Studies in *Galiella* (*Ascomycota*, *Pezizales*). V. Typification and study of *Galiella amurensis*

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Abstract: *Galiella amurensis* is typified and described based on macro- and microscopical characters upon the study of Russian collections. A lectotype and an epitype are selected in order to fix and circumscribe the species concept. A Chinese sample is also studied and confirmed by its genetic profile and morphology. Colour images of fresh specimens and microscopical features are also given.
Keywords: *Galiella rufa*, *Sarcosomataceae*, typification.

Резюме: Проведена типификация названия *Galiella amurensis*, с целью стабилизации концепции таксона выбраны лекто- и эпитип. Дано детальное описание таксона на основе макро- и микроскопических признаков. На основании изучения морфологии и генетического профиля коллекции из Китая подтверждена ее конспецифичность с российским материалом. Приводятся также цветные иллюстрации новой находки вида из типового локалитета.

Introduction

The genus *Galiella* Nannf. & Korf, typified by the American species *Galiella rufa* (Schwein.) Nannf. & Korf, was proposed by Nannfeldt and Korf (in Korf, 1957) to accommodate all the "*Bulgaria-Sarcosoma*-like" fleshy species having warted ascospores with cyanophilic markings. History of *Galiella*, its genetic independence, and several type studies regarding species previously assigned to this genus have been treated in detail by Carbone *et al.* (2013a, 2013b, 2015) and Carbone & Agnello (2015a, 2015b, 2015c).

Galiella amurensis (Lj.N. Vassiljeva) Raitv. was described from the Russian Far East (Vassiljeva, 1950, *sub nom. Sarcosoma amurense*) and transferred into the genus *Galiella* by Raitviir (1965) due to its macro- and micromorphological characters similar to *Galiella rufa*. It has been hardly reported in the mycological literature (Vassiljeva, 1960; Vassiljeva *et al.*, 1963; Nazarova, 1965; Tai, 1979; Cao *et al.*, 1992; Bi *et al.*, 1993; Teng, 1995; Bogachova, 1996, 1998, 2001; Zhuang & Wang, 1998; Li & Azbukina, 2010; Zhuang *et al.*, 2018), and only two colour photos have been published in hardly accessible Russian and Chinese books (Azbukina *et al.*, 2006; Mao, 2000).

The rediscovery of this species by the first author in the area of Russia from which it was first described, and the presence of a syntype and other collections in the herbaria of the Komarov Botanical Institute of Russian Academy of Sciences of Saint Petersburg (LE) and the Federal Scientific Centre of the East Asia Terrestrial Biodiversity of Vladivostok (VLA), allowed us to typify it and obtain genetic sequences useful for future phylogenetic investigations on its proper position in the *Sarcosomataceae* Kobayasi through a phylogenetic analysis.

Material and methods

Morphological study. — The colour designations when given are according to Kornerup & Wanscher (1978). The microscopical studies were based on both fresh and dried specimens. Two optical microscopes were used: Olympus CX41 trinocular and Zeiss Axio Lab A1 binocular with plan-achromatic objectives 4×, 10×, 40×, 60×, 100× in oil immersion. The following main reagents were used: Melzer's reagent, cotton blue, Congo red, erythrosine, 5% KOH. Water mounts were used for the observation of the pigmentation and measurements. At least 30 ascospores were measured from each apothecium.

Studied and sequenced collections

***Galiella amurensis*.** China. Sichuan, Xiangcheng Xian, vicinity of the town of Reda, N 29°6'11", E 99°37'55", 3500 m a.s.l., dry slopes with cut over forest of *Quercus*, *Pinus*, *Berberis*, *Cotoneaster*, on rotten wood of *Picea*, 16 Jul. 1998, *leg*. and *det*. Zh.-L. Yuang (FH 545002). Russia. Primorsky Krai, Shkotovsky District, Khualaza Mt., on dead branch of *Picea*, 18 Aug. 1947, *leg*. and *det*. Lj.N. Vassiljeva (lectotype of *Sarcosoma amurense*, LE 173085; duplicate in TUR-A 209477); Sikhote-Alinsky State Nature Reserve, Venera, in spruce forest, on trunk of *Picea jezoensis*, 31 Aug. 2012, *leg*. E.A. Pimenova, *det*. E.S. Popov (LE 236216); Shkotovsky District, near the top of the Litovka (Falaza) Mt., N 43°06'22.8", E 132°46'59.7", 1100 m a.s.l., on snag of *Picea jezoensis* at ca. 2.5–3 m above the ground, 26 Aug. 2020, *leg*. and *det*. E.S. Popov (LE 323822; duplicate in TUR-A 209476). Jewish Autonomous Oblast, Obluchensky District, south spurs of the Churbukondya Mt., N 49°19'03", E 132°20'01", in mixed fir forest, on fallen trunk of *Abies*, 06 Jul. 2014, *leg.* P.V. Budilov, *det*. E.A. Erofeeva (LE 323820; duplicate in TUR-A 209474). Amurskaya Oblast, Zeya State Nature Reserve, Kamenushka River basin, on fallen trunk and branches of *Picea jezoensis*, 18 Aug. 2015, *leg*. and *det*. E.A. Erofeeva (LE 323821; duplicate in TUR-A 209476).

