Notes on some Species of *Chloroscypha* Endophytic in Cupressaceae of Europe and North America

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Introduction

The genus *Chloroscypha* was proposed by Seaver in 1931, based on a fungus collected on *Thuja plicata* D. Don. He included *C. seaveri* Rehm ex Seaver as the type and three other species, viz. *C. chloromela* (Phill. & Hark.) Seaver, *C. jacksonii* Seaver [= *C. enterochroma* (Peck) Seaver] and *C. juniperina* (Ellis) Seaver [= *C. sabinae* (Fuck.) Dennis]. In 1938 Seaver added another species, *C. cedrina* (Cooke) Seaver. Later, in 1943, he transferred all *Chloroscypha* species to *Kriegeria* Rabenhorst. Seaver considered *Ombrophila kriegeriana* Rabh. to be the type-species of *Kriegeria* Rabh. However, as pointed out by Dennis (1954, 1956), *Kriegeria* has not been validly published by Rabenhorst, who only proposed to erect a new genus for *Ombrophila kriegeriana*, and suggested the name *Kriegeria* ad interim. In addition, at the time Seaver adopted it, this name was already occupied by *Kriegeria eriophori* Bresadola (1891), belonging to the Tuberculariaceae (Auriculariales according to von Höhnel, 1909). Thus the name *Chloroscypha* Seaver has been re-established by later authors. For a detailed account of the nomenclatural problem see Dennis (1954) and Kobayashi (1965). Ten species have been described, all of them occurring on Cupressaceae.

Species of *Chloroscypha* are suspected to be weak pathogens of Cupressaceae (Gremmen, 1963; Kobayashi, 1965), however, the extent of their pathogenicity is not known yet. Reinoculation experiments have not been conducted because it has not been possible to obtain species of *Chloroscypha* in pure culture. Petrini & Carroll (1981) repeatedly isolated species of *Chloroscypha* from healthy-looking needles and twigs of *Thuja plicata* D. Don, *Chamaecyparis lawsoniana* Parl. and *Sequoia sempervirens* (D. Don) Endl. Since that time, *C. sabinae* (Fuck.) Dennis was isolated from apparently healthy needles of *Juniperus nana* Willd. in Switzerland.

A brief account is presented of the cultural characteristics of *Chloroscypha*-species which were isolated, together with notes on the taxonomy of European and North American species.

Material and Methods

4% KOH was used as a rehydrating agent for dried herbarium specimens as well as to make microscopic mounts of the material studied. The iodine reaction of the ascus plug was studied after KOH (4%)-pretreatment by adding drops of Melzer's reagent to the microscopic mounts.

To study endophytism, small twigs or needles of *Chamaecyparis lawsoniana*, *Thuja plicata*, *Sequoia sempervirens* and *Juniperus nana* were surface sterilized following the method of CARROLL et al. (1977). Needles and twig segments were dipped for 1 min in 96% ethanol, immersed for 5 min in a solution of 1 part 14% v/v (aq) sodium hypochlorite to 2 parts water, then dipped again for 30 sec in 96% ethanol. Needles were cut into 2 segments and twigs into pieces 3—5 mm long and transferred to 12 cm Petri dishes containing 2% malt extract agar (MA). Plates were incubated at 18°—20° C; isolation of fungi from the plates to 2% Malt extract slants was carried out by direct transfer of mycelial fragments. Fruiting cultures were deposited at the ZT culture collections as well as at CBS, Baarn.

Developmental studies in culture

The development of the apothecia was studied directly on MA plates containing surface-sterilized foliage of the host plant. The apothecia generally formed within 3—4 weeks; primordia are formed within two weeks: the apothecial development of the species studied is gymnocarpous. From the beginning the apothecia are composed of elongate cells forming textura porrecta to textura oblita. The apothecia are at first surrounded by a gelatinous sheath or immersed in a gelatinous, green matrix. After the ripening of the asci and ascospores the apothecia become dry and olive-green to black at complete maturity.

C. chloromela remained sterile until about 4 months after isolation of the fungus in pure culture, but its development followed the patterns described for the other species. All attempts to obtain cultures from ascospores, either from freshly collected apothecia or from apothecia formed in culture failed: ascospores germinated readily on malt agar, producing germ tubes usually from the apex. However, the germinated ascospores failed to develop further.

Taxonomy

Chloroscypha SEAVER, Mycologia 23: 248. 1931.

= *Kriegeria* RABENHORST, Hedwigia 17: 32. 1878 (non *Kriegeria* BRESADOLA, Rev. Mycol. 49: 14. 1891).

Helotiales, Leotiaceae, Leotioideae, Leotieae.

Apothecia gregarious or scattered, sessile or stipitate, development gymnocarpous. Excipulum composed of long-celled hyphae,

textura oblita to textura porrecta. Apothecia fleshy, green to black at maturity, with gelatinized hyphae in the outermost layer. Asci 8-spored, cylindrical to slightly clavate, with a more or less distinct apical apparatus, blued or not blued by iodine. Ascospores large, one-celled or rarely one-septate, fusiform to broadly ellipsoidal, with granular contents, hyaline at first, sometimes slightly green when ripe. Paraphyses slender, simple or branched, surrounded by a green matrix.

Type species: *Helotium seaveri* SEAVER; On *Thuja plicata* Libby, Montana, USA, leg. Weir (BPI, FH, ZT, UPS), Fig. 5a.

Key to the *Chloroscypha*-species described in this paper

1. Ascospores ovoid, broadly fusoid to clavate-ovoid 2
1′. Ascospores ellipsoid-fusoid to almost acicular, sometimes slightly curved 4
2. Ascospores broadly ovoid to ellipsoid, (15)18—25×(6)9—11 µm; asci with a distinct apical plug, tapering rather abruptly at apex; ascus apex mainly blued, sometimes not blued by iodine after KOH-pretreatment. On *Juniperus* ..... 4. *C. sabinae*
2′. Ascospores broadly fusoid to clavate, sometimes slightly apiculate, 15—28×6—12 µm, ascus apex not blued by iodine after KOH-pretreatment. On *Thuja* and *Chamaecyparis* ..... 5. *C. seaveri* 3
3. Ascospores (18)20—28×8—12 µm. On *Thuja* ..... *C. seaveri* f. *seaveri*
3′. Ascospores 15—24×6—9 µm. On *Chamaecyparis* ..... *C. seaveri* f. *lawsoniana*
4. Ascospores ellipsoid to broadly fusoid, sometimes slightly asymmetrical, 16—23×5—8 µm; tip of the paraphyses often two-celled, constricted at the septum; ascus apex blued by iodine after KOH-treatment. Usually on *Calocedrus*, occasionally on *Juniperus* ..... 1. *C. alutipes*
4′. Ascospores narrowly fusoid to acicular 5
5. Ascospores narrowly fusoid to acicular, rarely slightly clavate, usually slightly curved, 17—30×4—6 µm; ascus apex blued by iodine after KOH-treatment. On *Sequoia* ..... 2. *C. chloromela*
5′. Ascospores narrowly fusoid, slightly pointed at one end, 19—24×6—9 µm; ascus apex not blued by iodine after KOH-treatment. On *Thuja* ..... 3. *C. enterochroma*

