Working document – *Lecidea lichenicola*

Lecidea lichenicola, hunted and found, on Dunstable Downs

Valentine's Day 2013

When trying to study this miniscule organism the literature is not a great deal of help. We are probably not even sure whether it is truly a species of lichen (even though it is listed in the lichen literature and see note 1 below) and we are certainly not sure what it is related to. Oliver Gilbert, in his New Naturalist Series classic "Lichens" (Gilbert 2000) writes at some length about the lichens associated with chalk pebbles brought to the surface by rabbits burrowing. He states that the chalk "pebble community is dominated by a rather small number of lichens, among which *Verrucaria hochstetteri*, *V. muralis* and *V. nigrescens* are the main species, although a persistent search will often reveal *Caloplaca citrina*, *Lecidea lichenicola*, *Polyblastia dermatodes*, *Protoblastenia rupestris*, *Sarcogyne regularis*, *Staurothele hymenogonia* and *Thelidium incavatum*." Apart from the *Protoblastenia* and the *Sarcogyne*, these species are either rarely recorded or misunderstood. Powell & Vondrák (2011) demonstrated that lichens of the *Caloplaca citrina* group in Britain appear to belong to five different taxa, none of which are *C. citrina* s.str.. Orange (2009) states that "*Polyblastia dermatodes* is said to differ [from *Thelidium incavatum*] in the submuriform ascospores but… it is difficult to make a clear distinction between the two species". So we are left with the (correct) impression that chalk pebbles support a small suite of lichens that are difficult to study but probably consist of interesting and under-recorded species.

My first encounter with *Lecidea lichenicola* was in February 2012 at Cliveden in Buckinghamshire where it occurs on chalk rubble above the River Thames in the Hanging Woods. I did not recognise the minute, translucent, dark red fruits in the field. Smith *et al*. (2009) is the current lichenologist's "bible" but *L. lichenicola* presents considerable difficulties when attempts are made to identify it from first principles using this modern "Flora". One gets used to sticking metaphorical fingers into dichotomous keys at points which appear to give ambiguous options but it is disconcerting when an unusual species provokes ambiguity near the start of the process. The first problem arises when one tries to decide whether the fruits are apothecia or perithecia. The Glossary of Smith *et al*. (2009) define a perithecium as "a subglobose or flask-like ascoma in which the hymenium is not exposed" while an apothecium is defined as "a cup- or saucer-like ascoma in which the hymenium is exposed at maturity. Most of the fruits of the Cliveden material are subglobose and could be considered to be perithecia by those definitions. This would lead to a fruitless diversion through the perithecioid key (Key 3). Smith *et al*. (2009) consider this species to have apothecia and the species keys out, on its own, at question 29 in Key 6. I only found my way back through the maze when I knew where I was heading; that is one of the common quoted problems with keys. They are useful for confirming how you should have arrived at a specimen's identification but often that was not the route that led you to the answer in the first place! Having decided to consider the fruits to be urceolate apothecia, one next encounters the problem of elucidating the nature of the photobiont. The thallus is thin and effectively endolithic, though the chalk is crumbly and consists of minute granules in the zone where

algal cells and hyphae are present. This layer of degraded chalk contains a heterogeneous collection of hyphae and photosynthetic cells in some specimens and appears virtually empty of living structures in other cases (see Note 1). Returning to the difficulty of keying this species out from first principles, if one makes the bold assumptions that the fruits are apothecia and that the photobiont is chlorococcoid, one ends up working through Key 6a where *L. lichenicola* is keyed out at couplet 29 but this relies on making the decision that the paraphyses are "unbranched or sparingly branched only towards the apex" rather than "branched or anastomosing" at couplet 22. In my early attempts to identify this lichen I mistakenly took the second option (finding that the paraphyses are sparsely branched and anastomosed) which leads to incorrect and implausible options. In my case I got to know *L. lichenicola* by borrowing a named specimen from an old collection and then the penny dropped.

Aptroot *et al*. (2009) state that "This species should probably be placed in *Trapelia*, from which it differs only in the immersed thallus and apothecia, and the unique habitat of chalk pebbles." With modern molecular techniques it ought to be possible to obtain genetic sequence information and hence work out its true relatives. Some of the Cliveden material was sent to Benjamin Stielow at Utrecht who attempted to do such an analysis but unfortunately this work was unsuccessful. The specimens came from a semi-shaded spot and were somewhat overgrown with miscellaneous algae and this contamination may have hindered the genetic work. David Hawksworth, who had enlisted Benjamin into our project, planned to return to Cliveden to search for more specimens while I planned a trip to look at the extensive chalk screes at Dunstable Downs and arrived there on 14th February 2013 with the sun attempting to emerge after heavy overnight rain.

