Hemiaustroboletus, a new genus in the subfamily Austroboletoideae (Boletaceae, Boletales)

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Abstract
The present study describes Hemiaustroboletus gen. nov. in the subfamily Austroboletoideae (Boletaceae). Hemiaustroboletus is supported by morphological and molecular data using LSU and RPB2 regions. Additionally, its geographic distribution and intraspecific variation were inferred using ITS sequences. The genus is characterised by pileate-stipitate basidiomata; purple, brown, reddish-brown, orange-brown to dark brown vinaceous pileus; whitish or lilac to vinaceous context and a subclavate stipe. Microscopically, it is characterised by ornamented, slightly verrucose, cracked to perforated brown basidiospores. Two species are described within the genus, Hemiaustroboletus vinaceobrunneus sp. nov. and H. vinaceus sp. nov. Hemiaustroboletus vinaceus sp. nov. is morphologically similar to Austroboletus gracilis, which suggests they may have been confused in the past. This study presents the phylogenetic placement, microscopic structures, detailed morphological descriptions and illustrations of both new species.

Keywords
Mexico, mycodiversity, neotropics, new taxa
Introduction

Boletaceae is the most diverse family within the Boletales; it has a wide distribution in both temperate and tropical regions (Binder and Hibbett 2006; Wu et al. 2014). Most species of this family are ectomycorrhizal with members of Betulaceae, Casuarinaceae, Dipterocarpaceae, Ericaceae, Fabaceae, Fagaceae, Mimosaceae, Myrtaceae, Pinaceae, Polygonaceae, and Salicaceae (Tedersoo et al. 2010; Smith et al. 2013; Wu et al. 2016). Currently, 98 genera are recognised in this family (He et al. 2019; Vadthanarat et al. 2019; Hosen and Yang 2021). Its members are characterised by fleshy, epigeous pileate-stipitate basidiomata or hypogeous to subhypogeous gastroid basidiomata, with tubular or lamellar hymenophore; elliptical, cylindrical, fusoid, subfusoid, ovoid, subglobose to globose, smooth or ornamented basidiospores; spore ornamentation ranging from striated, reticulate, echinulate, to verrucose to smooth (Singer et al. 1991; Halling et al. 2015; Ayala-Vásquez et al. 2018).

Wu et al. (2014) proposed six subfamilies for Boletaceae, of which Austroboletoideae includes *Austroboletus* (Corner) Wolfe, *Fistulinella* Henn., *Mucilopilus* Wolfe and *Veloporphyrellus* L.D. Gómez & Singer, with *Austroboletus* as the type genus. This subfamily is distinguished by pileate-stipitate basidiomes; smooth, furfuraceous, tomentose, dry or viscous pileus, with or without a marginal veil and whitish context that does not change colour when cut. The hymenophore is tubular, whitish or pink with purple tinge, immutable or rarely brown when cut. The stipe is smooth, reticulate or squamose with a whitish basal mycelium. The basidiospores are smooth or ornamented, perforated, verrucose to smooth, grey-violet, yellowish, yellow brown, ochraceous in potassium hydroxide (KOH) and yellow-brown, yellow-cinnamon to ochraceous in Melzer’s reagent. The pileipellis is formed by a trichoderm or ixotrichoderm. The hymenophoral trama is boletoid. Austroboletoideae species are mainly associated with Fagaceae and Pinaceae hosts in temperate, subtropical to tropical regions.

In recent years, various authors (Wu et al. 2014; Wu et al. 2016; Gelardi et al. 2020; Kuo and Ortiz-Santana 2020) have recognised the polyphyly of *Austroboletus*, which is divided into the *Austroboletus* s.s., *Austroboletus* s.l. and the *A. gracilis* s.l. independent clades. This study focuses on the phylogenetic placement and taxonomy of the *A. gracilis* s.l. clade, placing it in the new genus *Hemiaustroboletus* with two new species, *Hemiaustroboletus vinaceobrunneus* and *H. vinaceus*.

Materials and methods

To resolve the systematics and taxonomy of the new genus *Hemiaustroboletus*, we conducted an exhaustive sampling of an area with high bolete diversity according to García-Jiménez et al. (2013). The sampling was carried out over the last 10 years including the different biogeographic areas of Mexico: Nearctic, Neovolcanic Axis and Neotropic. The collection trips were conducted in the States of Chiapas, Chihuahua,
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Estado de Mexico, Jalisco, Michoacan and Oaxaca, in six vegetation types in temperate and subtropical forests during the rainy season from June to October from 2010 to 2019. The samples were characterised at macro- and micromorphological level and three genetic markers were sequenced and analysed.

Morphological study

Morphological characters were described according to Largent (1986) and Lodge et al. (2004). Chemical reactions with KOH and ammonium hydroxide (NH₄OH) were characterised. Photographs of basidiomata were taken in situ, as well as data on the botanical composition of the sites. The colours for taxonomic descriptions were based on Kornerup and Wanscher (1978). Microscopic characters of 30 basidiospores, basidia, pleurocystidia, cheilocystidia, pileipellis cells and stipitipellis were measured by optical microscopy (Carl Zeiss GmbH 37081, Germany). The Q index (length/width) was estimated for the basidiospores. Ornamentation of basidiospores was observed by scanning electron microscopy (SEM) (Hitachi Su 1510, Hitachi, Japan). The specimens were deposited at the “Herbario Nacional de México” of the “Instituto de Biología, Universidad Nacional Autónoma de México” (MEXU), at the “Herbario José Castillo Tovar del Tecnológico de Ciudad Victoria” (ITCV) and at the “Herbario del Instituto de Botánica, Universidad de Guadalajara” (IBUG).