Other collections seen: Russia: Primorsky Krai: Vorobei Mt., spruce forest, on bark of a dead *Picea* tree, 10 Aug. 1955, *leg*. Vorobiev (VLA D-1253); Terneysky District, Sikhote-Alinsky State Nature Reserve, upper reaches of the 4th Podnebesny stream, on trunk and branches of living *Picea ajanensis*, 1 Sep. 1957, *leg*. and *det*. L.N. Vasilyeva (VLA D-1252); Khualaza Mt., on a fallen *Picea* tree, Aug 1963, *leg*. V.N. Volkov, *det*. M.M. Nazarova (VLA D-1254); Khualaza Mt., *Abies nephrolepis* forest, on fallen trunk of *Abies*, 1963, *leg*. and *det*. M.M. Nazarova (VLA D-1258); Khualaza Mt., on a fallen coniferous tree, 27 Jun. 1964, *leg*. and *det*. M.M. Nazarova (VLA D-1257); Chuguevsky District, Upper Ussuri Research Station, spruce forest, on a fallen

Table 1 – Samples sequenced during this study and GenBank codes

Species	Herbarium voucher	GenBank Accession numbers	
		ITS	LSU
Galiella amurensis	LE 236216	MW879699	MW879115
Galiella amurensis	LE 323821	MW879700	MW879116
Galiella amurensis (epitype)	LE 323822	MW879701	MW879117
Galiella amurensis	FH 545002	MW879702	–

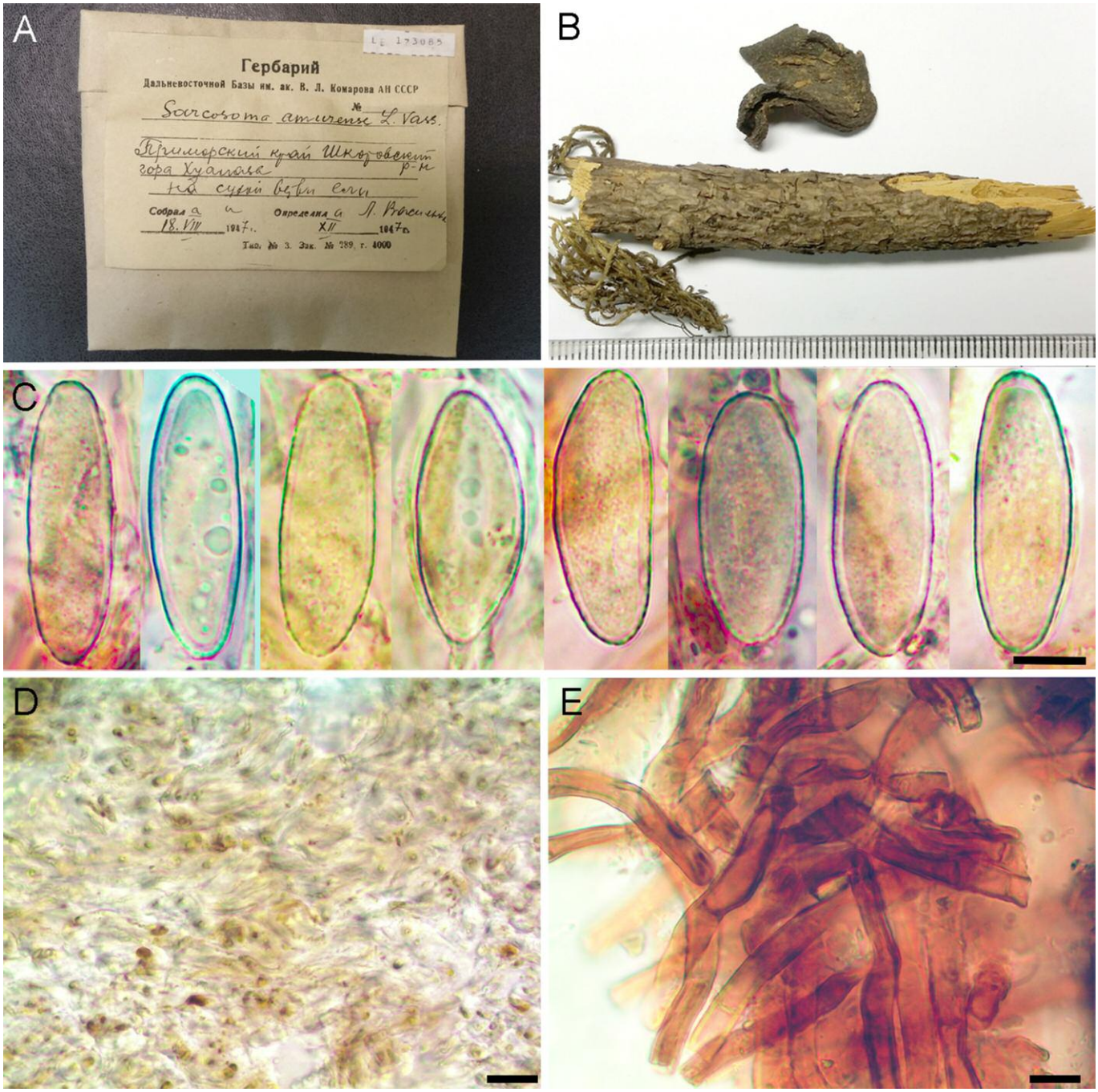

Plate 1 – *Galiella amurensis*

A, B: Lectotype of *Sarcosoma amurense* housed in LE Herbarium (LE 173085). Microscopical characters of the lectotype: C: Ascospores; D: Medullary excipulum; E: External hairs. All water mounts. Scale bars: C-E = 10 µm. Photos E. Popov.