My first hour or so on the Downs was spent examining chalk pebbles dislodged by rabbits within the various patches of scrub. Most of the pebbles had been too recently disturbed to have been colonised by lichens and most of them were blotched with free-living algae. Some were speckled with tiny black fruits belonging to members of the Verrucariaceae but, despite examining many dozens of pebbles, nothing resembling *L. lichenicola* was found. While walking from one patch of scrub to another my attention was drawn to numerous fragments of chalk rubble in more open conditions and the appearance of these reminded me of the Cliveden specimens. I had scrutinised less than half a dozen of these fragments when I found my first convincing candidate. I soon found another, then another, and started to realise that I had found a rich source of material. My only remaining concern was that these tiny fruits (just 0.1 to 0.2 mm diameter) might just turn out to be something else and I knew that I would have to wait until I got home to study them microscopically before I would know for sure. A recent collection of similar (but larger) dark reddish fruits on chalk rubble at Devil's Ditch near Newmarket had turned out to be *Steinia geophana*. These Dunstable specimens did look very convincing though (and were later confirmed). I now know that these chalk fragments were dislodged by a large mechanical excavator rather than by rabbits. This machinery was used to clear scrub from parts of the Downs about four or five years previously and had left a light scatter of chalk fragments, which, although out in the open, are lightly shaded by coarse herbaceous vegetation during the summer. These fragments are more stable than those associated with rabbit burrows and many have almost complete mosaics of crustose lichens. The appearance of these mosaics can be divided into "bright white with black convex dots" (members of the Verrucariaceae), "shades of brown with black convex dots" (Verrucariaceae again) and ochre patches which appear bare rock at a glance but which often turn out to contain the fruits of *L. lichenicola*. I mentioned the fact that there had been overnight rain. Wet lichens are usually more difficult to identify as they lose some of their characteristic colours and texture as the surface becomes plumped up and translucent (allowing the green colour of the algal partner to show through). With *L. lichenicola* the opposite appears to be the case. When dry the apothecia darken and shrink but in damp conditions they swell, and the translucence of their red-pigmented hymenium causes them to

"glow" with a perceptible reddish hue. A glance at the NBN Gateway shows that *L. lichenicola* has only been recorded once this century previous to the Cliveden discovery. Aptroot *et al*. (2009) give the habitat and distribution of *L. lichenicola* as follows: "On chalk pebbles in disturbed habitats (e.g. around rabbit burrows); localized. S. & E. England (Isle of Wight, Susses, Surrey, Hertfordshire, E. Yorkshire). Possibly endemic." It would be interesting if some of our Continental colleagues could search for this lichen; it seems strange that it should be rather widespread in England but not present in similar habitat on the Continent. I would slightly regret losing its endemic status, but we ought to find out. I suspect that British records will now start to trickle in once more people know more about its habitat range and access to photographic images.

The Dunstable material will be used for another attempt at genetic fingerprinting. The specimens are "cleaner" (less contaminated by algae) than the Cliveden material and better-developed so there is a hope that we may eventually find out this organism's true identity.

I am grateful to Jon Powell of the National Trust for his help on site and for information of past management practices. A debt of gratitude is owed to Benjamin Steilow (for many hours of ongoing laboratory analysis which will hopefully yield fruit in the near future) and to David Hawksworth (for past taxonomic work on this species and collaboration and encouragement to continue). Jon suggested that the ending of the specific name "cola" seems rather apt as at Dunstable this species grows in abundance on sunny slopes used for informal picnics and so empty bottles which formerly contained that beverage are a feature of its wider habitat. I don't suppose that is the true etymology of "lichenicola".

References

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Note 1: The photobiont. One of my opening comments (that we are probably not sure that *L. lichenicola* is truly a lichen) is perhaps deliberately provocative. Aptroot *et al*. (2009) say nothing about the photobiont. I have found it very difficult to decide which, if any, of various different hyphae and algal cells belong to this species and my comment will hopefully stimulate others to study this matter and prove to me that *L. lichenicola* is lichenized. The surface of the thallus (at least in the Cliveden material) usually has an orange hue due to the presence of spherical cells with a large chloroplast, the green colour of which is almost obscured by orange pigment. These are presumably a species of *Trentepohlia* and are probably just living on the surface of the thallus though they are absent on the thallus of other associated lichen species. Within the "thallus" there is an abundance of oblong cells with thick walls. These may be a species of cyanobacterium and I suspect that it is these that give the thallus a sub-gelatinous texture when wet. Even if the thallus immediately adjacent to the apothecia is examined it is still difficult to assign a particular type of photobiont as unambiguously occurring as the photosynthetic partner. Sometimes I have found clusters of green algal cells showing binary division. If these were in lichenized association it would be interesting

bearing in mind that in the genus *Trapelia* (to which *L. lichenicola* may be related) the photobiont cells often exhibit binary fission. I suspect, however, that such cells that I have found in the thallus of the Cliveden material are free-living, contaminant *Chlorella* cells.

