

Morphological description and new records of *Hygrocybe conica* var. *conica* and *H. nigrescens* var. *brevispora* (Hygrophoraceae) in Brazil

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ABSTRACT - (Morphological description and new records of *Hygrocybe conica* var. *conica* and *H. nigrescens* var. *brevispora* (Hygrophoraceae) in Brazil). *Hygrocybe conica* var. *conica* and *H. nigrescens* var. *brevispora* were collected in the Atlantic Rain Forest of the States of Paraná and Rio Grande do Norte States, respectively. These two taxa were reported in mushroom checklists of Brazil; however, their morphological features and variations have been hitherto poorly documented. Thus, it is provided for the first time a complete description for these two Brazilian varieties. *Hygrocybe conica* var. *conica* was reported in the last century for the southeast region, more precisely from the mixed ombrophilous forests of São Paulo State. Presently, this variety is a new record for the seasonal semideciduous forests of Paraná State, southern Brazil. *Hygrocybe nigrescens* var. *brevispora* was known from coastal ecosystems of the south and southeast regions; it is also being indicated here as a new record for the northeast region.

Keywords: Agaricales, brazilian mycodyversity, new record, restinga, seasonal semideciduous forest

RESUMO - (Descrição morfológica e novos registros de *Hygrocybe conica* var. *conica* e *H. nigrescens* var. *brevispora* (Hygrophoraceae) no Brasil). *Hygrocybe conica* var. *conica* e *H. nigrescens* var. *brevispora* foram coletados em floresta de Mata Atlântica, respectivamente nos Estados do Paraná e do Rio Grande do Norte. Esses dois táxons foram reportados em listas de diversidade de macrofungos no Brasil; entretanto suas características morfológicas e variações foram, até então, pouco documentadas. Em razão disso, é fornecida pela primeira vez, a descrição completa dessas duas variedades brasileiras. *Hygrocybe conica* var. *conica* foi reportada no século passado para o sudeste, mais precisamente em floresta ombrofila mista do Estado de São Paulo, e agora é um novo registro para floresta estacional semidecidual do Estado do Paraná, sul do Brasil. *Hygrocybe nigrescens* var. *brevispora* encontrada até o momento em ecossistemas costeiros do sul e sudeste do Brasil, agora é um novo registro também para a região do nordeste.

Palavras chaves: Agaricales, floresta estacional semidecidual, micodiversidade brasileira, novos registros, restinga

Introduction

Hygrocybe conica (Schaeff.) P. Kumm. and its varieties, *Hygrocybe nigrescens* (Quél.) Kühner and other nine related species form a complex of close and similar species of waxy caps with black staining reactions (Lodge *et al.* 2014). *Hygrocybe conica* var. *conica* and *Hygrocybe nigrescens* var. *brevispora* (Dennis) S.A. Cantrell & Lodge are known from

North to South America, based on previous fieldwork in these regions (Dennis 1961, Hesler & Smith 1963, Pegler 1983, Cantrell & Lodge 2000, Franco-Molano *et al.* 2010, Lodge *et al.* 2014).

Hygrocybe conica was described as *Agaricus conicus* by Schäffer (1774), based on a Dutch collection. Fries (1838) transferred it to the genus *Hygrophorus* Fr. Subsequently, Kummer (1871) raised the new genus *Hygrocybe* and consequently,

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proposes combine in *Hygrocybe*. Since then, new varieties have been described; *Hygrocybe nigrescens* var. *brevispora* is an American variety described by Dennis (1961, as *Hygrophorus nigrescens* var. *brevisporus* Dennis) based on Venezuelan collection. Pegler (1983) transferred it to *Hygrocybe nigrescens*, after synonymized by Cantrell & Lodge (2000) to *Hygrocybe conica* var. *brevispora* (Dennis) S.A. Cantrell & Lodge. Recently, molecular analysis recognized *H. conica* var. *brevispora* as distinct entity of *Hygrocybe conica* (Lodge *et al.* 2014, Latha & Manimohan 2018), considered into *H. nigrescens*.

In Brazil, 36 *Hygrocybe* species are reported (Singer 1965, Lodge & Pegler, 1990, Putzke 1994, Pegler 1997, Sobestiansky 2005, De Meijer 2006, Karstedt & Sturmer 2008, De Meijer 2010, Neves *et al.* 2013, Magnago *et al.* 2015, Maia *et al.* 2015, Vizzini *et al.* 2015). Among these, *H. conica* var. *conica* is known in the Atlantic forest of the Southeast region (Pegler 1997), and *H. nigrescens* var. *brevispora*, in the southeast and south (Pegler 1997 as *H. conica* var. *brevispora*, De Meijer 2006). Although these taxa reported in the Brazilian mycological literature, few works have presented a detailed description of the morphology (especially the microscopical ones) to support such identifications.

