

Geejayessia ruscicola (Hypocreales, Nectriaceae), a new species on *Ruscus aculeatus*

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Abstract: *Geejayessia ruscicola* sp. nov. is described and illustrated, based on many collections on *Ruscus aculeatus* L. (Liliaceae) from France and one from U.K. Four collections of this species were cultured and sequenced. The placement of this fungus in the genus *Geejayessia* and its status as a distinct species are based on the study of its sexual and asexual morphs, phylogenetic comparison of ITS sequences with species among nine genera in the *Nectriaceae* having a fusarium-like asexual morph as well as known *Geejayessia* species.

Keywords: Ascomycota, fusarium-like, ITS, ribosomal DNA, taxonomy.

Résumé : *Geejayessia ruscicola* sp. nov. est décrite et illustrée, d'après de nombreuses récoltes sur *Ruscus aculeatus* L. (Liliaceae) en France et une provenant du Royaume-Uni. Quatre récoltes de cette espèce ont été cultivées et séquencées. Le placement de ce champignon dans le genre *Geejayessia* et son statut d'espèce nouvelle reposent sur l'étude des stades sexué et asexué ainsi que la comparaison phylogénétique des séquences ITS avec des espèces dans neuf genres de *Nectriaceae* ayant une forme asexuée de type fusarium, ainsi que les espèces de *Geejayessia* connues.

Mots-clés : ADN ribosomal, Ascomycota, fusarium, ITS, taxinomie.

Introduction

In continuing the survey of the *Nectriaceae* in temperate areas (LECHAT & APLIN, 2016; LECHAT & FOURNIER, 2015, 2016, 2017, 2020; LECHAT & PRIOU, 2008; LECHAT & ROSSMAN, 2017; LECHAT *et al.*, 2010, 2014, 2015, 2018a, 2018b, 2019, 2021) a cosmospora-like species was repeatedly collected on dead stems of *Ruscus aculeatus*. It was assigned to the *Nectriaceae* based on its reddish-yellow to red ascomata changing colour in 3% KOH or lactic acid. Its placement in the genus *Geejayessia* Schroers, Gräfenhan & Seifert was confirmed by our phylogenetic analysis. We document herein the morphological and phylogenetic differences of this species from other species of *Geejayessia*, leading to the recognition of the new species *G. ruscicola* Lechat & J. Fourn. Possible confusion with *Pycnofusarium rusci* D. Hawksw. & Punith. is discussed.

Materials and methods

Dry specimens were rehydrated and examined using the method described by ROSSMAN *et al.* (1999). Microscopical observations and measurements were made in water or lactic cotton blue. The holotype specimen and paratypes were deposited in LIP herbarium (University of Lille, France) and living cultures at CIRM (Centre International des Ressources Microbiennes, Marseille, France). Cultures of living specimens were plated on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 5 cm diam., then incubated at 25°C. After growth of cultures for 7–10 days, genomic DNA was extracted from a portion of fresh mycelium using the Nucleospin plan II kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. For the cell lysis step, the mycelium was fragmented using FastPrep-24™ 5G Benchtop Homogenizer in a lysing Matrix A tube containing the lysis buffer PL1 and RNase. The sample obtained was purified following the Nucleospin plant II protocol (steps 3 to 7). ITS5 and ITS4 primers (WHITE *et al.*, 1990) were used for PCR amplification and sequencing reaction. The ITS1-5.8S rRNA gene-ITS2 was amplified from 1 µl genomic DNA in 50 µl PCR using CloneAmp Hifi PCR Premix (Takara). An automated thermal cycler (Mastercycler, Eppendorf, Germany) was used for amplification reactions. 35 cycles of 10 s denaturation at 98°C were followed by 5 s of annealing at 55°C and 5 s of elongation at 72°C. The PCR products were checked on FlashGel™ DNA System (Lonza, Switzerland) and sequenced by Genewiz (Leipzig, Germany). Chromatograms were reviewed searching for putative reading errors, and these were manually corrected. The nucleotide sequences were deposited in GenBank. Analyses were performed online at phy-

logeny.lirmm.fr (DEREEPER *et al.*, 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (ZWICKL, 2006), using the GTR+Γ+I model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (ANISIMOVA & GASCUEL, 2006). Nomenclature follows MycoBank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Geejayessia ruscicola Lechat & J. Fourn., sp. nov.