DNA Extraction, PCR and Sequencing

Samples of dehydrated basidiomata were used for DNA extraction. The DNA was extracted using the DNeasy Power-Soil kit (QIAGEN). Cell lysis was performed by grinding samples in mortar with liquid nitrogen. Three nuclear loci (ITS, LSU and RPB2) were amplified with Platinum Taq DNA Polymerase (Invitrogen-Thermo Fisher Scientific) and Taq & Load PCR Mastermix (MP Biomedicals) in a thermocycler (BIO-RAD). The PCR parameters were as follows: 95 °C initial denaturation for 4 min; 35 cycles of denaturation at 94 °C for 60 s, alignment at 54 °C for 60 s, extension at 72 °C for 60 s and a final extension at 72 °C for 10 min. The primers ITS1/ITS4 (White et al. 1990) were used for the ITS region; LROR/LR5 (Vilgalys and Hester 1990) for LSU; and RPB2-B-F2/RPB2-B-R (Wu et al. 2014) for the partial RPB2 gene. The amplification was examined by 1% agarose gel electrophoresis; gels were stained with GelRed (Biotium) and observed under an UVP Multidoc-It transilluminator (Analytikjena). Only PCR products generated with Taq-Platinum required LB loading buffer. PCR products with successful amplification were cleaned with ExoSAP-IT (Thermo Fisher Scientific) diluted 1:1 with ddH₂O and incubated at 37 °C for 45 min and 80 °C for 15 min. Sanger sequencing was performed at the “Laboratorio de secuenciación genómica de la biodiversidad y la salud, Instituto de Biología, Universidad Nacional Autónoma de México”. Samples were sequenced in both directions with PCR primers using BigDye Terminator v.3.1 (Thermo Fisher Scientific).
Phylogenetic analyses

Hemiaustroboletus species produce scarce fruit bodies; from 606 Boletales specimens collected, just eight (1.32%) belonged to this genus. Three materials corresponded to H. vinaceus, four to H. vinaceobrunneus and two were determined as Hemiaustroboletus sp. The three loci of the holotype of H. vinaceus (IBUG-AES334) and one more collection (ITCV-AV524, MEXU-30103) were sequenced; we only recovered ITS and RPB2 loci from a third specimen (IBUG-AES364) (Table 1). The three loci of the holotype of H. vinaceobrunneus (ITCV-AV868, MEXU-30051) and one additional material (ITCV-AV845, MEXU-30052) were sequenced; only the ITS and RPB2 loci were sequenced for a third collection (ITCV-AV1168, MEXU-30053). ITS locus was also sequenced for one Hemiaustroboletus sp. collection (ITCV-AK_3508) (Table 1).

We conducted two sets of phylogenetic analyses, the first one to reconstruct the phylogenetic relationships of Hemiaustroboletus gen. nov. and the second one to complement its taxonomic concept with biogeographic and ecological information. The first analysis used the LSU and RPB2 markers in a concatenated matrix, while the second used ITS in order to leverage GenBank data.

Individual LSU and RPB2 alignments were concatenated into a single matrix (83 taxa, 1335 characters) with GENEIOUS PRIME V.2019.0.4 (Biomatters Ltd). Alignments and concatenation were performed with the MAFFT algorithm (Katoh et al. 2002) using GENEIOUS PRIME V.2019.0.4. Sequences representing the subfamilies Austroboletoideae, Boletoideae and Xerocomoideae came from: 83 LSU sequences, 56 rpb2 sequences, 30 ITS sequences from published works and unpublished sequences available in GenBank (Table 1).

The best-fit evolutionary model was estimated with JMODELTEST 2 (Darriba et al. 2012) using CIPRES SCIENCE GATEWAY V. 3.3 (Miller et al. 2010) for each marker separately. For all three markers, the best model was GTR+G+I. We used the LSU-RPB2 dataset to make evolutionary inferences within Austroboletoideae and the ITS dataset to make biogeographic/ecological inferences for Hemiaustroboletus.

The phylogenetic hypotheses (LSU-RPB2) were constructed with Bayesian Inference (BI) and Maximum Likelihood (ML) on a partitioned alignment with same evolutionary model for both markers. Bayesian posterior probability phylogeny was performed using MrBayes algorithm (Ronquist et al. 2012) using two separate Monte Carlo four chains starting from random trees for 10 million generations each (final standard deviation ± 0.224), trees were sampled every 100 generations. The first 25% of samples were discarded as burn-in. ML analyses were performed using the RAxML algorithm (Stamatakis 2014) with 1000 bootstrap replicates. For both analyses, members of subfamilies Boletoideae and Xerocomoideae were used as outgroup. The second analysis (ITS) was performed with the same parameters including Veloporphyrellus and Austroboletus without outgroup. The resulting phylogenetic trees were edited with FIGTREE V.1.4.3 (Rambaut 2009).

Average intrageneric and intergeneric nucleotide similarities between the genera within Austroboletoideae were obtained separately for RPB2, LSU and ITS alignments.
as follows. For each alignment a nucleotide similarity matrix was computed in GENEIOUS 10.2.6 (Biomatters Ltd). Sequences belonging to genera outside Austroboletoidae were removed and then the mean nucleotide similarity was calculated amongst all pairwise comparisons between sequences of each pair of genera.

