A new species of *Amanita* sect. *Lepidella*, *A. heishidingensis*, is described based on both morphological and molecular evidences. It was compared with similar species and illustrated with line drawings and photographs. This species was found in Heishiding National Nature Reserve, Guangdong Province, South China.

**Keywords** Amanitaceae · Morphology · Phylogenetic analyses · Taxonomy

**Introduction**

The genus *Amanita* Pers. in China has been studied extensively in the last twenty years or so and over eighty species including several lethal ones caused many troubles and losses have been reported from China (Yang 1994, 1997, 2005; Zhang et al. 2010; Chen et al. 2014; Deng et al. 2014). Since November 2008, the first author has collected mushrooms in Heishiding National Nature Reserve (111°49′09″–111°55′01″E, 23°25′15″–23°30′02″N), Guangdong Province, China, and found more than 30 taxa of *Amanita* there. One of them, found in forests dominated by fagaceous trees, is described and illustrated herein as a new species.

**Materials and methods**

**Morphology**

The description of the new species is based on morphological studies of fresh material and exsiccatata. The photographs (Fig. 1) depict the holotype. Color code follows Kornerup and Wanscher (1978). Tissues were mounted in 5 % KOH and 1 % Congo red for microscopic examination and making of line drawings. Spores were mounted in Melzer’s reagent to test for amyloidity. The abbreviation (n/m/p) means n basidiospores measured from m basidiomata of p collections. Dimensions for basidiospores are given using a range notation of the form (a) b-c (d). The range b-c contains a minimum of 90 % of the measured values. Extreme values (a and d) are given in parentheses. Q = length/width ratio of a basidiospore in side view; Q̄ = average Q of all basidiospores measured ± sample standard deviation. The holotype collection of the new species was deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS), Kunming, China. Additional Collections were deposited in HKAS or in the Tottori Mycological Institute (TMI), Tottori, Japan.

**DNA extraction, PCR amplification and DNA sequencing**

Genomic DNA was extracted from fruiting bodies dried in silica gel according the modified CTAB protocol (Doyle and Doyle 1987). The large nuclear ribosomal RNA subunit of the nuclear ribosomal RNA (nrLSU) was amplified with primers LROR and LR5 (Vilgalys and Hester 1990). The PCR amplification followed those in Zeng et al. (2013) and references therein. The PCR products were then purified using a Gel Extraction and PCR Purification Combo Kit (Spin-colum) (Biotek, Beijing, China). Sequencing was performed with
an ABI 3730 DNA analyzer (Applied Biosystem, Foster City, CA, USA) using the same primer pairs used for the PCR.

Sequence alignment and phylogenetic analyses

Four nrLSU sequences of the new species and one nrLSU sequences of *Amanita japonica* extracted in this study were compared with 26 nrLSU sequences retrieved from GenBank (NCBI; http://blast.ncbi.nlm.nih.gov/). These sequences were aligned with MUSCLE v3.8.31 (Edgar 2004), and then manually optimised on BioEdit v7.0.5 (Hall 1999). Gaps were treated as missing data. The maximum likelihood analysis (ML) conducted on RAxML v7.2.6 (Stamatakis 2006) and Bayesian inference (BI) executed on MrBayes V3.2 (Ronquist and Huelsenbeck 2003) were implemented for the phylogenetic analyses. The optimal substitution model for ML and BI analyses was determined using the Akaike Information Criterion (AIC) as implemented in MrModeltest v2.3 (Nylander 2004). The statistical branch support values were evaluated using rapid non-parametric bootstrapping with 1000 replicates in RAxML and using posterior probabilities from BI analysis. The MrBayes analysis was automatically terminated using the stoprul and stopval commands when the standard deviation of the split frequencies fell below 0.01. Chains convergence was further verified using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to confirm sufficiently large ESS values (>200). Subsequently, the sampled trees were summarized after omitting the first 25% of trees as burn-in using the ‘sump’ and ‘sumt’ command implemented in MrBayes.
Results

Morphological analyses

Three collections with over thirty basidiomata of the new species were morphologically examined. For comparison, four collections of *Amanita japonica*, collected from Japan were examined. Our data indicated that the new species is morphologically different from *A. japonica* (see discussion below).