Note 2: Anatomical details. The fruits of the Cliveden material are mainly in the range 100 – 175 µm diameter. There is no thalline exciple, the true exciple is thin and remains peritheioid in almost all of the fruits. Aptroot *et al*. (2009) state that the apothecia of this species become "finally expanded with a ± flat disc, 0.1-0.2(-0.35) mm diam." Perhaps the apothecia of this species show a seasonal development and the lack of expanded discs in the Cliveden material is a result of the late winter season of collection. The exciple, in section, is pale orange-brown in colour becoming more densely pigmented near the upper (outer) edge. The epihymenium is bright orange-brown; otherwise the hymenium is colourless or pale straw. The hypothecium is shallow, only c. 10 µm deep and colourless or pale straw in colour. The hymenium is tall, c. 120-130 µm. The paraphyses are very long and slender and appear to be simple when sections are mounted in water or K. If the hymenium is suitably stained and squashed the paraphyses are found to be sparsely, but significantly, branched and anastomosed. The paraphyses extend well above the tops of the asci where they often seem to clump together in bundles, this may be an artefact produced when the hymenial gels gets disrupted during sectioning and mounting. The hymenial gel is weakly KI+ blue, especially in the upper part. The ascospores are narrowly ellipsoid to fusiform, sometimes slightly sinuous. The spores are spores are colourless and simple and contain conspicuous inclusions of various sizes. These inclusions (oil drops?) are very persistent even after the addition of K or N. The spores which I have measured so far are within the range stated by Aptroot *et al.* (2009) except that I have found some ejected spores which are as short as 13 µm. Some individual spores are somewhat attenuated at one end giving a narrow, lemon-shaped appearance. This attenuation can also be observed in the spores of some lichens in the genus *Trapelia*. Aptroot *et al*. (2009) state of the asci "apex thickened K/I-; outer coat K/I+ blue." I have found that using Lugol's iodine after pre-treatment using the ink-vinegar staining technique, to give superior results to the normal K/I staining technique. Using this method young asci show a distinctive amyloid layer, like a sheath, within the distal wall of the ascus though I have not yet observed an amyloid structure in more mature asci which are usually disrupted by the harshness of this method of staining. This disruption takes the form of lateral tearing close to the bases of the asci and also between half and two thirds of the way to the apex. The central portion of the asci are seen as collapsed-cylindrical remains which have a delicate appearance, transparent, non-rigid and with prominent pleat-like or seamed longitudinal thickenings. The disrupted hymenium appears as a "mown" sward of the short remains of ascus bases attached to the hypothecium.

Note 3: Some anatomical comparisons with the genus *Trapelia*. The pigments, including the lack of them, in the hymenium and hypothecium of *T. coarctata* are very similar to those of *L. lichenicola* and the hymenium stains weakly K/I+ blue in a similar manner. The spores share their lack of septa, occasionally attenuated ends and the presence of rounded inclusions which remain persistent in K. The asci of *T. coarctata*, when subjected to the ink-vinegar method, show a simple ragged rupture at the apex of asci which are mature enough to dehisce. Otherwise most asci remain intact and attached to the hypothecium. By contrast, the asci of *T. glebulosa* have a very similar appearance to those of *L. lichenicola* after ink-vinegar treatment, with a "pleated-cellophane" appearance and a fragile nature breaking off at the top and bottom to produce sleeve-like cylinders.

Note 4: I would like to include an extract from Gilbert (2000). The late Oliver Gilbert had a way with words and could make any habitat, whether urban debris, garden walls or chalk pebbles, sound compelling. It was this very section of his book which sparked an interest in this habitat, and in *L. lichenicola*, long before I explored and discovered.

“Chalk pebbles are brought to the surface by rabbits burrowing and scraping. On steep slopes sheep grazing can also be sufficiently heavy to disrupt the turf and unearth them. Around large warrens extensive fans of loose nodules are present, but these are mostly too mobile to support lichens. It is in the vicinity of abandoned warrens that the chalk pebble lichen community reaches its maximum development. Excavated pebbles collect in declivities, in folds in the hillside and at the foot of slopes, forming a fine scree. These are the richest sites, but old scrapings, no more than 10 cm across and containing only a dozen or so nodules, also repay examination. Eventually all these sites become overgrown and return to grassland. Pebbles associated with molehills, sheep tracks and terracettes are less interesting.”

Note 5: The following are some notes on the history of the taxonomy kindly supplied by David Hawksworth in a personal communication:
As regards "*Lecidea*" *lichenicola*, Peter James and I thought it was very *Trapelia*-like many years back, and I would have transferred it there before except that *Discocera* A.L. Sm. & Ramsb 1918 predates *Trapelia* M Choisy 1929. We mentioned this in the 1980 checklist (Lichenologist 12: 100, 1980) when I did the *Lecidea* account for the 1992 "Flora”. I discussed the typification of *Lecidea lichenicola* when I transferred it to that genus (Notes RBG Edinb. 36:184-185, 1978). Strange as it seems with hindsight, *Trapelia* was not accepted as separate from *Lecidea* in the then current checklist (James 1965).