

Preliminary statistical studies of the infraspecific variation in the ascospores of *Nesolechia oxyspora* growing on different genera of parmelioid lichens

Caroline J. DORÉ, Mariette S. COLE and David L. HAWKSWORTH

Abstract: In order to ascertain the extent of possible host-specialization in the *Nesolechia oxyspora* complex, as a pilot study ascospores from 20 collections from different parmelioid hosts representing seven genera were drawn, measured, and had length:breadth ratios calculated. The data were then subjected to multiple regression analysis using Huber-White sandwich estimators of standard errors (apparently not previously used in mycology) which take account of spores not necessarily being independent variables as they might come from the same ascus, and a statistical analysis. Significant differences between collections from seven genera were found. While the sample size was too small to reach definite conclusions, it is clear that there is a finer degree of host-relatedness than hitherto expected, which may be geno- or phenotypic. A more extensive study including species from a wider range of hosts and complemented by molecular methods will be necessary to further elucidate degrees of specificity and cryptic co-speciation in the complex. A list of the 63 reported lichen hosts is included; these are distributed through 19 genera.

Key words: co-evolution, co-speciation, cryptic species, host-specificity, lichenicolous fungi, lichens, *Parmeliaceae*, systematics

Introduction

The generic name *Nesolechia* A. Massal. was introduced by Massalongo (1855: 75) for “*Abrothallus oxysporum*” and other species excluded from *Abrothallus* De Not. in that they did not have brown 1-septate ascospores.¹ Later he was more specific (Massalongo 1856: 43) and applied the name to five lichenicolous fungi with emarginate apothecioid to arthonioid ascomata,

¹We accept this as a valid diagnosis as it was the absence of brown 1-septate ascospores that was the distinguishing character “in the opinion of its author” (Art. 32.2); the generic name thus dates from 1855 and not 1856 as commonly listed.

“excipulo quolibet destituta”, and producing simple colourless ascospores. The first listed species, in both publications, *N. oxyspora* (Tul.) A. Massal., has consistently been treated as lectotype (Clements & Shear 1931: 331; Nannfeldt 1932: 328; Triebel & Rambold 1988: 295; Greuter *et al.* 1993: 761).² The genus was treated as a synonym of *Phacopsis* Tul. by Triebel & Rambold (1988: 295), but this decision has not been universally accepted and has not been followed in many subsequent publications (e.g. Alstrup & Hawksworth 1990; Hafellner &

²However, we note that Lindsay (1869: 13) retained that species in *Abrothallus* while also accepting *Nesolechia* for four species, including one of the species included in the genus by Massalongo (1855), viz. *N. thallicola* (A. Massal.) A. Massal. (syn. *Phacopsis thallicola* (A. Massal.) Triebel & Rambold). It could therefore be argued that *N. thallicola* should have been regarded as already selected as lectotype by Lindsay, but we prefer not to make any change to the otherwise generally accepted lectotypification, although we recognize that a nomenclatural pedant might feel that a formal proposal to conserve the name with *N. oxyspora* as type species was necessary.

C. J. Doré: MRC (Medical Research Council) Clinical Trials Unit, 222 Euston Road, London NW1 2DA, UK.

M. S. Cole: 2017 Thure Avenue, St. Paul, MN 55116, USA.

D. L. Hawksworth (corresponding author): Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense, Plaza de Ramón y Cajal, Ciudad Universitaria, Madrid 28040, Spain.

TABLE 1. *Lichen species from which Nesolechia oxyspora s. lat. has been reported*

