Effects of hydration on the reproductive structures of *Illosporiopsis christiansenii* – the reliability of a simple water-drop test in a binary field key

Illosporiopsis christiansenii (B.L. Brady & D. Hawksw.) D. Hawksw. only catches the eye in the autumn/winter months when it produces shocking-pink cirrhi which often contrast vividly with nearby *Physcia* and *Xanthoria* (Fig. 1). It is a lichenicolous (lichen inhabiting) fungus (LF) and the pink cirrhi are the only part of the fungus that is generally observed. According to the British Lichen Society database, it is most commonly reported on *Physcia* species on the nutrient-rich bark of deciduous trees.

Ill. christiansenii is only known to reproduce asexually. The reproductive body is called a sporodochium which is a bundle of spore-bearing hyphae (conidiophores). The conidiophores in *Ill. christiansenii* look very like vegetative hyphae but they are specialised, bearing conidiogenous cells at the tips which produce the conidia (asexual spores). The spores are packed together to form the bright pink cirrhus (pl. cirrhi) observed in the field. The cirrhus is a mucus-bound, dense mass of conidia at the tip of the sporodochium. Cirrhi frequently join up to become confluent masses (Fig. 2). The gelatinous sporodochium was first described by Lowen *et al.* (1986) as 0.5–1 mm across and 0.1–2 mm high, and the conidia arising from it as 17–30 x 11–20 µm, each consisting of a helically coiled, transversely septate, multicellular filament. The conidia are initially hyaline (colourless), becoming pink as they mature (Gavériaux, 2015).

Ill. christiansenii is one of a number of LFs producing pink or orange-pink masses, that were previously thought to be related and are probably frequently misidentified in the literature because of a lack of morphological characters (Sikaroodi, 2001) and the minute size of the pigmented masses (< 1 mm).

Figure 1 *Illosporiopsis christiansenii* as typically observed: bright pink cirrhi in a nutrient-rich lichen community (here on *Malus*). Photo © M.L. Sisti

Identifying *Ill. christiansenii* in the field

The two species most likely to be confused with *Ill. christiansenii* are *Erythricium aurantiacum,* which is also parasitic on *Physcia*, and *Marchandiomyces corallinus* because its colour is similar (Fig. 3). All three have been reported in the UK as well as across Europe and in North America.

E. aurantiacum produces pale orange to orange-pink bulbils which erupt through the surface of the host thallus. These bulbils can be single or merged into clumps (Lawrey *et al.*, 2007). They do not contain spores, but are composed of large, spherical or ovoid, granular cells.