Descriptions

1. *Chloroscypha alutipes* (PHILL.) DENNIS, Persoonia **3**: 34. 1964. — Fig. 1.
   = *Peziza alutipes* PHILL., Grevillea *7*: 23. 1878.
   = *Phialea alutipes* (PHILL.) SACC., Syll. fung. *8*: 266. 1889.
   = *Kriegeria alutipes* (PHILL.) SEAVER, North American Cup-fungi (Inoperculates): 103. 1951 (invalid name).

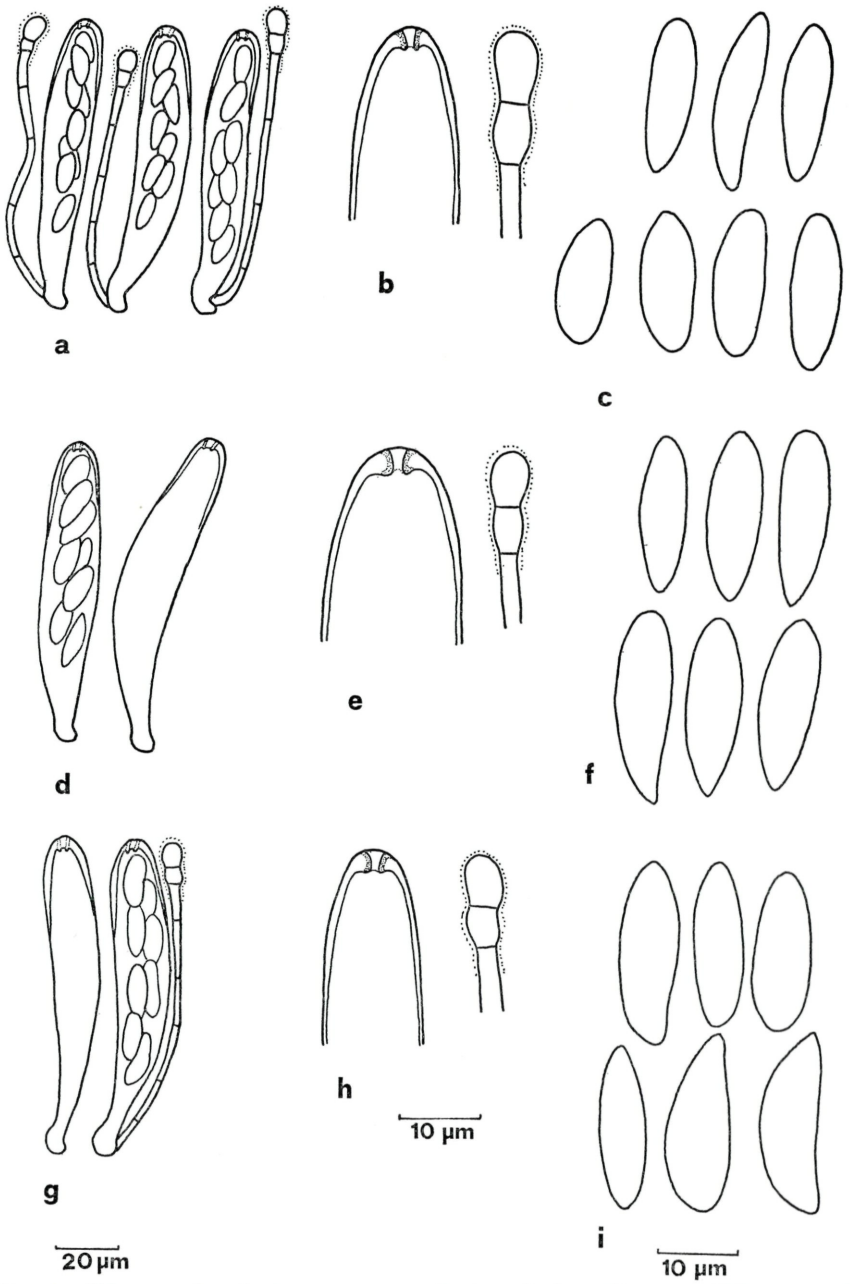

Fig. 1. *Chloroscypha alutipes*. a—c: as *Peziza alutipes* (Isotype, FH): a, asci and paraphyses; b, ascus tip as seen in the phase contrast microscope and tip of the paraphyses; c, ascospores. d—f: as *Kriegeria juniperina*, (Bonar, FH, GE). d, asci; e, ascus tip as seen in the contrast of phase microscope and tip of the paraphyses; f, ascospores. g—i: *Chloroscypha alutipes* (Horak 78/186, ZT). g, asci; h, ascus tip as seen in the contrast of phase microscope and tip of the paraphyses; i, ascospores

Apothecia gregarious, rarely solitary, stipitate or rarely subsessile, pale to darker brown, yellow-green in transmitted light, — —2 mm across; stipe up to 2—3 mm long, gradually expanding to form the apothecium. Asci clavate, with a distinct apical pore blued by iodine after KOH-treatment, 90—120×12—15 µm. Ascospores ellipsoid to broadly fusoid, sometimes slightly asymmetrical, becoming slightly yellow when aging, 16—23×5—8 µm. Paraphyses filiform, septate, abruptly enlarged above, tip of the paraphyses often two-celled, slightly constricted at the septum; the tips surrounded by a yellow-brown substance.

Host. — *Calocedrus decurrens* (Torr.) Florin, *Juniperus occidentalis* Hook. and *J. sabina* L.

