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Biological characteristics of the *Pleurotus* cultivars in southwestern Viet Nam

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Abstract The biological characteristics of *Pleurotus* cultivars in the southwestern region of Viet Nam were conducted by morphology, molecular phylogeny, spawn running and biological efficiency. Total 10 *Pleurotus* cultivars were belonged to 5 morphotypes as following: 5 cultivars GTG1, GBT1, GBT2, GVL1, GVL3 belonged to phoenix type, 2 cultivars WSG1, WVL1 belonged to oyster type, a cultivar YVL1 belonged to golden oyster type, a cultivar PVL1 belonged to pink oyster type, and a cultivar DgSG1 belonged to blue oyster type. By morphological and phylogenetic analysis, all 5 phoenix cultivars were identified to *P. pulmonarius*, both 2 oyster cultivars and a blue oyster cultivar were *P. ostreatus*, a golden oyster cultivar was *P. citrinopileatus*, and a pink oyster cultivar was *P. djamor*. By the model of 1.2 kg rubber tree sawdust in nylon bag, the spawn running of golden oyster cultivar (12.47±1.20 mm/day) and pink oyster cultivar (11.29±1.00 mm/day) was higher than all phoenix cultivars, and the spawn running of blue oyster cultivar was lowest (6.57±0.76 mm/day). The biological efficiency in conventional production of oyster cultivars was highest (37.58±5.00% of WSG1 and 34.84±3.90 of WVL1), opposite to blue oyster cultivar was lowest (12.93±3.93%). This the first study described the macro and micro morphological characteristic of commercial mushroom cultivars in Viet Nam. Combining the molecular and morphological identification, all these cultivars could be used as the parent in crossing due to their putative strain and standard characteristic of species. The results of cultivation under a standardized conditions should be a value data to screen and breed the new cultivar.

Keywords: *Pleurotus*, Spawn running, Biological efficiency, Identification, Cultivars

Introduction

Pleurotus spp. was the popular cultivated mushroom in the world as the second edible mushroom crop in the world with 19% of the world edible

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mushroom production (Royse *et al.*, 2017). Among suppliers and demanders, Asian countries are dominant in the world, mainly in China, Japan, RO Korea, Taiwan, Viet Nam and India. In Viet Nam, the southwestern region was one of the main suppliers with ca. 1500 tons/year production, a quarter of the total *Plerotus* spp. production in Viet Nam (Nguyen and Pham, 2013).

The numerous cultivars of *Pleurotus* spp. and alike were cultivated in the world (Ritota and Manzi, 2019). Among them, cultivars belonged to *P. pulmonarius*, *P. ostreatus*, *P. cystidiosus*, *P. citrinopileatus*, and *P. djamor* were popular in tropical countries. However, the identification of these cultivars was complicated due to the species complex. Oyster mushroom, *P. ostreatus s. l.*, was a complicated complex within at least 7 species inside such as *P. pulmonarius*, *P. ostreatus*, *P. abieticola*, *P. eryngii*, *P.* cf. *floridanus*, *P. placentodes*, and *P. tuoliensis* (Li *et al.*, 2017). Besides, the interspecies-cross abilities of *Pleurotus* spp. (Rajarathnam *et al.*, 1987) and the complexes of identification between several species (Buchanan, 1993) were barriers to recognize the origin of commercial cultivars in market. A study of 91 cultivars in China showed that the hybrid cultivars and the domestical name of alien cultivars also made the identification become more difficult (Li *et al.*, 2019). Comparing the inter-countries *P. cystidiosus* strains between Viet Nam and Japan also were not separated due to the inter-species hybrid cultivars (Truong *et al.*, 2008a).

On the other hands, the physiological characteristics including spawn running and biological efficiency (BE) were important to understand the vitality and agricultural potential of each *Pleurotus* cultivar. The most difficulty was the standardization of cultivation technology for each region or country, especially substrate and cultivated conditions. The only standardization example was recommended by The Plant Variety Protection Office at Ministry of Agriculture, Forestry and Fisheries of Japan. The lack of standardization in cultivation technology of *Pleurotus* spp. leaded to be impossible to comparing among different mushroom strains or cultivars.

In Viet Nam, studies about the characteristics of *Pleurotus* cultivars is scattered, especially in Southern Viet Nam. Most of studies were focused on cultivated technology in high land areas (Tay Nguyen region) (Truong *et al.*, 2008a, Truong *et al.*, 2008b, Nguyen *et al.*, 2019) with the subtropical weather condition, separated to the tropical weather in the other regions of Viet Nam. To understanding the *Pleurotus* mushroom cultivation in Viet Nam, this study tried to collect several cultivars in southwestern Viet Nam as a model. All collected cultivars were clarified the species difference in by both morphological and molecular analysis, as well as their physiological characteristics by mycelium growth, spawn running and biological efficiency.

Materials and methods

Mushroom cultivars

10 *Pleurotus* cultivars were collected from several mushroom farms in Ho Chi Minh City, Tien Giang Province, Vinh Long Province, Ben Tre Province, Viet Nam. The detail was showed in Table 1. All cultivars were checked the contamination by PDA plates (Potato Dextrose Agar) consisted of 4 g potato extract (Formedium, Norfolk, UK), 20 g Glucose (Himedia, Mumbai, India), 15 g agar powder (Himedia, Mumbai, India), and filled up to 1000 ml distilled water. All were also maintained in PDA slant agar in 20^{o}C under dark condition.

Table 1. Detail of 10 *Pleurotus* cultivars collected in southwestern Viet Nam

No.	Types	Cultivars	Collected location
1	Oyster	WVL1	Vinh Long Province
2		WSG1	Ho Chi Minh City
3	Phoenix	GTG1	Tien Giang Province
4		GVL1	Vinh Long Province
5		GVL3	Vinh Long Province
6		GBT1	Ben Tre Province
7		GBT2	Ben Tre Province
8	Golden oyster	YVL1	Vinh Long Province
9	Pink oyster	PVL1	Vinh Long Province
10	Blue oyster	DgSG1	Ho Chi Minh City

Cultivation

The grain spawn was prepared by mixing 200 g of boiled paddy rice (The OM 18 rice cultivars, Cuu Long Delta Rice Research Institute, Viet Nam), 4 g rice bran (Doanh Phu Ltd., Ho Chi Minh City, Viet Nam) and 2 g of food grade gypsum powder (Wah Loong Go Ltd., Hong Kong, China); packaged into a nylon bag with stopper; sterilized by autoclave at 121^{o}C in 45 minutes; and then let them to be as room temperature in at least 12 hours.

