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Tylopilus aquarius, comb. et stat. nov., and its new variety from Brazil

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We propose the new species and new combination, *Tylopilus aquarius* var. *aquarius*, by elevating *T. potamogeton* v. *aquarius* to species level. We also describe a new variety, *T. aquarius* var. *megistus*, from the Atlantic Forest states Paraiba and Rio Grande do Norte, Brazil, that produces larger, robust basidiomata and smaller basidiospores as compared to *T. aquarius* var. *aquarius*.

Keywords: Agaricomycetes, Boletaceae, Boletales, Neotropics, taxonomy.

Tylopilus P. Karst. was originally very poorly circumscribed by Karsten (1881) as "pink spored" boletes with "adnate tubes". The type species was designated as *T. felleus* (Bull.) P. Karst. (e.g., Singer 1986). The most traditional morphological concept of *Tylopilus* was given by Singer (1986: 793–794), and characterized by a dry pileus, a light colored or pallid at least when young hymenophore, usually depressed around the stipe, a sordid pink to dull flesh ocher spore print, melleous or pale basidiospores, well-developed cystidia internally pigmented; boletoid tube trama, and a whitish context. Recently Nuhn et al. (2013: 497) summarized several works and proposed a wider definition of *Tylopilus* as "smooth spored species, with or without a reticulum on the stipe, a spore print of pinkish to various brown hues, and a pore surface that is frequently white when young and turns pink, yellow, brown, grey or black with age".

The genus *Tylopilus* has been recently studied in the Guiana Shield (Henkel 1999, 2001; Fulgenzi et al. 2007). Other South American reports of the genus are mostly from the Brazilian Amazon where Singer (1978, 1989) and Singer et al. (1983) described five taxa: *T. acutesquamosus* Singer, *T. arenarius* Singer, *T. potamogeton* Singer, *T. potamogeton* var. *aquarius* Singer and *T. potamogeton* var. *mitis* Singer. In Northeast Brazil, only *T. balloui* (Peck) Singer is known from the State of Rio Grande do Norte (Neves 2016).

We propose the new species *Tylopilus aquarius* var. *aquarius*, by elevating *T. potamogeton* var. *aquarius* to species level and also describe a new variety, *T. aquarius* var. *megistus*, from the Atlantic Forest states Paraiba and Rio Grande do Norte, Brazil.

Material and methods

Sampling and morphological description

Collections were made at two localities: "Parque Estadual das Dunas de Natal", municipality of Natal (S 5°48′S–5°43′S and 35°09′W–35°12′W), State of Rio Grande do Norte, Northeast Brazil; and

"Reserva Biológica Guaribas", municipality of Mamanguape, an Atlantic Forest protected area of 4029 ha (S 6° 44.545', W 35° 08.533'). The Dunas habitat comprises elements of Atlantic Forest mixed with some species in common with Caatinga and Coastal Tableland, where species of Leguminosae, Myrtaceae, Poaceae, Asteraceae and Euphorbiaceae predominate (Freire 1990). Guaribas has 629 species of vascular plants of which the most diverse are Leguminosae (all subfamilies), Poaceae, Cyperaceae, Rubiaceae, Asteraceae, Malvaceae, Melastomataceae and Myrtaceae (Barbosa et al. 2011). Both habitats have sandy soil. The holotype of *T. potamogeton* var. *aquarius* was collected in an *"Igapó* Forest", a periodically inundated area in Amazonia, dominated by leguminous trees, particularly *Aldina latifolia* Spruce ex Benth. and *Swartzia* cf. *polyphylla* DC. in a soil with low mineral nutrient content (Singer & Aguiar 1986, Parolin et al. 2004).

Color codes follow Kornerup & Wanscher (1978) and Online Auction Color (2004), where KW is used in the description for the former one and OAC for the later one. Microscopic observations were made from material mounted in 3% KOH and Congo red solutions. Measurements and statistics are based on 30 spores. Abbreviations include L(W) = average basidiospores length (width), Q = the length: width ratio range as determined from all measured basidiospores, and Qm = the Q value averaged from all basidiospores measured. Translation from Latin under *T. potamogeton* var. *mitis* discussion is based on Borror (1960). The holotype and additional material analyzed are deposited at JPB, F, URM (Thiers 2016) and UFRN-Fungos (Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte, Brazil).