| Taxa                  | Voucher          | Country | ITS        | LSU        | RPB2       | Reference                          |
|-----------------------|------------------|---------|------------|------------|------------|------------------------------------|
| *Austroboletus betula*| MEXU:29006       | USA     | MK601736   | MK766298   |            | Kuo and Ortiz-Santana (2020)       |
| *A. garciae*          | 1839, AMV         | Colombia| KF937307   | KF714508   |            | Vasco-Palacios et al. (2014)       |
| *A. amazonicus*       | 1914, AMV         | Colombia| KF937308   | KF714509   |            | Vasco-Palacios et al. (2014)       |
| *A. austrovirens*     | BRI:AQ0795791    | Australia| KP242211   | KP242225   | KP242133   | Fechner et al. (2017)              |
| *A. austrovirens*     | BRI:AQ0794622    | Australia| KP242210   |            |            | Fechner et al. (2017)              |
| *A. austrovirens*     | MEL:2382920a     | Australia| KP242284   | KP242113   |            | Fechner et al. (2017)              |
| *A. austrovirens*     | BRI:AQ0794609    | Australia| KP242226   | KP242131   |            | Fechner et al. (2017)              |
| *A. austrovirens*     | BRI:AQ0794171    | Australia| KP242227   | KP242133   |            | Fechner et al. (2017)              |
| *A. eburneus*         | REH9487          | Australia| JX889668   |            |            | Vasco-Palacios et al. (2014)       |
| *A. dicrytopus*       | HKAS59804        | China   | JX901138   |            |            | Hosen et al. (2013)                |
| *A. fusisporus*       | HKAS75207        | China   | JX889719   | JX889720   |            | Hosen et al. (2013)                |
| *A. fusisporus*       | JXS0351          | China   | MK765810   |            |            | GenBank                            |
| *A. gracilis*         | 112-96           | USA     | DQ534624   |            |            | Binder and Hibbett (2006)          |
| *A. gracilis*         | TM03_434         | Canada  | EU522815   |            |            | Porter et al. (2008)               |
| *A. gracilis var. gracilis* | CFMR BOS-547 | USA     | MK601715   | MK766277   |            | Kuo and Ortiz-Santana (2020)       |
| *A. gracilis var. flavipes* | CFMR BOS-562 | USA     | MK601714   |            |            | Kuo and Ortiz-Santana (2020)       |
| *A. gracilis*         | ACAD11344F       | Canada  | MH465078   |            |            | Young et al. (2019)                |
| *A. gracilis*         | SFC2014823-02    | South Korea| MN794901  |            |            | GenBank                            |
| *A. gracilis*         | NAMA 2017-106    | USA     | MH79242    |            |            | GenBank                            |
| *A. gracilis*         | 310751           | México   | MH167935   |            |            | GenBank                            |
| *A. gracilis*         | CNV35            | USA     | MT345212   |            |            | Victoroff (2020)                   |
| *A. cf. gracilis*     | JLF6600          | USA     | MN174796   |            |            | GenBank                            |
| *A. lacunosus*        | REH9146          | Australia| JX889669   |            |            | Vasco-Palacios et al. (2014)       |
| *A. lacunosus*        | MEL2233764       | Australia| KC552056   |            |            | GenBank                            |
| *A. mucous*           | TH6300           | Guyana  | AY612798   |            |            | Drehmel et al. (2008)              |
| *A. mutabilis*        | BRI:AQ0795793    | Australia| KP242169   | KP242263   | KP242098   | Fechner et al. (2017)              |
| *A. mutabilis*        | BRI:AQ0669270    | Australia| KP242266   | KP242097   |            | Fechner et al. (2017)              |
| *A. mutabilis*        | BRI:AQ0796266    | Australia| KP242262   | KP242099   |            | Fechner et al. (2017)              |
| *A. niveus*           | 312              | New Zealand| DQ534622  |            |            | Binder and Hibbett (2006)          |
| *A. niveus*           | MEL2053830       | Australia| KC552016   | KC552058   |            | Orithara et al. (2016)             |
| *A. novae-zelandiae*  | PDD:72542        | New Zealand| HM060327  |            |            | GenBank                            |
| *A. rarus*            | BRI:AQ0794045    | Australia| KP242197   | KP242236   | KP242086   | Fechner et al. (2017)              |
| *A. rostrupii*        | TH8189           | Guyana  | JN168683   |            |            | Smith et al. (2011)                |
| *Austroboletus* sp.   | BRI:AQ0794156    | Australia| KP242235   | KP242115   |            | GenBank                            |
| *Austroboletus* sp.   | BRI:AQ0794222    | Australia| KP242234   | KP242106   |            | GenBank                            |
| *Austroboletus* sp.   | BRI:AQ0794271    | Australia| KP242259   | KP242102   |            | GenBank                            |
| *Austroboletus* sp.   | HKAS 57756       | China   | KF112383   | KF112764   |            | Wu et al. (2014)                   |
| *Austroboletus* sp.   | HKAS 59624       | China   | KF112485   | KF112765   |            | Wu et al. (2014)                   |
| *Austroboletus* sp.   | HKAS 74743       | China   | KT990527   | KT990367   |            | Wu et al. (2014)                   |
| *Austroboletus* sp.   | PERTH6658407     | Australia| KP242277   | KP242126   |            | GenBank                            |
| *Austroboletus* sp.   | BRI:AQ0794242    | Australia| KP242087   |            |            | GenBank                            |
| *Austroboletus* sp.   | OR0891           | Thailand| MH614753   |            |            | Vadhthanarat et al. (2019)         |
| Taxa                     | Voucher    | Country  | ITS  | LSU   | RPB2     | Reference                      |
|-------------------------|------------|----------|------|-------|----------|--------------------------------|
| Austroboletus sp.       | OTA-FUNNZ| New Zealand |      |       |          | GenBank                        |
| A. subflavidus          | JBSD130771| Dominican Republic | MT580902 | MT590754 | Gelardi et al. (2020)          |
| A. subflavidus          | JBSD130722| Dominican Republic | MT580903 | MT590755 | Gelardi et al. (2020)          |
| A. subflavidus          | CFMR-BZ-3178| Belize            | MK601716 | MK766278 | Kuo and Ortiz-Santana (2020)   |
| A. subflavidus          | KPM-NC-001783| Japan            | JN378518 |         | Oritara et al. (2012)          |
| A. viscidoviridis       | Perth 758862| Australia         | KP242282 | KP242128 | Fechner et al. (2017)          |
| Boletellus indistinctus| HKAS77623 | China            | KT990531 | KT990371 | Wu et al. (2016)               |
| Boletellus sp.          | HKAS80554  | USA              | KT990535 | KT990374 | Wu et al. (2016)               |
| Boletus harrisonii      | MIC: KUO-09071204| USA            | MK601718 | MK766280 | Kuo and Ortiz-Santana (2020)   |