The sawdust spawns were consisted of 88.7% sawdust (rubber tree sawdust, moister lower than 20%, and particle diameter from 0.5 mm to 4.0 mm), 1% of $CaCO_3$ (Himedia, Mumbai, India), 0.5% Urea (Himedia, Mumbai, India) and 0.2% $MgSO_4.7H_2O$ (Himedia, Mumbai, India). Each spawn was packaged by ca. 1200 g/nylon bag (175 mm – 180 mm high, 105 mm – 110 mm diameter) with plastic stopper; sterilized by steam water at 95^{o}C in 4 hours; and then let them to be as room temperature in at least 12 hours.

Each grain spawn was inoculated by three 6 mm-diameter circle pieces at the edge of the cultivar mycelial colonies. All grain spawns were incubated at 25^{o}C, under dak condition. After the mycelia cover more than 90% surface of

grain spawn, a spoon of grain spawn (3-5 g) was inoculated to a sawdust spawn. All sawdust spawns were incubated at room temperature ($27^oC – 33^oC$), humidity ca. 70%, under dark condition until the mycelia cover almost the surface of spawn. Then, all sawdust spawns were stimulated for fruiting mushrooms by opening the stopper at room temperature ($27^oC – 33^oC$), humidity 80% – 90%, light intensity 500 – 1000 lux, and light cycle 12/12 hours.

Each cultivar was conducted by 150 sawdust spawns. After incubation, 100 sawdust spawn were chosen without contamination for recorded the rate of spawn running and biological efficiency. The fruit bodies were harvested by 3 times in each sawdust spawn. The spawn running and biological efficiency (BE) were calculated following Stamets (2000). The contamination ratio of every cultivar was also recorded. The mature fruit bodies of each cultivar were collected for monokaryon isolation and morphological analysis.

Morphological analysis

The cultivated mature fruit bodies of each strain were dried at 60^oC for storage. The morphological characteristics were described following Largent (1977) and Largent *et al.* (1977). The description of each cultivar was conducted by at least 10 basidiomata. The size of spores was calculated by 50 spores (n=50). The size of basidia and cystidia were calculated by 10 units (n=10).

The identification was followed Pegler (1986), Corner (1981), Buchanan (1993), Boekhout *et al.* (1990), Segedin *et al.* (1995), and Lechner *et al.* (2004).

Phylogenetic analysis

In each cultivar, the spores from mature fruit bodies were collected and diluted into 50-100 spores/ml in distilled water. The portion of 0.5 ml of spore suspension was spread out on the PDA plate, then the plates were incubated at 25^oC in dark. Each mycelial colony grew from spore germination was isolated and maintained on PDA plates. All monokaryon strains were checked for confirming by the absence of mycelial clamp connections under microscope.

The fungal total DNA was from both cultivated fruit bodies and mycelia. A small myclelial/fruit body tissues were immersed in CTAB buffer (Saghai-Maroof *et al.*, 1984), lysed by Zirconia beads in Beadbug homogenier (Benchmark, New Jersey, USA), extracted by Chloroform/Isoamyl Alcohol 24:1 buffer (Merck, Darmstadt, Germany), purified by ethanol 70% (Merck, Darmstadt, Germany), and diluted in 100 µl TE buffer (Biobasic, Ontario, Canada). The ITS region was amplified by ITS1 – ITS4 pair primers (White *et al*, 1990). The polymerase chain reactions (PCR) were conducted by Mytaq HS

DNA polymerase (Meridian Bioscience, Tennessee, USA) following producer guide. The PCR products were check by electrophoresis, purified by ExoSAP-IT (Thermo Fisher, Massachusetts, USA), and then sent to the service of 1st Base (Selangor, Malaysia) for Sanger sequencing. All sequencing products were checked quality, fixed errors, and deleted primers by ATGC ver. 7 (Genetyx Corp., Tokyo, Japan).

In each cultivar, the ITS regions of dikaryon strain, fruit body, and 8 random monokaryon strains were sequenced. All ITS sequences of each cultivar were aligned by MEGA 7.0 (Kumar *et al.*, 2016) to check the possibility of inter-species hybrid cultivar then, the ITS representative sequences of each cultivar were retrieved. The ITS dataset of all cultivars was set up by adding the sequences from gene bank. Then, dataset was aligned; and analysed by MEGA 7.0 (Kumar *et al.*, 2016). The phylogenetic relationship was examined by the Maximum Composite Likelihood approach (MCL) (Tamura *et al.*, 2004) with Kimura two parameter model (Kimura, 1980).

Statistical analysis

Data analysed by StatDirect ver. 3.3 (StatDirect Ltd., Merseyside, UK).

Results

Cultivation

The sawdust spawns of all cultivars were fully covered by mycelia after 4-weeks incubation. The harvest period of all cultivars was 6 – 9 weeks after fruit body stimulating. All cultivars were harvest 3 times, except a blue oyster cultivar was fruited only one time. The rate of spawn running and biological efficiency (BE) of all cultivars were shown in Table 2.

Table 2. Rate of spawn running and biological efficiency of 10 cultivars

No.	Cultivars	Rate of spawn running (mm/day)*	Biological efficiency (%)**	Time from inoculation to fruiting (day)
1	WVL1	$10.60^{c} \pm 0.89$	$34.84^{\alpha\beta} \pm 3.90$	45
2	WSG1	$11.03^{bc} \pm 0.78$	$37.58^{\alpha} \pm 5.00$	35
3	GTG1	$9.87^{d} \pm 1.24$	$31.60^{\delta} \pm 3.46$	80
4	GVL1	$7.81^{f} \pm 1.05$	$35.96^{\alpha\beta} \pm 3.88$	43
5	GVL3	$8.81^{e} \pm 1.05$	$31.72^{\delta} \pm 3.64$	42
6	GBT1	$7.47^{f} \pm 2.31$	$20.86^{\varepsilon} \pm 6.20$	44
7	GBT2	$9.69^{d} \pm 0.71$	$22.67^{\varepsilon} \pm 4.58$	80
8	YVL1	$11.29^{b} \pm 1.00$	$33.09^{\gamma\delta} \pm 4.59$	32
9	PVL1	$12.47^{a} \pm 1.20$	$22.55^{\varepsilon} \pm 2.25$	32
10	DgSG1	$6.57^{g} \pm 0.76$	$12.93^{\zeta} \pm 3.93$	63

*Average ± Standard Deviation (SD). The different Romantic superscript letters indicated significant different (Turkey-Kramer test, $p \leq 0.05$).