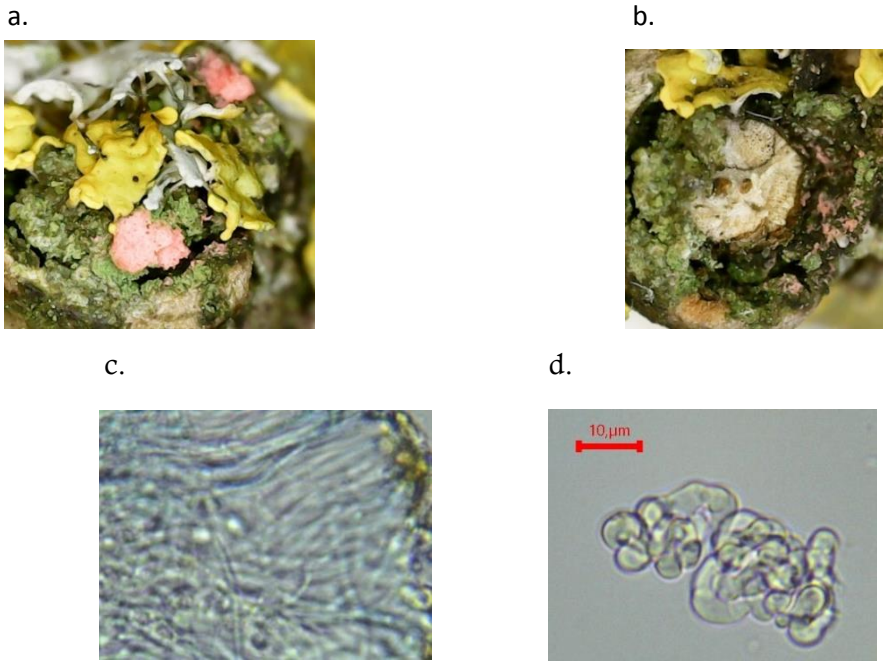

Figure 2 Anatomy of the *Ill. christiansenii* sporodochium a. Pink cirrhi surrounded by *Physcia adscendens* and *Xanthoria parietina* and necrotic thallus, growing at a senescent node of a *Fraxinus* twig. b. Point of excision of the lefthand mass of at least four confluent cirrhi, showing the bases of four cut bundles of conidiophores beneath the cirrhi c. Aqueous squash of the excised material showing (l. to r.) mycelium, conidiophores and conidia d. Helicoid, ribbon-like, multi-septate conidia. Photos © A.M. Claypole

M. corallinus also produces bright pink bulbils on bleached areas of thalli but is generally found on *Parmelia* species. First reporting *Ill. christiansenii* for Wales, Orange (2001) suggested it may previously have been ignored as *M. corallinus.* Lowen *et al.* (1986) showed the pink pigment is chemically identical to that in *Ill. christiansenii* and older cirrhi of *Ill. christiansenii* may appear a similar colour. However, the *Ill. christiansenii* cirrhi are irregular in shape compared to the rounded bulbils of *M. corallinus*.

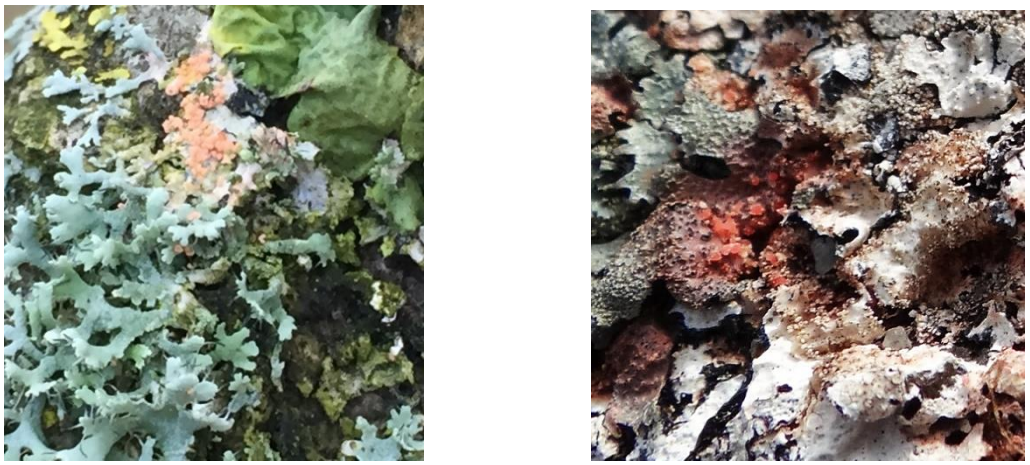

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Figure 3 Two look-alikes of *Ill. christiansenii*: *Erythricium aurantiacum* (left) orange-pink bulbils on the surface of *Physcia* thallus, and *Marchandiomyces corallinus* (right) bright pink bulbils on *Parmelia* thallus

Colour alone is an imprecise, subjective delimiter for the uninitiated, particularly when comparing photographs or observing under artificial illumination. Microscopic examination of the contents of the pink/orange material is required to show the presence of the distinctive, helicoid conidia of *Ill. christiansenii*.

Without a microscope, the simplest approach to identifying a lichenicolous fungus is to refer to a binary key for lichenicolous fungi known to be parasitic on the host lichen.