| Boletus sp.             | dd08055| China            | FJ810161 |         |         |
| Boletus sp.             | MHM165| Mexico           | EU569243 |         | Morris et al. (2008)           |
| Boletales sp.           | B0229| Canada           | KY825985 |         |         |
| Fistulinella campinaratae var. scrobiculata | AMV1980| Colombia          | KF714520 |         | Vasco-Palacios et al. (2014)   |
| F. gloeocarpa           | JBSD130769| Dominican Republic | MT580906 | MT590756 | Gelardi et al. (2020)          |
| F. gloeocarpa           | CFMR-B4| Bahamas          | MT580904 |         | Gelardi et al. (2020)          |
| F. gloeocarpa           | CFMR-B10| Bahamas          | MT580905 |         | Gelardi et al. (2020)          |
| F. prunicolor           | REH9502| Australia         | JX889648 | MG212630 | Halling et al. (2012)          |
| F. Olivaceoalba         | HKAS 53432| Vietnam          | MH745969 |         | GenBank                        |
| F. Olivaceoalba         | LE312004| Vietnam          | MH718396 |         | GenBank                        |
| F. ruschi              | CORT:TJ8-329| USA              | MT580907 |         | Gelardi et al. (2020)          |
| F. viscida              | 238 2S| New Zealand       | AF456826 |         | Vasco-Palacios et al. (2014)   |
| F. cinereoaalba         | TH8471| Guyana           | GQ477439 | KT339237 | GenBank                        |
| Hemiaustroboletus vinaceobrunneus | MEXU_30051| Mexico          | MN178797 | MN200222 | MT887617 | This study                     |
| H. vinaceobrunneus      | MEXU_30052| Mexico          | MN178798 | MN200223 | MT887618 | This study                     |
| H. vinaceobrunneus      | MEXU_30053| Mexico          | MN178799 | MT887619 | This study                     |
| H. vinaceobrunneus      | AV524| Paratype         | MN178802 | MN200225 | MT887622 | This study                     |
| H. vinaceus             | AES334| Holotype         | MN178800 | MN200224 | MT887620 | This study                     |
| H. vinaceus             | AES364| Isotype          | MN178801 | MT887621 | This study                     |
| Hemiaustroboletus sp.   | AK3508| Mexico           | MN178803 |         | This study                     |
| Hemsieccinum subglabripes | MIC: KOU-08301402| USA | MK601739 | MK766301 | Kuo and Ortiz-Santana (2020)   |
| Hortiboletus rubellus   | MIC: KOU-06081002| USA | MK601741 | MK766303 | Kuo and Ortiz-Santana (2020)   |
| H. amygdalinus          | HKAS54166| China           | KT990581 | KT990416 | Wu et al. (2016)               |
| Houtzangia chevi        | Tang572| China           | KP136953 | KP136985 | Zhu et al. (2015)              |
| Imleria badia           | MIC: KOU-09110404| USA | MK601743 | MK766305 | Kuo and Ortiz-Santana (2020)   |
| Mucidopus castaneiceps  | HKAS 75045| China          | KT990697 | KT990502 | Wu et al. (2016)               |
| M. castaneiceps         | HKAS50338| China           | KT990555 | KT990391 | Wu et al. (2016)               |
| M. Castaneiceps         | HKAS71039| China           | KT990547 | KT990385 | Wu et al. (2016)               |
| Paresteroomus pseudoobii | HKAS 80480| China          | KP658468 | KP658470 | Wu et al. (2016)               |
| Porphyrillus castaneus  | HKAS52554| China           | KT990697 | KT990502 | Wu et al. (2016)               |
| P. porphyrosporus       | MB97-023| Germany         | DQ534643 | GU187800 | Binder and Hibbett (2006)      |
| P. orientifumosipes     | HKAS53372| China           | KT990629 | KT990461 | Wu et al. (2016)               |
| Tengioboletus sp.       | HKAS 77869| China          | KT990658 | KT990483 | Wu et al. (2016)               |
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| Taxa                      | Voucher      | Country                | ITS        | LSU         | RPB2        | Reference                               |
|---------------------------|--------------|------------------------|------------|-------------|-------------|-----------------------------------------|
| Strobilomyces confusus    | CFMR:DR-3024 | Dominican Republic     | MK601809   | MK766365    | Kuo and Ortiz-Santana (2020)            |
| Tylopilus felleus         | CFMR: BOS-780| USA                    | MK601814   | MK766370    | Kuo and Ortiz-Santana (2020)            |
| T. sordidus               | MICH: KUO-06240801 | USA | MK601815 | MK766371 | Kuo and Ortiz-Santana (2020) |
| Tylopilus sp.             | HKAS 50229   | China                  | KF112423   | KF112734    | Wu et al. (2014)                        |
| Uncultured mycorrhizal    | BOLETE1      | USA                    | AY656925   |             | Walker et al. (2005)                    |
| Uncultured mycorrhizal    | clon N_1     | South Korea            | AB571507   |             | Obase et al. (2012)                     |
| Uncultured Boletus        | isolate: YM490| Japan                  | LC175482   |             | Miyamoto et al. (2018)                   |
| Uncultured Boletus        | Clon ZE2     | China                  | GU391428   |             | Ma et al. (2010)                        |
| Veloporphyrellus alpinus  | KUN:HKAS68301| China                  | JX984537   |             | Li et al. (2014)                        |
| V. pseudovelatus          | KUN: HKAS599444| China              | JX984542   |             | Li et al. (2014)                        |
| V. pseudovelatus          | KUN:HKAS52244| China                  | JX984531   |             | Li et al. (2014)                        |
| V. conicus                | CFMR: BZ1670 | Belize                 | JX984543   |             | Li et al. (2014)                        |
| V. conicus                | CFMR: BZ1705 | Belize                 | JX984544   |             | Li et al. (2014)                        |
| V. pantoreus              | F:Gomez21232 | Costa Rica             | JX984548   |             | Li et al. (2014)                        |
| V. velatus                | KUN:HKAS63668| China                  | JX984546   |             | Li et al. (2014)                        |
| V. aff. velatus           | HKAS 57490   | China                  | KF112380   | KF112733    | Wu et al. (2014)                        |
| V. vulpinus               | LE315544     | Vietnam                | MN511177   | MN511170    | GenBank                                  |
| V. vulpinus               | LE315549     | Vietnam                | MN511180   |             | GenBank                                  |
| V. vulpinus               | LE315546     | Vietnam                | MN511179   |             | GenBank                                  |
| V. vulpinus               | Vietnam      | MN511178               |             |             | GenBank                                  |
| Xerocomellus chrysenteron | HKAS:56494   | China                  | KF112357   | KF112685    | Wu et al. (2014)                        |