Fig. 2

Mycobank: MB 840450

Diagnosis: Differs from all known *Geejayessia* species by having the smallest ascomata 130–180 µm high, 100–140 µm diam. vs 240–350 µm high, 180–300 µm diam. for other species of *Geejayessia* and the smallest ascospores 9–10 × 2.8–3.5 µm as well as by its occurrence at base of stems of *Ruscus aculeatus*.

Holotype: FRANCE, Deux-Sèvres, Villiers-en-Bois, la Taillée, forêt de Chizé, at the base of dead, corticated stems of *Ruscus aculeatus*, 6 Nov. 2017, leg. C. Lechat, LIP-CLL17029, ex-type culture BRFM 2592, ITS GenBank sequence: MZ509541.

Etymology: *ruscus* and the suffix *-cola* (from the Latin verb *cōlĕre* = inhabit) referring to the substratum on which this species occurs.

Ascomata solitary or in small groups of 2–5(–10) on dead sporodochia of fusarium-like asexual morph forming a basal stroma, obpyriform, 130–160(–180) µm high, 100–120(–140) µm diam. (Me = 145 × 110 µm, n = 20), laterally pinched when dry, reddish-orange, dark red to nearly black with age, becoming purple in 3% KOH, yellow in lactic acid. Basal stroma erumpent, arising from plant tissue, 50–100 µm thick, composed of hyaline hyphal elements 4.5–6 µm diam., becoming ellipsoidal to subglobose, angular, thick-walled cells 5–15 × 4–8 µm with orange to red wall to form ascomatal wall. **Ascomatal surface** composed of angular cells up to 14 µm in greatest dimension, with orange wall 1–1.5 µm thick, sometimes covered by intertwined, reddish brown, hyphal, thick-walled elements arising from base, 4 µm wide, with wall 1–1.8 µm thick. **Ascomatal apex** rounded to conical with an acute papilla, composed of cylindrical, hyaline to pale yellow cells rounded at tip 8–15 × 1.5–1.8 µm. **Ascomatal wall** 20–30 µm thick composed of two regions; outer region 15–20 µm wide composed of globose to ellipsoidal, elongate, thick-walled cells, 6–13 × 5–7 µm with orange to reddish orange wall 1.5–2 µm thick, becoming pale yellow towards interior; inner region 5–10 µm thick composed of elongate