**Average ± Standard Deviation (SD). The different Greek superscript letters indicated significant different (Turkey-Kramer test, $p \leq 0.05$).

The rate of spawn running of all cultivars could be separated into 3 groups: the highest group included a golden oyster cultivar, a pink oyster cultivar and all oyster cultivars; the medium group included all phoenix cultivars; and the lowest group was a blue oyster cultivar. The blue oyster cultivar also had the lowest biological efficiency (BE). The BE of the other cultivars was separated into 2 groups: the higher group included all oyster cultivars, a golden oyster cultivar and 3 phoenix cultivars GTG1, GVL1, GVL3; and the lower group included 2 phoenix cultivars GBT1, GBT2, and a pink oyster cultivar. The time from inoculation to first fruiting of all cultivars was separated into 4 group: the fasted group as a month included a golden oyster cultivar, a pink oyster cultivar PVL1 and an oyster cultivar WSG1; the one and half month group included an oyster cultivar WSG1, 3 phoenix cultivars GVL1, GVL3, GBT1; the 2 months group included a blue oyster cultivar DgSG1; and the slowest of more than 2.5 month included 2 phoenix cultivars GTG1 and GBT2.

Morphological description

Oyster cultivars

Basidiomata medium to large, fleshy, pleurotoid, flabelliform, usually imbricate or subinfundibuliform. Pileus pleurotoid, ovoid; surface dry, entire; margin at first incurved; then flat or upturned, undulating when old. Stipe eccentric to lateral, equal, or tapered from apex to base; villous in old specimens. Lamellae decurent, crowded, rather narrow, often reticulous at the stipe apex, margin of lamellae smooth; concolorous with the stipe. Flesh soft, thick at the base of pileus, ca. 1 mm near the margin; white when fresh and pale cream when dry. Spores smooth, cylindrical, thin-walled, pale cream. Basidia clavate. Basisioles numerous, clavate. Cystidia absent. Subhymenium narrow, cellular. Trama irregular, composed of thick- or thin-walled hyphae, hyaline, septate, with clamp connections. Context hyphae dimitic, generative hyphae thin-walled, smooth, septate, branched. Skeletal hyphae thick-walled, unbranched.

Oyster cultivar WSG1 (Figure 1): Pileus 46.55-85.94 x 53.86-97.33 mm; pale brown to brownish grey in the central of pileus and fading near the margin. Stipe 17.75-50.24 x 4.03-9.47 mm. Flesh 8.53-16.03 mm thick at the base of pileus. Spores 6.20-10.78 x 2.08-3.78 μm, L=8.16, W=2.95, Q=2.76. Basidia 29.60-35.70 x 8.56-14.7 8 μm, four-spored.

Oyster cultivar WVL1 (Figure 2): Pileus 33.72-57.28 x 47.45-76.80 mm; pale white and pale cream in the middle of pileus. Stipe 25.86-76.40 x 4.59-18.83 mm. Flesh 5.15-9.70 mm thick at the base of pileus. Spores 4.48-7.50 x

1.92-2.86 μm, L=5.92, W=2.3, Q=2.57. Basidia 24.30-26.50 x 5.00-5.80 μm, clavate, four-spored, sometimes two-spored.

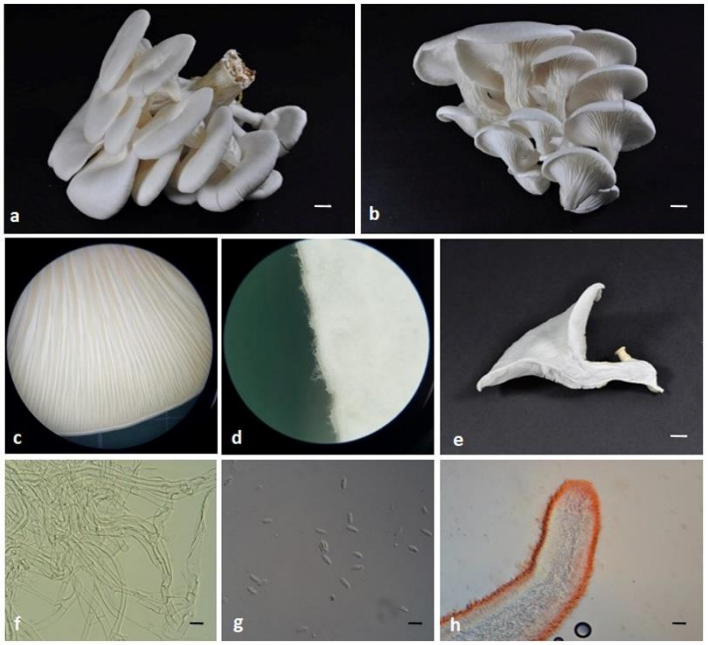

Figure 1. Morphological characteristics of cultivar WSG1. a, b. Fruiting body; c. Lamellae; d. surface of stipe; e. flesh; f. hyphae of flesh; g. basidiospores; h. cross section of lamellae. Scale bar: a, b, e: 1 cm; f, g, h: 10 μm

Phoenix cultivars

Basidiomata medium to large size, fleshy, pleurotoid, flabelliform to spathulate, usually imbricate or subinfundibuliform. Pileus pleurotoid; surface dry, entire; light grey, brownish grey; margin incurved when young and recurved, crenate, striate to undulate when old. Stipe long, clavate, equal, sometimes tapered from apex to base; villous in old specimens. Lamellae decurent, crowded, rather narrow, margin of lamellae smooth. Flesh soft, thick at the base of pileus, thin near the margin; white when fresh and pale cream when dry. Spores cylindrical, sometimes oblong-cylindrical, smooth, thin-walled, pale cream. Basidia clavate, four-spored, sometimes two-spored. Basisioles numerous, clavate. Subhymenium narrow, cellular. Trama irregular, composed of thick- or thin-walled hyphae, hyaline, septate, with clamp connections. Context hyphae dimitic, generative hyphae thin-walled, smooth, septate, branched. Skeletal hyphae thick-walled.