Thus in this paper, we provide a detailed description of *Hygrocybe conica* var. *conica* and *H. nigrescens* var. *brevispora* that occur in Brazil, expanding the known distribution of *H. conica* var. *conica* from the south and of *H. nigrescens* var. *brevispora* from the northeast of Brazil.

Materials and methods

Species were collected in two distinct areas - 1) Parque Estadual das Dunas, located in the municipality of Natal (24°18'26"S and 53°54'29" W), in the east region of Rio Grande do Norte State, Brazil. This region is predominantly composed of sandy coastal plain (restinga) vegetation, with sandy soil and presence of tree species of Convolvulaceae, Euphorbiaceae, Fabaceae, Myrtaceae, Poaceae, Rubiaceae, and Sapotaceae (Freire 1990); 2) RPPN Fazenda Açú situated in the municipality of Terra Roxa (24°11'28.05" S and 53°58'6.92" W), western region of Paraná State. This is a remaining of seasonal semideciduous forest, characterized by the presence of tree species of Anacardiaceae, Apocynaceae, Bignoniaceae, Boraginaceae, Caricaceae, Fabaceae, Malvaceae, Meliaceae, Moraceae, Rutaceae and Sapindaceae (Roderjan *et al.* 2002).

The samples were analyzed both macro- and micro morphologically, following standard procedures for the description of agaricoid mushrooms (Singer 1986). Color names and codes used in macroscopic descriptions are based on Kornerup & Wanscher (1978). All microscopic structures were observed in dried material. Freehand sections of rehydrated pieces of basidiomata were examined in KOH 5%, and Melzer's reagent was used to check amyloid reaction. Microscopic structure measurements were performed in 1000 × magnification. For statistics, 40 spores were measured. In basidiospores description: L = basidiospore length, average and W = basidiospore width, average from a single basidioma, Q is the quotient between length and width, Qm is the mean value of Q. Specimens are preserved at the herbaria of Universidade Federal do Rio Grande do Norte (UFRN-Fungos) and Universidade Federal do Paraná, *Campus Palotina* (HCP). Taxonomical concepts are following Lodge *et al.* (2014).

Results

Hygrocybe conica* var. *conica (Schaeff.) P. Kumm., *Der Führer in die Pilzkunde*: 111 (1871) ≡ *Agaricus conicus* Schaeff., *Fungorum qui in Bavaria et Palatinatu circa Ratisbonam nascuntur Icones* 4: 2 (1774) ≡ *Hygrophorus conicus* (Schaeff.) Fr., *Epicrisis Systematis Mycologici*: 331 (1838). Type locality: Europe, Germany.

Figures 1,3,7

Pileus 15-29 mm diam., at first conic, cuspidate in mature specimens; reddish-orange (7A8), orange (6B8), greyish yellow (4B4) and dark grey (1F1), surface smooth, viscid and slightly fibrillose at margin; margin slightly eroded, sometimes undulating, non-striate, decurved (figure 1a); context fleshy, 2-4 mm thick, concolorous with the pileus surface. Lamellae adnexed, subdistant, yellowish-white (1A2) to greyish white (1B1) with dark grey spots (1F1), waxy, up to 2.3 mm broad, edge eroded to crenate, slightly discolored and paler at the sides; lamellulae with two lengths (figure 1). Stipe 33-61 × 3-6 (apex), 4-10 (base) mm, central, terete to slightly compressed in older basidiomata, slightly flexuous, equal to slightly tapered at apex, pale yellow (1A3), greenish-yellow (1A8), light yellow (4A5) and dark grey (1F1), fistulous, surface longitudinally striate, texture rigid to pliable (figure 1). Spore print not observed.