One of the distinguishing properties of *Ill. christiansenii* is its reaction to water. *Ill. christiansenii* differs from *M. corallinus* 'in the formation of helicoid conidia that disperse readily in water' whereas the contents of *M. corallinus* bulbils are very difficult to separate (Lowen *et al.*, 1986 and Preece, 2011). This reaction to water has been used in binary field keys to help identify *Ill. christiansenii*. This study investigated the reliability of the water-drop test in a binary key to distinguish *Ill. christiansenii* from *E. aurantiacum* and *M. corallinus* in the field.

Resources and methodology

Fieldwork *Ill. christiansenii* and *E. aurantiacum* were tested *in situ* and sampled for laboratory study at three different sites across the UK. All specimens were growing in an upward-facing, horizontal habit, 1–2 m above ground level in regions of moderate pollution. The sites were visited throughout 2022 and into spring 2023.

Site 1. VC23, Oxfordshire, SP531052 with *Physcia adscendens* on healthy, well-lichenised *Malus* tree branches in an urban back garden
Site 2. VC63, South-west Yorkshire, SE063236 with *P. adscendens* on a senescent *Fraxinus* twig alongside a canal towpath
Site 3. VC38, Warwickshire, SP314546 with *Physcia tenella* on a painted metal, barred gate to a field alongside a country lane

The field studies enlisted the assistance of members of the Zoom Lichen Chat and Improvement Group (LCIG) and the post-LABS group, ABLE.

Laboratory work Time-lapse photography was used to record the effects of hydration. Sporodochia, bulbils and their contents were examined under the microscope.

Hydration of *Ill. christiansenii* compared with *E. aurantiacum*

The first step was to establish that *E. aurantiacum* could be distinguished from *Ill. christiansenii* in the same way as *M. corallinus* described in the literature.

Between January and March 2022, fourteen *Ill. christiansenii* cirrhi and ten *E. aurantiacum* bulbils from Sites 1 and 2 were tested. Each was mounted under a camera and hydrated with a drop of tap water. Photographs were taken at 10 second intervals for 30 minutes. Any change in the specimens was recorded. After the test, the species was confirmed microscopically by an aqueous squash of the entire cirrhus or bulbil. For comparison, the hydration reaction of the host thallus (*P. adscendens*) was also measured.

The *Ill. christiansenii* cirrhi were 0.8 mm across (mean value), but irregular in shape and the measurements varied considerably. The *E. aurantiacum* bulbils were much smaller (mean value 0.2 mm), smoother and spherical. The cirrhi expanded in all directions. Horizontal extension was

measured across the cirrhus at two or more points and expressed as a percentage of the initial measurement (% Δ^0).

On hydration, *Ill. christiansenii* cirrhi immediately extended rapidly, reaching a maximum of 63% Δ^0 at 45 s (mean values) (Fig. 4). Half the samples also showed a loss of colour. As the tissue dried, it reverted to its original size and colour. The reaction could be repeated when the cirrhus had recovered (after a matter of hours).

In contrast, the *E. aurantiacum* bulbils did not change colour on wetting and extended only 26% Δ^0. Healthy *P. adscendens* thalli extended to the same degree as *E. aurantiacum* bulbils. Indeed, it may be what was measured since it is extremely difficult to separate the bulbils from the thallus tissue that they are immersed in. This is similar to the handling of *M. corallinus* bulbils described by Lowen *et al*. (1986) and Preece (2011). *Ill. christiansenii* cirrhi could easily be separated as they did not appear to be attached to the host thallus.

The reaction of *Ill. christiansenii* cirrhi required further clarification. The documented 'dispersion' response had not been found. Colour loss was the nearest reaction, but only half the samples showed this. These samples were all taken in early spring just before the LF stopped producing cirrhi. Was it possible that cirrhi produced in autumn react differently?