Collections examined:

USA: California: blue Canyon, on dead Cedar leaves, leg Harkness, Ellis collection (Isotype, FH). — Oregon: Lane Co., Shotgun Creek Recr. Area, on *Libocedrus decurrens*, 13. 3. 1979, leg. Sherwood & Pike (FH). — California: Alpine Co., Hermit Valley, on dead shoots of *Juniperus occidentalis* Hook., 2. 8. 1952, leg. Bonar (FH, GE).

Switzerland: Graubünden, Ramosch (Plattamala), an faulenden Zweigen von *Juniperus sabina*, 8. 9. 1978, leg Horak (ZT, first collection in Europe).

2. *Chloroscypha chloromela* (Phill. & Hark.) Seaver, Mycologia 23: 250. 1931. — Fig. 2a—e.

= *Peziza chloromela* Phill. & Hark., Grevillea *13*: 22. 1884.
= *Chlorosplenium chloromelum* (Phill. & Hark.) Sacc., Sylloge Fung. *8*: 319. 1889.
= *Kriegeria chloromela* (Phill. & Hark.) Seaver, Mycologia *35*: 493. 1942. (invalid name).

Apothecia scattered, solitary, rarely gregarious, shortly stipitate, up to 0.8 mm across, green-black. Stipe up to 1 mm long. Hymenium plane, yellowish green. Asci clavate to cylindrical, with a distinct apical pore turning blue with iodine after KOH-treatment, 75—90(110)×(9)13—14 µm. Ascospores fusoid to broadly acicular, rarely slightly clavate, usually slightly curved, at first hyaline, green when ripe, (14)17—30×3—6 µm. Paraphyses hyaline, septate, simple to forked, tip of the paraphyses often swollen.

Host. — *Sequoia sempervirens* (D. Don) Endl.

Collections examined:

USA: California: Humboldt Co., Spruce Cove, Trinidad, on *Sequoia sempervirens*, 4. 1. 1947, leg. Parks, 6754 (BPI, FH). — California: Humboldt Co., Orick, Prairie Creek State Park, on *S. sem-*

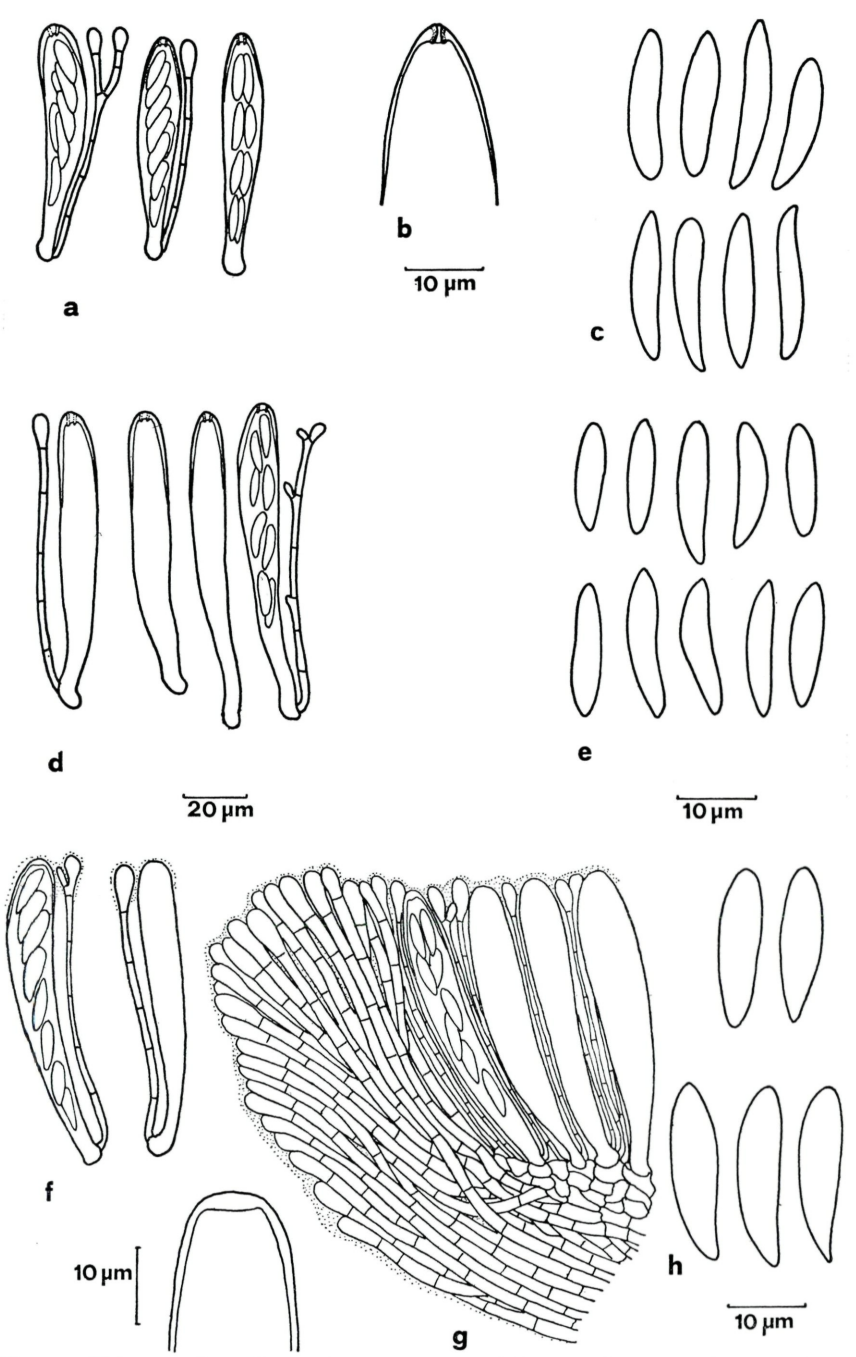

Fig. 2. *Chloroscypha chloromela* (a—e) and *Chloroscypha enterochroma* (f—i). a—c: *Chloroscypha chloromela* (PARKS no. 6754, FH). a, asci and paraphyses; b, ascus tip as seen in the contrast of phase microscope; c, ascospores. d—e: *C. chloromela* in culture, (coll. Sg 1.32, ZT). d, asci and paraphyses; e, ascospores. f—i: *Chloroscypha enterochroma* (DURAND Herbarium, no. 5697, Isotype, FH). f, asci and paraphyses; g, section of the ectal part of an apothecium; h, ascospores; i, ascus tip as seen in the phase-contrast microscope

pervirens, November 1935, leg. PARKS, 5714 (BPI, GE, UPS). — California: Marin Co., 3—5 miles west of Mill Valley, on panoramic road from Stinson Beach, on young tree of *S. sempervirens*, 9. 4. 1960, leg. TAVARES (UPS, GE): the spores of this collection are very small (18—20×5—6 µm). — California: Del Norte Park, on *S. sempervirens* twigs, 12. 5. 1937, leg. SMITH, 9409 (FH). — California: Humboldt Co., Spruce Cove, Trinidad, on *S. sempervirens*, december 1939, leg PARKS, 6379 (FH). —

USA, California: Cultures ZT 8980, 8981, 8982, 8983, 8984, 8985 isolated from needles of different plants collected in Klamath and Crescent city, december 1979, leg. KLIEBER & PETRINI (ZT, CBS). About 20 more collections were studied but not kept.

