

RESEARCH ARTICLE



Russulaceae of Ailawng forest with an emphasis on *Russula purpureoverrucosa* (Russulaceae): A first report for India

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This study presents *Russula purpureoverrucosa* marking the first record of its presence in India. Additionally, we have identified other fourteen species from the family Russulaceae and their taxonomic descriptions were given. This study was constrained to a small area, however, it hints at the potential for the discovery of more Russulaceae in Mizoram. The findings suggest that the region may host a rich diversity of these fungi, and further research in broader areas could unveil additional Russulaceae in Mizoram.

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Introduction

Russulaceae is one of the most numerous, stunning, and economically significant families of fleshy macrofungi,¹ and exhibits a worldwide distribution and represents over 1900 species.² The family Russulaceae serves a significant ecological role in terrestrial ecosystems by forming symbiotic relationships with higher plants and aids as human foods and medicine.³⁻⁶ *Russula* Pers. (1797) is the largest of these genera, with around 1100 species, followed by *Lactarius* Pers. (1797) with about 550 species, *Lactifluus* (Pers.) Roussel (1806) with about 120 species, *Boidinia* Stalpers & Hjortstam (1982) with 13 species, *Multifurca* Buyck & V. Hofst (2008) with 6 species, *Gloeopeniophorella* Rick (1934) with 6 species, and *Pseudoxenasma* K.H.Larss. & Hjortstam (1976) with just one species.²

Russula can be easily distinguished from its sister genera *Lactarius* and *Lactifluus* by its distinctive morphology, which includes a white to dark yellow spore print, free white gills, and absence of a partial veil or volva tissue on the stipe, absence of clamp connection, warted and light-colored amyloid basidiospores with varied ornamentations and a brittle fleshy basidiocarp due to the presence of sphaerocysts.⁷ However, several *Russula* species are

difficult to discern⁸⁻⁹ and DNA barcoding is increasingly used to resolve taxonomic issues, particularly, in these families. A recent analysis of genetic markers revealed that every milkcap is not *Lactarius*, therefore *Lactarius* are actually divided into three separate genera such as *Multifurca* Buyck & V. Hofst,⁵ *Lactifluus* (Pers.) *Russula* and *Lactarius* Pers.¹⁰⁻¹³ Unlike *Russula*, *Lactarius* and *Lactifluus* species emit a fluid when scratched, and similarities in appearance, such as the brittle flesh of their fruit bodies.¹⁴ The name *Lactarius* was kept for the larger group of mushrooms, including well-known ones from temperate regions and *Lactifluus* is now used for a smaller group of mushrooms, mainly from tropical areas, but it also includes some from temperate regions like *Lactifluus volemus* and *L. vellereus*.

In India, 158 different taxa of *Russula*, 83 taxa of *Lactarius*, and 29 taxa of *Lactifluus* have been reported.¹⁵ Furthermore, a more recent study listed 124 species of the genus *Russula* from India.¹⁶ Additionally, six species from the family Russulaceae (*Lactifluus corrugis*, *L. piperatus*, *Russula aurora*, *R. compacta*, *R. cyanoxantha*, and *R. subfragiliformis*) have been documented in Mizoram.¹⁷ Among these

species, *Lactifluus corrugis* and *L. volemus* is revered in Mizoram as famous wild edible mushroom due to their distinctive odor resembling dog meat. It is regularly sold in local markets for about Rs. 400 (4.8 USD) per kg.¹⁸ This mushroom is widely distributed in Champhai District of Mizoram, and local populaces often gather large quantities, sometimes up to 20 kg per collection. It plays a significant role in providing financial assistance to the local community and is often transported and sold in the state capital, Aizawl, further extending its economic impact beyond the local area.

In our study, we introduced a new report from the genus *Russula*, identified through a combination of morphological and molecular characteristics. *Russula purpureoverrucosa* Fang Li (subgenus - *Incrustatula*, section - Lilaceinae, subsection - Lilaceinae) originated from South China. It is characterized by a medium-sized fruiting body with a red to purplish-gray pileus.¹⁹ Interestingly, there have been no prior reports of this species in India, and we are the first to document its presence in the Indian region.