Molecular analysis

DNA extraction was performed on a dry fragment of basidiocarp, using the Wizard genomic DNA purification kit Promega (Charbonnières les Bains, France) as described in Rochet et al. (2011). The fungal ITS region was amplified using fungal specific and universal primers ITS-1F and ITS-4 (White et al. 1990, Gardes & Bruns 1993). PCR conditions were as in Rochet et al. (2011). The amplification product was sequenced by MilleGen company (Labège, France). The sequence was checked and cleaned using Geneious 6.1.8 (Kearse et al. 2012). It is deposited in GenBank under accession number KU692027. The sequence was compared to Genbank and UNITE by BLAST search (Altschul et al. 1990). In order to compare the level of ITS variation between distinct species of *Tylopilus*, an alignment of ITS sequences was produced using MAFFT 7.0 (Katoh & Standley 2013). Uncultured *Tylopilus* sequences from north temperate regions were not included. This alignment permitted to compare sequences from *T. aquarius* to the single sequence of *T. potamogeton* previously produced.

Taxonomy

Tylopilus aquarius (Singer) Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo var. *aquarius*, comb. et stat. nov.
MycoBank no.: MB 815800.

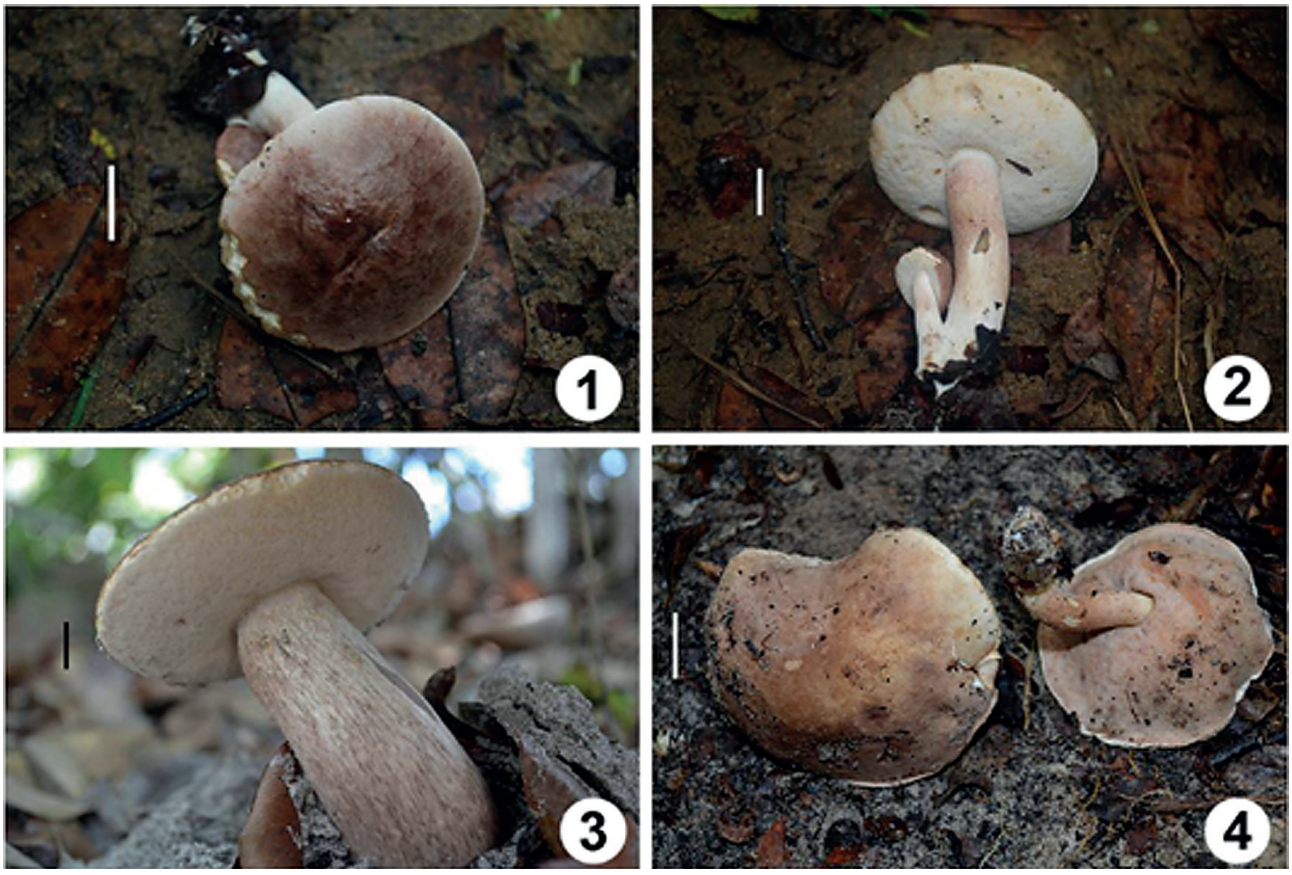
Basionym. – *Tylopilus potamogeton* var. *aquarius* Singer in Singer et al., Beih Nova Hedw. 77: 122. 1983.