Basidiospores (6.5-)7-10.5 × 4.5-6.5(-7) μm, L = 8 μm, W = 5.5 μm, Q = 1.14-2.12, Qm = 1.46,



Figures 1-2. Basidiomata. 1. *Hygrocybe conica* var. *conica* (A.G.S. Silva-Filho 728). 2. *Hygrocybe nigrescens* var. *brevispora* (A.G.S. Silva-Filho 890). Scale bars = 10 mm. Pictures by A.G.S. Silva-Filho.

subglobose to cylindrical, smooth, thin-walled, hyaline, inamyloid, with numerous refractive granules; hilar appendix conspicuous (figure 4). Basidia $29-42 \times 7.5-9 \mu\text{m}$, clavate, to broadly clavate, tetrasporic, rarely bisporic, thin-walled, hyaline, some with refractive content mixed with some brown pigmented. Pleurocystidia and cheilocystidia absent. Pseudocystidia $31.5-42.5 \times 5-8 \mu\text{m}$, clavate with mucronate apex, cylindrical-fusoid, thin-walled, hyaline; scarce, not projecting from hymenium, found in lamellar sides and gill edges (figure 7). Lamella edge fertile. Lamellar trama regular with hyphae up to $179 \mu\text{m}$ length, $5.5-12.5 \mu\text{m}$ diam., thin-walled, smooth sometimes brown incrustated, hyaline and brown pigmented. Subhymenium with filamentous, hyphae $2-3.5 \mu\text{m}$ diam., interwoven, smooth, thin-walled, hyaline. Pileus trama with inflated hyphae $10.5-40 \mu\text{m}$ diam., parallel and horizontally arranged, smooth, thin-walled, hyaline. Pileipellis a cutis of repent hyphae, hyphae subparallel to interwoven, $4.5-7 \mu\text{m}$ diam., smooth, sometimes brown incrustated, hyaline and light brown, slightly gelatinous at center. Stipitipellis a cutis of subparallel hyphae, $2.5-7 \mu\text{m}$ diam., hyaline and brown pigmented, predominantly smooth, sometimes brown incrustated. Stipititrama regular, composed of hyphae $2.5-27.5 \mu\text{m}$ diam., smooth sometimes incrustated, hyaline and brown. Caulocystidia absent. Clamp connection present in all tissue examined. Oleiferous hyphae

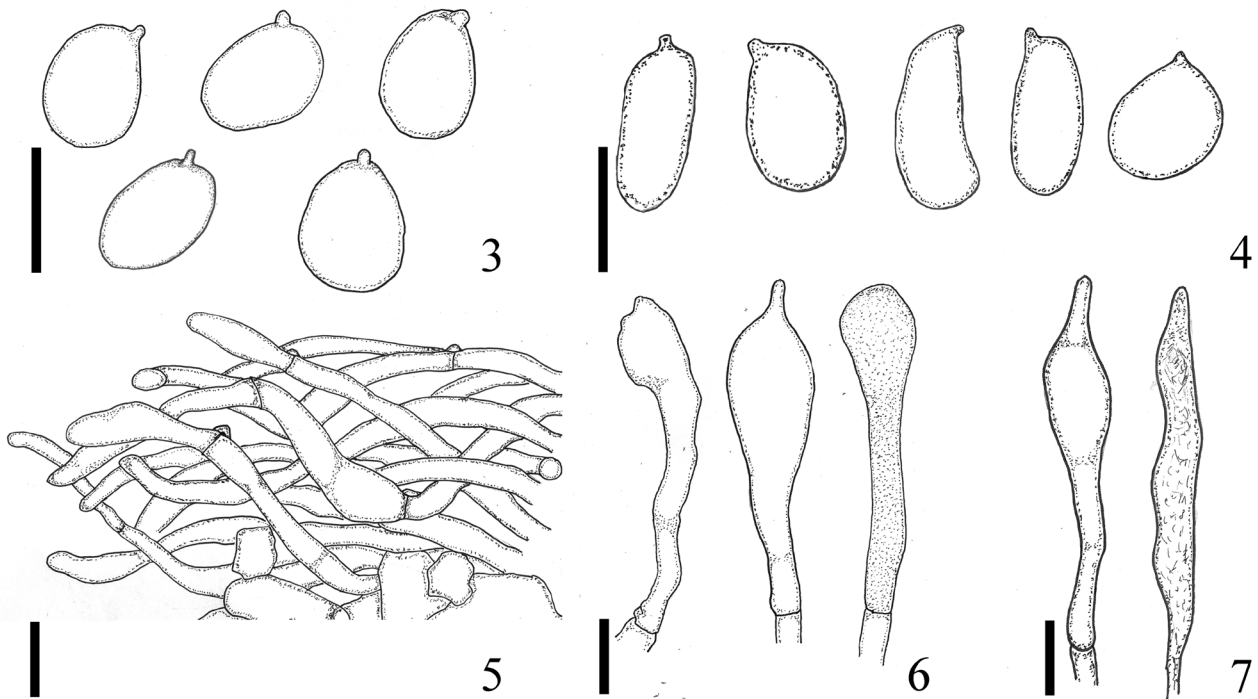
(thrombopleurous) observed in the lamellar trama, pileipellis, and stipitipellis.