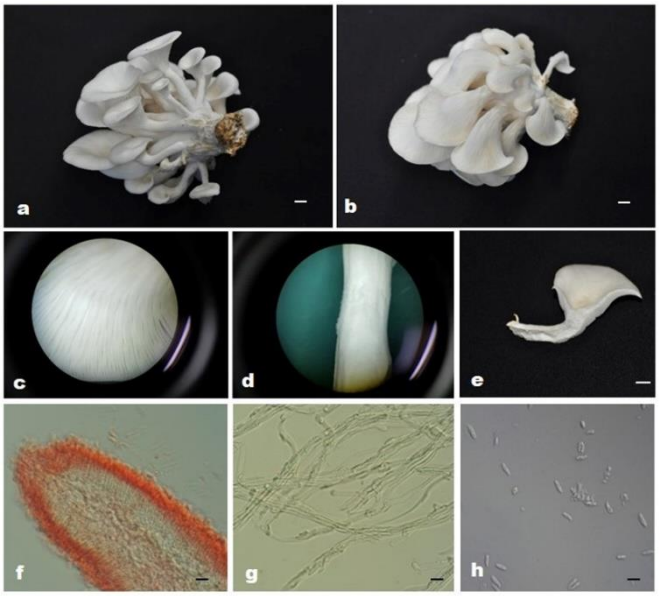

Figure 2. Morphological characteristics of cultivar WVL1. a, b. Fruiting body; c. Lamellae; d. surface of stipe; e. flesh; f. cross section of lamellae; g. hyphae of flesh; h. basidiospores. Scale bar: a, b, e: 1 cm; f, g, h: 10 μm

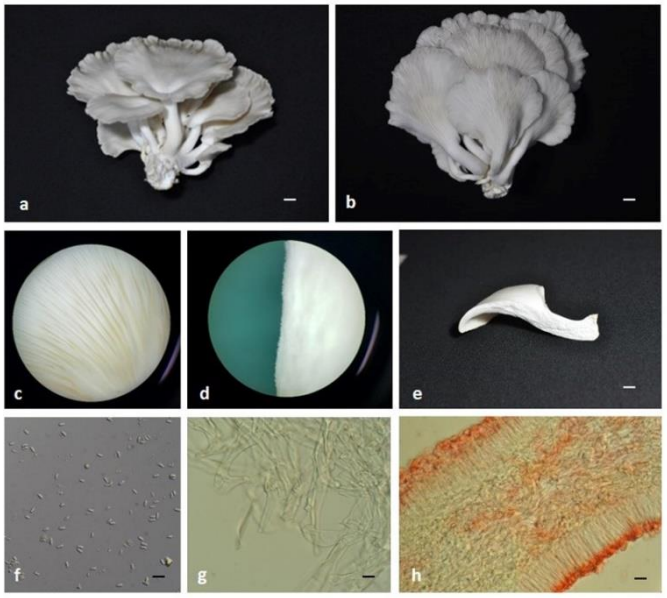

Figure 3. Morphological characteristics of cultivar GTG1. a, b. Fruiting body; c. Lamellae; d. surface of pileus; e. flesh; f. basidiospores; g. hyphae of flesh; h. cross section of lamellae. Scale bar: a, b, e: 1 cm; f, g, h: 10 μm

Phoenix cultivar GTG1 (Figure 3): Pileus 25.83-65.98 x 33.22-72.16 mm. Stipe 44.57-80.63 x 2.42-14.94 mm, eccentric to lateral. Lamellae usually broad, pale; concolorous with the stem. Flesh 3.66-9.10 mm thick at the base of pileus, 0.5-1.0 mm near the margin. Spores 4.63-6.73 x 2.01-3.57 µm, L=5.61, W=2.63, Q=2.13. Basidia 21.30-31.32 x 6.50-11.40 µm. Cystidia absent.

Phoenix cultivar GVL1 (Figure 4): Pileus 37.90-58.96 x 51.18-83.08 mm. Stipe 37.58-70.83 x 4.53-17.75 mm, central to lateral. Lamellae white, often reticulous at the stipe apex. Flesh 3.08-5.45 mm thick at the base of pileus, 0.5 mm near the margin. Spores 5.92-10.13 x 2.31-5.41 µm, L=7.86, W=3.64, Q=2.15. Basidia 33.52-39.25 x 13.59-16.71 µm. Cystidia absent.

Phoenix cultivar GVL3 (Figure 5): Pileus 30.14-50.47 x 43.91-70.65 mm; pale brown to brownish grey in the central of pileus and fading near the margin. Stipe 40.41-74.75 x 3.77-16.14 mm, eccentric to lateral. Lamellae white, often reticulous at the stipe apex. Flesh 2.03-5.48 mm thick at the base of pileus, 0.5 mm near the margin. Spores 5.34-9.79 x 2.69-4.82 µm, L=7.42, W=3.52, Q=2.10. Basidia 30.26-41.30 x 8.00-12.47 µm. Pleurocystidia absent. Cheilocystidia 22.49-36.09 x 47.80-15.30 µm, ventricose or clavate.