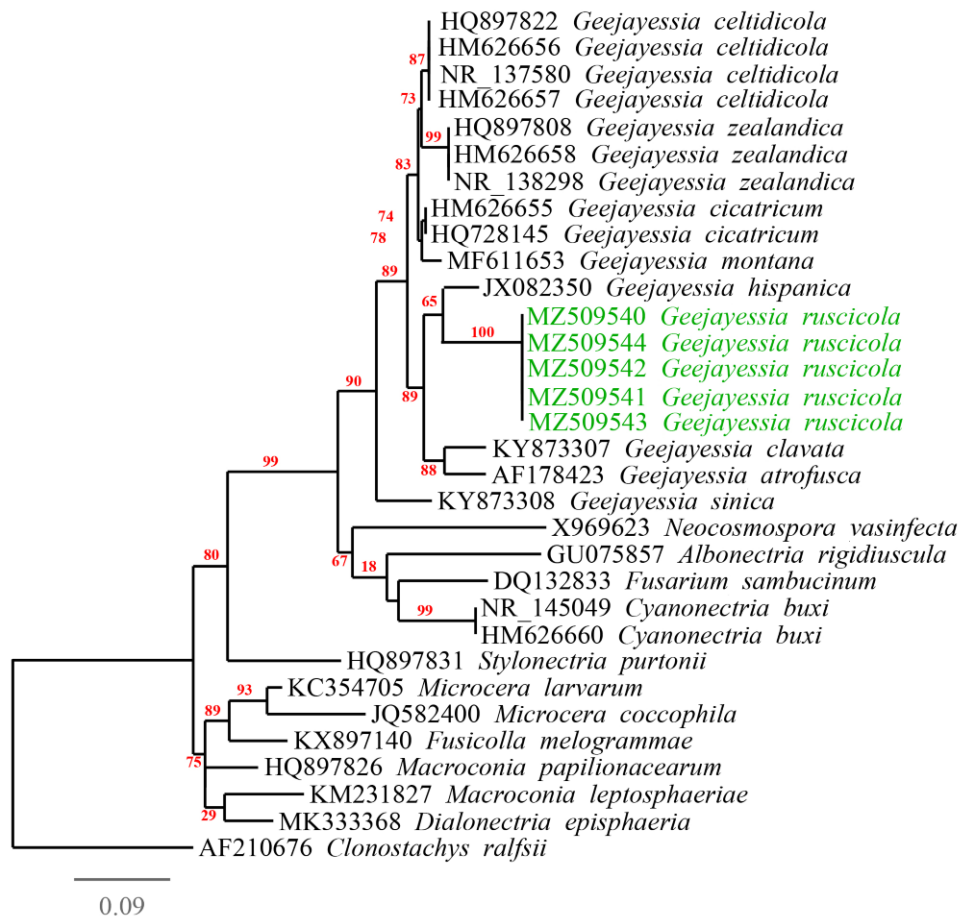


Fig. 1 – Maximum likelihood phylogeny ($-\ln L = 2972.13905$) of *Geejayessia ruscicola* inferred by PhyML 3.0, model HKY85 from a 556 bp matrix of ITS sequences, rooted with *Clonostachys ralfsii* Schroers.

cells $8\text{--}16 \times 5\text{--}8 \mu\text{m}$, with hyaline wall $1\text{--}1.5 \mu\text{m}$ thick. **Asci** narrowly clavate, short stipitate, $50\text{--}60 \times 6\text{--}8 \mu\text{m}$ ($Me = 55 \times 7 \mu\text{m}$, $n = 20$), apex flattened, with a refractive thickening, containing eight overlapping, uniseriate ascospores. **Paraphyses** narrowly moniliform, up to $8 \mu\text{m}$ wide at base, inserted between asci. **Ascospores** narrowly ellipsoidal with rounded ends, straight to slightly curved, $(8.5\text{--})9\text{--}10\text{--}(11) \times 2.8\text{--}3.5\text{--}(4) \mu\text{m}$ ($Me = 9.7 \times 3.1 \mu\text{m}$, $n = 30$), 2-celled, often with basal cell narrower than upper one, 2–3 guttules in each cell, hyaline, becoming pale yellowish-brown when mature, slightly constricted at septum, finely spinulose.

Cultural characteristics: After two weeks at 25°C , colony $2.5\text{--}3.5 \text{ cm}$ diam., producing a fusarium-like asexual morph; aerial mycelium whitish in centre, pale brown, slimy in middle area, evolving to whitish at margin. No microconidia produced; macroconidia produced in middle area, cylindrical, slightly curved, acute at tip with a narrow basal foot cell, 3-septate, $(30\text{--})42\text{--}50\text{--}(54) \times 3.5\text{--}4.2 \mu\text{m}$ ($Me = 47 \times 3.8 \mu\text{m}$, $n = 30$), identical to those from nature. Ascospores formed in culture after four weeks.

Additional specimens examined (paratypes): FRANCE. Deux-Sèvres, Souvigné, bois du Fouilloux, on dead corticated stems of *Ruscus aculeatus*, 8 Jul. 2007, *leg.* C. Lechat, LIP-CLL7172, ITS GenBank sequence: MZ509542; Villiers-en-Bois, forêt de Chizé, on dead corticated stems of *Ruscus aculeatus*, 4 Dec. 2009, *leg.* C. Lechat, LIP-CLL9238, ITS GenBank sequence: MZ509543; *Ibid.*, on dead corticated stems of *Ruscus aculeatus*, 22 Jul. 2013, *leg.* C. Lechat, LIP-CLL13146; Chizé, Le Bécassy, on dead corticated stems of *Ruscus aculeatus*, 14 Aug. 2016, *leg.* C. Lechat, LIP-CLL16064; Beauvoir-sur-Niort, Rimbault, forêt de Chizé, on dead corticated stems of *Ruscus aculeatus*, 10 Nov. 2016, *leg.* C. Lechat, LIP-CLL16098; Charente-Maritime, Vinax, forêt d'Aulnay, on dead, corticated stems of *Ruscus aculeatus*, 12 Apr. 2014, *leg.* C. Lechat, LIP-CLL14101; Ariège, Rimont,