Host*	Source**
<i>Cavernularia hulthenii</i>	Triebel <i>et al.</i> (1991, 1995) ³ , Diederich (2004) ³
<i>C. lophyrea</i>	Diederich (2004) ³
<i>Cetrelia olivetorum</i>	Vouaux (1913)
<i>Evermiasstrum nepalense</i>	Triebel <i>et al.</i> (1995) ¹ , Hafellner (1998)
<i>Evermiasstrum</i> sp.	Etayo (2002)
<i>Flavoparmelia caperata</i>	Vouaux (1913)
<i>Flavopunctelia flaventior</i>	This study
<i>Hypogymnia physodes</i>	Vouaux (1913), Hafellner & Sancho (1990)
<i>H. tubulosa</i>	Santesson (1988), Hafellner & Sancho (1990), Triebel <i>et al.</i> (1995) ³
<i>Hypotrachyna flavovirens</i>	Wedin (1994)
<i>H. imbricatula</i>	Aptroot <i>et al.</i> (1997)
<i>H. meridensis</i>	Hafellner <i>et al.</i> (2002)
<i>H. pulvinata</i>	Hafellner <i>et al.</i> (2002)
<i>H. reducens</i>	Aptroot <i>et al.</i> (1997)
<i>H. revoluta</i>	Santesson (2001)
<i>H. sinuosa</i>	Vouaux (1913), Triebel <i>et al.</i> (1995) ² , Aptroot <i>et al.</i> (1997), Etayo (2002)
<i>Melanelia sorediata</i>	Hafellner (1993), Santesson (1993), Santesson <i>et al.</i> (2004)
<i>Melanelixia glabra</i>	Kocourková (2000)
<i>M. subargentifera</i>	This study
<i>M. subaurifera</i>	Kocourková (2000)
<i>Melanohalea elegantula</i>	This study
<i>M. exasperata</i>	Keissler (1930)
<i>M. infumata</i>	Zhurbenko & Hafellner (1999), Kocourková (2000)
<i>M. olivacea</i>	Alstrup (1991), Triebel <i>et al.</i> (1995) ¹ , Hafellner (1998)
<i>Menegazzia terebrata</i>	Triebel <i>et al.</i> (1995) ⁴
<i>Menegazzia</i> sp.	Aptroot <i>et al.</i> (1997), Kocourková (2000)
<i>Parmelia fraudans</i>	Alstrup & Hawksworth (1990), Triebel <i>et al.</i> (1995) ^{1,2} , Santesson <i>et al.</i> (2004) ² , Zhurbenko (2004)
<i>P. hygrophila</i>	Diederich (2003)
<i>P. omphalodes</i>	Zhurbenko & Hafellner (1999), Kocourková (2000)
<i>P. saxatilis</i>	Tulasne (1852), Hafellner & Sancho (1990), Triebel <i>et al.</i> (1995) ^{1,2} , Zhurbenko & Hafellner (1999), Santesson <i>et al.</i> (2004) ²
<i>P. sulcata</i>	Hafellner & Sancho (1990), Triebel <i>et al.</i> (1991, 1995) ^{1,2} , Zhurbenko & Hafellner (1999), Santesson <i>et al.</i> (2004), Hafellner <i>et al.</i> (2004), Suija (2004)
<i>P. tenuirima</i>	Triebel <i>et al.</i> (1995) ²
<i>Parmelina tiliacea</i>	Vouaux (1913)
<i>Parmelinopsis damaziana</i>	Triebel <i>et al.</i> (1995) ³
<i>Parmotrema crinitum</i>	Triebel <i>et al.</i> (1995) ³
<i>P. reticulatum</i>	Kocourková (2000)
<i>P. tinctorum</i>	Etayo & Osorio (2004)
<i>Platismatia glauca</i>	Tulasne (1852), Santesson (1984), Triebel <i>et al.</i> (1995) ¹ , Hafellner (1998), Santesson <i>et al.</i> (2004)
<i>P. norvegica</i>	Santesson (1993), Diederich (2003), Santesson <i>et al.</i> (2004)
<i>Pseudevernia furfuracea</i>	Vouaux (1913), Triebel <i>et al.</i> (1995) ³
<i>Punctelia borrieri</i>	Vouaux (1913)
<i>P. lorentzii</i>	Santesson (1994)
<i>P. punctilla</i>	Triebel <i>et al.</i> (1995) ²
<i>P. rudecta</i>	Triebel <i>et al.</i> (1995) ^{1,2} , Diederich (2003)
<i>P. semansiana</i>	Triebel <i>et al.</i> (1995) ²
<i>P. subrudecta</i>	Triebel <i>et al.</i> (1995) ² , Santesson (1998)
<i>Rimeliella</i> sp.	Etayo & Osorio (2004)
<i>Xanthoparmelia angustiphylla</i>	Triebel <i>et al.</i> (1995) ³
<i>X. annexa</i>	Aptroot & Triebel (2002) ⁵
<i>X. conspersa</i>	Lindsay (1857), Vouaux (1913), Triebel <i>et al.</i> (1995) ³ , Aptroot & Triebel (2002) ⁵ , Santesson <i>et al.</i> (2004) ³ , Hafellner <i>et al.</i> (2004)