Picea tree, 27 Jun. 1974, *leg*. and *det*. E.M. Bulakh (VLA D-1255). Khabarovsky Krai: Grand Khekhtsir State Nature Reserve, summit of the Grand Khekhtsir Range, 949 m a. s. l., on *Pinus koraiensis*, 24 Jul 2004, *leg*. and *det*. A.V. Bogacheva (VLA D-2765); Grand Khekhtsir State Nature Reserve, Sosninsky ranger station, on *Pinus koraiensis*, 24 Jul. 2004, *leg*. and *det*. A.V. Bogacheva (VLA D-2886); Anyuysky National Park, right side of Anyuy River valley, downstream of Bogbasu River mouth, 49°22′37″ N, 137°42′58″ E, mixed *Pinus koraiensis* broadleaf forest, on a snag of *Picea*, 14 Aug. 2012, *leg*. and *det*. E.A. Erofeeva (LE 323839); Anyuysky National Park, valley of Sukhaya Pad' stream, 48°50′41″ N, 138°04′16″ E, mixed forest, on a fallen branch of *Picea*, 16 Aug. 2013, *leg*. and *det*. E.A. Erofeeva (LE 323840). Sakhalinskaya Oblast: Kunashir Isl., 17 km from Yuzhno-Kurilsk along the road to Mendeleevo, Stolbovskaya ecological track, mixed forest with *Abies*, *Betula*, *Quercus*, and *Magnolia*, on a fallen *Abies sachalinensis* tree, 19 Aug. 2017, *leg*. and *det*. E.M. Bulakh (VLA D-4124).

Taxonomy

Galiella amurensis (Lj.N. Vassiljeva) Raitv., *Eesti NSV Tead. Akad. Toim., Biol. Seer*, 14: 531 (1965).

Basionym: *Sarcosoma amurense* Lj.N. Vassiljeva, *Notul. syst. Sect. cryptog. Inst. bot. Acad. Sci. U.S.S.R.*, 6: 188 (1950).

Original diagnosis: *Ascomatibus gelatinosis hemisphaericis* 15-20 *mm altis,* 40-50 *mm latis, brunneo-castaneis, villosis, pilis septatis,* 180-500 × 6-8 *µ, brunneis obtusis tectis; disco plano, pallidi ore brunneo-ochraceo, margine sterili lato, lobato; ascis cylindraceis, circa* 400 × 16 *µ,* 8*-sporis; sporis ellipsoideis hyalinis vel fuscidulis, rugulosis,* 33-36-(40) × 12-16 *µ, guttulatis; paraphysibus hyalinis filiformi bus, furcatis. Statio*. USSR: *Extremus Oriens, prope* Chabarovsk, *jugo* B. Chechcir, VII 1946; *prope* Kangaus, *mons* Chualasa, VIII 1947, *leg.*