Las Muros, on dead corticated stems of *Ruscus aculeatus*, 26 Dec. 2012, *leg.* J. Fournier, LIP-JF12124; Rimont, Peyrau, on dead, corticated stems of *Ruscus aculeatus*, 17 Jan. 2013, *leg.* J. Fournier, LIP-JF13011, ITS Genbank sequence MZ509544. UNITED KINGDOM. West Sussex, Lynwood, Tortington Common, at base of dead corticated stem of *Ruscus aculeatus*, 6 Nov. 2020, *leg.* N. Aplin, LIP-NA6112020A, ITS Genbank sequence: MZ509540.

Discussion

Most species of cosmospora-like fungi having a fusarium-like asexual morph have been classified in the *Nectria episphaeria* group (SACCARDO, 1883; BOOTH, 1959; SAMUELS, 1976; ROSSMAN, 1983) and *Nectria* subgenus *Dialonectria* Sacc. (SAMUELS *et al.*, 1991). These species have since been placed in segregate genera in *Nectriaceae* such as *Dialonectria* Cooke, *Fusicolla* Bonord., *Geejayessia* Schroers, Gräfenhan & Seifert, *Macroconia* (Wollenw.) Gräfenhan, Seifert & Schroers, *Mariannaea* G. Arnaud ex Samson, *Microcera* Desm. and *Stylonectria* Höhn. (GRÄFENHAN *et al.*, 2011).

Geejayessia was introduced by SCHROERS *et al.* (2011) for five species: *G. atrofusca* (Schw.) Schroers & Gräfenhan, *G. celidicola* Gräfenhan & Schroers, *G. cicatricum* (Berk.) Schroers, *G. desmazieresii* (Becc. & De Not.) Schroers, Gräfenhan & Seifert and *G. zealandica* (Cooke) Schroers. Recently, five additional species were introduced: *G. clavata* Z.Q. Zeng & W.Y. Zhuang, *G. hispanica* (Lechat & Priou) Lechat & J. Fourn., *G. matuoi* (Hosoya & Tubaki) Lechat & Rossman, *G. montana* Lechat & J. Fourn. and *G. sinica* Z.Q. Zeng & W.Y. Zhuang. The fungus described above represents the eleventh known *Geejayessia* species, which suggests that there are still many species to be discovered. HAWKSWORTH & PUNITHALINGAM (1973) introduced the genus *Pycnofusarium* Punith. with *P. rusci* D. Hawksw. & Punith., an