TABLE 1. Continued

Host*	Source**
<i>X. fissurina</i>	Triebel <i>et al.</i> (1995) ³
<i>X. incerta</i>	Aptroot & Triebel (2002) ⁵
<i>X. loxodes</i>	Triebel <i>et al.</i> (1995) ³
<i>X. molybdiza</i>	Aptroot & Triebel (2002) ⁵
<i>X. mougeotii</i>	Kocourková (2000)
<i>X. perrugata</i>	Vouaux (1913)
<i>X. protomatrae</i>	Kocourková (2000)
<i>X. pulla</i>	Hafellner & Sancho (1990)
<i>X. stenophylla</i> ⁶	Hafellner & Sancho (1990), Triebel <i>et al.</i> (1995) ³ , Santesson <i>et al.</i> (2004) ³
<i>X. taractica</i>	Hafellner & Sancho (1990)
<i>X. tegeta</i>	Triebel <i>et al.</i> (1995) ³
	Navarro-Rosinés & Hladun (1987), Hafellner & Sancho (1990), Triebel <i>et al.</i> (1995) ³ , Santesson <i>et al.</i> (2004) ³
<i>X. tinctoria</i>	(1995) ³ , Santesson <i>et al.</i> (2004) ³
<i>X. verruculifera</i>	Kocourková (2000)
<i>X. xanthomelaena</i>	Triebel <i>et al.</i> (1995) ³

Excluded record: Olivier (1905) mentioned 'Evermia' as a host but no species name was indicated; the record may therefore refer to *Pseudevermia furfuracea*.

*Names used are the currently accepted ones, which are not necessarily the same as those adopted in the sources cited.

**Selected sources only are given in the case of commonly reported hosts, preference being given to accounts with descriptions, illustrations, and detailed bibliographic or specimen information.

¹As var. *oxyspora*.

²As var. *defecta*, a name treated as a synonym of the species here called *N. oxyspora* by Aptroot *et al.* (1997) and Diederich (2003), but still accepted by Peršoh & Kainz (2004).

³As var. *fusca* or *Phacopsis fusca*.

⁴As *Phacopsis menegazziae* Rambold & Triebel, a name treated as a synonym of the species here called *N. oxyspora* by Diederich (2003).

⁵As *Phacopsis australis* Aptroot & Triebel.

⁶The nomenclaturally correct name for *X. somloënsis* (Ahti & Hawksworth 2005).

Sancho 1990; Santesson 1993; Hafellner 1993, 1998; Zhurbenko & Hafellner 1999; Hawksworth 2003). Further, SSU nrDNA sequence data placed *N. oxyspora* in the same clade as *Xanthoparmelia conspersa*, although with only modest bootstrap support, and *P. huuskonenii* in a sister clade, also with modest support (Peršoh & Rambold 2002). ITS sequence data placed *P. huuskonenii* with two *Usnea* species, and the type species of *Phacopsis* (*P. vulpina* Tul.) did not group with *N. oxyspora* (Peršoh & Rambold 2002). According to this study, both *Nesolechia* and *Phacopsis* are separate genera, both of which belong to *Parmeliaceae*. The placement of the two genera in *Parmeliaceae* was unexpected, and we would like to see independent molecular work confirm this conclusion, although D. Peršoh (pers.

comm.) has assured us he was confident in the results.

Nesolechia oxyspora was originally described as *Abrothallus oxysporus* Tul. and stated to occur on thalli of *Parmelia saxatilis* and *Platismatia glauca* (Tulasne 1852: 116). However, in selecting as lectotype a specimen from Dolgelly in the UK collected by Ralfs and preserved in PC, Triebel *et al.* (1995) noted that the host that had been named *P. glauca* was actually also *Parmelia saxatilis*. Lindsay (1857) compared material from both these hosts, and *Xanthoparmelia conspersa*, concluding that they represented a single species. An extensive compilation of the various hosts reported in the literature was made by Kocourková (2000), which has been supplemented here (Table 1) and now includes 63 species in 19 genera.