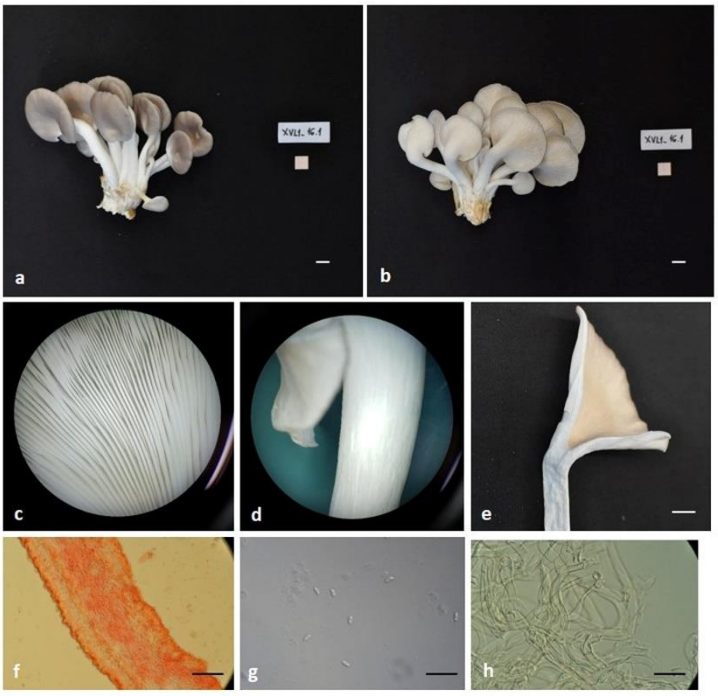

Figure 4. Morphological characteristics of cultivar GVL1. a, b. Fruiting body; c. Lamellae; d. surface of stipe; e. flesh; f. cross section of lamellae; g. basidiospores; h. hyphae of flesh. Scale bar: a, b, e: 1 cm; f, g, h: 10 µm

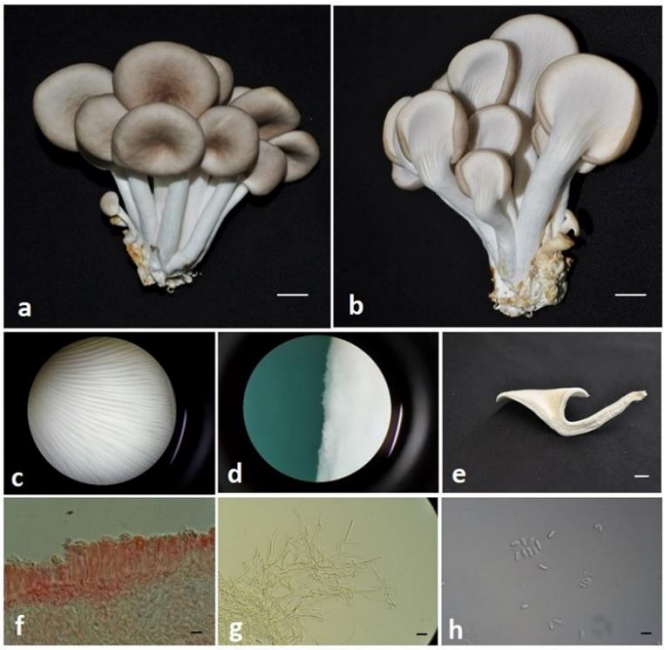

Figure 5. Morphological characteristics of cultivar GVL3. a, b. Fruiting body; c. Lamellae; d. surface of stipe; e. flesh; f. cross section of lamellae; g. hyphae of flesh; h. basidiospores. Scale bar: a, b, e: 1 cm; f, g, h: 10 μm

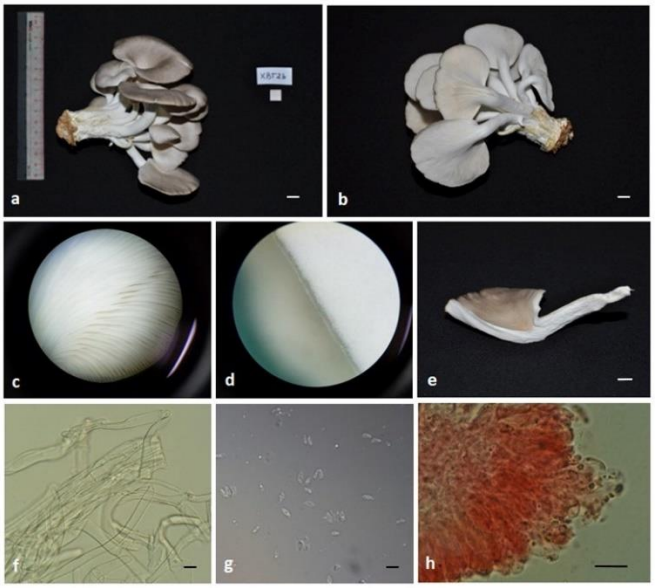

Figure 6. Morphological characteristics of cultivar GBT1. a, b. Fruiting body; c. Lamellae; d. surface of pileus; e. flesh; f. hyphae of flesh; g. basidiospores; h. cheilocystidia. Scale bar: a, b, e: 1 cm; f, g, h: 10 μm

Phoenix cultivar GBT1 (Figure 6): Pileus 42.12-70.54 x 63.49-90.63 mm. Stipe 50.94-.86.89 x 5.42-19.93 mm, eccentric to lateral. Lamellae usually broad, pale; concolorous with the stipe. Flesh 5.3-8.42 mm thick at the base of pileus, 0.5-1.0 mm near the margin. Spores 4.04-6.01 x 1.99-3.24 μm, L=5.04, W=2.53, Q=1.99. Basidia 24.30-26.50 x 5.00-5.80 μm. Pleurocystidia absent. Cheilocystidia 29.20-35.90 x 5.33-8.74μm, clavate.

Phoenix cultivar GBT2 (Figure 7): Pileus 45.68-89.85 x 52.57-104.42 mm. Stipe 62.74-112.78 x 7.24-15.99 mm, eccentric to lateral. Lamellae usually broad, pale; concolorous with the stipe. Flesh 7.89-10.62 mm thick at the base of pileus, 1.0 mm near the margin. Spores 4.57-7.19 x 1.97-3.25 μm, L=5.77, W=2.51, Q=2.29. Basidia 28.60-34.78 x 6.11-7.80 μm. Cystidia absent.