Known distribution - Worldwide (Boertmann 2010). In Brazil, it has been reported only from the Atlantic Forest of the Southeast (Pegler 1997, São Paulo) and South (this paper, Paraná) regions (figure 8).

Material examined: Brazil. PARANÁ: Terra Roxa, RPPN Fazenda Açú, 23-VI-2015, leg. AGS Silva-Filho 501 (HCP 1038).

Hygrocybe nigrescens* var. *brevispora (Dennis) Pegler, Kew Bulletin Additional Series 9:53 (1983) \equiv *Hygrophorus nigrescens* var. *brevisporus* Dennis, Kew Bulletin 15:69 (1961) \equiv *Hygrocybe conica* var. *brevispora* (Dennis) S.A. Cantrell & Lodge, Mycological Research 104: 876 (2000) Type locality: South America, Venezuela. Figs. 2,3,5,6

Pileus 12-28 mm diam., at first broadly parabolic, slightly umbonate at the disc, convex to slightly umbonate to conical in mature specimens, dark red (11C8), violet brown (11E8), orange (7A8), yellowish red (8A8), red (9B8), with dark grey (1F1) spots, surface smooth, viscid at center and slightly fibrillose at margin; margin entire, non-striate, incised, decurved (figure 2); context fleshy, 2-4 mm thick, concolorous with the pileus surface. Lamellae adnexed, subdistant, yellowish-white (1A2) to grayish-white (1B1) with dark gray spots (1F1), waxy, up to 2.5 mm broad, edge



Figures 3-7. Microscopic drawings. 3. Basidiospores of *Hygrocybe nigrescens* var. *brevispora* (A.G.S. Silva-Filho 890). 4. basidiospores of *Hygrocybe conica* var. *conica* (A.G.S. Silva-Filho 728). 5. Pileipellis of *Hygrocybe nigrescens* var. *brevispora* (A.G.S. Silva-Filho 890). 6. Pseudocystidia of *Hygrocybe nigrescens* var. *brevispora* (A.G.S. Silva-Filho 890). 7. Pseudocystidia of *Hygrocybe conica* var. *conica* (A.G.S. Silva-Filho 728). Scale bars = 10 μm . Drawings by A.G.S. Silva-Filho.

eroded to crenate, slightly discolored and paler at the sides; lamellulae with two lengths (figure 2). Stipe 32-41 \times 4-6 (apex), 4-6 (base) mm, central, terete, becoming later slightly compressed, slightly flexuous, equal to slightly tapered at apex, fistulous, pale yellow (1A3), greenish-yellow (1A8), light yellow (4A5), orange (5A6), yellowish red (8A8) with dark grey (1F1) spots, surface longitudinally striate, texture rigid to pliable (figure 2). Spore print not observed.

Basidiospores (8.5-)9-11 \times (6-)6.5-8(-8.5) μm , L = 9.6 μm , W = 6.7 μm , Q = 1.29-1.61, Qm = 1.46, broadly ellipsoid to ellipsoid, smooth, thin-walled, hyaline, with numerous refractive granules; hilar appendix conspicuous (figure 3). Basidia 34-44 \times 7.5-12 μm , clavate, to broadly clavate, tetrasporic, rarely bisporic, thin-walled, hyaline, brown pigmented sometimes with refractive content. Pleurocystidia and cheilocystidia absent. Pseudocystidia 37-53.5 \times 5-11 μm , cylindrical-clavate, clavate, sometimes with mucronate apex, thin-walled, hyaline some brown pigmented, scarce, not projecting from the hymenium, found in lamellar sides and gill edges (figure 6). Lamella edge fertile. Lamellar trama regular with hyphae up to 186 μm length, 5-11 μm diam., thin-walled, smooth,

sometimes brown incrustated, hyaline and brown pigmented. Subhymenium with filamentous hyphae 1.5-3.5 μm diam., interwoven, smooth, thin-walled, hyaline. Pileus trama with inflated hyphae 10-34 μm diam., parallel and horizontally arranged, smooth, thin-walled, hyaline. Pileipellis a cutis of repent hyphae, hyphae subparallel to interwoven, 4-7.5 μm diam., smooth, sometimes brown incrustated, hyaline and light brown, near at center, slightly gelatinous (figure 5). Stipitipellis a cutis of subparallel hyphae, 8-17 μm diam., hyaline and brown pigmented, predominantly smooth, sometimes brown incrustated. Stipititrama regular composed of hyphae 2-8 μm diam., smooth sometimes incrustated, hyaline and brown. Caulocystidia absent. Clamp connection present in all tissue examined. Oleiferous hyphae (thrombopleurous) observed in the lamellar trama, pileipellis and stipitipellis.