**Results**

Phylogenetic analyses of LSU-RPB2 concatenated alignment showed that *Hemiaustroboletus* is a supported monophyletic group, belonging to the Austroboletoideae (BPP = 0.98, MLB = 47%). Additionally, *H. vinaceobrunneus* (BPP = 1, MLB = 100%) and *H. vinaceus* (BPP = 1, MLB = 96%) were supported monophyletic species (Fig. 1). The ITS analyses showed that *Hemiaustroboletus* forms ectomycorrhizae with Fagaceae, particularly *Quercus* and also with *Pinus* in temperate, subtropical and tropical forests. It distributes in North America (Mexico, USA and Canada) and Asia (China, Japan and Korea) (Fig. 2). These analyses also showed that *Austroboletus gracilis* s.l. is a widely-used name mainly applied to designate *Hemiaustroboletus* species.

**Taxonomy**

*Hemiaustroboletus* Ayala-Vásquez, García-Jiménez & Garibay-Orijel, gen. nov.
MycoBank No: 838460

**Diagnosis.** *Hemiaustroboletus* is characterised by small and medium basidiomata with slightly ornamented pileus surface, stipe fibrillose to striated without veil, slightly verrucose or cracked to pitted basidiospores and pileipellis formed by an ixotrichoderm or trichoderm.
Figure 1. Phylogenetic placement of *Hemiaustroboletus* gen. nov. in the Austroboletoideae subfamily (Boletaceae) using LSU and RPB2 markers in a concatenated and partitioned matrix. The tree shows the topology of Bayesian analysis, with both MLB (≥ 70%) and BPP (≥ 0.7) clade support given. New genera and new species are indicated in the rectangles; taxa and/or branches in purple correspond to *Hemiaustroboletus* gen. nov.; remaining Austroboletoideae (blue); Boletoideae (green); Xerocomoideae (mustard). Background colours correspond to subfamilies; grey bars correspond to families.
Hemiaustroboletus, new genus with two new species

Figure 2. Phylogenetic tree of Hemiaustroboletus displaying geographic distribution using voucher and environmental ITS nrDNA sequences. The tree shows the topology of Bayesian analysis, with both MLB (≥ 70%) and BPP (≥ 0.7) clade support given. Taxa and branches in purple correspond to Hemiaustroboletus gen. nov. and those in blue to Veloporphyrellus and Austroboletus.
**Etymology.** From the Latin *hemi* “almost or half”, *Austroboletus* the generic epithet refers to the morphological affinities with this genus.

**Generic type.** *Hemiaustroboletus vinaceobrunneus* Ayala-Vásquez, García-Jiménez & Garibay-Orijel sp. nov.

**Generic Description.** Epigeous, stipitate-pileate basidiomata. *Pileus* reddish-brown, violet-brown, dark violet, reddish-brown, orange-brown, yellow-brown, cinnamon, dry surface, finely velvety, velutinous, rivulose, granular-tomentose, minutely areolate. *Hymenophore* tubular, circular to angular pores, whitish, pink-purple, lilac, magenta-grey, brown-violet to pinkish-brown, with or without change brown when cut. *Context* whitish to pale red. *Stipe* subclavate, tomentose, pruinose, granular furfuraceous, striate surface, longitudinally fibrous, very finely reticulated in tapering towards apex. Whitish basal mycelium. *Basidiospores* ornamented, slightly verrucose, cracked to pits, fusoid, oval-elliptical, cylindrical to subfusoid, oblong, ovoid-oblong. *Cystidia* clavate, sphaeropedunculate, subfusoid. *Pileipellis* an ixotrichoderm or trichoderm; terminal cells cylindrical, fusoid, ventricose-rostrate with or without encrustations in the wall. *Caulocystidia* fusoid, cylindrical to subclavate and tetrasporic caulobasidia.

**Distribution.** Canada, China, Japan, Mexico, South Korea and United States.

**Ecology.** Temperate and subtropical forests, with conifers and broadleaf trees (*Abies* spp., *Quercus* spp., *Pinus* spp.) from 2000 to 3000 m alt.

*Hemiaustroboletus vinaceobrunneus* Ayala-Vásquez, García-Jiménez & Garibay-Orijel, sp. nov.

MycoBank No: 838461

Figs 3, 4, 5B, D

**Diagnosis.** Pileus vinaceous to brown, pores whitish to pinkish at maturity, vinaceous context; longitudinally fribrillose stipe; basidiospores (10) 11–17 (–21) × 4–5 (–7) µm, slightly verrucose to cracked, fusoid to cylindrical; pleurocystidia ventricose-rostrate to fusoid, cheilocystidia sphaeropedunculate.

**Holotype.** Mexico. Oaxaca State, Santa Catarina Ixtepeji Municipality, La Cumbré Town, Peña Prieta site, 17°11’11.34"N, 96°38’00"W (DMS), 2800 m alt., 19 July 2017, Ayala-Vásquez (MEXU-30051; isotype ITCV-AV868).

**Etymology.** The name refers to the colour of the pileus, from the Latin “*vinosus*” vinaceous when young and “*brunneus*” brown when mature.

**Description.** Basidiomata stipitate-pileate. *Pileus* 36–40 mm diameter, convex when young becoming plano-convex, reddish-vinaceous (13B6) when young, orange brown (7C8), reddish-brown (8D8–8E8) to dark brown (7F8) with some ruby tones (12E8) at maturity, dry surface, subtomentose, rivulous to areolate, whitish context, de-curved margin. *Hymenophore* slightly depressed around the stipe to subadnate, pores 1–1.2 mm diameter, circular to subangular, whitish when young, pink to red-whitish (11A3–11A2) at maturity, tubes 6 mm length, of pores concolorous, unchanging when
Hemiaustroboletus, new genus with two new species

Context 4–8 mm thick, whitish, with some shades of pale red, vinaceous at the edge of the pileus and at the apex of the stipe at maturity. **Stipe** 45–65 × 8–10 mm, subclavate, reddish-vinaceous (13B6), orange-brown (7C8) to brown (7D8 -7E8) at the apex and part of the base, orange in the middle area (6B8) to orange-brown (6C8), rest of the base whitish; surface furfuraceous, longitudinally fibrillose. Whitish mycelium. **Chemical reactions** pileus negative in KOH, the context and the hymenophore slightly become pale violet (16A2) and the stipe becomes pale brown (6D4). When ammonium hydroxide (NH₄OH) is applied, the pileus becomes brown-violet (11F8-11F7), the hymenophore and context pale orange (5A2) and the stipe pale violet (16A2).

**Basidiospores** 10–15 (–20) × 4–5 (–7) µm, X = 14.04 × 4.96 µm, std = 3.46 × 0.99 µm, (n = 30, Q = (2.2) 2.4–2.5 (2.8), (holotype); (10–) 11–15 (–21) × 4.5–7 (–8) µm, X = 13.78 × 6.07 µm, std = 3.74 × 1.3 µm, Q = (2.2) 2.4–2.6 (2.8) (paratype MEXU-30052); (10–) 11–15 (–17) × (4–) 4.5–5.5 (–6) µm,

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**Figure 3. Hemiaustroboletus vinaceobrunneus** A, C basidiomata (MEXU-30052 Holotype) B, D pileus (MEXU-30053, MEXU-30051, Isotype) E hymenophore (MEXU-30052 Holotype) F, G context (MEXU-30052 Holotype). Scale bar: 10 mm (A–G).
X = 13.15 × 4 µm, std = 2.62 × 0.64 µm, Q = (2.2) 2.6–2.9 (3) µm, (paratype ITCV-AV1121), cylindrical to subfusoid, slightly verrucose to cracked, brown-orange in KOH, inamyloid in Melzer’s reagent. **Basidia** 30–33 (–49) × 9–11 (–12) µm, clavate, hyaline in KOH, pale yellow in Melzer’s reagent, with granular content, tetrasporic. **Pleurocystidia** 31–45 × 8–11 µm, ventricose to fusoid, some mammillate, hyaline in KOH, yellowish in Melzer’s reagent, thick walled (1–1.5 µm). **Cheilocystidia** 42–70 (–86) × 9–15 (–17) µm, clavate with septa (1–2 µm thick), sphaeropedunculate, some mammillate, hyaline in KOH, yellowish in Melzer’s reagent, thick-walled (1–1.5 µm).