Tylopilus aquarius var. *megistus* Wartchow, Barbosa-Silva, B. Ortiz & Ovrebo, var. nov. – Figs. 1–12.
MycoBank no.: MB 815801.

Diagnosis. – Differs from var. *aquarius* in larger, robust basidiomata and smaller basidiospores (7.6)7.9–9.5(9.7) × (3.8)4.1–6 (6.5) µm.

Typus. – BRAZIL, Rio Grande do Norte: Natal, Parque Estadual das Dunas, Trilha da Geologia, 24 July 2014, leg. F. Wartchow FW 19/2014 (JPB 60536 holotype, UFRN-Fungos 2677 isotype, URM 89204 isotype).

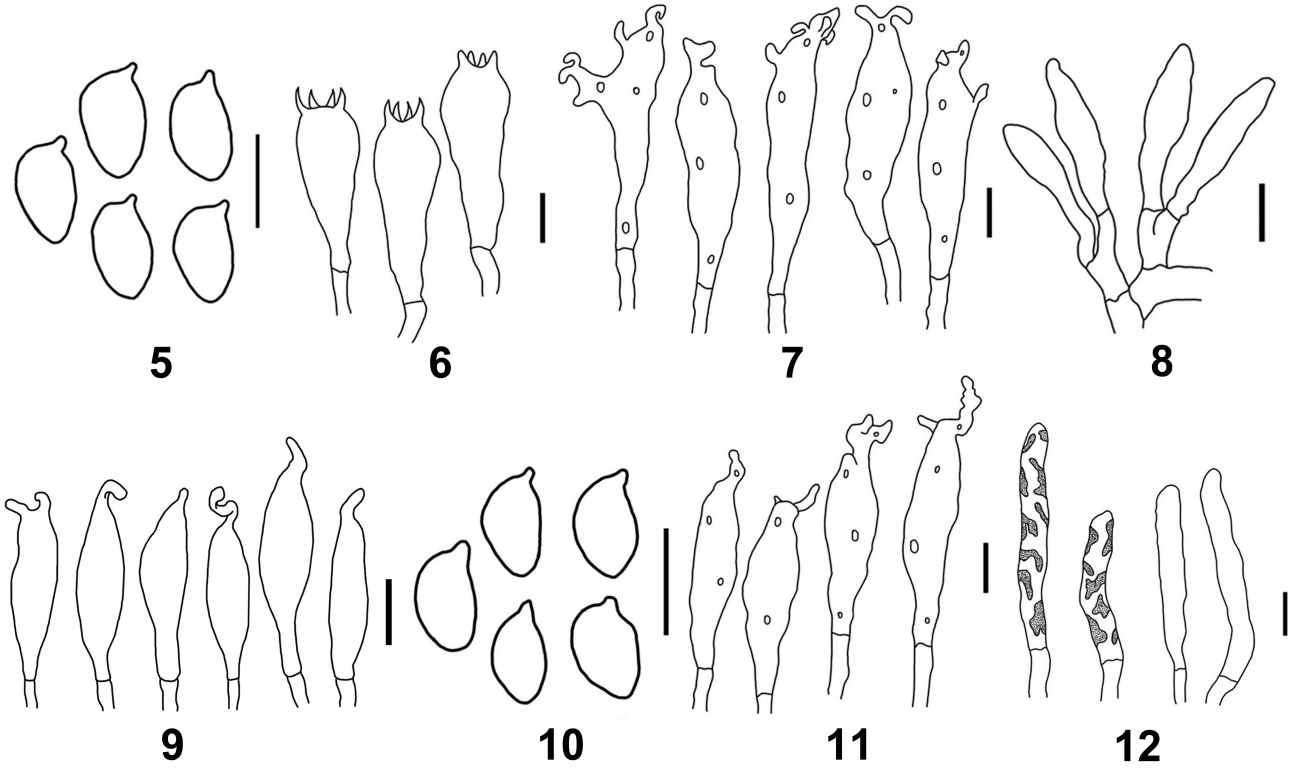
Description. – Pileus 20 mm wide and 30 m high on buttons, when mature 45–120 mm wide, hemispheric when young, then convex, broadly convex and finally plane, sometimes slightly depressed with margin slightly recurved; dark yellowish brown (KW 5E4), brown (OAC 637-640) then dull dark brown (KW 8E4, OAC 724), very edge nearly buff; surface dry, smooth to matted under 10× lens, slightly velutinous, cracking mostly at center; context thick 2–7 mm near margin and 8–21 mm at center, white (OAC 909), soft, unchanging. – Hymenophore tubulose, adnexed-adnate or slightly decurrent, tubes whitish to buff (OAC 717), pale cream (OAC 795) to orange white (KW 5B2) with 7–20 mm long, pores rounded to subpentagonal or hexagonal 0.5–1 mm diam., 1–2 pores per mm, more radially elongate near stipe, buff (off white) when young then pinkish brown when mature. – Stipe 25–152 × 8–31 mm (8–13 mm thick at apex, 10–15 mm middle, 12–31 mm base), central, subequal to gradually thickening toward base to clavate; surface whitish to buff with areas of vinaceous purplish brown, light brown (OAC 710) to reddish blond (KW 5C4) tints, longitudinally fibrillose, sometimes more reticulate at apex, striate at base, glabrous; context solid, white (OAC 909) to whitish sometimes buff, unchanging. – Macro-