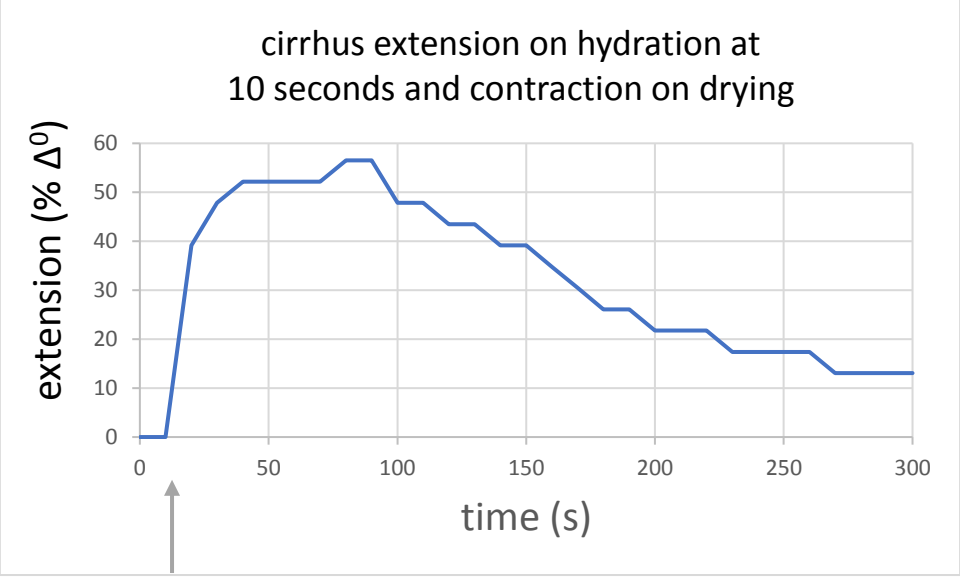

Figure 4 Extension of a typical *Ill. christiansenii* cirrhus on hydration at 10 seconds (indicated by the arrow) and subsequent contraction on drying; measurements taken at 10 s intervals, expressed as a percentage of the initial measurement (% Δ^0)

The search for 'dispersing' *Ill. christiansenii* cirrhi

After the sporodochia reappeared in September, the same hydration test was performed monthly. Two cirrhi were collected mid-month from Site 2 and each was tested, allowed to dry and then tested again (giving a total of 4 measurements on each cirrhus). The results were variable and the sample size small. Nevertheless, a pattern emerged:

September: both cirrhi extended by 46% and immediately lost colour, turning pink again after a few minutes.

October: one cirrhus extended by 36% and lost colour, becoming more translucent, then turned white and finally pink again on drying. The other immediately disappeared, reappearing white, then pink and smeared on drying.
November: both cirrhi disappeared immediately, reappearing first white, then pink on drying.
December: one cirrhus disappeared, reappearing white, turning pink. The other swelled, turned white and then pink on drying.

In short: between September and December, all specimens immediately swelled and lost colour on wetting. From October to December a more marked change of translucency was observed, being most striking in November. The cirrhi seemed to disappear but became visible again on drying. An example of a cirrhus that did not lose its colour (from February) and one that seemed to vanish are shown in Fig. 5.

Thus maturity of the cirrhus may be a factor in the dispersion response. Observations in other fungi show freshly formed cirrhi disperse more easily in water, while cirrhi that have been subjected to drying require squashing to separate the spores.