C. chloromela is a very common endophyte of *S. sempervirens*. CARROLL & CARROLL (1978) reported the infection of *Sequoia* needles by *C. chloromela* to be as high as 97%. I isolated this fungus from samples collected at different sites in northern California; however, only about 20% of the total isolates produced ripe asci and ascospores. The cultures are very slow-growing, reaching 4 cm diameter after 4—5 weeks; the aerial mycelium is scanty, white to green. Usually a green pigment is excreted into the agar very soon. Sterile apothecia are formed within 2 months. Incubation at 18° C induces ascus ripening and ascospores formation within 4 months. Apothecia, asci and ascospores produced in culture do not show major differences from those produced on host material; however, asci are generally more slender (100—110×9—12 µm) and ascospores tend to be smaller (14—20×3—4 µm) in culture than they are on the host plant.

3. *Chloroscypha enterochroma* (PECK) PETRINI, comb. nov. — Fig. 2f—i.

= *Peziza enterochroma* PECK, Ann. Rep. N. Y. State Museum *32*: 47. 1879.
= *Ombrophila enterochroma* (PECK) SACC., Syll. fung. *8*: 619. 1889.
= *Kriegeria enterochroma* (PECK) SEAVER, North American Cup-fungi (Inoperculates): 101. 1951.
= *Chloroscypha jacksonii* SEAVER, Mycologia *23*: 249. 1931.
= *Kriegeria jacksonii* (SEAVER) SEAVER, Mycologia *38*: 493. 1943.

Apothecia solitary, scattered, narrowly stipitate, up to 5 mm tall, stipe more or less curved, longitudinally wrinkled, dark brown with a dinstinct green cast; apothecial disc up to 2 mm across, hymenium slightly concave, waxy, very dark brown. Asci clavate, 100—110×10—12 µm, apical pore indistinct, not blued by iodine after KOH treatment. Ascospores fusiform, slightly pointed at one end, 19—24×6—9 µm, hyaline to very faintly green when ripe. Paraphyses filiform, simple to rarely branched, septate, swollen at the tip.

Host. — *Thuja occidentalis* L., *Calocedrus decurrens* (TORR.) FLORIN.

Collections examined:

CANADA: Ontario: Lake Temagami, T. F. R., on *T. occidentalis*, 9. 9. 1935, leg. JACKSON (FH, BPI). — Ontario: Bear Island, Lake Temagami, T. F. R., on *T. occidentalis*, 16. 9. 1930, leg. JACKSON (FH). — Ontario: Bear Island, Lake Temagami, T. F. R., 14. 8. 1937, leg. CAIN (UPS, BPI). — Ontario: Gull lake Portage, Lake Temagami, 9. 9. 1935, leg. OVERHOLTS (BPI). — Ontario: Spawning Bay, Lake Temagami, T. F. R., on fallen *T. occidentalis*, 29. 8. 1946, leg. CAIN (ZT). — Ontario: Island 340, lake Temagami, on *T. occidentalis* 28. 7. 1938, leg. CAIN (ZT).

USA: N. Y.: Adirondack Mts, on *Thuja*, leg. PECK, Durand herbarium, 5697 (ISOTYPE, FH). — Maine: Millinocket, on *T. occidentalis* leaves of fallen branches, august 1940, leg. LINDER & WHITE (FH). — Michigan: on fallen branches of cedar, 14. 9. 1935, leg MAINS (FH). — Oregon: Douglas Co., Comstock, on *Libocedrus decurrens*, 6. 11. 1937, leg. A. M. M. & D. P. ROGERS (with good drawings and notes by WHITE, FH). — California: del Norte Co., Darlinghtonia, Smith River, on *Libocedrus decurrens*, january 1948 (FH). — California, Trinity Co., South Fork Mt., on *Libocedrus decurrens*, 29. 8. 1941, leg. PARKS, 6957 (BPI). — California: Del Norte Co., Smith River, on *L. decurrens*, march and april 1936, leg. PARKS, 5603 (BPI). — California: Del Norte Co., Darlinghtonia, on *L. decurrens*, 3. 1. 1947, leg. PARKS, 6952 (BPI). — California, Del Norte Co., Smith River, on *L. decurrens*, january 1948, leg. PARKS, 7135 (BPI).

SEAVER (1943) and DENNIS (1964) suspected *Chloroscypha limonicolor* (BRES.) DENNIS (= *Helotium limonicolor* BRESADOLA, Fungi Tridentini 2: 81. 1898) to be an heterotypic synonym of *C. enterochroma*. *C. limonicolor* is separated from *C. enterochroma* only on the basis of the colour of the apothecium and of the iodine reaction. I have not been able to see the type material of *C. limonicolor*: therefore I prefer to consider *C. limonicolor* as a distinct taxon.

4. *Chloroscypha sabinae* (FUCK.) DENNIS, Kew Bull.: 410. 1954. — Fig. 3.

= *Peziza sabinae* FUCKEL, Fungi Rhenani no. 1867, 1866.
= *Dermatea juniperina* ELLIS, Amer. Nat. 17: 192. 1883.
= *Chloroscypha juniperina* (ELLIS) SEAVER, Mycologia 23: 250. 1931.
= *Kriegeria juniperina* (ELLIS) SEAVER, Mycologia 35: 493. 1943.
= *Cenangium helotioides* SACC. apud MOUTON, Bull. Soc. Bot. Belg. 28 (2): 81. 1889.

