O, Justo A, Hibbett DS. 2013. A phylogenetic overview of the antrodia clade (Basidiomycota, Polyporales). *Mycologia* 105:1391–1411.
- Patouillard NT. 1914. Quelques champignons du Congo. *Bulletin de la Société mycologique de France* 30:336–346.
- Rattan PS, Pawsey RG. 1981. Death of tea in Malawi caused by *Pseudophaeolus baudonii*. *International Journal of Pest Management* 27:225–229.
- Rehner SA, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1- $\alpha$  sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97:84–98.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574.
- Ronquist F, Teslenko M, Van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MRBAYES 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61:539–542.
- Ryvarden L. 1991. Genera of polypores, nomenclature and taxonomy. *Synopsis Fungorum* (Oslo) 5:1–373.
- Savić S, Tibell L. 2009. Taxonomy and species delimitation in *Sporodictyon* (Verrucariaceae) in Northern Europe and the adjacent Arctic—reconciling molecular and morphological data. *Taxon* 58:585–605.
- Sijaona MER. 2003. Pathology report. In: Cashew Research Coordinating Committee Meeting Report, 2003/2004. Naliende, Mtwara: Agricultural Research Institute. 112 p.
- Sijaona MER. 2007. Macro-fungi in association with cashew wilt disease in Tanzania. A poster presented at the 18th Congress of the Association for the Taxonomic Study of the Flora of Tropical Africa [AETFAT], 26 Feb to 2 Mar 2007, Yaounde, Cameroon.
- Sijaona MER, Shija B. 2005. Verification of Bayleton 25WP for the control of powdery mildew (*Oidium anacardii* F. Noack) on cashew in Tanzania. Registration report submitted to Registrar of Pesticides. Arusha, Tanzania: Tropical Pesticides Research Institute (TPRI).
- Song J, Sun YF, Ji X, Dai YC, Cui BK. 2018. Phylogeny and taxonomy of *Laetiporus* (Basidiomycota, Polyporales) with descriptions of two new species from western China. *MycKeys* 37:57–71.
- Song J, Cui B. 2017. Phylogeny, divergence time and historical biogeography of *Laetiporus* (Basidiomycota, Polyporales). *BMC Evolutionary Biology* 17:1–12.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.
- Tibuhwa DD, Kivaisi AK, Magingo FFS. 2010. Utility of the macro-micromorphological characteristics used in classifying the species of *Termitomyces*. *Tanzania Journal of Science* 36:3–46.
- Thiers B. [continuously updated]. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. [cited 2020 May 20]. Available from: <http://sweetgum.nybg.org/science/ih/>
- Vaidya G, Lohman DJ, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene

- datasets with character set and codon information. *Cladistics* 27:171–180.
- Van der Westhuizen GCA. 1973. *Polyporus baudoni* Pat. on *Eucalyptus* spp. in South Africa. *Bothalia* 11:143–151.
- Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172:4238–4246.
- White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. New York: Academic Press. p. 315–322.
- Zhao CL, Cui BK, Song, J, Dai YC. 2015. *Fragiliporiaceae*, a new family of Polyporales (Basidiomycota). *Fungal Diversity* 70:115–126.