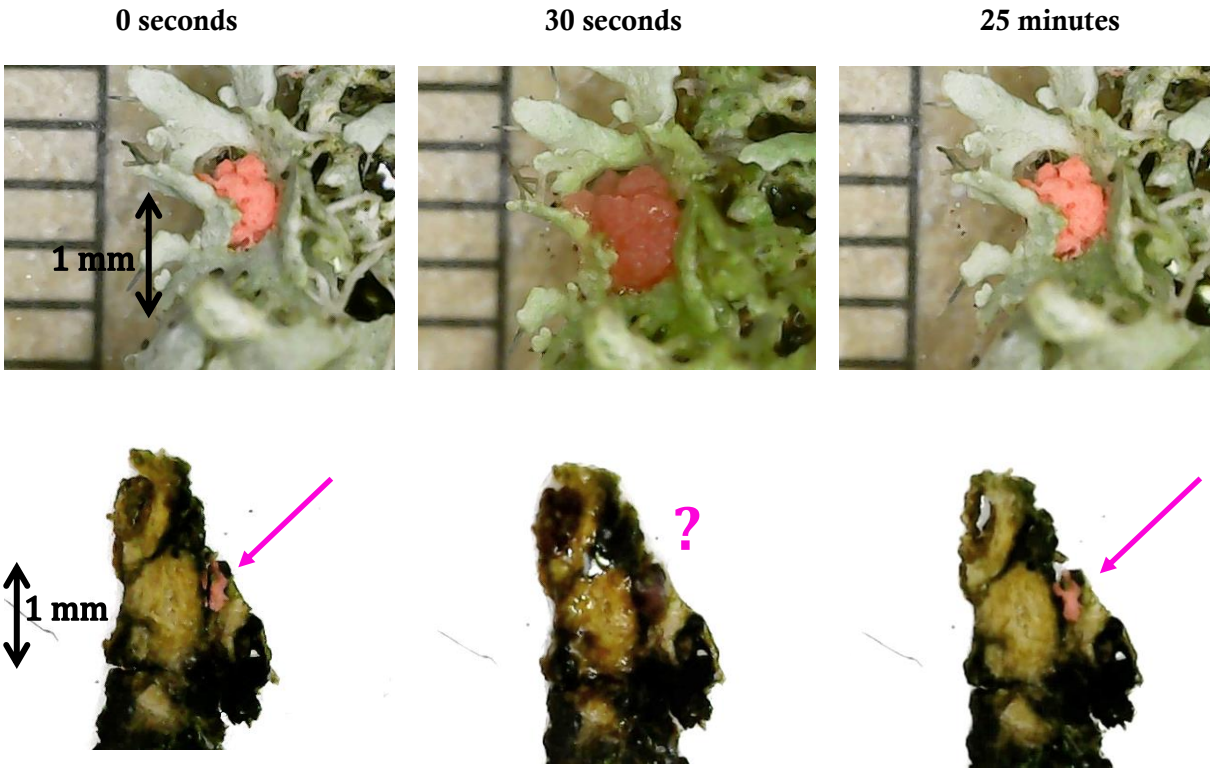

Figure 5 Colour and translucency change of cirrhi on hydration, samples from February (above) and November (below) The reaction ranges from shiny while wet but no colour change (above) to immediate disappearance, reappearing white, then pink on drying (below). Photographs taken immediately before hydration, 30 seconds later and after 25 minutes. Photos © A.M. Claypole

Field test trials

In autumn/winter 2022–3, a simple *in situ* hydration test (add a drop of water or spray) was carried out at the three observation sites and by LCIG colleagues across the UK. The results reflected those observed in the laboratory. All reported that *Ill. christiansenii* cirrhi swelled up immediately;

that roughly one-third lost some colour, and about half changed in consistency (dissipated, went glutinous or muculent). All observers with patience recorded the return to something like the initial state. No reaction was observed for *E. aurantiacum* bulbils. The results of one such field test are shown in Fig. 6.