Materials and Methods

Collection and identification of specimens

Basidiomes were collected from Ailawng Forest, Mamit during the monsoon season on 2021 – 22. The collected specimens were identified using standard methods based on macroscopic and microscopic characteristics.¹⁹⁻²² The specimens were submitted to the Natural History Museum of Mizoram, Mizoram University. Molecular methods were performed following Zothanzama et al.^{23,18} where DNA was extracted using a Cetyltrimethylammonium bromide (CTAB) method, followed by amplification of the internal transcribed spacer region (ITS) of the rDNA and sequenced with both primers (ITS1F and ITS4B).

PCR amplification

PCR reactions were set up using 25.5µl reaction volume comprising of 12.5µl GoTaq Green Mastermix (Promega, Madison, WI), 9.5µl nuclease-free water, 0.5µl bovine serum albumin (BSA), 1µl forward and reverse primers (5µM), 1µl and fungal DNA template. PCR was performed using primers ITS1-F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') ITS4-B (5'-CAG GAG ACT TGT ACA CGG TCC AG-3')²⁴ with the following parameters; 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 52°C for 1 minute and 72°C for 1 minute with a final extension step of 72°C. PCR amplicons were verified by electrophoresis on a 1% agarose gel with SYBR green and visualized on a Gel Documentation System. Sequencing was performed using both primers by using Sanger sequencing using an ABI 3730xl DNA sequencer. Consensus sequences for contigs were trimmed and aligned using Bioedit sequence alignment

editor. Sequences were then compared to those in GenBank database using the BLASTn²⁵ search tool for similarities and submitted to GenBank. The sequences were then aligned with Clustal W²⁶ and the phylogenetic tree was established using Maximum Likelihood in RaxML GUI software with the available sequences representing all the species.

Phylogenetic analysis

Phylogenetic analysis was conducted based on the ITS gene data using both Maximum Likelihood and Neighbor-Joining approaches. ML and NJ searches were carried out using RaxML GUI. Alignment gaps were treated as missing data. NJ trees were constructed based on the total character differences and bootstrap values were calculated from 1,000 replications.

Results

Fifteen species belonging to the Russulaceae family were identified. *Russula purpureoverrucosa* was reported in this study as a new record in India. Detailed morphological observations were thoroughly documented, comprising both macroscopic and microscopic characteristics.

Molecular identification

The nucleotide sequence of the mushroom blasted against sequences from GenBank database revealed the identification of *Russula purpureoverrucosa*, a fungal isolate from Mizoram. The ITS1-5.8S-ITS4 sequences of the fungal isolate were compared to 17 corresponding sequences of reference fungal taxa in the database and the list of species, voucher number and GenBank accession number used for the analysis is given in Table 1 and the phylogenetic tree in Figure 1.

Russula purpureoverrucosa Fang Li

Description of the specimen based on India (Figure 2)

Basidiomata medium-sized. Pileus 4–8.5 cm in diam., globose, then hemispherical when expanding, convex to plano-convex, sometimes uplifted with age; surface dry, sticky when moist; reddish, purplish red to rosy lilac with age, often with center area dark purpled or violet, sometimes dark, slightly areolate in small irregular warts from center to margin showing pale context in between, margin no striate. Gills adnate, closed, occasionally forked near the stipe, smooth edge; white to creamy white, without discoloring, occasionally same colored as the pileus. Stipe solid, slightly fibrous; 3–5.2 cm long, 0.7–1.3 cm thick, cylindrical, dry, same colored as the pileus. Context white, odor absent. Basidiospores 6–8 × 4.5–6 µm, ellipsoid, sometimes subglobose to globose, ornamented

