TRICHOCYTES IN LITHOPHYLLUM KOTSCHYANUM AND LITHOPHYLLUM SPP. (CORALLINALES, RHODOPHYTA) FROM THE NW INDIAN OCEAN

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The current diagnosis of the genus Lithophyllum includes absent or rare trichocyte occurrence. After examining holotype material, single trichocytes have been revealed to occur abundantly in Lithophyllum kotschyanum Unger, and in freshly collected specimens of Lithophyllum spp. from the Red Sea, Gulf of Aden and Socotra Island (Yemen). Trichocyte occurrence is not considered a diagnostic character at specific or supraspecific levels in the Lithophyloideae, and the ecological significance of trichocyte formation is discussed. The generitype species, L. incrustans Philippi, does not show trichocytes nor do many other Lithophyllum species from diverse geographic localities, but the presence of abundant trichocytes in other congeneric taxa requires emendation of the genus diagnosis. Therefore, the diagnosis of Lithophyllum is here emended by eliminating the adjective “rare” in the sentence concerning trichocyte occurrence, as follows: “Trichocytes present or absent, if present occurring singly.”

Key index words: coralline algae; holotype; Indian Ocean; Lithophyllum kotschyanum; Red Sea; TRH; trichocyte; Unger

According to the current description and delimitation of Lithophyllum Philippi (Harvey et al. 2009), trichocytes (=hair-bearing cells) are not a common feature, and when present, they occur singly (Keats 1995, 1997, Guiry and Guiry 2014). No trichocyte-bearing Lithophyllum has been described from the Red Sea and Indian Ocean. Among the most common Lithophyllum species recorded in this area, L. kotschyanum Unger is considered to be widely distributed across the tropical Red Sea and Indo-Pacific Ocean (Silva et al. 1996). In the framework of a large-scale investigation on corallines from these localities, we collected some common Lithophyllum specimens bearing abundant trichocytes. Ongoing morphological and molecular studies suggest multiple species are passing under L. kotschyanum and highlight the need to verify all previous reports of this species in the literature. This article presents the results and implications of an anatomical investigation of the holotype of L. kotschyanum and other freshly collected Lithophyllum specimens from Red Sea and Indo-Pacific and discusses the occurrence of trichocytes in Lithophyllum.

MATERIALS AND METHODS

Specimens were collected at several localities along the SE Sinai coast of Egypt, along the Yemeni coast of the Red Sea, Gulf of Aden, and Socotra, and in the Persian Gulf, and were deposited in MI (abbreviations after Thiers 2014). A selection of the new thin sections obtained from the holotype of L. kotschyanum were deposited in TRH.

Material examined. Red Sea: Nakhlet El Tall, Sinai peninsula, 0.6–1 m (leg. Basso: DB555-558, DB673, 5.x.2006); Ras Mohammed, Sinai peninsula, 0.6 m (leg. Basso: DB578, 9.x.2007); Ras Ghamila lagoon, Sinai peninsula, 1 m (leg. Caragnano: DB573, 18.x.2009; DB 637-638, 24.iv.2008); Yemen, Kamaran, 1 m (leg. Caragnano: DB574, 2.x.2009); 1.5 m (leg. Caragnano: DB575-576, 28.ix.2009); Indian Ocean: Persian Gulf, Kuwait, Qaro, 5 m (leg. Benzioni: DB672, 24.ix.2002); Aden Gulf, Yemen, Bahlab, 2–3 m (leg. Benzioni: DB559, 9.x.2006; DB563, xi.2006); leg. Caragnano: DB567-570, iii.2008; DB657, DB659, DB660, DB692, xi.2008; Yemen, Socotra Is.: Shalintaitin, 9 m (leg. Caragnano: DB612, iii.2010); Rosh, 16 m (leg. Caragnano: DB615-616, 12.iii.2010); Ras Adho, 22 m, 13 m (leg. Caragnano: DB636, 16.iii.2010). Corallines were air dried prior transport to the Milan facilities. The holotype of L. kotschyanum (leg. Kotschy; TRH A20-1272, Bahrain Gulf, Persian Gulf) was also examined (Fig. 1).

Air-dried specimens for SEM were fractured and mounted on aluminum stubs with silver glue. Samples were gold coated (15 nm in thickness) and examined in a SEM Vega Tescan TS5136XM at 20 kV at the Milano-Bicocca facilities.

For LM, pieces of thallus were decalcified with Telysiniczkyi’s solution (Bressan 1974) for 12–48 h, washed in distilled water, dehydrated through a graded ethanol series and embedded in methacrylate resin (Technovit 7100; Heraeus Kulzer, Wehrheim, Germany). Preparation of serial sections follows Basso and Rodondi (2006). Permanent slides for LM were examined and photographed with a Leica DMRB photomicroscope.