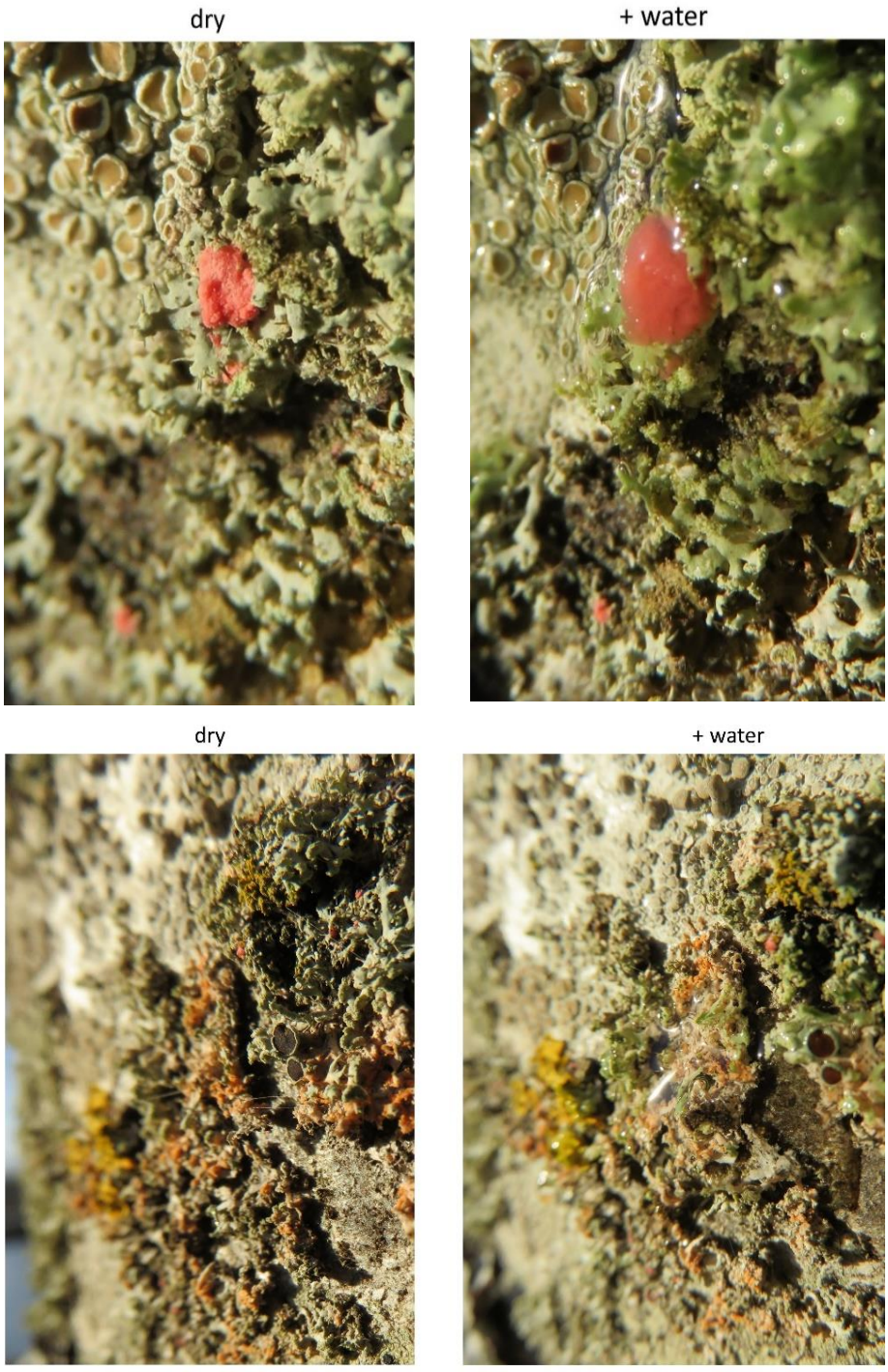

Figure 6 Field trial of the water-drop test in VC85, Fifeshire NO463285 conducted by M. Chapman in February 2023. The test compared the response to hydration of *Ill. christiansenii* and *E. aurantiacum* growing with *Physcia tenella* on the same *Sorbus aucuparia* bole. *Ill. christiansenii* cirrhi expanded but did not lose any colour (upper). No reaction (colour or swelling) was observed for *E. aurantiacum* (below). The weather was dry, cold and breezy. Photos © L. Bidau

Water-drop test in a binary field key

On wetting, *Ill. christiansenii* cirrhi extend rapidly by approximately 0.5 mm which is observable with a x10 hand lens or mobile phone camera. *E. aurantiacum* bulbils only expand about 0.05 mm which is barely visible and since there is no colour change, there appears to be no reaction at all.