Figs. 1–4. *Tylopilus aquarius* var. *megistus*. Basidiomata. **1–2.** CLO 5037. **3.** FW 108/2012. **4.** FW 19/2014 (holotype). Bars: 20 mm.

chemical test: KOH turning the pileus surface olivaceous, pileus context pinkish, and the stipe surface and context pink. – Odor none. – ***Taste*** bitter.

Basidiospores dark pinkish brown (OAC 666) in deposit; (7.6)7.9–9.5(9.7) × (3.8)4.1–6 (6.5) µm; L= 9 µm; W= 5.2 µm; Q= (1.38)1.42–2.33(2.38); Qm= 1.76, pale pink in H_2O, hyaline in KOH, inamyloid, smooth, thin-walled, amygdaliform or elliptic in profile, occasionally with adaxial face slightly flattened, elliptic in face view, with guttules; hilar appendage 1–1.5 µm long, subapical. – Basidia 23–33 × 8.5–12 µm, clavate, thin-walled, hyaline in H_2O and KOH, 4-sterigmate with sterigmata 2.5–5 µm long. – Cystidia as pleurocystidia 31.5–53 × 6.5–11.5 µm, versiform varying from ventricose, subventricose, fusoid, subfusoid, sometimes clavate to subclavate, with appendages on apex and sometimes with knobs or outgrowths appearing diverticulate; pale in H_2O and KOH but somewhat hyaline and densely packed with oil-drop contents; cheilocystidia 25–43 × 4.5–10 µm, ventricose, subventricose, ventricose-rostrate, fusoid to subclavate with mucronate apex sometimes with two appendages also at apex and recurved, hyaline, colorless in H_2O and KOH. – Tube trama boletoid, divergent with gelatinized mediostratum, hyphae parallel 2.5–5 µm wide, light yellow in H_2O and KOH; lateral stratum with strongly divergent hyphae 5–8 µm wide, hyaline in H_2O and KOH, non-gelatinized. – Pileipellis a trichoderm with terminal elements 4.5–7.5 µm wide, interwoven to anticlinal, uninflated, filiform to slender-cylindric, brown to golden brown in H_2O and KOH, thin-walled, frequently with parietal and internal brownish olivaceous content, smooth and non-incrusting. – Pileus trama hyphae 3.8–7.5 µm wide, interwoven; pale yellow in H_2O, a bit more paler in KOH, thin-walled, not gelatinized, oleiferous hyphae present. – Stipitipellis on most of the surface an undifferentiated cutis with tufts of caulocystidia 19.5–37.5 × 5–10 µm, subventricose, fusoid to subclavate, frequent, colorless but occasionally golden brown in KOH, pale in H_2O at apex, more scarce downward.