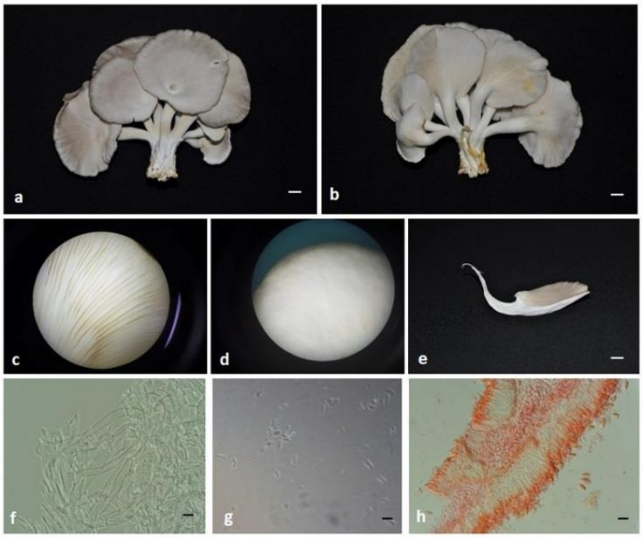

Figure 7. Morphological characteristics of cultivar GBT2. a, b. Fruiting body; c. Lamellae; d. surface of pileus; e. flesh; f. hyphae of flesh; g. basidiospores; h. cross section of lamellae. Scale bar: a, b, e: 1 cm; f, g, h: 10 μm

Golden oyster cultivar YVL1 (Figure 8)

Basidiomata usually small, fleshy, pleurotoid, usually imbricate, flabelliform, subinfundibuliform. Pileus 21.47-34.69 x 22.59-33.77 mm; surface dry, entire; shiny, pale yellow to dark yellow, fading to pale white when old, margin at first incurved, then flat. Stipe 22.72-41.76 x 3.05-6.65 mm, central or lateral, short, equal, or tapered from apex to base; villous in old specimens. Lamellae white, decurent, crowded, narrow, often reticulous at the stipe apex, margin of lamellae smooth. Flesh soft, 5.15-9.70 mm thick at the base of pileus, 1 mm near the margin; white when fresh and pale cream when dry. Spores 2.85-5.86 x 1.91-2.97 μm, L=4.88, W=2.42, Q=2.01; smooth,

oblong-cylindrical, thin-walled, pale cream. Basidia 28.53 x 13.16-17.53 µm, clavate, four-spored, sometimes two-spored. Basisioles numerous, clavate. Pleurocystidia 32.68-61.41 x 8.9-17.11 µm, clavate, to napiform, rarely cylindro-clavate. Cheilocystidia 24,62-26,73 x 12,36-13,72 µm, digitate. Subhymenium narrow, up to7 µm, cellular. Trama irregular, composed of thick- or thin-walled hyphae, hyaline, septate, with clamps. Context hyphae dimitic, generative hyphae thin-walled, smooth, septate, branched. Skeletal hyphae thick-walled, unbranched.

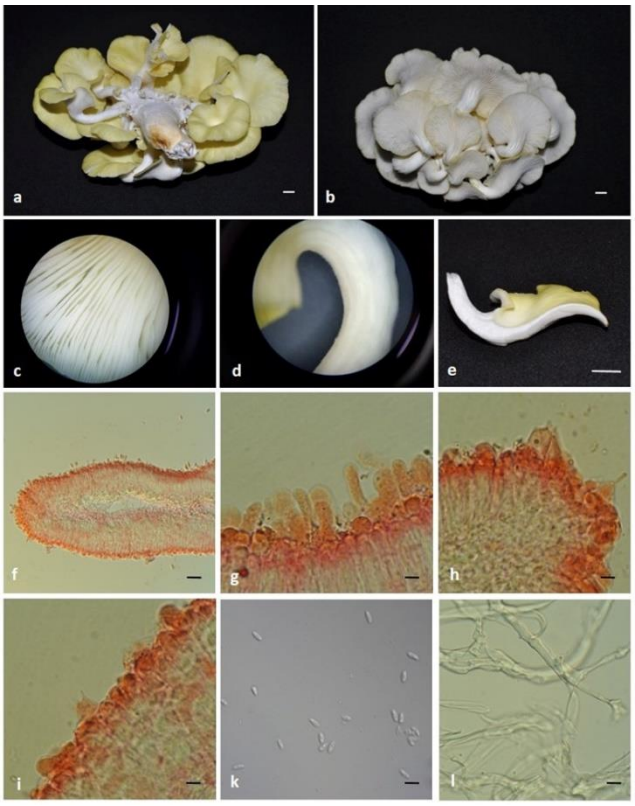

Figure 8. Morphological characteristics of cultivar YVL1. a, b. Fruiting body; c. Lamellae; d. surface of stipe; e. flesh; f. cross section of lamellae; g. pleurocystidia; h. cheilocystidia; i. basidia; k. basidiospores; l. hyphae of flesh. Scale bar: a, b, e: 1 cm; f, g, h, i, k, l: 10 µm

Pink oyster cultivar PVL1 (Figure 9)

Basidiomata medium to large size, fleshy, usually imbricate to dimidiate, pleurotoid, flabellate to spathe. Pileus 37.23-64.26 x 49.08-63.42 mm; surface at first entire, then often fibrillose, dry; often pink to salmon when young and pale white when old; margin at first incurved, then flat, undulating. Stipe 17.20-

40.70 x 2.28-15.25 mm, lateral, short, equal, or tapered from apex to base; more villous in old specimens. Lamellae pink, decurent, crowded, rather narrow, often reticulous at the stem apex, margin of lamellae smooth. Flesh soft, 3.50-8.00 mm thick at the base of pileus, 0.5-1.0 mm near the margin; white when fresh and cream when dry. Spores 4.24-7.06 x 1.61-2.61 μm, L=5.55, W=2.18, Q=2.54; smooth, cylindrical, thin-walled, pale cream. Basidia 24.30-26.52 x 5.04-5.80 μm, clavate, four-spored, sometimes two-spored. Basisioles numerous, clavate. Pleurocystidia absent. Cheilocystidia 20.32-25.60 x 6.20-6.83 μm, clavate. Subhymenium narrow, up to 8 μm, cellular. Trama irregular, composed of thick- or thin-walled hyphae, hyaline, septate, with clamps. Context hyphae dimitic, generative hyphae thin-walled, smooth, septate, branched. Skeletal hyphae thick-walled, unbranched.