The expansion reaction distinguishes *Ill. christiansenii* from both *E. aurantiacum* and *M. corallinus* and could usefully serve in a binary field key. The only limitation would be wet or humid conditions, but samples could also be tested in the laboratory after drying.

The changes in colour, texture and translucency also distinguish *Ill. christiansenii* from both *E. aurantiacum* and *M. corallinus* bulbils, neither of which change visibly. *Ill. christiansenii* cirrhi may become paler, and more translucent; or even seem to disappear. These are very striking reactions, but also highly variable according to season, rendering colour change less suitable as a binary field key test. A positive response could be of use only if supported by other characteristics. A comparison of the features of the three look-alike LFs is given in Fig. 7.

During the study (2022–2023) incidental reports were received from various members of the LCIG group that *E. aurantiacum* appeared to be displacing or replacing *Ill. christiansenii* at a number of sites across the country, making a quick identification test particularly valuable.

The variability of the hydration reaction means that the water test in identification keys for *Ill. christiansenii* may require rewording. While all dry cirrhi expand on wetting, not all give the impression of 'dissolving'. In the original references, the wording 'disperse readily in water' referred to conidia on a microscope slide. For a field test, 'become mucilaginous and spread' would better describe the reaction and this also describes cirrhi that retain their colour.

Feature	***Ill. christiansenii***	***E. aurantiacum***	***M. corallinus***
1. colour	bright pink to red	pale orange	coral to bright pink
2. habit	next to the host thallus or in fully necrotised areas, usually on *Physcia* spp.	on necrotised host thallus, commonly on *Physcia* spp.	on necrotised host thallus, commonly on *Parmelia* spp.
3. size/shape	0.6–1 mm across, 0.1–2 mm high, irregular shape	0.17–0.22 mm, ± spherical	0.08–0.25 mm, ± ellipsoidal
4. water test	immediate swelling or dissipation	no visible swelling or colour change	no visible swelling or colour change
5. content	helicoid, transversely septate conidia	± spherical, granular cells	chains of spherical to elongate cells

Data for *M. corallinus* from https://fungi.myspecies.info/all-fungi/marchandiomyces-corallinus and https://britishlichensociety.org.uk/sites/default/files/Marchandiomyces%20corallinus.pdf (both retrieved 30 April 2023)

Figure 7 Comparison of the pink or orange-pink masses of the three look-alike LFs. Features 1–4 are suitable for use in the field. Feature 5 serves as a confirmation in the laboratory.

Hydration of the cirrhi

Precipitation In the weeks before collection of the original February samples at Site 2, there had been 85 mm precipitation and an average relative humidity of 90%. Therefore, the cirrhi had already been subjected to multiple wettings and dryings.

Extreme hydration In autumn 2022 at Site 3, an attempt was made to disperse cirrhi *in situ.* Gentle spraying was gradually increased to forced irrigation and recorded on video. The cirrhi just became bloated, shiny and wet. At Site 1 cirrhi were filmed in torrential rain with the same result. *In situ* some cirrhi neither disperse nor wash away; they just become fat, shiny and wet (Fig. 8). This confirms that if the cirrhi are already wet, the water-drop test is unlikely to work in the field. The effect of successive expansion and contraction is to spread some of the pink conidia over the neighbouring tissue (Fig. 8, centre). After successive cycles of hydration and drying, the cirrhus is eventually reduced to a pink smear. A single soaking of the cirrhus does not achieve this.