Figs. 5–12. Microstructures of *Tylopilus aquarius* var. *megistus*. **5.** Basidiospores. **6.** Basidia. **7.** Pleurocystidia. **8.** Caulocystidia. **9.** Cheilocystidia. (FW 108/2012). **10.** Basidiospores. **11.** Pleurocystidia. (FW 19/2014, holotype). **12.** Terminal elements of the pileipellis. (Sulzbacher-453). Bars: 10 µm.

– Stipe context hyphae longitudinally oriented 3–7.5 µm wide, hyaline in H_2O and KOH, oleiferous hyphae present. – Clamp connections absent.

Etymology. – 'megisto' (Greek) – the largest, because of the larger size of the basidiomata of the Atlantic Forest collection, that corresponds to one of the diagnostic features of the variety.

Habitat. – In small groups of 2–4 basidiomata frequently on sandy soil in Atlantic Forest.

Distribution. – Forest of Paraíba and Rio Grande do Norte, Brazil.

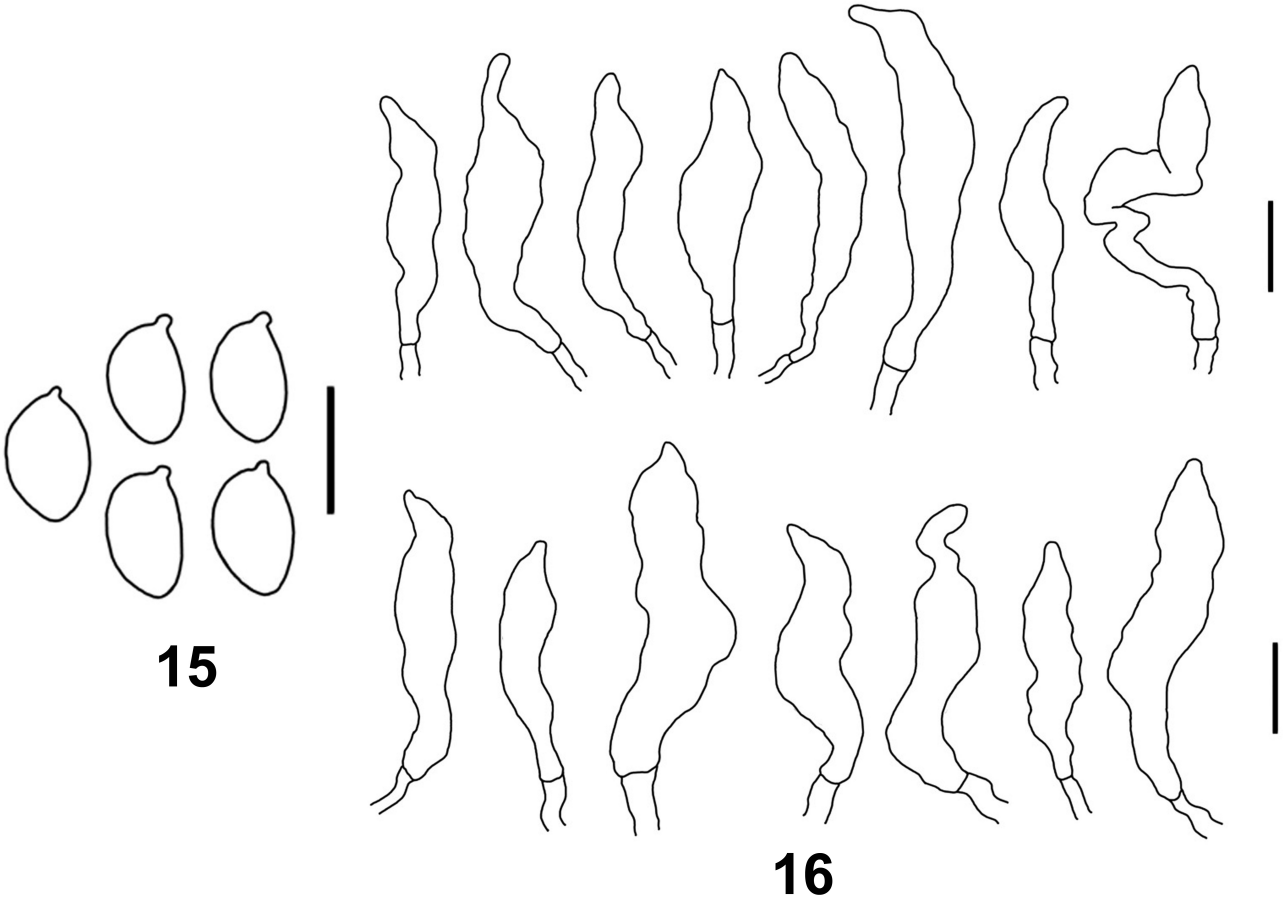
Material examined. – *Tylopilus aquarius* var. *aquarius*: BRAZIL, Amazonas, Igarapé do Tarumãzinho, 19 March 1980, *leg.* R. Singer B 12052 (F 1030901 holotype!). – *Tylopilus aquarius* var. *megistus* (besides types): BRAZIL, Paraíba, Mamanguape, Reserva Biológica Guaribas, SEMA II, 30 June 2012, *leg.* F. Wartchow FW 108/2012 (JPB 61780, UFRN-Fungos 2676, URM 89205); idem, 30 June 2013, *leg.* M.A. Sulzbacher 453 (JPB 61781, UFRN-Fungos 2493); 27 July 2012, *leg.* C.L. Ovrebo CLO 5037 (JPB 51100); Rio Grande do Norte: Natal, Parque Estadual das Dunas, Trilha da Peroba, 24 June 2013, *leg.* M.A. Sulzbacher 441 (JPB 61782, UFRN-Fungos 2489). – *Tylopilus potamogeton* var. *potamogeton*: BRAZIL, Amazonas, São Gabriel da Cachoeira, 20 km from São Gabriel da Cachoeira, 20 Jan 1978, *leg.* I. Araujo 938 (INPA 78694 paratypus!) .