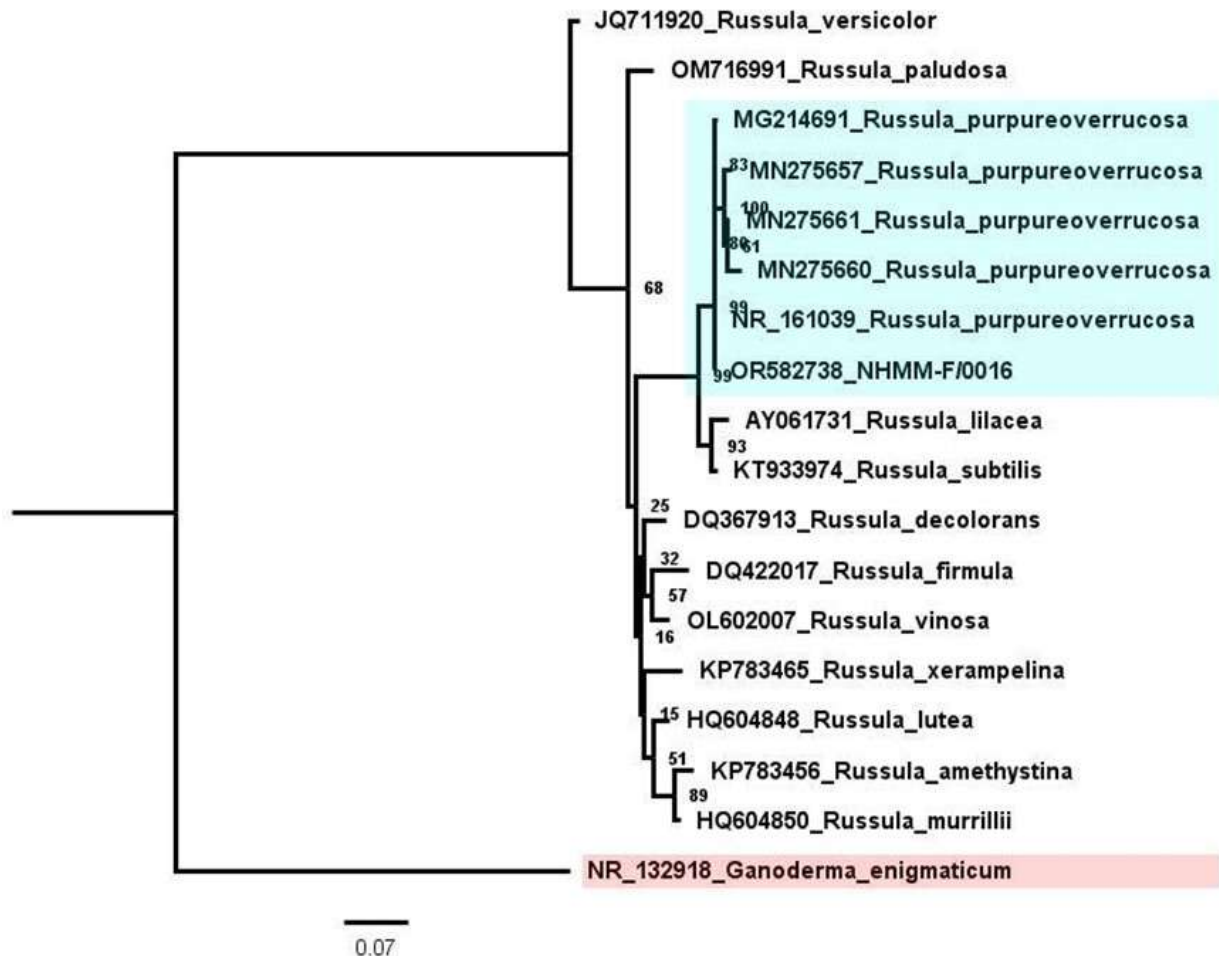


Figure 1: Phylogenetic tree. The evolutionary history was inferred by using the Maximum Likelihood method based on the GTR-GAMMA model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in RaxmlGUI 2.0. In the maximum Likelihood tree generated, the specimen, *Russula purpureoverrucosa* clustered with the related species with high support value. *Ganoderma enigmaticum* was used as the Outgroup which is highlighted in red colour. The fungal isolate collected from Mizoram, *Russula purpureoverrucosa* (NHMM-F/0016) along with the *R. purpureoverrucosa* clade is highlighted in blue colour.

densely with blunt to acute conic warts and short ridges 0.5–1 μm high; amyloid. Basidia 20–35 \times 7–11 μm , clavate, 4 spored, Pleurocystidia abundant; 36.5–71 \times 8–14.5 μm , clavate to subfusiform. Edibility – Unknown.