Movement This study showed that the effects of water are not limited to the cirrhus, but also result in a massive movement of any healthy host thallus nearby. The *Physcia* thrashes about dramatically as the water content of the thallus changes. The movements of the *Physcia* thallus may bring uninfected parts into closer proximity to the sporodochium, aiding the dispersal of the sticky *Ill. christiansenii* conidia onto it. Soralia of the host can also be seen nearby, suggesting the possibility of conidia of the LF and soredia of the host lichen being dispersed together.

very wet dry prolonged torrential rain

Photos © D.M. Napier Photo © M.L. Sisti

Figure 8 Effects of extreme wetting on cirrhi. Artificial irrigation showing a very wet state and the following day when dry (site 3) and during prolonged torrential rain (site 1)

Propagation and dispersal of *Ill. christiansenii*

Sporodochia produce and protect dense masses of conidia embedded in a gelatinous matrix, the cirrhi, which propagate the LF.

Dispersal is a limiting factor for any LF. They are most common at sites with long ecological continuity (Hawksworth, 2003) and most have a limited geographic range (Lawrey & Diederich, 2003). The community at Site 2 had only spread a few centimetres along a single twig, not even to *Physcia* on adjacent twigs. Similarly, at Site 3, the infection spread along a single bar of the gate, but not to the *Physcia* on the bar below.

Dry samples stored from the beginning of 2022 still responded to hydration at the end of the year. This may be an adaptation of the LF to the host lichen habit, but the conidia cannot propagate when dry. Instead, they remain protected, glued to the substrate in a layer of dried mucus. The cirrhi immediately expand on wetting, become gelatinous and sticky, and the conidia separate more easily.

A number of means of dispersal have been suggested, both physical and biological. Those that involve both wetting and mucilaginous spores are splash dispersal and animals.

Microarthropods may play a role as suggested by Fox (1997) either carrying the spores externally or eating them. Meier *et al.* (2002) demonstrated that faecal pellets of lichenivorous mites contain viable mycobiont and photobiont cells of *Xanthoria parietina*. Plenty of orabitid mites were observed at Site 2, mainly grazing in dark and damp conditions.

Stepanov (1935) compared mucilaginous spores from a number of fungi and concluded they were detached by raindrops and dispersed by splash. Rain, fog, dew, mist, overhead drip-splash etc. are all possible agents, particularly under canopies. Fitt *et al.* (1989) report that many splash-borne fungal spores appear to have adhesive properties when wet, enabling them to stick to surfaces.

Splash dispersal is considered characteristic of many mucilaginous fungal spores. The dry spores are glued to the substrate by the mucilage. An initial wetting (by rain or high humidity) causes the mucilage to swell and dissolve, leaving the spores suspended in a thin film of water on the surface. Subsequent rain drops propel them into the air in splash droplets (Gregory *et al.*, 1959). In the case of *Ill. christiansenii,* while a single wetting may release a batch of spores, the majority appear to remain in the cirrhi. If splash dispersal is occurring, then the cirrhus may actually serve to slow it down, lengthening the dispersal period. Some pink material containing conidia is smeared onto the adjacent tissue where it will remain until the next wetting.

The cirrhus is poikilohydric which means that its water status is completely dependent on the environment. The water vapour partial pressure of the cirrhus is in equilibrium with the humidity of the atmosphere. Except after rain, damp-air fungal spores only occur in quantity at night when dew is formed, reaching the highest concentration between midnight and dawn (Hirst, 1953). A single pre-dawn dewfall roughly fills most lichens' internal water-holding capacity, suggesting that lichens are optimized to use dew rather than rain (Gauslaa, 2014). Whatever the dispersal mechanism, a diurnal dew cycle would expand and contract the *Ill. christiansenii* cirrhi, dispersing the conidia over an extended period until the whole cirrhus smears away.

Summary

On wetting, *Ill. christiansenii* cirrhi expand rapidly and become muculent; they may also lose colour. On drying they contract and become pink again. The hydration response is most striking mid-season (late autumn) and is probably essential for propagation and dispersal.

Based on this expansion reaction, a simple water-drop test distinguishes *Ill. christiansenii* cirrhi from the bulbils of both *E. aurantiacum* and *M. corallinus* and is appropriate for use in a binary field key.

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