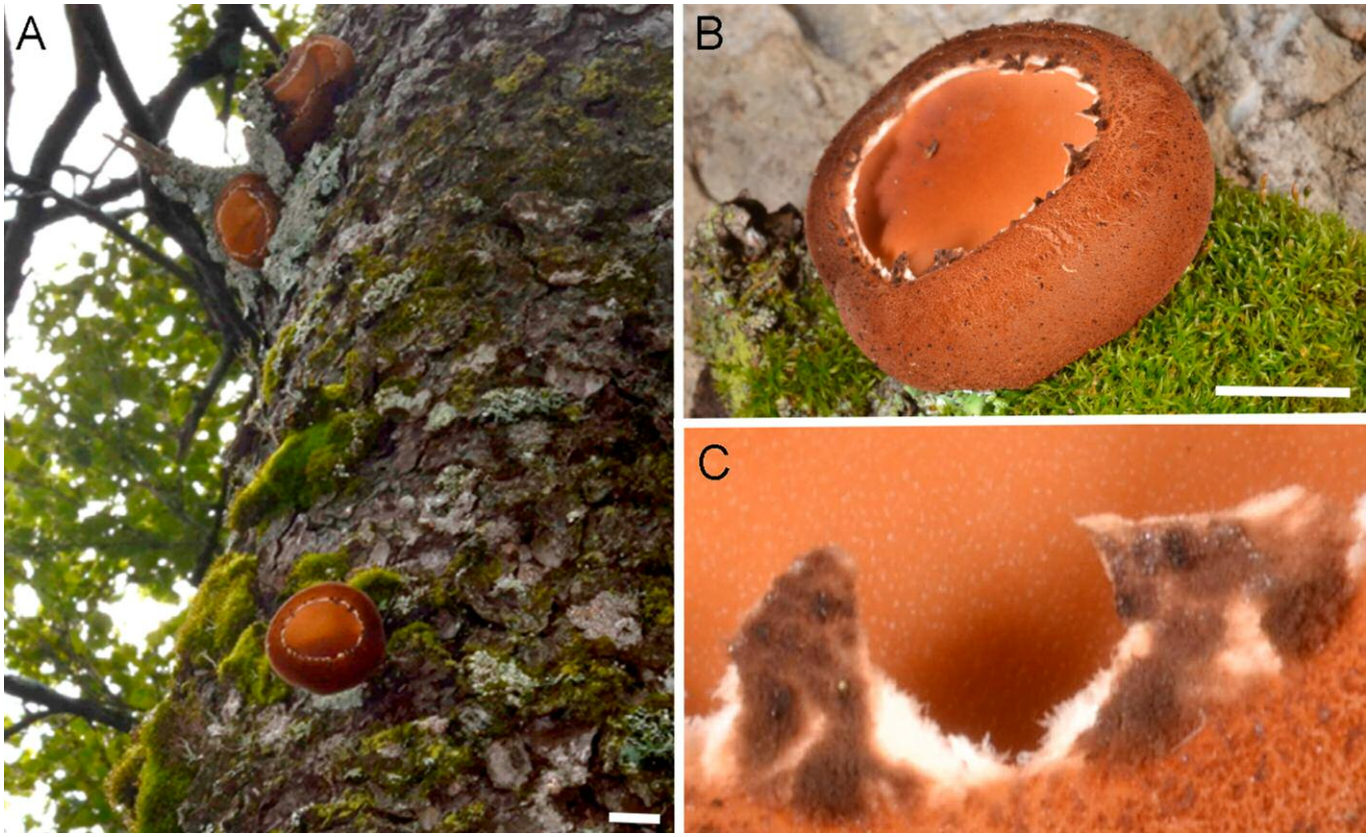

Plate 2 – *Galiella amurensis* (LE 323822)
A: Fresh fruitbodies *in situ*; B: Fresh fruitbody; C: Fragment of the margin. Scale bars: 1 cm. Photos E. Popov.

auctor; distr. Schkotovo, Maiche-Daubiche plato, VII 1947, *leg*. V.A. Rosenberg. *Ad truncos et ramis siccis Piceae jezoensis*, alt. 700-1200 m.

Typification: Vassiljeva (1950) indicated four distinct collections growing on wood of *Picea jezoensis* (Siebold & Zucc.) Carrière (syn. *P. ajanensis* Fisch. ex Carrière), made in two different years and from different stations without selecting a holotype: 1) in the vicinity of Khabarovsk, on the Grand Khechtsir Range at an altitude of 700 m, 28 Jul 1946; 2) in the vicinity of Kangauz Station, on Khualaza Mountain at an altitude of 800 m and 3) 1200 m above sea level, 19 Aug. 1947 (all three collected by herself), and 4) in the Shkotovo district, on the Maihe-Daubihe plateau at about 700 m above sea level, collected by V. A. Rosenberg in late July 1947. According to Art. 9.6 of I.C.N. (Shenzhen Code) those collections must be considered as "syntypes". Art. 9.11 indicates that "if no holotype was indicated by the author of a name of a species or infraspecific taxon […] a lectotype or, if permissible (Art. 9.7), a neotype as a substitute for it may be designated" and Art. 9.12 explains that "in lectotype designation, an isotype must be chosen if such exists, or otherwise a syntype if such exists".

The search for the Vassiljeva's original material of *Sarcosoma amurense* in herbaria of Vladivostok (VLA) and Saint Petersburg (LE) allowed us to locate only one of the four syntypes. The other three were probably lost (E.M. Bulakh, pers. comm.). We designate the only extant syntype as the lectotype of *S. amurense*.

Lectotype here designated: collection no. LE 173085 (Russia, Primorsky Krai, Shkotovsky District, Khualaza Mt.[1], on dead branch of Picea, 18 Aug. 1947, *leg*. and *det*. Lj.N. Vassiljeva), housed in the Mycological herbarium of the Komarov Botanical Institute of RAS (LE) of Saint Petersburg, Russia. MBT 10001204 (Plate 1).

Our attempts to obtain good DNA sequences from the lectotype failed repeatedly. From a phylogenetic point of view, the genus *Galiella* is nowadays represented only by the type species *G. rufa*. Its statistical support from the *Plectania* clade is still far from defined but our next study (in progress) will show that with a multigenic approach *Galiella* is definitely distinct. In this context, in order to fix the *amurensis* concept applying a clear genetic profile to this taxon, and to have a "type" available for future genetic studies, we choose to select the collection LE 323822 as an epitype.

Epitype supporting the above lectotype is here selected: collection no. LE 323822 (Russia, Primorsky Krai, Shkotovsky District, near the top of the Litovka (Falaza) Mt., 43°06'22.8"N 132°46'59.7"E, 1100 m a.s.l., on snag of *Picea jezoensis* at ca. 2.5–3 m above the ground, 26 Aug. 2020, *leg*. and *det*. E.S. Popov), housed in the Komarov Botanical Institute of Saint Petersburg (LE), Russia. MBT 10001207 (Plate 2 A-C, Plate 3 and Plate 4).

Macroscopical characters: Apothecia at first globose to urn-shaped, then concave to shallow cup-shaped, 2.5–4(–6) cm in diam. and 1–3 cm high, sessile, centrally attached to the substrate. **Hymenium** light orange to greyish orange (6A5–B6), smooth. **Margin** sterile, broad, membranaceous, incurved or more raised in age, torn into small triangular or irregular lobes as it stretches; the upper surface of the lobes light brown to brown (6D8–E8), the inner surface whitish. **Flesh** tough, rubbery and gelatinous, tan brown, brownish orange to blackish brown in age. **External surface** reddish orange

[1] In the protologue this specimen is said had been collected "*prope* Kangaus, *mons* Chualasa". Former Kangauz station is now part of Anisimovka, Primorsky Krai, and located 32 km north-east of the Khualaza Mt. (now Krinichnaya Mt.), but only 7.5 km north of the former Falaza Mt. (now Litovka Mt.) to which the station is directly connected by a road. Moreover, Khualaza Mt. is only 829 m high, while Falaza Mt. is 1279 m high which is in agreement with an altitude 1200 m stated in the protologue. It is also important to note that the Biological Station of the Far Eastern University was located in Kangauz. All of the above allows us to be confident enough that the syntype collection place was Litovka Mt. The confusion of the names of these two peaks by many Soviet and Russian biologists was also noted by Lafer (1996: 396–397). The second not so significant contradiction is the collection date, which is said to be 19 Aug. 1947 in the protologue, while on original label the collection date is 18 Aug. 1947.