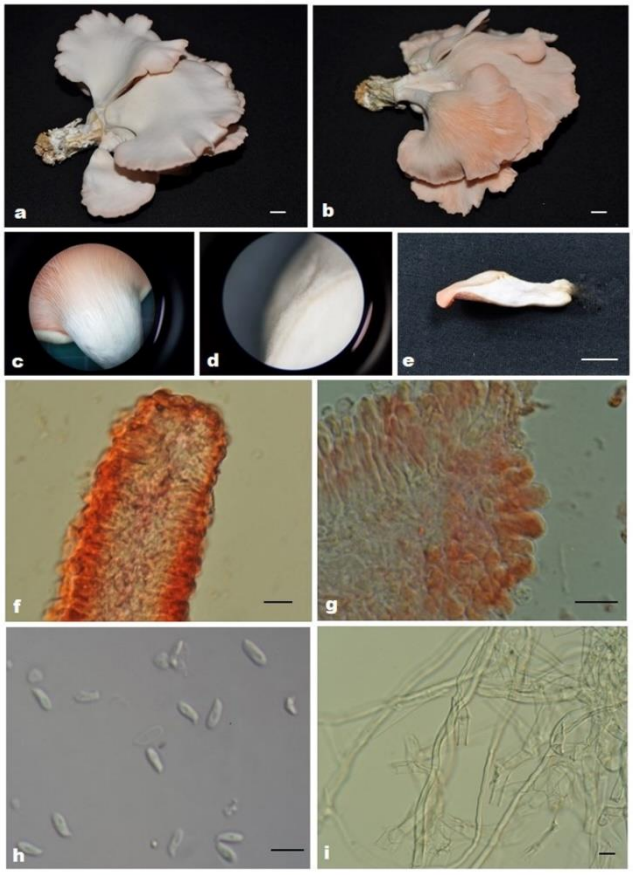

Figure 9. Morphological characteristics of cultivar PVL1. a, b. Fruiting body; c. Lamellae; d. surface of pileus; e. flesh; f. cross section of lamellae; g. cheilocystidia; h. basidiospores; i. hyphae of flesh. Scale bar: a, b, e: 1 cm; f, g, h, i: 10 μm

Blue oyster cultivar DgSG1 (Figure 10)

Basidiomata small to medium, fleshy, collybiod or clitocyboid, rarely imbricate, sub-infundibuliform, sometimes convex. Pileus 8.82-15.84 x 9.42-16.54 mm; surface dry, entire; light grey, brownish grey to purplish grey; margin incurved. Stipe 40.08-70.86 x 3.96-13.87 mm, long, central to eccentric, clavate, equal, sometimes tapered from apex to base; villous in old specimens. Lamellae decurent, crowded, rather narrow, often reticulous at the stipe apex, margin of lamellae smooth; concolorous with the stipe. Flesh semi-solid, 4.01-5.11 mm thick at the base of pileus, 0.5 mm near the margin; white when fresh and pale cream when dry. Spores 6.04-10.97 x 2.49-3.80 μm, L=8.68, W=3.09, Q=2.8; smooth, cylindrical, thin-walled, pale cream. Basidia 28.61-39.52 x 14.00-15.53 μm, clavate, four-spored. Basisioles numerous, clavate. Cystidia absent. Subhymenium narrow, up to 12 μm, cellular. Trama irregular, composed of thick- or thin-walled hyphae, hyaline, septate, with clamps. Context hyphae dimitic, generative hyphae thin-walled, smooth, septate, branched. Skeletal hyphae thick-walled, unbranched.

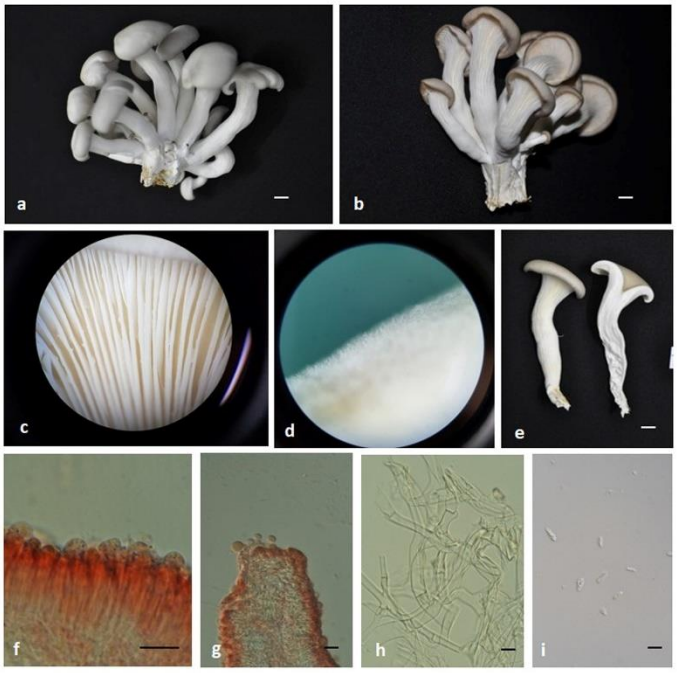

Figure 10. Morphological characteristics of cultivar DgSG1. a, b. Fruiting body; c. Lamellae; d. surface of pileus; e. flesh; f. basidioles; g. cheilocystidia; h. hyphae of flesh; i. basidiospores. Scale bar: a, b, e: 1 cm; f, g, h, i: 10 μm

Phylogenetic analysis

In all cultivars, the ITS sequences of dikaryon strain, fruit body, and 8 random monokaryon strains were 100% similar when aligned dataset. It conformed that all cultivars are not the inter-species hybrid. All representative ITS sequences were submitted to gene bank (Table 3).