Known distribution - Neotropical (Dennis 1961, Pegler 1983, Lodge & Pegler 1990, Cantrell & Lodge 2000). In Brazil, reported only from the Atlantic Forest of the Southeast (Pegler 1997, São Paulo), South (Meijer 2006) and Northeast (this Paper, Rio Grande do Norte State) regions (figure 8).



Figure 8. Known distribution of *Hygrocybe conica* var. *conica* and *H. nigrescens* var. *brevispora* in the Neotropical America.

Material examined: Brazil. RIO GRANDE DO NORTE: Natal, Parque Estadual das Dunas, 15-VIII-2008, leg. *I.M. Cocetino & J.J.S. Oliveira* s/n (UFRN-Fungos 844); *ibid*, 5-VII-2017, leg. *A.G.S. Silva-Filho* 890 (UFRN-Fungos 2949).

Discussion

Hygrocybe conica differs of *H. nigrescens* by its more robust habit, pileus shape and color and broader basidiospores (Pegler 1977). These features were observed in our samples: *H. conica* var. *conica* from Paraná has conical pileus in young specimens becoming cuspidate, orange to reddish-orange tints, and subglobose to cylindrical basidiospores ($Q = 1.14-2.12$); *H. nigrescens* var. *brevispora* from Rio Grande do Norte has broadly parabolic, slightly umbonated, convex slightly umbonated to conical pileus, predominance of dark red tints and broadly ellipsoid to ellipsoid basidiospores ($Q = 1.29-1.61$).

These mentioned features confirm the identification of these two entities, in which also agree with the descriptions of the literatures: *Hygrocybe conica* var. *conica* agrees with the brief morphological features indicated by Pegler (1997, from Brazil) and the description of Hesler & Smith (1963, as *Hygrophorus conicus* from USA) by the shape and color of pileus and basidiospore size $9-12 \times 5.7-8.6 \mu\text{m}$, and disagree only in smaller cap 15-29 mm; *Hygrocybe nigrescens* var. *brevispora* from Rio Grande do Norte State agrees with that described Pegler (1997, 1983) and Lodge & Pegler (1990), in its pileus shape and short broadly ellipsoid basidiospores $8.5-11 \times 5.5-8 \mu\text{m}$.

Cantrell & Lodge (2000) confronted morphological characters of *H. conica* var. *conica* from Central America, North America, and Europe and *H. nigrescens* var. *brevispora*, (as *H. conica* var. *brevispora*), from the Dominican Republic and Puerto Rico. Pseudocystidia were found more concentrated near to lamellar edge in *H. nigrescens* var. *brevispora*,

feature observed in the holotype analyzed by Pegler (1983) and Lodge & Pegler (1990). On the other hand, *H. conica* var. *conica* lacks pseudocystidia, or they concentrated only on the lamellar edge (Cantrell & Lodge 2000). In both collections analyzed by us, pseudocystidia are scarce on lamellar sides and edge.

Hygrocybe conica var. *conica* is known on all continents, except in Antarctica (Boertman 2010); it is reported in Neotropical America only at high elevations, such as in Costa Rica (3000-3500 a.s.l. alt., Cantrell & Lodge 2000) and Bolivia (2202 a.s.l. alt. Franco-Molano *et al.* 2010). *Hygrocybe nigrescens* var. *brevispora* is known in the coastal areas of tropical and subtropical humid forests of Central America (Martinique, Puerto Rico and the Dominican Republic) and South America (Venezuela, Brazil). *Hygrocybe conica* var. *conica* was recorded to São Paulo State (1600-1700 a.s.l. alt.), which is the only Brazilian occurrence to date (Pegler 1997). *Hygrocybe nigrescens* var. *brevispora* has been collected from resting vegetation in São Paulo by Pegler (1997) and in a dense ombrophilous forest of Paraná by De Meijer (2006). With the new findings, the distribution of *H. conica* var. *conica* is now expanded to seasonal semideciduous forest (346 m alt.) in the south, while *H. nigrescens* var. *brevispora* has a new record in the northeast of Brazil. New reports are expected from other areas of Brazil and, together with its molecular data, this may help answer questions related to the distribution pattern and evolutionary processes this species complex.

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