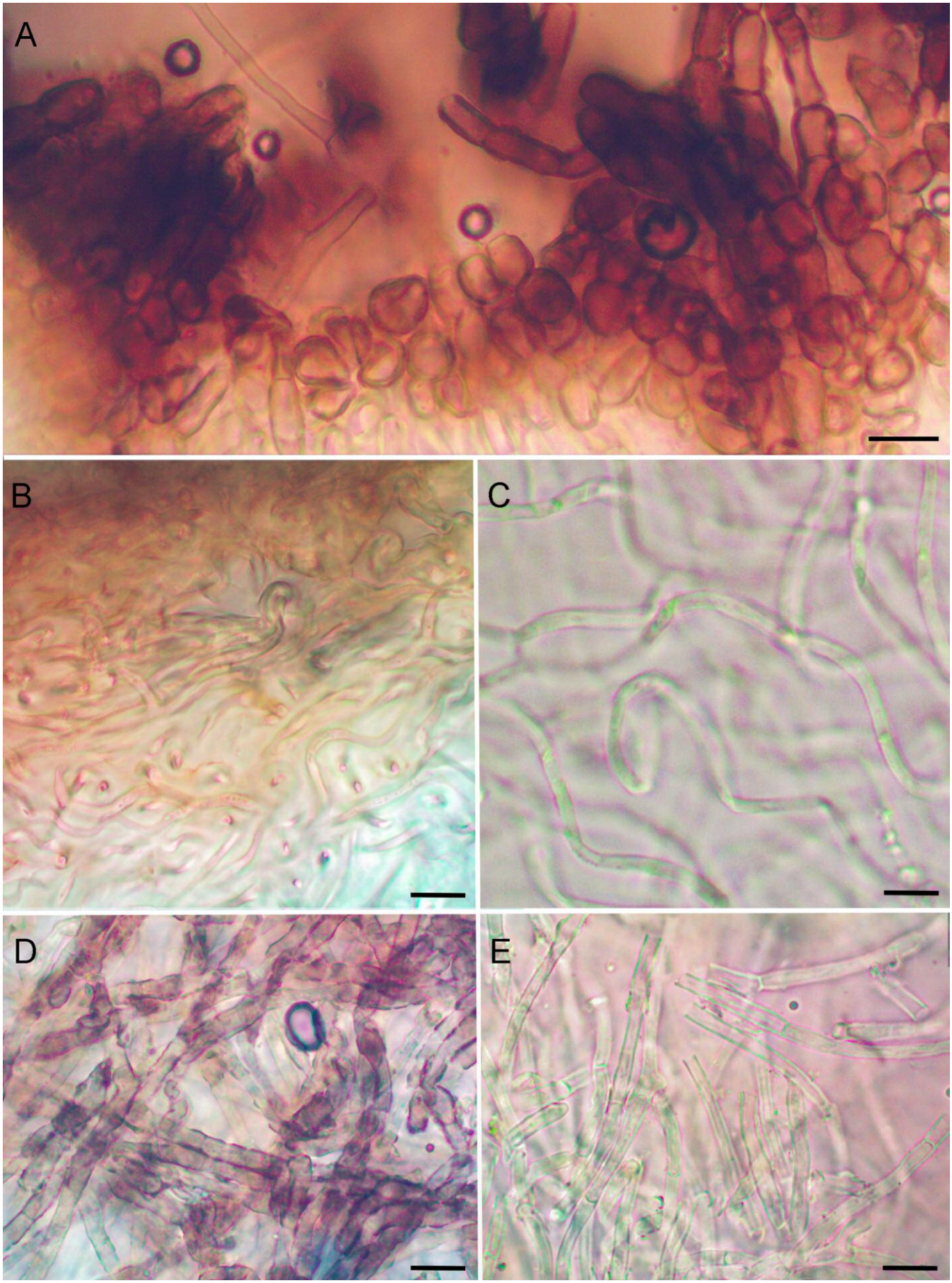

Plate 3 – *Galiella amurensis* (LE 323822)

A: Ectal excipulum; B: Subhymenium; C: Medullary excipulum; D: Brown incrusted hyphae from the upper surface of marginal lobes; D: Hyaline hyphae from the internal surface of marginal lobes. All water mounts. Scale bars: 10 µm. Photos E. Popov.

(6C7–C8) to blackish brown when old, often wrinkled in larger and older fruitbodies, with a dense covering of fasciculate hairs forming minute darker brown warts (Plate 2).