Triebel *et al.* (1995) sought to determine whether there were correlations between hosts and other characters, and concluded that three varieties could be recognized within the species, separated by the colour and iodine reaction of the hypothecium. However, in several cases more than one of their varieties occurred on the same host parmelioid genus or even species, a situation not to be expected if the hosts were not closely related. Indeed, within the parmelioid lichens there is often a strong correlation between the occurrence of lichenicolous fungi and some segregate genera (e.g. Diederich 1990; Hafellner 1998; Cole & Hawksworth 2001; Hawksworth *et al.* 2004). Aptroot *et al.* (1997) did not accept Triebel *et al.*'s var. *defecta*, based on the iodine reaction of the hypothecium, as they found that about 50% of the collections on *Parmelia* s. str. species growing in the same locality "belonged" to each of the "varieties". However, Aptroot & Triebel (2002) did accept var. *fusca*, with a brown hypothecium, when describing *P. australis* as new from *Paraparmelia* and *Xanthoparmelia* species in southern Africa; that species did not form galls and was characterized by the superficial ascomata, colourless hypothecium, and *N. oxyspora*-shaped ascospores. Later, Diederich (2003) raised var. *fusca* to species rank, but relegated *P. menegazziae*, that had been separated primarily on ascospore size, to synonymy with *P. oxyspora*. In contrast, Peršoh & Kainz (2004) accepted var. *defecta*, but did not think var. *fusca* should be treated as a species as the presumed difference in colour of the ascomata was related to that of the hypothecium.

The extent to which species of lichenicolous fungi are host-restricted to particular lichen genera or species varies greatly (Hawksworth 2003; Lawrey & Diederich 2003). While some species have wide host ranges, however, most are much more restricted, may have evolved along with the host lichens, and can act as indicators of phylogenetic relationships (Hawksworth 2003). Lawrey & Diederich (2003) estimated that 95% of the known lichenicolous

fungi were associated with particular host genera. The potential of obligately occurring fungi to act as plant taxonomists has long been recognized (e.g. Hedberg 1979), and we considered that a more critical examination of *N. oxyspora* was required as: (1) it is known from such a wide range of parmelioid lichens (Table 1); and (2) in the course of our studies on lichenicolous fungi, we examined many collections and became aware that there appeared to be considerable variation in ascospore shape and size within and between specimens. Aptroot & Triebel (2002) noted variations in the ascospore dimensions of *P. australis* collections from different hosts, and Diederich (2003) commented that "ascospores within the ... complex are highly variable in size". We therefore speculated that these differences might be correlated with either: (1) the hypothetical criteria used to recognize varieties in *N. oxyspora* by Triebel *et al.* (1995); or (2) with one or more of the segregated host genera. In order to determine whether the topic merited closer investigation, we made a preliminary study of 20 collections on different parmelioid hosts, representing seven species dispersed through six genera, and then analysed the data using a statistical approach apparently not previously used in mycology to explore these posited differences further.

Material and Methods

Specimens examined

The specimens used in this study were as follows:

Flavopunctelia flaventior: **USA**: California: Lake Co., Guenoc Winery, oak woodland, on *Quercus*, 7 v 2000, J. Roberston & R. Robertson 3616 (UC); Lake Co., serpentine rocks and cliffs near pond, Butts Canyon, on *Quercus*, 20 i 2000, R. Robertson 3094 (UC).

Melanelixia subargentifera: **USA**: Idaho Co., Hells Canyon National Recreation Area, Nez Pierce National Forest, south side of Klopton Creek, on *Celtis reticulata*, 25 x 2000, A. Mikulin AQ00-48695 (OSC); *ibid.*, north-east side of Klopton Creek, on *Celtis reticulata*, 27 x 2000, A. Mikulin AQ00-48855 (OSC); *ibid.*, north-west side of Klopton Creek, on *Celtis reticulata*, 24 x 2000, A. Mikulin AQ00-48657 (OSC); *ibid.*, north-west side of Klopton Creek, on *Celtis reticulata*, 24 x 2000, A. Mikulin AQ00-48658 (OSC); *ibid.*, west side of north fork of Klopton Creek, on *Celtis reticulata*,

24 x 2000, *A. Mikulin* AQ00-48671 (OSC); *ibid.*, north-east side of Kirkwood Creek, on *Celtis reticulata*, 27 x 2000, *A. Mikulin* AQ00-48833 (OSC), AQ00-48852 (OSC). **Oregon:** Wallowa Co., Hells Canyon National Recreation Area, Wallowa-Whitman National Forest, north-east side of Lightning Creek, on *Celtis reticulata*, 23 x 2000, *A. Mikulin* AQ00-48617 (OSC).