Phylogenetic analyses

A total of 34 nrLSU sequences were used in the phylogenetic analysis (Table 1). The phylogenetic tree inferred from the ML analysis was consistent with that obtained from the Bayesian

| Species               | Voucher    | Locality               | GenBank accession numbers | nrLSU   | ITS   |
|-----------------------|------------|------------------------|---------------------------|---------|-------|
| A. atkinsonia         | RET 301–1  | Connecticut, USA       | HQ539670                  | –       |       |
| A. abrupta            | BW_HP_101  | Massachusetts USA       | HQ539660                  | –       |       |
| A. conicobulb         | PSC 1368   | South Australia, Australia | HQ539683                  | –       |       |
| A. cokeri             | BW_STF 090506–19 | Massachusetts USA | HQ539682                  | –       |       |
| A. effusa             | PSC 2007   | South Australia, Australia | HQ539689                  | –       |       |
| A. eriophora          | RET 350–4  | Angkor, Cambodia        | HQ539672                  | –       |       |
| A. farinacea          | PSC 2529   | South Australia, Australia | HQ539692                  | –       |       |
| A. heishidingensis    | HKAS 76122 (holotype) | Guangdong, China | KC429045  | KC429051  |
| A. heishidingensis    | HKAS 81481  | Guangdong, China        | KJ922991                  | KJ922998|
| A. heishidingensis    | HKAS 81484  | Guangdong, China        | KJ922993                  | KJ922999|
| A. heishidingensis    | HKAS 82280  | Guangdong, China        | –                         | KJ922995|
| A. heishidingensis    | HKAS 82281  | Guangdong, China        | –                         | KJ922996|
| A. heishidingensis    | HKAS 82282  | Guangdong, China        | KJ922992                  | KJ922997|
| A. japonica           | TMI 26147 (duplicate HKAS 82328) | Tottori, Japan | KJ922990                  | KJ922994|
| A. kotohiraensis      | MHHNU 7112  | Hunan, China            | FJ011682                  | –       |       |
| A. longipes           | RET 360–1  | New Jersey, USA         | HQ539704                  | –       |       |
| A. macrocarp          | 31939 L    | Guangdong, China        | KC408378                  | –       |       |
| A. manginiana         | HKAS 26146  | Yunnan, China           | AF024463                  | –       |       |
| A. manginiana         | HKAS 56933  | Yunnan, China           | KJ466438                  | –       |       |
| A. modesta            | HKAS 75405  | Guangdong, China        | KJ466439                  | –       |       |
| A. modesta            | HKAS 79688  | Guangdong, China        | KJ466440                  | –       |       |
| A. onusta             | RET 297–3  | New Jersey, USA         | HQ539718                  | –       |       |
| A. oberwinklerana     | MHHNU 7113  | Hunan, China            | FJ011683                  | –       |       |
| A. oberwinklerana     | HKAS 77330  | Hainan, China           | KJ466441                  | –       |       |
| A. ochrophylla         | PSC 1127   | South Australia, Australia | HQ539717                  | –       |       |
| A. polybryonmas       | RET 159–8  | Maryland, USA           | HQ539723                  | –       |       |
| A. pseudoporphyria     | HKAS 26143  | Yunnan, China           | AF024471                  | –       |       |
| A. pseudoporphyria     | HKAS 56984  | Yunnan, China           | KC429047                  | –       |       |
| A. rhoadsi            | DD97/13    | North Carolina, USA     | AF097391                  | –       |       |
| A. rhopalopus          | BW_RET 386–3 | West Virginia, USA     | HQ539733                  | –       |       |
| A. smithiana          | RET 382–6  | California, USA         | HQ539740                  | –       |       |
| A. solitariiformis     | DD 97/12   | North Carolina, USA     | AF097390                  | –       |       |
| A. sublutea            | PSC 2401   | South Australia, Australia | HQ539749                  | –       |       |
| A. vestita             | HKAS 77277  | Hainan, China           | KC429044                  | –       |       |
| A. virgineoides        | HKAS 18394  | Sichuan, China          | AF024484                  | –       |       |
| A. virgineoides        | HKAS 77278  | Hainan, China           | KC429043                  | –       |       |

*Sequences produced in this study in bold*
inference, and thus only the ML tree was shown in Fig. 3. Our molecular phylogenetic analysis robustly supported that the new taxon was a separate species and was related to *A. japonica* with moderately statistical support.

**Taxonomy**

*Amania heishidingensis* Fang Li & Qing Cai, sp. nov. (Figs. 1–2).

**Etymology**: named for holotype locality.

**Holotypus**: China, Guangdong Province, Fengkai County, Heishiding National Nature Reserve, at 111°49′09″–111°55′01″E, 23°25′15″–23°30′02″N, alt. 190 m, 29 Feb 2012, Fang Li 33 (HKAS 76122); nLSU and ITS sequences generated from the holotype are KC429045 and KC429051, respectively.