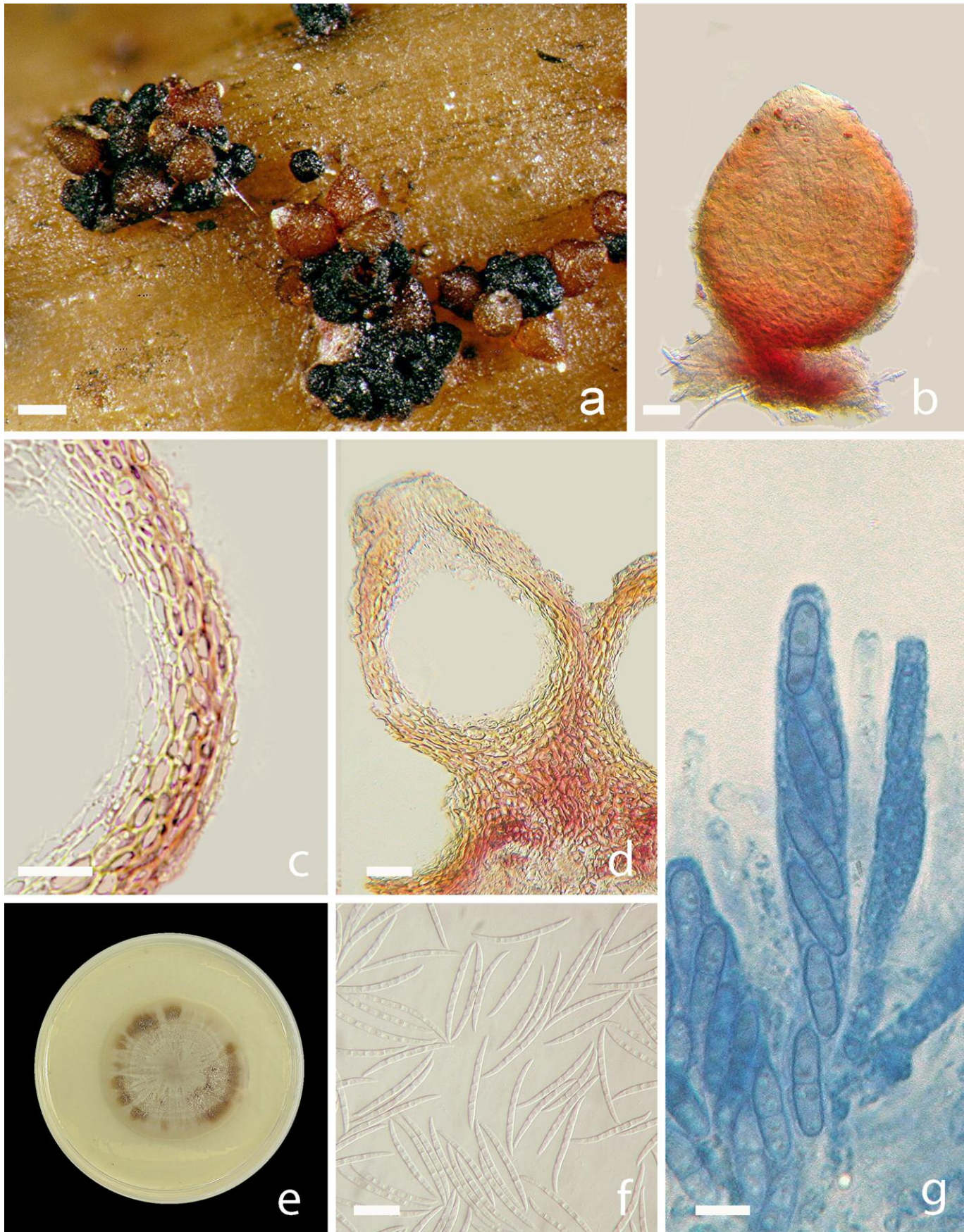


Fig. 2 – a-g: *Geejayessia ruscicola* (Holotype CLL17029); a: Red and old blackish ascomata in natural environment. b: Ascoma in water in side view; c: Vertical section of lateral ascomatal wall; d: Vertical section through ascomata and stroma in water. e: Culture at three weeks; f: Conidia from culture in water; g: Asci and ascospores in lactic cotton blue. Scale bars: a = 100 μ m; b-c, f = 20 μ m; d = 10 μ m; g = 5 μ m.

asexual morph occurring on cladodes of *Ruscus aculeatus*, which also has red sporodochia. However, *G. ruscicola* differs from it by occurring only at base of dead stems of *R. aculeatus* and having larger conidia (30–)42–50(–54) × 3.5–4.2 μm, 3-septate vs. 30–35(–45) × 2.5–4 μm, 1-septate in *P. rusci*. Most of the known *Geejayessia* species are morphologically similar and for some species host affiliation may be informative, but phylogenetic analysis is required to distinguish them accurately. *Geejayessia ruscicola* morphologically differs from known species by having the smallest ascospores. Our phylogenetic analysis (Fig. 1) shows that *G. ruscicola* is nested in the *Geejayessia* clade on a sister branch to *G. hispanica*, which primarily differs from it by having larger, smooth ascospores (LECHAT & PRIOU, 2008, as *Cosmospora hispanica*), with only 91% similarity of its ITS sequence to *G. ruscicola*. Also, *G. hispanica* occurs on *Phoenix canariensis*. Finally, *Geejayessia ruscicola* differs from known species in being a common saprobe, easily overlooked due to its small size.

Based on morphological characteristics, phylogenetic analysis of ITS sequence and comparison with known species, *G. ruscicola* appears to be a distinct species commonly encountered on *R. aculeatus* and likely host-specific for this plant. It is therefore proposed as a new species.

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