**Figure 4.** *Hemiaustroboletus vinaceobrunneus* (AV845-ITCV, MEXU-30052 Holotype) **A** basidiospores **B** basidia **C** pleurocystidia **D** cheilocystidia **E** pileipellis **F** caulocystidia. Scale bars: 10 µm (**A–F**).
Hemialastoletus, new genus with two new species

Hymenophoral trama boletoid; hyphae cylindrical 3–15 µm diameter, with gelatinous wall some with smooth walls, hyaline to yellowish in KOH and Melzer’s reagent. Pileipellis a trichoderm with terminal cells (22–) 35–75 (–105) × 8–14 (–21) µm, cylindrical to subclavate, hyaline in KOH, yellowish in Melzer’s reagent, embedded in a gelatinous substance and with visible contents in Melzer’s reagent, thick-walled (1–1.5 µm). Caulocystidia 20–64 (–140) × 6–14 (–16) µm, fusoid, cylindrical to sphaeropedunculate with one to two septa, hyaline to yellowish KOH with visible contents visible in Melzer’s reagent. Caulobasidia 25–30 × 7–8 µm tetraspore, concolorous with the caulocystidia. Clamp connections absent.

Habit and habitat. Solitary, in Abies guatemalensis, Pinus pseudostrobus and Quercus laurina mixed forest, putatively associated with Quercus laurina, from 2800 to 3000 m alt.

Known distribution. Currently only known from Oaxaca State, southeast Mexico.

Additional materials examined. Mexico, Oaxaca State, Santa Catarina Ixtepeji Municipality, La Cumbre Town, East of cottage site, 17°11'30"N, 96°38'18"W (DMS), 2903 m alt., 18 July 2017, Ayala-Vásquez (MEXU-30052; ITCV-AV845); Cabeza de Vaca site, 17°11'10"N, 96°38'28"W (DMS), 3038 m alt., 18 July 2017, Ayala-Vásquez (ITCV-AV1121), Cabeza de Vaca site, 15 August 2018, Ayala-Vásquez (MEXU-30053; ITCV-AV1168).

Remarks. Hemialastoletus vinaceobrunneus differs from H. vinaceus by its context with vinaceous tones especially at maturity and a whitish-pink to pale red hymenophore; the stipe is orange-brown; basidiospores are 10–15 (–20) × 4–5 (–7) µm, finely verrucose to cracked, lodged to sphaeropedunculate cheilocystidia, caulocystidia fusoid, cylindrical to sphaeropedunculate with a septum. In contrast, H. vinaceus has a whitish context with slight yellowish-brown tones near the epicutis, has shorter basidiospores (9–) 10–14.4 (–16) × 4–5(–8) µm, cylindrical to clavate cheilocystidia and caulocystidia fusoid or clavate. In the field, the former can be mistaken for Gyroporus purpurinus because of the colours and size of the basidiomata, but G. purpurinus has a hollow stipe (Davoodian and Halling 2013), while H. vinaceobrunneus has a compact context.

Hemiaustroboletus vinaceus Ayala-Vásquez, García-Jiménez & Saldivar, sp. nov.
MycoBank No: 838462
Figs 5A, C, 6, 7

Diagnosis. Pileus dark violet to dark brown, whitish context; hymenophore pink-purple to violet-brown; stipe surface tomentose to longitudinally fibrillose; basidiospores 9–13 × 4–5 µm, surface with cylindrical pits; pleurocystidia and cheilocystidia fusiform-ventricose to lanceolate.

Holotype. Mexico, Jalisco State, Tequila Municipality, Tequila Volcano site, between 11 and 12 km on the road uphill to the antenna station, 20°48'35"N, 103°51'46"W (DMS), 2144 m alt., 18 August 2019, Á.E. Saldivar (IBUG-AES334).

Etymology. The name refers to the colour of the pileus from the Latin “vinosus” vinaceous.
Description. Pileus 35–70 mm in diameter, convex when young, becoming plano-convex with age, dark violet (16F6-16F4), violet-brown (11F5-11F8), orange-brown (5E7), with lighter shades of dark brown (6F5-6F8) lighter towards margin, whole edge, straight, dry surface, finely scamose, slightly areolate at the centre. Hymenophore adnate, slightly depressed, pores 0.5–2 mm in diameter, subangular to angular, pink-purple (14A4), lilac (14B4–14C4), magenta-grey (14C4–14D4), ruby-grey (12C4–12D4), colour unchanging when injured, tubes 7–10 mm, concolorous with the pores. Context 7–12 mm thick, solid, whitish, with slight yellowish-brown tones near the epikutis. Stipe 62–77 × 8–9 mm, central, cylindrical, with wider base, surface with longitudinal striations, whitish at the apex, yellowish-brown (5D5-5E5), orange-brown (5C5) shades in the middle, base with yellowish (5B6) to whitish shades; whitish context, unchanged when cut. Whitish basal mycelium. Odour pleasant. Taste slightly acidic. Chemical reactions: KOH reddish-brown in pileus, brown in hymenophore, slightly pinkish in context, yellowish-brown in stipe. NH₄OH orange with violet tones on pileus, yellow in hymenophore, pale yellow in context, red-orange in stipe.