Apothecia solitary or gregarious, turbinate, sometimes seated on a very short stalk, dark olive to black when dry, 0.2 mm tall; hymenium up to 0.5 mm across. Asci clavate, 8-spored, with a distinct apical pore blued or (more rarely) not blued by iodine after KOH-treatment, 90—160 × 14—20 μm. Ascospores broadly ovate

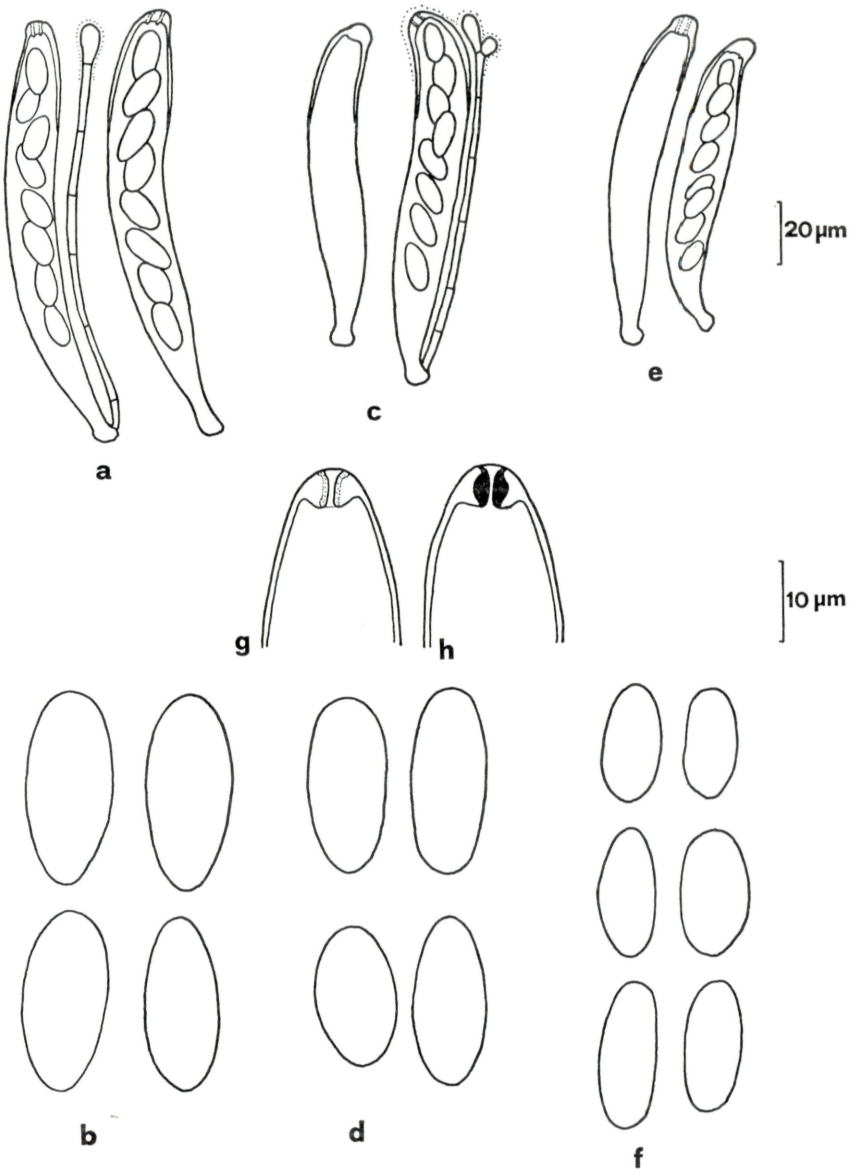

Fig. 3. *Chloroscypha sabinae*. a—b: as *Chloroscypha juniperina* (SHEAR 1933, BPI). a, asci and paraphyses; b, ascospores. c—d: *C. sabinae* (Val Grialetsch, ZT). c, asci and paraphyses; d, ascospores. e—f: *C. sabinae*, cultures, ZT. e, asci; f. ascospores. g—h: *C. sabinae* (MÜLLER 1962, ZT). Ascus tip as seen in the phase-contrast microscope. g, before and h, after coloration by Melzer's reagent

to ellipsoid- reniform, hyaline, pale green when ripe, (15) 18—25× (6)9—11 µm. Paraphyses slender, branched, brownish above, immersed in a gelatinous matrix.

Host. — *Juniperus* spp. (*J. communis* L., *J. nana* Willd., *J. sabina* L.).

C. sabinae was isolated only from healthy-looking needles of *Juniperus nana* Willd. from Val Grialetsch GR, Switzerland. The fungus grows very slowly in pure culture, attaining a diameter of ca 4 cm in 4 weeks and forming a scanty, greenish aerial mycelium. The agar is soon showing a greenish discoloration. Primordia are formed within 2—3 weeks; ripening of the asci and ascospores, however, set in only after about 4 months at 8° C and darkness. As for *C. chloromela*, the ascus and ascospore shapes are the same as for the dried herbarium material, however, the ascospores being significantly smaller (15—18×6—8 µm vs. 18—25×6—11 µm for collections produced on the host material). The iodine reaction of the ascal plug in *C. sabinae* is very peculiar and shall be discussed separatedly (See pag. 220).

C. sabinae is morphologically very close to *C. seaveri*, but it differs from this species in having more slender, tapering asci, in its iodine reaction, in the shape of the spores as well as in its habitat.

Collections examined:

SWEDEN: Uppland: Dalby parish, ca 400 m S of Jerusalem, on *J. communis*, 24. 9. 1978, leg. K. & L. Holm, 1507 (collection J^-. after KOH treatment, ZT). — Uppland: Dalby parish, ca. 400 m S of Jerusalem, on *J. communis*, 8. 8. 1981, leg. K. & L. Holm, 2438 a (also no iodine reaction, ZT).

SWITZERLAND: Graubünden: S. Bernardino, Lago Doss, 1500 m, on *Juniperus nana*, leg. Müller & Petrini (ZT). — Zürich: in den Fröbel'schen Baumschulen, auf *Juniperus* spp., november 1893, leg. von Tavel (ZT). — Graubünden: Schweizer Nationalpark, Val Tantermozza, bei der Hütte, auf *J. nana*, 29. 8. 1968, leg. Müller, Ouellette, Aebi & Harr (ZT). — Wallis: Aletschwald, Moränenweg unmittelbar bei Riederfurka, auf *J. nana*, 13. 9. 1962, leg. Müller (ZT). — Graubünden: Davos, on *J. nana*, 19. 9. 1963, leg. Müller (ZT). — Graubünden: Flüelapass, Val Grialetsch, on *J. nana*, 6. 9. 1981, leg. Petrini; cultures ZT 8987, 8988, 8989, 8990, 8991, 8992, have been all isolated from needles of *Juniperus nana* collected in the same site and are deposited at ZT and CBS. Some 20 more cultures were not kept after the end of the present study.