Melanelixia subaurifera: **USA:** Idaho: Idaho Co., Hells Canyon National Recreation Area, Nez Pierce National Forest, north-east side of Kirkwood Creek, 27 x 2000, *A. Mikulin* AQ00-48804 (OSC).

Melanohalea elegantula: **USA:** Oregon: Wallowa Co., Hell's Canyon National Recreation Area, grazed area northeast of Lightning Creek on *Celtis reticulata*, 23 x 2000, *A. Mikulin* AQ00-48593 (OSC).

Parmelia saxatilis: **Canada:** British Columbia: Spahats Creek Provincial Park, T. R. Goward (CANL).—**China:** Yunnan Province: Wuding Co., secondary conifer forest at Shizishan, Lion Mountain, on rock, *D. L. Hawksworth* DCH 40B (KUN).—**USA:** California: Marin County, oaks with serpentine outcrops near summit of Mt Tamalpias, Mt Tamalpias State Park, on *Quercus* bark, 30 vi 2000, R. Robertson 4520 (UC).

Platismatia glauca: **USA:** California: Marin County, Douglas fir forest on north slope of West Peak, Mt Tamalpias State Park, 30 vi 2000, R. Robertson 5749 (UC).

Punctelia ruddecta: **Canada:** New Brunswick: Charlotte Co., Grand Manandisland, on dead blown-down spruce branches, 14 viii 1996, M. Maxfield (FH).—**USA:** Maine: Penobscot Co., forest with *Tsuga canadensis*, *Thuja occidentalis*, and *Betula*, 24 vii 2000, L. Mann [M. S. Cole 9104] (MIN). Minnesota: Cook Co., fallen and standing *Thuja occidentalis*, *Picea glauca*, and *Abies balsamea* near shoreline of Jasper Lake within the Boundary Waters Canoe Area Wilderness, on fallen *Thuja occidentalis*, 10 viii 1999, M. S. Cole 7622 (MIN).

Measurements

Collections were examined macroscopically with a Nikon stereo-dissecting microscope with an eyepiece reticule at magnifications up to $\times 80$. Microscopic examination was carried out using hand-cut sections and squash preparations in an Olympus BH-2 microscope equipped with Nomarski differential interference contrast optics and a drawing tube. Measurements of ascospores free from the asci were made in water, with at least ten in each collection being measured and drawn with a drawing tube at $\times 3200$. Iodine reactions were performed using Lugol's solution after pre-treatment with 10% potassium hydroxide.

Statistics

The length and breadth of each spore were measured, and the ratio length:breadth of each spore was calculated. That different ascospores may not necessarily be independent variables as they may have arisen in the same ascus, was considered in the choice of analysis method. While this might be overcome if numerous ascospores were available for measurement, with low

numbers this could be a source of error. Multiple regression analysis was therefore performed using robust Huber-White sandwich estimators (White 1980) of the standard errors of the regression coefficients, to take account of the possible correlation between different ascospores from the same ascus. Dummy variables were used to compare every pair of specimen means. No adjustment was made for multiple comparisons. The statistical analysis was performed using Stata Release 8 (StataCorp 2000).

Results

The variation in ascospore shape in the collections studied is shown in Fig. 1; the total number of ascospores measured, mean, and standard deviation of the length, breadth, and length:breadth ratios are presented in Table 2; and a dot-plot for the observed length:breadth ratios is presented as Fig. 2. The differences between every pair of host genus means in length:breadth ratios are given in Table 3, where the most significant differences between pairs of genera are indicated. Highly significant differences ($P < 0.001$) were demonstrated between the *Flavopunctelia/Punctelia*, *Melanelixia/Platismatia*, *Melanelixia/Punctelia*, *Melanohalea/Punctelia*, *Parmelia/Platismatia*, *Parmelia/Punctelia*, and *Platismatia/Punctelia* pairs. There was also a somewhat less significant difference ($P < 0.01$) between the ascospores of specimens growing on species of *Flavopunctelia* and those on *Parmelia*. As the sample size varied considerably between genera, some of the differences in means of >0.40 were not statistically significant.