Habitat and distribution: Scattered on soil in forests dominated by Fagaceae such as *Castanopsis indica* (Roxb. Ex Lindl.) A. DC., *Quercus helferiana* A. DC., *Quercus glauca* Thunb. and *Lithocarpus xylocarpus* (Kurz) Markgr. etc). This species was originally described from South China¹⁹ and is now known from India. Coordinates - 23°41'0.1"N 92°37'42."E, 1168m asl.

Collection examined: Natural History Museum of Mizoram, Mizoram University. Accession number – NHMM-F/0016

Lactarius rufus (Scop.) Fr.

Cap 4-9 cm across, convex with an inrolled margin at first, flattens with shallow depression

having small umbo at centre with ages; smooth; surface dry; reddish brown, brick red or bray brown. Flesh creamy to white. Gills decurrent; crowded; creamy white, later same colored as cap. Latex abundant when young. Stem 4-8.5 \times 0.7-1.8 cm; subclavate, smooth, solid, often becomes hollow with age; concolorous with cap, but more paler and white at base. Spore print creamy white. Spores globose to ellipsoidal, hyaline, reticulate; 6.5-8.5 \times 5.5-7 μm . Edibility - Not recommended.

Lactarius subvellerus Peck var. *subdistans* Hesler & Smith

Cap 4-14 cm wide; convex, inrolled margin at first; becoming flat or shallowly depressed with age; soft, surface dry; velvety; whitish. Gills adnexed; distant or nearly, whitish, becoming yellowish; slowly bruising brownish. Stem 2.5-5 \times 3-3.5; dry, finely velvety; white to creamish white; sometimes bruising brownish. Flesh white; turning yellowish to brilliant yellow when cut. Latex white; changing to yellow on exposure. Spore print white. Spores ellipsoid; 7.5-9 \times 5-7.5 μm . Edibility – Inedible

Table 1: List of species, voucher, and GenBank accession nos.

Sl. No	Species	Voucher/Strain	GenBank Accession Number
1	<i>Russula purpureoverrucosa</i>	GDGM 32902	NR_161039
2	<i>Russula purpureoverrucosa</i>	LF2089	MG214691
3	<i>Russula purpureoverrucosa</i>	K16050805	MN275657
4	<i>Russula purpureoverrucosa</i>	K18090817	MN275661
5	<i>Russula purpureoverrucosa</i>	TLH18053016	MN275660
6	<i>Russula subtilis</i>	BPL275	KT933974
7	<i>Russula lutea</i>	040528-01OC	HQ604848
8	<i>Russula lilacea</i>	707IC54	AY061731
9	<i>Russula amethystina</i>	514	KP783456
10	<i>Russula murrillii</i>	041114-01	HQ604850
11	<i>Russula decolorans</i>	OUC99188	DQ367913
12	<i>Russula firmula</i>	AT2004142	DQ422017
13	<i>Russula vinosa</i>	iNAT:17704352	OL602007
14	<i>Russula versicolor</i>	FFP1034	JQ711920
15	<i>Russula paludosa</i>	ACAD21097F	OM716991
16	<i>Russula xerampelina</i>	368	KP783465
17	<i>Ganoderma enigmaticum</i>	CBS 139792	NR_132918

***Lactifluus corrugis* (Peck) Kuntze**

Cap 4-20 cm wide; convex, becoming shallowly depressed, sometimes with a distinctly wrinkled margin; notably velvety; dark reddish brown red to dark brick red but sometimes purplish brown or dark brown. Gills adnate to subdecurrent; close, pale buff when young but changing to orangish to yellowish or brownish; discoloring brown where injured. Stem 3-11 x 1.5-3 cm; colored same as the cap, more or less, or paler; solid. Flesh whitish to yellowish, changing slowly brown when cut. Latex white; copious, staining brown tissues. Spore print white. Spores globose or subglobose, amyloid; 9-12.5 x 8.5-12 μ . Edibility – Edible.

***Lactifluus piperatus* (L.) Roussel**

Cap 4-15 cm, broadly convex, becoming flat, broadly-shallowly depressed, surface dry; white, sometimes brownish yellow in age. Gills subdecurrent or decurrent, attached to the stem, forking; white then changing to pale cream. Flesh white, sometimes changing to whitish yellow. Stem 1.5-7.5 x 1-2 cm; solid, white. Milk white, changing slowly to cream white to yellow after reaction or exposure. Spore Print white. Spores ellipsoid; 5-10 x 5-8 μ . Edibility – Edible.