Figure 5. Basidiospore ornamentation of Hemiaustroboletus revealed by SEM A, C Hemiaustroboletus vinaceus (AV868-ITCV, MEXU-30051, Holotype) B, D Hemiaustroboletus vinaceobrunneus (AV1168-ITCV, MEXU-30053 Isotype).
Hemiaustroboletus, new genus with two new species

Basidiospores 9–13 (–14.5) × 4–5 (–8) µm, X = 12.14 × 5.2 µm, std = 2.08 × 1.36 µm, (n = 35), Q = (1.8) 2.1–2.2 (2.5) (holotype); (10–) 12–14 × 4–5 (–7) µm, X = 11.94 × 5.14 µm, std = 1.60 × 1.13 µm, (n = 35), Q = (2.2) 2.3–2.4 (2.5), (paratype MEXU-30103); (10–) 14–15 (–16) × (4–) 5–6 (–7) µm, X = 14.29 × 5.8 µm, std = 1.69 × 0.76 µm, (n = 40), Q = (2.2) 2.3–2.5 (2.6), (paratype colpos-CP5); subfusiform to cylindrical, slightly rough or dotted, apex rounded to subacute, with suprahilar depression, yellowish. Basidia 27–34 × 7–15.2 µm, claviform, bisporic, tetrasporic, with sterigma 2–4 × 0.5–1 µm, thin-walled, hyaline in KOH, yellow in Melzer's reagent. Pleurocystidia 28–50 × 6.4–11 µm, fusoid-ventricose, slightly lanceolate, with content hyaline in KOH, yellow in Melzer's reagent, with walls 0.5 µm thick. Cheilocystidia 25–61 × 6.4–11 µm, subclavate, hyaline in KOH, yellow in Melzer's reagent, thin-walled. Hymenophoral trama divergent, with central and lateral hyphae tubular, 2–6 µm wide, hyaline in KOH, yellow in Melzer's reagent, thin-walled; septa without clamp connections. Pileipellis a trichoderm with terminal cells 32–92 × 5–11 µm, cylindrical to subclaviform, hyaline in KOH, yellow in Melzer's reagent, thin-walled. Caulocystidia 29–95 × 14–17 (–19) µm, subclaviform to claviform, thin-walled, with yellow visible contents in Melzer's reagent, hyaline in KOH.
Habit and habitat. *Pinus-Quercus* forests and *Quercus* forests, associated with *Q. liebmani* and other *Quercus* spp.

Known distribution. Currently only known from Neovolcanic Axis and Sierra Madre del Sur, Mexico.

Additional material examined. Mexico, Jalisco State, Tequila Municipality, Tequila Volcano site, km 11–12 on the road uphill to the antenna station, 20°48’14”N,
Hemiaustroboletus, new genus with two new species

Remarks. Hemiaustroboletus vinaceus differs from H. vinaceobrunneus due to its dark violet pileus, lilac to violet hymenophore, yellow stipe in the basal area and whitish apex. It has short, perforated basidiospores 9–13 (–14.4) × 4–5 (–8) µm, caulocystidia clavate to fusoid and pileipellis formed by a trichoderm with terminal cell cylindrical or subclavate, thin-walled. In contrast, H. vinaceobrunneus has a pileipellis formed by a trichoderm with encrustations. Hemiaustroboletus vinaceus is easily confused with Austroboletus gracilis sensu Wolfe (1979), because of its macroscopic characteristics and basidiospore ornamentation, but A. gracilis differs by pileus red-brown, brown-orange, having a total or partial reticulum on the stipe surface; longer basidiospores 10–19.5 × 4.5–9 µm, rugulose-punctate, elliptical to ovoid-elliptical. Austroboletus var. gracilis (Peck) Wolfe differs from H. vinaceus by pileus surface dry, finely velvety, when young, sometime rimose, reddish-brown, cinnamon or yellow-brown; stipe surface anastomosing lines, narrow reticulation overall or at least on the upper half; basidiospores 10–17 × 5–8 µm, narrowly ovoid to subelliptical. Austroboletus gracilis var. laevipes is distinguished by the smooth stipe, pileus yellow-ochraceous to yellow-brown, stipe subclavate, striate, finely pruinose, neither ribs nor reticulated surface, pale yellow or yellow-brown, basidiospores 11.2–14 × 5–8µm, oval-elliptical in face view, inequilateral in profile (Bessette et al. 2000). Austroboletus gracilis var. pulcherripes Both & Bessette differs from H. vinaceus by a white hymenium when young, becoming pinkish to pale cocoa at maturity; stipe clavate, surface dry, coarsely reticulated on the upper two-thirds, reticulated, finely tomentose; basidiospores 13–19 × 5–8 µm, smooth to rugose-punctate, ovoid-elliptical, narrowly ovoid, inequilateral profile.

Discussion

According the phylogenetic analysis, our collections are nested within the Austroboletoideae close to Veloporphyrellus. Recognising the Hemiaustroboletus genus contributes to solving the systematics within Austroboletoideae since previous works have shown that Austroboletus and Veloporphyrellus, as currently morphologically circumscribed, are polyphyletic (Wu et al. 2016; Gelardi et al. 2020; Kuo and Ortiz-Santana 2020). For example, Wu et al. (2016) found two clades of Austroboletus, Austroboletus. s.s. and a second clade where Austroboletus gracilis s.l. (strain, 112/96) is nested with Veloporphyrellus gracilioides, this species being separated from the Veloporphyrellus s.s. clade. Gelardi et al. (2020) also recovered Austroboletus as polyphyletic with Austroboletus s.s. containing most of the species and other samples divided into four more clades. Particularly, in their analyses, most A. gracilis samples nested close to Veloporphyrellus; this is the clade we are erecting now as Hemiaustroboletus.
Our analyses show that *Hemiaustroboletus* is related to *Veloporphyrellus* (Fig. 1). This is supported by previous analyses (Gelardi et al. 2020; Kuo and Ortiz-Santana, 2020); indeed, they differ in several morphological characteristics. *Veloporphyrellus* has a veil which often embraces the apex of the stipe in younger basidiomata; hymenophoral surface white when young becoming pinkish to pink when mature; basidiospores smooth subfusiform to oblong. In contrast, *Hemiaustroboletus* has furfuraceous, tomentose to minutely areolate pileus surface; whitish, pink-purplish, lilac, magenta-grey to brown-violet hymenophoral surface; and slightly verrucose, cracked to pitted ornamented basidiospores (Table 2). Even while the phylogenetic relations between both genera are not statistically supported, nucleotide similarity demonstrated that

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**Table 2.** Comparative table of Austroboletoidae genera, based on Wolfe (1979) and Wu et al. (2016).