Molecular biology

Only one *T. aquarius* var. *megistus* specimen produced a readable sequence (CLO 5037). According to BLAST Search result, the ITS sequence of *Tylopilus aquarius* var. *megistus* was 83 % similar to *T. potamogeton* var. *irengensis* voucher TH8801 (over 77 % of the ITS sequence). These species were more similar than any other *Tylopilus*, but the high level of ITS variation suggested that these sequences clearly belonged to distinct species.

Discussion

Tylopilus aquarius and *T. potamogeton* belong to a small set of boletes with remarkable basidiospores up to 6 µm broad, grouped within *T.* sect. *Potamogetones* Singer described for Amazonia (Singer 1978: 434, Singer 1986: 795). Henkel (1999) and Fulgenzi et al. (2007) re-interpreted Singer's concept and considered in this group taxa with basidiospores Qm < 2.00 (Qm = 1.58 for *T. aquarius* var. *aquarius*). Chemical reactions are also frequently reported for species of this group. In our material due to lack of NH_4OH we used KOH, in which reacted the pileus



Figs. 15, 16. Microstructures of *Tylopilus potamogeton* var. *potamogeton* (paratype). **15.** Basidiospores. **16.** Pleurocystidia. Bars: 10 µm.

surface to olivaceous, pileus context pinkish, and the stipe surface and context pink.