***Lactifluus volemus* Fr. (Fr).**

Cap 4-12 cm, convex with an in rolled margin,

becoming flat in age, with a central depression; orangish brown, smooth to minute velvety, surface dry. Gills adnate, crowded, creamy white or pale white bruising brown where injured. Stalk 5-10 x 0.7-2.2 cm; same colored as cap or more paler; dry. Flesh brittle, white, staining brown when cut. Latex white, sticky, discoloring brown. Spore print white. Spores 7.5-9.5 x 7-9 μ ; subglobose, well develop amyloid reticulum. Edibility – Edible.

***Russula brevipes* Peck**

Cap size 6-22 cm across, convex with depressed center, non-striate margin with inrolled even at maturity, infundibuliform; smooth; dry; whitish or creamy stained, later discoloring to brownish. Gills adnate to slightly decurrent, crowded; white, becoming creamy to ochre-brown with age. Stem 2-7 cm long, 2-4 cm thick, solid, sturdy, surface dry; white stained brown with age. Flesh white. Spore print white to cream light. Spores 8-11 x 6.5-9.5m; ellipsoid to subglobose, ornamented with a broken to complete reticulum, with warts 0.8-1.7m high. Edibility – Inedible.

***Russula compacta* Frost**

Cap 3-18 cm across; convex, later flattened or depressed, often with a slightly sunken center; sticky when fresh; more or less smooth, white to



Figure 2: a-b Basidiocarp. c-e Microscopic characteristics of *R. purpureoverrucosa* (c – spore, d – pleurocystidia, e – basidia). Scale bars: a-b = 1cm, c = 5mm, d-e = 10mm.

whitish or orangish-yellow when young, but soon turning yellowish orange to reddish brown with age or on bruising. Gills adnate, fairly crowded, close or almost distant; white to pale cream yellowish; bruising reddish or rust-brown. Stem 2-10 cm long; 1.2-3.5 cm thick; becoming hollow, dry, smooth; whitish, but soon flushed with yellowish brown. Flesh brittle; white; discoloring yellowish to yellowish brown or reddish brown. Spores 7.5-10 x 6-8 μ; elliptical, with blunt, conic warts up to 1.2 μ high. Edibility - Edible but rather poor.

Russula crustosa Pk

Cap 3-10 cm wide; round or convex when young, becoming broadly convex or flat with ages, with a shallow depression; surface dry; yellowish-brown, more brownish than yellow. Stipe 3 - 8.5 cm, 1.5-3.5 cm thick; white; surface dry, smooth, slimy when wet. Flesh white. Gills closely attached to the stipe, occasionally forking; white to creamish

white. Basidiopores 6.5-9.5 x 5-6.5 μm, elliptical, round. Spore print creamish white. Scattered, solitary or gregarious in soil. Edibility – Edible.

Russula cyanoxantha (Schaeff.) Fr.

Cap 4-15 cm across; purple, green to olive green then pinkish white, convex, becoming flat or broadly convex with ages; surface dry, sometimes moist, smooth. Stem, 6-12 cm long, 1.5-3.5 cm thick, white; dry, smooth. Flesh white, thick. Spores 6.5-9 x 5.5-7.5 μ with isolated warts. Pseudocystidia narrow and fusiform to subcapitate. Gills attached to the stipe, closely, not forking, white when young and soft, softer when moist. Edibility – Edible.

Russula emetica (Schaeff.) Pers.

Cap up to 5-11 cm wide; convex to slightly depressed; brightly red, scarlet red or blood red, shiny; surface smooth, dry. Gills broad, medium spaced; attached to the stem ranging from adnate to adnexed; white to pale cream. Stem 5-8 x 1 -2.4cm, slightly scurfy, clavate or club shaped; white. Flesh white. Spore print white to yellowish white. Spores 8.5-11.5 x 7 - 8.5 μ; ovoid, large warts. Edibility - Inedible.

Russula foetens Pers.