| Genera            | Basidiomata                           | Basidiospores               | Cystidia               | Pileipellis                                      |
|-------------------|---------------------------------------|-----------------------------|------------------------|-------------------------------------------------|
| *Austroboletus*   | Pileus margin which embraces the stipe when young, Stipe surface distinctly reticulate, alveolate-lacunose | Ornamented, elongate to amygdaliform, with warths, reticulate ridges or shallow to irregularly furrowed pits | Cylindrical, clavate, fusoid | Trichoderm with filamentous interwoven hyphae, sometimes strongly gelatinous |
| *Fistulinella*    | Stipitate-pileate to occasionally sequestrate, with or without veil, usually viscid to strongly glutinous pileus | Smooth, elongate fusoid, inamyloid to dextrinoid | Fusiform to ventricose fusiform or lageniform | Trichoderm, ixotrichoderm or isocutis |
| *Hemiaustroboletus* | Pileus surface furfuraceous, tomentose, minutely areolate, stipe surface longitudinally fibrillose to striate | Slightly verrucose, cracked to pitted | Clavate, Rope-dunculate, subfusoid | Ixotrichoderm or trichoderm, terminal cells cylindrical, fusoid, ventricose-rostrate |
| *Mucilopilus*     | Viscid pileus, stipe without colour change, white to pinkish or pink hymenophore | Smooth, subfusiform to oblong | Fusoid, ventricose to subfusciform | Ixotrichoderm, composed of strongly gelatinous filamentous hyphae |
| *Veloporphyrellus* | Pileus margin with distinct membranous veil or appendiculate, stipe nearly glabrous or fibrillose | Smooth, subfusiform to oblong | Subfusciform to ventricose | Trichoderm composed of filamentous interwoven hyphae |

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**Table 3.** Average nucleotide similarity amongst genera of Austroboletoidae.

| Genus 1          | Genus 2          | Average nucleotide similarity (ITS) % | Average nucleotide similarity (LSU) % | Average nucleotide similarity (RPB2) % |
|------------------|------------------|---------------------------------------|---------------------------------------|----------------------------------------|
| *Hemiaustroboletus* | *Hemiaustroboletus* | 95.49                                 | 98.93                                 | 97.96                                  |
| *Hemiaustroboletus* | *Mucilopilus*     | 92.51                                 | 91.25                                 |                                        |
| *Hemiaustroboletus* | *Austroboletus*   | 71.27                                 | 85.94                                 | 87.75                                  |
| *Hemiaustroboletus* | *Fistulinella*    | 88.58                                 | 89.76                                 |                                        |
| *Hemiaustroboletus* | *Veloporphyrellus* | 74.75                                 | 94.01                                 | 93.45                                  |
| *Veloporphyrellus* | *Veloporphyrellus* | 95.49                                 | 100                                   |                                        |
| *Veloporphyrellus* | *Austroboletus*   | 85.64                                 | 86.66                                 |                                        |
| *Veloporphyrellus* | *Mucilopilus*     | 91.45                                 | 89.73                                 |                                        |
| *Veloporphyrellus* | *Fistulinella*    | 88.06                                 | 89.5                                  |                                        |
| *Fistulinella*    | *Fistulinella*    | 90.48                                 | 89.5                                  |                                        |
| *Fistulinella*    | *Mucilopilus*     | 87.61                                 | 89.5                                  |                                        |
| *Fistulinella*    | *Austroboletus*   | 83.03                                 | 86.87                                 |                                        |
| *Austroboletus*   | *Austroboletus*   | 86                                    | 92.06                                 |                                        |
| *Austroboletus*   | *Mucilopilus*     | 85.05                                 | 87.88                                 |                                        |
| *Mucilopilus*     | *Mucilopilus*     | 98.5                                  | 99.4                                  |                                        |
they are the closest genera within Austroboletidae. The overall nucleotide similarity between genera in Austroboletidae in RPB2 is 89.23%, in LSU it is 88.19%, and in ITS it is 72.55%. Between Hemiaustroboletus and Veloporphyrellus, the average nucleotide similarity is 93.45% in RPB2, 94.01% in LSU and 74.75 in ITS (Table 3). These amounts of variation in the three markers also support the conclusion of recognising both genera.

Hemiaustroboletus gen. nov. accomplishes the guidelines for the establishment of new genera proposed by Vellinga et al. (2015). It is a monophyletic group supported by morphological data and phylogenetic analyses (BPP = 0.98) (Fig. 1). When Hemiaustroboletus is recognised, the related clade Austroboletus s.s. (the clade including A. dictyotus, the genus type) becomes monophyletic. Additionally, the DNA sequence sampling is broad in taxonomic and geographic terms and uses ribosomal markers and protein coding genes. Indeed, holotypes for both species described are represented with the three markers included in the phylogenetic analyses.

Hemiaustroboletus is proposed as a new genus with two species H. vinaceobrunneus and H. vinaceus, including several of the revised material being previously identified as A. gracilis by Singer et al. (1991), Ayala-Vásquez et al. (2018) and Saldivar et al. (2021). The genus has at least one more known clade (Fig. 1) containing samples originally identified as A. gracilis (TM03-434) from Canada, A. gracilis var. gracilis (CFMR BOS-547) and A. gracilis var. flavipes (CFMR BOS-562) from USA. As found in our analyses and previous works (Wu et al. 2016; Gelardi et al. 2020; Kuo and Ortiz-Santana 2020), A. gracilis is a name widely applied to several clades. In our analysis, the sample A. gracilis 112/96 belongs to Austroboletus (maybe because it lacks RPB2 locus), while the rest of the sequences with this epithet belong to Hemiaustroboletus. As this species is polyphyletic, establishing the true identity of A. gracilis s.s. requires the sequencing of its type specimen, a task beyond the objectives of this study.

Hemiaustroboletus differs morphologically from Austroboletus sect. Austroboletus sensu Wu et al. (2016) (Austroboletus s.s. in this study) because the species of the latter have clearly reticulated to costate stipe, elongate, fusoid or amygdaliform basidiospores with warts, reticulate ridges, irregularly furrowed pits or shallow ornamentation and a subreptent to trichoderm pileipellis, composed of filamentous interwoven hyphae, sometimes strongly gelatinous. In contrast, Hemiaustroboletus is characterised by a subclavate, tomentose, pruinose, granular furfuraceous, striate surface, longitudinally fibrous, very finely reticulated stipe, oval-elliptical, cylindrical to subfusoid, oblong, ovoid-oblong basidiospores with slightly verrucose, cracked to pitted surface, its pileipellis is an ixotrichoderm or trichoderm with terminal cells cylindrical, fusoid or ventricose-rostrate with or without incrustations in the wall.

Finally, A. gracilis, described by Ortiz-Santana et al. (2007) from Central America, is probably Hemiaustroboletus vinaceus or a close species, because they match the description presented here. Further analysis of these collections and others, labelled as A. gracilis in subtropical regions of Central America and eastern Asia, are needed to fully understand the diversity and distribution of Hemiaustroboletus.
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