**Basidiomata** (Fig. 1) medium-sized to large. Pileus (4.5) 7–15.5 cm in diam., globose at first, hemispherical when expanding, later convex to plano-convex to flat with slightly depressed centre, with appendiculate smooth margin, white (2A1) when young, then dirty white (2A2-3A2), dingy cream to pale silvery grey or pale brownish grey (a little lighter than 5C3) especially near centre with age, subfretted when young and becoming smooth later, viscid, usually decorated with big, up to 6 mm wide and 6 mm high, grey to brownish grey (5B2, 5C3, 5D3), acute-pyramidal, truncate-pyramidal to verrucose, subfibrillose to felted adnate, subdetersile to detersile volval remnant warts, towards margin these sometimes passing into small, scattered flocks, flat fibrillose scales with somewhat raised amorphous tips or ridges; sometimes warts washed away by rains, leaving yellowish white (4A2–3) prints or scars on pileus (Fig. 1-c); context white, firm, unchanging.

**Lamellae** free, cream (3A2–4A2), rather crowded to moderately crowded, rather broad, up to 18 mm wide, subventricose

![Fig. 2 Microscopic characters of Amanita heishidingensis (a-c) and A. japonica (d-e). a. Hymenium and subhymenium (HKAS 81458). b. Basidiospores (HKAS 81458). c. Volval remnant on pileus in longitudinal section (HKAS 81458). d. Basidiospores (TMI 1323). e. Basidiospores (TMI 26147).](image)
to ventricose, often with smooth edge, occasionally with slightly decurrent edge. Lamellulae attenuate to rounded-truncate, broad, plentiful, in 2 to 3 ranks. Stipe (6) 8–20.5 × (0.6) 1.1–2.5 cm, subcylindric or slightly attenuate upwards, with apex slightly expanded, surface white to cream (2A1–3A2), with silks lustre, upper part often covered with white (2A1–2) floccose to farinose squamules, lower part often covered with pale yellow to pale brownish grey (3A2–3, SB2) floccose recurring squamules; context white to pale cream, solid to fistulose; bulb (1.7) 3–7 × (1.5) 2–4.7 cm, napiform, subclavate to ventricose, round based, covered with pale yellow, pale yellowish grey to brownish grey (3A2, 3B2–3, 5B2–5C2) subfelted to subtomentose volval remnants, often exhibiting some longitudinal splitting, with recurving scales 5–15 μm wide, conspicuous, branching. Stipe trama longitudinally acrophysalidic; filamentous hyphae 4–10 μm wide, abundant, septa often clamped; acrophysalides 140–230 × 20–25 μm, dominant; vascular hyphae 8–33 μm wide, locally conspicuous to abundant. Annulus composed of 2–9 μm wide, colorless, hyaline, fairly abundant to abundant filamentous hyphae, with septa often clamped; inflated cells very abundant, sphaeroedupunctate, pyriform to subglobose, 20–50 × 17–45 μm, usually single, colorless and with walls thin and hyaline; vascular hyphae locally conspicuous, sinuous, 3–10 μm wide.

*Habitat and distribution:* Gregarious or scattered on soil in forests dominated by *Fagaceae*, at 190–600 m alt. Presently known only from Heishiding National Nature Reserve, Guangdong Province, China.

**Additional specimens examined:** China, Guangdong Province, Heishiding National Nature Reserve, alt. 190 m, 18 February 2014, Fang Li 1580 (HKAS 81458–81480, HKAS 82279–82283); the same place, alt. 600 m, 5 March 2014, Fang Li 1581 (HKAS 81481–81484, HKAS 82284–82292).

**Specimens of Amanita japonica examined:** Japan, Tottori Prefecture, Katsurami, 01 October 2007, Yukihiro Nishio TMI 26147 (duplicate HKAS 82328); same location, 26 July 2011, Yukihiro Nishio TMI 26146 (duplicate HKAS 82329); Japan, Shiga Prefecture, Otsu City, Ishiyama-Terabe, 17 August 1973, Z. Sugiyama & E. Nagasawa TMI 1322 (duplicate HKAS 82330); Japan, Shiga Prefecture, Otsu City, Ishiyama-senjo, 14 September 1973, E. Nagasawa TMI 1323 (duplicate HKAS 82331).