Microscopical characters: Asci ca. 400–450 µm long, *pars sporifera* 250–270 × 12–17 µm, cylindrical, operculate, inamyloid, eight-spored, with wall up to 1.5 µm thick, with a tapered, flexuous, aporhynchous base. **Paraphyses** not exceeding the asci, 1.5–2 µm wide, hyaline, cylindrical, septate, sparsely anastomosing and branched, with a simple apex, slightly undulated, or, in few cases, lobed, glued with amorphous amber brown extracellular pigment in the upper part of dried specimens observed in water mounts. **Hymenial hairs** absent. **Ascospores** ellipsoid or subfusoid, (24.5–)29.2–38.2(–43.2) × (10.4–)12.4–14.5(–16.2) µm, Me = 34 × 13.5, Q = (1.7–)2.3–2.6(–3.6), Qm = 2.5 (n=270), hyaline, minutely warted (if seen in heated lactic Cotton Blue mounts), with granular content and two large oil drops, wall up to 1 µm thick. In young asci, developing ascospores are globose. **Subhymenium** ca. 75–125 µm thick, composed by a dense *textura intricata* of cylindrical, septate hyphae 5.0–7.5 µm wide, thick-walled (1.8–4 µm), light brown if seen at low magnifications. **Medullary excipulum** very gelatinous, of a very loose *textura intricata* with cylindrical, septate, hyaline, slightly thick-walled (0.5–0.8 µm), branched and anastomosing hyphae, 2.5–3.5 µm wide, immersed in a hyaline gelatinous matrix; crystalline inclusions absent. At upper flanks and at margin, medullary excipulum not gelatinous, of dense hyaline *textura intricata*. Upper surface of marginal lobes formed by loose light brownish hyphae, 2.3–4.6 µm wide, slightly thick-walled (0.3–0.5 µm), densely incrusted with brown amorphous epiparietal pigment; inner part of lobes of *textura intricata* with hyaline, non-incrusted slightly thick-walled (0.3–0.4 µm) hyphae 2.1–6.0 µm wide (Fig. 1). **Ectal excipulum** very thin, sometimes very difficult to observe, ca. 20 µm thick, of *textura subglobulosa* to *angularis* made up of elements up to 15 µm wide, dark brown due to the coloured thick wall (0.8–1.0 µm). **External hairs** single or fasciculate, especially at margin, cylindrical, septate, smooth, 6.5–9.0 µm wide, up to 500 µm long, sinuous or straight, with blunt tips. They are light brown due to the coloured thick wall, thickened up to 1.0 µm (Plate 3, 4).