The colour of the hypothecium varied from very pale tan to brown in all specimens, the intensity of the colour being related to the thickness of the sections; none had a completely colourless hypothecium. Iodine reactions of the hypothecium were negative in all but one collection, one of the seven specimens of *Melanelixia subargentifera* (L-48695).

Discussion

This pilot study supports the hypothesis that there are significant differences in the ascospore shapes between collections of

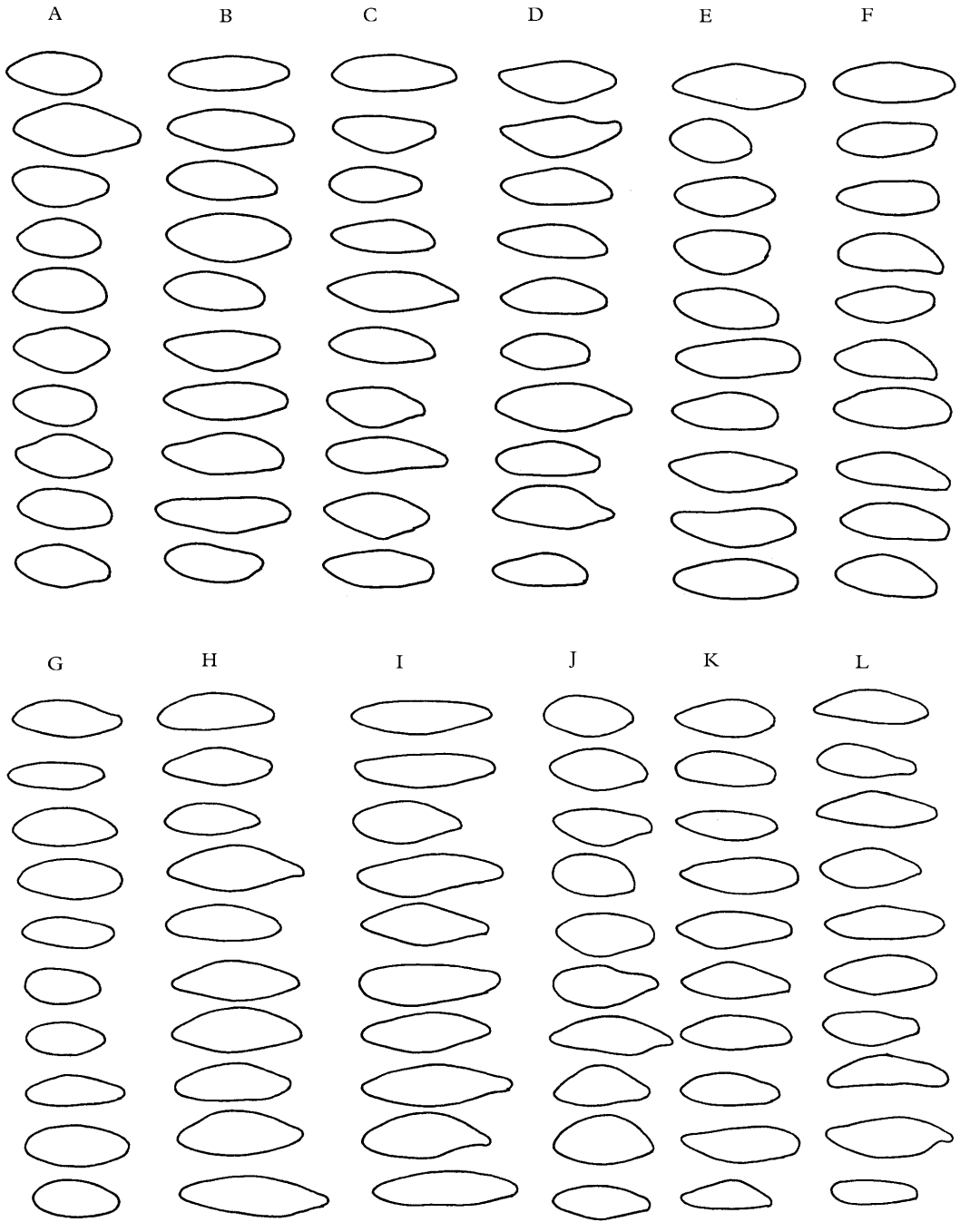
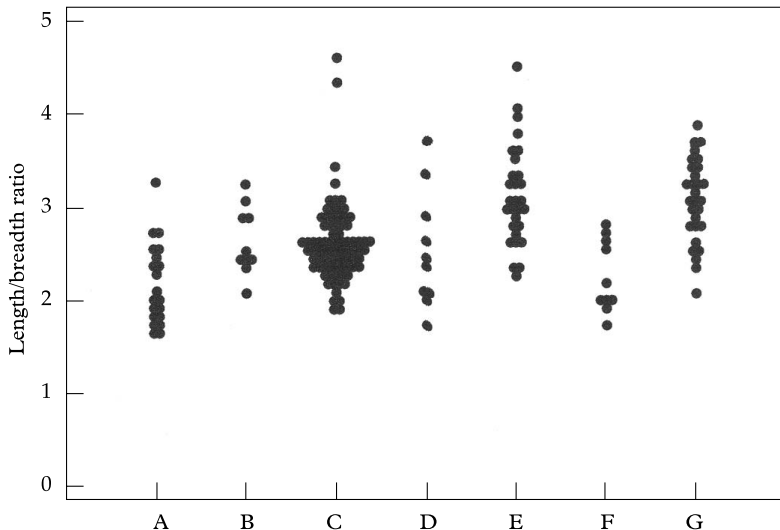