Table 2. Detail of ITS sequences using in phylogenetic analysis

No.	Cultivar	Species	Accession No.	References
1	WVL1		LC602510	
2	WSG1		LC602506	
3	GTG1		LC602502	
4	GVL1		LC602511	
5	GVL3		LC602515	
6	GBT1		LC602503	
7	GBT2		LC602504	
8	YVL1		LC602517	
9	PVL1		LC602508	
10	DgSG1		LC602519	
11		*P. pulmonarius*	AY450349	Shnyreva and Shnyreva 2015
12		*P. ostreatus*	AY450345	Shnyreva and Shnyreva 2015
13		*P. djamor*	MH862831	Vu *et al* 2019
14		*P. cornucopiae*	AY450341	Shnyreva and Shnyreva 2015
15		*P. citrinopileatus*	JX429936	Avin *et al* 2014
15		*A.auricularis-juda*	KX621136	Bandara *et al* 2017

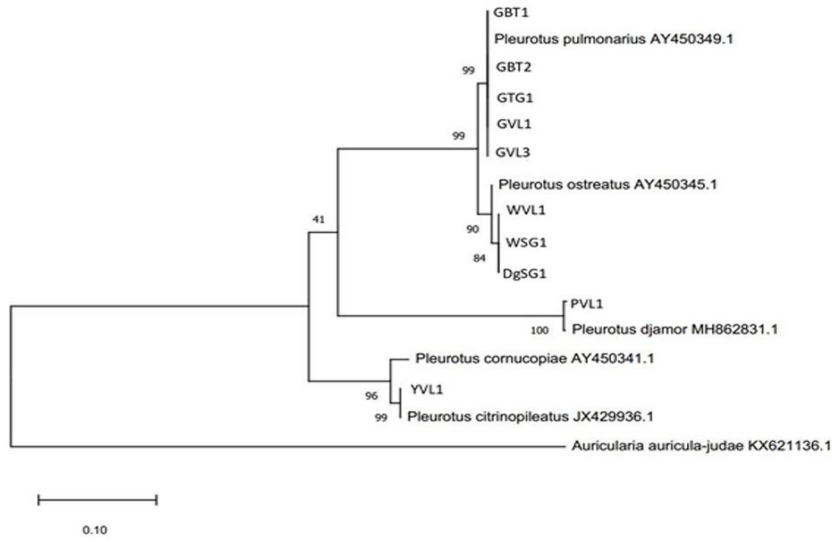

Figure 11. The phylogenetic tree from ITS dataset. The bootstrap consensus tree was inferred from 1000 replicates with collapsing all branches corresponding to less than 50% bootstrap replicates. The topology was retrieved by *Auricularia auricularis-juda* as outgroup

The aligned ITS dataset was 616 positions. The phylogenetic topology was shown in Figure 11. Following the topology, all 5 phoenix cultivars were belonged to *P. pulmonarius* clade; all 2 oyster cultivars and a blue oyster cultivar were belonged to *P. ostreatus* clade; a pink oyster cultivar was belonged to *P. djamor* clade; and the golden oyster cultivar was belonged to *P. citrinopileatus* clade. All clades were supported by the bootstrap ≥90%.

Discussion

From the morphological characteristics, all oyster and phoenix cultivars would be belonged to the complex *P. pulmonarius* and *P. ostreatus*. However, following Lechner *et al.* (2004), Corner (1981) and Boekhout *et al.* (1990), both oyster cultivars WSG1 and WVL1 should be belonged to *P. ostreatus* with the monomitic context hyphae, absence of cheilocystidia, and absence of pleurocystidia or pleurocystidia like basidia. Only 2 phoenix cultivars GBT1 and GVL3 were recorded the cheilocystidia, the characteristic for separating *P. pulmonarius* and *P. ostreatus* (Buchanan, 1993, Segedin *et al.*, 1995). However, all 5 phoenix cultivars and a blue oyster cultivar DgSG1 had the dimitic context hyphae, another characteristic of *P. pulmonarius* (Buchanan, 1993, Lechner *et al.*, 2004). Besides, the record of *P. pulmonarius* in Argentina (Lechner *et al.*, 2004) also showed the absence of cystidia. The golden oyster cultivar YVL1 should be belonged to the complex of *P. cornucopiae* and *P. citrinopileatus*. Even the original description of *P. citrinopileatus* (Singer, 1943) was not clear, following Boekhout *et al.* (1990), *P. cornucopiae* was not recorded the cystidia. In Corner (1981), *P. cornucopiae* was also separated with *P.* aff. *cornucopiae* recorded from Japan by the absence/presence of both pleurocystidia and cheilocystidia. *P.* aff. *cornucopiae* (Corner 1981) should be recorded as *P. citrinopileatus* which also collected from Japan and had both pleurocystidia and cheilocystidia (Buchanan, 1993). Therefore, the golden oyster cultivar YVL1 should identified as *P. citrinopileatus*. Following Lechner *et al.* (2004), the pink oyster cultivar should be identified to *P. djamor*. The morphological identification of all cultivars was confirmed by the topology of phylogenetic tree inferred from ITS dataset with high bootstrap clade. The alignment of intra-cultivar ITS dataset confirm that all cultivars were not inter-species hybrid. Combining the molecular and morphological identification, all these cultivars could be used as the parent in crossing due to their putative strain and standard characteristic of species.

In this study, all cultivars were cultivated in the disclosure model with room temperature in tropical area; and harvested only 3 times comparing with normally 5 times in farm. Therefore, the BE of all cultivars was lower than the results of other studies, especially in the case of *P. ostreatus* (Shah *et al.*, 2004,

Miah *et al.*, 2017). Among cultivars, the *P. ostreatus* cultivars (oyster mushroom) WSG1 and WVL1 had the better rate of spawn running as well as the biological efficiency and the short time to fruiting from inoculation. The physiological characteristics of *P. pulmonarius* cultivars (phoenix mushroom) GTG1, GVL1, GVL3, GBT1, GBT2 were complicated. By the time to fruiting from inoculation, those cultivars divided into the long-time cultivars included GTG1 and GBT2; and the short-time cultivars included GVL1, GVL3, GBT1. Recently, Ben Tre Province, Viet Nam was affected by the penetration of sea water. The lower BE of both cultivar from Ben Tre Province compared with the other in *P. ostreatus* group should be from the tolerant of salinity watering. The result also indicated that the blue oyster cultivar was not adapted to tropical climate in southwestern Viet Nam.

This the first study described the macro and micro morphological characteristic of commercial mushroom cultivars in Viet Nam. The combination between morphological and phylogenetic analysis supported to understand thoroughly the identification of *Pleurotus* cultivars. The results of cultivation under a standardized conditions should be a value data to screen and breed the new cultivar.

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