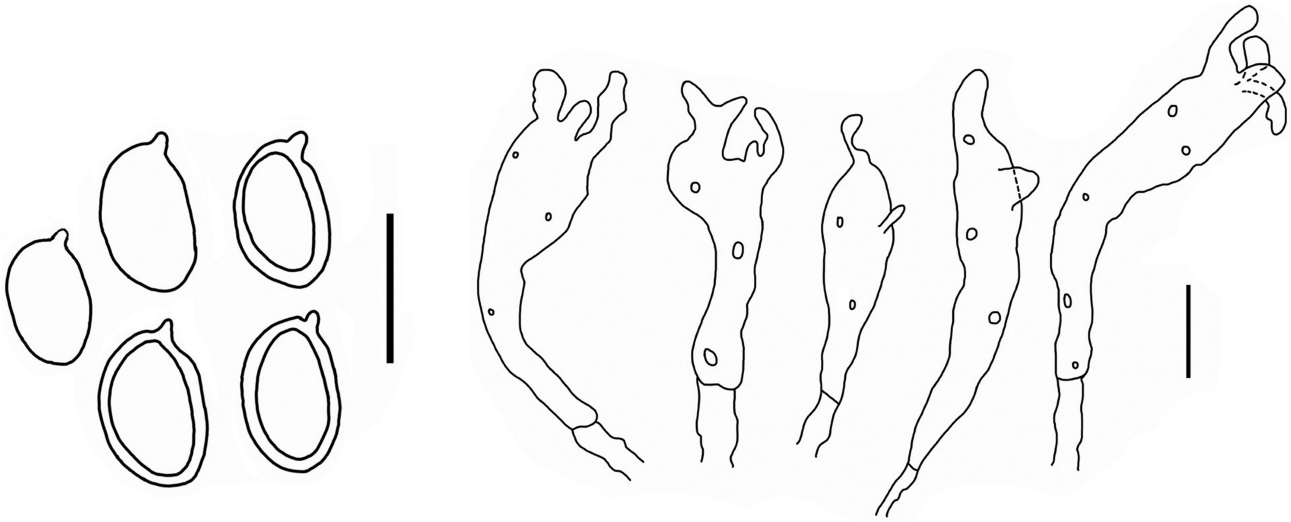
Tylopilus potamogeton was protologued as having brown tomentose then glabrescent 27–36 mm wide pileus, whitish then incarnate hymenophore; cinnamon then fuscous 33–75 × 4.5–16 mm stipe; basidiospores 9.5–12 × 6.5–8 µm; and hyaline cystidia (Singer 1978: 433).

Later, *Tylopilus potamogeton* var. *aquarius* was described (Singer et al. 1983: 122 with cystidia given as "versiform, often clavate, fusoid, utriform, ventricose, or cylindrical, often mucronate or even with two apical mucrons", and basidiospores "(8)9–11.5 × (5)5.5–7.5 µm (majority) and 11–16 × 5–6 µm (minority), with Q = 1.6–1.8 respectively Q = 2.2–2.7". Later, Singer (1989: 125) described *T. potamogeton* var. *mitis*, with 'brunneolo-livido' (pale/yellowish brown) pileus color, 'albis dein roseolis' (whitish then pinkish) hymenophore, 'carne alba, miti' (flesh white, mild) context, 'brunneo apice albo' (brown with white apex) stipe and microscopically similar to var. *aquarius*.

For comparison type materials of var. *aquarius*, var. *mitis* and var. *potamogeton* were requested. The type of var. *mitis* was not sent and we thus cannot address the nomenclatural situation.

Analysis of the holotype of *Tylopilus potamogeton* var. *aquarius* (F 1030901) revealed four mature basidiomata with pilei 27–36 mm in diam. and stipe 43–56 × 5–5 mm (in the dried state), basidiospores 10–11(12.3) × (5.5)6–7(7.5) µm, L = 10.7 µm, W = 6.8 µm, Q = (1.47)1.50–1.67(2.00); Qm = 1.58, and versiform cystidia, varying from subventricose, fusoid, clavate to subclavate, with appendages on apex and sometimes with knobs or outgrowths appearing diverticulate (Figs. 13, 14).

The material of our new variety *T. aquarius* var. *megistus* collected at the Atlantic Forest presents cystidia similar to those of *T. aquarius* var. *aquarius*, but distinctly larger, robust basidiomata (pileus 45–



Figs. 13, 14. Microstructures of *Tylopilus aquarius* var. *aquarius* (holotype). **13.** Basidiospores. **14.** Pleurocystidia. Bars: 10 µm.