USA: North Carolina, Highlands, Satulah Mt., on *Juniperus montana*, 19. 8. 1933, leg. Shear (BPI). —

5. *Chloroscypha seaveri* Rehm ex Seaver, Mycologia **23**: 249. 1931. — Fig. 4, 5.

= *Helotium seaveri* Rehm ex Seaver, nom. nud. in syn., op. cit.
= *Kriegeria seaveri* (Seaver) Seaver, Mycologia 35: 493. 1943.
= *Mollisia cryptomeriae* Sawada, Bull. Gov. For. Exp. Sta. (Tokyo) 45: 34. 1950.
= *Chloroscypha cryptomeriae* Terrier, Ber. Schw. Bot. Ges. 62: 422. 1952.

C. seaveri was isolated from both *Thuja plicata* D. Don and *Chamaecyparis lawsoniana* Parl. The cultures derived from *T. plicata* were very homogeneous and showed little variation in growth as well as in shape and size of the apothecia, ascospores and asci; the same applied to cultures derived from *C. lawsoniana*. Marked cultural differences were observed, however, between these two groups of cultures; collections or cultures derived from the one or the other host could also be distinguished on the basis of the size of the ascus and of the ascospores. Two forms can thus be recognized wich correlate with hosts.

5a. *Chloroscypha seaveri* f. *seaveri*. — Fig. 4.

Apothecia minute, scattered or in small, cespitose clusters, black, turbinate, sessile to shortly stipitate, 0.15—0.3 mm tall, rarely taller, hymenial disc 0.2—0.3 mm across. Asci clavate, 120—130 × 20—30 µm, with an indistinct apical pore not blued by iodine after KOH treatment. Ascospores broadly fusoid to clavate, sometimes almost apiculate at one end, pale yellow-green or subhyaline, 23—29 × 8—11 µm. Paraphyses filiform, septate, scarcely enlarged above, rarely branched, the tips surrounded by a gelatinous matrix.

Host. — *Thuja plicata* D. Don, *T. occidentalis* L., *Cryptomeria japonica* D. Don, *Chamaecyparis obtusa* Sieb. & Zucc.

Cultures very slow-growing, attaining a diameter of 3—4 mm in 14 days. In young cultures no aerial mycelium is formed; in older ones, as well as after some re-inoculations, the formation of a white, becoming cream colored aerial mycelium can be observed. After a few days, a black, cerebriform subiculum develops, on which sterile, slimy apothecia are formed after about 3 weeks; this subiculum is not always found after some re-inoculations. Ripening of the asci occurs in about 6 weeks on surface-sterilized pieces of the host plants to 2—3 months on 2% malt extract agar.

The apothecium, ascus and ascospore sizes are affected by the kind of medium the fungus is allowed to grow on. Strains cultivated on fully artificial media show greater variations and have generally larger asci and slightly smalle spores than those grown on natural substrate.