Anatomical terminology follows Adey and Adey (1973) and Woelkerling (1988). Conceptacle measurements follow the system of Adey and Adey (1973) and were made directly from the SEM or under LM using a calibrated eyepiece micrometer. Cell measurements follow Basso et al. (2004).
RESULTS

*Lithophyllum kotschyanum* Unger (1858: 22, pl. V, fig. 15)

Holotype: TRH, A20-1272 (Trondheim, Norway, Woelkerling 1993:133), Figs. 1 and 2, including thin section no. 1720 (Woelkerling et al. 2005).

Etymology: the specific epithet is dedicated to the collector, Mr. Theodor Kotschy (Unger 1858, Woelkerling 1993:133).

Illustrations of type material: Printz 1929: pl. 65, fig. 1; Verheij 1994: 129, fig. 8.

Type locality: Gulf of Bahrain, Persian Gulf

Habitat: Plants encrusting corals and mollusks (Unger 1858). Habitat details unknown.

Geographic distribution: *Lithophyllum kotschyanum* as circumscribed here is known only from the type locality. Other occurrences from the literature require further confirmation after critical revision based on morphological and molecular investigations of the field-collected material.

Habit and vegetative structure: The holotype of *L. kotschyanum* (TRH A20-1272, Fig. 1d) is apparently a broken fragment of a larger plant, nonendophytic, with fruticose growth-form. Protuberances have a smooth surface, are cylindrical or slightly compressed, branched, up to 20 mm long and about 2–5 mm wide. Most protuberances terminate with a blunt apex, and some are fused at their distal ends (Fig. 1d).

Thallus pseudoparenchymatous, dimerous, with a basal or ventral layer of cells (=hypothallus) running parallel to the substrate (Fig. 2, a and b). Hypothallial cells 7–13 μm long and 10–15 μm in diameter (Fig. 2c). Perithallus composed of filaments of cells rising from the hypothallus and curv-
ing outward toward the thallus surface, 8–31 μm long and 7–11 μm in diameter (Fig. 2b). Cells of adjacent filaments joined by secondary-pit connections, cell fusions not observed (Fig. 2, c and d). Palisade cells not observed. Single trichocytes 30–40 μm long and 12–14 μm in diameter, abundantly distributed in the perithallus and at the thallus surface (Fig. 2, a, b, d, e). Trichocytes not observed in the hypothallus and in the oldest 4–5 perithallial cell filaments (those corresponding to the early stages of thallus development). The holotype material includes a thin petrographic section 1720 (Fig. 1c) that does not show trichocytes. Single epithallial cells flattened, 10–12 μm in diameter and 2.5 μm long (Fig. 2e). Epithallial cells overlain by a cuticle (Fig. 2, b and e).

Reproduction: Empty uniporate conceptacle chamber in the holotype of Lithophyllum kotschyanum, presumed tetralsporangial on the basis of shape and size (in agreement with Verheij 1994), slightly protruding above the surrounding thallus surface, becoming buried in the thallus, 310–380 μm in diameter and 110–150 μm high, with pore-canal 70–80 μm long (Fig. 2f). Floor of the conceptacle chamber convex upward below a central columella. The conceptacle chamber floor is 16–17 cells below the thallus surface (Fig. 2f). Roof filaments 7 cells long, including the terminal epithallial cell (Fig. 2f). Gametangial and carposporangial conceptacles unknown.

Freshly collected Lithophyllum specimens. Freshly collected specimens of Lithophyllum spp. from the Red Sea, the Gulf of Aden, the Persian Gulf and Socotra show a pseudoparenchymatous structure and dimerous organization of the thallus, whose thickness ranges from about 100 μm to more than 1 cm of continuous thallus growth. Some specimens show superposition of conspecific thalli and have thickness exceeding 2 cm. Cells of adjacent filaments linked by secondary-pit connections, no fusions observed. Tetrasporangia contained in uniporate conceptacles. All thalli possess abundant, scattered trichocytes, but these are apparently lacking from the hypothallus and first few cells of perithallial filaments (about 2–4; Fig. 3, a–e). This pattern of trichocyte distribution appears unrelated to the total thickness of the thallus. SEM investigations of the same fresh material revealed very rare trichocytes in the perithallus (Fig. 3f), as well as at the surface of the algae (Fig. 3g).

**DISCUSSION**

Routine histological preparations of decalcified material, such as those used in the present study, easily allow the detection of trichocytes in coralline algae (Cabioch 1971, Johansen 1981, Chamberlain 1985, Woelkerling 1988, Afonso-Carrillo 1989, Penrose and Chamberlain 1993, Keats 1995, 1997). The type species of Lithophyllum is L. incrustans Pilippi, lectotypified with a specimen collected in Sicily (Woelkerling 1983). This lectotype was studied by histological sections, and no trichocytes were observed (Woelkerling 1983), in agreement with results from freshly collected plants of L. incrustans from the Mediterranean shallow subtidal of Ognina (Catania, Sicily; D. Basso, unpubl. data), and from the Atlantic (Cabioch 1969, Irvine and Chamberlain 1994).