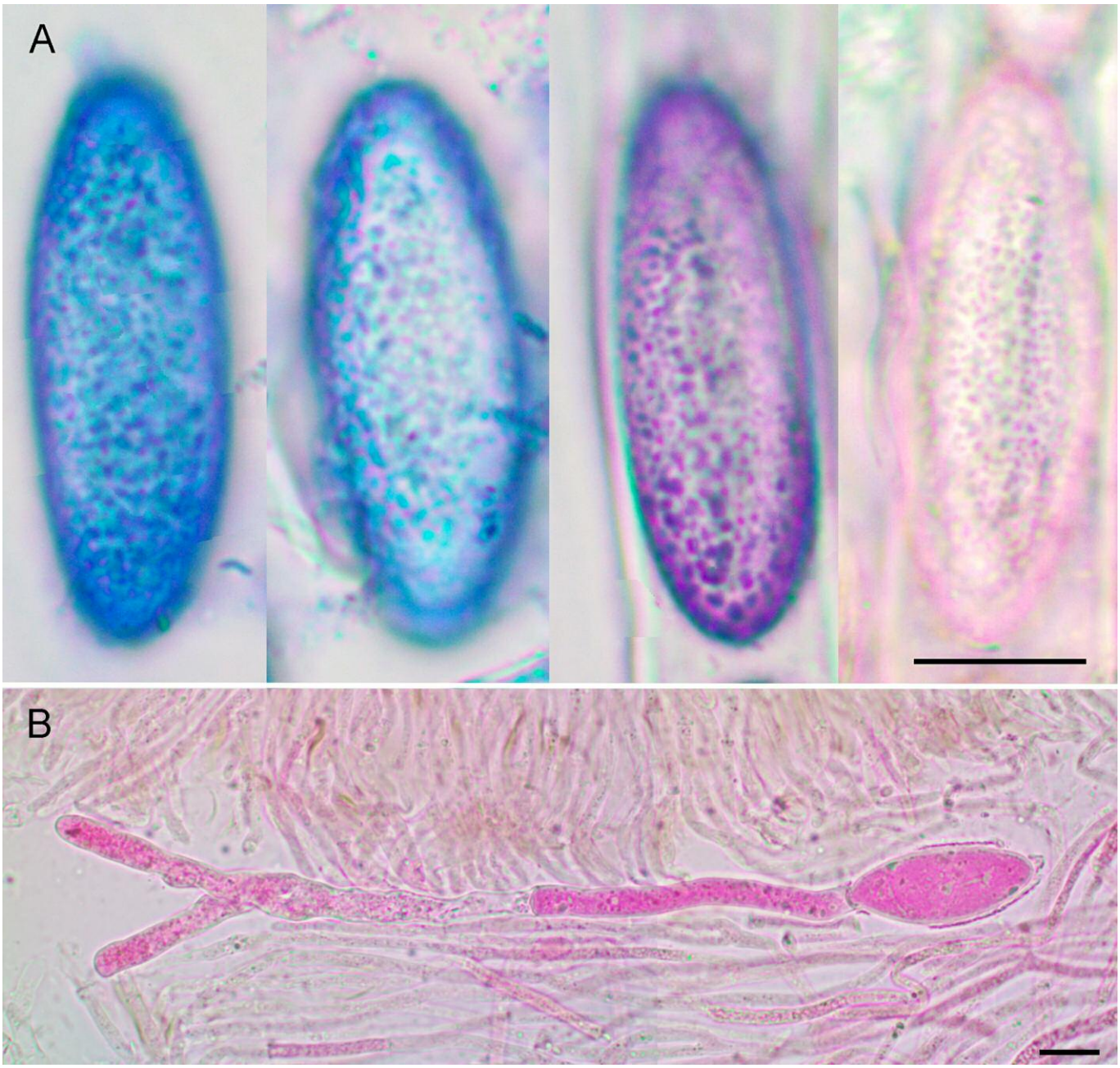

Plate 4 – *Galiella amurensis* (LE 323822)
A: Ascospores ornamentation stained in cotton blue (two on the left) and in erythrosine (two on the right); B: Germinating ascospore stained with erythrosine. Scale bars: 10 µm. Photos E. Popov.

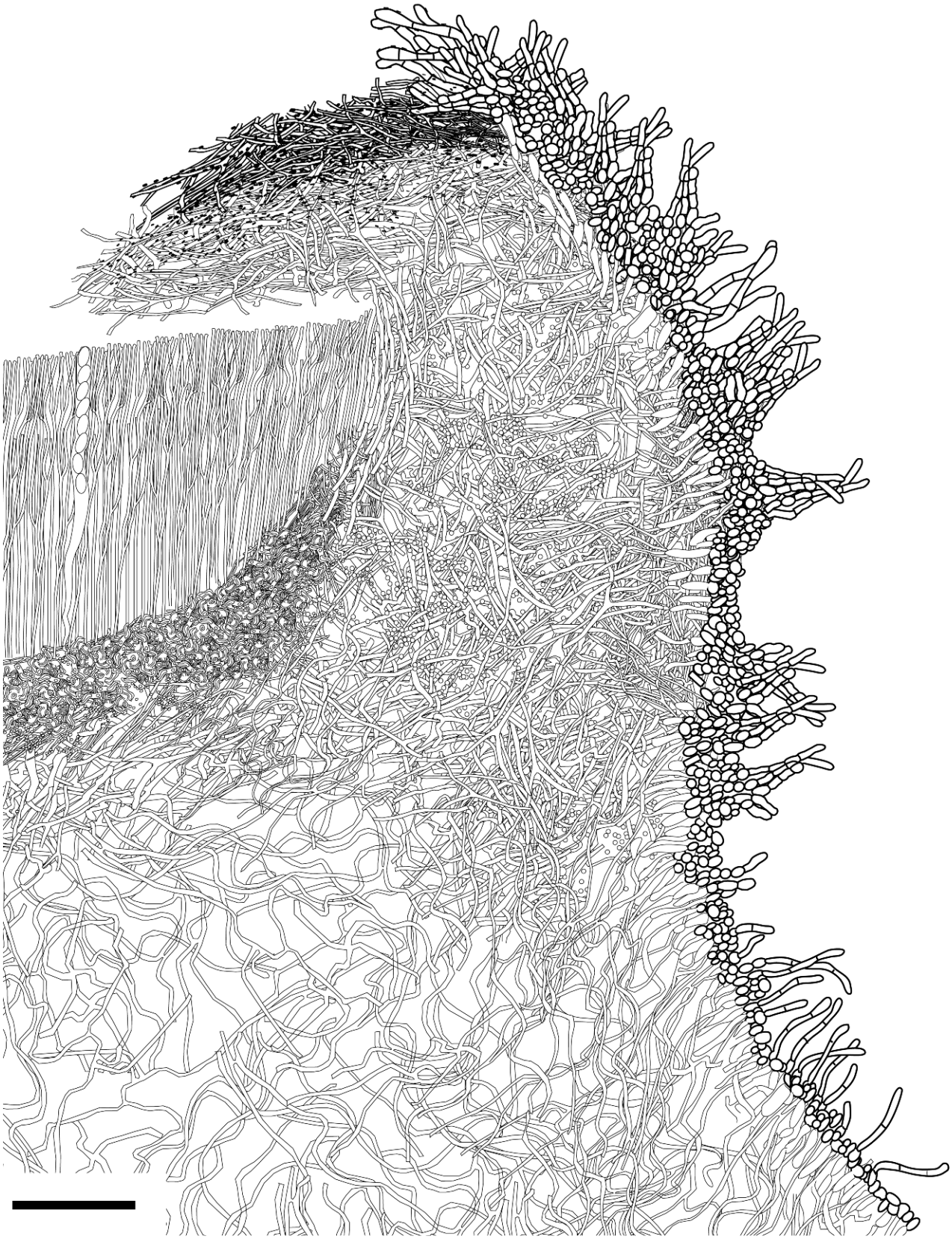

Fig. 1 – *Galiella amurensis*
Diagrammatic longitudinal section through the upper flank and margin of an apothecium. Scale bar = 200 µm. Drawing E. Popov.

Plate 5 – *Galiella amurensis* (FH 545002)

Up left: fresh samples *in situ* (photo by Dr. Zhuliang Yang). Remaining: dried samples and notes on the herbarium sheet (photo by M. Carbone).