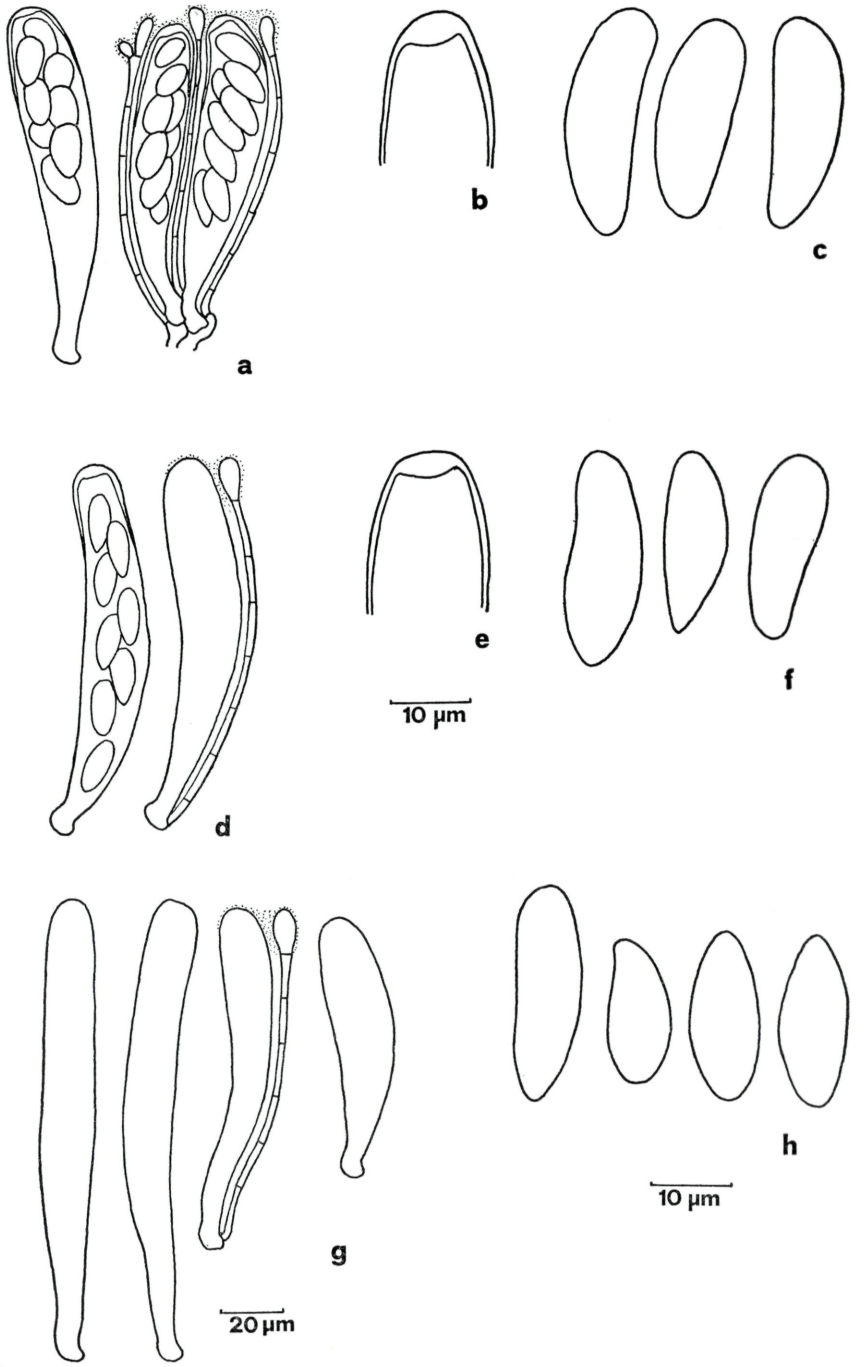

Fig. 4. *Chloroscypha seaveri* f. *typica*. a—c. *Chloroscypha seaveri* (Isotype, BPI). a, asci and paraphyses; b, ascus tip (phase-contrast microscope); c, ascospores. d—f: as *Chloroscypha cryptomeriae* (Terrier 1949, ZT). d, asci and paraphyses; e, ascus tip (phase-contrast microscope); f, ascospores. g—h: *C. seaveri* (in culture, ZT). g, asci and paraphyses; h, ascospores

Collections examined:

THE NETHERLANDS: Wageningen, Oranje Nassau Oord., on *C. japonica*, 20. 2. 1952, leg. GREMMEN (ZT).

SWITZERLAND: Vaud: Le Mont S/Lausanne, Bois des Gésiaux, on *Cryptomeria japonica*, 2. 4. 1949, leg. TERRIER (ZT).

USA: Montana: Libby, on *Thuja plicata*, leg. WEIR, (ISOTYPE, BPI, FH, UPS, ZT). — Idaho: Bonner Co., N slope of Nickleplate Mt., above Sta. 10, on *T. plicata*, leg. SLIPP (BPI). — Idaho: Bonner Co., Gravel Pit, Near East River, Priest River Exp. For., hypophyllous on *T. plicata*, leg. SLIPP, EHRLICH herbarium, 2547 (BPI). — Maine: Orono, on *Thuja occidentalis*, 20. 10. 1900, leg. RICKER (BPI). — Cultures: USA: Oregon: Lane Co., McKenzie River Campground, US HW 126, *T. plicata*, ZT 8997, 8999, december 1979, leg. PETRINI & CARROLL (ZT, CBS). — Oregon: Deschutes Co., Suttle Lake Jct., US HW 126, *T. plicata*, ZT 8998, november 1979, leg. PETRINI & CARROLL (ZT, CBS). — Oregon: Olallie Campground, US HW 126, *T. plicata*, ZT 9000, november 1979, leg. PETRINI & CARROLL (ZT, CBS). About 120 cultures were isolated and studied, however only 4 of them were kept after the end of the study.

5b. *Chloroscypha seaveri* f. *lawsoniana* f. n. — Fig. 5.

Differt a forma typica minoribus ascosporis ac culturae morphologia. Typus ad Chamaecyparidem lawsonianam, in horto universitatis oregonensis, Eugene, Oregon, USA, 20. 11. 1979, leg. Carroll et Petrini (ZT).

This fungus is distinguished from *C. seaveri* f. *seaveri* by its smaller ascospores (20—24 × 7—10 μm), by its different cultural characters and by its host specificity.

The cultures are slow-growing, attaining a diameter of about 4 cm in 4—5 weeks, with no aerial mycelium; the agar is soon discolored with a yellow pigment. The apothecia form within a few days, however mature asci and ascospores develop only after 6—8 weeks.

Collections examined:

USA: Oregon: Lane Co., Eugene, University campus, on *Chamaecyparis lawsoniana*, 20. 11. 1979, leg. CARROLL & PETRINI (ZT, TYPE). — Cultures: USA: Oregon: Coos Co., Coquille River Res. Nat. Area, on *C. lawsoniana*, ZT 8993, 8994, 8995, november 1979, leg. PETRINI & CARROLL (ZT, CBS). — Oregon: Coos Co., Boundary Campground, NW of Coquille River Res. Nat. Area, on *C. lawsoniana*, ZT 8996, November 1979, leg. PETRINI & CARROLL (ZT, CBS). About 40 more cultures were obtained and studied, but not kept after the period of this study.

List of other species of *Chloroscypha* described but not treated here.

1. *Chloroscypha cedrina* (COOKE) SEAVER, Mycologia 35: 493. 1941.
   = *Peziza cedrina* COOKE, Bull. Buffalo Soc. Nat. Sci. 2: 294. 1875.
   On branches of *Juniperus virginiana*.
   References: SEAVER (1941, 1951).