120 mm in the fresh state) and shorter and narrower basidiospores (7.6)7.9–9.5(9.7) × (3.8)4.1–6 (6.5) µm. However, we conclude that these differences are not sufficient to segregate the Atlantic Forest collections as a different species, but as a new variety, *T. aquarius* var. *megistus*.

The holotype of *T. potamogeton* var. *potamogeton* was not sent, but we analyzed the paratype (INPA 78694), which presents wider basidiospores (9.5)10–11 × 6–7(7.5) µm, L = 10.4 µm, W = 6.6 µm, Q = (1.36)1.43–1.69(1.83); Qm = 1.57, and exclusively fusoid, subfusoid to ventricose often mucronate

Tab. 1. Comparison among taxa of *Tylopilus potamogeton* and *T. aquarius* and their varieties (Singer 1978, Singer et al. 1983, Henkel 1999).

	T. aquarius var. *aquarius*	*T. aquarius* var. *megistus*	*T. potamogeton* var. *potamogeton*	*T. potamogeton* var. *irengensis*
Pileus size	(17–)20–39 mm	45–120 mm	27–36 mm	40–110 mm
Hymenophore color	white then "sandust"	whitish to buff, pale cream or orange white or pinkish brown	whitish then pale or light incarante or orange pink	incarnate when mature staining dull orange-tan upon pressure
context color	white, unchanging	white to whitish sometimes buff at stipe, unchanging	white, unchanging	white, then slowly yellow then olivaceous after exposure (at lower stipe's half)
Basidiospores	10–11(12.3) × (5.5)6–7(7.5) µm, L = 10.7 µm, W = 6.8 µm, Q = (1.47)1.50–1.67(2.00), Qm = 1.58	(7.6)7.9–9.5(9.7) × (3.8)4.1–6 (6.5) µm, L = 9 µm, W = 5.2 µm, Q = (1.38)1.42–2.33(2.38), Qm = 1.76	(9.5)10–11 × 6–7(7.5) µm, L = 10.4 µm, W = 6.6 µm, Q = (1.36)1.43–1.69(1.83), Qm = 1.57	9–9.7 × 4.8–6.4 µm, L, W, Q not reported, Qm = 1.69
Pleurocystidia	versiform from subventricose, fusoid, clavate to subclavate, with appendages on apex and sometimes with knobs or outgrowths appearing diverticulate	versiform from ventricose, subventricose, fusoid, subfusoid, sometimes clavate to subclavate, with appendages on apex and sometimes with knobs or outgrowths appearing diverticulate	fusoid, subfusoid to ventricose often mucronate	ventricose to mucronate

cystidia measuring 26.5–52 × 5.5–12.5(13.5) µm (Figs. 15, 16). We consider the versiform cystidia the most relevant feature for raising var. *aquarius* to species level, since *T. potamogeton* presents exclusively fusoid or mucronate cystidia (Fig. 16).

In addition to *T. potamogeton* var. *potamogeton*, two other taxa have Qm < 2.00 µm and dark tints on the pileus, viz. *T. potamogeton* var. *irengensis* T.W. Henkel and *T. pakaraimensis* T.W. Henkel. They differ easily from *T. aquarius* in the ventricose to mucronate or rostrate cystidia without diverticulate projections. In addition, *T. potamogeton* var. *irengensis* also can be segregated from *T. aquarius* as follows: lower half of stipe changing slowly to yellow then to olivaceous after exposure, basidiospores somewhat longer, 9–12 × 4.8–6.4 µm and Qm= 1.69 and ellipsoid with a slight suprahilar depression rather than amygdaliform (Henkel 1999). *Tylopilus pakaraimensis* differs by the reddish lilac to lilac pileus and by the slightly and rapidly bluing context when cut (Henkel 2001).

The molecular analysis confirms that *T. aquarius* var. *megistus* is a *Tylopilus*, closely related but differing from *T. potamogeton* var. *irengensis* by several nucleotide positions. We conclude that *T. aquarius* is a distinct species and does not belong to the *T. potamogeton* complex. Molecular studies in sect. *Potamogetones* will elucidate infrageneric classification, but more sequences of this section are necessary for such a study. Finally, we present a comparison of *T. potamogeton*, *T. aquarius* and their varieties (Tab. 1) providing characters to distinguish these distinct taxa.

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