Cap 5- 12 cm across; hemispherical, becoming more or less flat or slightly depressed with age; viscid; surface dry; honey brown to yellowish brown. Gills narrow, adnexed, close together; brittle; creamish, darken with age. Flesh thin, rather fragile. Stem 4-8.5 cm long, 1.5-3.5 cm thick; brittle; solid, becoming hollow; white. Spore print pale yellow. Spores 7-9.5 x 6.5-9μ; globose, warts up to 1.5μ high. Edibility – Not recommended.

Russula sanguinea (Bull.) Fr.

Cap 4-11 cm across; convex when young, becoming broadly convex to flat, saucer-shaped,



Figure 3: a. *Lactarius rufus* b. *Lactarius subvellereus* c. *Lactifluus corrugis* d. *Lactifluus piperatus* e. *Lactifluus volemus* f. *Russula brevipes* g. *Russula compacta* h. *Russula crustosa* i. *Russula cyanoxantha* j. *Russula emetica* k. *Russula foetens* l. *Russula sanguinea* m. *Russula subfragiliformis* n. *Russula vesca*

sometimes with a shallow depression; sticky when wet; smooth; bright red but sometimes fading in age; margin not lined or slightly lined at maturity. Gills adnate, slightly decurrent; close; whitish when young changing creamy to yellowish or yellow. Stem 3-10.5 x 1.3-2.5; colored like the cap or paler; dry; smooth. Flesh white. Spore print creamy to yellowish. Spores 7-9 x 6.5-7 μ ; with isolated warts 0.5-1 μ high. Edibility – Inedible.

Russula subfragiliformis Murr.

Cap 4-9 cm across; convex when young and slightly convex to flat with ages; smooth; jasper red, then pinkish red, surface dry, margin incurved; perforated sometimes broadly-shallowly depressed. Stem 2.5 - 5 x 1-2 cm, hollow, fleshy fibrous, solid, dry; white then slightly pinkish red. Spore print white. Spores elliptical to ovate, 6-8.5 x 5-7 μ . Edibility – Edible.

Russula vesca Fr.

Cap 5- 10 across; globose then convex or flattened with age; variable in colour but usually a pinkish buff, wine-red or sometimes paler. Gills adnexed, brittle and fairly crowded, narrow; whitish to pale cream. Stem 3-8 cm long, 2.5- 2.5 cm thick, firm, tapered at base; white. Flesh white. Spores print white. Spores 6-8 x 5-6 μ ; ovoid, warts up to 0.5 μ high. Edibility – Edible.

Discussion and Conclusion

Fifteen species of the Russulaceae family are described in this study using morphology and a molecular approach. Some of these species are edible and consumed in some parts of India, while others are poisonous. *Lactifluus corrugis*, *L. piperatus*, *L. volemus* and *Russula subfragiliformis* are among the species that are regularly consumed and highly valued in Mizoram. Among the identified species, *R. cyanoxantha* and *R. emetica* are the dominant species.

Several species, including the rare species *Ophiocordyceps mizoramensis*²⁷ have been described from Mizoram.²⁸ However, mushroom consumption in Mizoram is still often problematic due to limited knowledge of mycology. Locals tend to consume mushrooms that resemble *Lactifluus corrugis*, *L. piperatus* and *L. volemus*, which are milk-cap species, as well as that bear a resemblance to *Termitomyces heimii*, and often leads to the consumption of some *Amanita* species. Additionally, *Russula* species with a red cap are typically consumed by peeling off the red cap, locally known as 'Pa Lengsen' due to their distinct red color. 'Pa Lengsen' is the accurate local name for *Russula subfragiliformis*.¹⁸ Unfortunately, mushroom poisoning cases are often reported in rural areas of Mizoram, many people are now aware and choose not to consume certain species due to past incidents. Cultivated mushrooms have gained popularity while the consumption of wild edible mushrooms has gradually decreased over time.

R. purpureoverrucosa cap displays a range of colors from purplish-red to grayish-magenta, adorned with small warts extending from the center to the edge. The stipe shares the cap coloration, and the basidiospores feature amyloid warts and short ridges. Particularly in terms of cap color, shape, and spore ornamentation, it bears a resemblance to *R. subtilis* (More paler in cap color). Further to this, more research studies on the diversity of *Russula* mushrooms would be extremely valuable, and further research in this area should be explored.

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