FIG. 1. Ascospore outlines from specimens of the *Nesolecthia oxyspora* complex growing on different host lichens. A & B, *Flavopunctelia flaventior* (A, Robertson 3016; B, Robertson 3094); C, *Melanohalea elegantula* (Mikulín 48593); D–F, *Melanelixia subargentifera* (D, Mikulín 48855; E, Mikulín 48657; F, Mikulín 48695); G, *M. subaurifera* (Mikulín 48804); H & I, *Parmelia saxatilis* (H, Hawksworth DCH 40B; I, Goward); J, *Platismatia glauca* (Robertson 5749); K & L, *Punctelia nudecta* (K, Maxfield s.n.; L, Cole 7622). Scale = 10 μ m.

TABLE 2. Numbers of ascospores measured, mean and standard deviation of the length, breadth, and length:breadth ratio for *Nesolechia oxyspora* on each parmelioid species

Genus	<i>n</i>	Length (µm)	Breadth (µm)	Length/breadth ratio
<i>Flavopunctelia flaventior</i>	20	15.22 ± 1.88	7.11 ± 0.70	2.17 ± 0.43
<i>Melanohalea elegantula</i>	10	15.41 ± 1.84	5.88 ± 0.29	2.63 ± 0.36
<i>Melanelixia subargentifera</i>	80	15.62 ± 1.80	5.91 ± 0.92	2.60 ± 0.42
<i>Melanelixia subaurifera</i>	10	13.53 ± 1.50	5.58 ± 0.97	2.51 ± 0.61
<i>Parmelia saxatilis</i>	29	19.18 ± 2.09	6.23 ± 0.83	3.12 ± 0.53
<i>Platismatia glauca</i>	10	15.26 ± 1.32	6.88 ± 0.82	2.25 ± 0.38
<i>Punctelia rudecta</i>	29	17.51 ± 2.38	5.78 ± 0.70	3.07 ± 0.44

FIG. 2. Dotplot of the observed length:breadth ratios for the ascospores of *Nesolechia oxyspora* on each host species studied. A, *Flavoparmelia flaventior*; B, *Melanohalea elegantula*; C, *Melanelixia subargentifera*; D, *M. subaurifera*; E, *Parmelia saxatilis*; F, *Platismatia glauca*; and G, *Punctelia rudecta*.

Nesolechia oxyspora growing on species placed in different parmelioid lichen genera. While the conclusions would have been more robust if a similar number of ascospores and collections had been available and measured for each host genus, the suggestion that there may be a high degree of host specialization was not rejected by the statistical tests. Whether this is due to genotypic differences, implying cryptic cospeciation of the fungi along with the host lichens, or a phenotypic response to growth on different hosts, could not be resolved. However, this study demonstrates unequivocally that the complex merits a critical reassess-

ment using molecular approaches, using material from the fullest possible range of reported hosts (Table 1), and also with more specimens per host species.