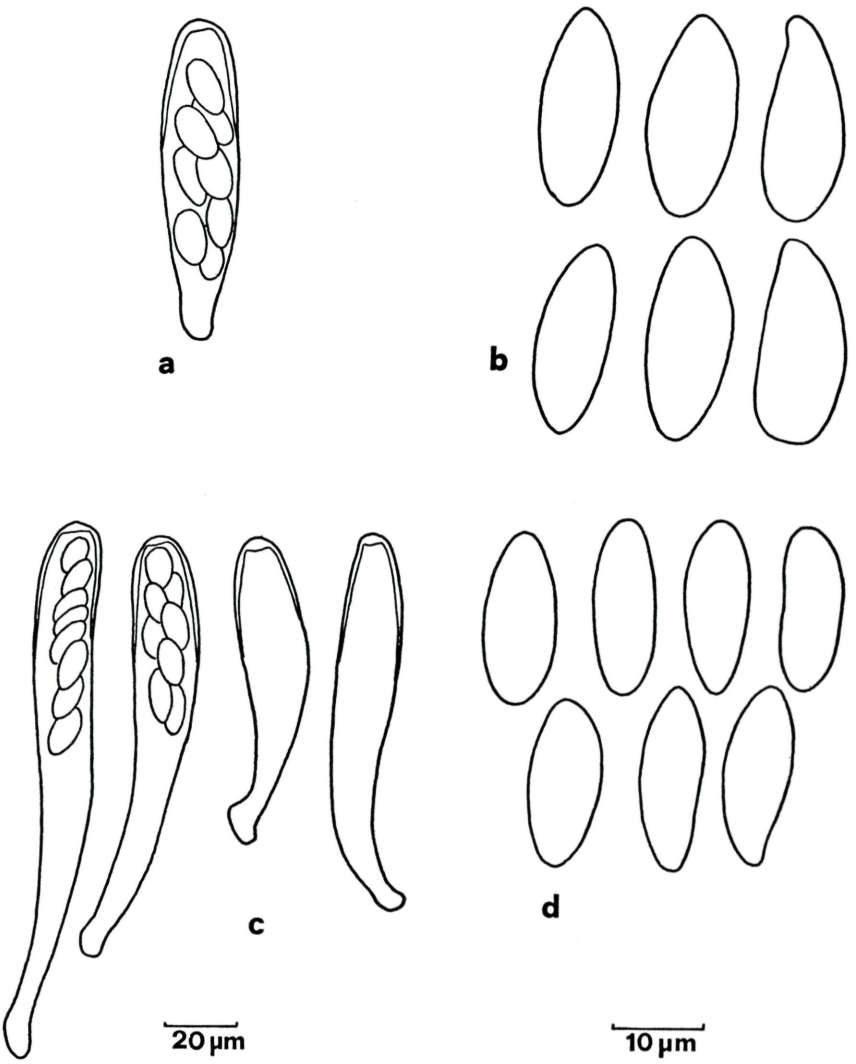

Fig. 5. *Chloroscypha seaveri* f. *lawsoniana*. a—b: *C. seaveri* f. *lawsoniana*, (CARROLL & PETRINI 1979, Typ, ZT). a, ascus; b, ascospores. c—d: *C. seaveri* f. *lawsoniana* (cultures, ZT). c, asci (coll. P 7232 and P. 8244); d, ascospores

2. *Chloroscypha chamaecyparidis* (Sawada) T. Kobayashi, Bull. Gov. For. Exp. Sta. (Tokyo) 176: 65. 1965.
   = *Mollisia chamaecyparidis* Sawada, Bull. Gov. For. Exp. Sta. (Tokyo) 46: 126. 1950.

   On *Chamaecyparis obtusa* Sieb., *Chamaecyparis pisifera* Endl., *Chamaecyparis lawsoniana* Parl.

   References: Kobayashi (1965).

3. *Chloroscypha limonicolor* (Bres.) Dennis, Persoonia 3: 50. 1964.
   = *Helotium limonicolor* Bresadola, Fungi Tridentini 2: 81. 1898.

   On *Thuja orientalis*.

   References: Dennis (1964); see also comments under *Chloroscypha enterochroma*.

4. *Chloroscypha thujopsidis* (Sawada) T. Kobayashi, Bull. Gov. For. Exp. Sta. (Tokyo) 176: 67. 1965.
   = *Mollisia thujopsidis* Sawada, Bull. Gov. For. Exp. Sta. (Tokyo) 46: 141. 1950.

   On *Thujopsis dolabrata* Sieb. & Zucc., *Thuja standishii* Carr., *Chamaecyparis obtusa* Sieb. & Zucc., *Chamaecyparis pisifera* Endl. and *Cryptomeria japonica* D. Don.

   References: Kobayashi (1965).

Discussion

1. The iodine reaction of the ascal plug in *Chloroscypha*-species.

The iodine reaction of the ascus tip has been used for a long time as an important taxonomic character. Korf (1962) considered the reactivity to iodine a constant character, important in defining whole families or even orders of Ascomycetes. He later modified his opinion (Korf, 1973) and eventually Kohn & Korf (1975) and Nannfeldt (1976) pointed out that the iodine reaction of the ascal plug in Ascomycetes is highly dependent upon pretreatment with or without KOH.

Parker & Reid (1969) reported that iodine-negative collections of *Rhabdocline pseudotsugae* Syd. lacked a thickened ascal apex and an apical pore, while iodine-positive collections possessed at their apices a starch-like cylinder perforated by a central pore. On the basis of this difference they erected a new species of *Rhabdocline*, *R. weirii*, to accomodate the J^+-collections. The different *Chloroscypha* species studied here showed the same reaction patterns discussed by Parker & Reid (1969) and Kohn & Korf (1975). No reaction was observed in KOH-untreated specimens of *C. alutipes*, while there was only a weak reaction in KOH-untreated specimens of *C. chloromela*. However, KOH-pretreatment induced a very pronounced blueing of the ascal plug in all the specimens of these two species. Only

Chloroscypha-species with a distinct apical pore, viz. *C. alutipes*, *C. chloromela* and most species of *C. sabinae* were iodine-positive with KOH-pretreatment, thus supporting the observations made by PARKER & REID (1969) on *Rhabdocline*.

MÜLLER & HÜTTER (1963) drew attention to a collection of *C. sabinae* which possessed J^+-asci and later PARKER & REID (1969) and KOHN & KORF (1975) discussed this observation arguing that the presence or the lack of iodine reaction could be connected with the existence of different races of the same species. The results reported here are rather contradictory: although most of the collections studied showed an unequivocal iodine reaction, KOH-pretreatment and subsequent mounting in Melzer's reagent did not induce any blueing in some strains. No explanation can be found for this anomalous Melzer's reaction; the difference between reactive and non-reactive ascal plugs can be purely chemical; non-reactivity, on the other hand, can also be due to morphological changes, the reactive structure being partly or wholly absent. In both cases, the taxonomic significance of the iodine reaction should not be over-emphasized, and its use in characterizing taxa limited to the species level.

2. The stability of differential characters.

One basic problem in Ascomycetes taxonomy is the stability of differentiating characters. Features such as ascus and ascospore shape and size, and iodine reaction of the ascal plug have been used to define species, and the size of the ascospores alone has been considered an important specific character.

The taxonomic significance of the iodine reaction in *Chloroscypha* has already been discussed; ascus and ascospore shapes are also stable characters in delimiting *Chloroscypha*-species. Ascospore size within the same species of *Chloroscypha* may vary strongly depending upon the substrate, the ascospores being smaller in material cultured on natural substrate. Ascospores formed in freshly isolated cultures are always smaller than they are in collections from the host plant, tending to get closer to the usual size after some re-inoculations. More reliable is the length/width ratio of the ascospores: little variation has been observed within collections and cultures of the same *Chloroscypha*-species. The consistency of this character offers a valuable approach to the taxonomic delimitation of species in this genus and may be a useful tool in the taxonomic treatment of similar ascomycetous genera.

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