**Discussion**

*Amanita heishidingensis,* a member of *Amanita* sect. *Lepiella* (Bas 1969), is characterized by its medium-sized to large basidioma with a dirty white to whitish viscid pileus covered with grey to brownish grey pyramidal to verrucose volval remnants, light cream lamellae, a whitish stipe covered with white to pale brownish grey floccose to farinose recurring squamules, usually with a big napiform, subclavate to ventricose bulb covered with pale yellow to pale brownish...
grey subfelted to subomentose volval remnants, a fugacious annulus, ellipsoid amyloid spores, and abundant clamps in all tissues.

*Amanita heishidingensis* keyed out in *Amanita* subsect. *Solitaria* Bas stirps *Solitaria* (Bas 1969). It resembles *A. japonica* Hongo ex Bas. *Amanita japonica*, originally described from Japan (Bas 1969; Imazeki and Hongo 1987), resembles *A. heishidingensis* by its similarly shaped basidioma, the greyish volval warts on the cap, and the similar basidiospores. But the main morphological and anatomical differences in basidioma can distinguish *A. japonica* from *A. heishidingensis*. On the morphological features, *A. japonica* generally has a medium-sized basidioma (cap 55–80 mm wide, stipe 80–170×7–15 mm); a small fusiform-rooting to subclavate bulb (up to 25 mm wide); a dry, moderately dark grey to pale buffy grey, felted-subflocculose pileus; a non-gelatinized pileipellis; small (about 2 mm wide and 1.5 mm high) subpyramidal warts adnate on the mature pileus; subflocculose edged lamellae (Bas 1969).

While *A. heishidingensis* usually has a big sized basidioma (cap 70–155 mm wide, stipe 80–205×11–25 mm) with a big napiform to subclavate bulb (20–47 mm wide), with the base always round, not rooting; a viscid, white to whitish (without greyish tint when young), glabrous pileus with the pileipellis always strongly gelatinized; the warts on the pileus are much larger (up to 6 mm in wide and high); the pileipellis under the warts is often strongly gelatinized-making the warts detestile and easily washed off in rainy weather; lamellar edge is always smooth. On the anatomical features, the pileipellis of *A. japonica* is difficult to delimited from the greyish volval remnants over it, not or very slightly gelatinized, while *A. heishidingensis* has a clear delimited, gelatinized to strongly gelatinized pileipellis; hyphae and inflated cells in the warts of *A. japonica* are much more darker colored than those in *A. heishidingensis*; the annulus of *A. japonica* has abundant to nearly abundant hyphae, with inflated cells mostly clavate shaped, and with yellowish vacuolar pigmentation in both hyphae and inflated cells, while *A. heishidingensis* has fewer hyphae in its annulus, with inflated cells mostly globose, subglobose to sphaeropedunculate, and has rare vacuolar

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**Fig. 3** Phylogenetic tree inferred from maximum likelihood (ML) analysis based on nrLSU sequences of *Amanita* sect. *Lepidella*. ML bootstrap over 50% and Bayesian posterior probabilities over 0.80 were reported.
pigments in both hyphae and inflated cells. Furthermore, *A. heishidingensis* grows in the early spring, when the temperature is average 5–12 °C, never over 20 °C, and can’t be found in late spring, summer or autumn; while *A. japonica* grows in late summer to early autumn, when the weather is much warmer. Our molecular phylogenetic analysis also suggested that *A. heishidingensis* and *A. japonica* are different species (Fig. 3).

*Amanita cokeri* (E.J. Gilb. & Kühner) E. J. Gilb., originally described from North America (Bas 1969), resembles *A. heishidingensis* in similar shape of basidioma and white pileus covered with rather larger white to brownish pyramidal warts. But *A. cokeri* can easily be differentiated from *A. heishidingensis* by its distinctive ample double ring, and somewhat larger basidiospores (11–13.5×7–9 μm) (Bas 1969). Additionally, warts of *A. heishidingensis* are never white at any stage of development, and always having a greyish tint; moreover the bases of warts are not radially fibrilllose; and the gills are never pinkish at any stage of development. The molecular phylogenetic analysis also indicated that the two species are distinct.

*Amanita miculifera* Bas & Hatanaka originally described from Japan (Bas and Hatanaka 1984), resembles *A. heishidingensis* in its similar sized and shaped basidioma, whitish-greyish (between 1A1 and 1B1) pileus covered with moderately grey subpyramidal warts, and similar sized and shaped basidiospores; but its pileus is conical with obtuse apex to plano-conical; its lamellae are rather narrow; its bulb is strongly rooting; its warts are much smaller and the arrangement of the inflated cells in its volval warts is quite irregular (Bas and Hatanaka 1984).

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