Lithophyllum has been considered a genus of trichocyte-free species for more than 150 years (Woelkerling 1988). Trichocytes do not occur in recently revised Lithophyllum species from Brazil (Villas-Boas et al. 2009), Gulf of California (Riosmena-Rodriguez et al. 1999), Mediterranean Sea (Basso et al. 1996, Chamberlain 1997, Athanasiadis 1999, Bressan and Babbini 2003), British Isles (Irvine and Chamberlain 1994), Spermonde Archipelago (Verheij 1994), Hawai‘i (Adey et al. 1982), south-eastern Australia (Harvey et al. 2009), and New Zealand (Harvey et al. 2005, Farr et al. 2009). Few exceptions have been recently documented from Fiji, viz. common solitary trichocytes in the semi-endophytic species Lithophyllum cuneatum Keats, and sporadic individual trichocytes in Lithophyllum insipidum Adey, Townsend et Boykins and Lithophyllum flavescens Keats (Keats 1995, 1997). In contrast, trichocytes occur abundantly in all Lithophyllum species from the Gulf of Aden, Persian Gulf and Socotra dealt in this article, although we could not find any literature report of trichocyte occurrence in L. kotschyanum. The holotype of L. kotschyanum was illustrated macroscopically by Printz (1929), and its anatomy was incompletely illustrated by Verheij (1994) based on a microphotograph of the petrographic section (=non decalcified material) that is part of the holotype (Verheij 1994, p. 129, fig. 8). Most subsequent reports are check-lists (Silva et al. 1996), and the few illustrated reports of L. kotschyanum are either based on petrographic sections (for example: Rasser and Piller 1997), or on material whose identity requires further investigation (such as Adey et al. 1982), since monophyly for this species is not supported by molecular data (Kato et al. 2011).

Three types of trichocyte development have been reported in coralline algae (Cabioch 1971, Johansen 1981). The Jania rubens type of trichocyte development (Johansen 1981, p. 33-35, fig. 18B) corresponds to our observations: an intercalary meristematic cell divides obliquely into two cells. The newly formed oblique wall does not become calcified (Fig. 3g), and the entire trichocyte is contained within the wall of the original meristematic cell. The distal cell of the trichocyte becomes modified by the formation of a hair (Fig. 3c) that grows out from the surface of the overlying epithallial cell which finally sloughs off (Fig. 3, c inset and h). When the hair withers, the proximal cell of the trichocyte becomes meristematic, producing new perithallial...
cells and a new epithallial cell that replaces the penetrated one. Within the Jania type of trichocyte development, the final steps may result in the complete obliteration of the old trichocyte (Jania), or, alternatively, in the conservation of the open-ended sac-like trichocyte walls that remain visible below the meristem (Metagoniolithon; Johansen 1981). The latter case corresponds to our observations in Lithophyllum kotschyanum and Lithophyllum spp., based on hystological decalcified sections enhanced by the Toluidine blue stain (Figs. 2b and 3, a and b). On the contrary, the calcified walls of the newly formed perithallial cells merge with the remains of the trichocyte walls (Figs. 2d and 3b) that are therefore obliterated or less apparent under SEM (Fig. 3) and in petrographic thin sections (Rasser and Piller 1997). This process explains why trichocytes were not detected in the petrographic section included in the holotype material and went overlooked (Verheij 1994, fig. 8). Given the difficulty to detect Lithophyllum trichocytes in petrographic sections and in SEM preparations, it is likely that they occur but were overlooked in other incompletely investigated species, besides those described in this article.


Within Lithophylloideae, besides Lithophyllum (sensu Bailey 1999) trichocytes are reported in Titanoderma fustulatum var. confine (Suneson 1943, Chamberlain 1991, Kjøsterud 1997) and in T. corallinae (as Lithophyllum corallinae, Woelkerling and
ing corallines, typical of low latitudes, would require trichocytes to satisfy their increased nutrient requirements in reef oligotrophic waters (Walker 1984). Intra- and interspecific comparisons of trichocyte production in the natural environment and under controlled conditions are necessary to test this hypothesis.

Implications for the diagnosis of the genus Lithophyllum. The anatomy of *L. kotschyanum* and *Lithophyllum* spp. fully corresponds to the circumscription of *Lithophyllum* as described by Harvey et al. (2009), with the exception of trichocytes occurring singly but very abundantly both in the type material and in the fresh collections from the Red Sea and Indian Ocean. Pending further studies, the occurrence of trichocytes is not considered a diagnostic character for the segregation of species or supraspecific taxa within the Lithophylloideae. The generic type species, *L. incrustans*, does not show trichocytes, as also found in many *Lithophyllum* species worldwide. However, the occurrence of abundant trichocytes in other species requires that they be included in the genus diagnosis. Therefore, the diagnosis of *Lithophyllum* provided by Harvey et al. (2009) is here emended solely in the sentence: “Trichocytes present or absent, if present rare and occurring singly,” by eliminating the adjective “rare,” as follows: “Trichocytes present or absent, if present occurring singly.”

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TRICHCYTES IN LITHOPHYLLUM