Ecology, phenology and known distribution: *Galiella amurensis* is a wood-inhabiting ascomycete growing chiefly on wood of *Picea* especially that of *P. jezoensis*. A few records on *Abies nephrolepis* (Trautv.) Maxim., *A. sachalinensis* (F. Schmidt) Mast., and *Pinus koraiensis* Siebold & Zucc. are also known (see in the "other collections seen") although misidentification of the substrate cannot be completely ruled out in these cases. Apothecia develop mainly on dead but still bark-covered logs, snags and larger branches, sometimes several meters above the ground, rarely on still living trees (Vassiljeva, 1960; Nazarova, 1965). Based on the studied collections and published data, fruiting of *G. amurensis* lasts from June until October, though most of the known records (36 of 42) fall in August and September.

Galiella amurensis is widely distributed in mountainous areas of temperate East Asia where it is confined to montane coniferous forests at an altitude of ca. 700–3500 m from the Tukuringra and Sikhote-Alin Ranges in the Russian Far East to the Hengduan Mountains in southwest China (Fig. 2). The absence of records from Japan is noteworthy given the fact that it has been recorded on the bordering Kuril Islands. According to Nazarova (1965), *G. amurensis* is quite common in the belt of coniferous forests of the Sikhote-Alin Range at an altitude of ca. 1000 m, where it grows often in large numbers. The species is also said to be common in China (Zhuang & Wang, 1998).

The record of *G. amurensis* "on the faded twigs and fallen leaves" from Nanxiong county (Guangdong, China) reported by Bi *et al.* (1993) is far beyond the range of *Picea* and *Abies* (Fig. 2) and appears to be based on misidentification. The same can apparently be said of the Vietnamese record by Kiệt (2011), which is most probably a *Trichaleurina* sp. judging from published illustrations.

Discussion

According to a multigenic study on the family *Sarcosomataceae* (in prep., data not shown here), *Galiella amurensis* definitely belongs to the genus *Galiella* due to its clustering with the type species *Galiella rufa*. At present it is the second species that could be genetically confirmed in this genus.

From a morphological point of view, *Galiella amurensis* and *G. rufa* share the general habit, colours, a reduced to very thin ectal excipulum, and ascospores which are finely warted on all their surface. Besides their different geographical distribution, *Galiella amurensis* differs also very much from *G. rufa* in definitely larger ascospores, (17–) 18–21 (–23) × 8–10 µm, in *G. rufa* (Carbone *et al.*, 2015), and the lack of hymenial hairs of *Sarcosomataceae* (i.e. a wider, not-septate second type of paraphyses). Lacking of hymenial hairs is really surprising if we consider that they are present in all the well-known and well-studied species of the family *Sarcosomataceae*, and it was suggested by Carbone *et al.* (2013a) to be a unique and distinguishing feature of the family *Sarcosomataceae*. We performed many microscopic observations but we were not able to find them in any of the collections studied.

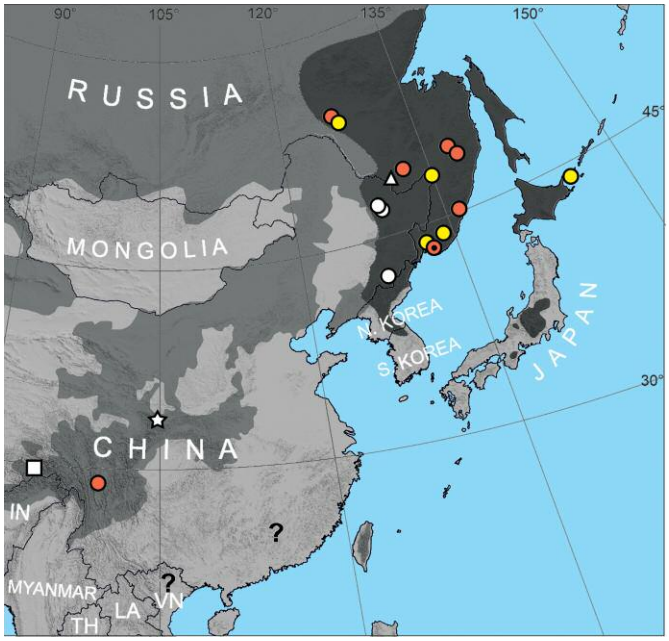

Fig. 2 – Map showing known distribution of *Galiella amurensis*. Red circles denote collections for which DNA and/or micromorphological data were examined (dot in the centre of an icon indicates lectotype locality); yellow circles denote collections for which only photos were available; white icons denote populations known only from published data (triangle: Raitviir, 1965; star: Tai, 1979; circle: Cao *et al.*, 1992; square: Zhuang & Wang, 1998); question marks designate dubious records by Bi *et al.*, 1993 and Kiệt, 2011. The shaded area corresponds to natural distribution range of *Picea*, the range of *P. jezoensis* is shown in darker grey (compiled after Fang *et al.*, 2011; Krestov & Nakamura, 2002; Nakamura & Krestov, 2005). Drawing E. Popov.

The ectal excipulum is very thin and in some cases it was very difficult to observe. This situation is reminisenct of the holotype *Galiella thwaitesii* (Berk. & Broome) Nannf. where the ectal excipulum was found to be absent or at least a simple extension of the medullary excipulum where darker hyphae are mixed with the basal part of the external hairs (Carbone & Agnello, 2015b).

The Chinese sample FH 545002 (Plate 5) possesses exactly the same microscopical characters found in the Russian samples, above all the lack of hymenial hairs and the difficulty in distinguishing a well-defined ectal excipulum. In addition its identity is confirmed by the ITS sequence successfully obtained after many attempts.

Acknowledgements

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