In addition, the results of our tests with iodine reactions on the hypothecium, support Diederich's (2003: 71) view that "this character is of a low taxonomic value" as only one positive was obtained, and that on a host where six other specimens examined gave no result.

We did not study any material on *Xanthoparmelia* (incl. *Neofuscelia*, *Paraparmelia*, etc.; Blanco *et al.* 2004), but note that the dimensions of the ascospores of *Phacopsis*

TABLE 3. Differences between all pairs of host genera in the length:breadth ratios for spores of *Nesolechia oxyspora*

	<i>Flavopunctelia</i>	<i>Melanohalea</i>	<i>Melanelixia</i>	<i>Parmelia</i>	<i>Platismatia</i>
<i>Melanohalea</i>	0.46				
<i>Melanelixia</i>	0.41	-0.05			
<i>Parmelia</i>	0.95*	0.49	0.53		
<i>Platismatia</i>	0.07	-0.39	-0.34**	-0.87**	
<i>Punctelia</i>	0.90**	0.44**	0.49**	-0.05	0.83**

Multiple regression analysis with Huber-White estimates of the standard errors of the regression coefficients was used to compare pairs of genus means.

*Differences in means with $P < 0.01$.

**Differences in means with $P < 0.001$.

australis, given as $(10.5-12-15(-18) \times (4.5-5.5-7(-7.5) \mu\text{m}$ (Aptroot & Triebel 2002), are to the lower end of the range found in our study.

Some further '*Phacopsis*' species are currently recognized on parmelioid lichens: *P. cephalothecoides* on *Hypogymnia physodes* with small ascospores $9-11(-12) \times (4-5-7(-8) \mu\text{m}$ and not curved (Triebel *et al.* 1995); *P. doerfeltii* Scholz on *Arctoparmelia centrifuga* characterized by shorter and broadly ellipsoid ascospores $11-13(-16) \times 7-8(-10) \mu\text{m}$ (Scholz 1998); *P. prolificans* (Müll. Arg.) Triebel & Rambold on *Platismatia interrupta* with narrowly fusiform ascospores $(16-16-17(-20) \times 3-4 \mu\text{m}$ (Rambold & Triebel 1992); and *P. thallicola* (A. Massal.) Triebel & Rambold on *Cetrelia sanguinea*, *Flavoparmelia caperata*, *Flavopunctelia praesignis*, *Rimelia cetrata*, and *Parmotrema eurysacum* with short broadly ellipsoid to almost subglobose ascospores $8-11 \times (4.5-5-6.5(-7) \mu\text{m}$ (Triebel *et al.* 1995). *Phacopsis menegazziae* Triebel & Rambold, described on *Menegazzia terebrata* with ascospores $(12-13-14.5(-16) \times (5-5.5-6(-6.5) \mu\text{m}$ and rounded and not attenuated ends (Triebel *et al.* 1995), was treated as a synonym of the species here called *N. oxyspora* by Diederich (2003) as a different collection on the same host genus had "ascospores similar in size to typical *P. [sic!] oxyspora*". In any future study of the complex, as many as possible of these described taxa, and on usneoid and alectoroid as well as parmelioid hosts, should be included.

This study appears to be the first to use Huber-White sandwich estimators to allow for possible within sample correlations, particularly that ascospores may not be independent variables if they have come from the same ascus. This might not be pertinent when large numbers of ascospores are available for measurement, but is potentially so when only small numbers of ascospores are found which may well have originated from the same ascus. This is clearly an approach that merits more attention in the analysis of measurement information in ascomycete fungi generally, not only those that are lichen-forming.

It is evident that a substantial amount of additional work on the *N. oxyspora* complex is required, but if this can be accomplished and utilized molecular approaches, the indications are that this could provide a model to explore the extent of host specialization to be expected within at least some groups of obligately lichenicolous fungi. The information presented here, showing statistically significant differences between the specimens on some pairs of parmelioid genera, also suggests that a more detailed analysis would generate information that could be used in supporting or opposing the recognition of some of those genera.

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